CHAPTER 1

Introduction

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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, which 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 analysis (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).
Figure 3: Diagram of steps to perform a biological traits analysis is based on the functional trait *relative adult size*. The trait includes 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.
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 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, it 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.
CHAPTER 1

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 main 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.