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JOÃO BOSCO LEITE GUSMÃO JUNIOR

SEDIMENTS AND FUNCTIONAL TRAITS: APPLYING A FUNCTIONAL TRAIT APPROACH TO ASSESS MARINE MACROBENTHIC FUNCTION

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Tese apresentada em co-tutela entre a Universidade Federal do Paraná, Programa de Pós-Graduação em Sistemas Costeiros e Oceânicos, Setor de Ciências da Terra, e a Rijksuniversiteit Groningen, Groningen Institute for Evolutionary Life Sciences, como pré-requisito para a obtenção do grau de Doutor em Sistemas Costeiros e Oceânicos (UFPR) e Doutor em Filosofia (RUG).

Orientadores: Prof. Dr. Paulo da Cunha Lana
Prof. Dr. Britas Klemens Eriksson

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The examining committee named to oppose the thesis defense of **João Bosco Leite Gusmão Junior**, PhD candidate of the Groningen Institute for Evolutionary Life Sciences, University of Groningen (RUG, the Netherlands) and the Post-Graduation in Coastal and Oceanic Systems, Federal University of Paraná (UFPR, Brazil), decided that the doctoral thesis **"Sediments and Functional Traits: Applying a functional trait approach to assess marine macrobenthic function"** was Approved.

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All have their worth and each contributes to the worth of the others

J. R. R. Tolkien

RESUMO

Na presente tese, investiguei como a função do macrobentos marinho de sistemas sedimentares rasos é afetada pela poluição ambiental, complexidade do habitat e mudanças na composição específica das assembleias. Utilizei uma abordagem analítica baseada em traços biológicos para estimar potenciais mudanças na função do macrobentos nos processos ecossistêmicos associados aos sedimentos. Meus objetivos de pesquisa incluem avaliações do papel dos gradientes ambientais naturais ou antropogênicos na estrutura funcional do macrobentos; da aplicabilidade das abordagens baseadas em traços biológicos para analisar a qualidade ambiental do bentos; dos efeitos das mudanças na composição específica das assembleias na função do macrobentos; do efeito da complexidade do habitat na diversidade de traços funcionais; e do impacto de espécies funcionalmente relevantes no funcionamento ecossistêmico. Os resultados e conclusões mais importantes desta tese foram:

1. Baixa qualidade do ambiente bêntico causada pelo enriquecimento orgânico prejudica o estabelecimento de espécies com determinados traços funcionais, resultando em assembleias com baixa diversidade funcional.
2. Abordagens baseadas em traços biológicos são uma forma válida de avaliação os efeitos gerais da poluição orgânica no macrobentos. Tanto a análise de traços biológicos quanto os índices multivariados de diversidade funcional representam boas ferramentas analíticas para a investigação de mudanças na estrutura macrobêntica ao longo de gradientes de contaminação orgânica.
3. As mudanças na estrutura das assembleias promovidas pelo estabelecimento de espécies exóticas podem levar a grandes mudanças na diversidade funcional do macrobentos. Essas mudanças podem aumentar o potencial de bioturbação das assembleias e ter implicações para o funcionamento do ecossistema nos sistemas sedimentares.
4. O aumento da complexidade do habitat promovido pelas gramas marinhas pode promover funções relacionadas com os sedimentos devido ao estabelecimento de organismos bioturbadores que habitam camadas superficiais do sedimento.
5. As espécies funcionalmente relevantes do macrobentos podem promover o microfitobentos, seja através do aumento da bioturbação dos sedimentos ou através da estabilização dos sedimentos devido à atenuação da hidrodinâmica local.

6. As espécies funcionalmente relevantes podem promover cascatas funcionais por favorecer o estabelecimento de organismos que desempenham outras funções. Nos casos abordados nesta tese, as gramas marinhas promovem a infauna que vive próximo à superfície do sedimento e indiretamente media processos relacionados à bioturbação; a bioturbação macrobêntica influencia os produtores primários por afetar a estrutura microfitobêntica e os bancos de mexilhões promovem a produção primária por favorecer o microfitobentos.
7. Os índices de diversidade funcional são úteis como uma ferramenta exploratória para avaliar os efeitos gerais de perturbações ou gradientes ambientais na estrutura do macrobentos. Por outro lado, a análise de traços biológicos ou o enfoque em identidades funcionais representa uma melhor abordagem para avaliar os efeitos funcionais gerais do macrobentos na estrutura e nos processos ecossistêmicos.

ABSTRACT

In this doctoral thesis, I investigate how the function of sediment living organisms is affected by environmental pollution, habitat complexity and changes in assemblage composition in shallow sedimentary systems. I used a trait-based approach to estimate potential changes in function of the macrobenthos in sediment-related ecosystem processes. My research subjects include: the role of natural and human induced gradients in shaping assemblage functional trait structure; the applicability of trait-based approaches to assess environmental status; the effects of changes in assemblage composition on macrobenthic function; the effect of complexity on functional diversity; and the impact of functionally relevant species on ecosystem functioning. The most important findings and conclusions of this thesis are:

1. Low benthic environmental quality driven by organic enrichment hinders the establishment of macrobenthic species with specific functional traits, resulting in assemblages with differentiated trait composition and decreased functional diversity.
2. Trait-based approaches are a reliable way to assess general effects of organic pollution on the macrobenthos. Both biological trait analysis and multivariate functional diversity are good analytical tools to analyze changes in macrobenthic structure across organic contamination gradients, although biomass- and abundance-based analysis can differ.
3. Changes in assemblage structure driven by establishing alien species can drive major changes in macrobenthic functional diversity. These changes can drive increased bioturbation potential, and have implications for ecosystem functioning in sedimentary systems.
4. Increased habitat complexity driven by seagrasses can promote sediment-related functions by favoring the establishment of shallow-living macrobenthic bioturbators.
5. Functionally relevant macrobenthic species can promote microphytobenthos either by increasing sediment bioturbation or by sediment stabilization via flow attenuation.
6. Functionally relevant species can promote functional cascades by favoring the establishment of organisms that perform other functions. In the cases addressed in this thesis, seagrasses promote shallow-living infauna and indirectly mediate bioturbation-related processes; macrobenthic bioturbation drives primary producers by affecting microphytobenthic structure; and mussel beds promote primary production by favoring microphytobenthos.

7. Functional diversity indices are useful as an exploratory tool to assess general effects of disturbances or gradients on macrobenthic structure. On the other hand, biological trait analysis or the focus on functional identities represents a better approach to assess the general functional effects of the macrobenthos on ecosystem structure and processes.

SAMENVATTING

In dit proefschrift onderzoek ik hoe de ecologische functie van bodemorganismen wordt beïnvloed door milieuvervuiling, habitatcomplexiteit en veranderingen in de soortensamenstelling binnen ondiepe sedimentaire ecosystemen. Ik heb gebruik gemaakt van een aanpak gericht op fenotypische kenmerken (een zogeheten trait-based approach) om een inschatting te maken van potentiële veranderingen in de functie van het macrobenthos binnen sedimentgerelateerde ecosysteemprocessen. Onderwerpen binnen mijn onderzoek zijn: de rol van natuurlijke en door de mens veroorzaakte gradiënten bij het vormen van de functionele structuur van ecologische gemeenschappen; de toepasbaarheid van de trait-based approach om de toestand van het milieu te beoordelen; de effecten van veranderingen in de soortensamenstelling op de functie van het macrobenthos; de effecten van complexiteit op functionele diversiteit; en de invloed van functioneel relevante soorten op het functioneren van ecosystemen.

De belangrijkste bevindingen en conclusies van dit proefschrift zijn:

1. Lage kwaliteit van de benthische omgeving, gedreven door organische verrijking, verhindert de vestiging van macrobenthische soorten met specifieke functionele kenmerken. Dit resulteert in samenstellingen met verschillende eigenschappen en verminderde functionele diversiteit.
2. De trait-based approach is een betrouwbare manier om de algemene effecten van organische vervuiling op het macrobenthos te beoordelen. Zowel een analyse van biologische kenmerken als multivariate indices van functionele diversiteit zijn nuttige analytische methoden om de veranderingen in macrobenthische structuur in verschillende gradiënten van organische vervuiling te analyseren, ondanks dat biomassa- en abundantie-analyse kunnen verschillen.
3. Veranderingen in de structuur van de soortensamenstelling gedreven door de vestiging van uitheemse soorten, kan grote veranderingen in de macrobenthische functionele diversiteit veroorzaken. Deze veranderingen kunnen een verhoogd bioturbatiepotentieel veroorzaken en hebben implicaties voor het functioneren van bodemecosystemen.
4. Toegenomen habitatcomplexiteit gedreven door zeegrassen kan sedimentgerelateerde functies bevorderen door de vestiging van ondiep levende macrobenthische bioturbators.

5. Functioneel relevante macrobenthische soorten kunnen het microfytobenthos begunstigen door bioturbatie van het sediment te verhogen of door het sediment te stabiliseren middels het verzwakken van de stroming.
6. Functioneel relevante soorten kunnen functionele cascades bevorderen door de vestiging van organismen die andere functies uitvoeren te begunstigen zoals in de gevallen besproken in dit proefschrift: zeegrassen bevorderen ondiep levende infauna en bemiddelen indirect bioturbatiegerelateerde processen; macrobenthische bioturbatie drijft primaire producenten door de microfytobenthische structuur te beïnvloeden; en mosselbedden bevorderen primaire productie door microfytobenthos te begunstigen.
7. Indicatoren van functionele diversiteit zijn nuttig als verkennend hulpmiddel om de algemene effecten van verstoringen of gradiënten op de macrobenthische structuur te beoordelen. Anderzijds vertegenwoordigen de analyse van biologische kenmerken of de focus op functionele identiteit een betere aanpak om algemene functionele effecten van het macrobenthos op de processen en de structuur van het ecosysteem te beoordelen.

SUMÁRIO

PREFACE

The first time I thought about the relationship between biodiversity and ecosystem function was back during my undergrad years, when I carried out field work in mangroves in Brazil. I was amazed by the diversity of crabs, their activities and life strategies. It was intriguing to see how different crab species behaved and the way they interacted with their environment. Questions like "*what they are doing there?"* or "*why do they behave like that*?" popped out in my mind. Years later, these first thoughts about species functions motivated me to investigate functional diversity. Although my current research subjects are not restricted to crabs or mangroves, I have still kept the interest on how species diversity and function relate, and the potential implications for marine benthic ecosystems. This thesis synthesizes my 4-year research on the functional diversity of marine macrobenthic assemblages. It is my professional contribution to the current understanding of marine benthic biodiversity in unconsolidated sediments, by focusing on functional traits of species rather than taxonomical categories.

CHAPTER 1

INTRODUCTION

Joao B. Gusmao

The study of how habitat variability affects species assemblages is one of the most recurrent topics in ecological literature. In the past century, ecologists dedicated time and efforts to investigate how environment shapes species assemblages. However, they soon realized that biological interactions also shape species assemblages. In the 60s, the concept that consumers control food webs was introduced, creating a new theoretical foundation and novel methodological approaches for community ecology (Hairston et al. 1960, Paine 1966). In the 90s, ecologists recognized the importance of positive biological interactions in determining assemblage species structure (Bertness and Callaway 1994) and introduced the concept of ecosystem engineers (Jones et al. 1994). Recently, non-trophic interactions started to be included in food web models (Kéfi et al. 2012) and described as the main drivers of certain ecosystem services (Donadi et al. 2013b). Such studies highlighted the importance of species functions in regulating assemblages, which are determined by their behavioral and morphophysiological biological traits (Bremner 2008). The rising number of studies addressing species roles on environmental structure and processes reflects these recent advances in ecological science.

Environment-assemblage relationship can be difficult to address when dealing with apparently homogeneous but highly dynamic environments, such as the soft bottom seascapes. Despite their monotonous appearance, marine soft habitats can be very variable in its physical and biogeochemical structure, and thus result in the establishment of diverse species assemblages (Gray and Elliott 2009). Biotic disturbances play an important role in promoting heterogeneity in such environments (Meysman et al. 2006b). Demersal and benthic species can promote bioturbation and alter the physical and biogeochemical characteristics of the substrate. Such changes impact not only the sediment-dwelling organisms, but also have implications for sediment metabolism and water-sediment chemical exchange (Lohrer et al. 2004, Meysman et al. 2006b, Kristensen et al. 2012). Therefore, the understanding of how benthic assemblages are structured would be favored by analytical approaches that include also the information about the functional role of the species in the sedimentary environment. Trait-based ecology has thus grown strong in the past years and recent research has produced relevant information in both aquatic and terrestrial systems (Gerisch et al. 2012, Schirmel and Buchholz 2013, D'Agata et al. 2014, Gusmao et al. 2016). In this thesis, I explore how changes in sedimentary habitats and assemblage structure determine the potential functional roles of macrobenthos in marine coastal soft bottoms.

The soft bottom macrobenthos

Marine soft bottoms are frequently described as vast and homogeneous sedimentary systems. Although apparently monotonous, they are diverse in their physical and biogeochemical structure and may sustain highly diversified species assemblages (Gray and Elliott 2009). Such sedimentary systems are largely controlled by hydrodynamic forces that shape their geomorphology and directly affect the biota (Rosenberg 1995, Paterson and Black 1999). Heterogeneity in such systems is commonly driven by differences in hydrodynamics, small-scale disturbances, presence of hard substrates, and local input of organic matter (Cusson and Bourget 1997, Airoldi 1998). The heterogeneity and complexity of sedimentary systems are also greatly affected by the presence of habitat-forming ecosystem engineers: organisms that can change the environmental conditions and affect direct or indirectly the availability of habitat and resources for other species (Jones et al. 1994). The so-called habitat-forming organisms are autogenic ecosystem engineers that change the environment via their physical structure and therefore create entirely new habitats for other species (Jones et al. 1994). Such organisms and the biogenic structures they produce contribute to increase environmental complexity in soft benthos systems. For instance, marine macrophytes such as seagrasses proportionate flow attenuation and decrease bedload transport, reducing sediment resuspension and increasing sedimentation rates, creating a new habitat for different kinds of animals and algae (Boström et al. 2006b). Bivalve beds on tidal flats introduce hard substrate into an soft-bottom ecosystem, allowing the establishment of fouling organisms and other associated biota (Borthagaray and Carranza 2007). The accumulation of empty bivalve shells in marine bottoms promote changes in sediment texture, affecting directly the infauna (Gutiérrez et al. 2003). Thus, the environmental gradients in biotic and abiotic together with the presence of habitat-forming organisms are the factors that control and promote the heterogeneity of marine sedimentary systems, having direct consequences for the sediment-dwelling organisms.

It is well known that macrobenthic species play a relevant role in the functioning of marine systems. They are important secondary and tertiary producers in the marine food webs, and a food source for economically relevant fisheries (Carlson et al. 1997). Local disturbance in sediment matrix by microbenthic organisms may modulate ecosystem services related to sediment processes by changing the vertical structure of the sediment layers, and modifying particle distribution and sediment stability (Kristensen et al. 2012). Bioturbation also produces biogeochemical changes in the sediments characteristics due to its effects on microbial activity (Mermillod-Blondin 2011). By exposing deeper sediment layers, bioturbation promote sediment aeration and stimulate aerobic microbial activity (Mermillod-Blondin 2011). These changes in sediment redox conditions have major implications for the cycling of nitrogen, sulfur, and organic carbon compounds in marine systems (Snelgrove 1998, Mermillod-Blondin 2011). The capacity of macrobenthic species to change sediment characteristics is directly related to their behavior and morphological traits (Figure 1). Epifaunal species can affect the sediment surface by selective deposit feeding, despite their limited capacity to disturb the subsurface sediment matrix (Alvarez et al. 2013). Conversely, sediment biodiffusers can disturb sediment matrix in all directions, destabilizing the substrate and increasing nutrient release (Kristensen et al. 2012). Tube and gallery-dwellers not only promote deeper oxygenation of the sediment, but also can change sediment microtopography (Kristensen et al. 2012, Berke 2012). Bioirrigators promote water flow through the sediment matrix, resulting in increased nutrient release (Chennu et al. 2015). Upward and downward conveyors transport material out or down into the sediment and may directly regulate the release and burial of organic matter in the sediment matrix (Kristensen et al. 2012). Besides, the size of the animals put weight in their capacity to change the substrate (Solan et al. 2004, Queirós et al. 2013). Considering the differences in bioturbation capacities among macrobenthic species, changes in assemblage structure have clear implications for sediment-related processes mediated by the fauna.

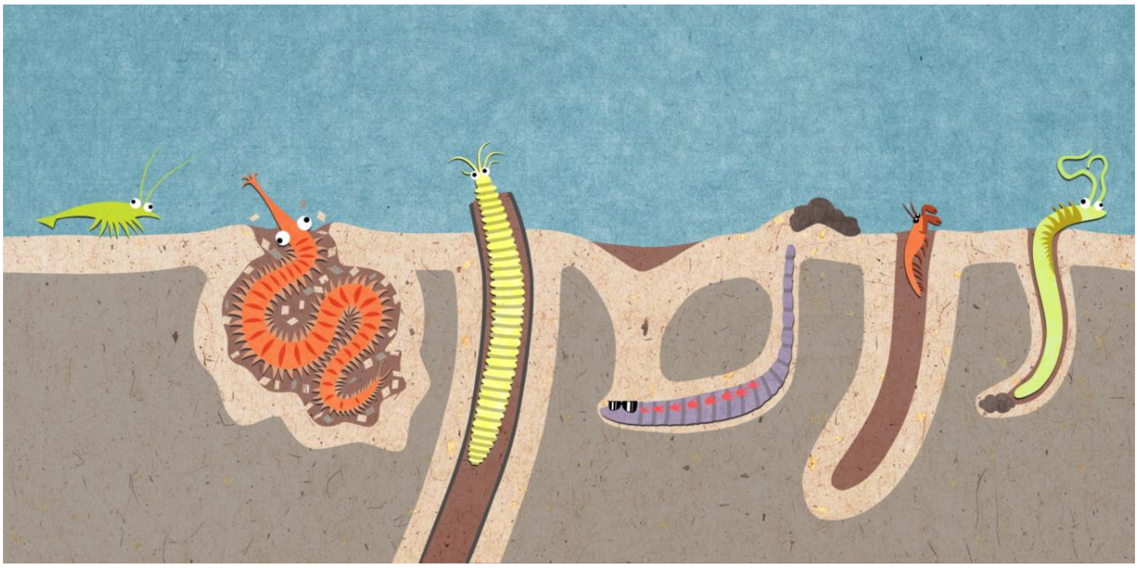


Figure 1: Macrobenthic species can process and modify soft-sediments in contrasting ways. From the left to the right: an epifaunal shrimp with limited bioturbation capacity; a goniadid polychaete disturbing sediment in all directions (biodiffusing); a tude-dwelling onuphid worm; a lugworm bioirrigating the substrate and depositing fecal material in the surface (upward-conveyor); an gallery-dweller shrimp; and a spionid polychaete depositing fecal material under the surface (downward-conveyor).

Disturbances are key factors in structuring marine benthos and one of the most important controlling drivers of ecosystem structure and functioning in marine sedimentary environments (Zajac et al. 1998, Ford et al. 1999, Dernie et al. 2003). Small scale disturbances driven by bioturbators play an important role in maintaining a mosaic of patches in different successional stages, promoting local heterogeneity and favoring increased beta and gamma diversities and ecological services (Thrush and Dayton 2002). However, natural or human-driven large-scale disturbances can have drastic effects on benthic biota and consequently affect process rates in sedimentary systems (Lohrer et al. 2004). Disturbances such as bottom trawling and dredging can eliminate habitat-forming organisms and remove big macrobenthic bioturbators from large areas in the seafloor, resulting in habitat losses and noticeable reductions in environmental heterogeneity (Thrush and Dayton 2002). The increasing pressure of human-driven large-scale disturbances on coastal seascapes, such as climate change and overfishing, have generated concern among researchers and marine managers about the functional consequences of benthic biodiversity loss (Thrush and Dayton 2002, Lohrer et al. 2004). Analytical tools that integrate information about macrobenthic species functional traits to estimate the relationship between species composition and community function are thus valuable to predict potential services losses in case of local or broader scale disturbances.

Functional trait approach

It is well known that biodiversity has major implications on ecosystem functioning (Hooper et al. 2005), mostly related to the variety of attributes presented by different species, which modulate ecosystem state and processes (Hooper et al. 2005). Trait-based approaches have been proposed to assess biodiversity effects on ecosystem states, on the assumption that trait diversity and variability are related to changes in functions across environmental gradients (Bremner et al. 2006a, Duffy et al. 2015). Such an approach allow for the direct comparison of putative ecological differences among species, and may thus assess their general functional role in an ecosystem, and estimate the potential function of the assembly (Norberg 2004, Gagic et al. 2015). Functional diversity is a measure based on trait distributions that gives a general overview of how variable are the functions performed by different species in an ecological system (Díaz and Cabido 2001, Petchey and Gaston 2006). It is expressed by the diversity of functional traits, whcih are any organismal characteristics related to individuals' performance (i.e. survival, growth, and reproduction success) directly or indirectly related to one or more ecosystem functions or processes (Mlambo 2014). For instance, in soft bottom marine benthos, the behavioral traits related to sediment bioturbation are considered functional traits since they affect sediment stability and biogeochemistry (Snelgrove 1998, Austen et al. 2002, Widdows and Brinsley 2002, Reise 2002, Bremner et al. 2006a). Functional diversity is highly related to the maintenance of ecosystem functions, such as productivity (Díaz and Cabido 2001, Tilman 2001, Hooper et al. 2005) and resilience to disturbances or invasions (Dukes 2001, Villéger et al. 2008, Mouillot et al. 2013). Other functional approaches focuses in trait turnover or the variation of specific functional identities (i.e. species with specific trait combinations) to explore assemblage roles in ecosystem function (Bremner et al. 2006a, Mokany et al. 2008, Gagic et al. 2015). Indices based on functional traits are considered more informative or sensitive than taxonomic-based indices to predict ecosystem function (Díaz and Cabido 2001, Mokany et al. 2008, Gagic et al. 2015). Therefore, trait-based approaches are currently considered a necessary analytical development to investigate the importance of biodiversity in maintaining ecosystem state and processes rates (McGill et al. 2006, Hillebrand and Matthiessen 2009).

Besides ecological studies, trait-based approaches are useful for impact assessment and environmental monitoring. Trait-based approaches produce novel information about the effect of natural and human-induced perturbations on species assemblages (Mouillot et al. 2013). By including information about the species functional differences, they may detect assemblage responses to disturbances that have functional consequences and would go undetectable if only taxonomic based approaches were used (Gerisch et al. 2012, Mouillot et al. 2013, Gagic et al. 2015). For instance, a disturbance that harms a dominant species that have unique and extreme trait combinations could have a positive effect on taxonomic diversity (i.e. due to increased species evenness), but the effects on functional diversity would be negative due to decreases in assemblage functional richness or divergence. These differences are visualized in a conceptual model depicting changes in taxonomic and functional trait diversity along a disturbance gradient (Figure 2a-b). In this model, species diversity describes a bell-shaped pattern along the disturbance gradient (Figure 2a), reflecting the intermediary disturbance hypothesis (Connell 1978), while functional diversity depicts a continuous decrease (Figure 2b), showing that assemblages in intermediary disturbances levels present increased functional redundancy. Models considering pulse disturbances can also depict differences in taxonomic and functional diversity (Figure 2c-d). A disturbance that promotes temporary decreases in species diversity may cause long-term effects on functional diversity due to increased susceptibility of certain species with specific functional trait combinations (Figure 2c-d). Therefore, trait-based approaches may assess the vulnerability of potential functions of macrobenthic assemblages and the potential consequences for certain ecosystem services, such as sediment related process. This also can give insights over the general consequences of functional services losses in case of reductions in functionally relevant species.

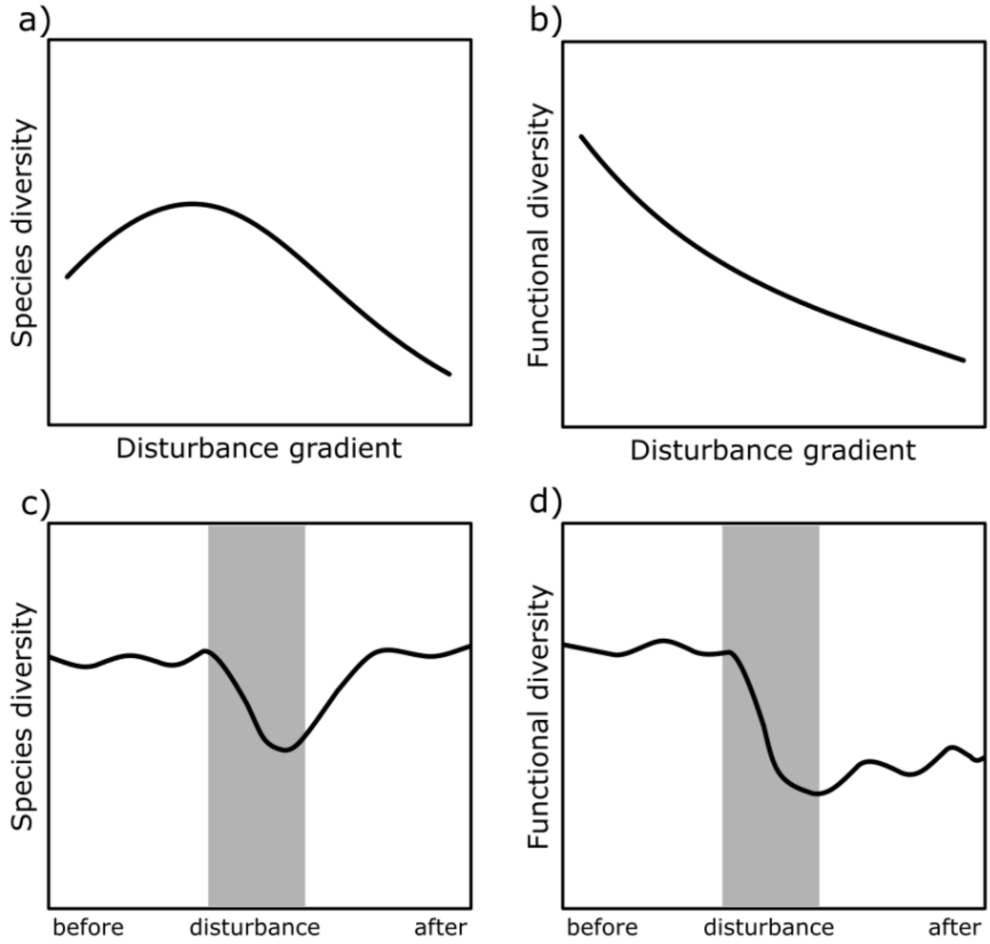
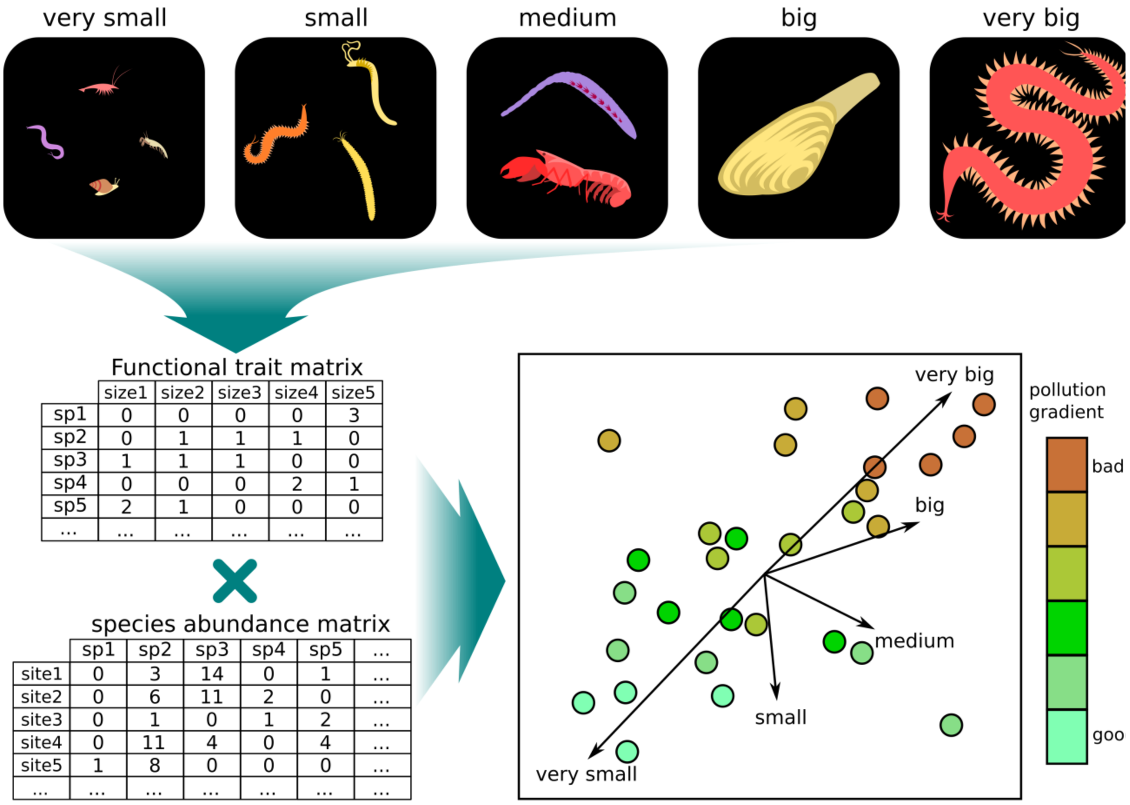


Figure 2: Distinct responses of taxonomic and functional diversity to environmental disturbances. Species diversity depicting an unimodal variation trend along a disturbance gradient (a), while functional diversity depicts a monotonic trend (b). After a pulse disturbance, the species diversity recover (c), while the effects on functional diversity stay (d), showing that the recovered assemblage has increased functional redundancy.

Different techniques and analytical tools are currently used to assess the functional trait diversity of ecological systems. The most popular approaches are analyses based on functional groups (Bolam and Eggleton 2014), biological traits analysis (BTA, Bremner, Rogers & Frid 2006b), and the quantification of indices for functional diversity (Villéger et al. 2008, Laliberté and Legendre 2010). BTA is a method to describe changes in assemblage trait composition (Box 1.1). By weighting trait frequencies to species abundances, the BTA can directly relate specific traits (or trait categories) to environmental gradients. Functional diversity indices have different abilities to describe the functional structure of an assemblage, including information about how functionally different are the species in an assemblage and how the abundances are distributed among them (Box 1.2). Both BTA (Box 1.1) and multivariate functional diversity indices (Box 1.2) have been largely used in the past decade to assess functional trait diversity and composition in different kinds of terrestrial and aquatic ecological systems (Gerisch et al. 2012, Schirmel and Buchholz 2013, Luck et al. 2013). Such trait-based approaches have produced promising results about the relationship between environmental factors and assemblage structure of benthic systems (Gusmao et al. 2016, Otegui et al. 2016, Linden et al. 2017).

BOX 1.1. Biological traits analysis

Biological traits analyses (BTA) is a method to describe variation in assemblage trait composition based on the abundances or frequencies of species functional traits. Assuming that the chosen functional traits are related to specific aspects of ecosystem functioning (e.g. feeding behavior traits indicate energy flow within an assemblage), BTA represent how individual traits are related to environmental factors and ecosystem functioning (Bremner et al. 2006a, 2006b, Pacheco et al. 2011, van der Linden et al. 2012). Multivariate ordinations, such as correspondence analysis, are the most common methods to perform a BTA. The analysis considers two different matrices: a species abundance per sample matrix; and a functional trait matrix (Figure 3). The last is a *fuzzy-coded* matrix that represent the species association degree to different categories of a functional trait by using a score rank system, usually ranging from 0 to 3 (Chevenet et al. 1994). For instance, considering the functional trait *feeding mode*, a herbivorous species would have the score 3 for the category ‘herbivore’ and 0 for the others, while an omnivorous species would have scores 1.5 for both categories ‘herbivore’ and ‘carnivore’. A third matrix is generated by multiplying the abundance matrix by the trait scores matrix, which represent trait frequencies weighted by the species abundances in each sample. A correspondence analysis based in this ‘trait per sample’ matrix can be used to depict changes in trait compositions along environmental gradients (Figure 3).



Functional trait matrix

	size1	size2	size3	size4	size5
sp1	0	0	0	0	3
sp2	0	1	1	1	0
sp3	1	1	1	0	0
sp4	0	0	0	2	1
sp5	2	1	0	0	0
...	...	...	...	...	...

species abundance matrix

	sp1	sp2	sp3	sp4	sp5	...
site1	0	3	14	0	1	...
site2	0	6	11	2	0	...
site3	0	1	0	1	2	...
site4	0	11	4	0	4	...
site5	1	8	0	0	0	...
...	...	...	...	...	...	...

Figure 3: Diagram of steps to perform a biological traits analysis is based on the functional trait *relative adult size*. The trait include 5 categories, varying from very small to very big. Fuzzy scores are assigned to each species depending on their association degree to each trait category. By weighting the trait frequencies by the species abundances in each sampling site, it is possible to represent the variation trends in trait composition across environmental gradients. In the above example, bigger species are associated to polluted sites.

BOX 1.2. Functional diversity indices

Functional diversity can be assessed by using indices that quantify how species differ from each other according to their functional traits. Such indices can be based in one or more functional traits and usually represent different aspects of the functional trait space of an assemblage. This space represents the general spectrum of trait values observed in an assemblage. If only two continuous functional traits are considered, the functional trait space can be represented in a two-dimensional graph. There are indices that are only based on continuous traits, but some can also deal with categorical, binary, or multiple types of trait information. Laliberté & Legendre (2010) proposed a set of multivariate functional indices that deal with different kinds of trait information at the same time. These indices are commonly used today and include Functional Richness (FRic), Functional Evenness (FEve), Functional Divergence (FDiv), and Functional Dispersion (FDis). FRic measures the total extension of the trait values in an assemblage and is largely affected by adding or removing species with extreme or unique trait values. FEve measures the regularity of the distribution of the species abundances in the functional trait space of in an assemblage. FEve can be affected by disproportional changes in abundances in species with contrasting trait values. FDiv and FDis measure how species abundances are distributed in the functional trait space. FDiv is a measure of how species abundances diverge from the general trend of the assemblage (average distance from the centroid of the functional space), while FDis measures how disperse are the species in the functional trait space (weighted by their abundances). Both indices are highly sensible to changes in number and abundance of species with average (located at the center of the functional trait space) or extreme trait values (located at the periphery of the functional trait space).

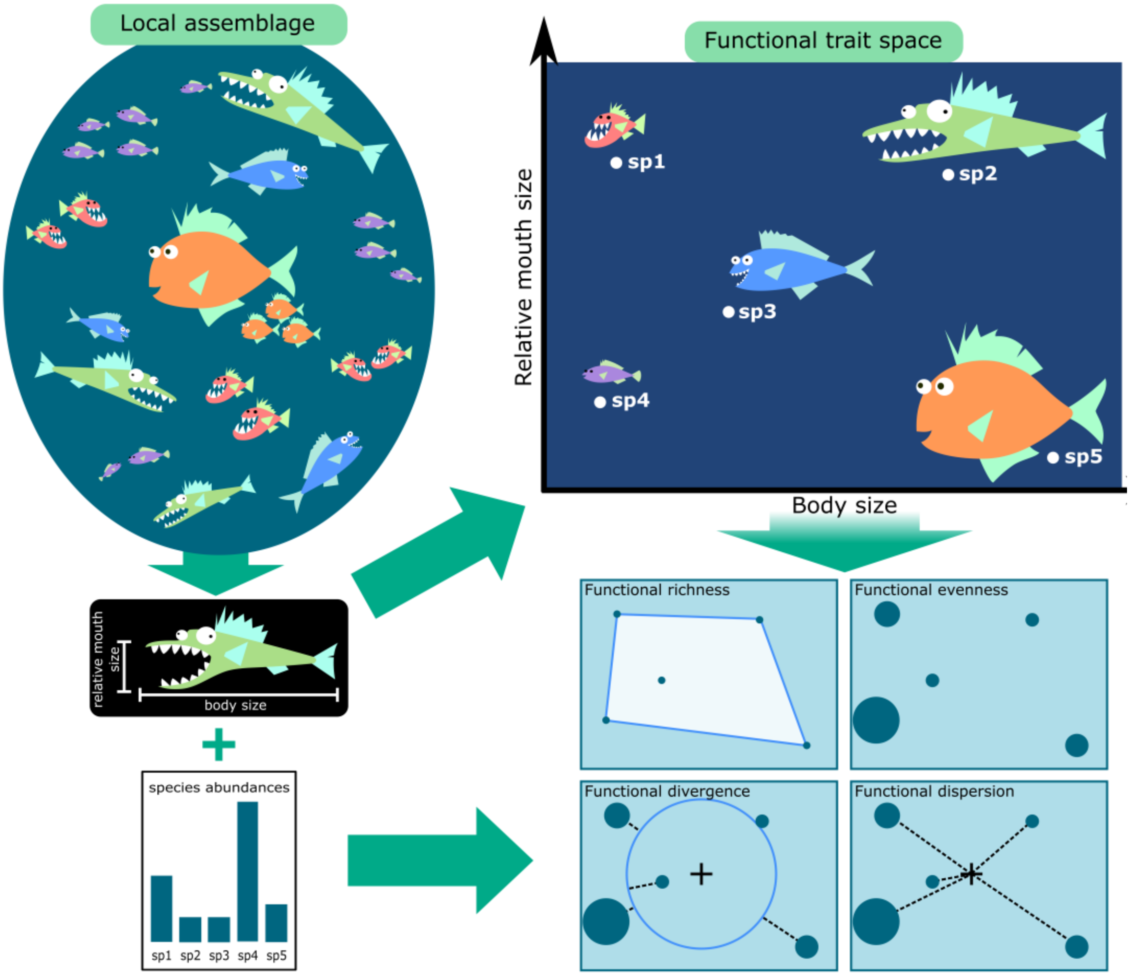


Figure 4: Diagram depicting the steps to represent the functional trait space of an assemblage and the different ways to quantify functional diversity. By estimating the average values of two functional traits of each species, is possible to construct a two-dimension functional trait space with each axis representing the values of one functional trait. The four different functional diversity indices complement each other by measuring different aspects of this functional space, and considering the information of species abundances or not in the calculations.

OUTLINE OF THE THESIS

This doctoral thesis investigate how macrobenthic function in shallow sedimentary systems is affected by both environmental variation and changes in assemblage composition. Species functional traits are used to determine the potential function of the macrobenthos in sediment-related ecosystem processes. The general addressed questions were:

(1) How do natural and human-induced environmental gradients affect the functional diversity of macrobenthic assemblages?
(2) Can we use functional trait distributions of species assemblages to assess environmental status?
(3) How do changes in species composition relate to functional diversity and changes in potential functioning?
(4) Does habitat complexity favor functional trait diversity?
(5) Can functionally important species such as habitat-forming species and ecosystem engineers induce functional cascades by determining the function of associated assemblages?

The research questions were addressed in five different studies, which include two sampling efforts conducted in tidal flats in Paranaguá Bay, southern Brazil (Chapters 2 and 5); data collected during fifteen years from tidal flats of the Dutch Wadden sea (Chapter 3); data collected in seagrasses from three continents and five different countries in the northern hemisphere (Chapter 4); and an experiment conducted in the Dutch Wadden sea (Chapter 6).

In **Chapter 2**, “*Functional diversity of macrobenthic assemblages decreases in response to sewage discharges”,* I investigated how macrobenthic functional diversity changes across different organic contamination conditions in a subtropical estuarine system. The study focused on testing the efficiency of different measures of functional diversity in analyzing the effects of pollution gradients on macrobenthic assemblages.

In **Chapter 3**, “*Effects of alien species on taxonomic and functional diversity of intertidal soft-bottom macrobenthic assemblages”,* I compared how large species composition shifts are reflected by different indices of functional diversity and measures of potential function, by analyzing temporal trends related to increasing number and biomass of alien species at a site in the the Dutch Wadden Sea.

In **Chapter 4**, “*Seagrass meadows induce a functional cascade by determining the functional trait composition of sediment fauna”,* I tested how potential functions related to sediment process performed by macrobenthos are influenced by the meadow structure of the seagrass *Zostera marina* across the northern hemisphere.

In **Chapter 5**, *"Effects of macrobenthic functional diversity on microphytobenthic assemblages",* I assessed how macrobenthic functional diversity affects the spatial trends in microphytobenthic chlorophyll *a* and diversity across tidal flats in a subtropical estuarine system. The aim was assess the relationship between functional trait diversity and an ecosystem service (primary production). The results of this study lead us to a field experiment that constitute the **Chapter 6**,

In **Chapter 6**, "*Mussel beds are biological power stations on intertidal flats*" we experimentally tested how a functionally important ecosystem engineer, the reef-forming blue mussel *Mytilus edulis*, influence the abundance of microphytobenthos in the sediment on a tidal flat. We hypothesized that mussel reefs, by decreasing bioturbation activities and hydrodynamic stress, increase the production of the biofilm.

Finally, the synthesis of the thesis‘ main findings are presented in **Chapter 7**. Here I discuss the general conclusions about the factors that shape macrobenthic function and the potential implications for ecosystem functioning.

CHAPTER 2

Functional diversity of macrobenthic assemblages decreases in response

Joao B. Gusmao, Kalina M. Brauko, Britas Klemens Eriksson, Paulo Lana

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, FDiv 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. FDiv (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 FDiv 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.

Ecological Indicators (2016) 66:65-75

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 (Violle et al. 2007, Mlambo 2014). 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 (Bremner et al. 2006a, Gerisch et al. 2012).

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, Rogers & Frid 2006b) and calculation of functional diversity indices (Villéger et al. 2008, Laliberté and Legendre 2010). 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, 2006b, Pacheco et al. 2011, Darr et al. 2014). 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, Villéger et al. 2008, Laliberté and Legendre 2010). FRic, FEve and 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, Borja & Bald 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.

METHODS

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 km², 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 (Figure 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 (Marone and Jamiyanaa 1997, Lana et al. 2001). Intertidal habitats cover about 34% of its surface area and include mangroves, marshes and tidal flats (Noernberg et al. 2006, Brauko et al. 2015).

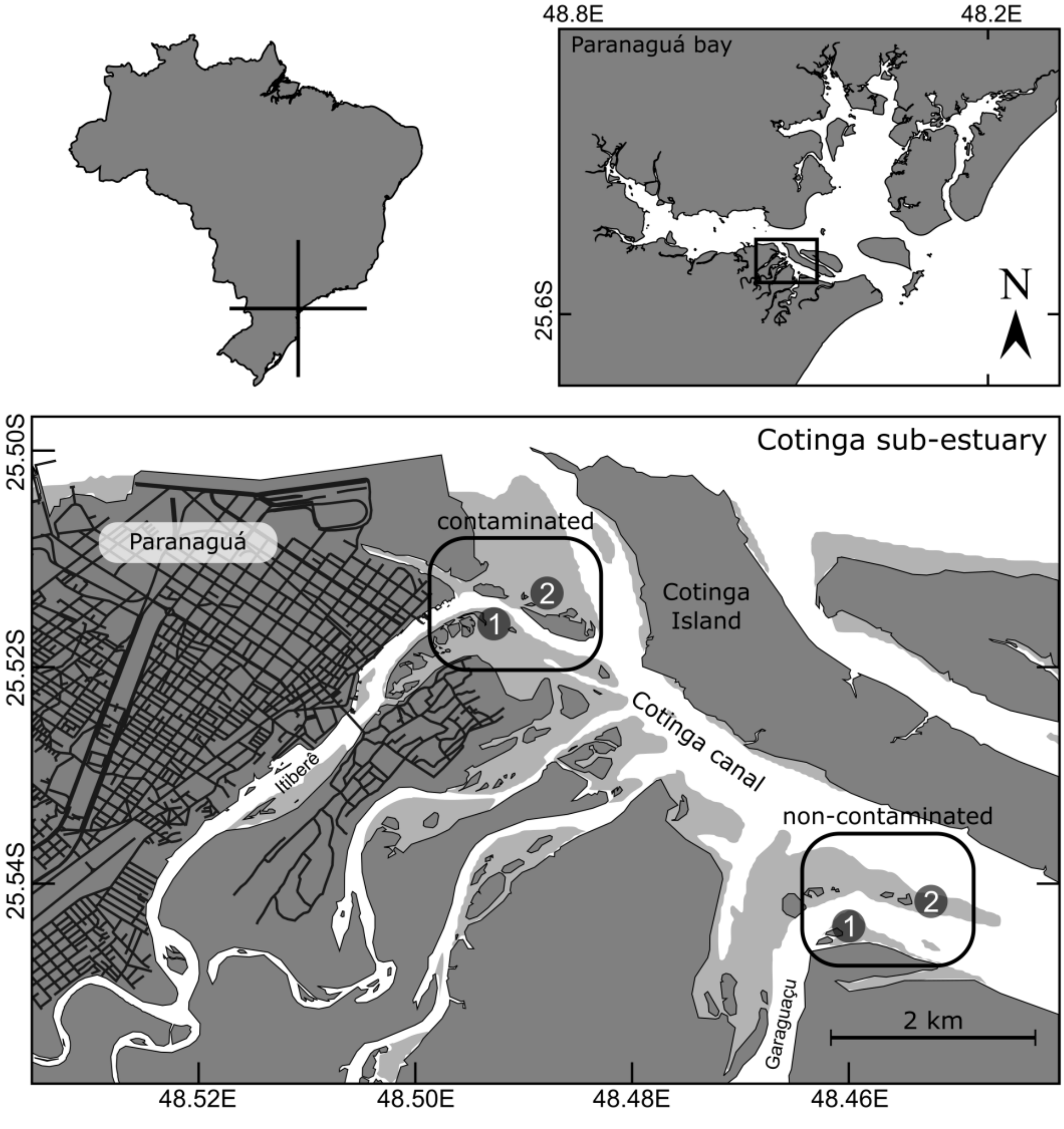


Figure 1: Location of the Cotinga sub-estuary in Paranaguá Bay. The distribuition of tidal flats are depicted in light grey. The contaminated and non-contaminated conditons and the sampled tidal flats (1 and 2) in each condition are highlighted.

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 (Martins et al. 2010, Barboza et al. 2013). 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).

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 & Montone (2002). Concentrations of N and P followed Grasshoff, Ehrhardt & Kremling (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.

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 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 & Frid (2009), the five traits were further sub-divided into several modalities (Box 2) 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 (MarLIN 2006, Faulwetter et al. 2014), literature (Arruda et al. 2003, Rios 2009, Queirós et al. 2013, Jumars et al. 2015) 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).

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 (Schleuter et al. 2010, Laliberté and Legendre 2010, Mouchet 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 to 1.2 corresponds to *High*; 1.2 to 3.3 for *Good*; 3.3 to 4.3 for *Moderate*; 4.3 to 5.5 for *Poor* and 5.5 to 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).

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 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.

RESULTS

Environmental variables

The PCA ordination of the 10 environmental variables revealed gradients within the first two axes (Figure 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.

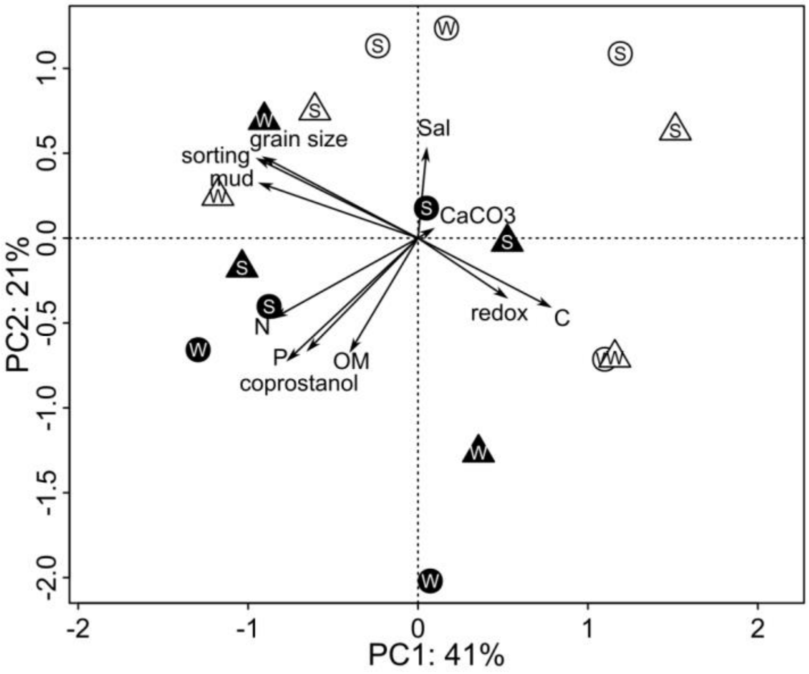


Figure 2: Principal Component Analysis (PCA) depicting the environmental variables for each season, condition and tidal flat. W = winter, S = summer, black circles = contaminated, white circles = non-contaminated, circles = tidal flat 1, triangles = tidal flat 2, OM = organic matter content, Sal = salinity,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

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

Environmental variables	Winter		Summer	
	Contaminated	Non-contaminated	Contaminated	Non-contaminated
Coprostanol (µg 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

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 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, Figure 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 (Figure 4c).

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 (Figure 3a to 3f). 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 to 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 (Figure 3g to 3l). All the BTAs also showed clusters of assemblages sampled in the same tidal flat and season.

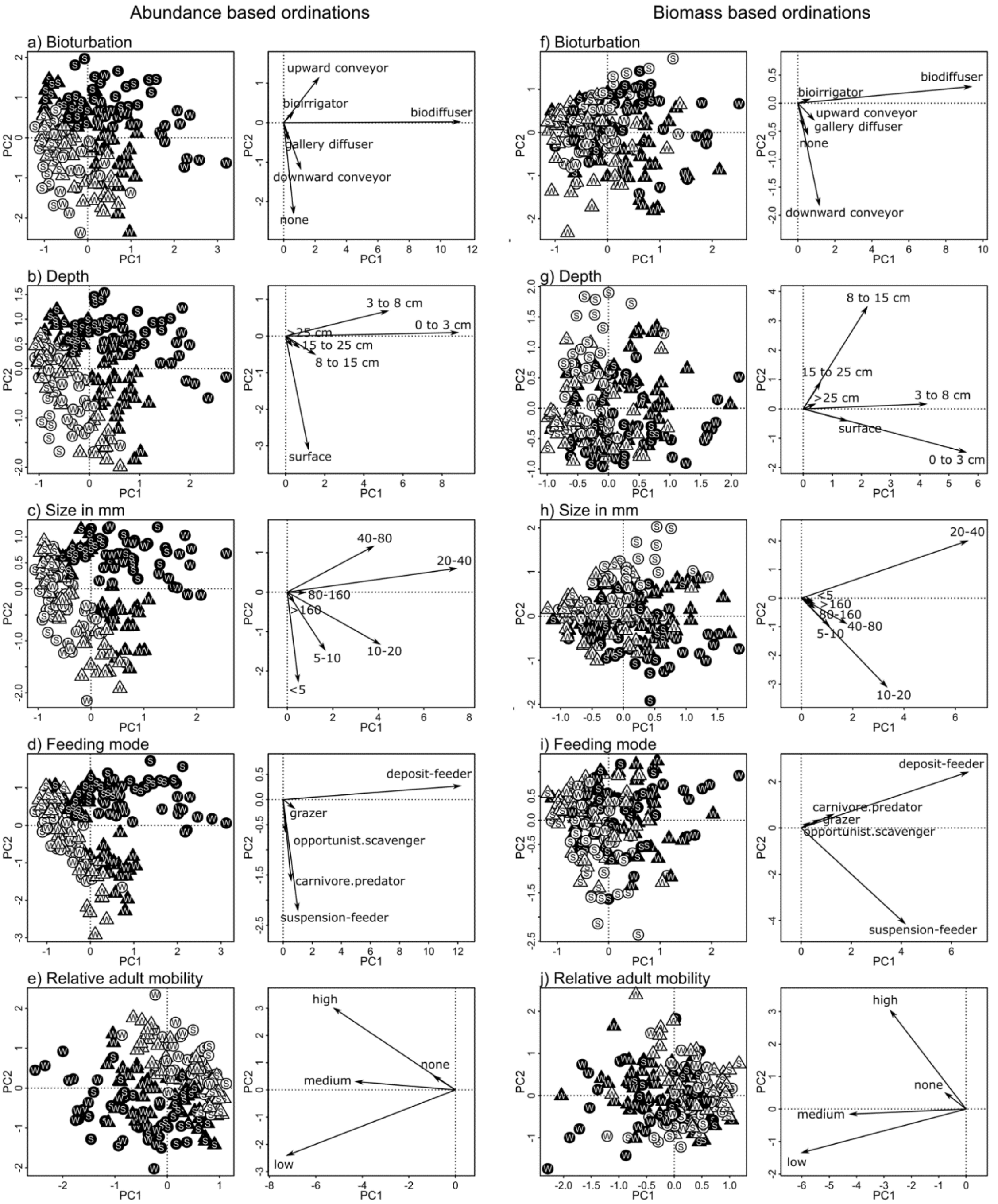


Figure 3: PCA ordinations depicting the variability in assemblage trait composition across season and condition. W = winter, S = summer, black symbols = contaminated, white symbols = non-contaminated, circles = tidal flat 1, triangles = tidal flat 2.

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

Abundance-based BTAs

	Bioturbation	Depth	Size	Feed	Mobility
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

	Bioturbation	Depth	Size	Feed	Mobility
PCA1	91.8%	73.0%	72.3%	70.0%	75.2%
PCA2	4.0%	18.0%	19.2%	25.6%	13.6%

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 Figure A1 and 2). The FRic (Table 3, Figure 4c) followed the trends observed for species richness (Figure 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, Figure 4d, 4g), which differences between conditions were dependent on the seasons and tidal flats. FDiv (Table 3, Figure 4e, 4h) 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, Figure 4f, 4i). Abundance-based Rao's Q showed significantly higher values in the non-contaminated condition (Figure 4f), while for the biomass-based index this differences were highly dependent on the season (Figure 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 and non-contaminated conditions depended on the season and tidal flat analyzed.

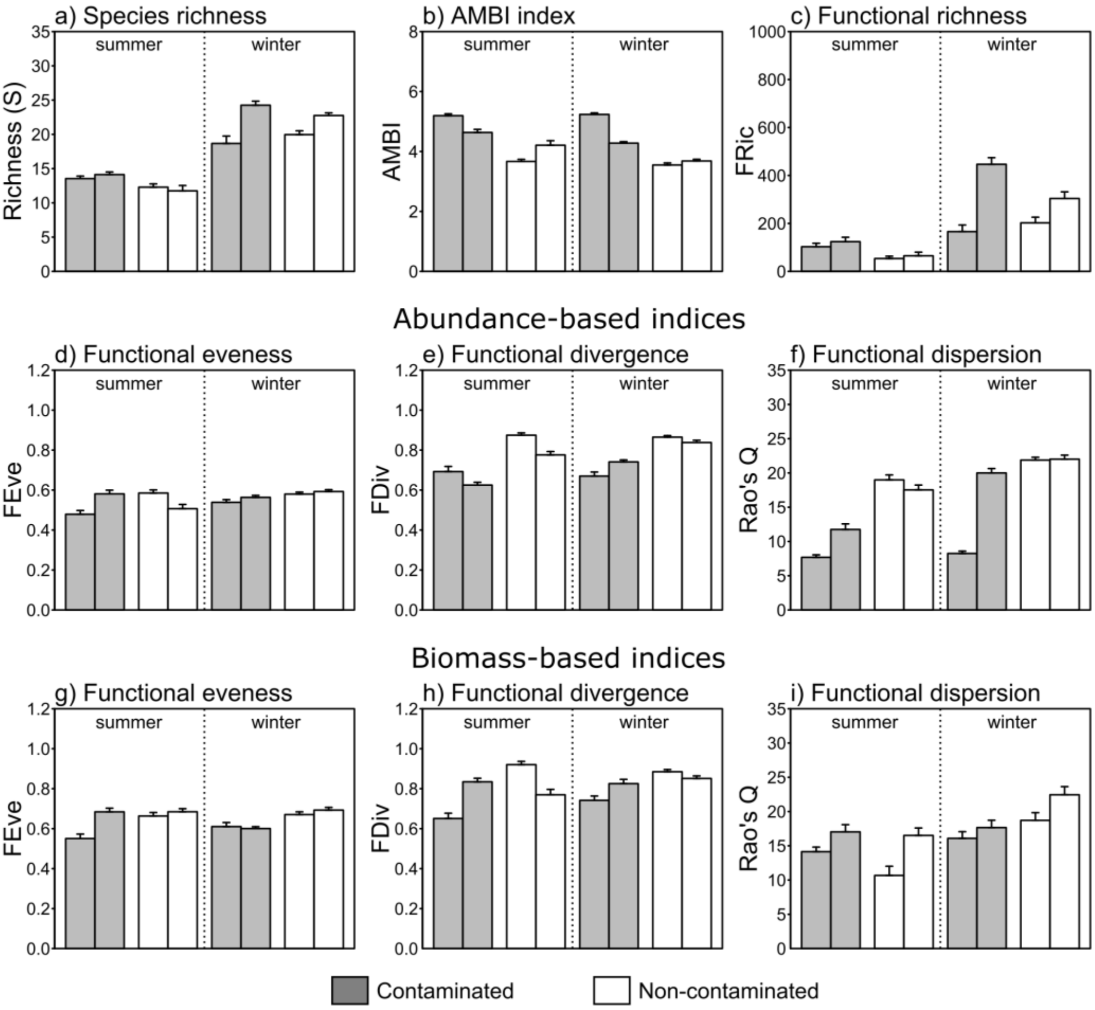


Figure 4: Mean values (± SE) of species richness (a), AMBI index for environmental health (b), functional richness (c), the abundance (d-f) and biomass-based (g-i) functional evenness, functional divergence and functional dispersion for tidal flat, condition and season.

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).

		Species richness[1]		AMBI[2]		FRic[1]	
Source	df	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						

Abundance-based indices

		FEve		FDiv[1]		Rao's Q	
Source	df	F-value	P-value	F-value	P-value	F-value	P-value
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						

Biomass-based indices

		FEve		FDiv		Rao's Q[1]	
Source	df	F-value	P-value	F-value	P-value	F-value	P-value
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

The relationship between the functional indices and the AMBI index depended on how the macrobenthos was quantified (Figure 5). All the abundance based indices were negatively related with the AMBI (i.e. increased with increasing environmental health Figures 5b, 5d and 5f). For the biomass-based indices, only FDiv was significantly related with AMBI, with a bell-shaped relationship (Figure 5e). FRic showed no significant relationship with AMBI (Figure 5a) but was the only functional index positively related with species richness ($p < 0.05$, $r^2 = 0.79$, Figure A3).

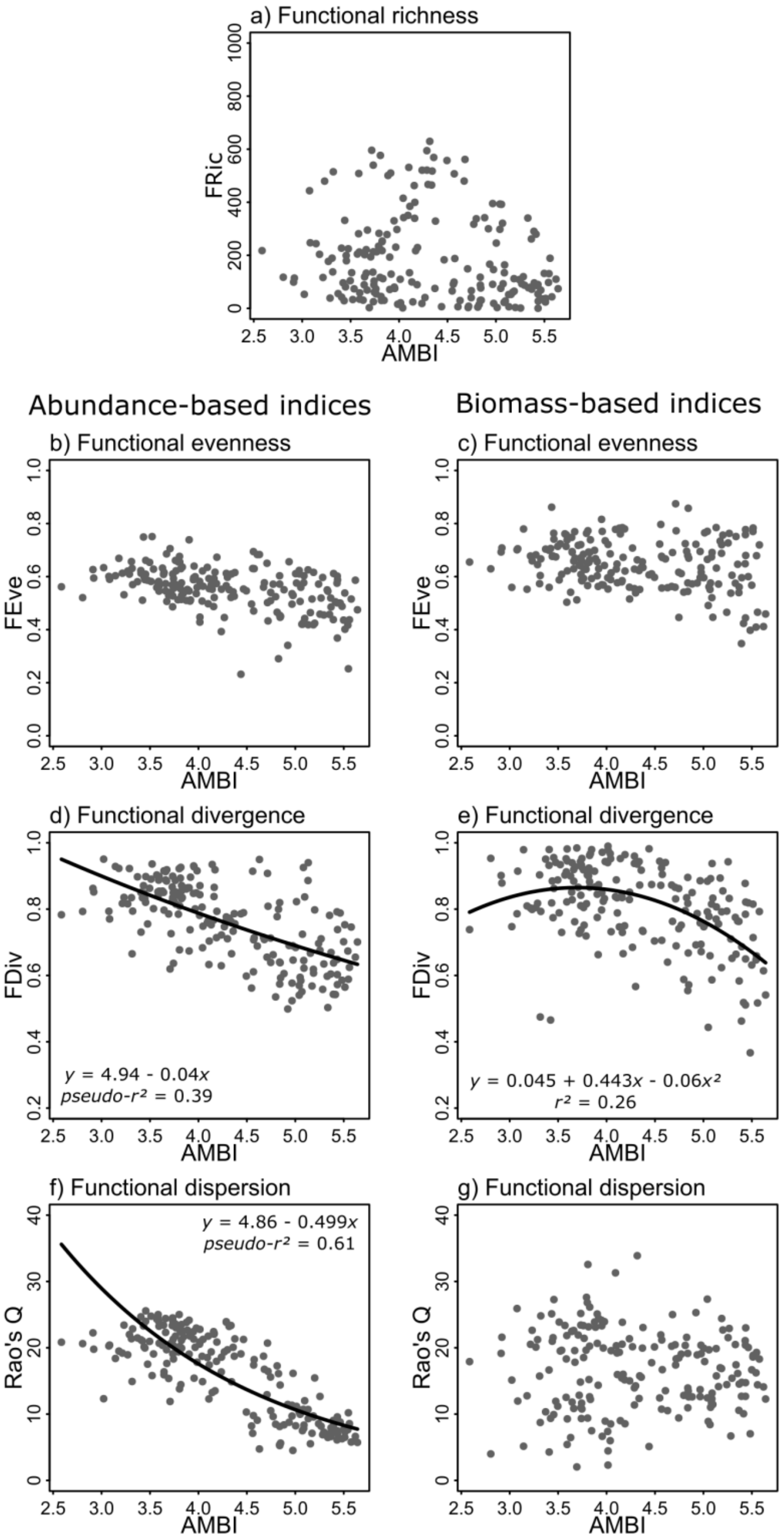


Figure 5: Relationship between the functional and AMBI indices.

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.

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

(Pagliosa and Barbosa 2006, Elliott and Quintino 2007, Brauko et al. 2015). Second, the dominance of carnivore/predators and suspension feeders in the non-contaminated condition could be related to increased sediment quality, which 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).

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.

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.

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, Kristensen et al. 2012, Jumars et al. 2015), 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.

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, Poos et al. 2009, Lefcheck et al. 2015). 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 (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 2011a), 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.

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, FDiv 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 FDiv 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

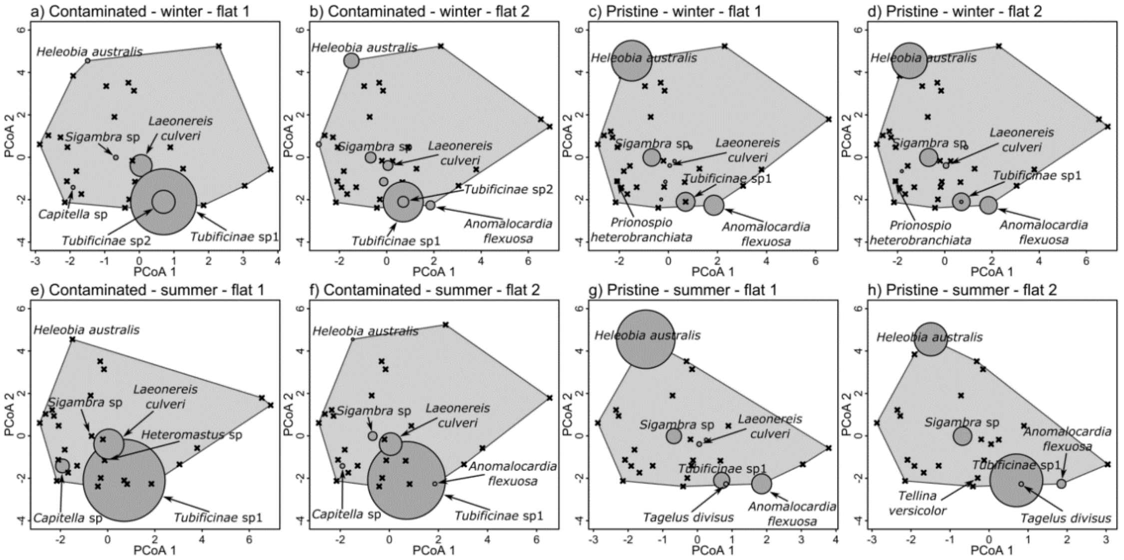

Figure A1: Abundance-based representation of the assemblage functional space for each condition and season. Circles sizes are proportional to species relative abundance.

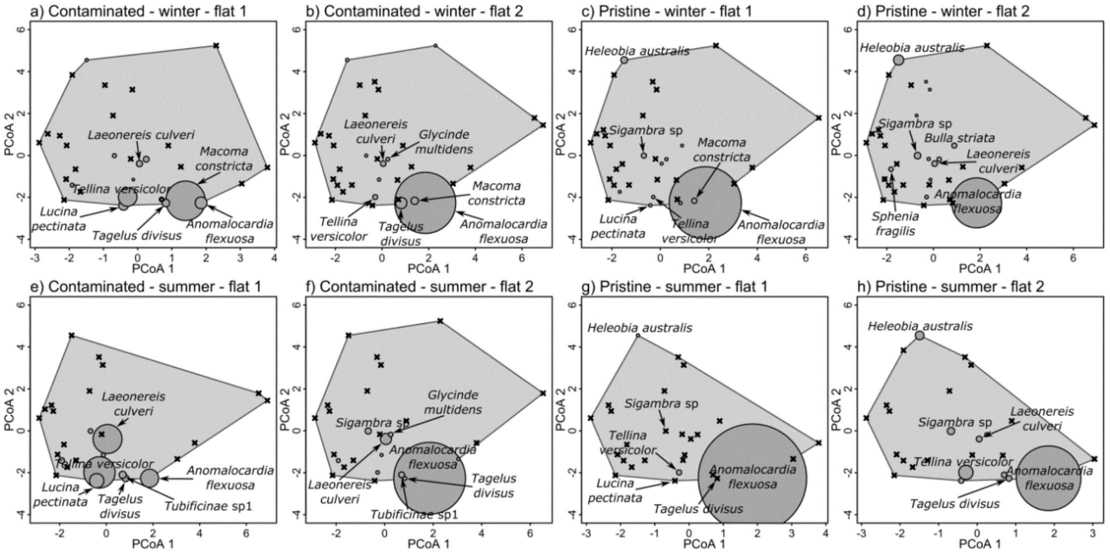

Figure A2: Biomass-based representation of the assemblage functional space for each condition and season. Circles sizes are proportional to the species relative biomass.

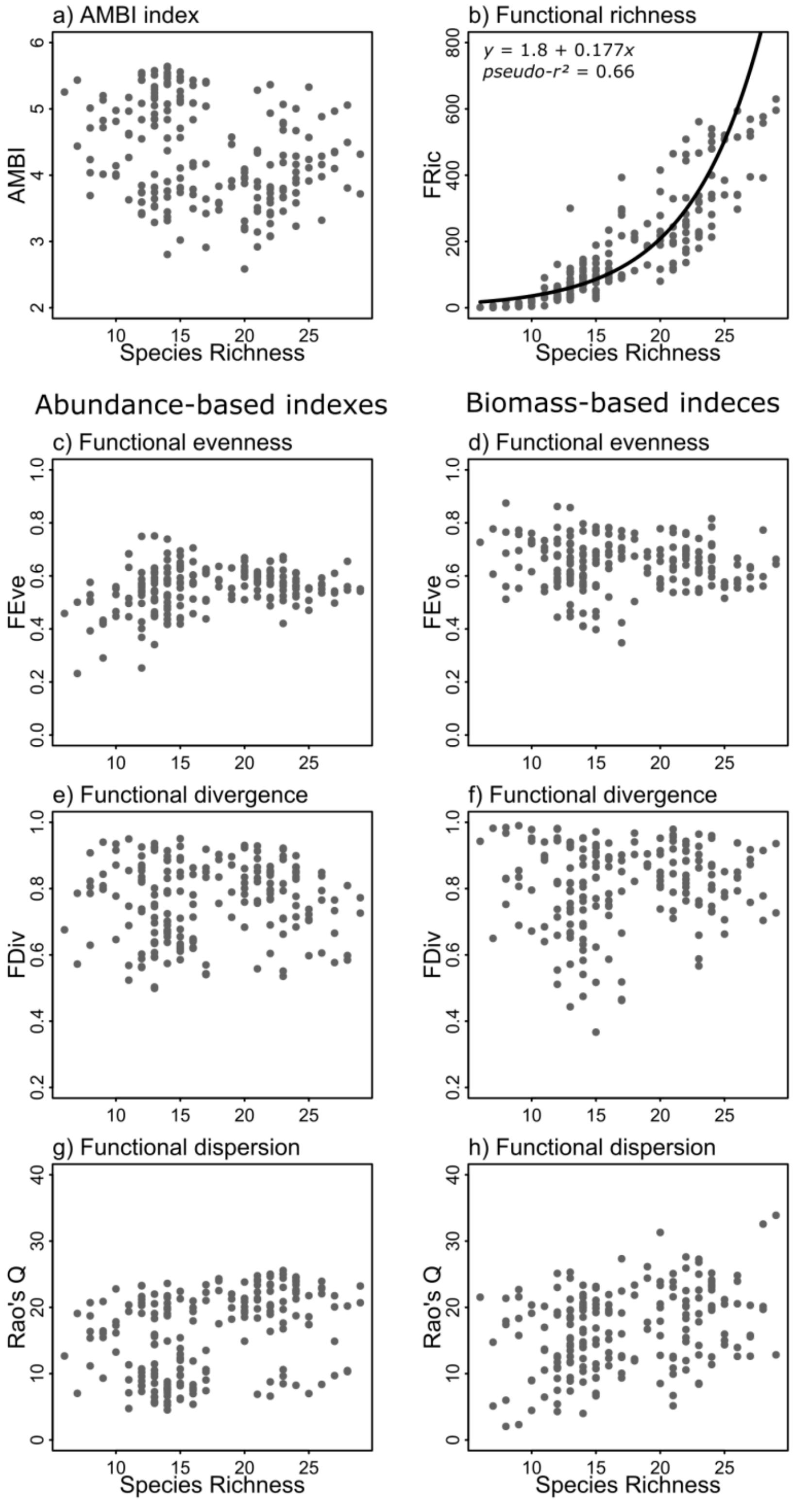

Figure A3: Relationship of functional diversity indices with species richness.

CHAPTER 3

Effects of alien species on taxonomic and functional diversity of intertidal soft-bottom macrobenthic assemblages

Joao B. Gusmao, David W. Thieltges, Paulo Lana, Britas Klemens Eriksson

ABSTRACT

Biological invasions affect the abundance and diversity of native species across the globe. However, the functional consequences of such species replacements are often unclear and highly variable. We investigated how increasing number and biomass of alien species affected both taxonomic and functional diversity of macrobenthos on tidal flats in the Dutch Wadden Sea, during a period (1994 to 2008) when many new species established in the area. The 15-year dataset was made up of samples collected twice a year (spring and fall) from six fixed transects in two different sites: Balgzand and Piet Scheveplaat. At Balgzand, the number and biomass of alien species increased strongly over time, making up almost 45 percent of the total biomass in 2008. At Piet Scheveplaat, the impact of alien species was persistently low. The community composition changed significantly at both sites, but diverged strongly over time, demonstrating different histories of species replacement between the sites. At Balgzand, the increase in alien species correlated with a general increase in species richness as well as in functional richness, eveness and divergence, while the Shannon diversity index decreased. Thus, the alien species increased richness by adding new species and relatively uncommon traits to the community, such as epifaunal supension feeders, but at the same time they changed community structure dramatically by becoming highly dominant species. The large influence of alien species traits also changed potential community function; the bioturbation potential increased in Balgzand because of an increased abundance of large, deeper living species such as *Ensis directus*; and decreased in Piet Scheveplaat where medium-sized, shallow living biodiffusers such as *Cerastoderma edulis* increased instead. This study showed that alien species can boost functional diversity, with potential effects on functioning of the new species assemblages, implying that further studies will be needed to develop a mechanistic relationship between functional diversity indices and actual ecosystem function.

INTRODUCTION

The establishment of alien species is a major environmental threat due to the potential impacts on local communities, food webs, and ecosystems (Mack et al. 2000, Sala et al. 2000, Ehrenfeld 2010). Alien species may be detrimental for native species by introducing additional ecological interactions, thereby intensifying competition and predation (Bøhn et al. 2008), with potentially strong effects on species diversity and abundance. For instance, alien species are among the most influencing factors driving local species extinctions on oceanic islands (Fritts and Rodda 1998, McKinney and Lockwood 1999, Gurevitch and Padilla 2004) and a recent meta-analysis showed that plant invasions in general decrease the abundance and diversity of native species (Vilà et al. 2011). Some alien species are ecosystem engineers that can physically change their environment, modulating the abiotic environment and availability of resources for other species (Sousa et al. 2009). Thus, alien species can cause functional changes in local species assemblages both by introducing new biological functions and/or by changing the abundance of native species with specific functional traits.

Studies addressing how benthic assemblage structure changes as a result of biological invasions are common in the literature, focusing mainly on negative effects on the taxonomic richness and biomass of native species (Thomsen et al. 2014b, 2014a). Effects of invasive alien species on native food-webs can be dramatic (e.g. Jormalainen *et al.* 2016), but in general, the impact on native species depends on the time scale and site-specific factors such as disturbances, environmental conditions, and community dynamics (Hewitt et al. 2016). The use of species functional traits to assess potential effects of disturbances on assemblage function in marine environments notably increased in the past 10 years and have produced fruitful information over how environmental conditions can affect macrobenthic functional diversity (Gusmao et al. 2016). However, studies using a functional trait approach to assess temporal changes in assemblages in marine systems are scarce (Villnäs et al. 2011, Hewitt et al. 2016). Hewitt *et al.* (2016) is the only study that assessed the consequences of biological invasions on benthic functional diversity along long time series (>40 years). They found different trends in trait beta diversity among locations as alien species increased in abundance, suggesting that the effects of the invaders on assemblage trait composition could be highly dependent on local community dynamics (Hewitt et al. 2016). Addressing how functional diversity is affected by increasing number and biomass of alien species in space and time, is fundamental to construct a more complete picture of how alien species affect local assemblage structure and ecosystem function.

The Wadden Sea is a suitable model environment to study the effects of alien species on spatial and temporal trends of macrobenthic functional diversity. Long-term benthic monitoring programmes have been conducted in different regions in the Wadden Sea. Such efforts generated an abundant literature over the ecology of the macrobenthos in the Wadden sea (Beukema 1976, Beukema and Cadée 1997, Philippart et al. 2003, Beukema and Dekker 2011b, Beukema et al. 2015). The macrobenthic assemblages of the Wadden sea are relatively poor in species diversity and the system has been subjected to major human disturbances in the past century, such as large-impact dredging, overfishing, and coastal engineering changing the structure of the food-web (de Jonge et al. 1993, de Jonge and van Beusekom 1995, Lotze et al. 2005). Besides the local impacts, food-web dynamics may have been largely impacted by climate change, for example decreasing reproduction success of bivalves by trophic mismatch (Beukema and Dekker 2014). Natural disturbances driven by currents, wind, and storms also play an important role in shaping the local biological assemblages, making it a very dynamic ecosystem (de Jonge et al. 1993, de Jonge and van Beusekom 1995). This combination of a highly disturbed system with low species diversity and changing environmental conditions suggest a fertile environment for the establishment of invaders, since vacant and new ecological niches may be available (Karlson et al. 2015). Accordingly, a number of dominant alien species such as the razor clam *Ensis directus* and the oyster *Crassostrea gigas* have been thriving in the Wadden Sea in the past decades (Troost 2010, Dekker and Beukema 2012).

In this study, we investigated how increasing numbers and biomass of alien species have affected both taxonomic and functional diversity of macrobenthos on tidal flats in the Dutch Wadden Sea from 1994 to 2008, a time period when many new species established in the area. We used a long-term monitoring dataset (MON*BIOLOGIE; Rijkswaterstaat and the Royal Netherlands Institute for Sea Research, the Netherlands) made up of samples collected twice a year (spring and fall) at two different sites that were very differently impacted by introduced species. To identify effects of the species invasions on macrobenthic assemblages we adapted a functional trait approach where each species is represented by an optimal trait matrix. First, we analyzed the effect of alien species on community composition in the Wadden Sea. Then we compared effects on taxonomic and functional diversity indices over time; and evaluated changes in potential community functions based on specific traits. The aim of the research was to identify potential effects of alien species on taxonomic and functional diversity of macrobenthic species assemblages in the Wadden Sea; and to identify how major changes in community composition and taxonomic diversity are reflected by different functional indices and potential functions calculated from species trait distributions.

METHODS

Study sites and sampling

The Wadden Sea is a sheltered coastal system characterized by a landscape of barrier islands and tidal flats that extends from the Netherlands to Denmark. The entire system has an area of approximately 8000 km^2, with more than a quarter of this area (~2500 km^2) in Dutch territory (Wolff 2000). The highly dynamic geomorphology of islands and tidal flats in the Wadden Sea is mostly affected by tides, wind and sediment particle transport from the North Sea (de Jonge et al. 1993). The Wadden Sea can be described as an estuarine system since it receives substantial inputs of fresh water from continental drainage. The salinity gradients vary depending on a number of factors, such as season, proximity to inlets, precipitation, and pumping for water regulation in mainland (de Jonge et al. 1993). The tides are semi-diurnal and driven by the tide in the North Sea. The tidal amplitude varies across the system, ranging from 1.4 to 3.4 m (Postma 1982).

The analyzed dataset comprised two different sites in the Dutch Wadden Sea (Figure 1): Balgzand (52°55’N 4°49’E), a 50 km^2 tidal flat area with average depth of -0.71 ± 0.3 m located next to the Marsdiep inlet in the westernmost part of the Wadden Sea; and Piet Scheveplaat (53°23’N 5°47’E), a wide tidal flat area with average depth of -0.07 ± 0.5 m located next to the island of Ameland. The dataset was generated by the environmental monitoring project MON*BIOLOGIE (dataholder: Rijkswaterstaat; conducted by the Royal Netherlands Institute for Sea Research: Monitoring Bodemfauna Waddenzee and Eems-Dollard). Samples were collected from three fixed transects distributed within each site (Figure 1). Samples were taken twice a year, during spring (February-April) and fall (August-September), using the same sampling design along 15 years (from 1994 to 2008).

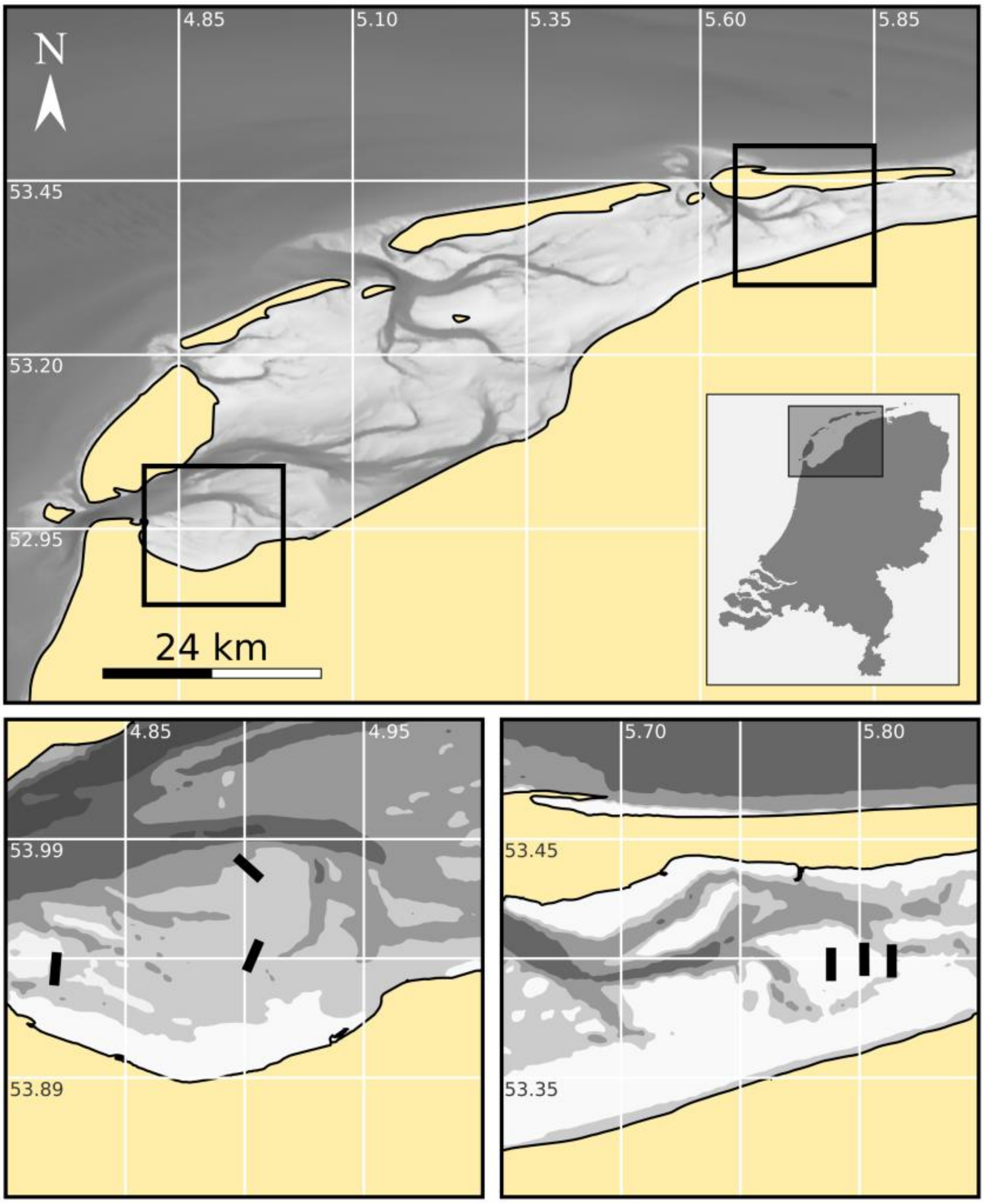

Figure 1: Above: map of the Dutch Wadden Sea showing the sampling sites (black frames) where the monitoring data that were used for the analyses were collected between 1994 and 2008: Balgzand (left) and Piet Scheveplaat (right). Below: maps of each site showing the position of each of the three transects placed within each site (black rectangles).

Transects at Balgzand were ~1000 m long in which 50 sampling stations separated by intervals of 20 m were distributed in line. PVC corers with different diameters were used to sample in spring (15.5 cm wide) and fall (10.7 cm wide). In both cases, the sampling depth was 35 cm. Samples from five successive sampling stations were taken together, thereby obtaining 10 composed samples per transect. In Piet Scheveplaat, transects were ~760 m long with 20 sampling stations distributed in line 38 m apart from each other. Two subsamples were taken at each station and then combined, resulting in 10 composed samples per transect . The samples were sieved out in the field through a 1 mm mesh sieve, and species were identified in the laboratory. Species were quantified using ash-free dry weight (AFDW) per m^2 for each transect.

An extra sediment sample (8 cm deep) was taken from each transect for analysis of sediment silt percentage, organic matter, and $CaCO_3$ concentrations. The total amount of carbon (*C total*) was determined using gas chromatography and thermal conductivity detection. The amount of organic carbon (*C organic*) was determined in the same way, but after pre-treatment of the sediment with HCl. The amount of $CaCO_3$ was calculated by subtracting *C organic* from *C total*. Silt content was estimated using the pipette method (Gee and Bauder 1986). All values are given as percentages of the total weight of sediment samples. No consistent trends were observed in environmental variables across sites, seasons and years (Figure A1).

Data analysis

Since we have *a priori* assumptions about differences in assemblage structure dynamics over different seasons (spring and fall), separated analyses were made for each season. Temporal changes in assemblage taxonomic and functional diversity were analyzed by fitting linear or polynomial regressions considering site as a fixed factor (since we knew that Balgzand and Piet Scheveplaat would present contrasting assemblages). Best model selection (linear or polynomial) was based on the smallest Akaike Information Criterion (AIC). Taxonomic alpha diversity was quantified (per transect) as species richness, number of native species, number of alien species, and Shannon diversity index based on biomass (H’). We also analyzed changes in native species biomass, and alien species biomass (g/m^2 per transect).

Changes in species composition across years and sites were analyzed using Constrained Analysis of Principal Coordinates (CAP) and PERMANOVA based on the same linear model used in the univariate analysis. Both CAP and PERMANOVA analysis were based on pairwise Bray-Curtis dissimilarities on square-rooted species biomasses.

For the functional diversity analysis, a functional trait matrix was generated by classifying the species according to six functional traits: bioturbation type, depth penetration in the sediment (in cm), body size (in mm), feeding mode, longevity (in years), and relative adult mobility (adapted from Jones & Frid 2009). These six traits were further sub-divided into modalities representing the range of variation for each functional trait (Box 2). Fuzzy coding (Chevenet et al. 1994), ranging from 0 to 3, was used to classify each species according to its association to each functional trait modalities. The scores within each functional trait were standardized in a way that the sum of the modalities of each functional trait was always 3. The information about species traits was collected from online data bases (MarLIN 2006, Faulwetter et al. 2014), literature (Queirós et al. 2013, Donadi et al. 2015a, 2015b, Jumars et al. 2015, van der Zee et al. 2015) and by consulting specialists.

Functional alpha diversity was analyzed by calculating three multivariate indices based on the Villéger, Mason & Mouillot (2008) framework: functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv). These indices are based on the assemblage functional trait space, a multivariate space represented by a principal coordinates analysis (PCoA) of the pairwise Euclidean distances of species functional traits. FRic estimates the total range of the functional traits in an assemblage by measuring the convex hull volume formed by the species of a given assemblage in the functional trait space. Since FRic is based on species incidence data, it only can be affected by adding/removing species with unique trait values/combinations (Villéger et al. 2008). FEve estimates the evenness of the biomass distribution across species in the trait space of a given assemblage. It is calculated as the biomass weighted sum of the branch lengths of the minimum spanning tree that connects all the species of a given assemblage (Villéger et al. 2008). FDiv estimates how dispersed the species biomasses of a given assemblage are in the functional trait space. It is calculated by measuring the biomass weighted distances of all species in relation to the mean distance to the gravity center of the functional trait space (Villéger et al. 2008). The relationship between functional diversity and taxonomic diversity was analyzed by fitting linear models.

Finally we calculated the bioturbation potential (BPc) of the assemblage according to Solan *et al.* (2004) and Queirós *et al.* (2013). Bioturbation potential takes into account three biological traits known to influence bioturbation activity: body size (expressed by the ratio biomass:abundance), mobility (Mi), and type of sediment reworking (Ri). Mi and Ri values (varying from 0 to 3 and 0 to 4, respectively) reflect the intensity the species can affect sediment structure. Bioturbation potential (BPc) was calcuted with the formula :

$$BPc = \sum_{i=1}^{n} \sqrt{Bi/Ai} \times Ai \times Mi \times Ri$$

Bi and *Ai* are the biomass and abundance of species/taxon *i* in a sample. Trait scores of *Mi* and *Ri* were obtained from Queirós *et al.* (2013).

Analyses were performed in R programing environment (R Development Core Team 2009) using the associated packages: *vegan* (Oksanen et al. 2009) for CAP, PCA, PERMANOVA, and taxonomic diversity indices; and *FD* (Laliberté and Shipley 2011) for functional diversity indices.

RESULTS

Species diversity

A total of 286,797 individuals from 105 taxa were identified along the entire study period. Eleven species were identified as non-native of the Dutch Wadden Sea (based on Buschbaum, Lackschewitz & Reise 2012), which include 3 bivalves (*Crassostrea gigas*, *Ensis directus*, *Petricolaria pholadiformis*), 1 gastropod (*Crepidula fornicata*), 5 polychaetes (*Alitta virens*, *Aphelochaeta marioni*, *Marenzelleria viridis*, *Marenzelleria wireni*, *Streblospio benedicti*), and 2 crustaceans (*Hemigrapsus takanoi*, *Austrominius modestus*). These species presented variable functional traits, occupying different parts of the functional trait space of the assemblages but generally overlapping with the trait distributions of native species (Figure 2). Three species, *C. gigas*, *C. fornicata* and *A. modestus* occupied a position in the upper left functional trait space (Figure 2), mostly related to sessile, surface dwellers with no bioturbator behavior; trait categories that are shared with the native blue mussel *Mytilus edulis*.

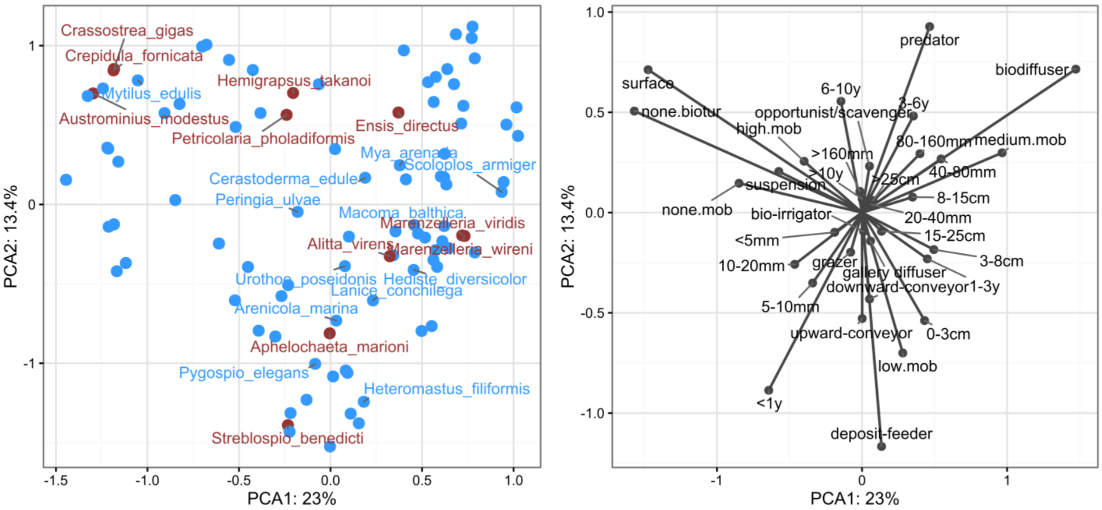

Figure 2: Functional trait space of macrobenthos assemblages from the Dutch Wadden Sea, constructed from a PCA based on fuzzy scores of assigned trait categories. Left: distribution of native (blue) and alien (red) species in the functional trait spaces. All the alien species and also the most common native species are named. Right: vectors of each functional trait category used for the analysis.

The macrobenthic community changed considerably across the years in both seasons, but the two sites diverged towards distinctly different species compositions. The CAP ordinations showed that the community composition at the two sites was located relatively close together in ordination space in the 1990s, and that they were clearly separated in the 2000s (Figure 3, Appendix tables 1 and 2). In general, the first axis explained more than 70% of the total variation and was related to differences between sites. The second axis explained more than 15% of the total variation and was related to changes across the years. Accordingly, the PERMANOVAs showed that trends across the years were significantly dependent on the analyzed site (Table 1). The divergence in community composition over the years was related to site specific changes in the biomass dominant species (Table A2). Balgzand was dominated by the bivalves *M. edulis* (14.9 ± 23.6 g/m²) and *Mya arenaria* (11.4 ± 12.6 g/m²) in 1994, but in 2008 the non-native razor clam *Ensis directus* became the dominant species (22 ± 34.4 g/m²). Piet Scheveplaat was dominated by *M. edulis* (18.3 ± 44.6 g/m²) and *M. arenaria* (10.6 ± 15.7 g/m²) in 1994, while in 2008 the dominant species were the cockle *Cerastoderma edule* (19.23 ± 18.34 g/m²) and the polychaete *Arenicola marina* (7.6 ± 4.7 g/m²).

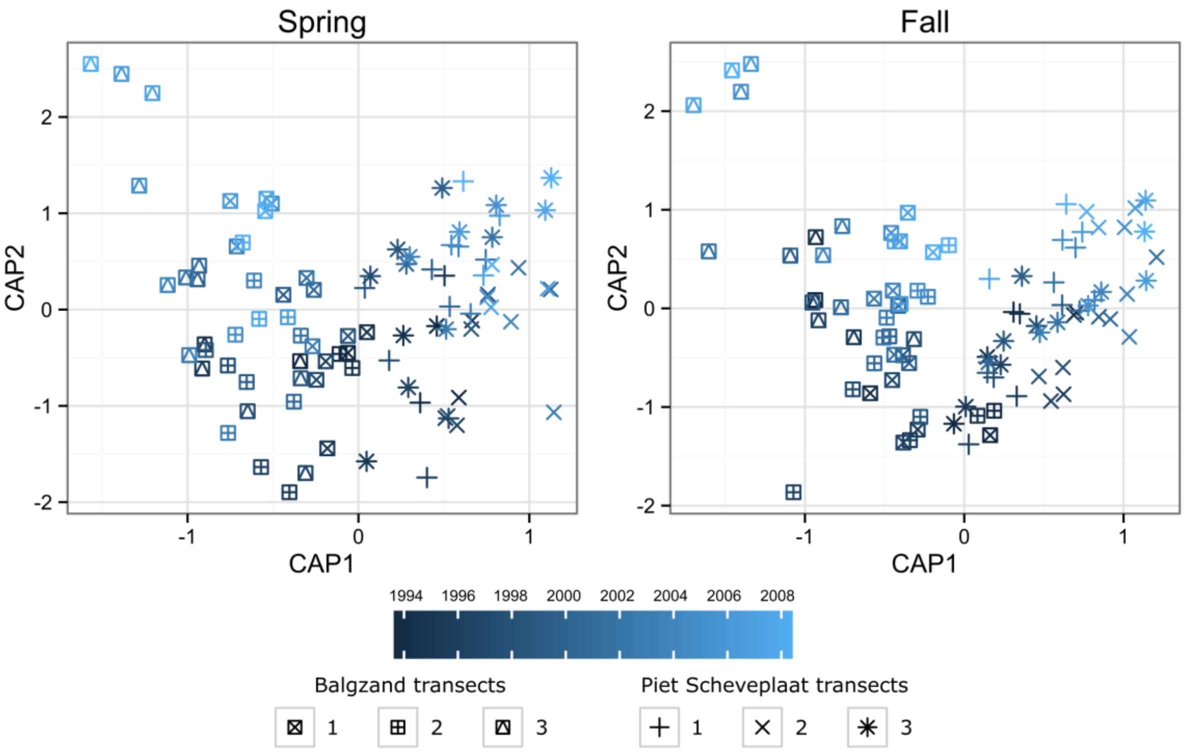

Figure 3: CAP ordinations depicting variation trends in assemblage species composition across years and sites for each season. Years are indicated from dark (1994) to lighter (2008) colors; Site Balgzand is indicated with symbols within a square; Site Piet Scheveplaat is indicated with symbols without a framing square.

Table 1: PERMANOVAs of each season comparing species composition across sites and years. Tests were based on Bray-Curtis dissimilarities on square-rooted species biomass and conducted on 9999 permutations.

		Spring			Fall		
	df	*Pseudo-F*	R^2	*p-value*	*Pseudo-F*	R^2	*p-value*
Year	1	3.52	0.03	**0.003**	4.10	0.04	**0.002**
Site	1	15.82	0.14	**0.0001**	15.18	0.14	**0.0001**
Year*Site	1	3.78	0.03	**0.003**	3.65	0.03	**0.003**
Residuals	86		0.79			0.79	

The divergence in community composition between the sites over the years were related to alien species rearranging the community structure in Balgzand, but not in Piet Scheveplaat. In Balgzand, there was a continuous increase in the number of alien species from 1 ± 0.6 in 1994 to 4 ± 1.1 species in 2008 (Figure 4a; Table 2) and alien species biomass increased dramatically from 2005 onwards (Figure 4b; Table 2); before 2005 the biomass of alien species on Balgzand was on average 9 % and between 2005 and 2008, the average was 47 %. At the same time, both total and native species richness increased (Figure 4c-d; Table 2), while there was a marginal decrease in native species biomass (Figure 4e; Table 2). At Piet Scheveplaat, alien species richness showed no directional changes for spring (~1.3 ± 0.9 species) and a continuous decrease in fall (from 3.6 ± 1.5 species in 1994 to 2 ± 0 species in 2008; Figure 4a; Table 2); and alien biomass showed low and temporally consistent values of less than 1 percent of total biomass (Figure 4b). Total species richness and the number of native species decreased at Piet Scheveplaat in fall (Figs 4c and 4d). Shannon diversity decreased across the years for both sites and seasons (Figure 4f).

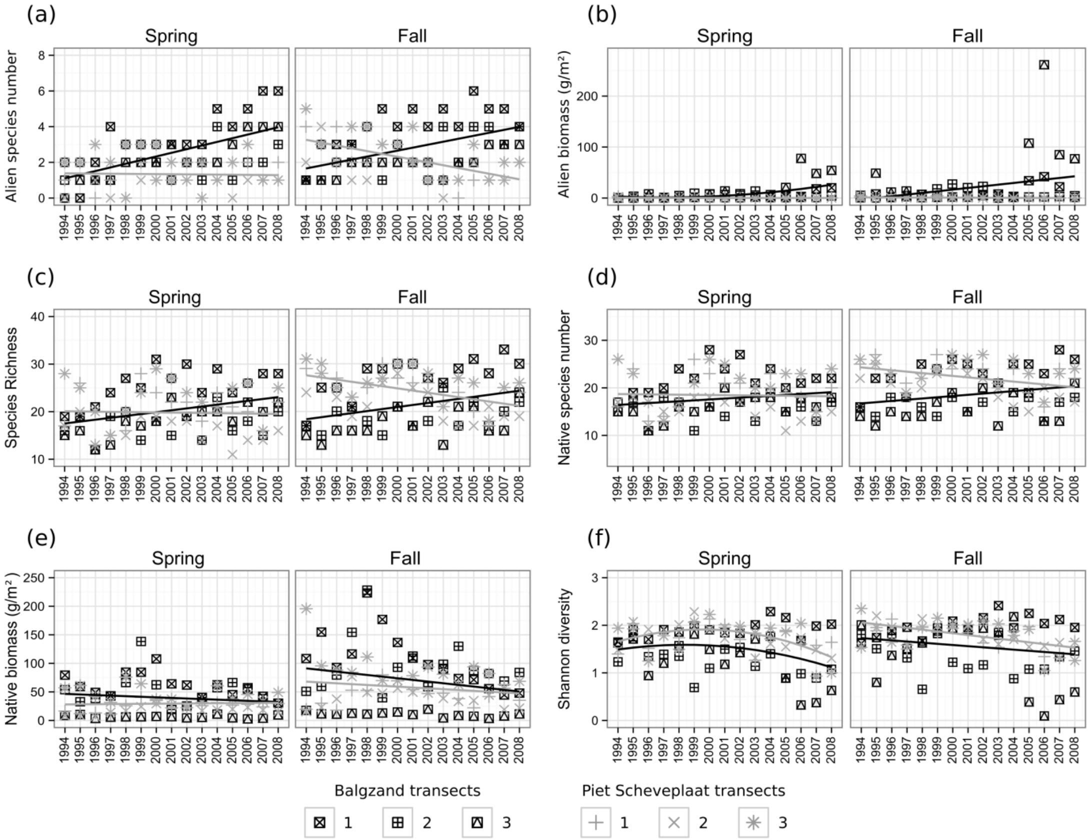

Figure 4: Trends in species diversity and biomass of alien and native species over the years for both spring and fall at Balgzand and Piet Scheveplaat. The regression lines show fit of the statistical models: black line show Balgzand, grey line show Piet Scheveplaat.

Table 2: ANCOVAs of the fitted linear models of taxonomic diversity across sites and years.

		Alien species number				Alien species biomass			
		Spring		Fall		Spring		Fall	
	df	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Site	1	30.98	**<0.0001**	8.42	**0.005**	15.97	**0.0001**	9.40	**0.003**
Year	1	15.68	**0.0002**	0.02	0.9	7.61	**0.0009**	4.87	**0.03**
Year*Site	1	18.03	**0.0001**	37.37	**<0.0001**	7.92	**0.0007**	5.43	**0.02**
Adjusted R^2:		0.41		0.32		0.32		0.16	
p-value:		**<0.0001**		**<0.0001**		**<0.0001**		**0.0005**	

		Species richness				Native species number			
		Spring		Fall		Spring[1]		Fall	
	df	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Site	1	0.24	0.6	10.40	**0.002**	0.69	0.4	22.22	**<0.0001**
Year	1	2.39	0.1	0.02	0.9	0.63	0.4	0.05	0.8
Year*Site	1	3.61	0.06	16.63	**0.0001**	1.28	0.3	9.54	**0.003**
Adjusted R^2:		0.04		0.21		<0.005		0.24	
p-value:		0.1		**<0.0001**		0.5		**<0.0001**	

		Native species biomass				Shannon diversity index			
		Spring		Fall		Spring[1]		Fall	
	df	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Site	1	3.58	0.06	1.83	0.2	12.87	**0.001**	6.86	**0.01**
Year	1	0.44	0.5	3.73	0.06	7.64	**0.001**	9.12	**0.003**
Year*Site	1	1.01	0.3	0.40	**0.05**	0.22	0.8	0.44	0.5
Adjusted R^2:		0.02		0.03		0.21		0.13	
p-value:		0.2		0.1		**0.0001**		**0.002**	

[1] Polynomial fit (2nd degree)

Functional diversity

The increased dominance of alien species enriched functional diversity and changed the potential bioturbation function of the community. At Balgzand, there was a significant increase in functional richness (FRic) and divergence (FDiv) across the years, while at Piet Scheveplaat there were no directional trends in spring and weak decreases in fall (Figure 5a-b, Table 3). Functional evenness (FEve) increased over the years across seasons and sites (Figure 5c, Table 3). For bioturbation potential there were no trends in spring, but in fall it increased at Balgzand and decreased at Piet Scheveplaat between 1994 and 2008 (Figure 5d, Table3). The increasing trend in functional divergence at Balgzand did not correlate with total or native species richness, but was associated with increasing number and biomass of alien species (Figure 6). The enrichment of the functional indices by alien species was in general related to non-native species with positions in the functional trait space related to sessile, surface dwellers with no bioturbator behavior, notably the Pacific oyster *Crassostrea gigas* (Figure 2 & 7, Figure A2 & 3). In contrast, the increase in bioturbation potential at Balgzand was linked to another non-native species – the biodiffuser *Ensis directus* (Figure 2 & 7, Figure A2 & 3) – that strongly increased the total biomass of large (above 80 mm) species (Figure A2 & 3). Thus, some alien species contributed more to the functional diversity indices than to the bioturbation potential function. At Piet Scheveplaat, the generally declining large native bivalves (*Mya arenaria* and *Mytilus edulis*; 80-160 mm) were replaced by medium-sized (5-20 mm), shallow living biodiffusers such as *Cerastoderma edulis*, that have intermediate positions in the functional trait space of the assemblage and contribute less to the bioturbation potential (Figure 2 & 7, Figure A2 & 3). This was reinforced by the changes in biomass distribution in the assemblage functional trait space (Figure 7, Figure A2 & 3).

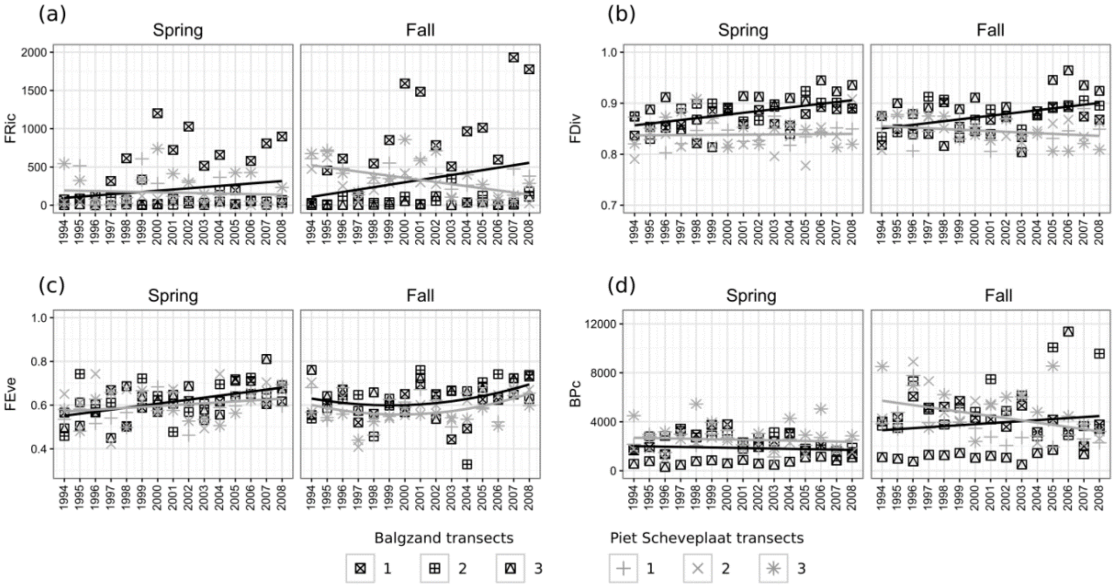

Figure 5: Trends in indices functional richness (FRic), functional divergence (FDiv), and functional evenness (FEve) and bioturbation potential (BPc) over the years for both spring and fall samples at Balgzand and Piet Scheveplaat. The regression lines show fit of the statistical models: black line show Balgzand, grey line show Piet Scheveplaat.

Table 3: ANCOVAs of the fitted linear models testing the effects of sites and years on functional diversity indices (FRic, FDiv, and FEve) and bioturbation potential (BPc).

		FRic				FDiv			
		Spring		Fall		Spring		Fall	
	df	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Site	1	0.54	0.5	0	1	606	<0.0001	285	0.0007
Year	1	0.84	0.4	0.06	0.8	74	0.0007	23	0.1
S*Y	1	23.89	0.1	101.01	0.002	76	0.007	145	0.0003
Adjusted R²:		0.009		0.07		0.45		0.32	
p-value:		0.3		0.2		<0.0001		< 0.0001	

		FEve				BPc			
		Spring		Fall[1]		Spring		Fall	
	df	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Site	1	10	0.3	67	0.01	10.58	0.002	1.63	0.2
Year	1	153	0.0002	62	0.003	0.94	0.3	0.85	0.4
S*Y	1	22	0.1	0.044	0.9	0.001	1.0	6.23	0.01
Adjusted R²:		0.15		0.14		0.087		0.06	
p-value:		< 0.001		0.003		0.01		0.04	

[1] Polynomial fit (2th degree)

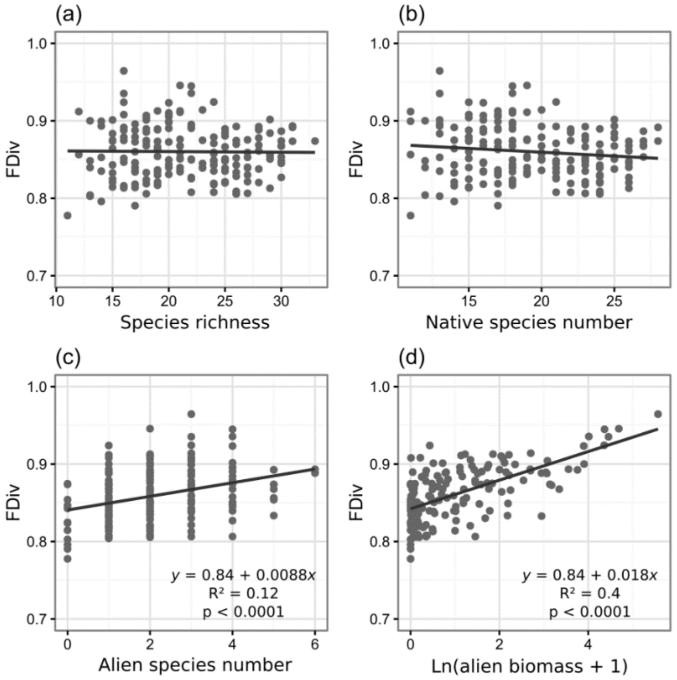

Figure 6: Relationship between functional divergence (FDiv), taxonomic diversity and the biomass of alien macrobenthic species at Balgzand in the Wadden Sea, from 1994 to 2008.

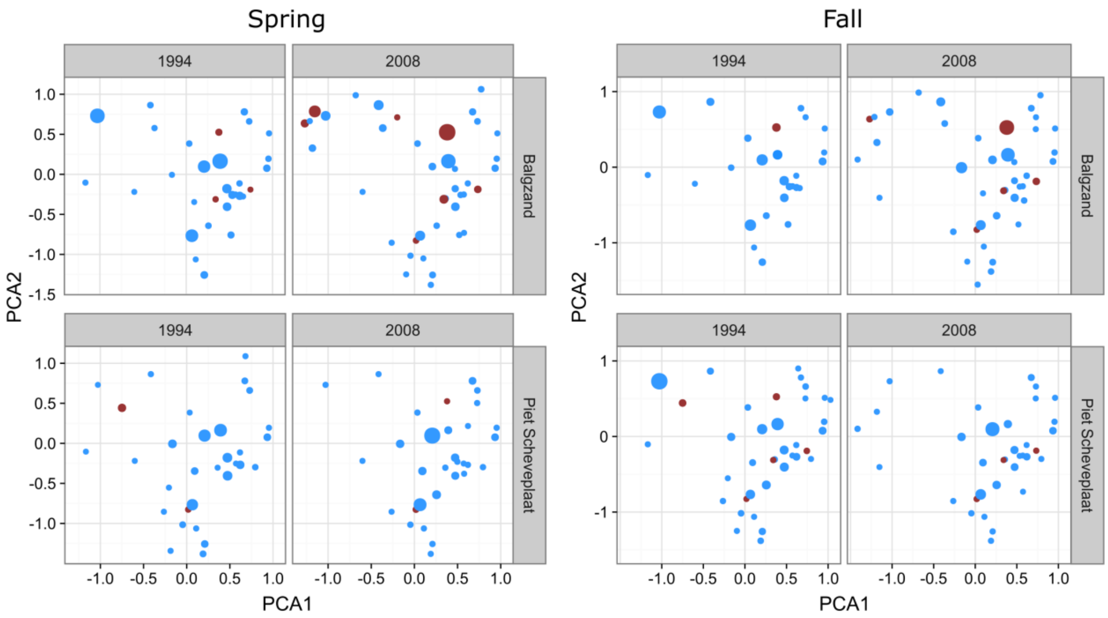

Figure 7: PCAs representing the macrobenthic functional trait space at Balgzand and Piet Scheveplaat in spring and fall in 1994 and 2008. Point sizes are proportional to species biomass. Red = alien species; blue = native species. See Figure 2 for species positions and Appendix 7 and 8 for all years.

DISCUSSION

Our analyses show that alien species are not only associated with major changes in macrobenthic community composition in the Wadden Sea, but also with changes in functional diversity and potential ecosystem function. The two study sites, Balgzand and Piet Scheveplaat, demonstrated large diverging changes in species composition over the years. Species composition and functional diversity were similar between sites in 1994, when both sites were dominated by the bivalves *Mytilus edulis* and *Mya arenaria*. Since 2005, at Balgzand several alien species have become dominant and restructured the community. At this site, the surge in non-native species has lead to increasing species richness and functional diversity by adding new species with distinct traits to the community, in particular *Crassostera gigas*, *Crepidula fornicata* and *Austrominius modestus*. At the same time, the strong increase in biomass of the large, deeper living species *Ensis directus*, which has become the dominating species in the community, increased the bioturbation potential of the assemblage. In addition, native species showed changes in composition and biomass over the years, either driven by interactions with alien species or due to environmental and other factors. Hence, it is not only specific functional trait profiles but also changes in dominance of alien species in an assemblage that underlie the changes in functional diversity of invaded assemblages. Interestingly, at

Piet Scheveplaat species richness, functional diversity and bioturbation potential all showed the opposite trends and declined. Also at Piet Scheveplaat the species composition changed dramatically over 15 years, but here losses of the native bivalves have been replaced by other native bivalves, and the precence of invaders was consistently negligable.

The diverging trends in species composition between the sites may depend on a number of environmental drivers and/or human impacts that modulate community dynamics. For instance, changes in recruitment rates due to disturbances can have site-specific effects on local communities (Zajac et al. 1998, De Grave and Whitaker 1999). Indeed, mechanical shellfish dredging and mussel extraction, as well as an increasing frequency of mild winters have all been associated to declines in the recruitment of native bivalves in the Wadden Sea, mostly driven by decreases in larval supply and recruitment success (Piersma et al. 2001, Philippart et al. 2003, Nehls et al. 2006, Eriksson et al. 2010). This might explain some of the general declines in species dominance; since for example the recruitment success of *Mytilus edulis* is highly dependent on winter temperature (Kautsky 1982, Myint and Tyler 1982) and both *Macoma baltica* and *Mya arenaria* have been shown to suffer strongly from the mechanical fishing disturbances that was common in the Wadden Sea in the late 1990s (Piersma et al. 2001). At the same time, some invaders such as the *Crassostera gigas* has been favored by the warmer winters during the past decades (Nehls et al. 2006). However, this does not explain why only Balgzand was enriched by a number of new non-native species, while Piet Scheveplaat was gradually losing species. We did not found any consistent changes in sediment properties across the years (Figure A1), suggesting that the trends in assemblage structure were not driven by changes or differences in the substrate. However, the tidal amplitude is higher at Piet Scheveplaat and these sampling transects may have been higher up in the intertidal than those at Balgzand. This may explain why Piet Scheveplaat became dominated by *Cerastoderma edule* instead of *E. directus*, because *C. edule* is a species that thrive higher up in the intertidal than *E. directus*.

Diverging trends in the current community composition betweem the two sites may also be related to biological factors such as differential effects of ecosystem engineers on the local assemblages. Many non-native bivalves are known to be ecosystem engineers that modify the habitats they are introduced to (Sousa et al. 2009). Ecosystem engineers modulate resource and habitat availability to other organisms, promoting or hindering the occurrence of certain species (Jones et al. 1994). In the Wadden Sea, the reef-forming blue mussel *Mytilus edulis* form hard-substrate habitats on the predominantly soft tidal flats (Ragnarsson and Raffaelli 1999) (Dittmann 1990, Ragnarsson and Raffaelli 1999, Buschbaum et al. 2009); changing the surface microtopography and local hydrodynamics, creating sheltered areas with increased sedimentation rates next to the reefs (van der Zee et al. 2012, Donadi et al. 2013b). Such effects allow the occurrence of hard-substrate epifauna within the reefs and also favors the occurrence of other benthic organisms in the surrounding areas (Dittmann 1990, Buschbaum et al. 2009, Donadi et al. 2013b). Thus, decreases in *M. edulis* populations would most likely result in large losses of associated macrobenthic diversity (Eriksson et al. 2010, van de Koppel et al. 2015). Accordingly, we observed in Piet Schveplaat that the large decrease in *M. edulis* was followed by a decrease in associated species, suggesting a cascading effect of the loss of *M. edulis* reefs. At Balgzand, on the other hand, the non-native Pacific oyster *Crassostera gigas*, which also is a reef-forming epifaunal species, increased strongly after the decline of *M. edulis* and replaced much of the vanishing hard-substrate. In addition, the deep digging razor clam, *Ensis directus*, that today dominates the community on Balgzand, engineer the substrate by increase oxygen penetration and interstitial water exchange to deep sediment layers (Armonies 2001). Accordingly, E. *directus* contributed strongly to an increase in the bioturbation potential of the community at Balgzand, and the improved aeration of the sediment may have favored other non-native species, in particular polychaetes, that live in the deeper layers of the sediment; such as *Alitta virens*, *Marenzelleria viridis* and *M. wireni.*

We have demonstrated that non-native species have modified the functional trait space of the macrobenthic community. At the same time, the non-native bivalves seem to counteract the losses of functional diversity and ecosystem engineering services performed by declining native species. In addition, they have become an important part of the food-web, where for example the razor clam *E. directus* have become an important food item for fishes and seabirds (Tulp et al. 2010). However, caution is necessary when deriving conclusions about ecological value of non-native species from increases in functional diversity and/or apparent replacement services when native species decline; because the complexity of trophic and non-trophic interactions among native and alien species makes it difficult to predict effects. For example, as *Mytilus edulis* and *Crassostera gigas* share more or less the same functional trait space, they may compete, suggesting that increases in *C. gigas* populations could influence the recovery of *M. edulis.* However, evidence for strong competition effects and negative impacts of oysters on mussel is poor (Markert et al. 2009, Troost 2010). Although not sharing trait space with native species, *E. directus, is* an important food item for predators in the Wadden Sea and may trigger indirect interactions such as apparent competition (Bonsall and Hassell 1997), which could drive a decline of native species that share the same predators. Therefore, despite the increased functional diversity and species number, negative feedbacks could emerge from the rearrangement in the functional trait space, potentially harming different components of the native macrobenthic assemblages.

In this study, we have shown that alien species can boost taxonomic richness and functional diversity of intertidal soft-bottom macrobenthic species assemblages. However, it is not clear how this translates into actual ecosystem functions, because at the same time, the alien species rearranged the functional trait structure and changed potential functioning of the species assembly. For infauna, bioturbation is an essential ecosystem service that influences key habitat characteristics of the sediment, such as grain size and sediment stability (de Deckere et al. 2001, Kristensen et al. 2012). By increasing sediment resuspension and interstitial water exchange, bioturbation also impact the above water column, increasing nutrient concentrations and the amount of sediment re-suspended in the water (Biles et al. 2002). Thus, by increasing the bioturbation potential, non-native species may be important for the perceived water quality of sedimentary coastal ecosystems, with potential impact on water clarity and algal bloom development. This highlights the need to develop functional diversity indices that have a clear mechanistic relationship with actual ecosystem functions which would allow to better evaluate the importance of different assemblages composition and functional diversity for specific ecosystem functions.

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APPENDIX

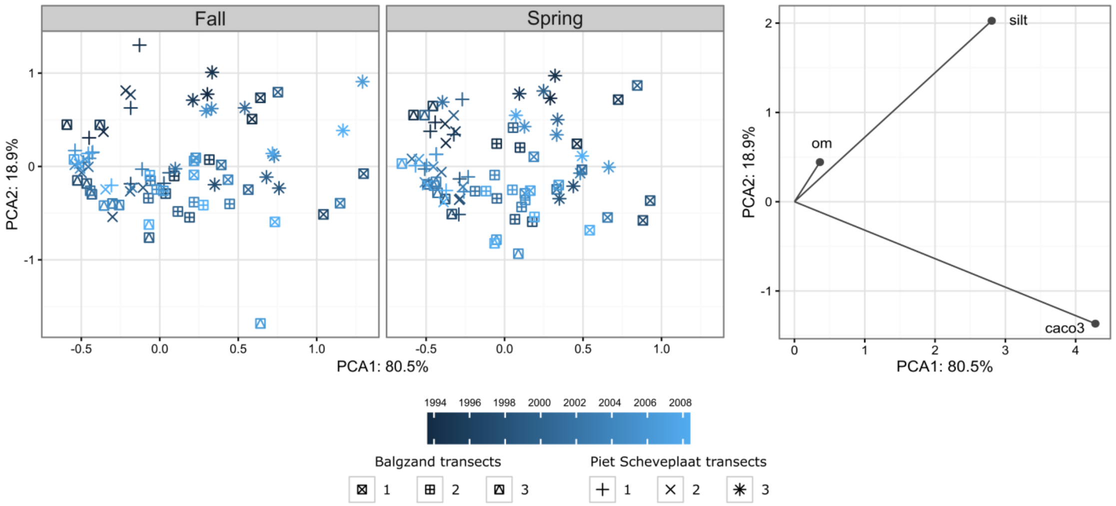

Figure A1: Principal Components Analysis (PCA) depicting variation trends in organic matter (om), silt percentage (silt), and $CaCO_3$ across sites, seasons, years.

Table A1: Accumulated constrained eigenvalues and importance of components of the CAP ordinations of Bray-Curtis dissimilarities based on square-rooted species biomass.

	Spring		Fall	
	CAP1	CAP2	CAP1	CAP2
Eigenvalue	2.16	0.46	2.00	0.51
Proportion Explained	0.73	0.16	0.70	0.18
Cumulative Proportion	0.73	0.89	0.70	0.87

Table A2: Sores of the 12 species that better explained trends in CAP ordinations of Bray-Curtis dissimilarities based on square-rooted species biomass.

	Spring		Fall	
Species	CAP1	CAP2	CAP1	CAP2
Arenicola marina	0.29	0.03	0.25	-0.01
Cerastoderma edule	0.61	0.20	0.65	0.08
Crassostrea gigas	-0.16	0.19	-0.18	0.15
Ensis directus	-0.97	0.83	-1.21	0.82
Hediste diversicolor	-0.02	-0.07	-0.09	-0.09
Heteromastus filiformis	-0.07	-0.13	-0.16	-0.07
Lanice conchilega	0.08	0.07	0.12	0.02
Macoma balthica	0.24	-0.06	0.20	-0.10
Marenzelleria wireni	-0.15	-0.02	-0.17	-0.02
Mya arenaria	-1.95	-0.32	-1.67	-0.38
Mytilus edulis	-0.37	-0.51	-0.39	-0.65
Peringia ulvae	0.19	0.01	0.25	0.06

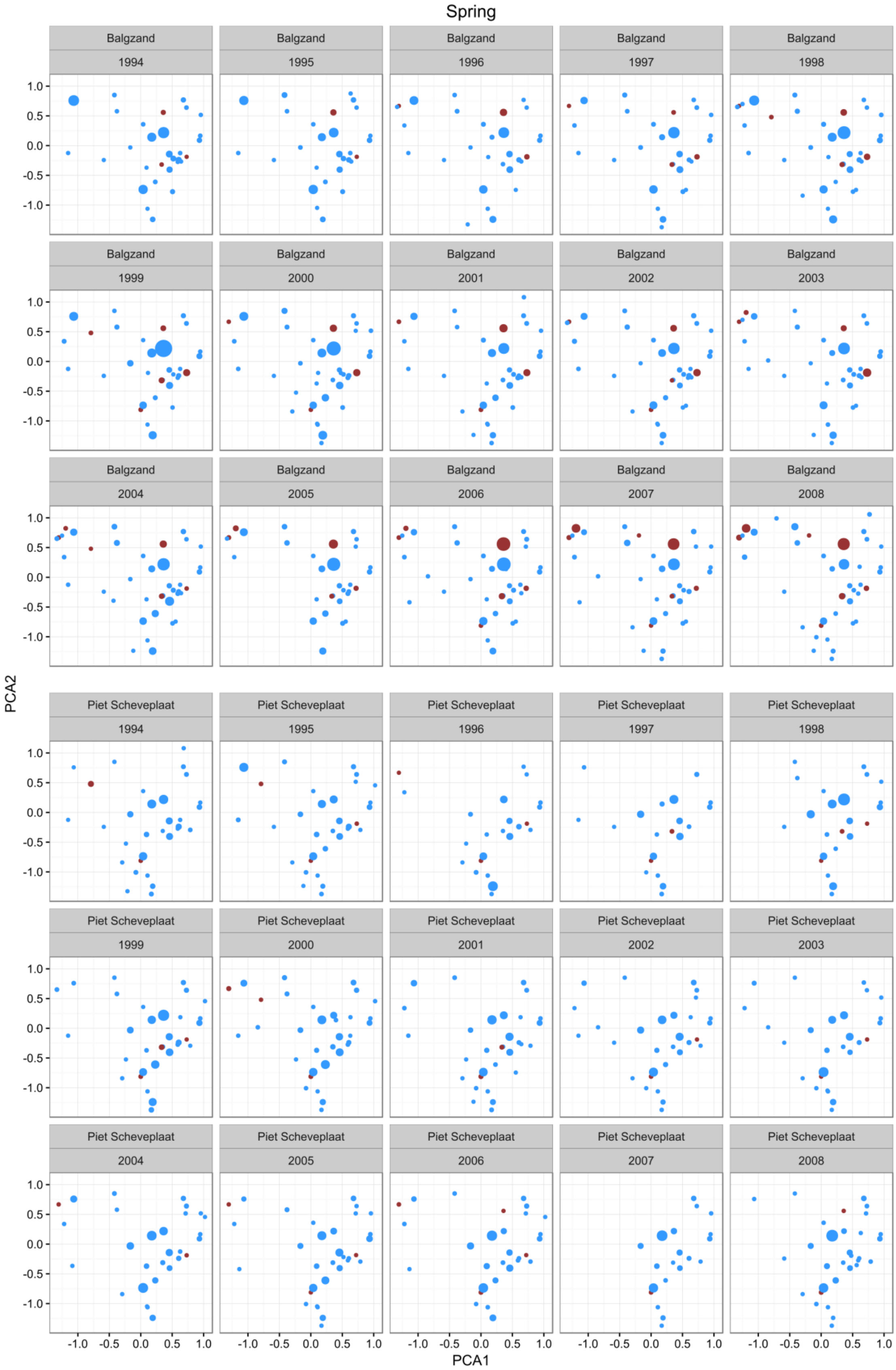

Figure A2: PCAs depicting changes in the functional trait space and species biomass (bubble size) for the season spring across years and sites. Blue = native species; red = alien species. See Figure 2 for species positions.

Figure A3: PCAs depicting changes in the functional trait space and species biomass (bubble size) for the season fall across years and sites. Blue = native species; red = alien species. See Figure 2 for species positions.

CHAPTER 4

Seagrass meadows induce a functional cascade by determining the functional trait composition of sediment fauna

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ABSTRACT

Seagrass meadows are also among the most productive ecosystem in temperate and tropical coastal seascapes and provide habitat for a variety of functionally important species, such as bioturbating macrobenthic species. In this study, we addressed the general function of the seagrass *Zostera marina* across the Northern Hemisphere by relating seagrass meadow structure to the functional diversity of the epifaunal and infaunal macrobenthos. We assumed that increasing complexity of *Zostera marina* meadows, expressed by above- and below-ground plant attributes, will be positively related to the macrobenthic trait diversity. Overall, seagrass presence promoted the macrobenthic species richness and functional diversity. However, both taxonomic and functional diversity decreased with seagrass above-ground biomass and shoot length. Thus, taxonomic and functional diversity was in general higher in the short-leafed, dense European meadows than in the long-leafed Pacific meadows. Compared to adjacent unvegetated habitat, assemblages within seagrass had a higher abundance of epifauna and shallow-living infauna. The shallow infauna was dominated by small biodiffusing organisms, suggesting that higher bioturbation related processes occur in the shallow layers of the sediment, such as sediment resuspension. At the same time, assemblages within seagrass had a lower abundance of deeper-living bioturbators. Our results suggest that the foundation role of seagrass may induce a functional cascade, where effects on sediment processes by the meadows themselves are mediated by changing the functional composition of bioturbating macroinvertebrates. Our findings show that we can use meadow characteristics to predict the ecosystem engineering function of seagrass across biogeographic regions. This highlights the need for seagrass conservation not solely for their own ecosystem engineering attributes, but also for their role as facilitators of other functionally relevant organisms.

INTRODUCTION

Ecologists over the past several decades have recognized that ecosystem functioning can be influenced by biodiversity, and therefore depends on the attributes of the biological communities (Cardinale et al. 2006, 2012, Bracken et al. 2008, Hooper et al. 2012). This fundamental advance of ecological science was preceded by an intense period of experimental ecological research driven by a need to understand the consequences of global biodiversity loss (Gamfeldt and Hillebrand 2008). Most of these experiments focused on understanding the role of species diversity and, how the identity and functional role of the species composing the assemblage were important in determining ecosystem functioning. For technical reasons, experiments were usually done over short periods of time, at small-scales and often in mesocosms with artificial species assemblages, thereby testing the effects of species richness and identity under simplified conditions (Brose and Hillebrand 2016). While these studies and others established the importance of biodiversity for ecosystem function, there is a general call to move further and test these results in real-world ecosystems (Gamfeldt and Hillebrand 2008, Emmett Duffy 2009, Brose and Hillebrand 2016).

Advances to better understand biodiversity effects in real ecosystems include trait-based approaches (Mokany et al. 2008, Lavorel 2013) and large scale coordinated sampling efforts, where species richness is related to predicted changes in functions across global environmental gradients (Duffy et al. 2015). Focusing on trait variation targets the actual ecological differences between species, presumably underlying relationships between diversity and ecological function (Norberg et al. 2001, Norberg 2004). Trait-based approaches have therefore been put forward as a better strategy to measure and understand the importance of biodiversity for ecosystem functioning (McGill et al. 2006, Hillebrand and Matthiessen 2009). As a response, Biological traits analyses have developed strongly the past years and there are a number of studies describing how traits and trait variation of taxa that characterize community structure translate into ecosystem functions (Bremner et al. 2006a, Lavorel et al. 2011). Many studies addressed how habitat-forming and/or ecosystem engineering organisms can indirectly influence ecosystem function by facilitating assemblages of associated organisms with their own engineering role (see Passarelli *et al.* 2014). For instance, habitats created by mangroves (foundation species) promote the occurrence of highly bioturbating ecosystem engineers such as crabs (Kristensen 2008). Here, we use a coordinated sampling of the habitat-forming seagrass *Zostera marina*, across the northern hemisphere to evaluate a functional cascade by estimating the influence of meadow structure on the trait distribution of sediment-dwelling macrobenthos known to affect sediment biogeochemistry.

Seagrass meadows are vital habitats of many coastal systems and provide numerous ecosystem goods and services (de la Torre-Castro & Rönnbäck 2004). Being both a food source and habitat for many organisms, seagrasses sustain highly productive food-webs and facilitate the presence of a variety of organisms across trophic levels (Heck et al. 2008). The high productivity and physical effects of seagrass meadows contribute to nutrient cycling and carbon sequestration (Duarte et al. 2005, McGlathery et al. 2007). The exchange of organic matter and energy between seagrasses and other ecosystems also has a major role in sustaining marine biodiversity (e.g. coral reefs, Dorenbosch *et al.* 2005). In addition, seagrasses are ecosystem engineers that progressively attenuate currents and waves with increasing shoot density, trapping suspended organic matter and sediment particles (Gambi et al. 1990, Granata et al. 2001). This together with the structural effect of the rhizome-root system promote sediment stabilization and carbon sink capacity (Duarte et al. 2005, de Boer 2007, Hendriks et al. 2008), with implications for coastline protection and management (Ondiviela et al. 2014). Seagrasses are threatened by a number of human-driven stressors, primarily eutrophication, climate change, overfishing, pollution, and urbanization of coastal zones (Waycott et al. 2009).

The structural complexity of a seagrass meadow, defined as the abundance of physical structures, can be considered at a range of spatial scales (from leaf to seascape) and have direct implication for the associated fauna (Prado et al. 2009, Staveley et al. 2016). Seagrass provides habitat for benthic fauna living both on the grass blades and surface of the sediment (epifauna) and taxa that live within the sediment (infauna) (Orth et al. 1984, Boström and Bonsdorff 1997). In general, there is ample experimental evidence from within-meadow field studies that demonstrates that the presence of seagrass increase the diversity and abundance of benthic fauna (reviewed in Orth *et al.* 1984; Boström, Jackson & Simenstad 2006) and change the distribution of functional traits (Bostrom and Bonsdorff 2000, Boström et al. 2006a, 2010). The infauna is favored by the aboveground shoots, which decrease hydrodynamic stress and change sediment conditions and thereby favor their recruitment (Boström and Bonsdorff 1997); and the belowground structures roots and rhizomes that provide refuge from predators (Orth et al. 1984). However, increasing densities of roots and rhizomes may also prevent larger and/or tube building species that bury deep in the sediment (Stoner 1980, Brenchley

1982). Thus, while seagrass shoots and roots both contribute to sediment accretion and stabilization through reducing currents and binding sediments, the plants may further influence sediment dynamics by facilitating the occurrence of species with certain life history traits related to sediment reworking capacity (Figure 1). We addressed this conceptual hypothesis of a functional cascade of seagrass meadow structure on the functional diversity of benthic fauna, by relating changes in meadow structure of *Z. marina* across its global range to the functional diversity of associated fauna. We tested two general hypothesis based on the described meadow-specific effects of presence and complexity of seagrass meadows: (1) the presence of seagrass increase the overall taxonomic and functional diversity of the macrobenthos; (2) increasing seagrass complexity (expressed as above- and belowground biomass components) is positively related to epibenthic and shallow living bioturbators, and negatively related to deep-burrowing infauna.

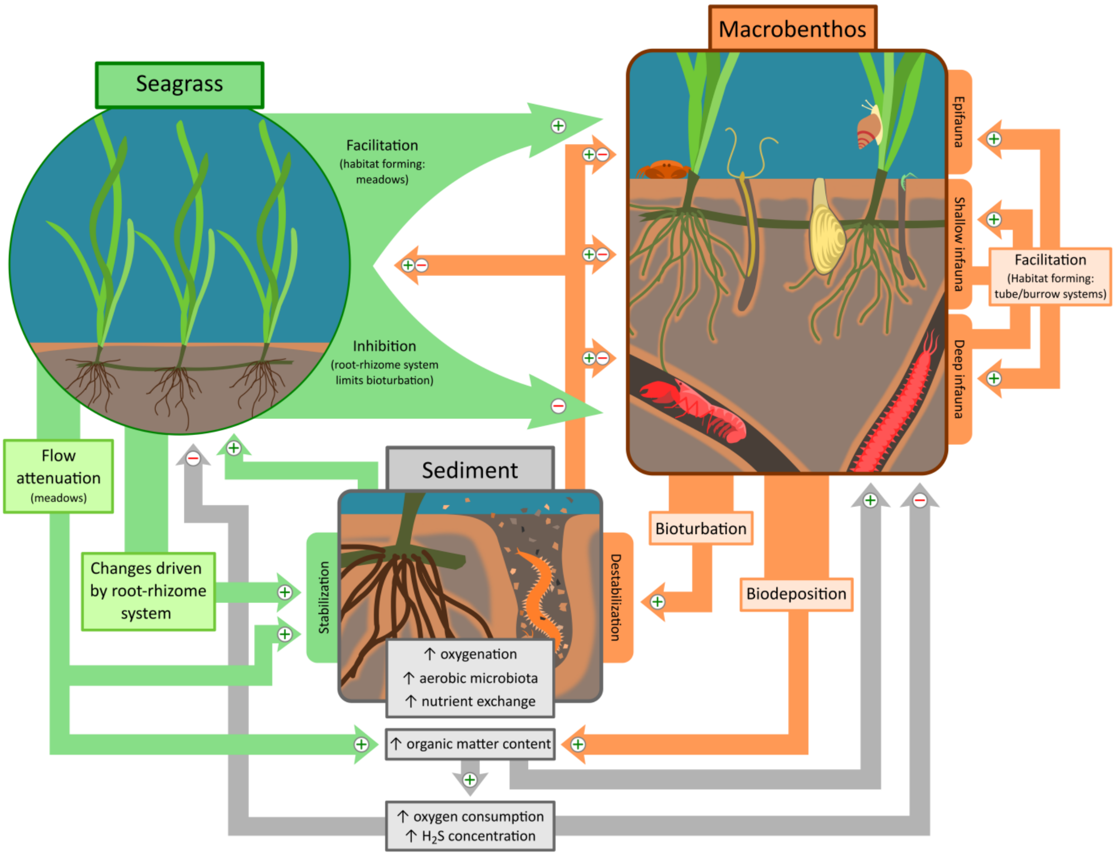


Figure 1: Conceptual model of the functions and effects on sediment performed by seagrass (green arrows and boxes) and by the macrobenthos (orange). Effects on the sediment biogeochemistry and associated feedbacks are denoted in gray boxes and arrows. Positive signs indicate promotion/increase; negative sings indicate inhibition/decrease.

METHODS

Study area

Samples were collected during the summer of 2014 at a total of 9 monospecific subtidal meadows of *Zostera marina* located in in France, Finland, Norway, Japan, and the United States of America. France sampling sites were located in two Mediterranean lagoons (Étang de Bages-Sigean 43°05N 3°00’E; Étang de Thau 43°23’N 3°38’E); Finland at Ängsö in the Archipelago Sea (60°06’N 21°43’E); Norway at three locations within a large fjord complex in Bodø (67°13’N 14°58’E); Japan in Akkeshi Bay (43°02’N 144°51E); and U.S.A. in two locations of the Bodega Bay, California (38°19’N 123°03’W). The mean values of water depth, temperature, and salinity of each sampling site are shown in Table 2.

Table 2: Sampling sites codes and *in situ* measurements of average water temperature (°C), water depth at high tide (m), water salinity (ppt); and estimated sediment organic matter content (mean ± SD).

Country	Site name	Side code	Temp. °C	Depth at high tide (m)	Salinity (ppt)	Sediment O.M. (%)
U.S.A	Bodega Bay A	Bod.1	13.5	2	33	0.73 ± 0.12
U.S.A	Bodega Bay B	Bod.2	14	2	33	0.56 ± 0.22
Finland	Ängsö	Fin.1	13	2.75	6	0.26 ± 0.05
France	Bages-Sigean	Fra.1	19.7	1.5	38.8	3.63 ± 3.24
France	Thau	Fra.2	27.3	0.5	32.5	6.8 ± 1.13
Japan	Akkeshi Bay	Jap.1	26	0.75	21	3.41 ± 0.4
Norway	Misvaersfjorden	Nor.1	11	1.42	22.8	5.37 ± 2.84
Norway	Rovika	Nor.2	8	0.42	24.5	1.46 ± 0.46
Norway	2 Bridge	Nor.3	10.5	0.65	18.5	1.56 ± 0.73

Sampling and laboratory procedures

Samplings were made by SCUBA diving, snorkeling or wading. At each site, ten sampling plots (0.5 x 0.5 m PVC squares) were randomly distributed within a meadow at two visually-identified shoot density levels (high and low; n = 5). Distinctions between shoot density levels were made visually at the site and intended to capture the range of shoot density found at that site. Additionally, five plots were also placed in the unvegetated sediment between meadows, for a total of fifteen plots per site. All plots were placed $\geq$2 m apart from each other and at least 2 m distant from the meadow border to avoid edge effects.

Within each plot, shoot density was estimated by counting individual shoots. In case of very high densities, the shoot counting was made only in a subset of 0.25 x 0.25 m in the plot. To quantify the macrobenthos, seagrass shoot length, and seagrass above- and below-ground biomass, a sediment sample was taken using a ~10 cm deep core (∅ 10 cm) in the center of each plot. Two additional 5 cm deep cores (∅ 2 cm) were taken on opposite sides of the plot within undisturbed sediment (~ 10 cm from the big core) to quantify sediment organic matter. Sediment samples were stored on ice until further laboratory processing. All the measured variables were extrapolated for the plot scale.

Macrobenthic samples, including both epifauna and infauna, were washed on 1 mm mesh sieves and all animals sorted and stored in 70% ethanol, and counted and identified to species. The macrobenthos were also classified according their degree of association with the categories of six bioturbation related functional traits (Box 2): *bioturbation mode* (6 categories), *depth in the sediment* (6 categories), *body size* (7 categories), *feeding mode* (5 categories), *adult movement* (4 categories), and *relative adult mobility* (4 categories). Information about the species traits was collected from the literature, online databases (e.g. www.marlin.ac.uk, www.genustraithandbook.org.uk, www.polytraits.lifewatchgreece.eu), and opinion of specialists based on the species behavior and morphology. The classification was made using fuzzy coding (Chevenet et al. 1994) ranging from 0 (no association) to 3 (total association). The codification was standardized for each trait such that the sum of all categories of a functional trait was always 3. This standardization reflected how exclusively the species related to a given trait category. For instance, a macrobenthic species that can be both suspension- and deposit-feeder would have the scores values of 1.5 for the category suspension-feeding, 1.5 for deposit-feeding, and 0 for the others.

Seagrass plant characteristics were also quantified to assess their relationship with the microbenthic community. Five seagrass shoots were collected from each plot (four in the corners and one in the center) to estimate average shoot length. The average shoot length of each plot was estimated by measuring the length (mm) of the tallest blade of each separated shoot using a measuring tape (from the bottom of the meristem to the blade tip; n = 5 per site). After removing all the epiphytic algae, shoots, roots and rhizomes from each plot were separated dried in an oven at 60°C for 24 hours. Shoot dry weight was used as an estimate of above-ground biomass, while the combined root and rhizome dry weights estimated below-ground biomass. Sediment organic matter content was quantified from ~5 g of sediment and calculated as the differences between the dry sediment samples before (~5 g) and after ignition in a muffle furnace at 550°C for 5 hours.

Data analysis

Macrobenthic functional diversity was estimated by calculating two multivariate indices, Functional Richness (FRic) and Functional Divergence (FDiv) (Villéger et al. 2008). We did not include Functional Evenness (FEve) since, based on preliminary exploratory analyses, it was highly affected by changes in the number and type of functional traits included in the calculation. FRic estimates the total range of species traits in an assemblage trait space by calculating the total volume of a convex hull that comprises all the species observed in an assemblage (Villéger et al. 2008). This index only accounts for species presence/absence and is affected by adding or removing species with unique and/or extreme trait values combinations. FDiv measures the distribution of species' relative abundances in a functional trait space in relation to the gravity center of the functional trait space (Villéger et al. 2008). The indices calculations were made in R programing environment (R Development Core Team 2009) using the package *FD* (Laliberté and Legendre 2010).

The relationship between macrobenthic taxonomic and functional diversity, and seagrass meadow structure; expressed by shoot density, shoot average length, above-ground biomass, and below-ground biomass, was analyzed by fitting linear models (LMs). The differences among sites were also included as a random factor. General differences among bare sand and plots with low and high shoot densities were analyzed using ANOVAs. Values of shoot density, above-ground biomass, and below-ground biomass were square root transformed prior the analysis. All LMs were run using the R package *nlme* (Pinheiro et al. 2016).

Qualitative changes in macrobenthic functional trait composition along gradients in seagrass attributes were assessed using Biological Traits Analysis (BTA) based on the abundance of functional trait categories. Functional trait abundances per plot were obtained by multiplying the species abundance matrix (transformed to the squared-root) by the fuzzy coded functional trait matrix. In other words, trait abundances are trait frequencies weighted by species abundances at each plot. Then LMs were fitted to model changes in trait abundances across seagrass gradients. Following Anderson (2008), quantile regressions based on the 95th percentile were fitted to determinate the peaks in trait abundance along seagrass gradients. Polynomial models were chosen to describe the optimum values along the gradient (i.e. where the highest abundances were observed). Alternative polynomial models with varying degrees were compared by the corrected Akaike Information Criterion (AICc) using the R package *qpcR* (Spiess 2014). Quantile regressions were made using the R packages *quantreg* (Koenker 2016).

RESULTS

The results indicate that seagrass presence had a major impact on macrobenthic species richness across the northern hemisphere (Figure 2). Both above and below-ground biomasses correlated positively with infauna richness overall (above-ground: $R^2 = 0.61$, $F_1=4.67$, $p<0.05$; below-ground: $R^2 = 0.65$ $F_1=6.55$, $p<0.01$; Figure 2a-b). However, only below-ground biomass models retained the significant effects after deleting the bare plots ($R^2 = 0.67$, $F_1=6.14$, $p<0.05$). At Scandinavian sites, seagrass shoot density correlated strongly with macrobenthic richness ($R^2 = 0.65$, $F_1=4.03$, $p<0.05$; Figure 1c; Table A1). In general, site-specific positive relationships were mostly related to European meadows (Table A1).

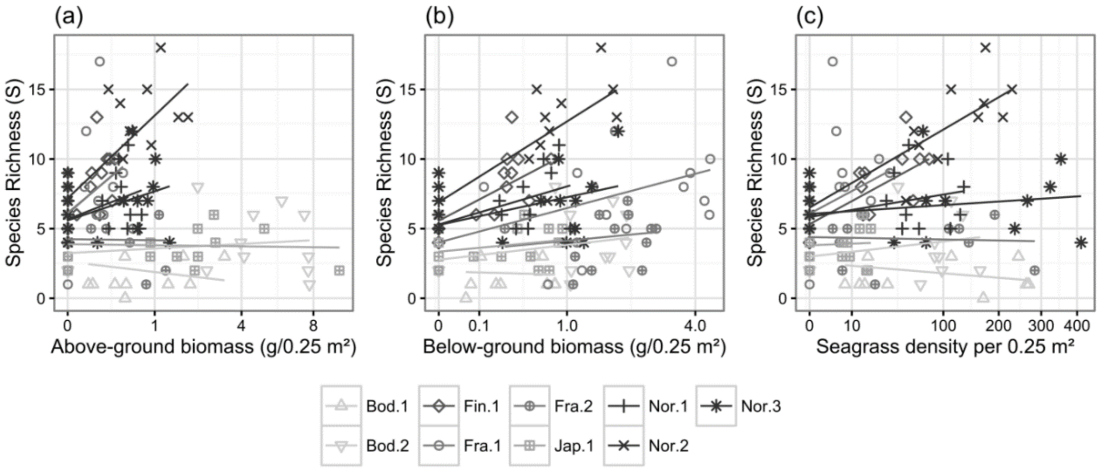


Figure 2: Relationship between the seagrass properties and macrobenthic species richness across the northern hemisphere. (a) Above-ground biomass, (b) below-ground biomass, and (c) shoot density. Lines show fitted models for each site.

Macrobenthic species richness was highly dependent on the structure of the seagrass meadows, and thus varied across sites. In general, European and Pacific meadows presented contrasting meadow structures (Figure 3a-b, Appendix 2). The relationship between shoot density and total seagrass biomass shows that biomass/shoot density ratio was much higher at Pacific sites, which contained long-leafed sparse meadows, compared to European sites, which were predominantly short-leafed and denser meadows (Figure 3a-b; Table A2). Across sites, macrobenthic richness was negatively correlated to above-ground meadow attributes, such as average shoot length (Figure 3c) and the ratio between above- and below-ground biomasses (Figure 3d). As expected, increasing macrobenthic taxonomic richness was related to an increased functional diversity of the macrofauna (Figure 3e-f). Accordingly, the presence of seagrass increased functional richness (FRic) of the macrofauna significantly (ANOVA: $F_2=3.7$, $p<0.05$; Figure 4a; Table A3), although the within site trends were highly variable ($R^2=0.64$, $F_1=1.45$, $p>0.05$; Figure 4b; Table A3). FRic decreased across sites with the size of the shoots (Figure 4c). Consequently, both macrobenthic richness and functional diversity tended to be higher in European than in Pacific seagrass meadows.

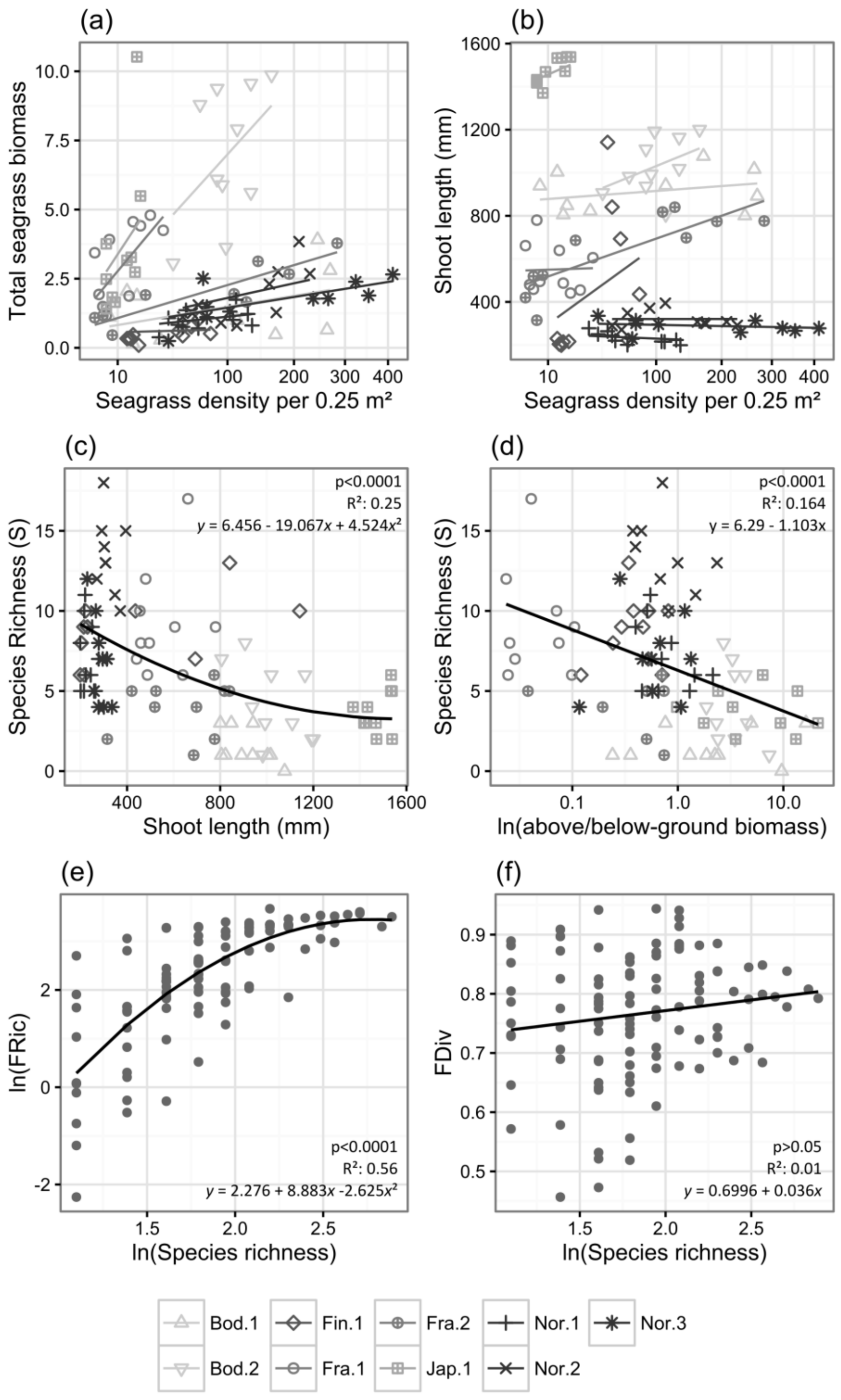


Figure 3: Relationship between (a) shoot density and seagrass biomass; (b) shoot density and mean shoot length; (c) mean shoot length and macrobenthic species richness; and (d) the ratio below/above-ground biomass and macrobenthic species richness; and the relationship among species richness and the functional diversity indices FRic (e) and FDiv (f).

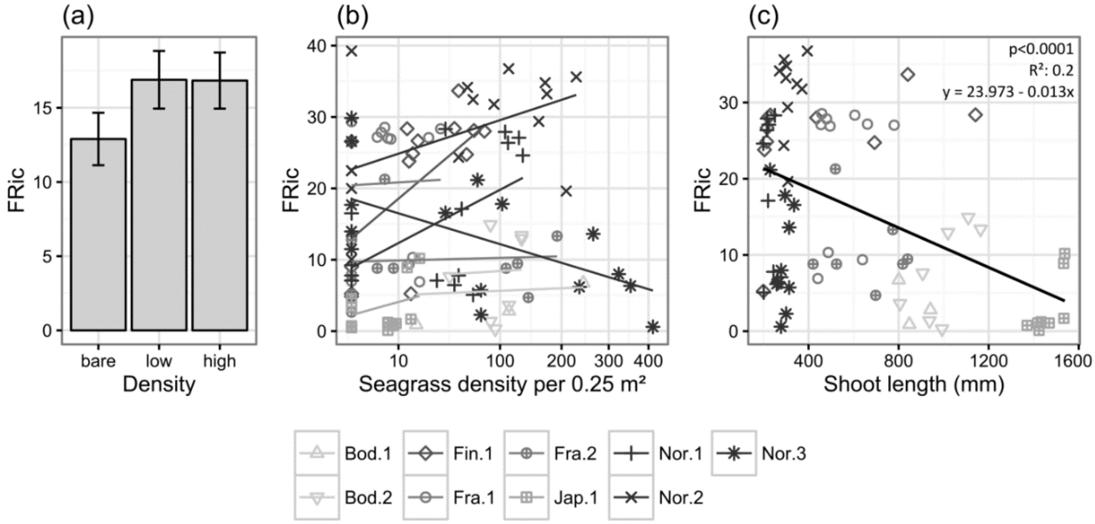


Figure 4: Changes in functional richness (FRic) along shoot density levels (a) and gradients in seagrass shoot density (b) and above-ground biomass (c).

Changes in functional trait composition were mostly driven by the functional traits *depth in the sediment* and *bioturbation type*. The presence of seagrass increased the abundance of epifauna and the shallow infauna (0-3 cm depth), but decreased the abundance of the deep infauna (Figure 5a-b; Table A4). However, within meadows, increasing above-ground biomass of seagrass correlated with a decrease in shallow infauna (Figure 5b). This was confirmed across sites where the abundance of shallow infauna peaked at low above-ground seagrass biomasses (Figure 5b). The presence of seagrass increased the abundance of non-bioturbating epifaunal species, but also the abundance of biodiffusers significantly (Figure 6, Table A5). Globally, biodiffusers peaked at low seagrass above-ground-biomass, and decreased strongly at higher seagrass biomass (R^2=0.74; F_1=5.3; p<0.05; Figure 6b). Conveyors were negatively influenced by seagrass biomass, both within and across meadows globally (R^2=0.63; F1=3.81; p=0.05; Figure 6c).

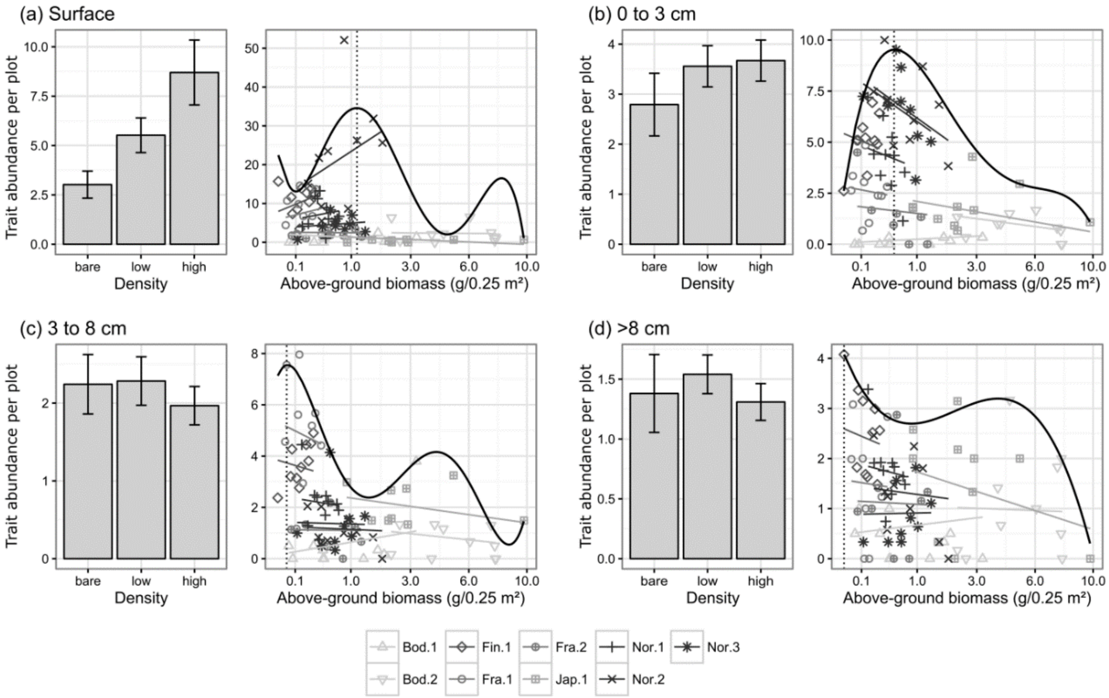
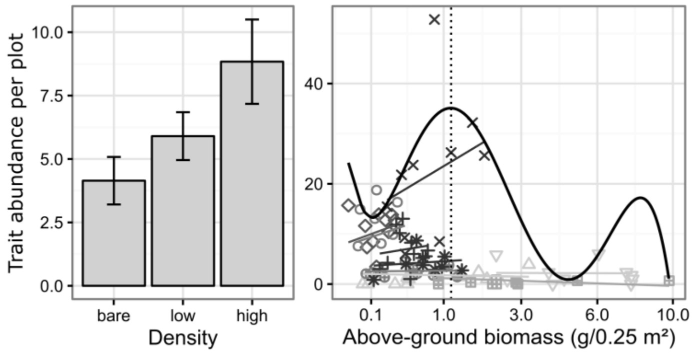
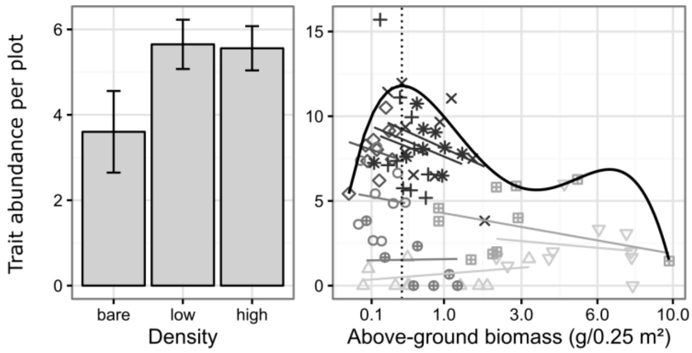


Figure 5: Changes in abundance of the categories of the trait *Depth in the sediment* along shoot density levels (bar plots) and gradients in above-ground biomass (histograms). Lines indicate linear models fitted for each site. Thick black lines represent the best polynomial model fitted by the quantile regressions (on the 95th quantile). Vertical dotted lines indicate the optimum found by the quantile regression (value along the gradient that holds the highest abundance).

(a) None



(b) Biodiffuser



(c) Up-Down conveyours

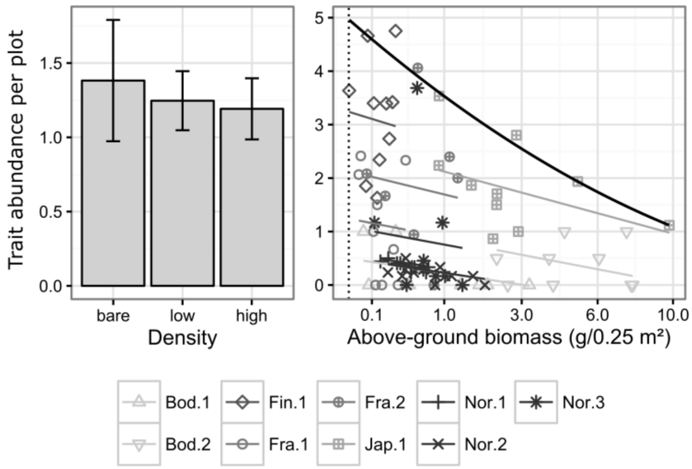


Figure 6: Changes in abundance of Bioturbation trait categories (*a*. none, *b*. biodiffuser, *c*. up-down conveyor) along shoot density levels (bar plots) and gradients in above-ground biomass (histograms). Lines indicate linear models fitted for each site. Thick black lines represent the best polynomial model fitted by the quantile regressions (on the 95th quantile). Vertical dotted lines indicate the optimum found by the quantile regression (value along the gradient that holds the highest abundance).

DISCUSSION

Our results support that *Z. marina* meadows have the potential to induce a functional cascade, where the effects of the facilitation promoted by plants on sediment processes are likely to occur through changes in the functional composition of bioturbating macroinvertebrates. Across the northern hemisphere, both the taxonomic richness and functional diversity of the sediment fauna was higher in the presence of seagrass meadows as compared to the bare sediment. This was mostly driven by epifauna and shallow-living biodiffusing infauna, whose abundances increased strongly in the presence of seagrass. This indicates increased sediment reworking rates at shallow layers of the sediment within the meadows, potentially affecting sediment resuspension and other bioturbation-related processes (Kristensen et al. 2012). However, peaks in macrobenthic functional diversity were mostly related to low or medium levels of shoot length and above-ground biomass, suggesting a nonlinear response of the macrobenthic functional structure to increasing seagrass meadow complexity. Therefore, seagrasses promote sediment-dwelling macrobenthos, but it is dependent on meadow above-ground complexity. European meadows in general had a lower above-ground biomass and smaller denser shoots, while Pacific meadows had a higher above-ground biomass and larger shoots. The differences in meadow structure across European and Pacific meadows suggest that the role of *Z. marina* in promoting sediment-dwelling macrobenthic functions is variable across these biogeographic regions.

Upward-downward conveyors decreased with the presence and the increased complexity of the meadow. Conveyors are infaunal species with high bioturbating capacity that increase the exchange of nutrients and organic carbon across the vertical sediment-water axis, by feeding head down or head up the sediment and defecating on the other side (Kristensen et al. 2012). This suggests that the potential effect of seagrass on sediment reworking processes change with meadow structure. Meadows with low above-ground biomass indirectly facilitate higher bioturbation related processes in the shallow layers of the sediment, which should promote sediment resuspension, while meadows with high above-ground biomass decrease macrobenthic bioturbating capacity. Thus, the *sediment stabilizing* function of seagrass meadows with dense, short shoots seems to be weakened by their facilitation function (i.e. more bioturbators within meadows), while the foundation function in meadows with long broad shoots seem to reinforce their *sediment stabilizing* engineering function.

The importance of meadow structure for the functional composition of the sediment fauna suggests that the seagrass has indirect, but complex effects on sediment processes. Highly bioturbating macrobenthos can promote sediment oxygenation of deep layers and facilitate colonization of deeper species, transforming the sediment in a high 3D dimension matrix (Rosenberg 2001, Meysman et al. 2006a). Through bioirrigation, oxygenated interstitial water reaches deep sediment and allows oxidation of decomposing organic matter, avoiding the production of toxic compounds such as H_2S and somewhat promoting the release of mineralized carbon from the sediment to the water column (Rosenberg 2001, Mermillod-Blondin 2011). However, Rossi *et al.* (2013) described that the exclusion of bioturbators increased CO_2 mineralization of macroalgal detritus, which was related to the capacity of highly bioturbating species (lugworms) to enhance burial of vegetal detritus and consequently reduce decomposition rates. This has effects on the whole ecosystem metabolism since bioturbation promotes heterotrophic compartments of the benthic microbiota and also facilitate the occurrence of other macrobenthic species (Rosenberg 2001, Reise 2002, Mermillod-Blondin 2011), although it can also negatively affect other species such as algae (Brustolin et al. 2016) and other macrobenthos (Alvarez et al. 2013).

These biogeochemical changes might counteract some of the seagrass ecological services, such as carbon sequestration and sediment stability (Figure 1). Also, highly bioturbating species can have negative effect on seagrass plants (Suykerbuyk et al. 2012). So far, the general consequences of the interplay between seagrass and macrobenthic function on the general state of the ecosystem are still poorly studies (Maxwell *et al.* in press). Our results indicate that seagrass meadows promote increased variety of ecosystem services by allowing a functionally diverse sediment-living assemblage, but at the same time the influence on important functions, such as sediment resuspension, seem to change depending seagrass meadow structure. This demonstrates that we need coordinated experimental approaches across large biogeographic regions to understand complex biodiversity effects on ecosystem functioning.

The importance of meadow structure for the functional composition of the sediment fauna is reinforced by the engineering effects on flow rates. Seagrass attenuate flow, which increases sedimentation and also prevents particle resuspension (Gambi et al. 1990, Granata et al. 2001). This promotes higher recruitment rates and favor macrobenthic post-settlement establishment; probably contributing to the global increase in taxonomic and functional diversity within meadows. However, accumulation of fine organic-rich sediments also promote sediment anoxia and sulfide toxicity (Terrados et al. 1999), which may smother organisms in the sediment (Vaquer-Sunyer and Duarte 2010). This may explain why the abundance of both biodiffusers and shallow infauna was so strongly promoted by low levels of above-ground seagrass biomass, while they decreased at higher levels of above-ground biomass. It also is consistent with the general decrease in functional diversity with increasing shoot length. Local environmental conditions that determine the meadow structure may also have negative effects on macrobenthic diversity. For example, long shoot leaves are frequently associated with stressed conditions induced by light limitation events (Longstaff and Dennison 1999), and the same stressed conditions could cause the apparent correlation between long leaves and low macrobenthic diversity. However, the consistency of the general trends of the infauna along above-ground biomass gradients both within and between meadows suggests that seagrass structure is a driver of macrobenthic taxonomic and functional diversity in Z. marina meadows.

The response of bioturbating infauna to seagrass biomass across meadows was non-linear; the abundances of fauna increased strongly with seagrass presence but peaked at relatively low levels of above-ground biomass. Studies have described positive relationships among macrobenthic taxonomic diversity and increasing seagrass biomass within regions (Attrill et al. 2000). Our results indicate such monotonic relationships are not present when comparing macrobenthic functional traits across larger biogeographical regions. This suggests that indirect effect of seagrass on sediment function has a threshold where it changes and that local relationships cannot be generalized without the regional context. This is key information for the development models to describe the cascading effect of Z. marina on ecosystem functions. Incorporating non-linear and indirect effects to the development of predictive models relating seagrass structure, benthic functional diversity, and sediment process rates would be a useful tool for marine managers to assess a more detailed picture of the seagrass ecological state as well as its associated services.

In conclusion, our study indicates that Z. marina meadows may indirectly promote ecologically relevant processes across the northern hemisphere by facilitating functionally relevant macrobenthic species; resulting in a potential functional cascade. This highlights the need to preserve and restore seagrasses not only for their directly generated services and engineering functions but also for their role as facilitators of other functionally relevant organisms such as bioturbators. The results also show that changes in functional trait distributions depend on seagrass morphology, but that they are not linearly related. Different functional traits reacted very differently to seagrass morphology, showing the high complexity of the relationship between biodiversity and function in these systems. Therefore, we suggest that to understand the macrobenthic effects on sediment-related processes in seagrass ecosystems, analyzing the relationship between specific functional traits and process rates would be a more reliable analytical tool than the diversity of functional traits.

ACKNOWLEDGEMENTS

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APPENDIX

Table A1: Pearson product-moment correlation coefficient (*r*) and *p-values* of the relationship between species richness and the seagrass attributes (shoot density, and above- and below-ground biomasses) for each analyzed site.

		Shoot density		Above-ground		Below-ground	
Site	df	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*
Bod.1	7	0.7	0.17	0.2	0.50	0.96	-0.02
Bod.2	13	0.1	0.44	0.2	0.37	0.1	0.44
Fin.1	13	**<0.001**	0.83	**<0.001**	0.77	**<0.001**	0.82
Nor.1	13	0.5	0.17	0.6	0.13	0.3	0.27
Nor.2	13	**<0.0001**	0.87	**<0.01**	0.73	**<0.001**	0.83
Nor.3	13	0.97	-0.01	0.5	0.17	0.6	0.14
Fra.1	13	0.2	0.38	0.07	0.49	0.08	0.46
Fra.2	13	0.5	-0.18	0.3	-0.31	0.6	0.14
Jap.1	13	0.8	-0.06	0.6	-0.14	0.8	0.09

Table A2: Linear models results of the relationship between total seagrass biomass and shoot density; and shoot length and shoot density. Pearson's correlation results for each site are also presented.

Linear model results						
	Total biomass ~ Density			Length ~ Density		
	$R^2 = 0.74$			$R^2 = 0.92$		
	df	*F-value*	*p-value*	df	*F-value*	*p-value*
Intercept	1	6.38	**0.01**	1	18.70	**<0.0001**
Fixed effects (Density)	1	6.43	**0.01**	1	2.33	0.1
Pearson's correlation	Total biomass ~ Density			Length ~ Density		
	site	*p-value*	*r*	site	*p-value*	*r*
	Bod1	0.2	0.46	Bod1	0.8	0.09
	Bod2	0.1	0.52	Bod2	0.2	0.49
	Fin1	**0.02**	0.70	Fin1	0.1	0.53
	Nor1	0.2	0.45	Nor1	**0.01**	-0.76
	Nor2	**0.01**	0.74	Nor2	0.6	-0.16
	Nor3	**0.03**	0.67	Nor3	0.4	-0.32
	Fra1	**0.04**	0.65	Fra1	0.6	-0.21
	Fra2	**<0.001**	0.88	Fra2	**<0.01**	0.83
	Jap1	0.07	0.60	Jap1	**<0.01**	0.83

Table A3: Anova results of the index FRic for functional diversity comparing sites and shoot density levels (bare, low, and high).

	df	SS	MS	F-value	p-value
Site (S)	7	6060.5	865.8	17.23	**<0.0001**
Treatment (T)	2	371.6	185.8	3.70	**<0.05**
S*T	13	2038.3	156.8	3.12	**<0.001**
Residuals	80	4019.6	50.3		

Table A4: Anova results of the categories of the trait *Depth in the sediment* comparing sites and shoot density levels (bare, low, and high).

		Surface		0 to 3 cm	
	df	*F-value*	*p-value*	*F-value*	*p-value*
Site (S)	7	26.11	**<0.0001**	29.24	**<0.0001**
Density (D)	2	5.82	**<0.01**	1.14	0.33
S*D	10	4.40	**<0.0001**	2.87	**<0.01**
Residuals	71				

		3 to 8 cm		> 8 cm	
	df	*F-value*	*p-value*	*F-value*	*p-value*
Site (S)	7	17.80	**<0.0001**	4.66	**<0.001**
Density (D)	2	1.71	0.19	0.78	0.46
S*D	10	5.08	**<0.0001**	0.81	0.62
Residuals	71				

Table A5: Anova results of the categories of the trait *Bioturbation* comparing sites and shoot density levels (bare, low, and high).

		None		Biodiffusers		Up- and downward	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Site (S)	7	24.35	**<0.0001**	25.65	**<0.0001**	12.02	**<0.0001**
Density (D)	2	4.61	**<0.05**	0.07	0.93	0.06	0.94
S*D	10	4.73	**<0.0001**	3.42	**<0.01**	1.16	0.33
Residuals	71						

APPENDIX 2

We explored the variation trends of seagrass attributes across sites and countries using Principal Components Analysis on standardized data (performed in R programing environment using the *vegan* package). Since some of the attributes were highly collinear (Pearson correlation >0.8), only five attributes were used in the PCA: shoot density, shoot length, above-ground biomass, below-ground biomass, and number or leaves. Bare sediment plots were not included in the PCA.

The PCA depicting the variation trends of seagrass attributes across countries and sites showed gradients in seagrasses attributes associated to the first two axes (Figure 1). The first axis explained 34.6% of the total variation and was mostly associated to changes in above-ground biomass, shoot mean length, and number of leaves. The second axis explained 28.9% of the total variation and was associated to changes in seagrass shoot density, below-ground biomass, and shoot length. The mean number of leaves per shoot was also associated to the second axis, but in a smaller degree. European seagrass meadows were characterized by shorter shoot leaves and smaller above-ground biomass comparing to the Japanese and American meadows. Differences between American and Japanese meadows were related to the second axis. Internal variability in European meadows was mostly related to the second axis. Although the clear superposition of European meadows, Finland tended to present lower shoot density and above-ground biomass.

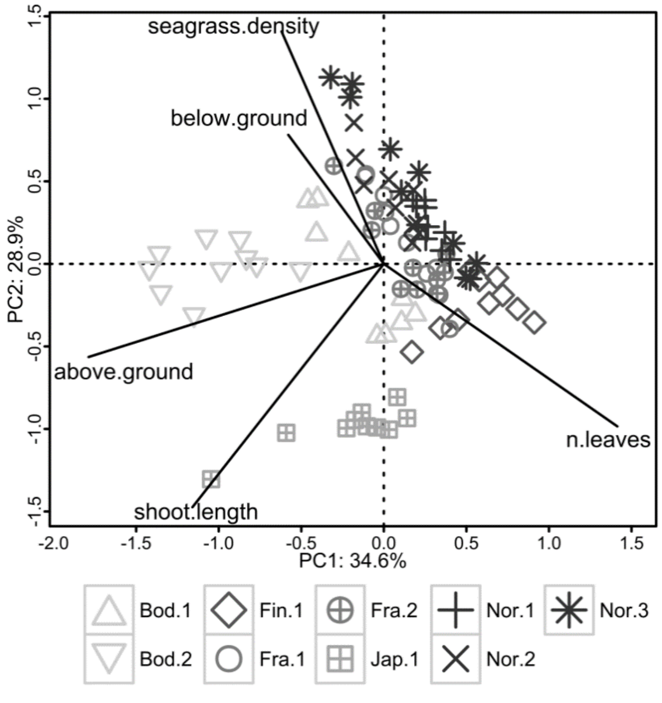

Figure 1: Principal Components Analysis depicting the variation trends in the seagrass attributes for each site within each country. Symbol legends: Bod=Bodega Bay (U.S.A.); Fin = Finland; Fra = France; Nor = Norway; Jap = Japan. Vector codes: n.leaves = average number of leaves.

CHAPTER 5

Effects of macrobenthic functional diversity on microphytobenthic assemblages

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ABSTRACT

Microphytobenthos are the most important primary producers in unvegetated intertidal sedimentary systems. They are highly influenced by hydrodynamics, but biotic disturbances are also major drivers of their structure and productivity. Although sediment-dwelling macrobenthos are known to disturb sediment matrices, most studies are restricted to a few bioturbating species. There is scant information on the functional effect of macrobenthic assemblages as a whole on microphytobenthos. In this study, we analyzed the functional relationships between macrobenthos and microphytobenthos on tidal flats across a subtropical estuarine gradient, from high-energy saline (i.e. outer sector) to low-energy less saline areas (i.e. inner sectors). We used functional diversity indices and biological traits analysis to assess the effects of macrobenthic functional diversity and composition on microphytobenthic structure. The relationship between macrobenthic functional diversity and microphytobenthic biomass and diversity was spatially inconsistent and highly depended on the analyzed sector. A gradient in macrobenthic traits was observed along the estuarine system, varying from dominance of large-sized, highly bioturbating infauna at the outer sector to dominance of small-sized, nonbioturbating epifauna at the inner sectors. This gradient explained trends in microphytobenthic species composition across sectors, showing that microalgal assemblages in the outer sector were mostly related to dominance of bioturbating macrobenthos. Microphytobenthic biomass and diversity showed a bell-shaped relationship across the macrobenthic trait gradient, while species evenness increased as bioturbating macrobenthos became dominant. These results indicate that, instead of functional diversity, the functional identities of the macrobenthic species, expressed by species with different capacities to bioturbate the substrate, controlled microphytobenthic structure in this estuarine system.

INTRODUCTION

Macrobenthic species play critical roles in marine coastal ecosystems, as the main secondary producers in bottom food webs, serving as food for human populations, and sustaining economically relevant fisheries (Ambrose Jr. 1984, Carlson et al. 1997, Chen et al. 2016). Soft-bottom macrobenthic species also play a key role in regulating sediment properties and biogeochemistry (Snelgrove 1998, Mermillod-Blondin 2011), mostly through their feeding strategies, which may increase oxygenation, enhance vertical particle movements and alter sediment stability (Kristensen et al. 2012). Benthic bioturbation can also affect nitrogen, carbon, and sulfur cycling in marine sediments and thus directly or indirectly affect other organisms living in and on the sediment, such as the microphytobenthos (Mermillod-blondin and Rosenberg 2006, Kristensen et al. 2012, Birchenough et al. 2012).

Microphytobenthos are an assemblage of eukaryotic microalgae and cyanobacteria species, often the main primary producers in tidal flats and an important food source for benthic invertebrates (Underwood and Kromkamp 1999, Kromkamp et al. 2006). They may form extensive microbial films on the sediment surface that can be visually identified by patchy color patterns. Microphytobenthic assemblages can also have a significant impact on sediment biogeochemistry through nutrient consumption and production of extracellular polymeric substances (or exopolysaccharides) that have cohesive effects on individual particles, contributing to sediment stability by decreasing erosion rates (Widdows and Brinsley 2002). Therefore, variation in microphytobenthos across space and time affect not only the local primary production, but also the general functioning of the sedimentary system.

Physical forces are the main drivers of microphytobenthic variation in sedimentary systems. The hydrodynamic stress driven by wind, waves, and tidal currents promote major resuspension events, affecting microphytobenthic assemblages along large spatial scales (de Jonge and van Beusekom 1995). The vulnerability to such disturbances can be modulated by macrobenthic species themselves due to increased sediment destabilization via bioturbation (de Deckere et al. 2001, Widdows and Brinsley 2002). Experimental studies described changes in microphytobenthos structure driven by selective grazing and bioturbation (Brustolin et al. 2016), showing that macrobenthic feeding behavior and bioturbation capacity also play a role in explaining microphytobenthic variation. Selective grazing on dominant microalgal species can promote microphytobenthic diversity and evenness (Hagerthey et al. 2002), while bioturbation effects on chemical flow in sediments can boost microalgae production (Chennu et al. 2015). In addition, habitat forming organisms such as mussels and oysters can have drastic effects on local hydrodynamics, promoting sheltered low energy patches where microphytobenthos can thrive (Donadi et al. 2013b). These studies suggest that effects of sediment-dwelling fauna on microphytobenthos depends mostly on their functional effects on the sediment matrix, mostly explained by their behavioral and morphophysiological traits.

Functional analyses of macrobenthic assemblages have gained popularity in recent years (Gusmao et al. 2016, Otegui et al. 2016, Van Der Linden et al. 2016) and have produced novel and promising information on the ecology of marine sediments. Most of the proposed functional approaches are trait-based techniques that relate species functional traits to the structure or functioning of ecological systems. Functional traits are any organismal characteristics related to an individual's performance (i.e. survival, growth, and reproduction success) that can directly or indirectly affect one or more ecosystem functions or processes (Violle et al. 2007, Mlambo 2014). Two widely used trait-based approaches are the biological traits analysis (BTA) and calculation of functional diversity indices (Villéger et al. 2008, Laliberté and Legendre 2010). BTA assesses variation trends in the frequency of one or more functional traits and associated subcategories (also known as attributes), usually based on multivariate ordinations (Bremner et al. 2006b). Functional diversity indices quantify the variety of functional traits in an assemblage, and may also be weighted by species abundances (Villéger et al. 2008, Laliberté and Legendre 2010). Multivariate indices of functional diversity proposed by (Villéger et al. 2008) are relatively recent analytical tools to assess the functional diversity of ecological systems and have been applied both to terrestrial and aquatic environments (Gerisch et al. 2012, Gusmao et al. 2016). The combined usage of BTA and functional diversity indices may provide pragmatic and applied approaches to the functional structure of benthic assemblages, as in the assessment of benthic assemblage variation along natural and human-induced environmental gradients (Bremner et al. 2006a, Gusmao et al. 2016).

Although macrobenthic bioturbation is known to affect overall benthic diversity (Alvarez et al. 2013, Brustolin et al. 2016), a major gap remains in the knowledge of how the diversity of their functional strategies can influence other benthic compartments such as the microphytobenthos. To address this issue, we carried out a field survey to assess the functional relationships between macrobenthic and microphytobenthic assemblages in tidal flats from high-energy haline to low energy and less saline sectors of Paranaguá Bay, a subtropical estuarine system in southern Brazil. Considering that functional diversity based on bioturbation related traits would reflect the variety of modes macrobenthic species explore and modify their sedimentary environment, we tested two alternative predictions: (1) increasing macrobenthic functional diversity would provide a more heterogeneous environment for the microflora and thus would increase microphytobenthic biomass and diversity; and (2) increasing macrobenthic functional diversity would be associated to a more efficient exploitation of sediment food resources, resulting in decreased microphytobenthic biomass and diversity. Whether the microphytobenthic response to macrobenthos be positive or negative depending on the microalgal species, we expect that (3) gradients in macrobenthic trait composition would co-vary with microphytobenthic chlorophyll *a* and diversity. The first and second predictions were tested by fitting linear models between macrobenthic functional diversity indices and microphytobenthic chlorophyll *a* and diversity. The third prediction was tested by fitting linear models between gradients in macrobenthic trait composition and microphytobenthic chlorophyll *a* and diversity.

METHODS

Study area

Sampling was carried out in Paranaguá Bay, an estuarine system in Paraná State in southern Brazil, in June 2014 (Figure 1). The bay has an average depth of 5.4 m, tidal amplitude is up to 2 m, and water residence time about 3.49 days; estuarine hydrodynamics is highly influenced by tidal currents (Lana et al., 2001).

Four sampling sectors were defined were defined in tidal flats across the northern axis of Paranaguá Bay (Figure 1): one at the euhaline mouth of the bay (sector 1: Limoeiro, 25°33,61’S 48°18,96’O); one at the central polyhaline region (sector 2: Perigo, 25°28,35’S 48°25,92’O); and two located in the inner mesohaline region (sector 3: Mariana, 25º23’26”S 48°24’48”W; and sector 4: Guapicu, 25°23’20”S 40°20’00”O). The tidal flats at sector 1 are adjacent to an island located at the mouth of the bay (*Ilha do Mel*). The salinity in this sector ranges from 27 to 33 psu and the sediment is mainly composed by fine and very fine sand (Couto & Savian 1998). Sector 2 includes the tidal flats of *Baixio do Perigo*, a wide sedimentary system characterized by well-sorted fine sand and relatively low organic matter content (Noernberg et al. 2006) with salinity ranging from 24 to 28 psu. Sectors 3 and 4 have tidal flats with sediment characterized by well to moderately sorted fine sand (Lamour et al 2004, Krug & Noernberg, 2007). The salinity in these sectors is much more variable and ranges from 16 to 27 psu. In the first three sectors, empty shells of the cockle *Anomalocardia brasiliana* are abundant and contribute to the complexity of the sediment matrix.

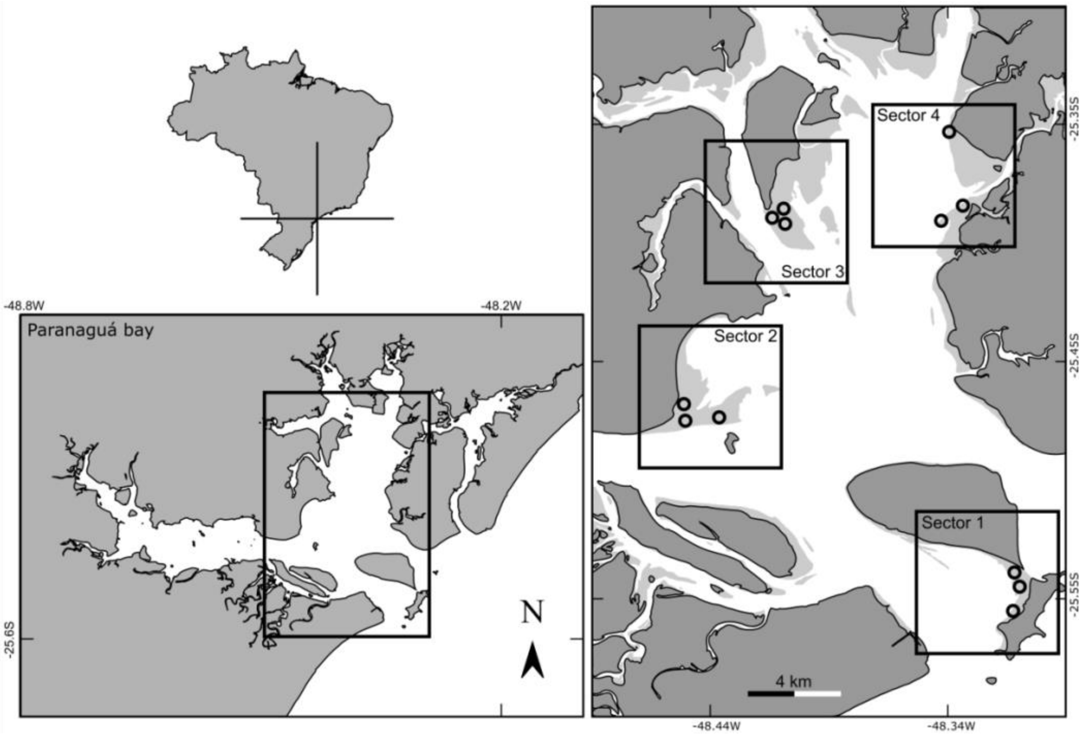

Figure 1: Left: map of Paranaguá bay showing the northern axis (black rectangle) where the samples were collected. Right: map of the northern axis showing sampling sectors (black squares) and the position of each sampling area within each sector (black circles). Tidal flats are highlighted in light gray.

Sampling and laboratory procedures

At each sector, three areas of 300 x 300 m were stablished at least 500 m apart from each other. Within each area, three locations of 50 m x 50 m were randomly delimited, in which two sampling sites of 2 m x 2m were established 20 m apart from each other. At each site, two squares of 0.5 m x 0.5 m were randomly distributed. Macrobenthos was sampled by taking sediment cores (PVC tubes with 15 cm diameter and 10 cm height) in the center of each square, resulting in 156 samples. Microphytobenthos were sampled twice in each quadrat using plastic syringes as corers (19 mm wide, 30 mm deep). One sample was taken to analyze the microphytobenthic species composition, which was immediately fixed in a 4% formaldehyde solution. The sample from the second core was taken to quantify the chlorophyll *a* concentrations. These samples were deposited in amber glass bottles and immediately stored in a cooling box. Two additional sediment samples were collected with a PVC core (4 cm diameter and 7 cm height) in each site to determine sediment texture (grain size and sorting coefficient), nutrient concentrations (P, N, and total organic C), and contents of organic matter (OM) and calcium carbonate ($CaCO_3$).

After the field sampling, macrobenthic samples were fixed in 7% formalin solution and later washed in 0.5 mm sieves to separate the organisms. Residues retained in the sieves were conserved in 70% ethanol solution and stained with Rose Bengal. Species identification and counting were performed with a stereomicroscope. The macrobenthic species were also classified according to their association degree to different categories of five functional traits (Box 2). The functional traits were: *adult size*, *bioturbation type*, *adult mobility*, *feeding mode*, and *depth in the sediment*. The classification was made using a fuzzy coding ranging from 0 to 3, in which 0 represents ‘no affinity’ and 3 ‘total affinity’ (Chevenet et al. 1994). Intermediary values between 0 and 3 were used when one species showed affinity to two or more categories of a trait. For instance, omnivorous species would be assigned with scores 1.5 for both categories *carnivore* and *herbivore* of the trait feeding mode. Therefore, the sum of the scores of all categories of a trait was always 3. The classifications were based on information collected from regional and international literature (Arruda et al. 2003, Rios 2009, Pagliosa et al. 2012, Jumars et al. 2015, Gusmao et al. 2016); online databases such as BIOTIC (www.marlin.ac.uk/biotic), Polytraits (polychaetes.lifewatchgreece.eu), and Marine Macrofauna Genus Trait Handbook (www.genustraithandbook.org.uk); and *ad hoc* opinion of local specialists. As a result of this classification, a fuzzy coded functional trait matrix was generated.

The fixed microphytobenthos samples were subsampled and analyzed under a microscope using 400x magnification. The species were identified and quantified considering their relative abundances. This was done by counting and identifying the first 100 individual algae cells observed in each subsample. Shannon diversity (H) and Pielou’s evenness (J) were calculated from relative abundances. Samples for pigment content were frozen at -20° C prior to the laboratory analysis. Pigments were extracted from 1 g of the frozen sediment by adding 10 ml of acetone (100%) and storing in dark freezing conditions (-20° C) for 24 hours. Then the samples were centrifuged at 2500 rpm for 20 minutes. Chlorophyll *a* was quantified by measuring absorbance differences of the centrifuged solutions before and after addition of HCl (1N) using a spectrophotometer

(Plante-Cuny 1978). Chlorophyll *a* content was used as a proxy for microphytobenthos abundance/biomass.

Sediment texture was evaluated by a Microtrac Bluewave laser granulometer that uses light refraction technology. Organic matter content (OM) was determined by measuring weight variation before and after burning a 5 g sample of dry sediment in a muffle kiln at 550° C for 60 minutes. Calcium carbonate content was estimated by measuring changes in weight of 10 g of dry sediment before and after addition of 20 ml of a hydrochloric acid solution (10% concentration). Nutrient concentrations were estimated following the protocols proposed by Grasshoff et al. (1983) and Strickland & Parsons (1972).

Data analysis

For both macrobenthos and microphytobenthos, core abundance values within each site were averaged to get one replicate per site. Therefore, the total number of replicates was 72 for macrobenthos, microphytobenthos, and environmental variables.

The functional diversity of the macrobenthos was quantified using the framework proposed by Villéger et al. (2008) and Laliberté & Legendre (2010) based on four multivariate indices: functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), and functional dispersion (FDis). These indices have been used to describe the functional diversity of biological assemblages in terrestrial and aquatic environments (Gerisch et al. 2012, Gusmao et al. 2016), and are complementary since they measure different dimensions of assemblage functional structure (Laliberté and Legendre 2010). Their calculations were based on the multidimensional trait space constructed from an Euclidean resemblance matrix of the species traits (i.e. the fuzzy coded scores). FRic is a measure of the total range of the functional traits observed in an assemblage and does not account for abundances in its calculations. It is mostly affected by adding or removing species with extreme or unique trait combinations. FEve estimates how evenly species abundances are distributed in the functional trait space and is mostly affected by disproportional changes in abundances of species with contrasting trait values. FDiv is an estimate of how species traits and associated abundances diverge from the average trait values of an assemblage. It is affected by changes in abundances of species with extreme or average trait values. Finally, FDis measures the average distances of the species and associated abundances to the centroid of the functional trait matrix. It can be affected by both changes in abundances and adding or removing species with extreme functional trait values. The four indices were calculated using the R package *FD* (Laliberté and Legendre 2010). Rare taxa (less than 10 individuals in total) were not included in the indices calculations.

The effect of macrobenthic taxonomic and functional diversity on microphytobenthic chlorophyll *a* and diversity was tested using analysis of covariance (ANCOVA) considering sector as a spatial fixed factor. This analysis was also used to test the effect of sediment texture on macro- and microphytobenthic diversity. Normality of error distribution, homogeneity of variances, and presence of outliers were analyzed visually by Q-Q plots, fitted versus square-rooted standardized residuals plots, and Leverage versus standardized residuals. ANCOVAs were performed using R 3.3.2 software (R Development Core Team 2009).

Variation trends in species composition of the microphytobenthos and macrobenthos were analyzed using Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarities of square-rooted species abundances. Macrobenthic trait composition was analyzed using correspondence analysis (CA) applied to a trait abundances. This trait abundance matrix was constructed by multiplying the species abundance matrix (transformed to the squared-root) by the fuzzy coded functional trait matrix. Finally, a Principal Components Analysis (PCA) was made to visualize trends in spatial variation of the sediment biogeochemical variables, including grain size (µm), sediment selection coefficient (µm), total organic carbon (mg C/g), nitrogen (mg N/g), phosphorus (mg P/g), organic matter content (%), and shell fragments (mg). Mantel tests were performed to correlate environmental variables and macro- and microphytobenthic dissimilarities. All the ordinations were performed using the R package *vegan* (Oksanen et al. 2009).

The relationship between microbenthic species composition and gradients in macrobenthic trait composition was assessed using canonical analysis for principal coordinates (CAP, Anderson & Willis 2003), considering the first axis of the CA of macrobenthic functional trait composition as an independent variable. This produced a visual picture of how changes in macrobenthic trait composition affected variation trends in microphytobenthic trait composition. Linear models were fitted to test the relationship between trait gradient (i.e. first CA axis) on microphytobenthic chlorophyll *a* and diversity. CAP was performed in R using the function *capscale()* of the package *vegan* (Oksanen et al. 2009).

RESULTS

In total, 55,503 macrobenthic individuals from 92 species were identified and counted. The gastropods *Heleobia australis* and *Bulla striata*, and unidentified species of Ostracoda sp. and Caprellidae sp. were numerically dominant, accounting for 14.1%, 10.1%, 12.9%, and 11.6% of the total macrobenthic abundance, respectively. For the microphytobenthos, we identified 14,400 individual cells from 11 species, with the diatoms *Coscinodiscus gigas*, *Cyclotella* sp., and *Diploneis* sp. numerically dominant (25.3%, 24.6%, and 11.9% of the total abundance, respectively).

Macro- and microphytobenthic species composition varied similarly across sectors (Figure 2a and 2b; Mantel test: $p<0.01$, Mantel's $r=0.35$). Except for sector 3, differences across areas were also observed for both macro- and microphytobenthos. Variation trends of the environmental variables did not follow the same trends as macro- and microphytobenthos assemblages, showing clear separation of sector 2 and from the other sectors mostly related to gradients in shell weight, CaCO3 content and the concentrations of P and N (Figure 2c, Table 2). Environmental variables also showed relatively high correlation with the microphytobenthic assemblages ($p<0.01$, Mantel's $r=0.43$). This was mostly driven by the sediment texture gradient which increasing grain size have an positive effect on microphytobenthic chlorophyll *a* and diversity in all sectors (Figure A1 and Table A1).

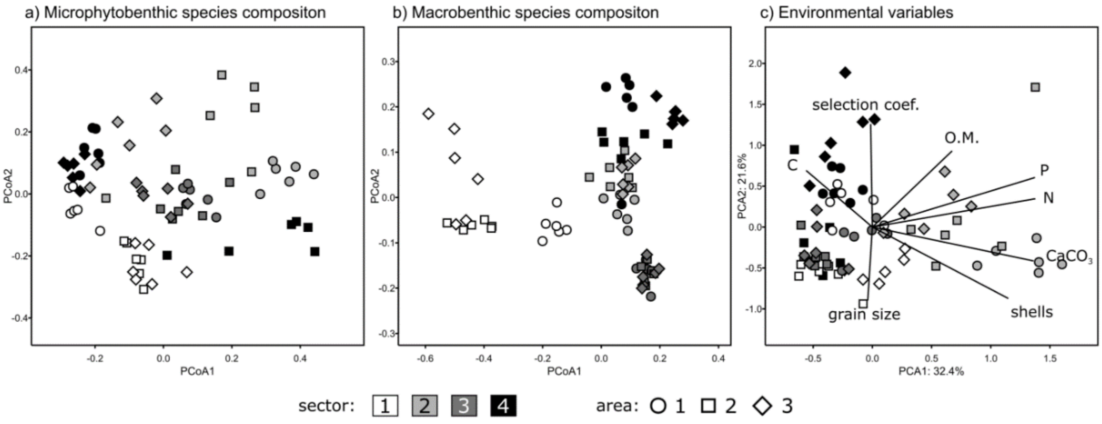

Figure 2: Multivariate ordinations depicting variation trends in species composition in microphytobenthic (a) and macrobenthic (b) assemblages; and gradients in environmental variables (c).

Table 2: Averaged environmental variables measured at each sector.

Variables	Sector 1	Sector 2	Sector 3	Sector 4
shells (mg/corer)	96.78 ± 64.09	135.51 ± 75.56	80.37 ± 33.48	13.06 ± 30.22
Phosphorus (mg P/g)	2.69x10^{-3} ± 0.6	4.78x10^{-3} ± 0.9	2.72x10^{-3} ± 0.5	3.14x10^{-3} ± 1.1
Nitrogen (mg N/g)	4.47x10^{-2} ± 2.6	4.72x10^{-1} ± 1.3	5.56x10^{-2} ± 1.7	2.07x10^{-1} ± 0.7
Carbon (mg C/g)	8.40 ± 1.84	3.79 ± 2.56	5.83 ± 0.93	7.74 ± 4.48
Organic matter (%)	1.89 ± 0.76	2.03 ± 1.30	1.56 ± 0.91	1.94 ± 0.77
$CaCO_3$ (%)	5.77 ± 2.52	7.72 ± 3.11	2.64 ± 1.57	2.13 ± 0.81
Grain size (µm)	191.78 ± 13.30	184.13 ± 24.13	178.69 ± 15.17	194.57 ± 35.93
Selection coefficient (µm)	56.34 ± 5.85	64.39 ± 9.92	56.29 ± 6.45	75.95 ± 9.67

The correspondence analysis depicting variation trends in macrobenthic trait composition shows a clear gradient in bioturbation capacity across sectors (Figure 3) from small-sized, nonbioturbating epifauna (sectors 2, 3 and 4) to large-sized, highly bioturbating infauna (sector 1). Such differences were mostly related to the first axis, that explained a huge portion of the total variation (61%). This trait gradient across sectors was related to the dominant taxonomic groups in each sector. More precisely, sector 1 was dominated by infaunal annelids (Oligochaeta sp. and *Armandia* sp.), while the other sectors were dominated by shallow-infaunal and epifaunal snails (*Heleobia australis* and *Bulla striata*) and caprellid amphipods.

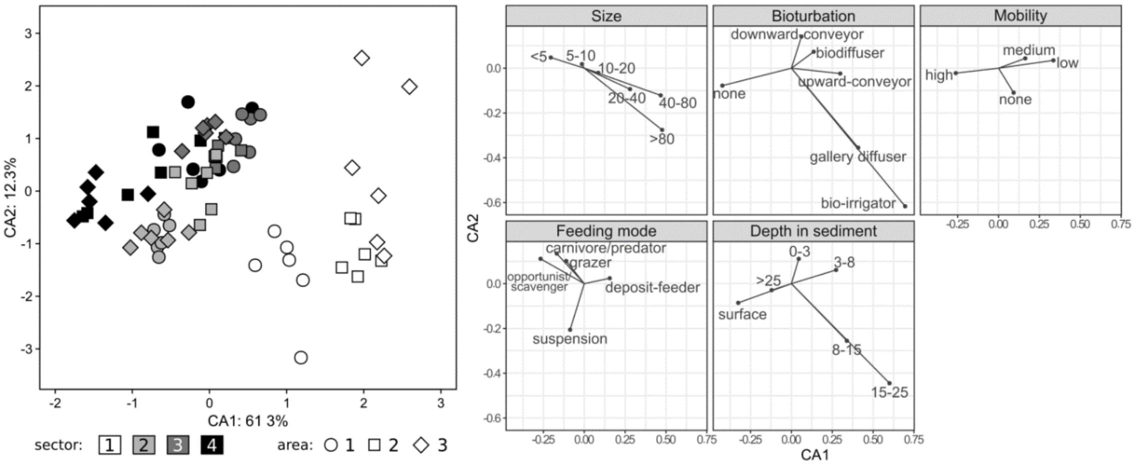

Figure 3: Correspondence analysis depicting variation trends in trait composition of macrobenthic assemblages (left). Vectors of the functional trait categories (titles with gray boxes) are shown (right).

The CAP score depicting variation trends in microphytobenthic species composition along the macrobenthic trait gradient (expressed by the first axis of the CA, Figure 3) shows a clear separation of sector 1 from the others (Figure 4a), mostly related to increases in the relative abundance of *Cyclotella* sp. and *Melosira sulcata* (associated to sector 1) and decreases in *Biddulphia* spp (associated to sectors 2, 3, and 4) along the trait gradient. This indicates that microalgae species in this sector were mostly related to the presence of highly bioturbating macrobenthic species. Microphytobenthic chlorophyll *a* and species diversity depicted a bell-shaped trend along the trait gradient, with peaks associated to sectors 2 and 3 (Figure 4b-c). These trends indicate that both microphytobenthic biomass and diversity are promoted by bioturbating macrobenthos, but only at low to intermediary levels. Microphytobenthic species evenness showed a gradual increase along the trait gradient (Figure 4d), suggesting that increasing bioturbation mostly impacts dominant microalgae.

Although significant effects were detected between macrobenthic functional diversity indices FRic, FDiv, and FEve and microphytobenthic chlorophyll *a* and diversity, the effects were highly dependent of the analyzed sector (Figure A3, Table A2). These trends indicate that gradients in macrobenthic trait composition are more important than functional diversity in explaining microphytobenthic variation across the estuarine system.

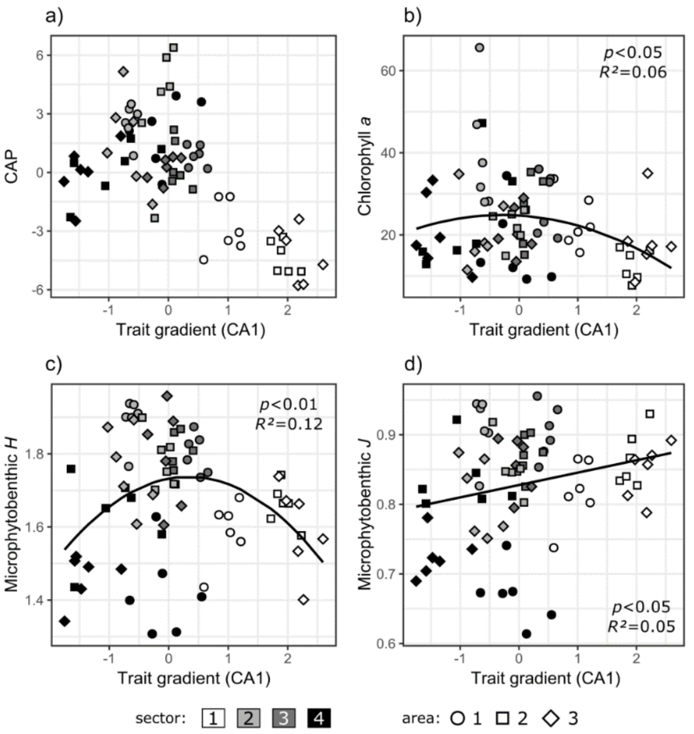

Figure 4: Effects of the macrobenthic trait gradient (from low bioturbation to high bioturbation; CA axis 1 of Figure 3) on microphytobenthic assemblage composition (a), chlorophyll *a* (b), species diversity (c), and evenness (d).

DISCUSSION

Our results indicate that macrobenthic trait composition affects microphytobenthic biomass and structure, which is in line with our third prediction. The variation of macrobenthic trait composition across the estuarine system described a gradient in bioturbation capacity, which had varying effects on different microphytobenthic species. The bell-shaped trend depicted by microphytobenthic biomass and diversity along such bioturbation gradient suggests that macrobenthic bioturbating activity might promote microphytobenthos at intermediary levels of bioturbation. Increasing bioturbation was positively related to microphytobenthic evenness, indicating that bioturbation has a major impact on dominant microalgal species. At the highest levels of the bioturbation gradient, the disturbance promoted by the macrobenthos possibly became too intense for the establishment of a diverse and productive microphytobenthic assemblage. Macrobenthic functional diversity had highly variable effects on microphytobenthic structure depending on the sectors, showing no consistent trend along the estuarine system. This lead us to reject our first and second predictions. Therefore, macrobenthic effects on microphytobenthos were mostly related to the abundance of specific functional identities, i.e. species with specific combinations of functional traits related to their bioturbation capacity.

The trait gradient observed across sectors reflected differences in macrobenthic bioturbation capacity along the estuarine gradient, having species-specific effects on microphytobenthic assemblages. More precisely, two microalgae species (*Cyclotella* sp. and *Melosira sulcata*) were related to increased presence of bioturbators, while one species (*Biddulphia* spp.) was mostly related to non-bioturbators. This reflects the different responses of microalgal species to disturbances in the substrate and explains the differences in assemblage structure in sectors with high and low macrobenthic bioturbation capacity. Similar responses of the microphytobenthos to the presence of bioturbating macrobenthos were described by other studies in the same estuarine system (Brustolin et al. 2014, 2016). These different affinities of microalgal species to bioturbation also explain the bell-shaped trend in microphytobenthic biomass and diversity along the trait gradient, reflecting the intermediary disturbance hypothesis (Connell 1978). Thus, intermediary bioturbation levels would represent conditions that allow the occurrence of species with both high and low affinity to disturbances in the substrate, while the extreme levels of the trait gradient favor specialist species. Besides, although it is known that deposit-feeders and biofilm-grazers can affect microphytobenthic biomass and diversity (Sommer 2000, Alvarez et al. 2013, Brustolin et al. 2016), it is unlikely that macrobenthic feeding modes could be driving observed trends in microphytobenthic structure since deposit-feeders were dominant in both inner and outer estuarine sectors. Confounding effects related to gradients in environmental variables are also unlikely to be occurring since gradients in substrate characteristics were more related to variation within sectors (i.e. across areas). Therefore, our results indicate a major effect of the macrobenthic trait composition on microphytobenthic assemblage structure in the studied estuarine system.

The low performance of functional diversity indices in explaining microphytobenthic structure possibly is related to the way microalgae respond to macrobenthic bioturbation capacity. The diversity of bioturbation-related functional traits does not reflect bioturbation capacity, but the variety of ways species can explore and disturb the sedimentary substrate. In the other hand, the bioturbation capacity of an assemblage depends on the abundance of species with specific functional traits, such as large-sized infaunal biodiffusers (Solan et al. 2004, Queirós et al. 2013). Assemblages dominated by such highly bioturbating species would have increased bioturbation capacities than more functionally diverse assemblages. Studies have recognized the importance of specific functional identities to explain certain ecosystem processes, such as plant resource use and primary production (Mokany et al. 2008, Gagic et al. 2015). Macrobenthic species with different feeding and bioturbating traits can have contrasting effects on sediment biogeochemistry and associated microphytobenthic assemblages (Mermillod-Blondin 2011, Alvarez et al. 2013). In some cases, the presence of ecosystem engineers with increased capacity to disturb or stabilize the sediment matrix have been associated with higher primary production in tidal flats (Donadi et al. 2013b, Chennu et al. 2015). Therefore, we argue that focusing in functional identities should be prioritized in assessing the functional effects of the macrobenthos on microphytobenthic structure.

Although this study covered a large area of Paranaguá bay, including the largest tidal flats across its north-south axis, the lack of temporal replication limits our power to develop broader generalizations. It is well known that the occurrence of both macro- and microphytobenthic species can be influenced by short- and long-term temporal variations (Underwood and Kromkamp 1999, Beukema and Dekker 2007). Major changes in temperature, light incidence, and nutrient input across seasons are known to drive the temporal patterns in microphytobenthic species composition (Oppenheim 1991, Montani et al. 2003, Méléder et al. 2007). The temporal variation in frequency of disturbances, recruitment peaks, and other environmental conditions such as increased number of predators/grazers can affect both microphyto- and macrobenthic assemblages (Arntz and Rumohr 1982, Diaz and Rosenberg 2008). Besides, seasonal variations can also affect biogeochemical process mediated by the macrobenthos via bioturbation (Braeckman et al. 2010), which could affect microphytobenthic assemblage structure. Thus, we strongly suggest that temporally replicated sampling designs would improve the analytical power to describe the relationship between macro- and microphytobenthos.

We demonstrated that macrobenthic functional identities, expressed by species with different capacities to disturb the substrate, are more important than functional trait diversity in explaining the spatial trends of microphytobenthos variation along an estuarine system. Microphytobenthic species variously reacted to the increasing presence of highly bioturbating infauna, highlighting the importance of sediment disturbance in shaping microphytobenthic assemblage structure. Therefore, quantifying the bioturbation capacity of macrobenthic assemblages by using indices such as bioturbation potential (BPc; Solan et al. 2004, Queirós et al. 2013), or the abundance of specific species with high capacity to affect the substrate, represent a valuable approach to assessing the macrobenthic effects on microphytobenthic species. Experimental manipulation of the abundance of macrobenthic species with varying abilities to affect the sediment, either through bioturbation or sediment stabilization, are clearly need to better assess their effects on microphytobenthic assemblage structure.

ACKNOWLEDGEMENTS

We thank the Federal University of Paraná (UFPR), the Post-Graduation Program in Coastal and Oceanic Systems (PGSISCO-UFPR), and the Brazilian research funding agency CAPES (Coordination for the Improvement of Higher Education Personnel) for the support in developing this study. We also thank Benthos Lab team for their help during field samplings. This study was financed by a grant from the National Council for Scientific and Technological Development (CNPq, grant no. 457336/2014-5).

APPENDIX

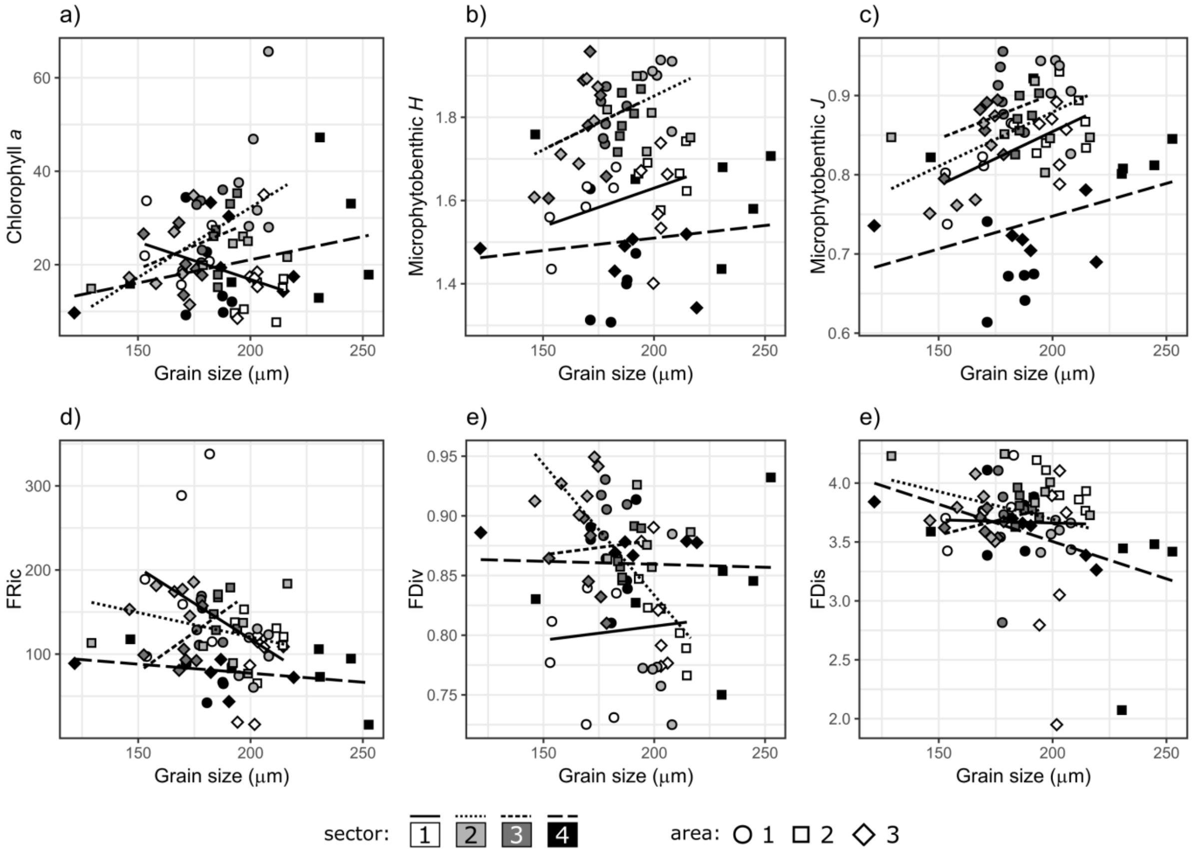

Figure A1: Fitted linear models describing the effect of grain size gradients in the microphytobenthos (a-c) and macrobenthic functional diversity (d-e).

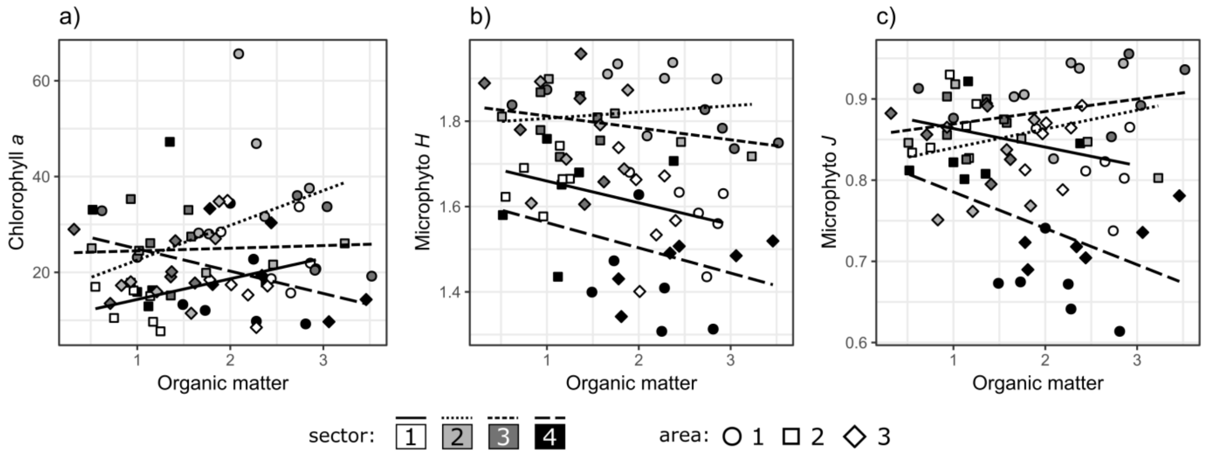

Figure A2: Fitted linear models describing the relationship between microphytobenthos and sediment organic matter content.

Table A1: Result of the ANCOVAs testing the effects of sediment grain size and organic matter on microphytobenthos and macrobenthic functional diversity.

Microphytobenthos

		Chlorophyll *a*		Microphyto *H'*		Microphyto *J'*	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	3.68	**0.02**	40.37	**<0.0001**	21.88	**<0.0001**
Grain size	1	5.23	**0.03**	5.62	**0.02**	14.20	**0.0004**
Grain:Sector	3	3.20	**0.03**	0.97	0.41	0.28	0.84

		Chlorophyll *a*		Microphyto *H'*		Microphyto *J'*	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	3.86	**0.01**	37.40	**<0.0001**	20.30	**<0.0001**
O.M.	1	1.11	0.30	4.01	**0.05**	0.48	0.49
O.M.:Sector	3	2.76	**0.05**	0.94	0.43	3.29	**0.03**

Macrobenthic fucntional diversity

		FRic		FDiv		FDis	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	6.00	**0.001**	7.89	**0.0002**	1.08	0.37
Grain size	1	3.94	**0.05**	2.43	0.12	4.57	**0.04**
Grain:Sector	3	3.11	**0.03**	4.09	**0.01**	0.65	0.59

		FRic		FDiv		FDis	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	5.38	**0.002**	7.53	**0.0002**	0.89	0.45
O.M.	1	0.41	0.52	0.18	0.67	1.84	0.18
O.M.:Sector	3	1.62	0.19	3.66	**0.02**	1.96	0.13

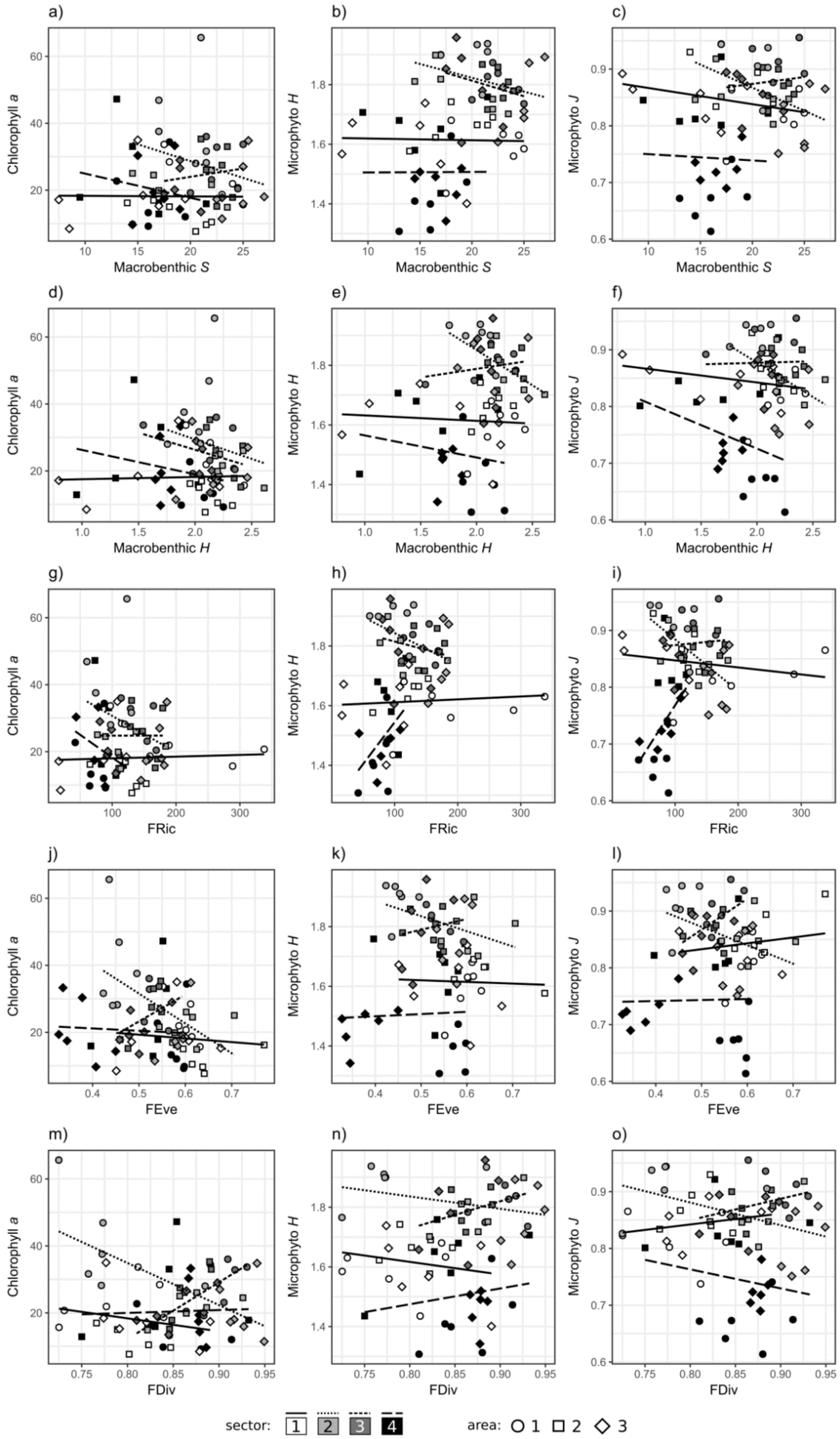

Figure A3: Fitted linear models describing the relationship between microphytobenthos and macrobenthic taxonomic and functional diversity.

Table A2: Result of the ANCOVAs testing the effects of macrobenthic functional diversity indices on microphytobenthos.

		Chlorophyll *a*		Microphyto *H'*		Microphyto *J'*	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	3.17	**0.03**	41.37	**<0.0001**	24.34	**<0.0001**
FRic	1	0.64	0.43	0.07	0.80	1.42	0.24
Sector:FRic	3	1.07	0.37	3.09	**0.03**	4.97	**0.004**

		Chlorophyll *a*		Microphyto *H'*		Microphyto *J'*	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	4.17	**0.009**	35.38	<0.0001	19.44	**<0.0001**
FDiv	1	6.37	**0.01**	0.20	0.66	1.73	0.19
Sector:FDiv	3	6.31	**0.0008**	1.24	0.30	1.50	0.22

		Chlorophyll *a*		Microphyto *H'*		Microphyto *J'*	
	df	F-value	p-value	F-value	p-value	F-value	p-value
Sector	3	3.5277	**0.02**	34.82	**<0.0001**	19.35	**<0.0001**
FEve	1	2.6497	0.11	0.30	0.58	0.07	0.79
Sector:FEve	3	2.9475	**0.04**	0.84	0.47	1.94	0.13

CHAPTER 6

Mussel beds are biological power stations on intertidal flats

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ABSTRACT

Intertidal flats are highly productive areas that support large numbers of invertebrates, fish, and birds. Benthic diatoms are essential for the function of tidal flats. They fuel the benthic food web by forming a thin photosynthesizing compartment in the top-layer of the sediment that stretches over the vast mudflats during low tide. However, the abundance and function of the diatom film is not homogenously distributed. Recently, we have realized the importance of bivalve reefs for structuring intertidal ecosystems; by building structures on the intertidal flats they provide habitat, reduce hydrodynamic stress and modify the surrounding sediment conditions, which promote the abundance of associated organisms. Accordingly, field studies show that high chlorophyll *a* concentrations in the sediment co-vary with the presence of mussel beds. Here we present conclusive evidence by a manipulative experiment that mussels increase the local abundance of benthic microalgae; and relate this to increasing abundances of diatoms as well as productivity of the biofilm across a nearby mussel bed. Our results show that the ecosystem engineering properties of mussel beds transform them into hot spots for primary production on tidal flats, highlighting the importance of biological control of sedimentary systems.

Submitted for publication

INTRODUCTION

Benthic microalgae are important primary producers in intertidal soft-sediment habitats where they contribute up to 50% of total primary production (Underwood and Kromkamp 1999). In these highly productive areas that have a great ecological and economical value across the globe (Heip et al. 1995), benthic microalgae fuel the benthic food web by forming extensive biofilms that support a vast array of organisms (Decho 2000, Stal 2003, Kromkamp et al. 2006, Markert et al. 2013, Rigolet et al. 2014). The abundance and productivity of benthic microalgae is commonly attributed to abiotic conditions, where increasing hydrodynamic stress decrease benthic microalgae biomass by resuspension of the sediment (de Jonge and van Beusekom 1995, van der Wal et al. 2010). Recently, we have recognized the importance of biological control over local hydrodynamic conditions on intertidal flats (van der Zee et al. 2012, Donadi et al. 2013a) and shown that the high abundances of benthic microalgae correlate strongly with the occurrence of mussel beds (Donadi *et al.* 2013b, Nieuwhof et al. *in prep.*).

Organisms that modify their habitats can facilitate complex food-webs by for example providing structural complexity and improving environmental conditions for many organisms (Olff et al. 2009, Kéfi et al. 2015, van der Zee et al. 2016). On tidal flats, bivalve reefs are of fundamental importance for biological control of ecosystem structure and properties (Commito et al. 2008, Gutiérrez et al. 2011, van der Zee et al. 2012, Donadi et al. 2013a). By forming large structures in the otherwise bare landscape, bivalve reefs create habitat for many other species that live in or on the sediment (van der Zee et al. 2012, Nieuwhof et al. 2015). The reefs physically protect the surface sediment against erosion and resuspension, and furthermore increase organic matter content via suspension feeding and biodeposition (Widdows and Brinsley 2002). The habitat modifying properties and ecosystem effects extend up to several hundred meters around reefs (van der Zee et al. 2012, Donadi et al. 2013a, 2013b, van de Koppel et al. 2015), which is reflected by a conspicuous increase of benthic microalgae biomass in the vicinity of intertidal mussel beds (Donadi et al. 2013b). Due to these impacts on a large spatial scale, it can be assumed that the interaction between benthic microalgae and intertidal bivalve reefs contribute significantly to coastal production. However, the assumed regulatory importance of bivalve reefs for microalgae biomass is based on observational data and statistical modelling only, while the causal link of (living) bivalves facilitating benthic diatoms have not been extensively examined. Consequently, we lack conclusive empirical evidence of the facilitation effect. In addition, due to limited measurements of actual productivity, we have a poor understanding of how the increased biomass of microalgae (commonly estimated by chlorophyll *a* concentrations) around reefs relate to productivity of the system.

In this study, we tested whether the biomass of benthic microalgae was consistently elevated across a mussel bed using transect data from two different habitats (sand, mussel bed) and related this to primary productivity in the same transects. We then used empirical evidence from a small scale field experiment to demonstrate that the addition of live mussels to bare plots facilitates benthic microalgae.

METHODS

Set-up Transects

We set up two parallel transects spanning a distance of 1 km each on the tidal flat South of the island Schiermonnnikoog (Friesland, The Netherlands; Figure 1a-b). The two transects were 300 m apart. One transect crossed a *Mytilus edulis* (blue mussel) bed that was ca. 100 m wide and extended for approximately 250 m along the coast; the other one was in a sand habitat without mussels present. We established the first point of each transect 350 m coastward of the mussel bed (about 500 m from the shore) and placed subsequent points every 50 m in seaward direction up to 100 m behind the mussel bed (last point ca. 1000 m from the shore).

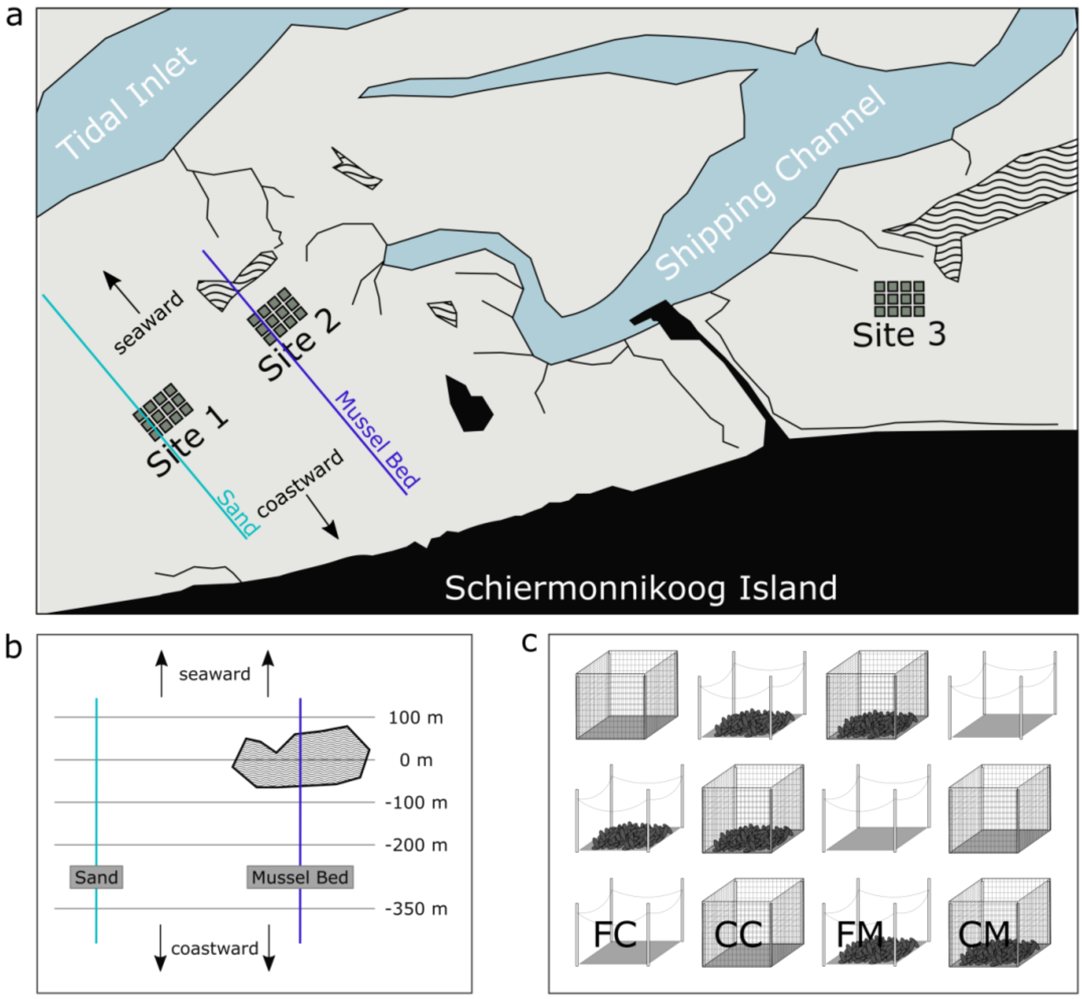

Figure 1: a) Overview of the study areas for the transect measurements as well as for the experiment; b) close-up of transect set-up; c) close-up of experimental set-up with the four treatments fenced control (FC), semi-caged control (CC), fenced mussel addition (FM), and semi-caged mussel addition (CM).

In June 2012 we sampled chlorophyll *a* concentration at six transect points in both transects (-300 m, -200 m, -100 m, 0 m, + 100 m, + 150 m distance to the mussel bed/ the corresponding tidal elevation in the sand habitat, where negative values mean distances coastward of the mussel bed/ the corresponding tidal elevation in the sand habitat and positive distances seaward of the mussel bed/ the corresponding tidal elevation in the sand habitat). Distance to the mussel bed is hereafter referred to collectively as distance to the mussel bed in both habitats. Sampling was replicated spatially, by including samples 50 m to the right and 50 m to the left of each transect point (N=36). In 2015-16, we took chlorophyll *a* and organic matter samples at five similar transect points in both transects (-350 m, -200 m, -100 m, 0 m, + 100 m distance to the mussel bed, where negative distances are coastward and positive distances are seaward of the mussel bed), but instead of two spatial replicates we repeated the sampling six times in total (October 2015, October 2016, April 19 2016, April 29 2016, May 2016, June 2016). Due to unexpected weather conditions, we could not sample the two last transect points for the sand habitat in October 2015 (0 m, + 100 m) and had to abandon the last sampling point (+ 100 m) in both habitats in April 2016 (N=56).

We measured photosynthetic yield of the sediment as proxy for benthic microalgae productivity at two different time points. In June 2012 and June 2016, we took triplicate samples in five transect points per habitat.

Set-up Field Experiment

We set up the mussel facilitation experiment on the tidal flat South of Schiermonnikoog Island in three different sites (Figure 1a,c). We selected the three sites along a gradient of influence by mussel beds: Site 1 in the sandy habitat included in the transect without a mussel bed; Site 2 coastward of the mussel bed included in the mussel bed transect (300 m to the east of Site 1); and Site 3 coastward of another larger mussel bed that is 100-200 m wide and extends almost 1000 m along the coast (2000 m east of Site 1; Figure 1a, Figure A1).

In each site, we tested the effects of adding mussels on benthic microalgae biomass. For this, we prepared four different treatments with three replicates in each site, leading to 36 experimental units (plots) in total. Each individual plot had an area of 0.25 m^2 (plot dimension: 0.5 m by 0.5 m) and each corner of the plot area was marked with a plastic pole. The poles were 66 cm long and inserted about 30 cm deep into the sediment. The distance between the plots was 5 m on each side. All three sites have similar tidal exposure times, meaning that all plots fell dry at the same time during low tide.

The experiment combined two mussel addition treatments and two controls in a factorial design with: a fenced control (FC; Figure A1b), a fenced mussel addition treatment (FM; Figure A1c), a semi-caged control (CC; Figure A1d), and a semi-caged mussel addition treatment (CM; Figure A1e). For the two mussel addition treatments (FM, CM), we collected live *Mytilus edulis* and distributed them evenly in the plots so that the surface of the entire plot was covered. Placing experiments on an intertidal flat may lead to critical artifacts because of changes to water flow caused by equipment rather than treatments, but also because of changed predation rates since the experiment may hinder or attract natural predators. This is critical when placing bivalves on the tidal flat, since they become islands of food for both birds and crabs that quickly consume the treatments. We therefore constructed two types of experimental controls: one semi-caged control (CC) that excluded both crab and bird predation but may have strong effects on flow attenuation; and one fenced control that only excluded bird predation but with minimal effects on flow. The semi-caged control consisted of a coarse plastic coated metal net that was wrapped around the marking poles. The net (mesh size: 1.2 cm) was placed directly on the seafloor with no space below it and had a height of 25 cm. The fenced control (FC) had a string attached to the poles at about 25 cm height, wrapping around the plots as protection against predation from birds (Van Gils et al. 2012). The distribution of the plots was randomized within sites. Deployment of all plots disturbed the sediment in the same way, as all treatments had the same basic built with the four plastic poles in each corner. The experimental plots were set-up in three rows perpendicular to the tidal currents; the most seaward row of treatments was hit by the tides first in all three sites (Figure 1a,c, Figure A1a).

To test for experimental artifacts, we also included a control without a fence that was only marked in the corners of the plot (no string between the poles; randomized among the other treatments at each site; N=9; Figure A2).

Deployment of the experimental plots took place in the end of April 2015. After more than a month, in June 2015, we took chlorophyll *a* and organic matter samples in all experimental plots. We also collected data on hydrological conditions by using dissolution plasters as proxy for hydrodynamic stress (for methods see Donadi *et al.* 2013b). We deployed two dissolution plasters in each experimental plot for two tidal cycles before the final sampling.

Due to a severe storm in the end of May 2015, we lost three replicates of the semi-caged control (CC; two in Site 1 and one in Site 3), so that the total N=6 for this treatment.

Sampling and Analysis

Chlorophyll *a* concentration in the sediment was measured as proxy for benthic microalgae biomass. For the chlorophyll *a* samples we collected three cores (diameter: 2.6 cm, depth: 0,2 cm) in the respective transect points. We pooled the sediment from all three cores and wrapped them onto a 10x10 cm piece of aluminum foil to prevent exposure to light. The sediment in the foil was placed into small labeled plastic bags which were sealed and stored on ice in the dark immediately after collection. The samples were transported in cool boxes to the laboratory (<24h). Samples were taken within a few hours on the same day in all plots. Once returned to the lab, we freeze-dried the samples and determined chlorophyll *a* content by acetone extraction (90%, dark, -20°C, 48 h) and methods described by Jeffrey & Humphrey (1975). The 2012 chlorophyll *a* concentration was measured with a spectrophotometer, whereas the 2015-16 samples were measured with a fluorometer.

For the organic matter samples, we took one sediment core (diameter: 2.6 cm, depth: 5 cm) at each transect point and in each experimental plot. The samples were placed into labeled plastic bags and stored in cool boxes on ice until they were transported to the laboratory. Upon return to the laboratory, the samples were frozen at -20°C until further processing. Organic matter content was measured by Loss on Ignition (LOI; 4h, 550°C) of oven dried (48h, 60°C) samples.

The dissolution plasters were collected after exposure to two tidal cycles. The relative plaster loss was calculated by subtracting the dry weight of the plaster after exposure to the tides from the dry weight before exposure. Submersion time in the different plots only varied marginally, therefore no standardization was deemed necessary.

Photosynthetic yield was measured as the maximum quantum yield of photosystem II with a Pulse Amplified Modulation fluorometer (Mini-PAM, Walz) as a proxy for benthic microalgae productivity. To avoid differences caused by different light conditions and changes in weather and timing of the tide, we collected triplicate samples of sediment at each transect point and transported them back to the lab (some were lost in 2012, reducing replication to duplicates at some points, N=28). The samples were placed in petri dishes which were randomly distributed on the same shelf in a climate room and allowed to acclimatize for half a day (light level: 11 $\mu mol \cdot m^{-2} \cdot s^{-1}$; temperature: 16°C). The photosynthetic yield was then measured using the PAM after 30 minutes dark adaptation. Fibers were fixed 10 mm above the sediment.

Statistical Analysis

All statistical analyses were performed and graphs were created in R v 3.3.1 (R Development Core Team 2009). If necessary, the data were log-transformed to meet the assumptions of homogeneity of variances and normality of data distribution.

Since the methods for the transect chlorophyll *a* data varied slightly between 2012 and 2015-16, we analyzed them separately. 2012 transect chlorophyll a did not meet the assumption of homogeneity of variances, even after data transformation. Therefore we analyzed differences in chlorophyll *a* concentrations between the habitat types (mussel bed, sand) using a Kruskal-Wallis rank sum tests. We then performed two separate

Spearman rank correlations for each habitat type, testing if chlorophyll a concentrations changed with distance to the mussel bed (300 m, 200 m, 150 m, 100 m, 0 m). We averaged all three transects per habitat for the analysis. For the 2015-16 transect data, we performed a two-factorial ANOVA to test the effect of habitat type (mussel bed, sand) and distance to the mussel bed (continuous) on chlorophyll *a* concentration and organic matter content. For the analyses, we used data from all the six different sampling times in 2015-16. We pooled the 2012 and 2016 PAM data for the analysis, because lost samples of 2012 led to poor replication. We performed a three-factorial ANOVA including the factors habitat type (mussel bed, sand), distance to the mussel bed (continuous) and year (2012, 2016) with the triplicates per sampling point.

For the experiment, we analyzed the effect of the two different controls and mussel addition treatments on chlorophyll *a* concentration, organic matter content and hydrodynamic stress (plaster loss) using a fully crossed three-factorial ANOVA with site, cage and mussel addition as fixed factors.

RESULTS

Transect Results

The mussel bed increased benthic microalgae biomass and productivity across the intertidal flat; both in the 2012 and the 2015-16 samplings (Figure 2a-d).

On average, the chlorophyll *a* concentration was significantly higher in the mussel bed habitat compared to the sand habitat in both sampling periods (Figure 1a-b; 2012: $\chi^2 = 9.62$, df=1, p =0.002; 2015-16: $F_{1,52}=25.55$, $p<0.001$). In 2012, the chlorophyll *a* concentration significantly increased with increasing proximity to the mussel bed in the mussel bed transect (n=18, Spearman R=0.82; t=5.8, $p<0.001$). In the sand habitat in 2012, there was no similar increase of chlorophyll a with distance to the tidal level of the mussel bed (n=18, Spearman R=0.24; t=0.97, p=0.35). The increase in benthic microalgae biomass directly on the mussel bed was on average more than 25 times stronger in the mussel bed transect compared to points at the same tidal elevation in the sand habitat (2012; Figure 2a). In 2015-16, the chlorophyll *a* concentration significantly increased with increasing proximity to the mussel bed and corresponding tidal level in both transects (Figure 2b; significant main effect of distance: $F_{1,52}=25.62$, $p<0.001$). The interaction between habitat type and distance to the mussel bed was statistically not significant (2015-16: $F_{1,52}=1.76$, p=0.19), but the increase was nearly four times stronger in the mussel bed transect compared to points at the same tidal elevation in the sand habitat (Figure 2b).

Photosynthetic yield was overall higher in 2016 compared to 2012 (Figure 2c-d; Year: $F_{1,50} = 36.41$, $p < 0.001$) and there was a marginal trend towards an average higher yield in the mussel bed habitat in both years (Habitat type: $F_{1,50} = 3.30$, $p = 0.08$). The highest yield overall was recorded in the mussel bed habitat for both years: in 2012 the highest yield was measured directly on the mussel bed (Figure 2c); whereas in 2016 it was measured 100 m seaward of the mussel bed (Fig 2d). Accordingly, there was a significant interaction between year, habitat type and distance to mussel bed ($F_{1,50} = 5.09$, $p = 0.03$), which demonstrated that the effect of the mussel bed depended on the year the photosynthetic yield was measured. In 2012, the photosynthetic yield decreased gradually in seaward direction across the sand transect, while it increased strongly with proximity to the mussel bed in the mussel bed habitat (Figure 2c). In contrast, in 2016 the photosynthetic yield increased gradually in seaward direction across the sand transect (Figure 2d). The yield in the mussel bed transect in 2016 was in general higher than in the sand transect, but had an abrupt minimum 100 m coastward of the mussel bed, where the yield was even lower than in the sand habitat at a comparable tidal elevation (Figure 2d). This area was characterized by the presence of macrofaunal structures (tube worms) that covered the entire area and possibly disturbed the biofilm.

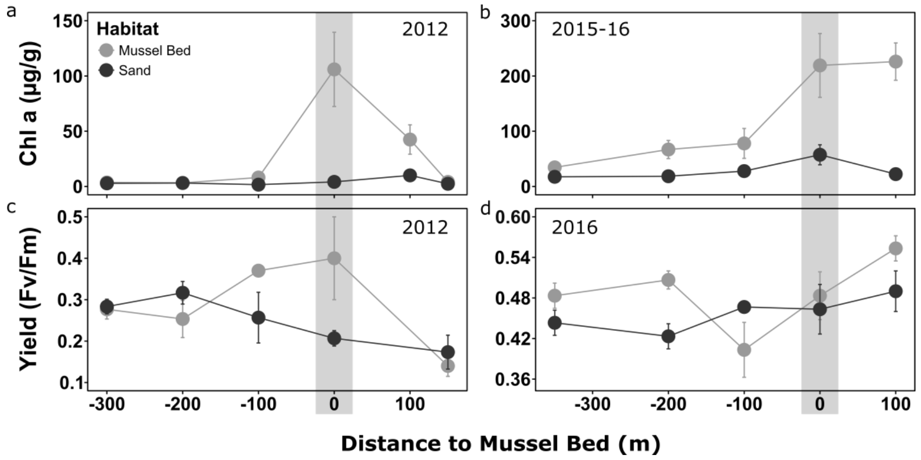

Figure 2: Chlorophyll a concentration 2012 (a) and 2015-16 (b), photosynthetic yield 2012 (c) and 2016 (d) in the transects. Negative distances to the mussel bed indicate positions coastward of the mussel bed, whereas positive values indicate a position seaward of the mussel bed. In the sand habitat, the distances represent similar tidal elevations as in the mussel bed habitat. The gray circles represent averages of transects in the mussel bed habitat and the black circles represent averages of transects in the sandy habitat. Error bars denote standard errors of the mean.

Results Field Experiment

The mussel additions doubled chlorophyll *a* concentrations in the sediment (Figure 3a; $F_{1,21}$=12.59, p=0.002). There was an indication of a stronger effect in the caged treatments, but there was no significant effects of caging, and no interaction effect between caging and mussel addition (Figure 3a; $F_{1,21}$=1.71, p=0.21 and $F_{1,21}$=0.37, p=0.55, respectively). The mussel additions had no significant effects on organic matter content or plaster loss (Figure 3b-c; $F_{1,21}$=0.52, p=0.48 and $F_{1,21}$=0.66, p=0.43, respectively). However, there was a marginal trend for the cage treatment to decrease plaster loss ($F_{1,21}$=4.53, p=0.05), indicating that the cages decreased hydrodynamic stress in a way that the mussels alone did not (Fig 2c).

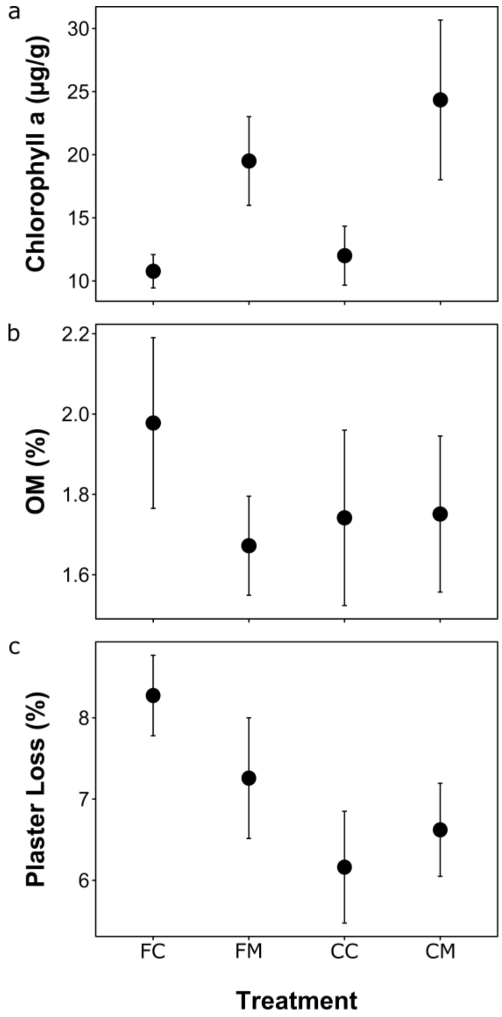

Figure 3: Average chlorophyll a concentration (a), organic matter content (OM; b), and plaster loss (c) in the different treatments (FC = fenced control, FM = fenced mussel addition, CC = semi-caged control, CM = semi-caged mussel addition) of the field experiment. Bars denote standard errors of the mean.

DISCUSSION

We demonstrate that mussel beds increase the abundance of primary producers in the sediment up to 200 m in their vicinity (Figure 2a-b). Previous research had suggested this facilitative effect of mussel beds (Donadi et al. 2013b), but did not provide conclusive evidence. Our results are also the first to actually link the long distance interaction of mussel beds, to both increasing abundances of primary producers (Figure 2a-b) and higher levels of productivity (Figure 2c-d). In general, the areas on the tidal flat with the highest biomass of benthic microalgae also had high values of photosynthetic yield and these were the sampling points in proximity of the mussel bed (Figure 2a-d). These results confirm and highlight the importance of reef-building bivalves for the functioning of coastal ecosystems. Specifically, in soft-sediment habitats reef-building bivalves act as ecosystem engineers and thereby are essential for associated communities (van de Koppel et al. 2015). By creating a three-dimensional habitat structure, bivalve reefs are important structuring components of many coastal ecosystems. In soft-bottom intertidal areas, bivalve reefs are often the only hard surfaces that can be used by other sessile organisms for attachment, favoring habitat forming algae and rich communities of epifauna (Albrecht and Reise 1994). Bivalve reefs are also important nursery grounds for many economically important organisms such as fish (Kristensen et al. 2015). In addition to locally modifying the habitat with their physical presence, they also have long distance effects by creating sediment conditions around them that are beneficial for many benthic organisms including infauna species (van der Zee et al. 2012, Donadi et al. 2015b). This shows that bivalves are autogenic and allogenic ecosystem engineers at the same time (Jones et al. 1994). Our study demonstrates that mussel beds not only facilitate animal biodiversity on many different trophic levels, as shown in previous studies, but they also provide excellent growing conditions for benthic primary producers across the intertidal. Together, this demonstrates that mussel beds are hot spots that fuel primary production in intertidal areas and support a productive and diverse food web far beyond its physical borders.

The abundance of benthic microalgae in intertidal sediments strongly depends on sediment erosion. Increased hydrodynamic stress leads to higher sediment erosion, which in turn re-suspends the microalgae (de Jonge and van Beusekom 1995). Larger biofilms of benthic microalgae are therefore often found in areas that are protected from hydrodynamic stress. Previous research shows that mussel beds reduce hydrodynamic stress (Donadi et al. 2013b) and therefore productivity of benthic primary producers is expected to increase in the vicinity of these structures. Our transect results confirm an increase in primary producer biomass closer to the mussel bed, but in our experiment we found that protection from hydrological forces alone does not increase the standing stock of primary producers. Although shelter by the cages decreased the hydrodynamic stress more than the mussels, it was only when mussels were physically present in the plots we could demonstrate an increase in primary producer biomass. Yet, there was no effect of mussel addition on organic matter content in our experiment. Generally, in addition to reducing hydrodynamic stress, mussels excrete feces and pseudo-feces that are rich in organic matter and nutrients which also may promote growth of the biofilm (van Broekhoven et al. 2015) and support higher abundances of other associated flora and fauna (Norling and Kautsky 2007). The duration of our experiment may simply have been too short to significantly modify the organic matter content of the sediment. This is supported by the significant increase in organic matter content in the mussel bed transect compared to the sand transect (Figure A4, Table A7). Also, since we only used live mussels in our treatments and not empty shells, we do not know to what extent biological (i.e. biodeposition by the bivalves) or mechanical (i.e. protection from hydrological forcing) facilitation contribute to the increased biomass of benthic microalgae.

Scale is important for the magnitude of engineering effects. Comparing the experimental results of mussel addition to the field measurements across the mussel bed indicates that the facilitative effect on primary producers was dependent on the extent of the mussel aggregation and also on time. Our small addition plots doubled the biomass of benthic microalgae, but this was still less than half of the biomass on the natural mussel bed. The importance of the size of mussel patches was previously observed for associated macrofauna (Norling and Kautsky 2007). Even though the presence of single mussels was shown to increase biomass and species richness of associated macrofauna, the increase was much higher in larger patches of *Mytilus edulis* (Norling and Kautsky 2007). The mussel bed from the transect data had been present in the same location for multiple years before we took the measurements. This means that there was much more time for organic matter to be accumulated in the sediment and we saw a large increase of organic matter content on the mussel bed. In contrast, we did not see any significant effect of the mussel additions on organic matter content in the experimental plots, where the mussels only had about one month time to produce and accumulate organic matter. It would be interesting to study, whether these small patches of mussels can increase the sediment organic matter content if given more time. Worldwide, we have observed a decrease in bivalve reefs due to overfishing and habitat degradation (Jackson et al. 2001, Edgar and Samson 2004, Eriksson et al. 2010), which can have detrimental effects on coastal ecosystems. Protection and restoration efforts should not only focus on the presence of the species but also needs to consider the scale of reefs.

Our results demonstrate the importance of bivalve reefs for primary production on intertidal flats. On a global scale, we see a decline in ecosystem engineering species in coastal areas (Jackson et al. 2001, Lotze et al. 2006). Bivalves are therefore already of an importance to conservation and restoration efforts in many areas of the world (Schulte et al. 2009, McLeod et al. 2012, de Paoli et al. 2015). Our research shows that establishing and protecting reefs in soft-bottom habitats is a key conservation priority and an essential strategy to restore and manage coastal production.

ACKNOWLEDGEMENTS

We thank Vereniging Natuurmonumenten and the Province of Friesland for granting us permission for field work on the tidal flats. Special thanks to Luna van der Loos and Just van der Endt for their help setting up the field experiment; Stella Boele-Bos for help in the laboratory; the 2012 Marine Research students Reinier Nauta, Karsten Roelfsema, Melanie Timmerman, Bart Zwart and the 2016 Marine Biology Research students Alex Hanewacker, Sjoerd den Hertog, Titus Hielkema, Jorick Hiemstra for help collecting the transect data. This study was financed by a grant from the ZKO program of the Netherlands Organization of Scientific Research (NWO) to BKE (grant no. 839.08.310).

APPENDIX

Figure A1: a) Example of experimental set-up (depicted is part of Site 1); b) fenced control treatment (FC); c) fenced mussel addition treatment (FM); d) semi-caged control; e) semi-caged mussel addition treatment.

Table A1: Coordinates and attributes of sampling points along the transects for 2015-16 data.

Transect	Plot	Plot ID	Habitat	Latitude (°)	Longitude (°)	Distance MB (m)
2	A	2A	Mussel Bed	53.47090	6.22400	-350
2	D	2D	Mussel Bed	53.46955	6.22418	-200
2	F	2F	Mussel Bed	53.46865	6.22438	-100
2	H	2H	Mussel Bed	53.46776	6.22462	0
2	J	2J	Mussel Bed	53.46686	6.22469	100
5	A	5A	Sand	53.47101	6.23025	-350
5	D	5D	Sand	53.46967	6.23041	-200
5	F	5F	Sand	53.46876	6.23050	-100
5	H	5H	Sand	53.46788	6.23058	0

Table A2: Coordinates for set-up sites of the field experiment.

Site	Latitude	Longitude
1	53.28590	6.13474
2	53.28720	6.13293
3	53.28775	6.11262

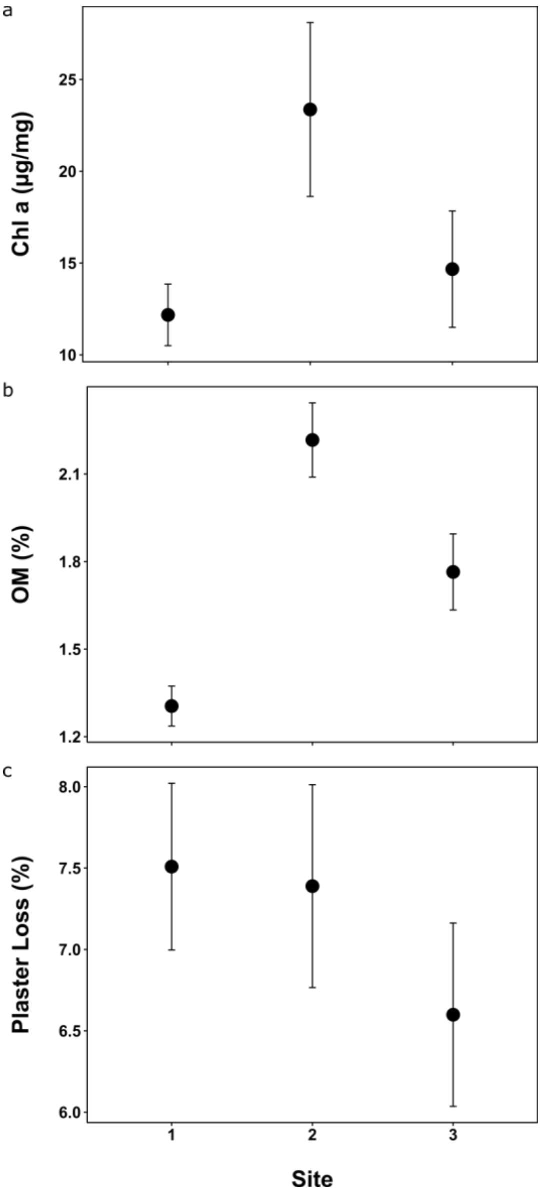

Figure A2. Average chlorophyll *a* concentration (Chl *a*; a), organic matter content (OM; b), and plaster loss (c) in the different sites of the field experiment. Bars denote standard errors of the mean.

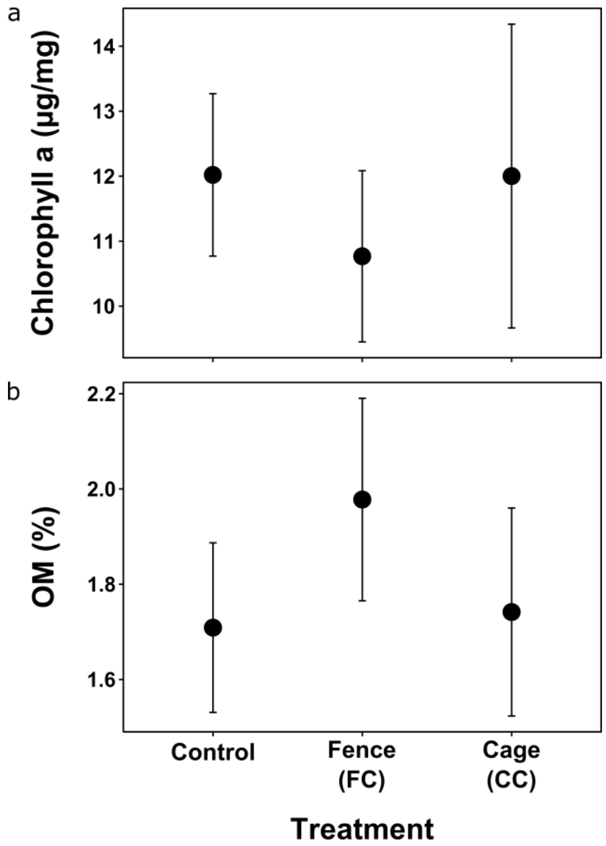

Figure A3: Average chlorophyll *a* concentration (a) and organic matter content (OM; b) in the different cage treatments of the experiment (Control, FC = fenced control, CC = semi-caged control) of the field experiment. Bars denote standard errors of the mean.

Table A3: Results of two-factorial ANOVA for field experiment. We tested the influence of the factors Treatment (Tr; three levels – Control, Fence, Cage) and Site(S; three levels) on the response variables chlorophyll *a* and organic matter content. Significant results with p<0.05 are depicted in bold.

Response Variable	Factor	df	SS	F	p
***Chlorophyll* a**	Tr	2	8.72	0.355	0.71
	S	**2**	**200.16**	**8.14**	**<0.01**
	TrxS	4	16.59	0.34	0.85
	Residuals	15	184.35		
Organic Matter	Tr	2	0.37	0.62	0.55
	S	2	1.65	2.73	0.10
	TrxS	4	0.79	0.65	0.64
	Residuals	15	4.53		

Table A4: Results of two-factorial ANOVA for transect chlorophyll *a* data 2015-16. We tested the influence of the factors habitat type (HT); two levels – sand and mussel bed) and distance to mussel bed (D); continuous) and the interaction of the two factors (HTxD) on the response variable chlorophyll *a* (log-transformed, six sampling times). Significant results with $p<0.05$ are depicted in bold.

Response Variable	Factor	df	SS	F	p
Chlorophyll a *2015-16*	**HT**	**1**	**2.47**	**25.55**	**<0.01**
	D	**1**	**2.47**	**25.62**	**<0.01**
	HTxD	1	0.17	1.76	0.19
	Residuals	52	5.02		

Table A5: Results of two-factorial ANOVA for transect PAM data 2012 and 2015-16. We tested the influence of the factors habitat type ((HT); two levels – sand and mussel bed), distance to mussel bed ((D); continuous), year ((Y), two levels – 2012 and 2016)) and all interactions of the factors on the response variable photosynthetic yield (triplicate samples per transect point). Significant results with $p<0.05$ are depicted in bold.

Response Variable	Factor	df	SS	F	p
Photosynthetic Yield	HT	1	0.02	3.30	0.08
	D	1	<0.01	0.02	0.89
	Y	**1**	**0.20**	**36.41**	**<0.01**
	HTxD	**1**	**0.03**	**5.10**	**0.03**
	HTxY	1	0.02	3.30	0.08
	DxY	1	<0.01	0.02	0.89
	HTxDxY	**1**	**0.03**	**5.10**	**0.03**
	Residuals	50	0.27		

Table A6. Results of three-factorial ANOVA for field experiment. We tested the influence of the factors mussel (M; two levels – no mussel addition, mussel addition), cage (C; two levels – cage, no cage) and site (S; three levels) on the response variables chlorophyll *a* (log-transformed), organic matter content, and plaster loss. Significant results with $p<0.05$ are depicted in bold.

Response Variable	Factor	df	SS	F	p
***Chlorophyll* a**	**M**	**1**	**0.49**	**12.59**	**<0.01**
	C	1	0.02	0.52	0.48
	S	**2**	**0.43**	**5.45**	**0.01**
	MxC	1	0.01	0.37	0.55
	MxS	2	0.02	0.21	0.81
	CxS	2	0.06	0.73	0.49
	MxCxS	2	0.05	0.66	0.53
	Residuals	21	0.82		
Organic Matter	M	1	0.24	1.71	0.21
	C	1	0.03	0.22	0.65
	S	**2**	**4.51**	**16.00**	**<0.01**
	MxC	1	0.44	3.14	0.09
	MxS	2	0.36	1.28	0.30
	CxS	2	0.20	0.70	0.51
	MxCxS	2	0.23	0.82	0.46
	Residuals	21	2.96		
Plaster Loss	M	1	1.96	0.66	0.427
	C	1	13.54	4.53	0.05
	S	2	5.23	0.88	0.43
	MxC	1	4.38	1.46	0.24
	MxS	2	5.52	0.92	0.41
	CxS	2	20.33	3.40	0.05
	MxCxS	2	1.36	0.23	0.80
	Residuals	21	62.80		

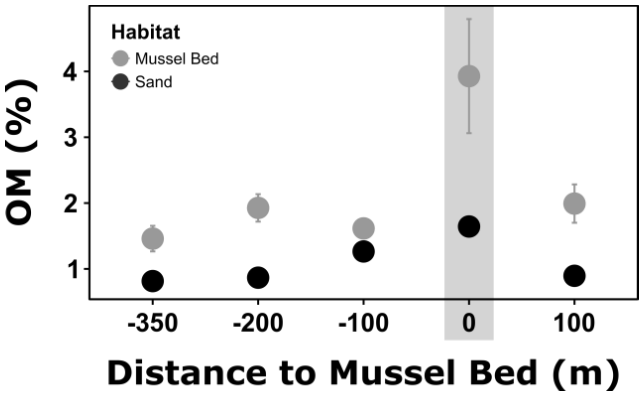

Figure A4. Organic matter content (OM) in the transects at different sampling points. Displayed are means of the six sampling times in 2015-2016 with standard errors.

Table A7. Results of two-factorial ANOVA for transect data 2015-16. We tested the influence of the factors habitat type (HT); two levels – sand and mussel bed), distance to mussel bed (D); continuous) and the interaction of both factors (HTxD) on the response variable organic matter content (log-transformed, six sampling times). Significant results with p<0.05 are depicted in bold.

Response Variable	Factor	df	SS	F	p
Organic Matter	**HT**	**1**	**0.42**	**21.45**	**<0.01**
	D	**1**	**0.60**	**30.41**	**<0.01**
	HTxD	1	<0.01	0.11	0.74
	Residuals	52	1.03		

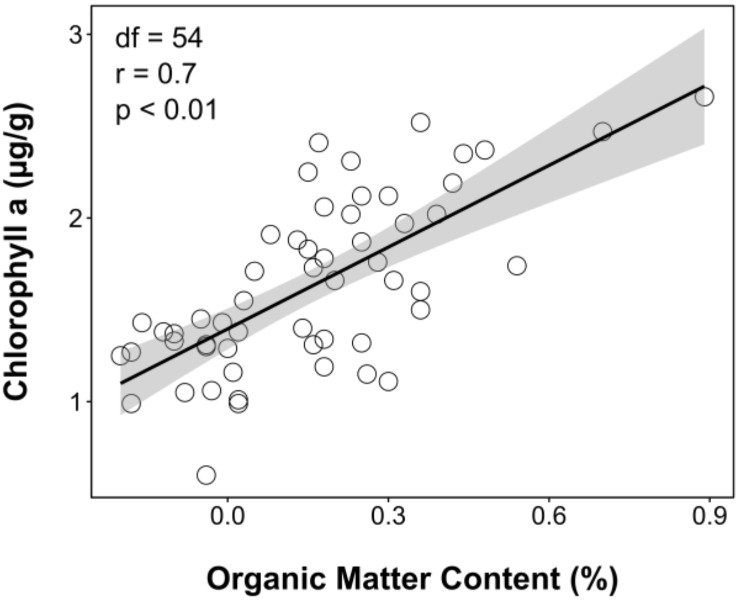

Figure A5. Overall correlation between organic matter content and chlorophyll *a* concentration in the transects. Displayed are values from all sampling times in 2015-2016 and all sampling points along both transects in those years. Both variables are log-transformed.

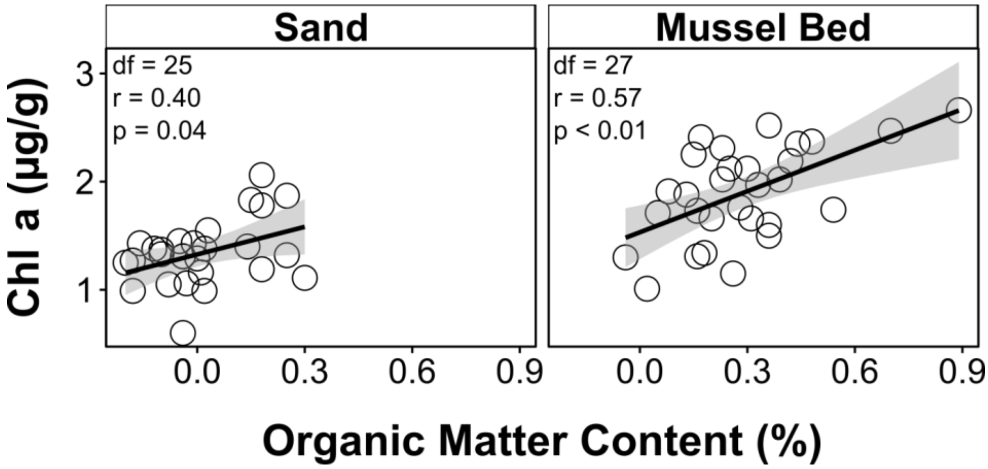

Figure A6. Correlation between organic matter content and chlorophyll *a* concentration in the transects, split up by habitat type. Displayed are values for all sampling times in 2015-2016 and all transect points. Both variables are log-transformed.

CHAPTER 7

SYNTHESIS

Joao B. Gusmao

Ecologists are increasingly realizing that the properties of individual species are essential to promote and maintain ecosystem functioning (Pawar et al. 2015). The ability of species to affect ecosystem properties and processes can be assessed through analyses of their functional traits, which reflect behavior and morphophysiology (Díaz and Cabido 2001, Pawar et al. 2015). By using trait-based approaches, ecologists can relate species assemblage structure to environmental variability and thus explore their potential implications for ecosystem function (Díaz and Cabido 2001, Bremner et al. 2006a, Mouillot et al. 2013). As a result of increasing concern regarding the effects of worldwide losses in biodiversity on ecosystem function and services (Cardinale et al. 2012, Hooper et al. 2012), trait-based instead of taxonomical approaches are getting more and more common in the ecological literature.

Environmental variability and trophic and non-trophic biological interactions play a major role in shaping macrobenthic assemblages in sedimentary systems (Gray and Elliott 2009). Macrobenthos are important secondary and tertiary producers in marine food webs, and are major drivers of sediment-related processes (Ambrose 1984, Braeckman et al. 2010, Mermillod-Blondin 2011). Macrobenthic species have different capacities to affect physical and biogeochemical properties of marine bottoms and can directly shape the sedimentary matrix (Kristensen et al. 2012).

This thesis aimed to investigate how macrobenthic function is affected by environmental variation and assemblage composition in shallow soft bottoms, by using trait-based approaches. Five studies were carried out to address the following research questions: (1) *How do natural and human-induced environmental gradients affect the functional diversity of macrobenthic assemblages?* (2) *Can we use functional trait distributions of species assemblages to assess environmental status?* (3) *How do changes in species composition relate to functional diversity and potential changes in ecosystem functioning?* (4) *Does habitat complexity favor functional trait diversity?* (5) *Can functionally important species such as habitat-forming species and ecosystem engineers induce functional cascades by determining the function of associated assemblages?*

My main findings are listed as follow:

A. Macrobenthic functional diversity changes predictably along an organic enrichment gradient

A field survey to assess the relationship between macrobenthic functional diversity and organic enrichment in the subtropical Paranaguá Bay, Brazil (Chapter 2) showed that the macrobenthic trait structure changes predictably across sites with different contamination levels. Such trends were mostly driven by changes in species abundances, demonstrating the need for abundance-based analyses to detect changes in potential community function caused by pollution. Both functional diversity and trait composition varied along a pollution gradient. Most notably, at contaminated sites functional divergence was lower than at unpolluted reference sites. Also, the AMBI benthic quality index (AZTI Marine Biotic Index), which is based on species life history traits, indicated a poorer environmental health at the contaminated sites. This indicates that a contamination gradient effectively imposes restrictions to the establishment of species with certain combinations of functional traits. Thus, abundance-based functional diversity indices and Biological Trait Analyses (BTAs) are useful and informative tool to assess impacts of organic pollution in macrobenthic assemblages, with high potential for application in environmental assessment and monitoring.

B. Changes in species composition driven by increases in alien species affect macrobenthic functional diversity

A survey based on a 15-year data series from the Dutch Wadden Sea (Chapter 3) revealed that increases in the number and biomass of alien species reshaped macrobenthic assemblage structure in one of the analyzed sites, and caused major increases in overall species richness and functional diversity. The establishment of large-sized bivalves such as the reef-forming oyster *Crassostrea gigas* and the infaunal razor clam *Ensis directus* rearranged the assemblage trait space, promoting increased functional divergence. The increasing dominance of *E. directus* also promoted decreases in species diversity and changed the assemblage bioturbation potential, indicating potential changes in assemblage function on sediment-related processes such as increasing instability, resuspension, and nutrient exchange. This highlights that we need to develop functional indices based on better mechanistic connections to actual ecosystem processes to better assess consequences of changes in macrobenthic assemblages for the functioning of benthic systems.

C. Changes in seagrass complexity can affect sediment related functions by determining the distribution of bioturbating species

An assessment of the effects of *Zostera marina* meadows on macrobenthic functional structure along the northern hemisphere (Chapter 4) revealed that seagrasses have a large impact on functionally relevant macrofauna, resulting in indirect effects on the potential function of the macrobenthic community. The presence of seagrasses increased species richness and functional diversity, although negative effects were observed at high levels of seagrass above-ground biomass and shoot length. Epifauna and shallow-living infauna were favored by seagrasses, especially at low to medium levels of above-ground biomass, suggesting that higher bioturbation related processes occur in the shallow layers of the sediment, such as sediment resuspension. At the same time, seagrass presence decreased deeper-living bioturbators. This indicates that the foundational role of seagrass may induce a functional cascade, in which effects on sediment processes by the meadows themselves are mediated by changing the functional composition of bioturbating macroinvertebrates. Therefore, we highlight the need for seagrass conservation not solely for their own ecosystem engineering attributes, but also for their role as facilitators of other functionally relevant organisms.

D. Macrobenthic functional identities determines microphytobenthic production in tidal flats

A second field survey in Paranaguá Bay, Brazil (Chapter 5), revealed that a gradient in macrobenthic trait composition correlates with variation in microphytobenthic biomass, diversity, and species composition across estuarine sectors. This trait gradient varied from highly bioturbating infauna at the outer sector to non-bioturbating epifauna at the inner estuarine sectors. Changes in microphytobenthic species composition reflected different tolerances of microalgal species to bioturbation. The bell-shaped trend depicted by microphytobenthic biomass and diversity along such bioturbation gradient suggests that macrobenthic bioturbating activity might promote microphytobenthos at intermediate levels of bioturbation. However, four multivariate indices for measuring functional diversity of the macrobenthos (FRic, FEve, FDiv, and FDis) had a poor performance in explaining variation trends in microphytobenthic structure. Our results indicate that, instead of functional diversity, the functional identities of the macrobenthic species, expressed by species with different capacities to bioturbate the substrate, control the microphytobenthos in this estuarine system. These results lead us to develop a field experiment in the Dutch Wadden Sea (Chapter 6) to assess the effect of one functionally relevant species, the reef-forming mussel *Mytilus edulis*, on microphytobenthic production. This species was chosen due to its effects on sediment stabilization and for limiting bioturbation within the mussel beds. Aggregations of mussels increased microphytobenthic biomass strongly, confirming the importance of these sediment stabilizing bivalves for primary production on intertidal flats

CONCLUDING REMARKS

This thesis demonstrates that shifts in the structure of macrobenthic assemblages in marine soft-bottom coastal systems related to biological structures, natural environmental gradients and human-induced disturbances; all relate to predictable changes in trait distributions and potential functions of the macrofauna communities. The changes in macrobenthic functional trait structure driven by such factors has clear implications for the functioning of sedimentary systems; and for the methods we need to use to assess environmental health.

Chapter 2 showed the potential of trait-based approaches to assess impacts of organic pollution on macrobenthic assemblages, giving clues about the general health status of the benthic environment. Decreases in functional diversity indices in contaminated sites reflected the deleterious effects organic pollution on species with certain trait combinations. Chapter 3, however, described that alien species promoted rearrangements in assemblage trait structure, which increased the functional diversity, but also changed the potential function of the macrobenthic community. This study highlights that increases in functional diversity only describes the variety of functional types present in the assemblage. Thus, functional diversity indices are a valuable exploratory tool to assess the general state of an assemblage when facing disturbances that act on the community by selecting for a limited set of traits; but they cannot be directly translated to specific changes in ecosystem functioning caused by shifts in species composition. Therefore, we need to be careful and motivate predictions using a mechanistic framework based on ecological theory, when we use functional diversity indices to asses environmental quality.

Chapter 4 confirmed the foundation effect of *Zostera marina* meadows on the belowground community across the northern hemisphere by increasing the general assemblage taxonomic and functional diversity. Interestingly, indirect effects of the meadows have the potential to promote sediment resuspension by facilitating shallow-living infauna that contribute to bioturbation-related processes in the uppermost sediment. The potential functional consequences of such functional cascade mean that indirect effects of seagrass on infauna function, may weaken their well-recognized direct engineering effect of promoting sediment stability. However, further investigations are necessary to unveil how much of these potential effects translate in real function for the system.

Chapter 5 and 6 highlight the importance of macrobenthic structure for primary production in tidal flats. Chapter 5 showed a relation between macrobenthic functional identities and microphytobenthic variation. A gradient of increasing bioturbation, presumably reflecting sediment disturbance rates, explained changes in microphytobenthic assemblages, suggesting that sediment stability is the controlling factor for microphytobenthos. Chapter 6 tested and confirmed this affirmation experimentally, and also highlight that key species in macrobenthic assemblages – such as ecosystem engineers – are essential drivers of ecosystem functions in soft sediment habitats. Therefore, analytical tools such as biological traits analysis, bioturbation potential indices, or focusing on functionally relevant species or functional identities, represent reliable approaches to assess the functional relationships between macrobenthos and the actual function in sedimentary ecosystems.

CONCLUSIONS

1. Low benthic environmental quality driven by organic enrichment hinders the establishment of macrobenthic species with specific functional traits, resulting in assemblages with differentiated trait composition and decreased functional diversity.
2. Trait-based approaches are a reliable way to assess general effects of organic pollution on the macrobenthos. Both biological trait analysis and multivariate functional diversity are good analytical tools to analyze changes in macrobenthic structure across organic contamination gradients, although biomass- and abundance-based analysis can differ.
3. Changes in assemblage structure driven by establishing alien species can drive major changes in macrobenthic functional diversity. These changes can drive increased bioturbation potential, and have implications for ecosystem functioning in sedimentary systems.
4. Increased habitat complexity driven by seagrasses can promote sediment-related functions by favoring the establishment of shallow-living macrobenthic bioturbators.
5. Functionally relevant macrobenthic species can promote microphytobenthos either by increasing sediment bioturbation or by sediment stabilization via flow attenuation.
6. Functionally relevant species can promote functional cascades by favoring the establishment of organisms that perform other functions. In the cases addressed in this thesis, seagrasses promote shallow-living infauna and indirectly mediate bioturbation-related processes; macrobenthic bioturbation drives primary producers by affecting microphytobenthic structure; and mussel beds promote primary production by favoring microphytobenthos.
7. Functional diversity indices are useful as an exploratory tool or to assess general effects of disturbances or gradient on macrobenthic structure; while biological traits analysis or focusing in functional identities represent a better approach to assess the general functional effects of the macrobenthos on ecosystem structure and processes.

BOX 2: Functional traits and associated categories based on Jones & Frid (2009)

Trait	no.	Modality
Bioturbation mode	1	None.
	2	Biodiffuser: fauna which move sediment in a random manner over short distances causing diffusive mixing (François et al. 1997).
	3	Gallery diffuser: gallery or tube dwellers that promote biodiffusion via construction/maintance of tube/gallery system (Michaud et al. 2005).
	4	Upward-conveyor: head-down oriented fauna which cause active movement of sediment from depth to the surface (François et al. 1997).
	5	Downward-conveyor: head-up oriented fauna which cause active movement of sediment from the surface to depth through their gut (François et al. 1997).
	6	Bio-irrigator: fauna which actively irrigate burrows by drawing down surface water past their gills (Chennu et al. 2015).
Depth in the sediment/burrowing capacity	1	Surface
	2	0-3 cm
	3	3-8 cm
	4	8-15 cm
	5	15-25 cm
	6	> 25 cm
Body size (adults)	1	≤ 5 mm
	2	5-10 mm
	3	10-20 mm
	4	20-40 mm
	5	40-80 mm
	6	80-160 mm
	7	> 160 mm
Feeding mode	1	Deposit-feeder
	2	Suspension-feeder
	3	Opportunist/scavenger
	4	Grazer (including selective feeding on benthic microalgae)
	5	Predator/carnivore
Longevity	1	< 1 year
	2	1-3 years
	3	3-6 years
	4	6-10 years
	5	> 10 years
Adult movement	1	Sessile
	2	Swimmer
	3	Crawler
	4	Burrower/Tube dweller
Relative adult mobility	1	None
	2	Low
	3	Medium
	4	High

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