BIVALVE LOOPS

THE IMPORTANCE OF ECOSYSTEM ENGINEERING

FOR SOFT-BOTTOM INTERTIDAL ECOSYSTEMS

Serena Donadi

RIJKSUNIVERSITEIT GRONINGEN

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Serena Donadi

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Promotores: Prof. dr. B.K. Eriksson

                                 Prof. dr. J.L. Olsen

Beoordelingscommissie: Prof. dr. P. Herman

                                 Prof. dr. R. Elmgren

                                 Prof. dr. U. Sommer

To my father

Paranimfen: Els M. van der Zee

Karin de Boer

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Chapter 1

1

GENERAL INTRODUCTION

Serena Donadi

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I perfectly remember my astonishment when I walked on the mudflats for the first time. It happened four years ago, on the Dutch island of Schiermonnikoog. The place was so flat and empty, featureless for kilometers at a glance. Strong winds and tides seemed to govern what looked like a wet desert. Yet, in this apparently desolated place, life not only thrives, but also shapes the surrounding environment. Many species hide in the substrate and modify sediment properties; others form dense epibenthic aggregations that provide shelter from predators and hydrodynamic forces. In such harsh conditions, these species create habitat, and by doing so, they structure the local community. What is the importance of these species for this type of intertidal, soft-sediment ecosystem? Can biogenic habitat transformation overrule environmental forcing? With these questions in mind I started an exciting four-year-long scientific adventure, whose main discoveries are described in this thesis.

ECOSYSTEM ENGINEERS: A NEW PERSPECTIVE

Some of the most challenging questions in ecology concern species co-occurrence and distribution. Research has long been driven by the idea that abiotic factors, such as the physical and chemical properties of the habitat, and biotic processes, especially competition and predation, are the primary determinants of species composition and diversity. Until the 1990s, the traditional view was that species survive when environmental conditions meet their physiological requirements ("fundamental niche") in combination with the influence of their competitors and predators ("realized niche", Hutchinson 1957). Jones et al. (1994, 1997) augmented the traditional view by introducing the concept of "ecosystem engineers"; defined as "*organisms that directly or indirectly modulate the availability of resources (other than themselves) to other species, by causing physical state changes in biotic or abiotic materials*". That species not only respond to habitat conditions but also modify them was not fundamentally new (i.e. Darwin 1881, Shaler 1892), but was formalized for the first time. Since then the concept has fuelled debate (Wilby 2002) and led to a remarkable number of experimental studies and modeling efforts designed to assess the role of ecosystem engineering in structuring natural communities (e.g. Gurney and

Lawton 1996, Wright and Jones 2006, Hastings et al. 2007 and references therein). Thus, the basic question of how abiotic conditions influence species distribution and community structure was enhanced by the addition of how biotic processes dually influence the environment and the members of the community; a type of reciprocal causality (e.g. Cardinale 2006).

By changing physical conditions, ecosystem engineers modulate niche space for the surrounding species, thereby facilitating certain species while inhibiting others (Bruno et al. 2003). Therefore, at a local scale, ecosystem engineers typically produce shifts in community composition, while at a landscape scale the coexistence of engineered and unmodified habitats enhances environmental heterogeneity, eventually increasing species diversity (Jones et al. 1997, Wright et al. 2002, Erwin 2005). Furthermore, incidental modifications to the environment created by organisms can increase the fitness of the engineers themselves. Thus, ecosystem engineering may create positive feedbacks between habitat changes and ecosystem engineer populations. This type of engineering has been called "*extended phenotype engineering*" (Jones et al. 1994) or "*niche construction*" (Laland et al. 1999, Odling-Smee et al. 2003), and is a powerful evolutionary force influencing community dynamics and species distribution and diversity (i.e. Laland 2004, Erwin 2008, Post and Palkovacs 2009).

Although a number of controversies remain to be solved (Reichman and Seabloom 2002, Wilby 2002, Wright and Jones 2006), the importance of ecosystem engineering is nowadays well recognized among ecologists. Habitat-mediated interactions have been suggested to be at least as important as trophic interactions in structuring natural communities (Wilby et al. 2001). As a consequence, in the last decade there has been an increased research effort to include habitat-mediated and other non-trophic interactions into traditional food-web ecology that have commonly only included predator - prey interactions (Reise 2002, Bruno et al. 2003, Olff et al. 2009, Kefi et al. 2012). In this thesis I contribute to this body of research by investigating: 1) the influence of interactions between coexisting ecosystem engineers both on the physical environment and associated communities, and 2) scale-dependent effects of such engineering networks.

1

THE TIDAL FLATS OF THE WADDEN SEA

A biologically-driven balance between sediment stabilization and destabilization

The tidal flats of the Wadden Sea (Fig. 1.1) are highly dynamic and productive areas that support large numbers of shorebirds and other animals (Beukema 1976, van de Kam et al. 2004, Piersma 2007). For this reason, the Wadden Sea is protected by international agreements, i.e. the Ramsar Convention on Wetlands (1971), a *Natura 2000* area by the European Community, and a World Heritage Site (2009). Despite their seemingly desolate appearance, tidal flat systems are sculpted by two types of biogenic habitat transformation. Autogenic engineers (Jones et al. 1994) transform the environment through alteration of their own structure and remain in the system as part of the new state. In contrast, allogenic engineers change the environment but do not remain part of the new physical state. Examples of autogenic ecosystem engineers on the tidal flats include seagrasses and reef-building bivalves, which reduce current velocities and alter topography through their physical structure, thereby enhancing the deposition of fine sediment and larvae (Fonseca and Cahalan 1992, Commito et al. 2005). Reef builders such as mussels and oysters also accumulate large amounts of organic matter through biodeposition of faeces and pseudofaeces (Graf and Rosenberg 1997, Hartstein and Rowden 2004), thus also acting as allogenic engineers. Examples of allogenic engineers include those species that live within the sediments and alter habitat through bioturbation (Rhoads 1974, Cadée 2001, Kristensen 2001, Reise 2002) such as arenicolids (e.g. Beukema 1976, Reise 1985, Volkenborn et al. 2007), thalassinid shrimps (Swift 1993, Atkinson and Taylor 2005, Berkenbusch and Rowden 2007) and infaunal bivalves (e.g. Marinelli and Williams 2003, Montserrat et al. 2009, Weerman et al. 2010). Bioturbation is the displacement and mixing of sediment particles and is caused by various faunal activities, such as sediment ingestion and defecation, burrowing and construction and maintenance of galleries. All these activities alter the physical structure as well as the chemical nature of the sediments. Some organisms may further enhance chemical exchange by flushing their burrows with the overlying water, a process termed bioirrigation. Among the most relevant effects of bioturbation and bioirrigation are the increases in oxygen content and decomposition rate in
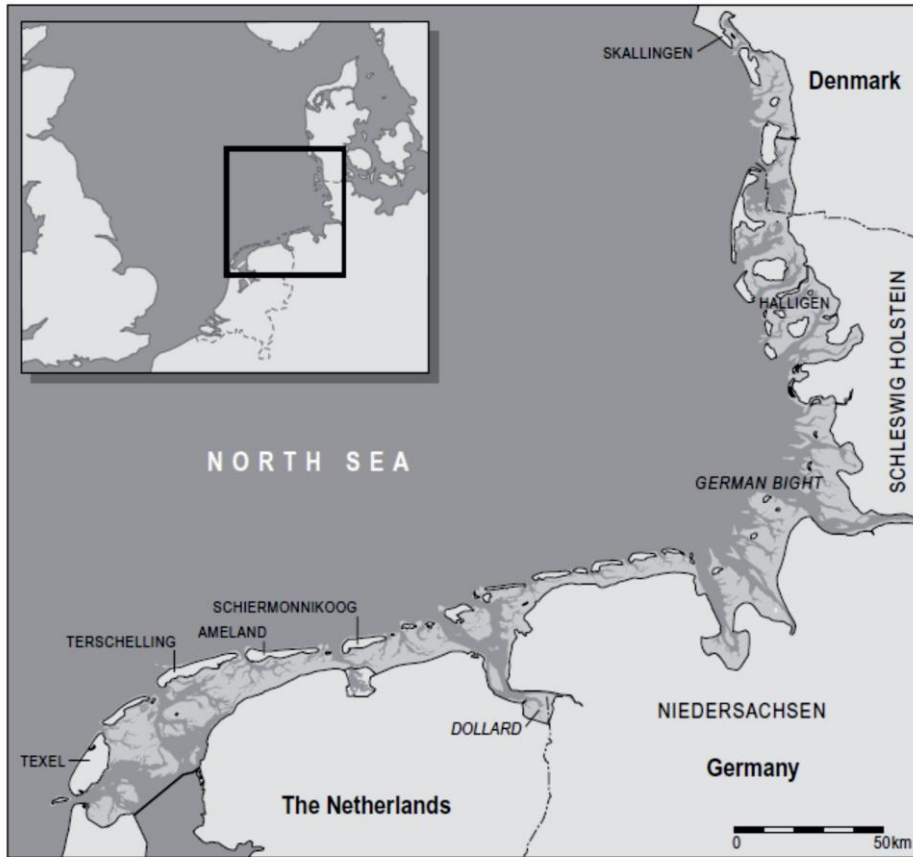
Fig. 1.1. The Wadden Sea (reproduced by permission from Dirk Visser).

the sediment (Anderson and Kristensen 1991, Banta et al. 1999, Volkenborn et al. 2007). Organic matter and electron acceptors are moved from the top layer of the seabed to deeper layers, where they are used by anaerobic bacteria, while aerobic bacteria in surface sediments are furnished with refractory carbon substrates (Kristensen and Holmer 2001). Furthermore, bottom-dwelling invertebrates create burrows that are microhabitats for small infauna (Reise 1985, 1987) and act as 'hot spots' for degradation of organic matter (Kristensen 2000).

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Thus, autogenic and allogenic processes affect intertidal sediments in opposite ways. Sediment stabilization processes such as reef-building, biodeposition and mucus-formation alleviate hydrodynamic stress and/or increase sediment accumulation and cohesiveness; whereas sediment destabilization mechanisms such as bioturbation promote sediment erosion, solute exchange within the substrate and loss of fine particles (Reise 2002, Widdows and Brinsley 2002). The balance between these antagonistic engineering effects may interact with sediment properties and physical processes to influence the vulnerability of the sea bed to erosion by waves and currents. For these reasons it is essential to investigate interactions between sediment stabilizing and sediment de-stabilizing ecosystem engineers in order to understand the ecology and morphology of coastal ecosystems (Eriksson et al. 2010).

Loss of key engineers in the western Wadden Sea

Ecosystem engineers have declined dramatically in the western Wadden Sea over the past century. Extensive reefs of European oysters (*Ostrea edulis* L.) disappeared in the 19th century due to overfishing (Lotze 2005). Around 1930 eelgrasses (*Zostera marina* L. and *Z. noltii* Hornem.) declined radically due to the combined effects of the wasting disease (caused by a protist that swept the North Atlantic) and changes in the hydrodynamic and physical properties of the water following the closure of the 'Afsluitdijk' (Wolff et al. 1979, Giesen et al. 1990). Subtidal eelgrass beds (*Z. marina*) have never re-established themselves. In 1989-1990, most intertidal reefs of the common blue mussel (*Mytilus edulis* L.) were wiped out by a combination of extensive beam-trawl fisheries, severe storms and recruitment failures (Beukema and Cadee 1996, Eriksson et al. 2010). Thereafter, the intertidal mussel fishery was forbidden and intertidal mussel beds only partially recovered (Beukema and Cadee 1996). Also, in the same period (1980-90s), industrialized bottom trawl fisheries expanded to target cockles (*Cerastoderma edulis* L.) (Dijkema 1997, Kesteloo et al. 2004, Kraan et al. 2011), with major consequences not only for the cockle populations, but also for the shorebirds that fed on them (van Gils et al. 2006b). Given the role that the aforementioned species play in the food web and as ecosystem engineers, their decline has significantly affected ecosystem function (Eriksson et al. 2010). In this thesis I focus on joint effects of three ecosystem engineers that are notable for

their widespread occurrence on the tidal flats of the Wadden Sea: cockles (*Cerastoderma edule* L.), lugworms (*Arenicola marina* L.) and blue mussels (*Mytilus edulis* L.). Cockles and lugworms are endobenthic species that commonly coexist at the local scale (<0.1 m), while mussels typically form large (>1 ha) epibenthic aggregations.

Cockles (*Cerastoderma edule*)

Cockles lives in the upper few cm of the sediment and can occur in dense assemblages of many thousands m^{-2} (Jensen 1992; Fig. 1.2). The vertical burrowing and the horizontal ploughing behavior of cockles, as well as the valve adductions, destabilize the sediments by loosening the surface layers and may negatively affect several tube-building species (Flach 1996). Shells and individuals that are not fully buried increase bed roughness leading to a density-dependent alteration of the near-bed hydrodynamics. For example, Ciutat et al. (2006) found that sediment erosion generally increased with cockle population density, but at high density (312 ind. m^{-2}) the suspended sediment concentration was lower than at medium density (141 ind. m^{-2}), probably due to a binding effect resulting from higher mucus secretion and pseudofaeces production. Indeed, cockles are filter feeders and can produce large amounts of biodeposits (Loo and Rosenberg 1989). Therefore, although cockles have been primarily described as bioturbating organisms that increase sediment disturbance and erodibility (Flach 1996, Neumeier et al. 2006, Ciutat et al. 2007, Montserrat et al. 2009), high densities of cockles can also promote sediment stability (Ciutat et al. 2006, Andersen et al. 2010; Fig. 1.3).

Lugworms (*Arenicola marina*)

The deposit-feeding lugworm is a well-known ecosystem engineer that occurs in relatively stable densities of 20-40 adults m^{-2} in roughly 90% of the tidal flats of the Wadden Sea (Beukema and Devlas 1979). Lugworms live in 20-40 cm deep, J-shaped burrows in the upper sediment layer. Their ingesting activity at the end of the head-shaft causes the surface sediments to slide down, thus forming a funnel (Flach 1992), which provides new surface for growth of microorganisms, an effect referred to as 'gardening' (Hylleberg 1975). Above the tail-shaft of the burrow, a

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Fig. 1.2. (A) High densities of cockles (*Cerastoderma edule*, bottom left) coastward of a bed of blue mussels (*Mytilus edulis*, top left) in the intertidal flats of Schiermonnikoog, The Netherlands. (B) Fecal casts produced by lugworms (*Arenicola marina*, bottom right) increase the roughness of the seabed. Sampling frame is 0.5×0.5 m size. (Photo credits: S. Donadi. Small photo mussels: E. Weerman. Small photo lugworm: B. K. Eriksson).

characteristic cast made by a coiled up string of sediment is the faecal mound of the worm (Volkenborn and Reise 2007). Feeding funnels and faecal casts result in a tip-and-mound topography that increases the roughness of an otherwise smooth seabed (Fig. 1.2). Lugworms preferentially ingest sediment particles smaller than 300-400 µm (Cadée 1976), which are deposited in the fecal mounds at the surface and are subsequently washed away by the tidal currents. This results in a loss of fine particles that is essential for maintaining a sandy habitat and preventing geomorphological transformation towards muddy flats and salt marshes that commonly occurs in a depositional coastal environment such as the Wadden Sea (Reise 1985; Fig. 1.3). Sediment erosion is further enhanced by the pit-and-mound topography (Graf and Rosenberg 1997), as well as the removal of sediment-binding diatom films, due to deposit feeding (Volkenborn et al. 2007). Finally, by flushing their burrows for respiration (bioirrigation), lugworms enhance the exchange of solutes between sediments and the overlying water, thereby influencing the rates of benthic metabolism by increasing oxygen penetration in to the substrate (Riisgård and Banta 1998, Banta et al. 1999). While the sediment disturbance induced by lugworm bioturbation has negative effects on many tube-building organisms, enhanced pore water advection due to bioirrigation can promote free-burrowing subsurface species (Flach 1992, Volkenborn and Reise 2006).

Blue mussels (*Mytilus edulis*)

The common blue mussel is a reef-forming, suspension-feeding bivalve that can be found in intertidal or subtidal aggregations where layers of mussels, accumulated sediment, faeces, pseudofaeces and shell debris form a mussel matrix (Norling and Kautsky 2008; Fig. 1.2). The biogenic structure of living mussels and their shells provide hard substrate for the settlement of algae and epifauna, as well as refuge for several other invertebrates (Buschbaum et al. 2009). Mussel reefs alter the near-bed hydrodynamic regime by reducing water flow speed (Gutierrez et al. 2003), which can enhance the accumulation of sediments, organic particles and post-larvae (Commito et al. 2005; Fig. 1.3). The organic content of the bottom is further increased by large amounts of faeces and pseudofaeces that mussels produce (Kroncke 1996, Graf and Rosenberg 1997) and that constitute a source of food for deposit feeders (Norkko et al. 2001). However,
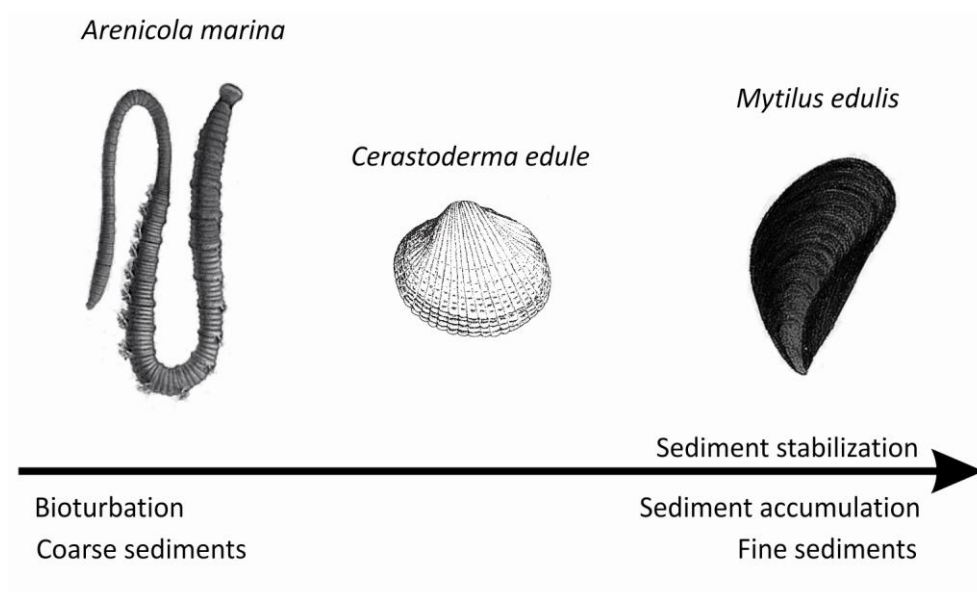
Fig. 1.3. Effects of different ecosystem engineers on the Wadden Sea tidal flats. Lugworms (*A. marina*) reduce sediment stability through bioturbation, thus increasing coarse sediments, while blue mussels (*M. edulis*) enhance the settlement of fine particles through biodeposition and alleviation of hydrodynamic stress. Cockles (*C. edule*) can act both as sediment stabilizers and destabilizers (see references in the text; species drawings modified from Jeffreys 1869 and Ashworth 1912).

strong biodeposition can also result in low oxygen and high sulfide and ammonia levels in the sediments, eventually hindering the survival of many macrofauna and meiofauna species (Hartstein and Rowden 2004, Norling and Kautsky 2008).

OUTLINE OF THIS THESIS

The central aim of this thesis was to investigate how ecosystem engineers such as cockles, mussels and lugworms modify the environment and how these habitat changes affect the structure of soft-bottom communities and the function of the intertidal ecosystem.

The general questions of this thesis were:

1) **How do coexisting ecosystem engineers interact to influence the physical environment? What is the importance of such engineering networks for the intertidal communities and the ecosystem functions?**

2) **Do positive feedbacks occur between engineered habitat and ecosystem engineers, so that ecosystem engineers favor their own survival?**

3) **What is the spatial scale of engineering effects in the intertidal?**

To address these questions, I set up a large-scale experiment from March 2010 to November 2011, in which I manipulated cockle and lugworms densities on the tidal flats of Schiermonnikoog, in the Dutch Wadden Sea (**Chapters 2**, **3**, **4**) (Box 1.1). Additional data come from smaller-scale experiments and multiple field surveys (transects and grid sampling) performed between April 2009 and May 2012 in different locations of the tidal flats of Schiermonnikoog (**Chapters 3**, **5**, **6**).

In **Chapter 2** I investigated the importance of habitat-mediated changes by cockles, lugworms and mussels for microphytobenthos abundance - which are the most widespread primary producers on the tidal flats in the Wadden Sea. Microphytobenthos is mainly composed of diatoms and is negatively affected by waves and currents, which cause sediment resuspension and erosion (de Jonge and van Beusekom 1995, van der Wal et al. 2010). I therefore hypothesized that at high level of hydrodynamic stress, sediment-stabilizers such as cockles would promote microphytobenthos growth, while sediment-destabilizers such as lugworms would increase sediment resuspension, thereby hindering the formation of algal mats. To test these hypotheses I used data from the large-scale experiments related to sediment parameters and chlorophyll *a* content.

In **Chapter 3**, I tested the occurrence of positive feedbacks between engineering processes by cockle banks and cockle recruitment. Many engineering species can act as '*extended phenotype engineers*' (Jones et al. 1994) and thereby influence the fitness of their own populations through habitat alteration ('*niche construction*', Laland et al. 1999, Odling-Smee et al. 2003). To explore niche

construction processes by cockles, I analyzed cockle juvenile abundances recorded in the large-scale experiments together with sediment organic matter content and bed level change, and I tested whether adult cockles could promote recruitment of the future generations by increasing sediment stability. Loss of biological feedbacks (due to overfishing and human-induced changes in environmental conditions) is suggested to be partially responsible for the lack of recovery of many overexploited shellfish populations (Eriksson et al. 2010).

In **Chapter 4** I provide an example of an engineering network, where multiple engineering species interactively alter environmental conditions and influence the surrounding species. As cockles, mussels and lugworms modify sediment parameters in opposite directions and at different spatial scales, I explore how their combined activity affects the functional composition of the macrobenthic community at a landscape level by modulating niche space. For this purpose, community data from the large-scale experiments were analyzed using Biological Trait Analysis (BTA), a trait-based approach that has mainly been applied to assess anthropogenic impacts on natural communities, but that has hardly been used to explore ecosystem engineering effects.

Given that ecosystem engineers often have impacts at much larger spatial scales than the organisms themselves (Hastings et al. 2007), in **Chapter 5** and **Chapter 6** I examined the implications of long-range engineering of mussel beds for the communities of the tidal flats. The occurrence of cross-habitat interactions between reef-forming species and sedentary benthic organisms is discussed in **Chapter 5**, where I tested the hypothesis that beds of blue mussels can promote the abundances of cockles at relatively long distances from the reefs. For this purpose, I used data from field experiments and from transects across three intertidal mussel beds and nearby areas without mussels. Next, effects of long-range engineering by bivalve reefs on higher trophic levels were investigated in **Chapter 6**, where spatially-explicit field data on sediment properties, macrobenthos abundances and shorebird distribution collected within and around bivalve reefs were analyzed by Structural Equation Modeling (SEM).

Finally, in **Chapter 7**, I synthesize the main insights developed form this thesis. My aim was to discuss the findings from earlier chapters in relation to the existing literature and highlight the novelties of my research.

Box 1.1. Large-scale experiments set up

On the intertidal flats south of the island of Schiermonnikoog (Dutch Wadden Sea), cockle-dominated communities and lugworm-dominated communities were set up in a randomized block design within the pseudofaeces plumes of two mussel beds (distance to the reef was 100 and 350 m, 53°28'8.15"N, 6°13'27.95"E; 53°28'2.44"N, 6°10'59.83"E) and at two unaffected sandy sites without mussel beds, at the same tidal elevation (53°28'7.42"N, 6°13'55.95"E; 53°28'7.54"N, 6°11'33.48"E). Each site was assigned two blocks of twelve 5×5 m plots, for a total of ninety six plots. Each block contained a random full factorial combination of (1) defaunation treatment (two levels) and (2) engineer addition (three levels). The defaunation treatment consisted of an anoxia-induced kill off of macrobenthos in half of the plots by covering the sediment with plastic tarps for a period of 28 days (April - May 2010). Two weeks after removing the tarps, we performed the engineer addition: one-third of all plots was enriched with high densities of cockles (1000 individuals per m^2), one-third with high densities of lugworms (80 individuals per m^2) and the remaining plots served as a control (no addition). Thus, in total we added 800 000 cockles and 64 000 lugworms to the experiment. Treatment densities of cockles and lugworms were chosen to mimic maximum observed densities in the study area. Cockles were collected from a nearby intertidal flat by professional fishermen through hand-raking and were 2 to 4 years old. Lugworms were harvested by a commercial lugworm fisheries company (Arenicola BV, Oosterend, The Netherlands) from an intertidal flat in the Western Wadden Sea. Abundances of both lugworms and cockles were monitored periodically throughout the whole duration of the experiments.
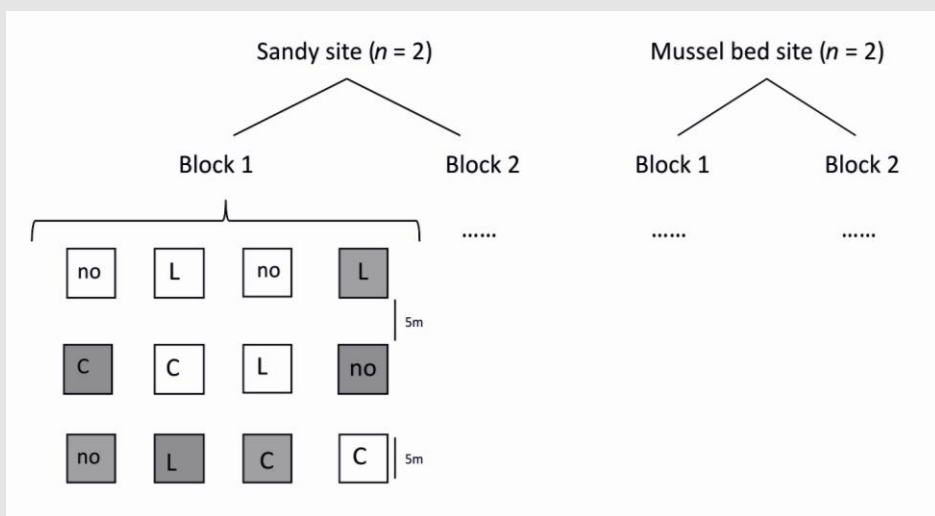
Diagram of the experimental design. Dark and white squares represent defaunated and not-defaunated plots, respectively. Letters indicate the three levels of the engineer addition treatment: (C) cockle addition, (L) lugworm addition, (no) no addition.
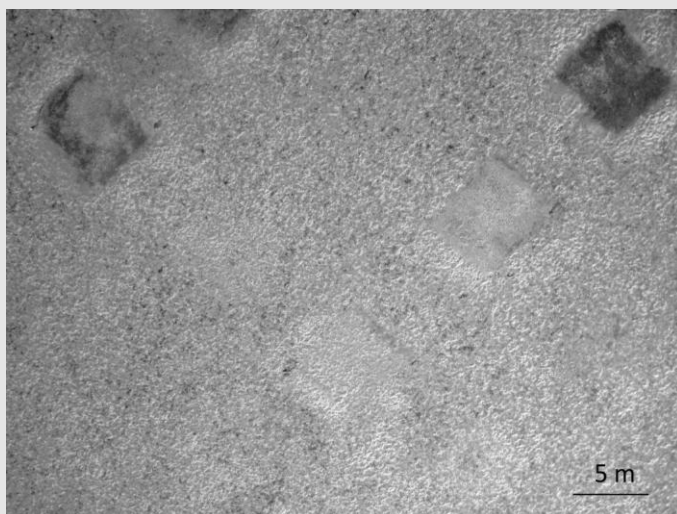
Kite aerial photograph of the experimental plots. Dark squares are cockle-addition plots, where diatom mats covered the surface sediment. Photo credit: E. Weerman.
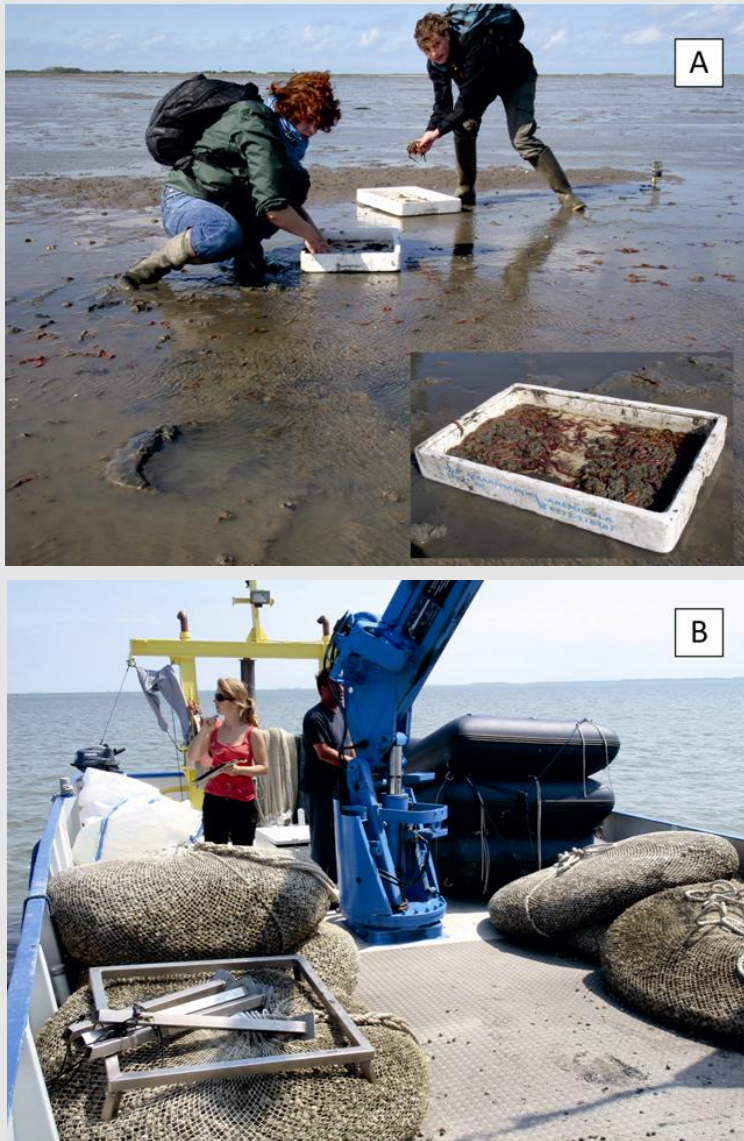
Setting up the large-scale experiments on the tidal flats of Schiermonnikoog, The Netherlands. (A) A total of about 64 000 lugworms and (B) 800 000 cockles were added to the experimental plots (engineer addition treatment). Photo credits: B. K. Eriksson.

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(C) To defaunate half of the experimental plots, plastic tarps covered the substrate for at least four weeks (defaunation treatment). Photo credits: B. K. Eriksson.

Chapter 2

2

NON-TROPHIC INTERACTIONS CONTROL BENTHIC PRODUCERS ON INTERTIDAL FLATS

Serena Donadi, Joëlle Westra, Ellen J. Weerman, Tjisse van der Heide, Els M. van der Zee, Johan van de Koppel, Han Olff, Theunis Piersma, Henk W. van der Veer & Britas Klemens Eriksson

ABSTRACT

The importance of positive effects of ecosystem engineers on associated communities is predicted to increase with environmental stress. However, incorporating such non-trophic interactions into ecological theory is not trivial since facilitation of associated species is conditional on both the type of engineer and the type of abiotic stress. We tested the influence of two allogenic ecosystem engineers (lugworms, *Arenicola marina* L. and cockles, *Cerastoderma edule* L.) on the main primary producers (microphytobenthos) of the tidal flats, under different abiotic stresses controlled by reefs of blue mussels (*Mytilus edulis* L.). We added 25.000 cockles or 2000 lugworms to 5×5 m plots, both in a muddy site with high sedimentation rates located coastward of a mussel bed, and in a sandy site without mussels and characterized by high hydrodynamic stress. After a year, cockles increased algal biomass in the sandy area, but not in the mussel bed site, where high values were measured in all plots. However, lugworms did not affect algal biomass in any of the sites. Field measurements suggest that cockles outweighed negative effects of water currents in the site without mussels by locally increasing sediment stability, while mussels overruled the effects of cockles in the wake of the reefs through hydrodynamic stress alleviation and/or biodeposition. Our results suggest that non-trophic interactions by ecosystem engineering bivalves control primary production of intertidal areas, and that the sediment stabilizing effect of cockles plays a crucial role where the overruling effects of mussel beds are not present.

2

INTRODUCTION

Ecosystem engineers can facilitate the survival of other species in hostile environments by modifying habitat conditions (Jones et al. 1994; Bruno and Bertness 2001; Crain and Bertness 2006). The relative importance of facilitation is predicted to increase with environmental stress (Bertness and Callaway 1994; Bruno et al. 2003) and accordingly, studies from both marine and terrestrial ecosystems show that dominant interactions among species can shift from negative or neutral to positive when harshness of abiotic conditions increases (Callaway 1997; Callaway and Walker 1997; Bulleri et al. 2011). Most of these studies have focused on foundation species, species which create and modify habitats through their own physical structure (Dayton 1972; Bertness and Callaway 1994; Crain and Bertness 2005). However, important facilitative effects in stressed environments also come from species that modify the environment through their activity rather than their physical shape, the so-called allogenic ecosystem engineers (Jones et al. 1994, Reise 2002; Volkenborn et al. 2007). Despite the broad literature on ecosystem engineers, experimental evidence of the relative importance of allogenic ecosystem engineering under different stress intensities is still scarce (but see Daleo and Iribarne 2009).

In the last decade there has been an increased research effort to include habitat-mediated and other non-trophic interactions into ecological theory by developing ecological network analyses away from the traditional food-webs studies that only include predator - prey interactions (Reise 2002; Bruno et al. 2003; Olff et al. 2009; Kefi et al. 2012). Yet, understanding consequences of ecosystem engineering in natural ecosystems is not trivial, because facilitative effects in stressed environments vary with the engineering species under study and the nature of the stress factor involved (Choler et al. 2001; Kawai and Tokeshi 2007; Maestre et al. 2009). Ecosystems are often subjected to different types of abiotic stress and host engineering species that modify habitat conditions in contrasting ways. Therefore, facilitative effects of engineering species on local communities may alternate predictably along different stress gradients (Fig. 2.1A). It is thus important to consider multiple ecosystem engineers and multiple abiotic stresses to better understand the importance of facilitation as a structuring force in natural communities.
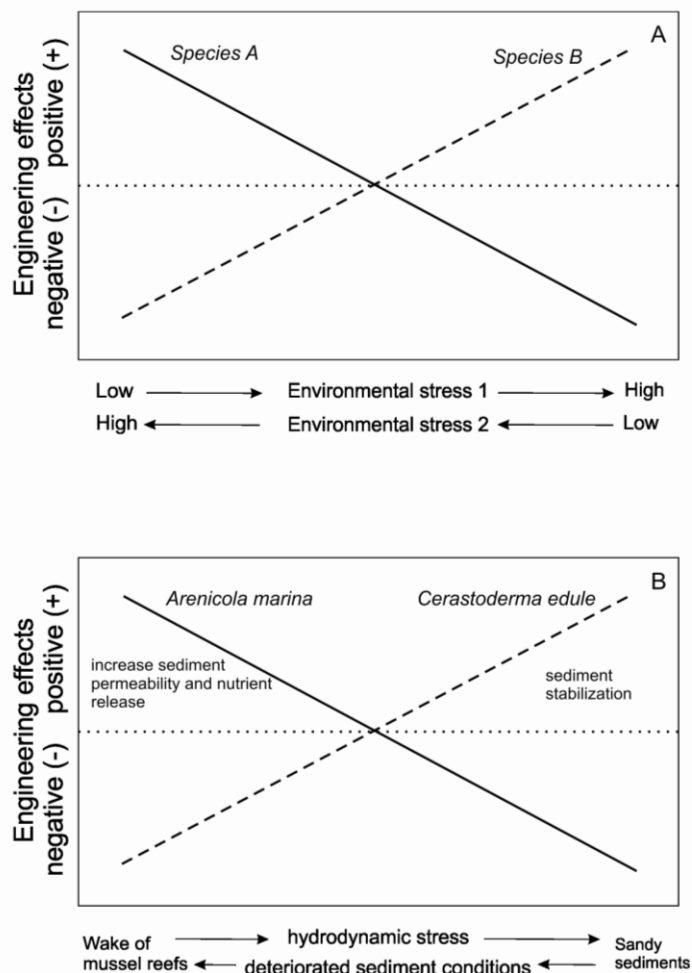
Fig. 2.1. (A) Conceptual model illustrating a scenario where two ecosystem engineers (species A and species B) have opposite effects on the surrounding species under different abiotic stresses (stress 1 and stress 2) which covary along an environmental gradient. (B) The same conceptual model is used to illustrate our hypotheses: cockles (*C. edule*) have positive effects on microphytobenthos through sediment stabilization under high hydrodynamic stress, but not in the wake of mussel reefs, where low current velocities and biodeposition lead to deteriorated sediment conditions (low sediment permeability and rate of solute exchange). On the opposite, lugworms (*A. marina*) have positive effects on microphytobenthos under deteriorated sediment conditions by increasing sediment permeability and nutrient availability, but the effects are negative in high-energy environments.

In this study we investigated the effects of two allogenic ecosystem engineers (lugworm: *Arenicola marina*, and common cockle: *Cerastoderma edule*) on microphytobenthos, under different abiotic stresses controlled by reefs of blue mussels (*Mytilus edulis*). Blue mussels form dense epibenthic reefs that enhance sedimentation of fine suspended matter by reducing water current velocities and by biodeposition, thus creating a plume of fine cohesive sediments coastward of the reefs (Graf and Rosenberg 1997; van der Zee et al. 2012; Donadi et al. 2013a). Previous studies showed that microphytobenthos biomass decreases with wave energy, which induces resuspension and subsequent transport by currents (de Jonge and van Beusekom 1995; van der Wal et al. 2010), and is positively associated with fine sediments where both grazer pressure and resuspension are generally lower (de Jong and de Jonge 1995; Herman et al. 2001). However, the settlement of large amounts of fine particles can result in hypoxic sediments, with very low sediment permeability and rate of solute exchange (Winterwerp and van Kesteren 2004). Thus, the presence or absence of mussel reefs exposes coastal soft-sediment habitats to different types of stresses: in areas with no mussel reefs high hydrodynamic energy triggers sediment erosion, while in the lee of mussel reefs low hydrodynamic energy and biodeposition cause deteriorated sediment conditions.

Cockles and lugworms are dominant species on tidal flats with opposite engineering effects on sediment stability. Cockles (*Cerastoderma edule*) are filter-feeding bivalves that can decrease sediment grain size and increase sediment stability through the production of mucus-rich faeces and pseudofaeces (Andersen et al. 2010). Lugworms are well-known sediment de-stabilizers which promote erosion of fine material through bioturbation (Volkenborn et al. 2007). Furthermore, by flushing their burrows (bioirrigation) lugworms enhance the exchange of solutes between sediments and overlying water and influence the rates of benthic metabolism by increasing oxygen penetration in the substrata (Banta et al. 1999). Recent studies have shown that positive effects on primary producers due to nutrient release by macrofaunal bioturbation can offset negative effects of grazing and subduction, especially in cohesive sediments where diffusion of solutes is slow (Lohrer et al. 2004; Thrush et al. 2006; Lohrer et al. 2010; Needham et al. 2011).

To investigate if cockles and lugworms have contrasting effects on benthic primary producers and if these effects depend on hydrodynamic and sediment

conditions, we performed a manipulative field experiment. In this experiment we created a total of forty-eight 5×5 m plots with either high densities of cockles, high densities of lugworms, or no manipulation (control). The experimental treatments were replicated in a muddy site sheltered from the tidal currents by a large mussel bed and in a sandy area without mussels on a tidal flat in the Wadden Sea. We hypothesize that at high levels of hydrodynamic stress (in the sandy site without mussels) cockles enhance algal biomass by increasing sediment stability, while lugworms hinder microphytobenthos growth by increasing sediment erosion. In deteriorated sediment conditions (in the wake of mussel reefs), we expect lugworms to increase algal abundances by increasing sediment permeability and nutrient availability and cockles to have negative effects on microphytobenthos through biodeposition (Fig. 2.1B).

METHODS

Field experiments

The study was conducted on the intertidal flats south of the island of Schiermonnikoog (Dutch Wadden Sea). On these tidal flats, cockles and lugworms commonly co-occur at the local scale (<0.1 m), while mussels typically form large (>1 ha) epibenthic reefs. Pilot surveys conducted in 2009 in the study area showed relatively stable densities of 20-40 lugworms per m^2 and a patchy distribution of cockles, with abundances ranging from 0 to several hundred individuals per m^2. These values are in agreement with natural occurring densities of cockles and lugworms typically found on the tidal flats of the Wadden Sea (Beukema and Devlas 1979; Jensen 1992).To evaluate the effects of these species on microphytobenthos, a large-scale experiment was set up in May 2010 in two selected sites: the first one located at about 350 m coastward of a large mussel bed (53° 28' 2" N, 6° 10' 59" E) and characterized by fine sediment (muddy) and lower current velocities; the second one at 500 m from the first site, at the same tidal elevation (53° 28' 7" N, 6° 11' 33" E), and characterized by coarser sediment (sandy) and higher current velocities. At each site, twenty-four 5×5 m experimental plots grouped in two blocks were randomly assigned to one of the following treatments: (i) addition of high densities of cockles (1000 per m^2); (ii) addition of high densities of lugworms (80 per m^2) and (iii) no addition (control).

Treatment densities of cockles and lugworms were chosen to mimic maximum observed densities in the study area. Cockles were collected from a nearby intertidal flat by professional fishermen through hand-raking and were 2 to 4 years old (mean mm lenght ± SE = 32.40 ± 0.78, n = 15). Lugworms were harvested by a commercial lugworm fishery company (Arenicola BV, Oosterend, The Netherlands) from an intertidal flat in the Western Wadden Sea (mean g. dry weight ± SE = 0.97 ± 0.12, n = 20). Persistence of transplanted specimens was monitored throughout the experiment by periodic counts of cockles (one replicate per plot) and lugworm fecal casts (three replicates per plot) within a 0.5×0.5 m frame. Abundances of both cockles and lugworms were always significantly higher in enriched plots compared to the other treatments, the only exception being lugworm abundances in the mussel bed site in July 2011, when there was no difference between the treatments (Appendix 2A).

To estimate microphytobenthos biomass in the experimental plots we analyzed chlorophyll *a* content of the sediment, a commonly used proxy for algal biomass. Two replicates per plot of the upper sediment layer (2 mm depth) were collected by means of cut-off disposal syringes (2.4 cm internal diameter) in May, June and July 2011. Samples from all plots of both sites were taken within few hours on the same day and stored in a dark and cold place. After freeze-drying the sediment, chlorophyll *a* content was determined by acetone extraction following Jeffrey and Humphrey (1975).

Hydrodynamic stress was estimated in the experimental plots in June 2011 as % weight loss of plaster dissolution cylinders. Laboratory experiments showed that the dissolution rate of plaster is a good (relative) measurement of water flow speed (Thompson and Glenn 1994). However, under field conditions the loss of plaster may also indicate the magnitude of the abrasion effect by sediment suspended in the water column, and is therefore a general proxy for the erosive power of the currents. Dissolution cylinders (6.3 cm long; 2.4 cm diameter) were molded with model plaster (Knauf Modelgips, Knauf B.V., Utrecht, The Netherlands), dried, weighted (mean g. dry weight ± SE = 41.22 ± 1.54, n = 48) and placed in the centre of each plot for four tidal cycles (about 46h), after which they were retrieved and dried until constant weight. To account for differences in the exposure time between sites, plaster weight loss values were divided by the average tidal submersion time of each site (mean minutes Δt ± SE = 406 ± 4 coastward of the mussel bed; Δt ± SE = 370 ± 6 in the sandy site, n = 3), estimated

in June 2011 by means of Sensus Ultra pressure loggers (Reefnet, Missisauga, Canada).

To investigate effects of ecosystem engineers on sediment accumulation and erosion, we assessed changes in bed level using a Trimble Spectra Precision LL500 Laser Level (Trimble, California, USA). At each site, measurements were calibrated against fixed reference metal poles. Five replicates were randomly taken in all plots in July 2010 and July 2011, and averages per plot were calculated. The difference of the averages between the two years was computed to evaluate bed level change over time (from here on *bed level change*), while the averages of July 2011 were used as a measurement of relative bed level at the time of the experimentation (from here on *relative bed level height*). Measurements of erodibility estimated in the sandy sites in September 2011 by means of a cohesive strength meter (CSM, Partrac, Glasgow, UK) following Tolhurst et al. (1999) showed a positive correlation between critical erodibility stress (CSM jet pressure at which sediment erosion occurs) and relative bed level height (r = 0.51, *P* = 0.018, n = 21), and between critical erodibility stress and bed level change (r = 0.65, *P* = 0.001, n = 21). These results, combined with the evidence that differences in relative bed level height were due to sediment accumulation or erosion in the experimental plots rather than to the site topography (see Results), suggest that bed level height can be reasonably considered as a good proxy for sediment stabilization or erosion.

Finally, to investigate sediment properties, two sediment samples were taken from each plot in June and in July 2011. Sediment was collected to a depth of 5 cm using a PVC corer (3 cm internal diameter) and average organic matter content was estimated from oven-dried (48 h, 75 °C) samples as Loss On Ignition (LOI; 4 h, 550 °C). Silt content of sediment samples (fraction < 63 μm) collected in July 2011 was determined with a Malvern particle size analyzer (Malvern Particle Sizer 2000, United Kingdom) after freeze-drying the sediment samples. Concentration of ammonium, which is one of main limiting nutrients for microphytobenthos and a proxy for anaerobic conditions, was measured from pore-water samples collected in July 2011 in airtight 50 ml syringes using ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, The Netherlands). Average pore-water reduction-oxidation potential (RedOx) was estimated from two samples collected in each plot in March 2011 and September 2011 with a Sentix ORP probe connected to a Multi 340i voltmeter (WTW, Germany).

Data analysis

Effects of cockles, lugworms and mussels on all measured parameters were evaluated with statistical linear models including the fixed factors Engineer addition (three levels: Cockle addition, Lugworm addition, No addition), Site (two levels: Mussel bed site, Sandy site) and their interaction. Effects on average chlorophyll *a* content (n = 6), average bed level change (n = 5) and average redox potential (n = 4) were assessed by general linear models, while effects on % plaster weight loss, silt content, average % organic matter content (n = 2) and ammonium concentration were estimated by generalized least squares (GLS) to account for heteroscedasticity (Zuur et al. 2009). Independence of observations taken within the same block was investigated by comparing models with and without a residual compound symmetry correlation structure (Zuur et al. 2009). The addition of a correlation structure led to a significant improvement only in the analysis of % silt content, therefore mixed-effect models were used to assess the significance of fixed factors. Treatment effects on relative bed level height were assessed separately per each site, as measurements were calibrated against two different reference poles (see above). A linear model was used for this analysis including Engineer addition and Block as fixed factors, as the number of levels for block in the site-separated analysis did not allow us to include it as a random factor. Normality assumption was checked with the Shapiro-Wilks test (P = 0.05) and homoscedasticity was assessed with the Bartlett test (P = 0.05) and by comparing GLS (Generalized Least Squares) models with or without a residual variance structure (Zuur et al. 2009). Independence, normality and homogeneity of variance of residuals were further confirmed by graphical validation of the final models. When needed, multiple comparisons to identify the source of any significant differences were performed using Fisher's LSD post-hoc test.

To investigate the relationship between primary producer biomass, hydrodynamic stress and sediment accumulation, correlations between chlorophyll *a* content and plaster weight loss, and between chlorophyll *a* content and relative bed level height, were calculated using Pearson correlation coefficient and Spearman's rank correlation coefficient for parametric and non-parametric data respectively. When the Pearson correlation coefficient was significant, the parameters of the functional relationship between variables were estimated using standard major axis regression (SMA), as both variables were

random and measured with error (Legendre and Legendre 1998). All statistical calculations were carried out in R (CRAN, R: A language and environment for statistical computing. 2010. R Foundation for Statistical computing. Vienna, Austria).

RESULTS

We observed a reduction of the erosive power of the water currents coastward of the mussel bed compared to the sandy area, as indicated by lower plaster weight loss in the lee of the reefs (Site effect: Likelihood Ratio test statistic = 20.23, $P <$ 0.001; Fig. 2.2A). Bed level measurements revealed that cockles and lugworms influenced sediment stability in opposite directions, but only in the absence of mussel reefs (interaction effect between Engineer addition and Site; Table 2.1). Cockles significantly favored sediment accumulation in the sandy area compared to lugworm-addition plots (Fisher LSD post-hoc test; $P <$ 0.001) and no-addition plots (Fisher LSD post-hoc test; $P =$ 0.003), while lugworms enhanced sediment erosion (Fisher LSD post-hoc test; lugworm addition vs cockle addition in sandy site, $P <$ 0.001; lugworm addition vs no addition in sandy site, $P =$ 0.019; Fig. 2.2B). This resulted in a more elevated substrate in the cockle-addition plots: on average 4.6 ± 1.4 cm (mean ± SD; n = 8; Fisher LSD post-hoc test; $P <$ 0.001) and 2.7 ± 1.7 cm (mean ± SD; n = 8; Fisher LSD post-hoc test; $P <$ 0.001) higher than in the

Table 2.1. Statistical results of linear models examining effects of mussel bed vicinity (Site) and high densities of cockles and lugworms (Engineer addition) on chlorophyll *a* content and bed level change.

Source of variation	Df	Bed level change			Chorophyll *a*			Redox potential		
		MS	F	P	MS	F	P	MS	F	P
Site	1	173.96	134.85	<0.001	569.54	51.87	<0.001	3464	41.641	<0.001
Engineer addition	2	4.50	3.49	0.039	53.30	4.85	0.012	68	0.812	0.451
Site * Engineer addition	2	18.11	14.03	0.002	75.89	6.91	0.002	7	0.084	0.920
Residuals	42	1.29			10.98			83		

Significance level (α) was set at 0.05 of probability
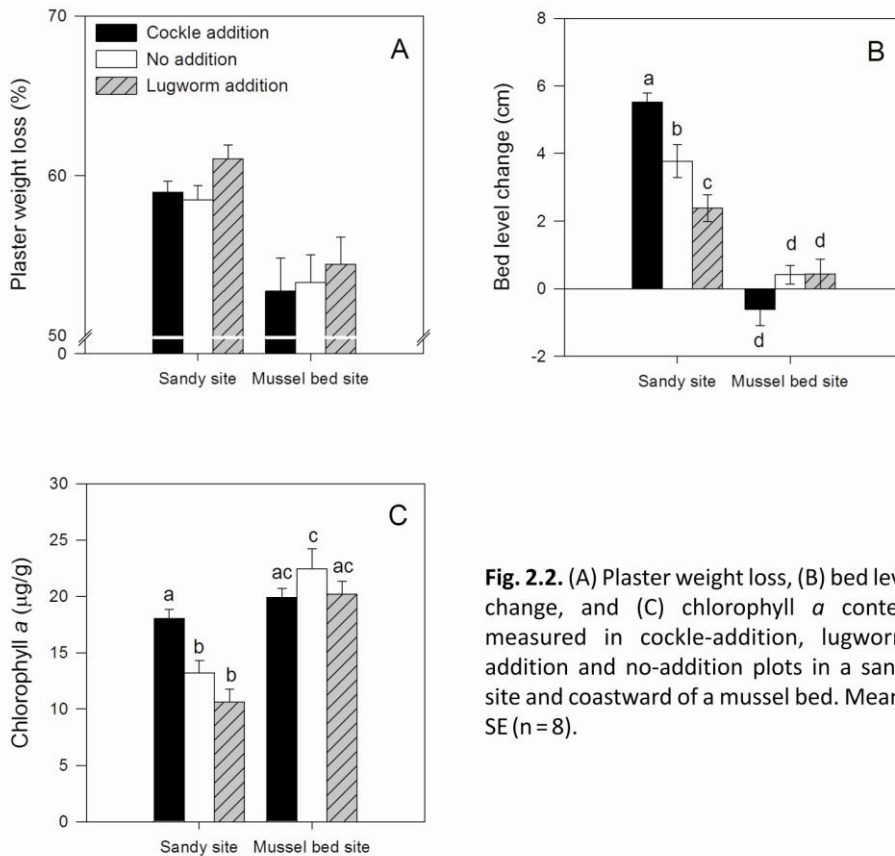
Fig. 2.2. (A) Plaster weight loss, (B) bed level change, and (C) chlorophyll *a* content measured in cockle-addition, lugworm-addition and no-addition plots in a sandy site and coastward of a mussel bed. Mean ± SE (n = 8).

lugworm-addition and no-addition plots, respectively (Table 2.2).

We found a significant effect of the interaction between Engineer addition and Site (Table 2.1) on chlorophyll *a*: in the sandy area, cockle addition resulted in a 70% and 37% higher microphytobenthos growth compared to lugworm-addition (Fisher LSD post-hoc test; *P* < 0.001) and no-addition plots (Fisher LSD post-hoc test; *P* = 0.005), respectively, while lugworms did not significantly decrease algal biomass (Fig. 2.2C). In the muddy site in the wake of the mussel bed, chlorophyll *a* content was overall 49% higher than in the sandy site, but we did not detect any effects of either cockles or lugworms (Fig. 2.2C; Table 2.1).

2

Table 2.2. Statistical results of linear models examining effects of high densities of cockles and lugworms (Engineer addition) and block on relative bed level height in the sandy site and in the mussel bed site.

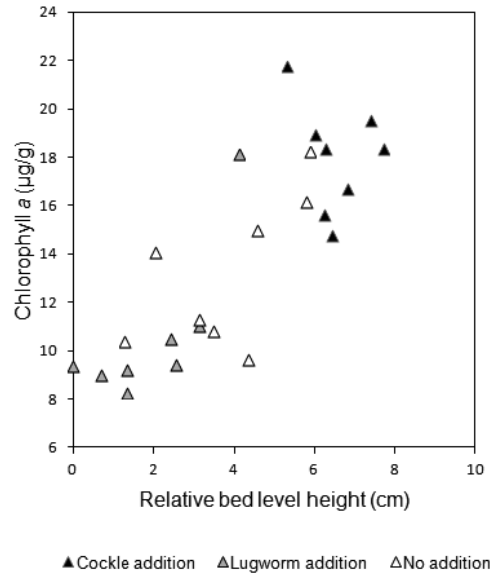|		Relative bed level height			Relative bed level height		
		SANDY SITE			MUSSEL BED SITE		
Source of variation	Df	MS	F	P	MS	F	P
Engineer addition	2	41.89	28.41	**<0.001**	0.08	0.10	0.905
Block	1	1.33	0.90	0.353	17.95	20.18	**<0.001**
Block * Engineer addition	2	4.08	2.76	0.089	0.83	0.93	0.411
Residuals	18	1.47			0.89		

Significance level (α) was set at 0.05 of probability

Accordingly, we found an overall negative correlation between chlorophyll *a* and plaster weight loss (r_s = -0.54, *P* < 0.001, n = 47), supporting the idea that erosion by currents limits microphytobenthic growth in the intertidal flats where we conducted our study. However, while hydrodynamic stress and sediment transport negatively affected microphytobenthos growth in a similar way in the lugworm-enriched plots (r = -0.62, n= 16, *P* = 0.011, SMA slope = -1.16, 95% confidence interval: -1.80 to -0.75) and in the no-addition plots (r = -0.71, n= 15, *P* = 0.003, SMA slope = -1.38, 95% confidence interval: -2.07 to -0.92), such negative relationship was not detected in the cockle-addition plots (r_s = 0.01, n= 16, *P* = 0.969; Fig. 2.3). In the sandy area, higher values of algal biomass were found in more elevated plots, as shown by the positive correlation between chlorophyll *a* content and relative bed level height (r_s = 0.83, n = 24, *P* < 0.001; Fig. 2.4).

Contrary to our expectations, cockle and lugworm addition did not have any significant effect on organic matter, silt content, pore-water ammonium concentration and redox potential. Yet, organic matter and silt content were at least three and four times higher respectively in the mussel bed area than in the sandy site (Site effect on organic matter content: Likelihood Ratio test statistic = 83.34, *P* < 0.001; Appendix 2B; Site effect on silt content: Likelihood Ratio test statistic = 19.03, *P* < 0.001; Appendix 2B), pointing to accumulation of biodeposits
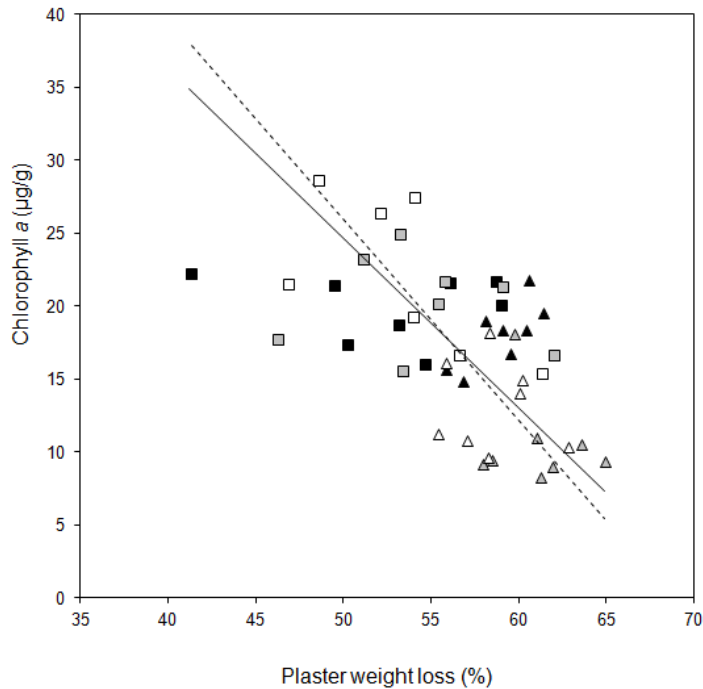
Fig. 2.3. Scatter plot of chlorophyll *a* content and plaster weight loss measured in cockle-addition, lugworm-addition and no-addition plots in a sandy site and coastward of a mussel bed (n = 47). Black symbols show cockle-addition plots, grey symbols show lugworm-addition plots and white symbols show no-addition plots. Squares are plots from the mussel bed site, while triangles are plots from the sandy site. SMA regression lines indicate significant correlations in the lugworm-addition (*solid line*) and no-addition plots (*dashed line*).

in the wake of the mussel reefs. Ammonium concentration was higher in the mussel bed site than in the sandy site, although this difference was only marginally significant (Site effect: Likelihood Ratio test statistic = 3.80, P = 0.051, Appendix 2B). Also, we found a significant effect of Site on pore-water redox potential, with lower values coastward of the mussel reef suggesting oxygen depletion in the mussel biodeposition plume (Table 2.1; Appendix 2B).

2

Fig. 2.4. Scatter plot of chlorophyll *a* content and relative bed level height measured in cockle-addition, lugworm-addition and no-addition plots in the sandy site (n = 24). Bed level measurements are relative values to the minimum, which was set as zero. Black triangles show cockle-addition plots, grey triangles show lugworm-addition plots and white triangles show no-addition plots.

DISCUSSION

We demonstrate that cockles facilitate benthic primary producers on intertidal flats by increasing sediment stability, but this effect is conditional on hydrodynamic and sediment conditions. At high levels of hydrodynamic stress, high densities of cockles accumulated sediment and thereby compensated for the negative effects of waves and currents on microphytobenthos, leading to increased chlorophyll *a* content in the sediment. Cockles have been primarily described as a bioturbating species, which increases sediment disturbance and erodibility (Flach 1996; Neumeier et al. 2006; Ciutat et al. 2007; Montserrat et al. 2009). We are the first to show through a large-scale manipulation that high densities of cockles can actually enhance sediment stability (as recently suggested

by Andersen et al. 2010) and that this in turn favors primary producers. At lower hydrodynamic stress in the wake of a mussel bed, cockles and lugworms neither affected sediment parameters nor algal biomass. In the lugworm-addition plots, this might be due to low lugworm abundances measured at the end of the experimental period. However, in the cockle-addition plots high cockle abundances rather point to the overwhelming effects of the mussel bed, which likely enhanced algal biomass by a combination of hydrodynamic stress alleviation and biodeposition. Hence, the vicinity of a mussel bed shifted the balance between abiotic and biotic control, overriding engineering effects of cockles. In fact, interactions between primary producers and cockles shifted from facilitative far from the mussel bed to neutral coastward of the mussel bed, where environmental conditions were more benign. Thus, our large-scale manipulation demonstrates that not only foundation species but also allogenic ecosystem engineers play an increasingly important role at higher intensities of environmental stress. As the intertidal flats of the Wadden Sea host relatively few mussel beds if compared with historical data, the persistence of cockle populations likely contributes significantly to maintain high level of productivity in these habitats. In light of this consideration, we suggest that the simultaneous exploitation of mussels and cockles in the Wadden Sea and the consequential decline of their abundance over the last half century (Lotze et al. 2005; Kraan et al. 2011) might have contributed to a decrease in benthic primary production (Eriksson et al. 2010). Because benthic primary producers (such as algae and seagrasses) play an important role as sediment stabilizers (Widdows and Brinsley 2002; van der Heide et al. 2007) and constitute an essential source of food for many birds and benthic species (for example, Nacken and Reise 2000; Lebreton et al. 2011), their loss may entail severe consequences for the habitat and for different trophic levels of coastal ecosystems.

Cockles facilitated microphytobenthos by stabilizing the sediment at hydrodynamic conditions where sediment erosion otherwise limited microphytobenthic growth. Previous evidence demonstrated that waves and currents can drastically reduce microphytobenthos biomass through sediment resuspension and subsequent transport (de Jonge and van Beusekom 1995; van der Wal et al. 2010). Accordingly, we observed a negative correlation between plaster weight loss and chlorophyll *a* content, where high chlorophyll *a* values corresponded to low erosive power of water currents coastward of the mussel

bed. However, such relationship was not significant on cockle-addition plots, demonstrating that high densities of cockles overruled hydrodynamic control of the primary producer community. Production of faeces and mucus by high densities of cockles can reduce vulnerability to erosion (Ciutat et al. 2006), and might have promoted sediment accretion in the cockle-addition plots in the sandy area, as suggested by the positive correlation between critical erosion threshold and bed level change. Alternatively, enhanced nutrient availability (Swanberg 1991) and/or reduced sediment resuspension due to cockle biodeposition might have favored diatom growth, which in turn further decreased vulnerability to erosion and promoted sediment accumulation (Widdows et al. 2004; Andersen et al. 2010). Also, protruding shells of cockles from the sediment might have slowed down current velocity, causing local sediment accretion. Although our experiments clearly show that cockles can positively affect sediment stability, they don't allow the separation of structural and functional effects of cockles, and further experiments are needed to unravel the mechanisms underlying facilitation of microphytobenthos by cockle beds.

Our study suggests that cockles trigger positive feedbacks between sediment accumulation, microphytonbenthos growth and the cockles themselves. Positive feedbacks are essential for high benthic primary production in intertidal soft-bottom ecosystems (van de Koppel et al. 2001). Indeed, productive tidal flats might persist through positive feedbacks (1) between microphytobenthos growth and sediment accumulation: low water content in locally elevated areas on tidal flats allows the persistence of colloidal carbohydrates, water-soluble diatom excretions which bind sediment particles (Blanchard et al. 2000; Weerman et al. 2010); (2) between diatoms and cockles, as microphytobenthos constitutes a food source for cockles (Sauriau and Kang 2000; Filgueira and Castro 2011); (3) between cockles and sediment stability, as coarser sediment may attract smaller densities of bivalve spat (Thrush et al. 1996b; Piersma et al. 2001; Beukema and Dekker 2005). This suggests that removal of cockles or sediment disturbance on large scales following anthropogenic activities may entail severe and unexpected consequences for the ecosystem functioning.

The high levels of algal biomass measured in the surface sediments coastward of the mussel bed together with the negative correlation between chlorophyll *a* and plaster erosion and the observed reduction of erosive energy coastward of the reefs suggest that mussels facilitated microphytobenthos by

alleviating hydrodynamic stress. However, also intense biodeposition indicated by high sediment organic matter and silt content and high ammonium concentration in the mussel bed site might have contributed to enhance microphytobenthos growth. Besides decreasing the suspended sediment concentration and therefore the erosive power of water currents, filter feeding and production of mucus-rich biodeposits can result in (1) higher cohesiveness of the substrata (Widdows et al. 1998), (2) lower sediment grain size (Ysebaert et al. 2009), as confirmed by our results, which in turn alleviates grazer pressure and sediment disturbance by bioturbators (Herman et al. 2001), (3) increased light availability for primary producers (see Newell and Koch 2004 for review), and (4) enhanced nutrient availability (Bracken 2004). Therefore, both hydrodynamic stress alleviation and biodeposition by mussel reefs are likely to play a role in the facilitation of microphytobenthos, and their relative contribution remains to be confirmed by further studies providing conclusive experimental evidence of such mechanisms as well as spatial replication of the patterns observed.

In previous studies we showed that engineering effects of mussel reefs on hydrodynamic and sediment properties extend far from the reefs, thereby affecting the distribution of benthic species as well as bird populations on long distances (van der Zee et al. 2012; Donadi et al. 2013a). In the current study we found that microphytobenthos growth was facilitated several hundred meters coastward of a mussel bed, suggesting that mussel reefs may influence multiple trophic levels at large spatial scales. Thus, our results highlight both the general importance of non-trophic interactions for ecosystem function, as well as the specific importance of sediment-stabilizing bioengineers in conserving and promoting the primary productivity of soft-bottom intertidal ecosystems. Our findings are critical to improve coastal management and conservation policies of intertidal flats, eventually leading to the preservation and restoration of healthy and productive ecosystems.

Acknowledgements

APPENDICES

Appendix 2A. Cockle and lugworm abundances during the experimental period.

Fig. 2A. Cockle abundances (individuals/m^2) and lugworm abundances (fecal casts/m^2) in cockle-addition, lugworm-addition and no-addition plots measured in the mussel bed site and in the sandy site in September 2010, March 2011 and July 2011. Mean ± SE (n = 8 and n = 24 for cockle and lugworm abundances, respectively).

Appendix 2B. Sediment and pore-water parameters in the experimental plots.
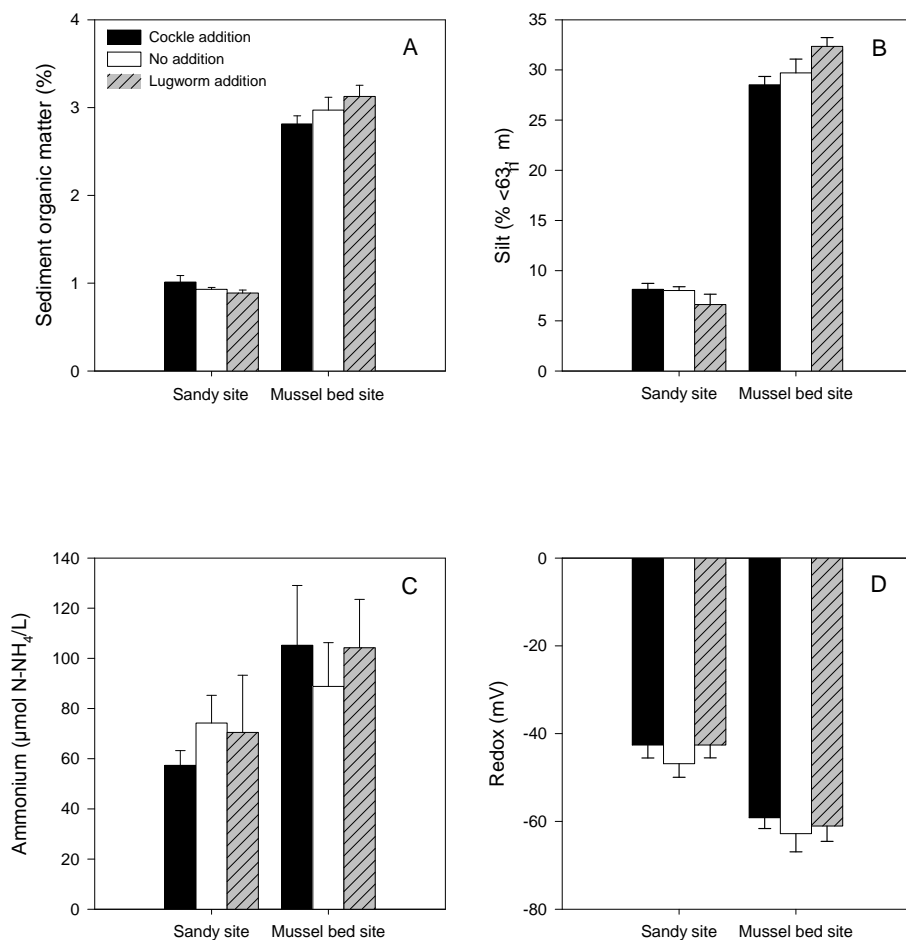
Fig. 2B. (A) Sediment organic matter content, (B) silt content, (C) ammonium concentration, and (D) redox potential measured in cockle-addition, lugworm-addition and no-addition plots in a sandy site and coastward of a mussel bed. Mean ± SE (n = 8).

Chapter 3

3

THE BIVALVE LOOP: THE ROLE OF POSITIVE FEEDBACKS FOR RECOVERY OF EXPLOITED POPULATIONS

Serena Donadi, Els M. van der Zee, Tjisse van der Heide, Ellen J. Weerman, Theunis Piersma, Johan van de Koppel, Han Olff, Marc Bartelds, Imke van Gerwen & Britas Klemens Eriksson

ABSTRACT

Human exploitation of bivalve populations has changed intertidal landscapes worldwide. Many bivalves are ecosystem engineers that modify the physical environment, affecting the conditions for their survival. Here we argue that lack of recovery of overexploited intertidal bivalve beds may be caused by the loss of important biological feedbacks from depleted populations. In a large-scale experiment we investigated engineering effects of cockles (*Cerastoderma edule* L.) and lugworms (*Arenicola marina* L.) on cockle recruitment by adding high densities of either species to 5×5 m plots in areas with different hydrodynamic and sediment conditions in the intertidal flats of the Wadden Sea. Cockles enhanced the recruitment of conspecifics in sandy areas with high wave and current energy and this was likely due to increased sediment stability in the cockle-addition plots. Lugworms did not have an effect on bivalve recruitment. In muddy sites sheltered from the tidal currents by mussel reefs (*Mytilus edulis* L.), cockle juvenile densities were generally high, demonstrating the general importance of biological engineering for recruitment processes in the intertidal. Thus, acknowledging positive feedbacks between bivalves and sediment stability is essential to achieve long-term restoration goals in coastal ecosystems.

INTRODUCTION

Overfishing and habitat destruction have led to dramatic reductions in bivalve populations all over the world (Jackson et al. 2001, Airoldi and Beck 2004, Lotze et al. 2006 and references therein). The rate of decline has accelerated dramatically within the last 200 years, bringing several species close to collapse (e.g. Kirby 2004, Lotze 2005). Although the results of restorations efforts are encouraging (e.g. Arnold et al. 2005, Schulte et al. 2009, Marsden and Adkins 2010), large-scale restoration of shellfish stocks often remains a faraway goal. Lack of recovery has been attributed to the cumulative effects of food web changes (i.e. the arrival of new competitors or predators, Walters and Kitchell 2001), habitat destruction (e.g. Lenihan and Peterson 1998, Medina et al. 2007), climate change (e.g. Schuckel and Kroncke 2013), and pollution (Worm and Lotze 2000). However, a possibly underestimated cause preventing re-establishment of former abundance levels is the loss of biological feedbacks from pre-existing populations. Many bivalve species act as ecosystem engineers that modify the surrounding environment often for their own benefit (Jones et al. 1994, Bertness and Leonard 1997, Jones et al. 1997). Hence, the eradication of natural populations can break these positive feedbacks between ecosystem engineers and environment and result in degraded habitat conditions that are hard to reverse (Nÿstrom et al. 2012, Thrush et al. 2009). Here we investigate positive feedbacks by sediment-living bivalves and suggest that the loss of adult populations may be responsible for the slow recovery of many bivalve species.

Positive feedbacks have been suggested to be crucial for the stability and resilience of ecological communities (Gurney and Lawton 1996, Scheffer and Carpenter 2003), as they can lead to the occurrence of alternative stable states in ecosystems (May 1977, Scheffer et al. 2001). Habitat modification by species may affect the dynamics of future generations ("ecological imprint") by increasing the probability of successful recruitment (Laland et al. 1999, Cuddington et al. 2009). Ecological imprints may thus lead to the segregation of species, resulting in self-organized spatial heterogeneity (Hui et al. 2004). Hence, positive feedbacks can strongly influence the distribution, persistence and dynamics of natural populations, and the appreciation of these mechanisms has profound implications

for conservation and restoration of ecosystem engineers (Boogert et al. 2006, Byers et al. 2006).

In this study we investigated the effects of sediment-stabilizing bivalves (*Cerastoderma edule* – cockle) and sediment-destabilizing worms (*Arenicola marina* – lugworm) on cockle recruitment on the tidal flat of Schiermonnikoog (Wadden Sea, The Netherlands). Cockles live in the upper few cm of the sediment and can occur in dense assemblages of many thousands m^{-2} (Jensen 1992). Although several studies emphasized the bioturbating effects of cockles (Flach 1996, Neumeier et al. 2006), high densities of cockles can also increase sediment stability through the production of mucus-rich biodeposits and facilitation of sediment-binding diatom colonies (Ciutat et al. 2007, Donadi et al. 2013b). Sediment stabilization may affect cockle recruitment and post-larval processes (Bouma et al. 2001, Piersma et al. 2001, Kater et al. 2006), as it might enhance the ability of recruits to anchor themselves to the substrate, thus buffering the negative effects of waves and currents (Armonies and Hellwigarmonies 1992, Armonies 1996). A widespread species with high abundances on tidal flats is the deposit feeding lugworm *Arenicola marina* (Flach and Beukema 1994). Through bioturbation, lugworms cause the loss of fine particles thus decreasing sediment stability (Volkenborn et al. 2007). The cohesiveness of sediments can be further reduced by the removal of diatom mats due to deposit feeding (Volkenborn et al. 2007). Consequently, lugworms might increase resuspension of recruits of macrozoobenthos by enhancing sediment erosion. Previous studies reported negative effects of lugworms on juveniles of *C. edule* and of several other species, which were attributed to sediment re-working activities but also to repeated burying by the fecal castings (Brey 1989, Flach 1992).

Here, we hypothesize that (1) cockles can facilitate the recruitment of conspecifics by increasing sediment stability and that (2) lugworms can disrupt these positive feedbacks through sediment disturbance. As the intensity of ecosystem engineering by cockles and lugworms likely depends on environmental conditions (Volkenborn et al. 2007, Montserrat et al. 2009), we tested our hypotheses in habitats with different hydrodynamic and sediment properties controlled by reef of blue mussels (*Mytilus edulis*) by adding a total of 800 000 cockles and 64 000 lugworms to 5×5 m plots on the intertidal flats of the Wadden Sea. In addition, to explore the relative importance of ecosystem engineering by

cockles and lugworms versus the effects of the local species, half of the plots were defaunated before the additions.

METHODS

Experimental treatments

The study was conducted on the intertidal flats of Schiermonnikoog, in the Dutch Wadden Sea. On these tidal flats, blue mussels form several large (>1 ha) epibenthic reefs, cockles can form dense assemblages within the sediment of several hundred individuals m^{-2} and lugworms occur in populations with relatively stable densities of 20-40 individuals m^{-2} (Beukema and Devlas 1979, Jensen 1992), as confirmed by pilot surveys conducted in 2009 in the study area (*pers. obs.*).

We used a large-scale experiment to investigate the effects of cockles and lugworms on cockle recruitment. Cockles commonly settle in the Wadden Sea in late spring (May - June) and in late autumn (October - November, van der Veer et al. 1998; R. Dekker, *pers. comm.*). A total of ninety-six 5×5 m plots arranged in a randomized block design were assigned to two muddy sites (53°28'8.15"N, 6°13'27.95"E; 53°28'2.44"N, 6°10'59.83"E), sheltered from the tidal currents by reefs of blue mussels (located at 100 and 350 m seaward of the experimental sites), and two more exposed sandy sites (53°28'7.42"N, 6°13'55.95"E; 53°28'7.54"N, 6°11'33.48"E), at the same tidal elevation, respectively. In the lee of mussel reefs low current velocities and biodeposition from mussels result in muddy sediments, with high organic matter and silt content and low redox potential, as shown by previous research in the same study area (van der Zee et al. 2012, Donadi et al. 2013a). Each site was assigned two blocks of twelve plots with a random full factorial combination of (1) defaunation treatment (two levels) and (2) engineer addition (three levels) (Fig. 3.1). The defaunation treatment consisted of an anoxia-induced kill off of macrobenthos in half of the plots by covering the sediment with plastic tarps for a period of 28 days (April - May 2010). After this period, the tarps were removed and sediment cores were taken to confirm the absence of living macrobenthic organisms. Two weeks after removal of the tarps, we performed the engineer addition: one-third of all plots was enriched with high densities of cockles (1000 individuals m^{-2}), one-third with
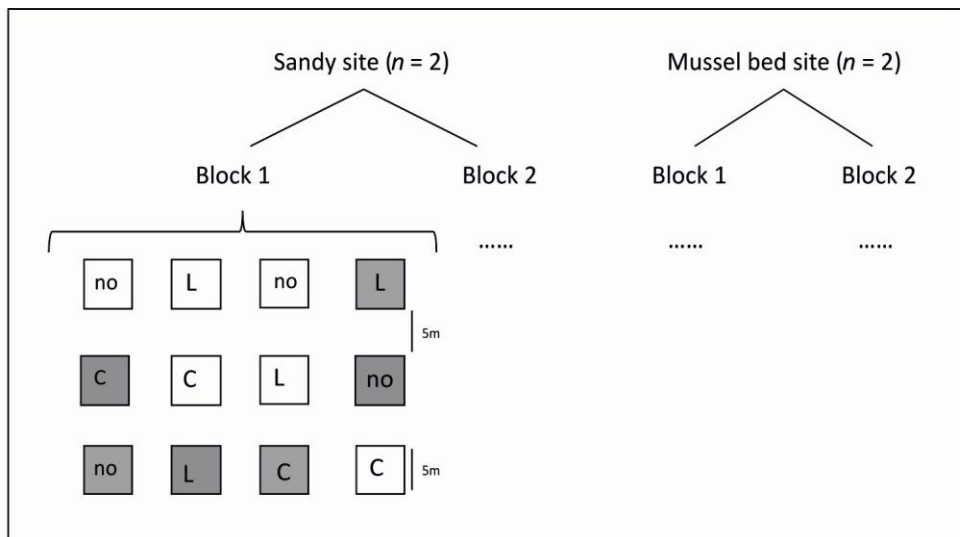
Fig. 3.1. Diagram of the experimental design. At each of two sandy sites (with no mussels) and two mussel bed sites (located coastward of mussel reefs), two blocks were assigned twelve 5×5 m plots with a random full factorial combination of (1) defaunation treatment, two levels: defaunated (dark squares) and not-defaunated (white squares) plots, and (2) engineer addition, three levels: cockle addition (C), lugworm addition (L), no addition (no).

high densities of lugworms (80 individuals m^{-2}) and the remaining plots served as a control (no addition). Treatment densities of cockles and lugworms were chosen to mimic maximum observed densities in the study area. Cockles were collected from a nearby intertidal flat by professional fishermen through hand-raking and were 2 to 4 years old (mean mm lenght ± SE = 32.40 ± 0.78, n = 15). Lugworms were harvested by a commercial lugworm fishery company (Arenicola BV, Oosterend, The Netherlands) from an intertidal flat in the Western Wadden Sea (mean g. dry weight ± SE = 0.97 ± 0.12, n = 20).

Abundance of added engineers was monitored every six months by counting cockles (one replicate per plot) and lugworm fecal casts (three replicates per plot) within a 0.5×0.5 m frame. Abundances of both cockles and lugworms generally decreased after the additions (also because of a winter freeze between 2010 and 2011) but remained considerably higher in enriched plots compared to the other treatments, the only exception being lugworm abundances in September 2011, when there was no difference between cockle- and lugworm-addition plots (Fig. 3.2).

Cockle juveniles and engineering effects

Abundance of cockle juveniles was estimated within a 0.5×0.5 m frame which was randomly placed on each plot in September 2010, March 2011, July 2011 and September 2011. Numbers of cockle juveniles (3-12 mm long) were counted after sieving the upper sediment layer (0-3 cm depth) through a 1 mm mesh. To minimize potential effects on the boundary flow, the sediment was put back in place after sieving and leveled with the surrounding bed level. For each plot the average of the counts from different dates was used for graphical representation and statistical analysis.

To investigate engineering effects of transplanted species on sediment accretion/erosion bed level height was estimated in March 2011 and September 2011 using a Trimble Spectra Precision LL500 Laser Level (Trimble, California, United States of America). At each site, measurements were calibrated against fixed reference metal poles. At each sampling time, five replicates per plot were collected and averaged and the differences between the two dates were used to calculate bed level changes (a proxy for net sediment accumulation or erosion) during the summer, when bioengineering activity of cockle and lugworms is more conspicuous.

To explore effects of cockles and lugworms on sediment properties, sediment samples were collected in each plot in September 2010, March 2011 and September 2011 to a depth of 5 cm through cut disposal syringes (2.4 cm internal diameter). Organic matter content was estimated from oven-dried (48 h, 75 °C) samples as Loss On Ignition (LOI; 4 h, 550 °C), and silt content was measured with a Malvern particle size analyzer (Malvern Particle Sizer 2000, United Kingdom) after freeze-drying the sediment samples.

Hydrodynamic conditions and field surveys

Differences in hydrodynamic conditions between the experimental sites were assessed in October 2010, April and June 2011 as percentage of weight loss of plaster cylinders. Cylinders (6.3 cm long; 2.4 cm diameter) were molded with model plaster (Knauf Modelgips, Knauf B.V., Utrecht, The Netherlands), dried, weighted and placed in the centre of each plot for four tidal cycles (about 46h), after which they were retrieved and dried until constant weight. Laboratory

Mussel bed sites   **Sandy sites**

Cockles (individuals m^{-2})

Lugworms (fecal casts m^{-2})

Sept 2010 March 2011 Sept 2011   Sept 2010 March 2011 Sept 2011

●— Cockle addition   —○— No addition   ▼— Lugworm addition
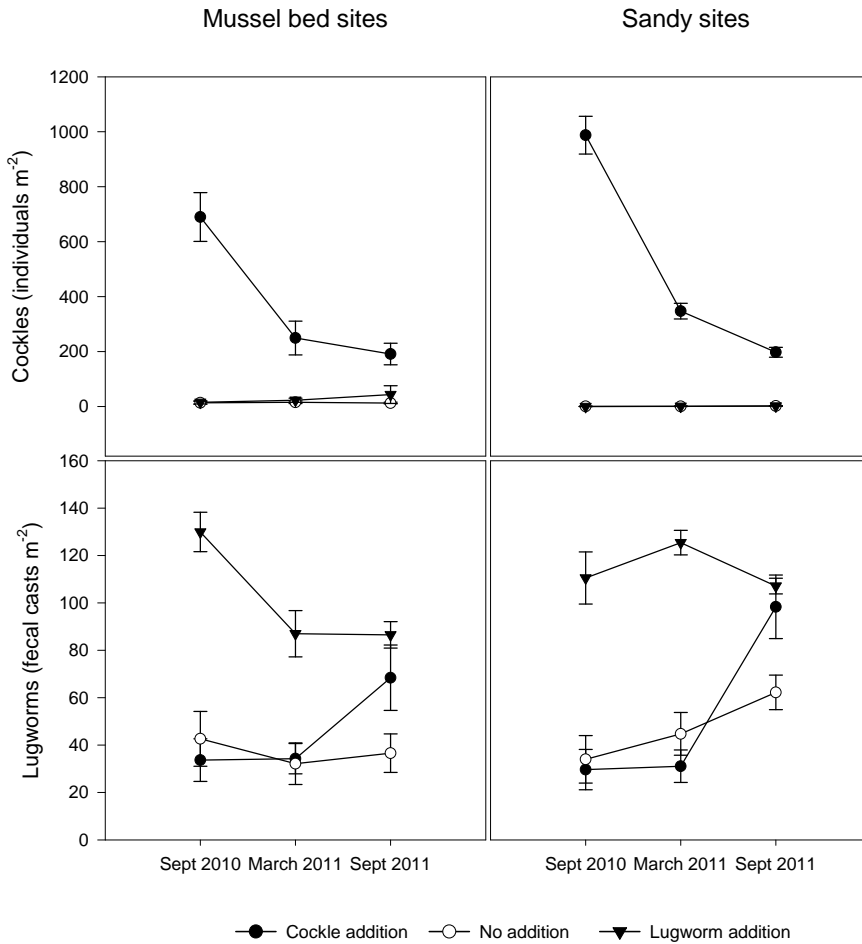
Fig. 3.2. Cockle and lugworm abundances in the experimental plots over one year

experiments showed that the dissolution rate of plaster is a good (relative) measurement of water flow speed (Thompson and Glenn 1994). However, under field conditions the loss of plaster will also indicate the magnitude of the abrasion effect by sediment suspended in the water column, and is therefore a general proxy for the erosive power of the currents.

      The influence of hydrodynamic stress and the erosive energy of the currents on cockle recruitment was investigated in May 2012 by measurements of

plaster dissolution (see above) and cockle juvenile abundances (estimated within a 0.5×0.5 m frame) along three transects perpendicular to the shore in an nearby area (53°28'5.92"N, 6°13'47.39"E) without any mussel bed.

Data analysis

Effects of cockles and lugworms on cockle juvenile abundance (averages across all sampling dates) were explored by a generalized linear mixed-model including the fixed factors Engineer addition (three levels: cockle addition, lugworm addition, no addition), Habitat (two levels: sandy sites, mussel bed sites), Defaunation (two levels: defaunated, not defaunated) and their interactions. A two-nested random-effect structure, taking into account sites (four levels) and blocks (eight levels) within a site, following a normal distribution, was considered for each plot. A Negative Binomial error distribution with log-link function was selected among other distributions based on the lowest AIC. A linear mixed-model including the same fixed and random factors listed above was used to explore effects on plaster weight loss, bed level height, sediment organic matter and silt content percentages (averages across all sampling dates). For all response variables, including the random effects improved significantly the model fit, as indicated by AIC values and likelihood ratio tests, after *P* values were corrected for testing on the boundary (Zuur et al. 2009). To account for heteroscedasticity, different residual variance structures were included in each model (Zuur et al. 2009). Comparisons based on AIC values revealed that the best models were those where the variance was allowed to differ between either blocks or habitats. Significant treatment effects were assessed through a stepwise backward elimination method, where nested models were compared through a likelihood ratio test (Diggle et al. 2002). Independence, normality and homogeneity of variance of residuals were confirmed by plotting relationships between residuals vs random factors and fitted values, and observed vs theoretical quantiles (QQ-plots), respectively. Where appropriate, multiple comparisons were performed and the familywise error rate was controlled with the Bonferroni correction.

    To explore the effect of hydrodynamic stress on cockle recruitment correlations between densities of cockle juvenile and plaster weight loss measured in the experimental plots (averages of all treatment combinations considered) and along transects (all replicates considered) were calculated using

Spearman's rank correlation coefficient. All statistical calculations were carried out in R (CRAN, R: A language and environment for statistical computing. 2010. R Foundation for Statistical computing. Vienna, Austria).

RESULTS

Cockle juveniles and engineering effects

Our experiment demonstrates that cockles enhanced the survival of their own recruits (main effect of Engineer addition: Chi-squared test statistic = 24.07, $p < 0.001$; Fig. 3.3A). The numbers of cockle juveniles in cockle-addition plots were on average 2 and 1.5 fold higher than in no-addition plots and lugworm-addition plots, respectively (post-hoc test, $p < 0.001$ for both comparisons). In the lee of the mussel beds cockle juveniles were overall between 4 and 5 times more abundant than at the sandy sites but abundances decreased in defaunated plots (interaction effect between Habitat and Defaunation: Chi-squared test statistic = 5.83, $p = 0.016$).

Bed level measurements showed that cockles enhanced sediment accretion, and this effect was stronger at the sandy sites than at the mussel bed sites (interaction effect between Engineer addition and Habitat: Likelihood Ratio test statistic = 6.78, $p = 0.034$; Fig. 3.3B and Fig. 3.4). Bed level in the cockle-addition plots raised by on average 2.10 ± 0.91 cm (mean ± SD; $n = 16$) over the summer (March – September 2011) at the sandy site, while no-addition plots and lugworm-addition plots raised only by on average 0.50 ± 0.60 cm (mean ± SD; $n = 16$) and 0.61 ± 0.35 cm (mean ± SD; $n = 16$), respectively. Defaunation reduced sediment accumulation by 61% overall (main effect of Defaunation: Likelihood Ratio test statistic = 26.73, $p < 0.001$;). The effects of defaunation were particularly clear at the mussel bed sites, where reduction of macrofauna resulted in a trend towards sediment erosion in both the no-addition and addition plots (Fig. 3.3B).

Measurements of silt and organic matter content in the sediment indicated that engineering of cockles and lugworms prevented the change of abiotic conditions in the lee of the mussel reefs caused by the reduction of biological activity. Coastward of mussel reefs, where silt and organic content were respectively more than 3 and 2 times higher than at the sandy sites, defaunation
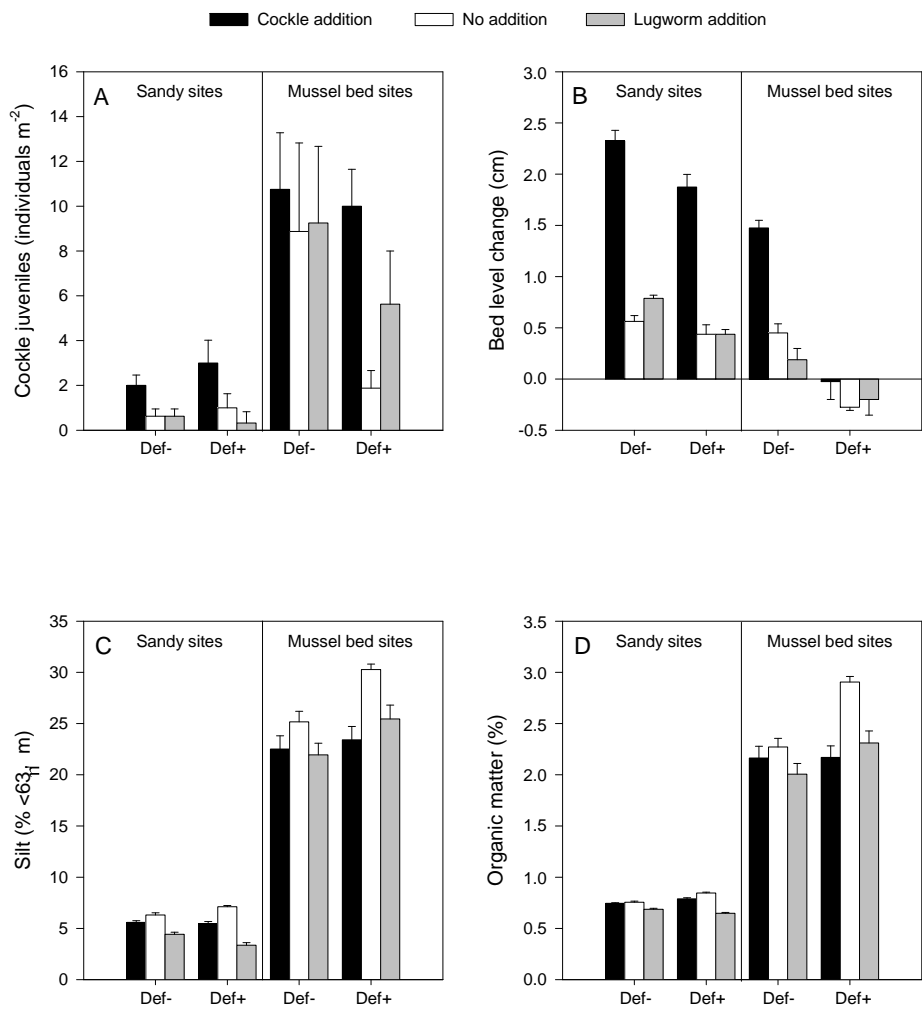
Fig. 3.3. (A) Cockle juvenile abundance, (B) bed level change, (C) silt content and (D) organic matter content measured in defaunated (Def+) and not-defaunated (Def-) cockle-addition, lugworm-addition and no-addition plots at sandy sites and mussel bed sites. Mean ± SE (n = 8).

Fig. 3.4. Photograph showing one block of twelve 5×5 m experimental plots at one of the sandy sites. Cockle-addition plots appear as dark squares, as they are more elevated and therefore dry sooner than the other plots and the surrounding.

led to further enrichment of silt content (interaction effect between Defaunation and Habitat: Likelihood Ratio test statistic = 9.36, p = 0.002; Fig. 3.3C) and organic matter content, which was significantly attenuated by both cockle and lugworm additions (interaction effect between Defaunation, Habitat and Engineer addition: Likelihood Ratio test statistic = 7.58, p = 0.023; Fig. 3.3D). At the sandy sites, the bioturbating activity of lugworms significantly reduced silt content (interaction effect between Habitat and Engineer addition: Likelihood Ratio test statistic = 7.68, p = 0.022; Fig. 3.3C) and organic matter content compared to the other treatments, although for the latter this effect was significant only in defaunated plots (interaction effect between Habitat, Engineer addition and Defaunation: Likelihood Ratio test statistic = 7.58, p = 0.023; Fig. 3.3D).

Hydrodynamic conditions and field surveys

Alleviation of hydrodynamic stress in the lee of mussel reefs was indicated by an overall 8% decrease in average plaster dissolution at the mussel bed sites compared to the sandy sites (main effect of Habitat: Likelihood Ratio test statistic = 5.02, p = 0.025), and is corroborated by other studies performed in the same area (Donadi et al. 2013a, 2013b). We found no evidence of experimental treatment effects on plaster dissolution in any of the sites.

Field transects data collected in a nearby tidal flat showed a negative relationship between cockle juvenile abundances and the erosive power of waves and currents (r_s = -0.71, n = 21, p < 0.001; Fig. 3.5A), suggesting that hydrodynamic stress was likely a major limiting factor for cockle recruitment. A similar negative relationship between numbers of cockle juveniles and plaster weight loss was mirrored in the averages from the experimental treatment combinations (r_s = -0.68, n = 12, p = 0.014; Fig. 3.5B). However, in defaunated plots coastward of the mussel beds where neither cockles nor lugworms were added, abundances of cockle juveniles were still very low despite reduced plaster dissolution, pointing to limiting factors other than hydrodynamic stress (Fig. 3.6).
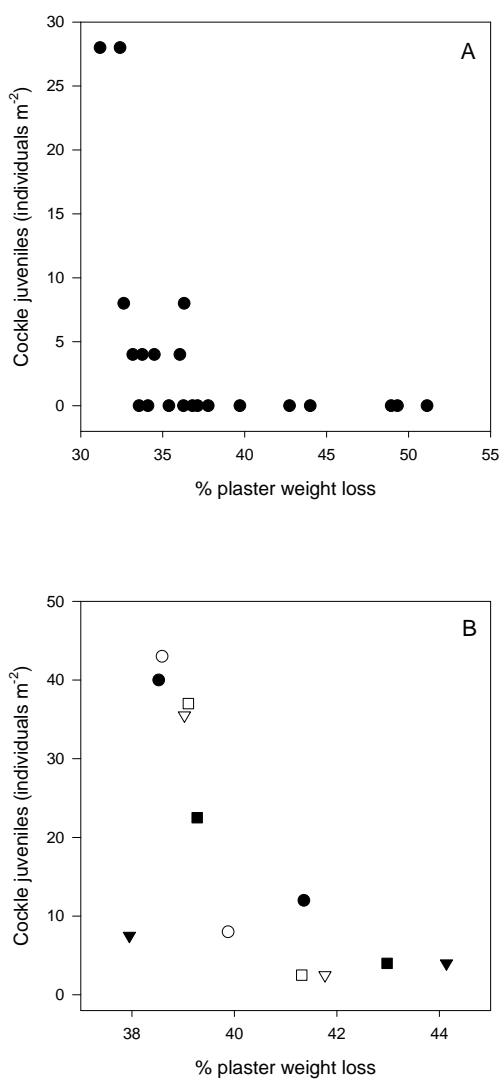
Fig. 3.5. (A) Scatter plot of cockle juvenile abundance and plaster weight loss measured along three transects perpendicular to the shore in a tidal flat with no mussel reefs (n = 21). (B) Scatter plot of means of cockle juvenile abundance and plaster weight loss (n = 8) measured in defaunated (black) and not-defaunated (white) cockle- addition (circles), lugworm- addition (squares) and no-addition plots (triangles).

3
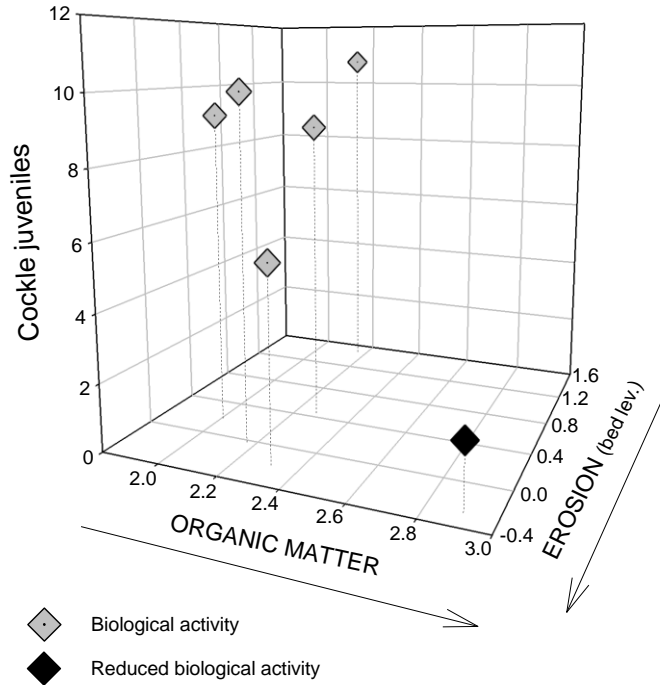
Fig. 3.6. Scatter plot showing how reduced biological activity in the defaunated no-addition plots (black) results in high stress along two environmental gradients and reduced cockle recruitment compared to the other treatments (gray). Values are averages of organic matter content (%), bed level change (cm) and cockle juvenile abundance (individuals m^{-2}) from different treatments at the mussel bed sites.

DISCUSSION

Our results show that positive feedbacks between cockles and habitat changes are fundamental for the survival of cockle populations on the tidal flats of the Wadden Sea. We found that the amount of cockle recruits was enhanced in the presence of high densities of adults and that this effect was due to the ability of cockles to increase sediment stability. Our findings bring new evidence of non-

linear dynamics in soft-bottom intertidal systems and have important implications for both the stability and management of the bivalve communities that live in these habitats.

To date, many shellfish populations worldwide have shown limited recovery following human overfishing (Jackson et al. 2001, Lotze et al. 2006). With our work we suggest that the lack of recovery of overexploited shellfish populations could largely be explained by the loss of positive feedbacks between engineering species and habitat changes. Many bivalve species show evidence of positive feedbacks mediated by habitat amelioration (Gutierrez et al. 2003). Aggregations of shell-producing organisms can provide protection from predation (Peterson and Black 1993, Dolmer 1998) or physical disturbance (Stiven and Gardner 1992), thus reducing mortality rates of conspecifics. Blue mussel reefs create substrata that facilitate the attachment and survival of mussel larvae (Bertness and Grosholz 1985, Largaespada et al. 2012), and alleviate hydrodynamic stress which enhances the recruitment of cockles (Donadi et al. 2013a, this study). A recent study on oyster restoration showed that spat recruitment correlated positively with density of adult oysters (Schulte et al. 2009), which facilitate spat settlement and provide refuge from predation (O'Beirn et al. 2000). These studies demonstrate that several bivalve species are co-operative ecosystem engineers, as their ability to thrive depends on the habitat modification promoted by many individuals. Therefore, there might be density thresholds below which recovery of shellfish populations is not possible. While evidence of positive feedbacks mediated by engineering exists for epibenthic and reef-forming bivalves, our results suggest that similar mechanisms are essential for the persistence and recovery of infaunal bivalves as well. The existence of such non-linear dynamics entails that depletion of bivalve stocks below the level necessary for habitat amelioration can result in further declines and slow recovery rates of target populations.

We found that cockle beds promoted cockle recruitment by increasing sediment stability. This is in contrast with previous evidence of negative effects of adult cockles on cockle larvae due to ingestion (e.g. André and Rosenberg 1991, Flach 1996). Indeed, among the variety of interactions demonstrated between adults and juveniles in soft-bottom communities (e.g. Woodin 1991; Olafsson et al. 1994; Thrush et al. 1996a), density-dependent inhibition of recruitment by adults has been frequently showed by both manipulative and monitoring studies

(e.g. Thrush et al. 1997; Turner et al. 1997). However, this apparent inconsistency could be explained by habitat characteristics (i.e. hydrodynamic conditions) interacting with local biotic processes (Thrush et al. 1996a, 2000) and the small spatial scale or low experimental density of previous studies (Hewitt et al. 1997), which might have prevented infaunal bivalves from displaying significant engineering effects. Recruitment on tidal flats can be strongly limited by water current velocities and sediment resuspension (Abelson and Denny 1997, Bouma et al. 2001, Jonsson et al. 2004), as supported by the negative correlation between cockle juvenile abundance and plaster weight loss observed at the study area. We found that at sandy sites characterized by rough hydrodynamic conditions, high densities of cockles caused a 2 cm increase in bed level compared to control plots during summer time. This effect was previously documented in the experimental plots and could be due to the joined effect of functional and structural traits of cockles (i.e. biodeposition and protruding shells from the substrate) and facilitation of sediment-binding diatoms mats (Donadi et al. 2013b). Hence, despite of the high erosive power of tidal currents and waves at the sandy sites, cockle recruitment was likely promoted by sediment stabilization caused by conspecifics. However, alternative mechanisms explaining positive adult-juvenile interactions in cockle beds, such as the release of detectable chemical cues from conspecifics (e.g. Butman et al. 1988; Woodin 1991) or different effects of predators (e.g. Watzin 1983), i.e. lower predation pressure in cockle-addition plots than in lugworm-addition and no-addition plots, cannot be dismissed. Also, our experiments were not able to distinguish between settlement and post-settlement survival, and further studies are needed to investigate the relative importance of settlement and post-settlement processes in explaining the observed patterns and corroborate the mechanisms involved.

Higher survival of cockle juveniles at the mussel bed sites is in agreement with previous evidence of enhanced cockle recruitment and settlement of macrozoobenthos larvae due to declining hydrodynamic stress in the lee of the mussel reefs (Widdows and Brinsley 2002, Commito et al. 2005, Donadi et al. 2013a). However, in defaunated plots coastward of mussel reefs, sediment erosion occurred in all treatments, likely impairing cockle recruitment. This might be surprising, as defaunation of soft bottoms often results in the increase of diatom abundance (Volkenborn et al 2007, Van Colen et al. 2010), which can enhance sediment stability through the formation of sediment-binding colonies at

the surface sediment (van de Koppel et al. 2001). However, the high water content observed in the mussel bed sites even during low tide (*pers. obs.*) might have hindered the production of extracellular polymeric substances (EPS), water-soluble colloidal carbohydrates excreted by diatoms that attach sediment particles (Blanchard et al. 2000; Weerman et al. 2010). Thus, coastward of mussel reefs, the lack of diatom mats combined with the alteration of the near-bed flow caused by the removal of animal tubes in defaunated plots might have exacerbated sediment erosion by waves and currents (Eckman 1983). Besides higher sediment instability, defaunated plots at the mussel bed sites where neither cockles nor lugworms had been added experienced a strong increase of organic matter and silt content. Decomposition of mussel biodeposits can increase sediment oxygen uptake, leading to anoxia and accumulation of sulphides (Dahlback and Gunnarsson 1981, Christensen et al. 2003), which in turn may negatively affect the abundance and biomass of many benthic species (Hyland et al. 2005, Callier et al. 2009). Therefore, the eradication of macrobenthos coastward of mussel reefs might have drastically reduced cockle recruitment due to a combination of sediment erosion and oxygen depletion caused by organic enrichment (Fig. 3.6). Our results suggest that cockles facilitated their own recruits by increasing sediment stability at the sandy sites and by alleviating the effects of organic enrichment at the mussel bed sites, thus acting as both sediment stabilizators (Andersen et al. 2010, Donadi et al. 2013b) and bioturbators (Flach 1996, Neumeier et al. 2006, Montserrat et al. 2009). Contrary to our hypothesis, lugworms did not affect abundance of cockle juveniles at the sandy sites, but they had positive effects on cockle recruits in defaunated plots coastward of mussel reefs. As for cockles, this is likely due to alleviation of anoxic stress through bioturbation (Banta et al. 1999, Norkko et al. 2012).

Our results imply that human activities that modify sediment conditions on large scales may severely impair the recovery of cockles and other shellfish species. Degradation of shells of living and dead bivalves due to dredging has been suggested to reduce the substrate available for settlement of blue mussels (Hiddink 2003). Likewise, the loss of fine silt after dredging events may have reduced the settlement of bivalve spat, causing an 8-year long decline of the stocks of cockles (*Cerastoderma edule*) and Balthic tellins (*Macoma balthica*) in the Wadden Sea (Piersma et al. 2001). With our work, we bring experimental evidence that sediment stabilization is an important factor for the successful

recruitment of cockles. We therefore argue that overexploitation of bivalve stocks combined with the changes in sediment properties and hydrodynamic conditions undergone by the Wadden Sea in the last century (Lotze et al. 2005, Reise 2005) have a role in explaining some of the long-term declines of shellfish populations (Eriksson et al. 2010, Kraan et al. 2011).

Positive feedbacks between environment and engineering species have been proposed to lead to the emergence of self-organized spatial heterogeneity (Hui et al. 2004, Rietkerk and van de Koppel 2008). In intertidal habitats, patterns in the distribution of mussels (van de Koppel et al. 2005), seagrass (van der Heide et al. 2010), diatoms (Weerman et al. 2010) and salt marsh vegetation (van Wesenbeeck et al. 2008) emerge from the tied interplays between organisms and abiotic conditions, which are crucial for the productivity and resilience of these communities (Bianchi et al. 1989, van de Koppel et al. 2001). Our results support that soft-bottom tidal communities are complex systems governed by non-linear dynamics where productivity and stability might depend on a high degree of self-organization. The incorporation of such knowledge into current management strategies can certainly aid the restoration and conservation of these threatened ecosystems.

Acknowledgements

Chapter 4

4

ENGINEERING NETWORKS STRUCTURE COASTAL ECOSYSTEMS THROUGH NICHE PARTITIONING

Serena Donadi, Tjisse van der Heide, Theunis Piersma, Els M. van der Zee,
Ellen J. Weerman, Johan van de Koppel, Han Olff, Clare Devine,
Udhi E. Hernawan, Myra Boers, Linda Planthof
& Britas Klemens Eriksson

ABSTRACT

By changing habitat conditions, ecosystem engineers increase niche diversity and have profound effects on the distribution and abundances of other organisms. Research on ecosystem engineering has mostly focused on effects of single dominating ecosystem engineers. However, many ecosystems contain multiple engineering species, whose effects may interact in complex ways to control habitat conditions and local communities. Based on a large-scale field experiment in an intertidal soft-sediment ecosystem, we here demonstrate that three intertidal engineers together increase habitat spatial heterogeneity at a landscape level by modifying the physical environment along different abiotic gradients, thereby leading to niche space partitioning. By using biological trait analysis (BTA), we show that changes in sediment properties caused by the identified three-species engineering network translate into changes in the distribution of functional traits within the macrobenthic community. Our results emphasize the need to incorporate engineering networks into classical ecological research to better understand community assembly rules. In practical terms, this knowledge can improve conservation and management efforts.

4

INTRODUCTION

Ecosystem engineers are organisms that have profound effects on the environment and the associated communities (Jones et al. 1994, Hastings et al. 2007, Olff et al. 2009). By modifying abiotic conditions, ecosystem engineers alter the amount of space that meets the requirements of the species fundamental niches, thus facilitating certain species while inhibiting others (Bruno et al. 2003). At the spatial scale of the engineered habitat, ecosystem engineering typically causes a shift in the community composition, which, depending on the engineer and the local conditions, may either increase or decrease community diversity (Crooks 2002). At a landscape level, however, ecosystem engineering often enhances environmental heterogeneity and niche partitioning, thereby increasing the total number of ecological niches and eventually the diversity of the community (Jones et al. 1997, Wright et al. 2002, Erwin 2005).

In the last few decades, studies from a wide range of ecosystems have demonstrated strong effects of a single engineering species on the local community structure and ecosystem properties (e.g. Wright and Jones 2006, Hastings et al. 2007 and references therein). Although these studies have been an essential step forward in our understanding of how ecosystem engineering affects communities, they may poorly describe the inherent complexity of real ecosystems. Natural ecosystems may host multiple engineering species that synergistically or antagonistically interact at various spatial scales, thereby leading to different effects from those of single engineering species (Davidson and Lightfoot 2006, Baiser et al. 2008, Eklof et al. 2011, Dijkstra et al. 2012). Thus, at present, our understanding of the importance of ecosystem engineering is limited by the fact that very few studies acknowledge interaction networks of engineering species and their impacts on ecosystem properties (Jones et al. 1997, Olff et al. 2009).

Here, we focus on a three-species engineering network in a soft-sediment intertidal ecosystem consisting of three well-known engineers: cockles (*Cerastoderma edule* L.), lugworms (*Arenicola marina* L.), and blue mussels (*Mytilus edulis* L.). Whereas previous research has mainly investigated effects of each of these three ecosystem engineers individually (e.g. Gunther 1996, Volkenborn and Reise 2007, Jones and Frid 2009), we tested how these species

interactively alter their environment across space and how the resulting modulated niche space affects the community composition. For this purpose, we set up a large-scale field experiment where we manipulated a total of 2 400 m^2 of intertidal mudflat by adding 800 000 cockles and 64 000 lugworms to different 25-m^2 plots. Rather than analyzing effects on species composition, we examined how the engineering network affected the functional trait diversity of the community. Functional traits of species are related to life-history attributes that are specifically sorted by the environment (Bremner et al. 2006). Hence, compared to species composition, the investigation of the community's functional composition can provide more direct insights into the underlying mechanisms by which ecosystem engineers affect the community. Nevertheless, very few studies used trait-based approaches to explore ecosystem engineering effects (Volkenborn and Reise 2007, Cesar and Frid 2009). Our results not only aid the general understanding of the importance of engineering networks in shaping natural communities, but can also contribute to improving ecosystem-based management and conservation actions.

STUDY SYSTEM AND METHODS

Study system

Our experiments were conducted on the intertidal flats of the island of Schiermonnikoog, in the Dutch Wadden Sea, one of the world's largest intertidal soft-sediment ecosystems (Compton et al. 2013). On these tidal flats, cockles and lugworms commonly co-exist at the local scale (<0.1 m), while mussels typically form large (>1 ha) aggregations, which cause long (several hundred meters) spatial gradients in sediment composition (van der Zee et al. 2012, Donadi et al. 2013a). Lugworms occur with relatively stable densities of 20-40 individuals m^{-2}, while cockle abundances vary widely from zero to several hundred individuals m^{-2} (Beukema and Devlas 1979, Jensen 1992, pers.obs.).

The lugworm *A. marina* is a sediment-destabilizer polychaete that promotes erosion of fine material through bioturbation and increases oxygen penetration in the soil by flushing its burrows (Banta et al. 1999, Volkenborn et al. 2007, Kristensen et al. 2012). On the opposite, blue mussels (*M. edulis*) can enhance sediment stability at large spatial scales by forming dense aggregations that

provide shelter from the tidal currents (Donadi et al. 2013a) and produce big amount of faeces and pseudofaeces (Kroncke 1996). The filter-feeder cockle (*C. edule*) has been mainly described as bioturbator (Flach 1996, Neumeier et al. 2006, Ciutat et al. 2007, Montserrat et al. 2009). However, recent studies suggest that high densities of cockles can locally increase sediment stability through biodeposition and mucus-production (Ciutat et al. 2006, Andersen et al. 2010, Donadi et al. 2013b). Through habitat alteration, all these three species affect the community composition (Volkenborn and Reise 2007, Cesar and Frid 2009, van der Zee et al. 2012). We hypothesize that by affecting sediment stability in opposite directions and at different spatial scales, cockles, lugworms and mussels together will increase environmental heterogeneity and therefore niche diversity, thus favoring niche partitioning among different functional groups of macrofauna (Fig. 4.1). More specifically, we predict that certain traits such as small body-size and shallow living-depth will be promoted in more stable sediments, which are less vulnerable to erosion, while larger and deep-living species will be best adapted to habitats with low sediment stability, where oxygen availability is typically higher.

Experimental treatments

To test the effects of our three-species engineering network on both environmental conditions and the associated community of macrofauna, we created plots with cockle-dominated communities and lugworm-dominated communities in a randomized block design within the pseudofaeces plumes of two mussel beds (distance to the reef was 100 and 350 m, 53°28'8.15"N, 6°13'27.95"E; 53°28'2.44"N, 6°10'59.83"E) and at two, unaffected sandy sites without mussel beds at the same tidal elevation (53°28'7.42"N, 6°13'55.95"E; 53°28'7.54"N, 6°11'33.48"E). Each site was assigned two blocks of twelve 5×5 m plots for a total of 96 plots. In June 2010, one-third of the plots was enriched with high densities of cockles (1000 individuals m^{-2}), one-third with high densities of lugworms (80 individuals m^{-2}) and the remaining plots served as a control (no addition; Fig. 4.2). Treatment densities of cockles and lugworms were chosen to mimic naturally occurring communities dominated by either species (Beukema 1976). Cockles were collected by fishermen from a nearby intertidal flat and were 2 to 4 years old. Lugworms were harvested by a commercial lugworm fisheries

A) NO ECOSYSTEM ENGINEERS

Sediment stability

B) ENGINEERING NETWORKS

Deep-living species   Shallow-living species

Larger species   Smaller species

Sediment stability

Blue mussel reefs - - - - - - - - - - - - ▸

- - - - - - - - - - - - ▸ Cockles ◂ - - - - - - - - - - - - -
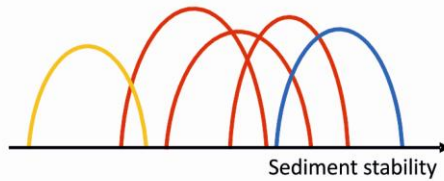
◂ - Lugworms
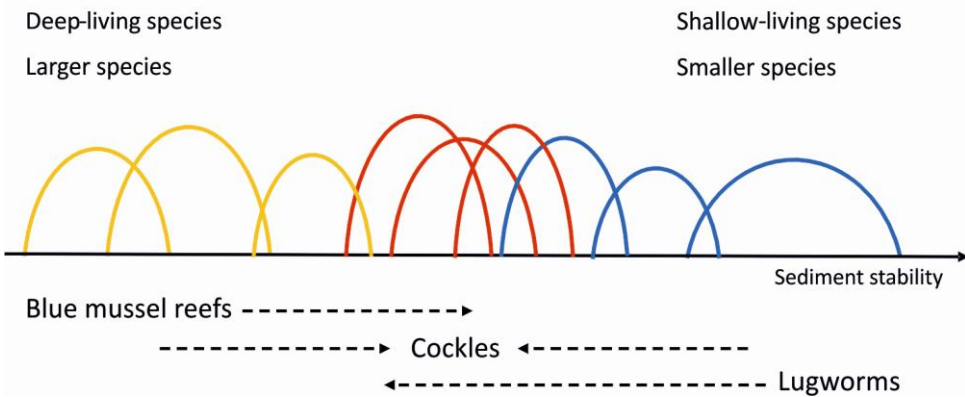
Fig. 4.1. Conceptual model illustrating different scenarios: (A) When sediment stability is determined by external forcing only and is not affected by ecosystem engineering, fewer niches are available (different colors indicate different functional groups). (B) In an engineering network, multiple ecosystem engineers interact to increase the number of niches available and lead to niche partitioning. By affecting sediment stability in different directions (see text), cockles, lugworms and mussels promote the segregation of different functional groups of macrofauna.

company (Arenicola BV, Oosterend, The Netherlands) from an intertidal flat in the Western Wadden Sea.

Abundance of transplanted specimens was monitored every six months by counting cockles (one replicate per plot) and lugworm fecal casts, as a measure for lugworm abundance (three replicates per plot), within a 0.5×0.5 m frame. Abundances of both cockles and lugworms generally decreased after the transplantations but remained considerably higher in enriched plots compared to

the other treatments, the only exception being lugworm abundances at the end of the experiment in September 2011, when there was no difference between cockle and lugworm addition plots (Appendix 4A).
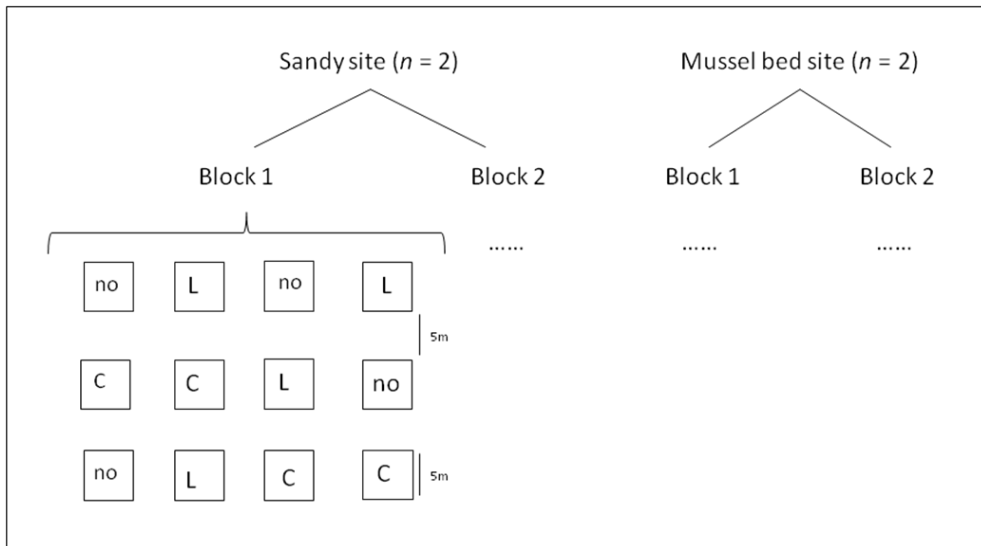
Fig. 4.2. Diagram of the experimental design. In each of two mussel bed sites (located coastward of mussel reefs) and two sandy sites without mussels twenty-four 5×5 m plots were allocated in two blocks. Within each block, the plots were randomly assigned to one of three treatments: cockle addition (C), lugworm addition (L) and no addition (no) (n = 4 per block, 96 plots in total).

Field sampling and laboratory analyses

Macrofauna. In September 2011 (after 16 months since the start of the experiments), sediment cores (diameter = 15 cm, depth = 20-25 cm) were extracted from the center of each plot and sieved over a 1 mm mesh. In the lab, macrofauna was fixated in 10 % formalin and stained with Rose Bengal Red. All organisms were identified (mostly to species level) under a 10× magnification stereo and the dry biomass of each taxon was measured after drying for 48 h in an oven at 60 °C. In total we collected 14084 individuals belonging to 29 taxa.

Sediment properties. Sediment samples were collected from each plot in September 2011 through cut disposal syringes (diameter = 2.4 cm, depth = 0-5 cm) and organic matter content was estimated from oven-dried (48 h, 75 °C) samples as Loss On Ignition (LOI; 4 h, 550 °C). Bed level height was estimated in March 2010 and September 2011 using a Trimble Spectra Precision LL500 Laser Level (Trimble, California, USA). At each site, measurements were calibrated against fixed reference metal poles. Five replicates per plot were collected and averaged and the differences between the two dates were used to estimate bed level changes over the course of the summer, when bioengineering activity of cockles and lugworms is most conspicuous. Positive values of bed level changes indicate net sediment accumulation while negative values indicate net sediment erosion.

Data analysis

We applied Biological Trait Analysis (BTA) to detect changes in the pattern of trait expression caused by the engineering effect of cockles, lugworms and mussels. BTA uses life history, and behavioral and morphological characteristics of the species to describe the ecological functioning of an assemblage (Bremner et al. 2006). Five biological traits were selected and each trait was divided into several categories, following Jones and Frid (2009) (Table 1). Individual taxa were scored for the extent to which they display the trait category using a 'fuzzy coding' procedure (Chevenet et al. 1994), which allowed taxa to exhibit trait categories to different degrees. Traits were scored from 0 to 3, with 0 being no affinity and 3 being total affinity to a trait category (Appendix 4B). When trait information for a taxon could not be obtained (in only 3% of cases), 0 scores were used for each category and hence did not influence the analysis (Chevenet et al. 1994). Fuzzy scores were standardized in such a way that within each trait, the category scores for each taxon summed to one. Information on biological traits was obtained from published peer-reviewed literature and online databases for European marine invertebrates. *Cerastoderma edule* and *Arenicola marina* were excluded from the analysis, as they were experimentally manipulated.

Trait category scores per taxon were multiplied by the biomass of each taxon for every sample and subsequently summed across all taxa to provide a sample-by-trait matrix containing a total score for each functional category and

Table 4.1. Functional traits and categories used in the fuzzy coding of taxa (adapted from Jones and Frid 2009).

Trait	Category	Abbreviation
Bioturbation	none	none
	biodiffuser	biodif
	gallery diffuser	gall
	upward-conveyor	upw
	downward-conveyor	down
	bio-irrigator	bioirr
Depth	surface	surf
	0 – 3 cm	d0_3
	3 – 8 cm	d3_8
	8 – 15 cm	d8_15
	15 – 25 cm	d15_25
	> 25 cm	d>25
Body size (mm)	< 5	s<5
	5 < > 10	s5_10
	10 < > 20	s10_20
	20 < > 40	s20_40
	40 < > 80	s40_80
	> 80	s>80
Feeding mode	deposit-feeder	dep
	suspension-feeder	susp
	opportunist/scavenger	opp
	grazer	graz
	predator	pred
Longevity	< 1 year	<1y
	1 – 2 years	1_2y
	3 – 5 years	3_5y
	6 – 10 years	6_10y
	> 10 years	>10y

for each sample (Charvet et al. 1998). This sample-by-trait matrix, containing a total of 96 samples and 28 trait categories, was analyzed using Principal Component Analyses (PCA) in Canoco for Windows version 4.55 (ter Braak and

Smilauer 2002). Gradient lengths were first estimated in a detrended correspondence analysis (DCA). As the lengths of DCA axes 1 and 2 were both lower than 3, we used PCA (ter Braak and Smilauer 2002). Matrix data were square-rooted and centered by species prior to ordination (covariance-based PCA).

To explore whether cockles, lugworms and mussels interactively affected the structure of the community and the environmental conditions, a linear mixed-model including the fixed factors Engineer addition (three levels: cockle addition, lugworm addition, no addition), Habitat (two levels: sandy sites, mussel bed sites) and their interaction was used to explain variation in the sample scores of the first and second principal component axis, and in the values of sediment organic matter content and bed level change. A two-nested random-effect structure, taking into account sites (four levels) and blocks (eight levels) within a site, significantly improved the model fit, as indicated by AIC values and likelihood ratio tests, after *P* values were corrected for testing on the boundary (Zuur et al. 2009). To account for heteroscedasticity, a residual variance structure was included in the models (Zuur et al. 2009). Comparisons based on AIC values indicated that the best models were those where the variance was allowed to differ between sites. Significant treatment effects were assessed through a stepwise backward elimination method, where nested models were compared through a likelihood ratio test (Diggle et al. 2002). Independence, normality and homogeneity of variance of residuals were confirmed by plotting relationships between residuals vs random factors and fitted values, and observed vs theoretical quantiles (QQ-plots), respectively. When either fixed factors or their interaction was found significant, multiple comparisons were performed and the familywise error rate was controlled with the Bonferroni correction.

To explore the relationship between functional traits and sediment properties, values of organic matter content and bed level change for each plot were weighted by the scores of the functional categories to give weighted averages for each functional category. Weighted averages therefore indicate sediment conditions at which each category was observed most frequently. Standard errors of weighted averages were calculated following Cochran (1977) as suggested by Gatz and Smith (1995). All statistical calculations were carried out in R (CRAN, R: A language and environment for statistical computing. 2010. R Foundation for Statistical computing. Vienna, Austria).

RESULTS

Macrofauna. Cockles, lugworms and mussels had significant joint effects on the functional trait distribution of the macrofauna (Fig. 4.3A & B). The first two PCA axes explained 66% of the total variance, with 40% of variance across principal component axis 1 (PC1) and 26% across axis 2 (PC2). Along PC1, cockles and lugworms had contrasting effects on the functional trait composition behind the mussel beds, while they did not have any effects at the sandy sites (significant interaction effects between Engineer addition and Habitat; Likelihood Ratio test statistic = 10.42, P = 0.006; post-hoc test, cockle addition vs lugworm addition at the mussel bed sites, *P* = 0.0207; Fig. 4.3A). Along PC2, cockle-addition plots significantly separated from lugworm-addition plots (main effect of Engineer addition: Likelihood Ratio test statistic = 9.07, *P* = 0.011; post-hoc test, *P* = 0.019; Fig. 4.3B) and no addition plots (post-hoc test, *P* = 0.038; Fig. 4.3B), both behind the mussel beds and on the sand. These results suggest that interactive rather than additive effects of multiple ecosystem engineers determine the functional composition of the local macrobenthic communities.

Sediment properties. Cockles, lugworms and mussels affected sediment organic matter content in different directions and with different intensities (interaction effect between Engineer addition and Habitat: Likelihood Ratio test statistic = 10.26, *P* = 0.006; Fig. 4.3C). Coastward of mussel reefs both cockles and lugworms decreased percentage of organic matter compared to no-addition plots (post-hoc test, *P* = 0.002 and *P* < 0.001, respectively), where organic matter content was 3 times higher than at the sandy sites (post-hoc test, *P* = 0.017; Fig. 4.3C). At the sandy sites a significant reduction in organic matter content was observed only in lugworm-addition plots relative to no-addition plots (post-hoc test, *P* < 0.001; Fig. 4.3C).

Bed level measurements indicated that the cockles induced sediment accumulation (main effect of Engineer addition: Likelihood Ratio test statistic = 52.75, *P*< 0.001; Fig. 4.3D). In September 2011 bed level was on average 1.41 ± 0.23 cm (mean ± SE; n = 32) higher than winter levels in cockle-addition plots, while in no-addition and lugworm- addition plots bed level raised on average only 0.29 ± 0.11 cm (mean ± SE; n = 32) and 0.30 ± 0.15 cm (mean ± SE; n = 32), respectively.

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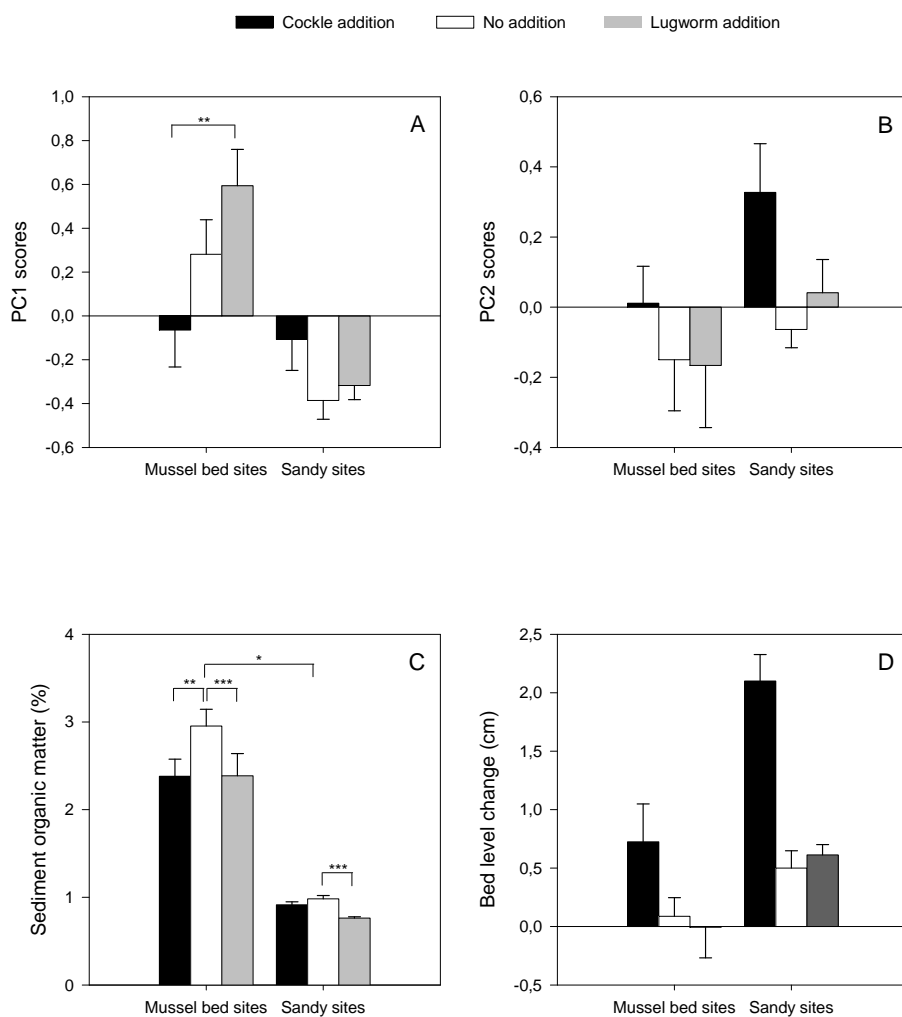
Fig. 4.3. (A) PC1 scores, (B) PC2 scores, (C) sediment organic matter content, and (D) bed level change for cockle-addition, lugworm- addition and no-addition plots in sites located coastward of mussel reefs and sandy sites without mussels. Mean ± SE (n = 16). Post hoc analysis: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Relationship between functional traits and sediment properties. Combined engineering effects of cockles, lugworms and mussels resulted in niche partitioning among different functional groups of macrofauna. Averages of organic matter content and bed level change weighted on the scores of each functional category (see Methods) showed that the functional categories were separated in a bi-dimensional space defined by gradients of organic matter content and bed level change created by the joint effects of cockles, lugworms and mussels (Fig. 4.4). Functional categories related to the trait "body size" sorted in such a way that small species occurred predominately at high levels of sediment accumulation, while larger species preferred sediments enriched with organic matter (Fig. 4.4A). Accordingly, due to collinearity among functional categories (Appendix 4C), habitats with high rates of sediment accumulation typically hosted shallow-living species, suspension feeders, biodiffusers, and organisms with a relatively long life-span, whereas sediments enriched with organic matter were mainly inhabited by deep-living species, grazers, downward conveyors, bioirrigators, and organisms with a relatively short life-span (≤ 2 years) (Appendix 4D). Our results show that changes in organic matter content and bed level stability caused by the combination of species-specific engineering effects (Fig. 4.4B) translated to changes in the distribution of functional traits within the macrobenthic community (Fig. 4.4A, Appendix 4D).

4

4
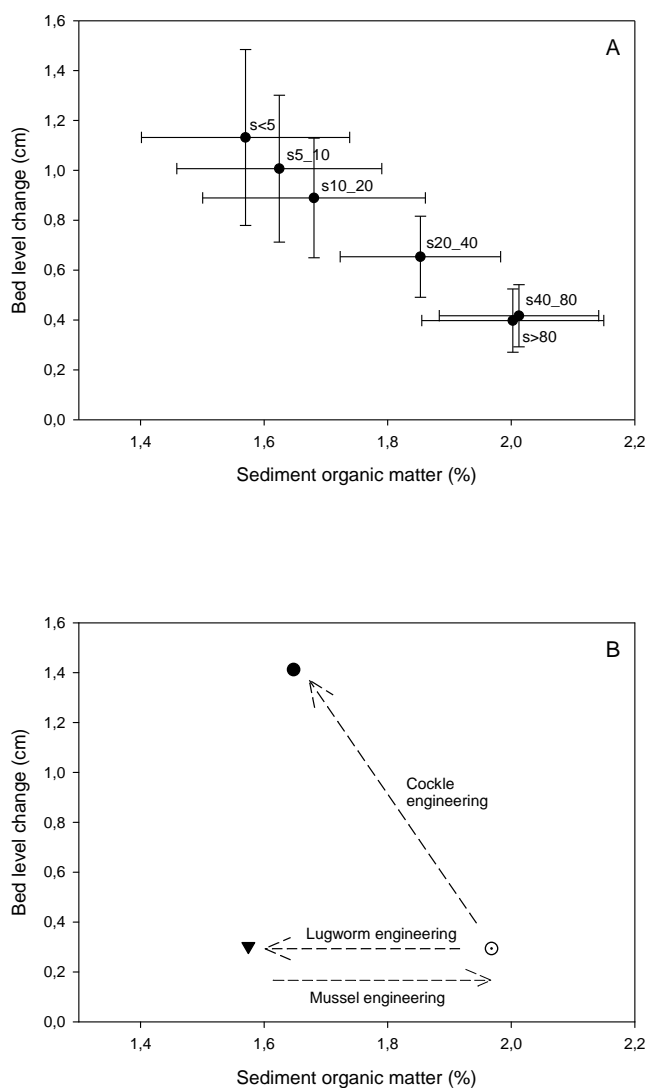
Fig. 4.4. Different functional categories are associated to different sediment conditions determined by the joint engineering effects of cockles, lugworms and mussels. (A) Scatter plot of weighted averages of sediment organic matter content and bed level change for each category of the functional trait "body size" (see abbr. in Table 1). (B) Averages of organic matter content and bed level change in cockle-addition plots (black dot), lugworm-addition plots (triangle) and no-addition plots (white dot). Arrows indicate the species-specific engineering effects of cockles, lugworms and mussels.

DISCUSSION

We found that a three-species engineering network determined the structure of the macrobenthic community at a landscape scale on an intertidal flat. By modifying sediment organic matter content and stability in opposite directions and at different spatial scales, cockles, mussels and lugworms together increased habitat heterogeneity, which led to niche partitioning among different functional groups of the macrobenthic community.

Our results demonstrate that multiple engineering species can contribute simultaneously to the structuring of ecosystems. Although our large-scale experiment merely provides one example of such a network, it is very likely that similar engineering networks shape the community in other types of ecosystems as well. For example, in grassland systems the interaction between engineering effects of prairie dogs *Cynomys ludovicianus* and kangaroo rats *Dipodomys spectabilis* greatly enhanced landscape heterogeneity and biodiversity (Davidson and Lightfoot 2006). In southern California, the co-occurrence of two epibenthic engineers, the snail *Cerithidea californica* and the crab *Pachygrapsus crassipes*, determined the structure and functioning of *Salicornia* marshes (Boyer and Fong 2005). In intertidal soft-sediment systems several studies reported on individual effects of cockles, lugworms and mussels on the surrounding habitat and communities (i.e. Flach 1996, Widdows et al. 2000, Volkenborn and Reise 2007, Commito et al. 2008, Ysebaert et al. 2009). However, our results suggest that these species act synergistically to shape the environment and their combined effects are not necessarily predictable from simple additive rules. For example, cockles are primarily known as bioturbators, which mobilize fine material from the sediment thereby decreasing sediment stability (Flach 1996, Montserrat et al. 2009). Indeed, we found that cockles significantly reduced organic matter content in the biodeposition plume of the mussel beds. However, in sandy areas out of the influence range of the reefs, cockles instead increased sediment accumulation, thus acting as sediment stabilizators. This illustrates the strong context-dependence of the engineering effects of cockles in our system. Overall, our results emphasize the need for an integrative approach that incorporates parallel networks of non-trophic relationships such as ecosystem engineering into classic community ecology (Ings et al. 2009, Olff et al. 2009, Kefi et al. 2012).

Our results suggest that ecosystem engineers by affecting sediment stability and by modulating availability of resources structure the macrobenthic intertidal communities through a size-based selection. We found that small-sized species (i.e. *Aphelochaeta marioni*, *Capitella capitata*, *Urothoe poseidonis*, Appendix 4B) were preferably associated to habitats with higher sediment accumulation, caused by the engineering effects of cockles. This suggests that cockles facilitated small-sized species by decreasing sediment erosion, which likely prevented the resuspension and transport of small organisms from the surface sediments by waves and tidal currents (see Donadi et al. 2013b). Contrary to our expectations, big-sized species, such as *Scrobicularia plana*, *Hediste diversicolor*, *Lanice conchilega* (Appendix 4B), were mainly found in sediments with high organic matter content. Many of these species are primarily deposit-feeders and may be favored by the large amount of organic matter in the sediment, indicating a possible facilitation effect by mussels through the production of biodeposits. Hence, we suggest that the observed pattern is the result of a size-driven tradeoff between the nutritional requirements of organisms and their ability to anchor themselves in sediments to resist dragging forces of the water currents. However, an alternative explanation could be that competition for space prevented large species from settling in cockle-dominated communities. Also, the engineered habitat might have selected on functional traits that covary with body size (de Roos and Persson 2013). For example, shallow-living species could have been promoted by the sediment stabilization caused by cockles. Nevertheless, the observed sorting of different body-size groups from small-sized to big-sized organisms along gradients of sediment stability and food availability strongly suggests that body size is a good indicator for the spatial structuring of the macrobenthic community on the intertidal flats.

Up to now, analysis of functional traits has been mainly used by plant ecologists to investigate how changes in biodiversity translate to changes in ecosystem functioning (see Diaz and Cabido 2001 and references therein). In marine systems, few studies applied BTA to assess the anthropogenic impacts on the community (i.e. fishing, Tillin et al. 2006, Kenchington et al. 2007, Cesar and Frid 2009) or for monitoring and management purposes (Bremner 2008, Frid et al. 2008, van der Linden et al. 2012). Nevertheless, hardly any study used trait-based approaches to explore ecosystem engineering effects (Volkenborn and Reise 2007, Cesar and Frid 2009). Our study shows that the analysis of functional traits

can provide strong insights into the underlying mechanisms of facilitation and inhibition by which ecosystem engineers control the community. Furthermore, understanding how the distribution of functional traits changes within the assemblages under the influence of engineering species can improve our ability to predict impacts of human activities that affect ecosystem engineer populations.

In summary, we show that an engineering network composed by cockles, mussels and lugworms determines the large-scale structure of an intertidal macrobenthic community. Given that ecosystem engineering is a common and an important type of biotic interactions in ecosystems worldwide, we suggest that the occurrence of engineering networks could be a rule rather than an exception. Hence, it is essential to identify the players in such networks and their net effects if we wish to understand, preserve and restore natural ecosystems.

Acknowledgements

APPENDICES

Appendix 4A. Cockle and lugworm abundances in the experimental plots over one year.

Fig. 4A. Cockle abundances (individuals/m^2) and lugworm abundances (fecal casts/m^2) in cockle-addition, lugworm- addition and no-addition plots measured in mussel bed sites and sandy sites in September 2010, March 2011 and September 2011. Mean ± SE (n = 16 and n = 48 for cockle and lugworm abundances, respectively).

Appendix 4B. Fuzzy scores for each trait category and taxon.

| Taxon	Bioturbation none	biodif	gall	upw	down	bioirr	Depth surf	d0_3	d3_8	d8_15	d15_25	d>25	Body size s<5	s5_10	s10_20	s20_40	s40_80	s>80	Feeding mode dep	susp	opp	graz	pred	Longevity <1y	1_2y	3_5y	6_10y	>10y
Ampharete acutifrons	0	0	0	0	3	0	2	1	0	0	0	0	0	0	2	1	0	0	2.5	0.5	0	0	0	0	2.5	0.5	0	0
Aphelochaeta marioni	0	0	0	0	3	0	0	2.75	0.25	0	0	0	0	1.5	1.5	0	0	0	2	0.5	0	0.5	0	0	1	1	1	0
Balanus spp.	3	0	0	0	0	0	3	0	0	0	0	0	0.5	2	0.5	0	0	0	0	3	0	0	0	0	3	0	0	0
Capitella capitata	0	0.5	0	2	0	0.5	0	1.5	1.5	0	0	0	0	3	0	0	0	0	3	0	0	0	0	1	2	0	0	0
Carcinus maenas	3	0	0	0	0	0	3	0	0	0	0	0	0	0	1.5	1	0.5	0	0	0	1	0	2	0	0	1.5	1.5	0
Corophium spp.	0	1.5	0	0	1.5	0	0	3	0	0	0	0	1	2	0	0	0	0	2.5	0.5	0	0	0	3	0	0	0	0
Crangon crangon	0	3	0	0	0	0	1	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	0	0	3	0	0
Eteone spp.	0	3	0	0	0	0	1.5	1.5	0	0	0	0	0	1.5	1	0.5	0	0	0.25	0	0	0	2.75	0	3	0	0	0
Gammarus spp.	3	0	0	0	0	0	3	0	0	0	0	0	0	1	2	0	0	0	2	0	0	1	0	3	0	0	0	0
Harmothoe spp.	3	0	0	0	0	0	3	0	0	0	0	0	0	0.75	2	0.25	0	0	0	0	0	0	3	0	1	2	0	0
Hediste diversicolor	0	0	3	0	0	0	0	0	0	1	2	0	0	0	0	1	1	1	1.5	0.5	0.5	0	0.5	1	2	0	0	0
Heteromastus filiformis	0	0	0	2	0	1	0	0	0	0	3	0	0	0	3	0	0	0	3	0	0	0	0	1	2	0	0	0
Hydrobia ulvae	2	1	0	0	0	0	2	1	0	0	0	0	2	1	0	0	0	0	3	0	0	0	0	0	3	0	0	0
Lagis koreni	0	0	0	0	0	3	0	1.5	1.5	0	0	0	0	0	1	2	0	0	2.5	0	0.5	0	0	3	0	0	0	0
Lanice conchilega	0	0	0	0	0	3	0	0	0	3	0	0	0	0	0	0.5	1.5	1	2.5	0.5	0	0	0	0.25	1	1.75	0	0
Littorina littorea	3	0	0	0	0	0	3	0	0	0	0	0	0	0	1	2	0	0	0	0	0	3	0	0	0	0.25	1.75	0
Macoma balthica	0	3	0	0	0	0	0	1.3	1.3	0.2	0.2	0	0.5	1	1	0.5	0	0	1.5	1.5	0	0	0	0	0	0	3	0
Magelona mirabilis	0	1.5	0	0	1.5	0	0	3	0	0	0	0	0	1.5	1.5	0	0	0	2.5	0.25	0	0	0.25	0	0	3	0	0
Marenzelleria viridis	0	2	0	0	1	0	0	0	3	0	0	0	0	0	3	0	0	0	1	1	0	1	0	0	3	0	0	0
Microphthalmus similis	0	0	0	0	0	0	1.5	1.5	0	0	0	0	0	1.5	1.5	0	0	0	1	0	1	1	0	0	0	0	0	0
Mya arenaria	0	3	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1.5	1.5	0	3	0	0	0	0	0	0	0	3
Mytilus edulis	3	0	0	0	0	0	3	0	0	0	0	0	0	1	2	0	0	0	0	3	0	0	0	0	0	0	0	3
Nemertea spp.	3	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1.5	1.5	0	0	0	0.5	0	2.5	0	0	0	0	0
Oligochaeta spp.	0	3	0	0	0	0	0	2.5	0.5	0	0	0	0	0	1	2	0	0	1.5	0	0	1.5	0	0	1	2	0	0
Phyllodoce spp.	1.5	1.5	0	0	0	0	1.5	0.5	0.5	0.5	0	0	0	0	0	0	2	1	0	0	2.5	0	0.5	0	0	0	0	0
Polydora spp.	2	0	0	0	0	1	0	3	0	0	0	0	0	0.5	2	0.5	0	0	0.75	2	0	0.25	0	0	3	0	0	0
Polyplacophora spp.	3	0	0	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	1.5	0	0	1.5	0	0	0	0	0	0
Scolelepis bonnieri	0	0	0	3	0	0	0	1	1	1	0	0	0	0	0	2.5	0.5	0	2	0.3	0	0.7	0	0	3	0	0	0
Scoloplos armiger	0	0	0	3	0	0	0	1	1	1	0	0	0	0	0.25	0.75	1.5	0.5	3	0	0	0	0	0	0	3	0	0
Scrobicularia plana	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0
Spionida spp.	0	1	0	0	2	0	0	2	0.5	0.5	0	0	0.17	1	2	0	0	0	2	1	0	0	0	0	3	0	0	0
Streblospio benedicti	0	1	0	0	2	0	0	3	0	0	0	0	0.5	2.5	0	0	0	0	2	0.75	0	0.25	0	0	3	0	0	0
Urothoe poseidonis	2	1	0	0	0	0	0	0	0	0.5	1	1.5	2.5	0.5	0	0	0	0	3	0	0	0	0	0	3	0	0	0

Appendix 4C. Spearman correlation coefficients for each pair of functional trait categories.

	none	biodif	gall	upw	down	bioirr	surf	d0_3	d3_8	d8_15	d15_25	d>25	s<5	s5_10	s10_20	s20_40	s40_80	s>80	dep	susp	opp	graz	pred	<1y	1_2y	3_5y	6_10y
none	1.00																										
biodif	0.06	1.00																									
gall	-0.32	-0.27	1.00																								
upw	-0.23	-0.31	0.11	1.00																							
down	0.13	0.31	-0.22	-0.44	1.00																						
bioirr	0.09	0.15	0.12	-0.42	0.56	1.00																					
surf	0.79	0.07	-0.23	-0.40	0.40	0.34	1.00																				
d0_3	0.01	0.89	-0.27	-0.10	0.42	0.15	0.07	1.00																			
d3_8	-0.02	0.87	-0.23	0.07	0.13	0.00	-0.06	0.92	1.00																		
d8_15	-0.10	0.10	0.54	-0.15	0.11	0.62	0.08	0.15	0.12	1.00																	
d15_25	-0.29	0.05	0.85	-0.04	-0.06	0.18	-0.21	0.01	-0.01	0.57	1.00																
d>25	0.00	-0.21	-0.10	0.71	-0.49	-0.53	-0.34	-0.14	0.04	-0.31	-0.17	1.00															
s<5	0.01	0.93	-0.27	-0.11	0.17	0.02	-0.07	0.87	0.93	0.03	-0.02	0.02	1.00														
s5_10	-0.02	0.91	-0.20	-0.22	0.42	0.19	0.04	0.92	0.85	0.12	0.07	-0.21	0.89	1.00													
s10_20	0.08	0.87	-0.19	-0.25	0.49	0.23	0.18	0.90	0.79	0.13	0.09	-0.27	0.81	0.95	1.00												
s20_40	-0.09	0.33	0.59	-0.20	0.11	0.37	0.04	0.35	0.33	0.83	0.73	-0.31	0.26	0.36	0.39	1.00											
s40_80	0.06	-0.11	0.53	-0.09	0.07	0.53	0.22	-0.07	-0.09	0.86	0.58	-0.24	-0.21	-0.13	-0.07	0.71	1.00										
s>80	-0.13	-0.15	0.72	-0.10	0.03	0.54	0.07	-0.12	-0.12	0.92	0.65	-0.24	-0.20	-0.11	-0.08	0.74	0.88	1.00									
dep	-0.13	0.49	0.34	-0.10	0.23	0.42	-0.01	0.59	0.52	0.79	0.58	-0.26	0.42	0.52	0.53	0.86	0.64	0.61	1.00								
susp	-0.07	0.76	0.18	-0.33	0.32	0.35	0.03	0.74	0.69	0.52	0.44	-0.37	0.70	0.82	0.79	0.75	0.27	0.32	0.80	1.00							
opp	0.16	-0.15	0.70	-0.30	0.12	0.39	0.29	-0.18	-0.24	0.60	0.62	-0.43	-0.26	-0.13	-0.02	0.61	0.64	0.73	0.33	0.24	1.00						
graz	0.15	0.34	-0.15	-0.46	0.91	0.52	0.42	0.41	0.14	0.14	0.02	-0.50	0.18	0.43	0.50	0.15	0.09	0.07	0.25	0.36	0.16	1.00					
pred	0.13	-0.18	0.75	-0.22	0.05	0.33	0.29	-0.21	-0.25	0.57	0.68	-0.36	-0.27	-0.15	-0.05	0.62	0.66	0.69	0.32	0.21	0.91	0.10	1.00				
<1y	-0.24	-0.16	0.89	-0.12	0.00	0.43	-0.05	-0.18	-0.19	0.76	0.83	-0.28	-0.21	-0.10	-0.07	0.71	0.71	0.90	0.51	0.32	0.77	0.07	0.77	1.00			
1_2y	-0.06	-0.04	0.67	-0.32	0.29	0.59	0.19	-0.02	-0.14	0.78	0.69	-0.43	-0.14	0.03	0.09	0.72	0.73	0.85	0.61	0.43	0.74	0.31	0.71	0.88	1.00		
3_5y	0.13	-0.03	0.02	0.41	0.11	0.36	0.22	0.17	0.19	0.48	-0.04	0.17	0.02	0.03	0.08	0.27	0.54	0.41	0.39	0.08	0.07	0.06	0.09	0.14	0.21	1.00	
6_10y	-0.04	0.94	-0.21	-0.21	0.29	0.11	-0.03	0.88	0.89	0.08	0.04	-0.16	0.95	0.94	0.89	0.34	-0.16	-0.15	0.45	0.77	-0.13	0.30	-0.16	-0.14	-0.07	0.00	1.00
>10y	0.21	0.12	-0.04	-0.10	0.13	0.11	0.26	0.13	0.12	0.05	0.00	0.09	0.12	0.28	0.29	0.11	0.04	0.07	0.09	0.29	-0.01	0.11	0.01	0.00	0.10	0.10	0.13

Appendix 4D. Association between functional categories and sediment conditions for different functional traits.
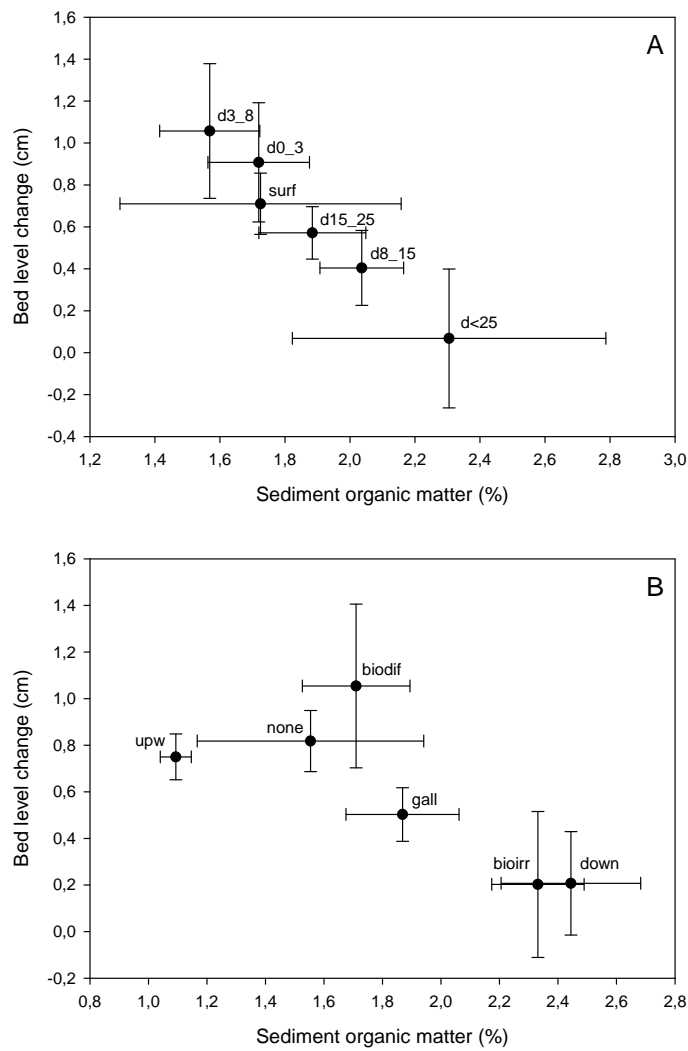
Fig. 4D1. Scatter plots of weighted averages of sediment organic matter content and bed level change for each category of the functional trait (A) "depth" and (B) "bioturbation" (see abbr. in Table 1).

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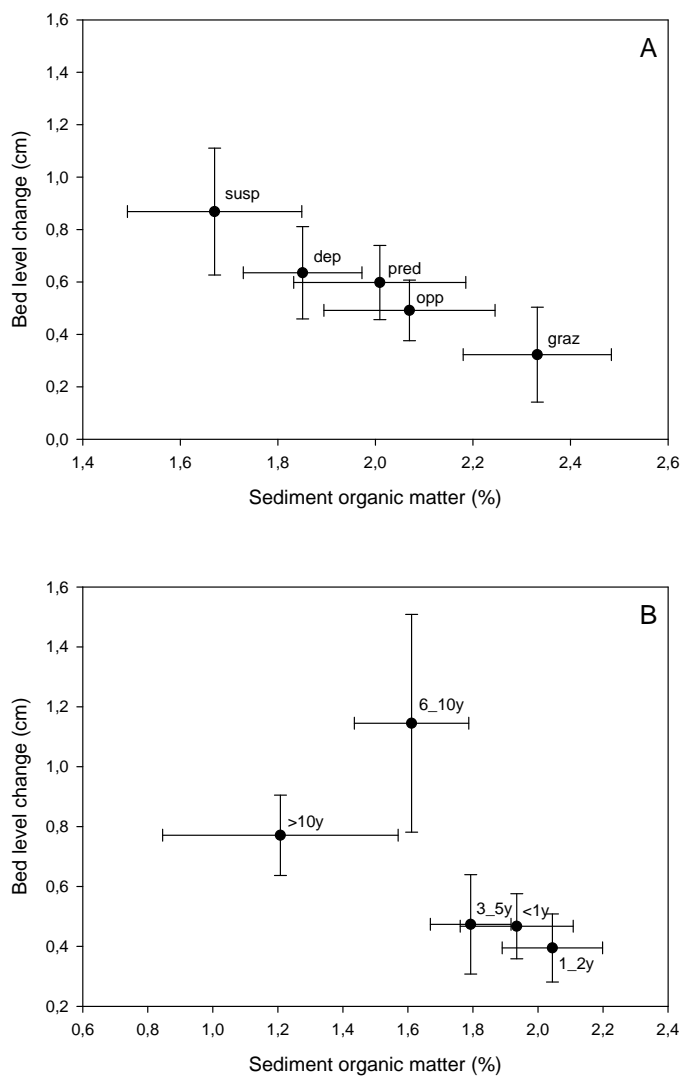
Fig. 4D2. Scatter plots of weighted averages of sediment organic matter content and bed level change for each category of the functional trait (A) "feeding mode" and (B) "longevity" (see abbr. in Table 1).

Chapter 5

5

CROSS-HABITAT INTERACTIONS AMONG BIVALVE SPECIES CONTROL COMMUNITY STRUCTURE ON INTERTIDAL FLATS

Serena Donadi, Tjisse van der Heide, Els M. van der Zee, Johan S. Eklöf,
Johan van de Koppel, Ellen J. Weerman, Theunis Piersma, Han Olff
& Britas Klemens Eriksson

ABSTRACT

Increasing evidence shows that spatial interactions between sedentary organisms can structure communities and promote landscape complexity in many ecosystems. Here we tested the hypothesis that reef-forming mussels (*Mytilus edulis* L.), a dominant intertidal ecosystem engineer in the Wadden Sea, promote abundances of the burrowing bivalve *Cerastoderma edule* L. (cockle) in neighboring habitats at relatively long distances coastward from mussel beds. Field surveys within and around three mussel beds showed a peak in cockle densities at 50-100 m towards the coast from the mussel bed, while cockle abundances elsewhere in the study area were very low. Field transplantation of cockles showed higher survival of young cockles (2-3 yr old) and increased spat fall coastward of the mussel bed compared to within the bed and to areas without mussels, whereas growth decreased within and coastward of the mussel bed. Our measurements suggest that the observed spatial patterns in cockle numbers resulted from (1) inhibition effects by the mussels close to the beds, due to preemptive algal depletion and deteriorated sediment conditions, and (2) facilitation effects by the mussels further away from the beds, due to reduction of wave energy. Our results imply that these spatial, scale-dependent interactions between reef-forming ecosystem engineers and surrounding communities of sedentary benthic organisms can be an important determinant of the large-scale community structure in intertidal ecosystems. Understanding this interplay between neighboring communities of sedentary species is therefore essential for effective conservation and restoration of soft-bottom intertidal communities.

INTRODUCTION

In the last two decades, studies from a wide range of terrestrial and marine ecosystems have demonstrated that interactions between sedentary organisms can generate spatial patterns at a landscape level (e.g. Connell 1961, Paine 1974, Dayton 1975, Maron and Harrison 1997; Klausmeier 1999, Guichard et al. 2003). The interplay between small-scale facilitation and long-range inhibition of organisms generate regularly patterned ecosystems, a form of spatial self-organization (Rietkerk and van de Koppel 2008). An opposite interplay between local competition and large-scale facilitation has been observed on cobble beaches, where various forbs species were facilitated behind stands of the cordgrass *Spartina alterniflora* Loisel. (Bruno 2000), but were outcompeted within the dense *Spartina* beds (van de Koppel et al. 2006). These studies have in common that intra- and interspecific interactions between sedentary species are scale-dependent, as they change in intensity and even sign with distance among the organisms under consideration.

The concept of scale-dependent interactions has mainly been used to explain spatial patterns of vegetation. Little is known about the importance of scale-dependent interactions between sedentary animals (van de Koppel et al. 2005). Moreover, to what extent such interactions determine the structure of neighboring communities has rarely been investigated. Nevertheless, understanding such spatial interactions may be necessary for the ecological conservation of ecosystems in which the structure of the community living in one habitat depends on interspecific interactions with species in neighboring habitats (Gaines and Roughgarden 1987, Rilov and Schiel 2011).

In this study, we investigated whether negative effects on food availability and sediment properties combined with positive effects on hydrodynamic conditions by reef-forming blue mussels (*Mytilus edulis*) result in scale-dependent effects of mussel beds on edible cockles (*Cerastoderma edule*) on intertidal mudflats in the Dutch Wadden Sea. Blue mussels are well-known ecosystem engineers that decrease near-bed hydrodynamic stress (Widdows et al. 1998, Widdows and Brinsley 2002, Widdows et al. 2002, Gutierrez et al. 2003) and increase sediment organic content through production and deposition of faeces and pseudofaeces; processes that affect both the mussel bed itself and the

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surrounding tidal flat (Graf and Rosenberg 1997, Bergfeld 1999, van der Zee et al. 2012). While cockle recruitment and persistence may be positively affected by decreased hydrodynamic stress (Bouma et al. 2001), cockle survival may be negatively affected by adverse low-oxygen sediment conditions created by the accumulation of organic material (Wegeberg and Jensen 1999). Furthermore, by their filter-feeding, mussels locally reduce algae concentrations in the benthic boundary layer (Frechétte et al. 1989). As cockles are also filter-feeders, blue mussels can compete with cockles for food over larger distances due to the directional flow of water in intertidal habitats (Asmus et al. 1992, Kamermans 1993). There is a rich literature on the formation of spatial patterns within mussel beds due to the scale-dependent interplay of facilitation and competition between mussels (Gascoigne et al. 2005, van de Koppel et al. 2005, van de Koppel et al. 2008). However, no study has reported on how scale-dependent effects extent beyond the boundaries of mussel bed to affect neighboring communities at larger distances.

We hypothesized that cockle abundances may be enhanced at a distance from mussel beds because mussel beds: 1) inhibit cockle growth locally by competition for food and by creating adverse sediment conditions (anoxia), and 2) promote the settlement of cockle spat at larger distances through reduction of hydrodynamic stress. As the size and protective status of mussel beds precludes manipulative experiments on the effects on their surroundings, we apply a comparative approach in which we confront our hypotheses with multiple lines of evidence, following Hollings "Adaptive inference" methodology (Holling and Allen 2002). To test our hypotheses, we measured cockle abundances and abiotic variables along transects across three intertidal mussel beds and nearby areas without mussels. At one of the sites where these patterns were observed, we carried out field transplantations of cockles where we investigated if cockle settlement, survival and growth rates within and close to the mussel reef correlated to changes in food availability, sediment chemistry and hydrodynamic conditions. Our results suggest that an interplay between local inhibition and longer-range facilitation result in maximal cockle abundance at a distance from mussel beds where positive effects on larval settlement outweigh negative effects on cockle growth and survival.

METHODS

Field surveys

The first field survey was conducted in April-May 2009 in the intertidal zone of the Dutch Eastern Wadden Sea, just south of the island of Schiermonnikoog. The intertidal mudflats in the area are mostly dominated by the bioturbating lugworm (*Arenicola marina*), but also harbor several large mussel beds (each > 1 ha). To study the effect of blue mussels on cockles, we first sampled three transects perpendicular to the coast across a 100×150 m mussel bed (53° 28' 8" N, 6° 13' 27" E) and three additional transects at the same elevation in a nearby area without mussels (53° 28' 7" N, 6° 13' 52" E) without mussel beds. Along each transect, we measured cockle (*Cerastoderma edule*) abundance, sediment characteristics and sediment pore water every 50 m. Local cockle abundances were determined by counting individuals within a 0.5×0.5 m frame, and sediment samples were collected for two depth ranges (0-1 and 0-5 cm) using a PVC corer (with an internal diameter of 3 cm). Pore water samples were collected in airtight 50 ml syringes using ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, The Netherlands). Within two hours after collection, pore water reduction-oxidation potential (RedOx) was measured with a Sentix ORP probe connected to a Multi 340i voltmeter (WTW, Germany). Silt content of the 0-1 cm sediment samples (fraction < 63 μm) was determined with a Malvern particle size analyzer (Malvern Particle Sizer 2000, United Kingdom) following freeze-drying of the sediment samples. Organic matter content was estimated from oven-dried (48 h, 95 °C) 0-5 cm samples as Loss On Ignition (LOI; 5 h, 550 °C).

In May 2011, we performed additional surveys at two other mussel beds (53° 28' 2" N, 6° 10' 59" E; 53° 29' 44" N, 6° 19' 20" E) and corresponding areas without mussels (53° 28' 7" N, 6° 11' 33" E; 53° 29' 51" N, 6° 19' 48" E) in Schiermonnikoog to test the generality of the observed patterns. In each survey, variation in cockle abundances was determined as described above along three transects from each mussel bed towards the coast and three transects at the same elevation in a nearby area without mussel beds.

Field transplantation experiments

The initial field surveys showed a peak in cockle abundance at about 100 m coastward from the mussel bed, while cockle abundances within the mussel bed and in the area without mussels were extremely low (see Results). To test if this pattern was caused by scale-dependent effects of mussels on cockles, a field transplantation experiment was carried out following the field surveys. Due to logistical constraints, we had to limit our experiment to a single mussel bed. We chose four experimental locations: (1) "mussel bed" (bare patches between the mussel aggregations inside the mussel bed), (2) "coastward of the mussel bed", at a distance of approximately 100 m coastward from the mussel bed, where we determined the peak in cockle abundance, and, in a sandy area without mussels, (3) at the same elevation as the mussel bed ("lower sandy site"), and (4) at the same elevation relative to mean sea level as the area of the cockle peak ("upper sandy site"). At each location we randomly designated four 1×1 m experimental plots (Appendix 5A). Naturally occurring densities of *Cerastoderma edule* varied greatly among sites. We found an average density of 225 cockles per square meter (n = 20) coastward of the mussel bed, while in all other locations densities were lower than 1 per square meter. In order to minimize potential density-dependent effects due to the presence of cockles (e.g. competition for space, settlement facilitation/inhibition), 225 adult cockles were transplanted in May 2009 from the area coastward of the mussel bed to the plots at each of the two sandy sites and inside the mussel bed. Persistence of high density of cockles in the plots was checked and confirmed at the end of the experiments.

To test whether the vicinity of mussels affected cockle growth and survival, we added 10 tagged cockles to the 0.9×0.9 m center area of each plot at the end of May 2009. Young cockles (2 to 3 yr. old; 12 to 26 mm shell length) were collected from a nearby mudflat, transferred to the lab, measured (shell length, using a vernier calliper), tagged with a plastic label glued to the shell with cyanoacrilate and added to the experimental plots. Nearly four months later (September 2009), all cockles were collected by hand-raking. To avoid edge effects, only the 0.9×0.9 m center area of each plot was sampled. The number of recaptured tagged cockles was used to compare *in situ* survival of juveniles, while cockle spat (3-12 mm long individuals), which settle in the Wadden Sea from end of May until end of June (van der Veer et al. 1998; R. Dekker, *personal*

observation), were counted to examine effects on recruitment and settlement success.

Measurements of chlorophyll *a* content and hydrodynamic conditions

Since mussel beds may facilitate settlement of cockle spat in the wake of the reef through reduction of water flow velocities, we investigated the effect of the mussel bed on hydrodynamic conditions. For this, we simultaneously measured current velocity at the site coastward of mussels (at 100 m from the reef) and in its corresponding site without mussels (upper sandy site), at 5 cm above the sediment bed in 1 Hz intervals during four consecutive tidal cycles in July. For this purpose, we fixed two Acoustic Doppler Current Profilers (ADCP; Nortek Aquadopp, Norway) in the sediment with a stainless steel frame.

Furthermore, we measured water chlorophyll *a* content as a proxy for concentrations of suspended algae and suspended sediment at each site. Water samples were taken at 10 cm above the sediment in the middle of incoming tide, when water speed was highest. In total, we simultaneously sampled both sites three times across a two-week period in August. After collection, each sample was filtered over two pre-combusted (3 h; 450 °C), pre-weighed GF/F glass-fiber filter (Whatman, United Kingdom) in standardized aliquots in a dark room. Suspended sediment concentrations were determined from the first filter as the weight difference between the initial filter and the dried filter sample (24 h; 70 °C). Chlorophyll *a* content was determined from the second filter by acetone extraction following Jeffrey and Humphrey (1975).

Measurements of sediment erosion and cockle spat abundance

Reduction of water flow velocity coastward of mussel reefs in the study area was confirmed in March 2012 by additional measurements of plaster dissolution along four transects perpendicular to the coast across a mussel bed and four transects at the same elevation in a sandy area without mussels. Dissolution cylinders (6.3 cm long; 2.4 cm diameter) were molded with model plaster (Knauf Modelgips, Knauf B.V., Utrecht, Netherlands), dried, weighted and placed in the field for four tidal cycles (about 46h), after which they were retrieved and dried until constant

weight. To account for difference in the exposure time between sites, plaster weight loss values were divided by the average tidal submersion time of each site estimated by means of Sensus Ultra pressure loggers (Reefnet, Missisauga, Canada). Cockle spat abundances along the transects were measured by counting individuals (3-12 mm long) within a 0.5×0.5 m frame.

Statistical analyses

The effect of site on the number of recaptures and cockle recruits was analyzed by fitting Generalized Linear Models (GLM). Error distributions were selected based on the lowest AIC or on the comparison of residual patterns. We assumed Poisson and Negative Binomial error distributions for the number of recaptures and spat fall densities, respectively, both with log-link functions. When possible, multiple comparisons were performed via contrast coefficients through the "glht" function from the Multcomp package in R.

To compare growth rate of recaptured tagged cockles, the effect of initial size on growth was removed by fitting an equation to the pooled data set of initial and final lengths and residuals were calculated. We used the von Bertalanffy growth model as modified by Ramón (2003):

$$L_2 = L_1 + (L_\infty - L_1) [1 - e^{-k\Delta t}]$$

where L_1 and L_2 are respectively the initial and final lengths and Δt is the time elapsed between L_1 and L_2. The parameter Δt was assigned a value of 0.8 year because the experiments were carried out during the growth season. The parameters L_∞ and k were estimated by a non-linear least-squares method using the Gauss-Newton algorithm, that yielded L_∞ = 29.711 mm and k = 1.423 year^{-1} (n = 28, Residual Sum of Squares = 8.599, r = 0.945). Residual averages of each plot were compared by one-factor analysis of variance (ANOVA) to investigate effects of site on cockle growth and Tukey's HSD test was used for post-hoc comparisons. As tagged cockles in the Lower Control were recaptured in only one plot, this site was excluded from the analysis (see Results).

Effects of site and sampling time on chlorophyll *a* content, suspended sediment matter and water current velocity were tested in three separate models. The direction of the water current (incoming vs. outgoing) was also

included as explanatory variable when analyzing water current velocity data. Independence of observations taken at the same site or time interval was tested by comparing GLS (Generalized Least Squares) models with and without a residual correlation structure (Zuur et al. 2009). For current velocity data, a significant correlation (Likelihood Ratio Test statistic = 14.06610, $P < 0.05$) was found between values observed within the same time interval. We calculated an intraclass correlation of 0.96 and used this value to calculate the design effect and the effective sample size (Snijders and Bosker 1999).

To confirm the effect of mussel reefs on hydrodynamic conditions, a one-way ANOVA was used to compare values of plaster dissolution measured in four locations along transects: at 100 m seaward of the mussel bed, at 50 m coastward of the mussel bed (where a peak in cockle abundance was previously observed) and in the sandy site without mussels at the same elevation respectively. Tukey's HSD test was used for post-hoc comparisons. The relationship between plaster weight loss and cockle spat abundance in the absence of mussel beds was investigated for data collected in the site without mussels by means of a quantile regression on the natural-log transformed data.

When needed, normality assumption was checked using both a normal quantile plot and Shapiro-Wilks test ($P = 0.05$) and homoscedasticity was assessed by comparing GLS models with different residual variance structures (Zuur et al. 2009). All statistical calculations were carried out in R (CRAN, R: A language and environment for statistical computing. 2010. R Foundation for Statistical computing. Vienna, Austria).

RESULTS

### Field surveys: spatial pattern of abiotic parameters and cockle abundance

The combined surveys from 2009 and 2011 strongly suggested that the mussel bed affected the structure of the intertidal community both at the site of the bed and on its surroundings. Most importantly, the presence of the mussel bed was associated with the occurrence of cockle beds at a distance; the survey of 2009 showed that cockle densities in the mussel bed transects peaked 100 m coastward from the bed (at 225 ± 62.53 SE individuals/m^2), sharply declined to zero within

the bed, and then slightly increased seaward from the bed (Fig. 5.1A). In contrast, cockle abundance along the transects in the area without mussels did not change with distance from land. The spatial pattern in cockle distribution relative to the mussel bed was confirmed by the surveys conducted in 2011 at two other mussel beds (Fig. 5.1B & C). In both mussel areas, cockle abundance peaked at 50 m coastward from the beds, while little variation was observed in the corresponding areas without mussels. Hence, our surveys suggested a spatial covariation of mussels and cockles between neighboring communities of these sedentary species.

A clear difference in abiotic parameters was found between the mussel transects and the area without mussels. We found a strong increase in silt content and organic matter towards the mussel bed with maximum values inside of 46.92 (± 7.74 SE) % and 6.98 (± 0.74 SE) %, respectively (Fig. 5.2A & B). In contrast, sediment pore water redox decreased sharply towards the mussel bed with a minimum value of -382 (± 6.08 SE) mV inside the bed (Fig. 5.2C). Silt content, organic matter and redox showed little variability in the area without mussels (Fig. 5.2A-C).

Field transplantation of cockles: effects on cockle growth and survival

Our experiments show that survival and recruitment of cockles were higher on the coastward side of the mussel bed, compared to within the bed or in any of the sandy areas without mussels. Over 50% of the tagged cockles were found back coastward of the mussel bed, while ≤ 8% in the mussel bed or in any of the sandy sites (Site effect, Likelihood Ratio test statistic = 26.81, df = 3, $P < 0.001$; Fig. 5.3A). Likewise, we found higher densities of recruits (juvenile cockles of 3-12 mm) coastward of the mussel bed (ca. 10 m^{-2}) compared to the other sites, where hardly any juveniles were collected (Site effect, Likelihood Ratio test statistic = 64.00, df = 3, $P < 0.001$; Fig. 5.3B). Contrastingly, cockle growth was generally negatively affected by the mussel bed. Analysis of variance of mean growth residuals revealed a significant effect of site ($F_{2,7}$ = 8.23, $P < 0.015$), such that growth in the upper sandy site was significantly higher compared both to coastward of the mussel bed (Tukey's HSD post-hoc test; P = 0.046) and inside the mussel bed (Tukey's HSD post-hoc test; P = 0.014; Fig. 5.3C). This suggests that
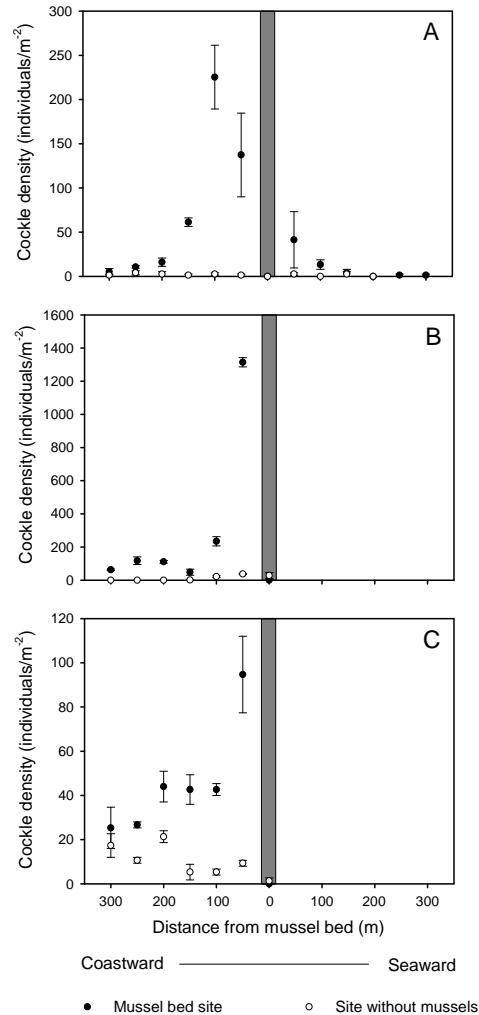
Fig. 5.1. Cockle (*Cerastoderma edule*) density along transects perpendicular to the coast across three intertidal mussel (*Mytilus edulis*) beds (closed circles) and three corresponding areas without mussels at the same tidal elevation (open circles) in 2009 (A) and 2011 (B, C) in Schiermonnikoog. The x axis represents the distance from the mussel reef along the mussel-bed transects. Grey area indicates the position of the mussel bed. Mean ± SE (n = 3).

improved survival of cockles at the spat or juvenile stage is the main driver of the high abundance that we observed coastward of the mussel beds.

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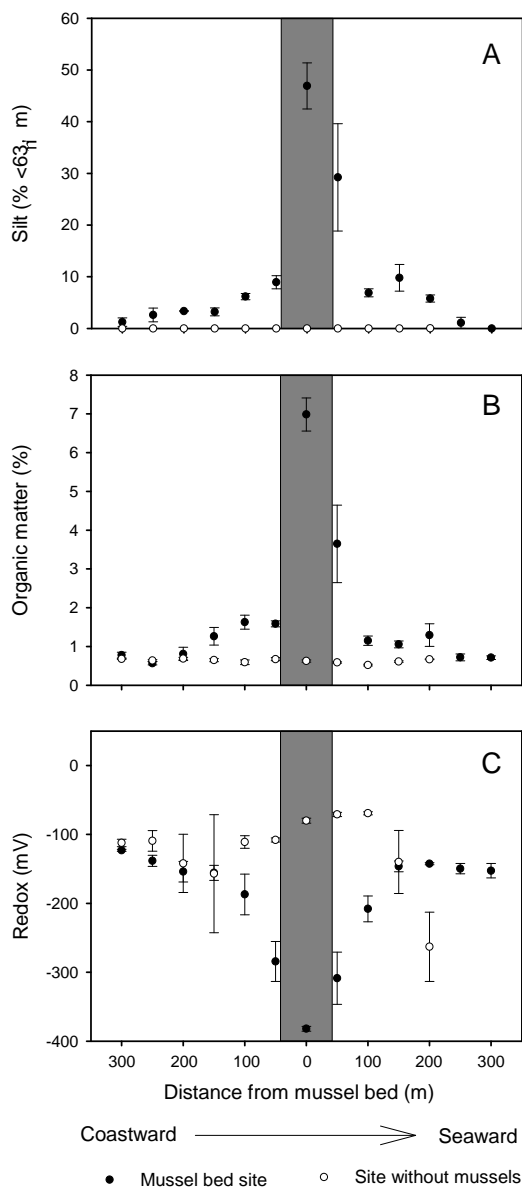
Fig. 5.2. A) Silt content, (B) organic matter content and (C) redox potential along transects perpendicular to the coast across an intertidal mussel bed (closed circles) and a corresponding area without mussels at the same tidal elevation (open circles). The x axis represents the distance from the mussel reef along the mussel-bed transects. Grey area indicates the position of the mussel bed. Mean ± SE (n = 3).

Variation in chlorophyll *a* content and hydrodynamic conditions

We found that the mussel bed likely influenced both food availability for other filter feeders and hydrodynamic conditions. Chlorophyll *a* content ($F_{1,4}$ = 17.17, *P* = 0.014), suspended sediment concentration ($F_{1,4}$ = 17.08, *P* = 0.014) and water current velocity ($F_{1,6}$ = 69.05, *P* < 0.001) were lower coastward of the mussel bed than in the site without mussels (Fig. 5.4A-C), and no differences were observed between sampling times (main effect and interactions of time were not significant; *P* > 0.05). For current velocity, we found an interaction between site and water current direction ($F_{1,6}$ = 167.90, *P* < 0.001), revealing a 21% lower current velocity coastward of the mussel bed compared to the area without mussels with rising tide, but no significant difference during outgoing water. Our results therefore imply that mussels may affect cockle survival in two opposite directions, by depleting food availability in the water column and by providing shelter to new recruits from waves and water currents.

Sediment erosion and cockle spat abundance

Reduction of hydrodynamic forces coastward of mussel beds in the study area was confirmed by data collected along transects: plaster weight loss measured at 50 m coastward of the reef (where a peak in cockle abundance was previously observed) was decreased by 20% compared to seaward of the reef (significant one-way ANOVA result, $F_{3,12}$ = 16.18, *P* < 0.001; Tukey's HSD post-hoc test; *P* = 0.001) and by 21% compared to the same tidal elevation in the sandy site (Tukey's HSD post-hoc test; *P* < 0.001; Appendix 5B). Our measurements strongly suggest that cockle spat settlement and survival observed in an area without mussels are affected by hydrodynamic stress and sediment erosion: we found a negative relationship between high spat densities (quantile 0.90) and plaster weight loss (n = 32, *P* = 0.023), with high spat densities never occurring at high values of sediment erosion (Appendix 5C). This indicates that cockle bed settlement and survival may be possible only below a certain threshold of hydrodynamic stress, which is lower than the values measured in the area without mussels (43.91 ± 1.60 SE % plaster weight loss) and higher than the values observed coastward of the mussel bed (32.30 % ± 1.16 SE % plaster weight loss).

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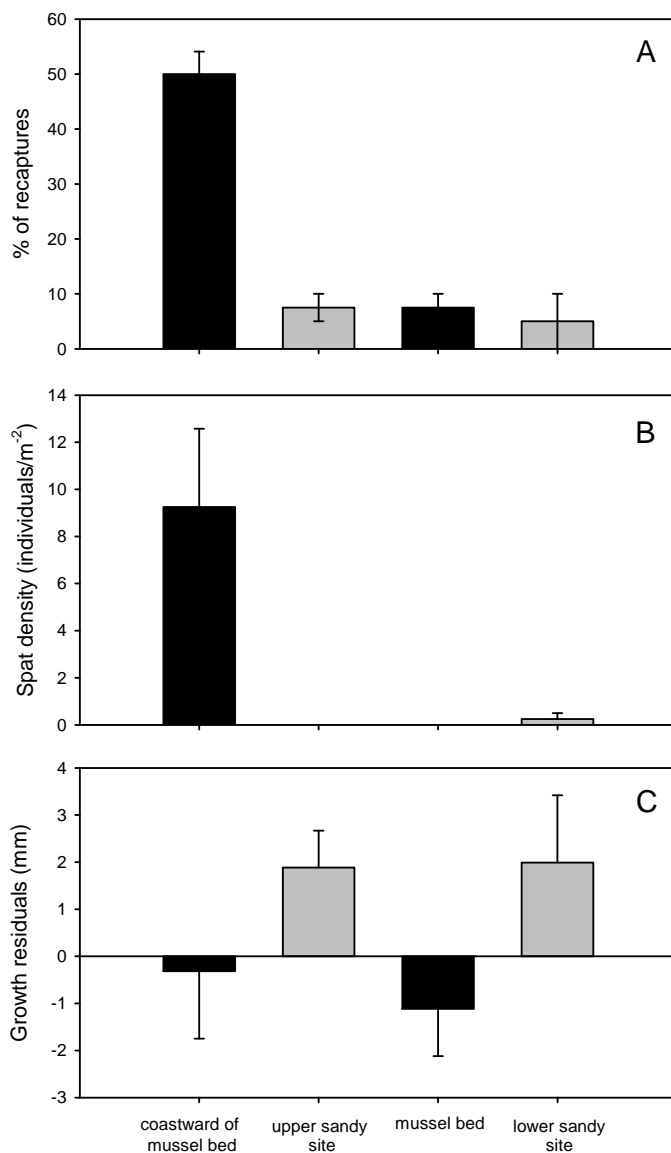
Fig. 5.3. A) Percentage of recaptured tagged cockles, (B) spat density and (C) growth residuals measured coastward of the mussel bed, at a comparable tidal elevation in the area without mussels (upper sandy site), within the mussel bed and at a comparable tidal elevation in the area without mussels (lower sandy site). Mean ± SE (A and B: n = 4. C: n = 20 coastward of the mussel bed, n = 3 in the upper sandy site, n = 3 within the mussel bed, n = 2 in the lower sandy site).
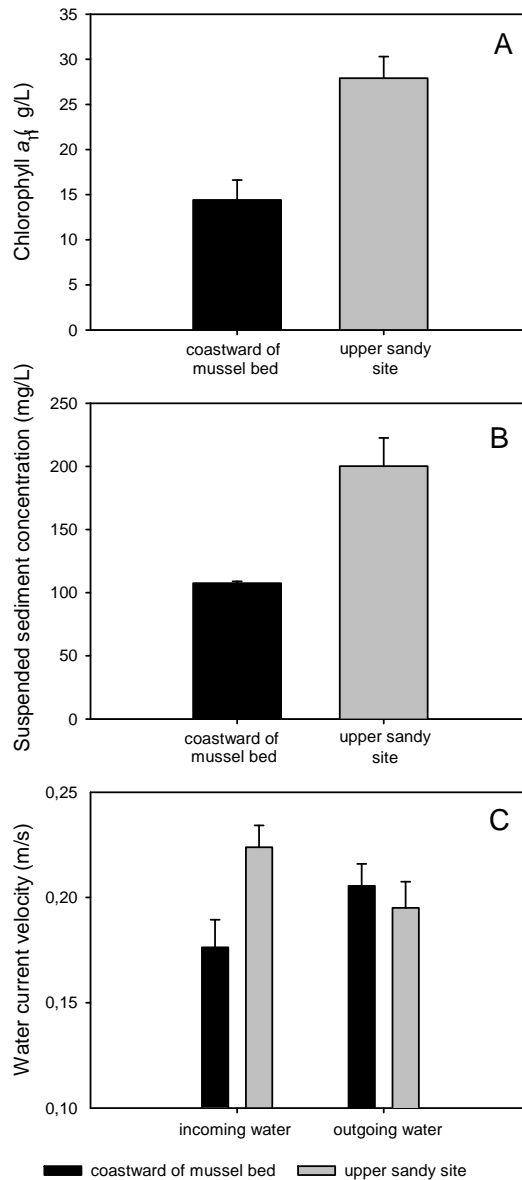
Fig. 5.4. A) Chlorophyll a content and (B) suspended sediment concentration in the water column coastward of the mussel bed and at a comparable tidal elevation (upper sandy site). (C) Water current velocity measured during incoming and outgoing water coastward of the mussel bed and at a comparable tidal elevation (upper sandy site). Mean ± SE (A and B: n = 3. C: n = 4).

DISCUSSION

We found that cross-habitat interactions can play an important role in determining the community structure of sedentary species in soft-bottom intertidal systems. Enhanced densities of cockles were observed coastward of reef-forming mussels, but not within the reef itself or in the areas without mussels. Our experiments suggest that this pattern of cockle distribution is caused by a scale-dependent influence of the mussel bed on the survival and growth of cockles: cockles experienced strong competition for food by mussels within the mussel bed, while spat fall and juvenile survival were profiting from reduced hydrodynamics stress coastward of the mussel bed. These results emphasize the importance of mussels as ecosystem engineers that shape soft-bottom intertidal communities, and reveal that their influence may extend well beyond the boundaries of the actual reefs. Moreover, it emphasizes that intertidal ecosystems can be structured by long-distance interactions, suggesting that protection of specific intertidal habitats requires consideration of its linkages to other habitats in the surrounding.

When studying the effects of mussel reefs on the surroundings, strong inference methods, implying replicated experimental removal of entire beds, are not feasible because of the scale and protective status of mussel reefs. Therefore, we confronted our hypotheses to multiple lines of supportive evidence, following Hollings "Adaptive inference" approach. First, we found a decline in chlorophyll *a* and low oxygen content in the sediment within and coastward of the mussel bed, relative to the site without mussels at the same tidal elevation. This points at the proposed short-range negative effects of mussels on cockles imposed by the combined effects of intense competition for food and deteriorated sediment conditions. These observations are in close agreement with other studies revealing reduced cockle growth close to mussel beds due to algal depletion (Kamermans 1993, Ramón 1996). Also, deteriorated sediment conditions (e.g. low oxygen content, high organic matter) have been previously reported to hinder survival of many benthic species (Pearson and Rosenberg 1978, Diaz and Rosenberg 1995, Gray et al. 2002, Hyland et al. 2005, Magni et al. 2008).

A striking opposite effect of mussels on cockles appears to occur at a larger spatial scale. Our results show that cockle densities were greatly enhanced coastward of mussel beds, compared to sites without mussels, and that this may

be explained by reduced hydrodynamics stress caused by the mussel bed. Hydrodynamic forces are known to have a strong influence on the settlement and recruitment of larvae, though in contrasting ways. Flow speed can have positive effects on larval settlement (i.e by increasing larval supply and the contact rate with the substrate) (Judge and Craig 1997, De Montaudouin et al. 2003). However, evidence from the field often showed opposite results (Armonies and Hellwig-Armonies 1992; Bouma et al. 2001, Jonsson et al 2004). High flow speed decreases the time available for larval adhesion and increase the drag force detaching the larvae from the substrate (Abelson and Denny 1997). Reef-forming mussel beds can enhance the settlement of larvae and post-larvae by alleviating hydrodynamic stress imposed by waves and water flow (Widdows and Brinsley 2002, Gutierrez et al. 2003, Commito et al. 2005). In agreement with these studies, we measured higher recapture rates of tagged cockles and spat densities at 100 m coastward of a mussel bed, where field measurements revealed a peak in cockle abundance and reduced water flow, suggesting an increased survival relative to the sites without mussels. Hence, at a larger spatial scale, the positive effects of reduced wave action on spat fall seem to outweigh the negative effects of increased competition, explaining the high densities of cockles coastward of the mussel bed.

An alternative explanation for the observed high cockle survival in the wake of the mussel bed is that predation by birds, flatfishes and crustaceans (Sanchezsalazar et al. 1987, Norris and Johnstone 1998, van der Veer et al. 1998) is reduced here for some reason. Yet, we documented much higher abundances of cockle predators, such as oystercatchers (*Haematopus ostralegus*) and crabs (*Carcinus maenas*), within and coastward of the mussel bed (van der Zee et al. 2012) rather than in the area without mussels, where numbers of cockles recaptured and spat fall were lower. This leaves the alleviation of hydrodynamic stress as the best explanation for enhanced cockle settlement and survival in the larger neighborhood of mussel beds. However, our conclusions remain to be confirmed by further studies providing conclusive experimental evidence of the mechanisms investigated.

When these lines of evidence are put together, our results strongly suggest that the interactions between mussels and cockles are scale-dependent: competition dominates at a small scale, while facilitation predominates at larger scales. A similar type of interaction has previously been proposed to explain

zonation on cobble beaches (van de Koppel et al. 2006), where forbs were facilitated by dense stands of *Spartina alterniflora*, but only in their wake, at a distance up to 10 meters. Within the dense *S. alterniflora* stands, competition predominated, and no forbs could persist. Our results now suggest that such scale-dependent interactions can also structure soft-bottom intertidal communities at scales up to 250 meters, linking mussel bed habitats to the cockle-dominated tidal flat that lies in their wake. In this way, long-range effects of reef builders may allow the persistence of productive intertidal communities under conditions that would normally not support such a high spatial complexity and associated biodiversity.

Scale dependence has been proposed as the basic mechanism causing self-organized, regular spatial patterns in ecosystems all over the world, ranging from arid bushlands to boreal peat bogs (see review in Rietkerk and van de Koppel 2008). Self-organization is a process whereby spatial patterns at the global level of a system emerge solely from local interactions among the components of a system (Camazine et al. 2001). Even within mussel beds, regular spatial patterns are found, both at sub-meter scale (van de Koppel et al. 2008) and at 10 meter scale (van de Koppel et al. 2005), that seem crucial in minimizing competition, promoting mussel biomass and increasing mussel bed resilience. Other studies have found self-organized spatial patters in other intertidal habitats such as mudflats (van der Heide et al. 2010), tidal freshwater wetlands (van de Koppel and Crain 2006) and salt marshes (van Wesenbeeck et al. 2008). The current study shows that effects of spatial self-organization may scale up to much larger spatial scales than previously shown, affecting the presence and distribution of sedentary communities and species across distances up to several hundred meters. Although at this scale regularity is not prevalent, it emphasizes the importance and reach of self-organization processes in intertidal ecosystems, determining distribution patterns of individuals at meter scale (van de Koppel et al. 2008) to habitats (e.g. cockle beds) at sub-kilometer scale.

Our study suggests that reef forming organisms can change the balance between abiotic and biotic control over species settlement, growth and survival over different spatial scales. In the sites without mussels, adverse hydrodynamic conditions likely caused low settlement of juvenile cockles, explaining the lack of cockles in these locations. This confirms that reef builders are indeed important ecosystem engineers that alter hydrodynamic energy and sedimentation

processes, both locally (within the reef) and at the tidal flat that lies in their wake (Kroncke 1996, Graf and Rosenberg 1997, Gutierrez et al. 2003). Hence, while abiotic stress potentially inhibits cockles in the absence of the mussel reef, biological processes, such as competition for food and interactions through ecosystem engineering, may dominate within and coastward of the mussel bed.

Our results point to the importance of spatial interactions among neighboring communities. As a consequence, intertidal ecosystems can be particularly vulnerable to disturbances that destroy such spatial interactions and the resulting structures (Weerman et al. 2011). Because intertidal communities seem to rely on a spatial network of facilitative and competitive interactions at different scales, human perturbations (e.g. the overfishing of ecosystem engineers) can cascade through the ecosystem, leading to unexpected and drastic changes on large spatial and temporal scales (Lotze 2005, Eriksson et al. 2010). On the other hand, our results suggest that successful restoration of soft-bottom ecosystem engineers can have beneficial cascading effects on ecosystem over large areas, increasing spatial complexity and associated biodiversity. Therefore, better understanding of cross-scale interactions among neighboring habitats is essential for effective conservation and restoration of intertidal ecosystems, such as the Wadden Sea.

Acknowledgements

APPENDICES

Appendix 5A. Schematic of the field transplantation design.
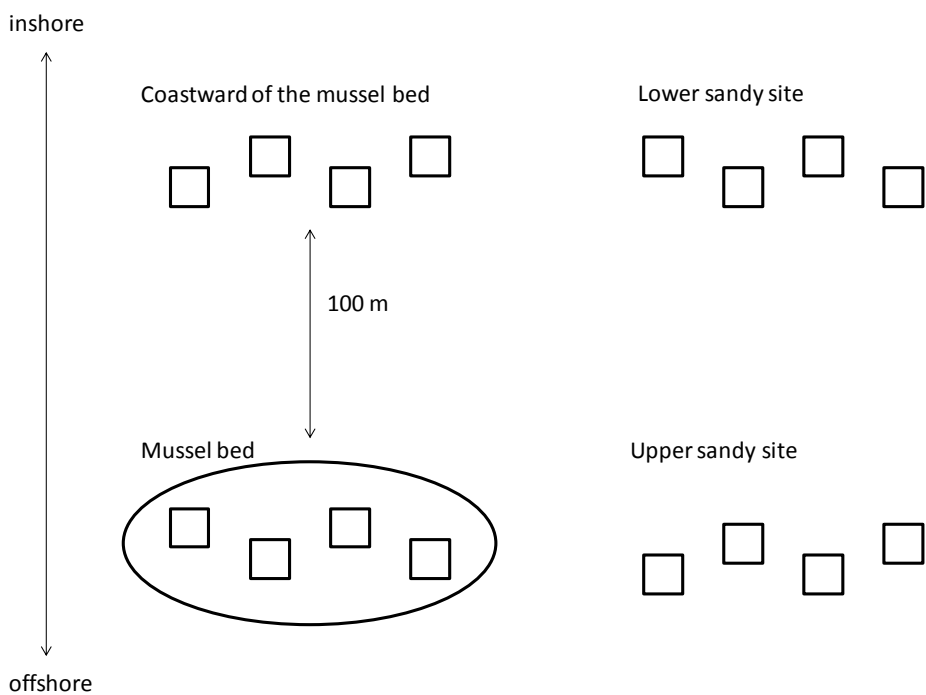
Fig. 5A. Schematic showing the four sites of the field transplantation where four 1×1 m plots were randomly assigned. Distance between plots was at least 5 m.

Appendix 5B. Plaster weight loss along transects across a mussel bed and in an area with no mussel beds.
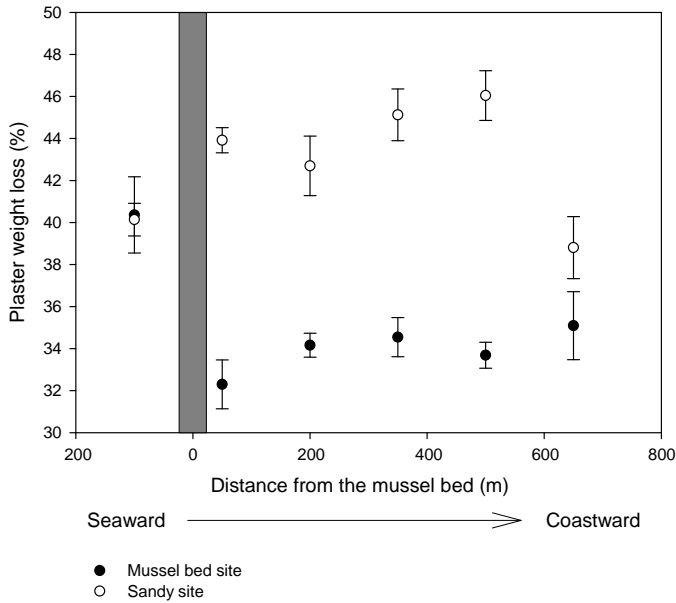
Fig. 5B. Plaster weight loss along transects perpendicular to the coast across a mussel bed (closed circles) and in a sandy site without mussels at the same tidal elevation (open circles). The x axis represents the distance from the mussel reef along the mussel-bed transects. Grey area indicates the position of the mussel bed. Mean ± SE (n = 4).

Appendix 5C. Relationship between cockle spat densities and plaster weight loss.
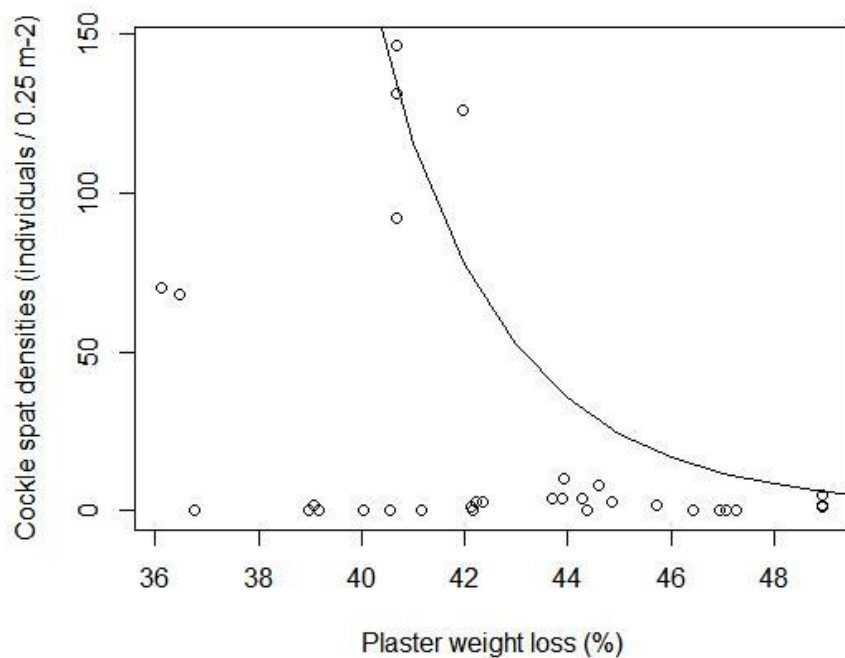
Fig. 5C. Scatter plot of cockle spat densities and plaster weight loss measured along transects perpendicular to the coast in an area without mussel beds (n = 32). The line indicates regression for quantile 0.90 (P = 0.023).

Chapter 6

6

Spatially extended habitat modification by intertidal reef-building bivalves has implications for consumer-resource interactions

Els M. van der Zee, Tjisse van der Heide, Serena Donadi, Johan S. Eklöf,
Britas Klemens Eriksson, Han Olff, Henk W. van der Veer & Theunis Piersma

ABSTRACT

Ecosystem engineers can strongly modify habitat structure and resource availability across space. In theory this should alter the spatial distributions of trophically interacting species. Here, we empirically investigated the importance of spatial extended habitat modification by reef-builders in explaining the distribution of four avian predators and their benthic prey in the Wadden Sea – one of the world's largest intertidal soft-sediment ecosystems. We applied Structural Equation Modeling (SEM) to identify important direct and indirect interactions between the different components of the system. We found strong spatial gradients in sediment properties into the surrounding area of mixed blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) reefs, indicating large-scale (100s of m) engineering effects. The benthic community was significantly affected by these gradients, with the abundance of several important invertebrate prey species increasing with sediment organic matter and decreasing with distance to the reefs. Distance from the reef, sediment properties and benthic food abundance simultaneously explained significant parts of the distribution of oystercatchers (*Haematopus ostralegus*), Eurasian curlews (*Numenius arquata*) and bar-tailed godwits (*Limosa lapponica*). The distribution of black-headed gulls (*Chroicocephalus ridibundus*) – a versatile species with many diet options – appeared unaffected by the reefs. These results suggest that intertidal reef builders can affect consumer-resource dynamics far beyond their own boundaries, emphasizing their importance in intertidal soft-bottom ecosystems like the Wadden Sea.

INTRODUCTION

Over the last decades it has become well established that some organisms can have disproportionally strong effects on their abiotic environment, indirectly affecting other species. Such species, often called 'ecosystem engineers' by Jones *et al.* (1994), typically promote their own preferred conditions at the local ('patch') scale (Bertness and Leonard 1997, Rietkerk et al. 2004 and references therein). However, ecosystem engineering is often not only important locally, but may also have strong impacts at landscape scales (Wright et al. 2002, Kefi et al. 2007, Scanlon et al. 2007). Apart from altering the spatial structure of the environment, ecosystem engineers may affect the spatial distribution and abundance of their resources (e.g. nutrients, water, light). This also alters resource availability for other species (Gutierrez et al. 2003, van de Koppel et al. 2006), which should in turn affect the spatial distribution of their consumers (e.g. Hassell and May 1974, Folmer et al. 2010, Piersma 2012). Although effects of prey-patchiness and ecosystem engineering on the distribution of species have been documented separately (e.g. Hassell and May 1974, Wright et al. 2002), assessments of the spatially extended effects of ecosystem engineers on resources and its consumers have remained largely theoretical (Bagdassarian et al. 2007, Olff et al. 2009).

Reef-builders like blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) are striking examples of ecosystem engineers that impact their environment through habitat modification (Kröncke 1996, Gutierrez et al. 2003, Kochmann et al. 2008). At a local scale, mussels and oysters create hard substrate and increase habitat complexity, reduce hydrodynamics, and modify the sediment by depositing large amounts of pseudo-feces and other fine particles (Kröncke 1996, Hild and Günther 1999, Gutierrez et al. 2003). However, in soft-bottom systems, their effects on sediment conditions typically extend beyond the direct surroundings of the reefs and may be detectable up to several hundreds of meters (Kröncke 1996, Bergfeld 1999). Many studies have demonstrated that reef builders have an important effect on the local benthic community (Dittmann 1990, Norling and Kautsky 2008, Markert et al. 2009) and that the reefs themselves are important foraging grounds for avian consumers (e.g. Nehls et al.

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1997, Caldow et al. 2003). However, the spatially extended effects of such reef builders on this community remain largely unstudied.

Furthermore, possible implications of such spatially extended habitat modification on the community may also be important from a management perspective. In many intertidal soft-bottom systems, like the Wadden Sea, ecosystem engineers have disappeared due to multiple anthropogenic disturbances and many associated species disappeared with them (Piersma et al. 2001, Lotze et al. 2005, Kraan et al. 2007, Eriksson et al. 2010). For instance, in the Wadden Sea, 150 km^2 of seagrasses disappeared in the 1930s (van der Heide *et al.* 2007) and mussel beds were almost completely removed in the beginning of the 1990s and have only partly recovered thus far (Beukema and Cadee 1996). If spatial effects of ecosystem engineers are not recognized, such dramatic changes might result in unexpectedly strong losses in these ecosystems.

Here we investigate the effects of spatial habitat modification by mixed blue mussel and Pacific oyster reefs on the distribution of benthic prey and their consumers (shorebirds) at a sandy intertidal flat. We collected spatially explicit data on important abiotic variables and the biota in and around two reefs in the Dutch Wadden Sea. We used Structural Equation Modeling (SEM) to infer the relative importance of ecosystem engineering on the spatial distribution of recourses and consumers. Based on sediment and benthos data of 119 sampling stations at varying distances from the reefs and the spatial mapping of shorebirds, we constructed default models for four of the most commonly observed bird species that included all possible interactions between the birds and their environment. Next, we determined the relative importance of each interaction, using an approach with stepwise exclusion of variables.

MATERIAL AND METHODS

Study area

Our study area covered about 44 ha of intertidal mudflats, south of the island of Schiermonnikoog in the eastern Dutch Wadden Sea (53°28'15.75" N, 6°13'20.06" E). These intertidal flats contain a variety of macrobenthic invertebrate species (Beukema 1976) that are accessible to shorebirds twice a day (van de Kam et al. 2004, van Gils et al. 2006a). The area contained two mixed reefs of blue mussels

and Pacific oysters, established in 2002 (Goudswaard *et al.* 2007 and unpublished data of our research group). The main cohort of bivalves was 7 years old, with several younger cohorts. Prior to the establishment of the two reefs, our study area consisted of a sandy intertidal flat without patches of hard substrata (van de Pol 2006 and unpublished data of our research group). The spatial relationships of the reef builders with the local and surrounding benthic community and associated shorebirds was examined at two adjacent study areas of 22 ha each (see Fig. 6.2).

Benthic sampling

Sediment, pore water and benthic samples were collected in August 2009 on a predetermined 100 m grid with 46 additional random points. In total, 119 station points were sampled across the two study sites. All stations were identified during low tide using a handheld GPS. At each sampling station, we sampled and pooled three 5 cm deep sediment cores with a PVC corer with area of 7.1 cm^2. Sediment organic matter content in dried sediment (24 h, 70 °C) was estimated as weight Loss On Ignition (LOI; 5 h, 550 °C). Silt content (% sediment fraction < 63µm) was determined by a Particle size Analyzer (Malvern). Redox potential was measured immediately after sampling with a multi-probe meter (556 MPS, YSI) in pore water that was extracted from the sediment with a ceramic cup into a vacuumized 50 ml syringe. Benthic samples were taken with a stainless steel core with area of 179 cm^2 down to a depth of 20-25 cm. Samples were sieved over a 1 mm mesh and all fauna fixed in 4% formalin. In the laboratory, samples were stained with Rose Bengal and fauna was identified to species level. Ash free dry mass (AFDM) of each species was determined by Loss On Ignition (5 h, 550 °C) after drying for 48 h in a stove at 60 °C.

Bird mapping

A 3.2 m high observation platform was constructed 100 m away from each of the two study sites in such a way that the platforms covered the respective sampling grids, i.e. a reef and the associated gradient towards a sandy area, all within a radius of 500 m. The spatial distribution of shorebirds was determined during four tidal cycles between 18 August and 8 September 2009. Positions of individual

birds were determined using the newly developed Telescope-Mounted Angulator (TMA) described by van der Heide et al. (2011). This was done from an hour before to an hour after time of low water, i.e. when the areas were completely exposed and tidal movement would not affect their spatial distribution. With the TMA, using trigonometry, we were able to determine a bird's spatial position with high accuracy (maximum prediction error of 8.7 m at 500 m; van der Heide *et al.* 2011).

We mapped the spatial distribution of four common shorebird species: oystercatcher (*Haematopus ostralegus*), Eurasian curlew (*Numenius arquata*), bar-tailed godwit (*Limosa lapponica*) and black-headed gull (*Chroicocephalus ridibundus*). These focal species were chosen for three reasons. First, due to their body size, all four species are easy to follow and clearly visible which prevented double counting and inaccurate positioning (van der Heide *et al.* 2011). Second, all four species form sparse flocks, a feature that represents a degree of sensitivity to interference of conspecifics (Goss-Custard 1980, Piersma 1985). In contrast to social and interference-insensitive species, the distribution of such interference-sensitive species should mostly be determined by the distribution of food resources (Folmer *et al.* 2010). Thirdly, each of these species should differ in its degree of association with mussel and oyster reefs. For example, as blue mussels form a substantial part of their diet, oystercatchers tend to be highly associated with reef builders (Goss-Custard 1996). Eurasian curlew typically respond to an increased abundance of crabs and shrimps in and near reefs compared to sandy intertidal flats, but they also feed on bare mudflats (Goss-Custard and Jones 1976, Petersen and Exo 1999). The degree of association for bar-tailed godwits is probably lower, since they feed on a large variety of benthic animals often along the edge of the receding and advancing tide (Goss-Custard et al. 1977, Scheiffarth 2001). Black-headed gulls feed on a large variety of prey and can be found in many different habitats (Dernedde 1994, Kubetzki and Garthe 2003).

Data analysis

Both study sites were subdivided by Thiessen polygons (Thiessen 1911) in ArcGIS (Environmental Systems Research Institute, Redlands, California, USA). Each polygon defines a discrete area around each sampling station (both random and predetermined) in such a way that any location inside the polygon is closer to that

point than to any of the neighboring points. No great differences were detected between shorebird numbers during the four tidal cycles, so data were pooled to calculated densities. Densities of each bird species (# ind. m^{-2}) were calculated for each polygon and merged into a single master dataset that now contained data on abiotic variables (sediment organic matter, silt and redox), biomass of all benthic species and bird densities for each sampling station. To approach a normal distribution for analyzed variables, organic matter content was reciprocally transformed ($y = 1/x$), redox potential was log transformed ($y = \log_{10}(x)$) and all other variables were square root transformed ($y = \sqrt{x}$).

Next, we used Structural Equation Modelling (SEM; in Amos v18) to test the spatial effects of the reefs on abiotics and the possible direct and indirect effects on the distribution of macrobenthic and bird species. For each bird species, we created default models that included all potentially important causal relationships between straight-line distance to the centre of the reef (calculated in ArcGIS), directional effects that may arise from strong winds or currents (calculated in ArcGIS as the deviation of each station from the north-south axis through the centre of the reef), sediment conditions (organic matter, silt fraction & redox), macrobenthos biomass and bird density (Fig. 6.1). These models focus on

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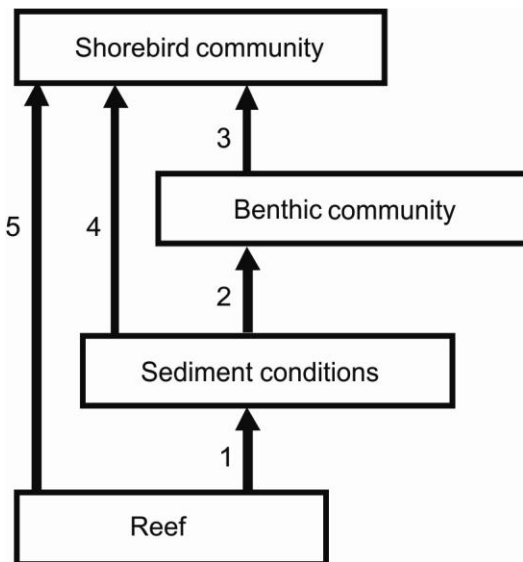
Fig. 6.1. The conceptual path analysis model. Arrows depict direct effects of one variable (boxes) on another. Numbers represent specific mechanisms described in *Materials & Methods: Data analysis*.

Table 6.1. Variables included in the model to test the default model for each focal bird species. General prey preference of the four common shorebird species (Dernedde 1994, Kubetzki and Garthe 2003, Folmer et al. 2010 and references therein) used in SEM is listed together with sediment conditions and distance and direction to the centre of a reef. Shore crabs (*Carcinus maenus*) and brown shrimps (*Crangon crangon*) were clustered as crustaceans.

	Model			
Prey species	Oystercatcher	Curlew	Bar-tailed godwit	Black-headed gull
Arenicola marina		+	+	+
Lanice conchilega		+	+	
Hediste diversicolor	+	+	+	+
Heteromastus filiformis			+	
Scoloplos armiger			+	+
Cerastoderma edule	+			+
Macoma balthica	+	+	+	+
Mytilus edulis	+			
Scrobicularia plana	+	+		
Crustaceans (*Carcinus maenas* & *Crangon crangon*)		+	+	+
Sediment conditions				
Organic matter	+	+	+	+
Silt	+	+	+	+
Redox	+	+	+	+
Reef builder presence				
Distance to centre of reef	+	+	+	+
Direction to centre of reef	+	+	+	+

explaining shorebird distribution from information on underlying resources. Therefore, each model only included macrobenthos species that are known prey items for that particular bird species (Table 6.1). Apart from modelling the effect of prey density on shorebird distribution, the models also tested for possible relationships between sediment variables, distance to the reef, and bird density. Sediment conditions can, directly or indirectly, affect bird distribution (Myers et al. 1980, Yates et al. 1993, Johnstone and Norris 2000). Furthermore, distance to the reef might influence bird distribution because birds may be attracted to these areas in anticipation of altered sediment conditions and prey densities. In summary, all four default models include (Fig. 6.1): (1) effect of distance and direction to the reef on sediment variables, (2) the effect of sediment variables on macrobenthos, (3) effects of macrobenthos variables on bird density, (4) direct effects of sediment variables on bird density, and (5) effect of distance to the reef on bird density.

To test whether the identified relationships extended beyond the reefs themselves, we analysed each model twice – once with all data points included (119 stations) and a second time with the stations inside the reefs excluded (111 stations). Models were analysed with stepwise backward elimination of relations included in the default model (threshold significance for elimination: p<0.05). After each elimination step, we used the χ^2 test (probability level > 0.05) to test for an adequate fit (i.e. that observed data did not differ significantly from those predicted by the model), and compared the model to previous models using Akaike's Information Criterion (AIC). Unidentified models were excluded from the results. We also excluded macrobenthic species from the model if they were not correlated with the modeled bird species, while sediment conditions were omitted if they were not related with either macrobenthic species or bird density. Furthermore, when abiotic or benthic variables exhibited strong significant collinearity (r>0.4) without one explaining the other (e.g., different proxy's for sediment conditions), we only included the variable with the highest explained variation in our models. The latter was done because SEM models become notoriously unreliable when relations with very strong covariance are included (Petraitis et al. 1996, Grewal et al. 2004).

RESULTS

Organic matter, silt content and redox were all highly correlated (r-values for OM-silt, OM-redox and silt-redox were 0.9, 0.5, and 0.5 respectively) and exhibited strong spatial gradients, with organic matter and silt increasing and redox decreasing in the direction of the reef. A map overlay of organic matter and the distribution of the four shorebird species suggest that oystercatchers, and to a lesser extent also curlews and bar-tailed godwits, tend to aggregate in these organic matter-rich areas in and around the reefs (Fig. 6.2). In contrast, the spatial distribution by black-headed gulls appears much less affected by the presence of the reefs.

Organic matter was included as a proxy for sediment conditions in the SEM models instead of silt content or redox because of its highest explained variation (R^2's were 0.45, 0.31 and 0.43 respectively). The distributions of several macrobenthic species were strongly affected by sediment organic matter, which in turn explained a significant part of the distribution of all four shorebirds (Fig.

Sediment organic matter content (%) 0 - 1   1 - 2   2 - 3   3 - 4   4 - 6

Meters
0   95 190   380   570   760
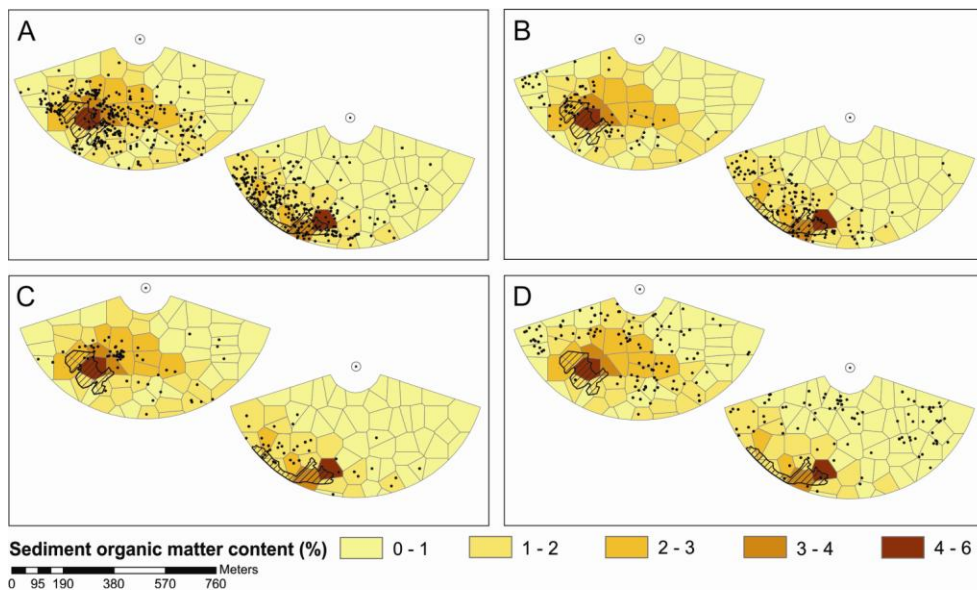
Fig. 6.2. Overview of the two reefs and their surrounding intertidal flats, showing Thiessen polygons (each polygon contains one sampling station), the position of the reefs (striped black areas) and the distribution of sediment organic matter content in relation to the distribution of (A) oystercatchers (B) curlews, (C) bar-tailed godwits and (D) black-headed gulls. Black dots represent the positions of the birds. Circles with a black dot indicate the position of the observation platforms.

6.3; Appendix 6A & B). The correlations suggest that organic matter had a positive effect on the biomass of *Lanice conchilega, Hediste diversicolor*, *Cerastoderma edule* and crustaceans (explaining 7%, 12%, 39 % and 11% of their variance, respectively).

All default models based on the fully saturated model (Fig. 6.1) and species-specific feeding relations (Table 6.1) demonstrated poor model-data fits (Table 6.2). After stepwise backward elimination and removal of non-significant relations, all final models demonstrated a strong fit. In contrast to the default models, final models demonstrated low Chi-square values, a probability level above 0.05 and low AIC's (Table 6.2; Appendix 6A). After removing the sampling stations within the reefs from the dataset, all final models still had an adequate fit and the structure of the models remained nearly identical (Table 6.2; Fig. 6.3). The models including the sampling stations on the reefs yielded a slightly better fit for

Table 6.2. Model fit summary from SEM for the default model and the final modified model for the dataset with all sampling stations included and for the dataset wherein the sampling stations inside the reefs were excluded.

	Oystercatcher		Curlew		Bar-tailed godwit		Black-headed gull	
	default	final	default	final	default	final	default	final
All stations								
χ^2	46.383	0.220	70.176	3.753	113.654	0.937	97.784	0.767
df	11	1	28	4	38	2	31	2
Probability level	<0.001	0.639	<0.001	0.441	<0.001	0.626	<0.001	0.682
AIC	96.383	18.220	146.176	25.753	193.654	16.937	167.784	16.767
Stations inside reefs excluded								
χ^2	32.494	1.378	46.505	1.210	92.908	1.742	72.309	1.387
df	11	1	28	2	38	2	31	2
Probability level	0.001	0.240	0.015	0.546	<0.001	0.419	<0.001	0.500
AIC	82.494	19.378	122.505	17.210	172.908	17.742	142.309	17.387

oystercatchers, bar-tailed godwits and black-headed gulls, whereas the model for curlews improved after removing the reef stations.

The final models for each bird species revealed significant correlations with macrobenthic species, but also with abiotic variables. Distance to the reef, organic matter and *Cerastoderma edule*, were significant predictors of oystercatcher density (Fig. 6.3A & E), with the final model explaining 62 (including local effects) to 59 (excluding local effects) % of the variance. The standardized effect of distance to the reef on oystercatcher density (-0.417 to -0.380) was stronger than the effect of organic matter (0.338 to 0.331) and biomass of *Cerastoderma edule* (0.152 to 0.179). For curlews (51 to 44 % of the variance explained), crustaceans and distance to the reef were significant predictors for both models (Fig. 6.3B), while *Hediste diversicolor* was dropped in the model that excluded the reef effect (Fig. 6.3F). The standardized effect of distance to the reef on curlew density (-0.597 to -0.617) was larger than the effect of crustacean biomass (0.195 to 0.168) and biomass of *Hediste diversicolor* (0.141, only in the model which included local effects). *Lanice conchilega* and organic matter were the two significant predictors of densities of bar-tailed godwits (Fig. 6.3C & G). The standardized effect of organic matter on bird density (0.370 to 0.386) was larger than that of the biomass of *Lanice conchilega* (0.227 to 0.187) and the final models both explained
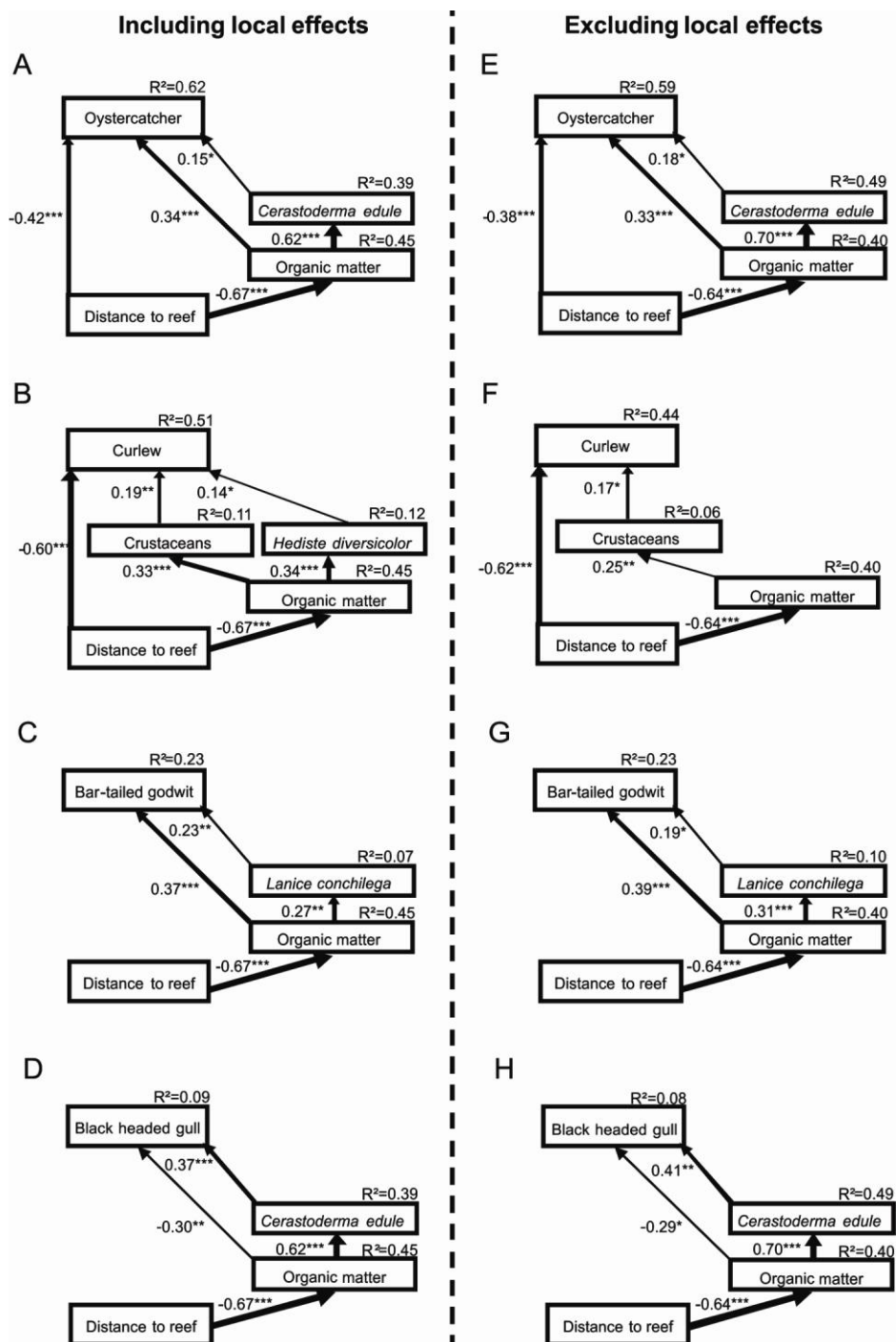
Fig. 6.3. Diagram of the SEM results with all sampling stations for oystercatchers, curlews, bar-tailed godwits and black-headed gulls (**A** to **D**) and the sampling stations inside the reefs excluded for the same bird species (**E** to **H**). Straight arrows indicate significant direct effects. The line thickness of each arrow indicates the magnitude of the standardized path coefficient, which is presented numerically next to each path. The R^2 values adjacent to the boxes represent the total variance explained by all significant predictors (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

23% of the observed variance. Finally, for black-headed gulls, organic matter and *Cerastoderma edule* were significant predictors of density (Fig. 6.3D & H). The standardized effect of *Cerastoderma edule* on black-headed gull density (0.372 to 0.405) was larger than the effect of organic matter (-0.303 to -0.289). The final models explained 9 to 8 % of the variance.

DISCUSSION

Although ecosystem engineering can determine the spatial distribution of resources (e.g. Gutierrez et al. 2003, van de Koppel et al. 2006) and resources in turn importantly control the distribution of consumers (e.g. Nachman 2006, Folmer et al. 2010, Piersma 2012), the interaction between these two processes so far has rarely been examined (Olff *et al.* 2009). Here we demonstrate that ecosystem engineers can affect consumer-resource interactions far beyond their own physical spatial boundaries in intertidal soft-sediment systems. Reef building bivalves like mussels and oysters cover a relatively small part of the intertidal mudflats of the Wadden Sea (± 1%). Our results, however, imply that their ecological impact is much larger than their size may suggest.

We found strong spatial gradients of increasing sediment organic matter and silt fraction and decreasing redox potential in the direction of mixed mussel- and-oyster reefs, which in turn affected the distribution of benthic species. Moreover, distance from the reefs, sediment characteristics, and prey abundance simultaneously affected the distribution of the three studied species that have more or less specific prey requirements (oystercatchers, curlews and bar-tailed godwits). This is most likely because the birds feed in the modified areas in anticipation of higher prey abundances. Black-headed gulls, the only species that did not cluster on and around the reefs, are versatile foragers with many diet

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options and this may explain why the reefs and the modified areas did not affect their spatial distribution. When the data points for the reefs themselves were excluded from the statistical analysis, the outcomes did not change, thus emphasizing the importance of the spatial extended effects of reefs. Only the ragworm *Hediste diversicolor* was excluded from the model as predictor for the distribution of curlews. This was, however, understandable as ragworms were mostly found in muddy sediments in and around the mixed reefs.

Community structure alteration by ecosystem engineers through spatially extended habitat modification seems to occur in many different ecosystems including beaver-inhabited wetlands (Wright *et al.* 2002) and cordgrass-inhabited cobble beaches (Bruno 2000). However, the relevance of habitat modification by ecosystem engineers on its surrounding and higher trophic levels may vary with environmental conditions. For instance, while our results show that habitat modification by reef-builders can be pronounced and exceed the spatial boundaries of the reefs themselves, spatial engineering effects by the same species on rocky shores are typically more limited. In these systems, blue mussels modify environmental conditions mainly by providing structural protection for associated fauna (Thiel and Ullrich 2002, Gutierrez et al. 2003). Hard substrate is already present and fine particles produced by mussels (faeces and pseudofaeces) are washed away by more intense hydrodynamics, resulting in more limited modifications at larger spatial scales (Thiel and Ullrich 2002). Furthermore, the effect of habitat modification by reef builders may also interact with the presence of other ecosystem engineers. For example, the tube-worm *Lanince conchilega* is also considered as an ecosystem engineer in soft-sediment systems, as their tubes provide substrate and facilitate the deposition of fine sediments (Friedrichs et al. 2000, Zühlke 2001). Since the presence of *L. conchilega* is positively correlated with the abundance and richness of the benthic community (Zühlke 2001, Callaway 2006, Godet et al. 2011), *L. conchilega* may locally enhance the engineering effect of the reefs on the benthic and shorebird community.

In our study, Structural Equation Modeling proved to be a useful tool for disentangling the relative importance of consumer-resource interactions and spatial habitat modification by ecosystem engineers. Using stepwise backward elimination of significant relations, we obtained models with reliable fits of multiple ecologically relevant variables. The method is correlative and does therefore not provide any direct evidence. Ideally, this method should be

complemented with other, more direct approaches like smaller-scale manipulative experiments. However, before the reefs established themselves seven years ago the study the area was sandy and homogeneous, and in this respect the study reported here can be regarded as experimental (but in want of detailed description of the re-establishment situation).

In conclusion, our results indicate that consumer-resource interactions can be affected by reef-builders far beyond the spatial boundaries of the reefs. This implies that these reefs have a much larger ecological impact on the intertidal community than their actual size suggests, which in turn means that loss of ecosystem engineers may result in disproportionally large consequences for biodiversity values in protected intertidal areas, like the Wadden Sea. Although the Pacific oyster is an alien species that invaded the Wadden Sea in the late 1970s (Troost 2010 and references therein), recent studies showed that oyster reefs might compensate for the large loss of mussels in 1990-1991 by replacing the ecological function of blue mussel reefs (Kochmann et al. 2008, Markert et al. 2009, Troost 2010). Nevertheless, the effects of Pacific oysters on the intertidal community and trophic interactions should be further investigated. Overall, our study emphasizes that conservation and restoration of reef builders should be considered a crucial step in the restoration of such systems.

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Acknowledgements

APPENDICES

Appendix 6A. Results from structural equation modeling after stepwise backward elimination of relations for each shorebird model. All sampling stations were included in these models. Distance represents distance to the centre of a reef.

	Model			
	Oystercatcher	Curlew	Bar-tailed godwit	Black-headed gull
Squared multiple correlations (R^2)				
Organic matter	0.450	0.450	0.450	0.450
Lanice conchilega			0.072	
Hediste diversicolor		0.117		
Cerastoderma edule	0.387			0.387
Crustaceans		0.111		
Oystercatcher	0.616			
Curlew		0.511		
Bar-tailed godwit			0.233	
Black headed gull				0.090
Standardized regression weights (p-value)				
Distance > Organic matter	-0.671 (<0.000)	-0.671 (<0.000)	-0.671 (<0.000)	-0.671 (<0.000)
Organic matter > *L. conchilega*			0.268 (0.003)	
Organic matter > *H. diversicolor*		0.342 (<0.000)		
Organic matter > *C. edule*	0.622 (<0.000)			0.622 (<0.000)
Organic matter > Crustaceans		0.333 (<0.000)		
L. conchilega > Bar-tailed godwit			0.227 (0.007)	
H. diversicolor > Curlew		0.141 (0.033)		
C. edule > Oystercatcher	0.152 (0.037)			
C. edule > Black headed gull				0.372 (0.007)
Crustaceans > Curlew		0.195 (0.003)		
Distance > Oystercatcher	-0.417 (<0.000)			
Distance > Curlew		-0.597 (<0.000)		
Organic matter > Oystercatcher	0.338 (<0.000)			
Organic matter > Bar-tailed godwit			0.370 (<0.000)	
Organic matter > Black headed gull				-0.303 (0.007)

Appendix 6B. Results from structural equation modeling after stepwise backward elimination of relations for each shorebird model. Sampling stations inside the reefs were excluded in these models. Distance represents distance to the centre of a reef.

	Model			
	Oystercatcher	Curlew	Bar-tailed godwit	Black-headed gull
Squared multiple correlations (R^2)				
Organic matter	0.404	0.404	0.404	0.404
Lanice conchilega			0.097	
Cerastoderma edule	0.495			0.495
Crustaceans		0.060		
Oystercatcher	0.590			
Curlew		0.441		
Bar-tailed godwit			0.229	
Black headed gull				0.083
Standardized regression weights (p-value)				
Distance > Organic matter	-0.636 (<0.000)	-0.636 (<0.000)	-0.636 (<0.000)	-0.636 (<0.000)
Organic matter > *L. conchilega*			0.311 (0.003)	
Organic matter > *C. edule*	0.704 (<0.000)			0.704 (<0.000)
Organic matter > Crustaceans		0.245 (<0.008)		
L. conchilega > Bar-tailed godwit			0.187 (0.034)	
C. edule > Oystercatcher	0.179 (0.037)			
C. edule > Black headed gull				0.405 (0.002)
Crustaceans > Curlew		0.168 (0.020)		
Distance > Oystercatcher	-0.380 (<0.000)			
Distance > Curlew		-0.617 (<0.000)		
Organic matter > Oystercatcher	0.331 (<0.000)			
Organic matter > Bar-tailed godwit			0.386 (<0.000)	
Organic matter > Black headed gull				-0.289(0.025)

Chapter 7

7

Discussion and Conclusions

Serena Donadi

DISCUSSION AND CONCLUSIONS

The central aim of this thesis was to investigate how ecosystem engineers such as cockles, mussels and lugworms modify the environment and how these habitat changes affect the structure of soft-bottom communities and the function of the intertidal ecosystem.

The general questions of this thesis were:

1) **How do coexisting ecosystem engineers interact to influence the physical environment? What is the importance of such engineering networks for the intertidal communities and the ecosystem functions?**

2) **Do positive feedbacks occur between engineered habitat and ecosystem engineers, so that ecosystem engineers favor their own survival?**

3) **What is the spatial scale of engineering effects in the intertidal?**

Conclusion 1) Networks of ecosystem engineers shape the tidal flat ecosystem

My research demonstrates that engineering effects of cockles, mussels and lugworms interact with each other and with external conditions to affect community structure and ecosystem functioning at large scales on the Wadden Sea tidal flats. The results suggest that intertidal soft-sediment ecosystems are structured by *networks of engineering species*.

On the tidal flats of the Wadden Sea, cockles, lugworms and mussels live in close contact together. So far, however, previous research focused on individual effects of these species on the surrounding habitat and communities (e.g. Flach 1996, Widdows et al. 2000, Volkenborn and Reise 2007, Commito et al. 2008, Ysebaert et al. 2009). I found that cockles, lugworms and mussels act synergistically to determine the structure of the macrobenthic communities at a landscape level (**Chapter 4**). The combination of species-specific engineering effects expanded the ecological space (Fig. 7.1A), thus leading to niche partitioning among different functional groups of organisms. This suggests that
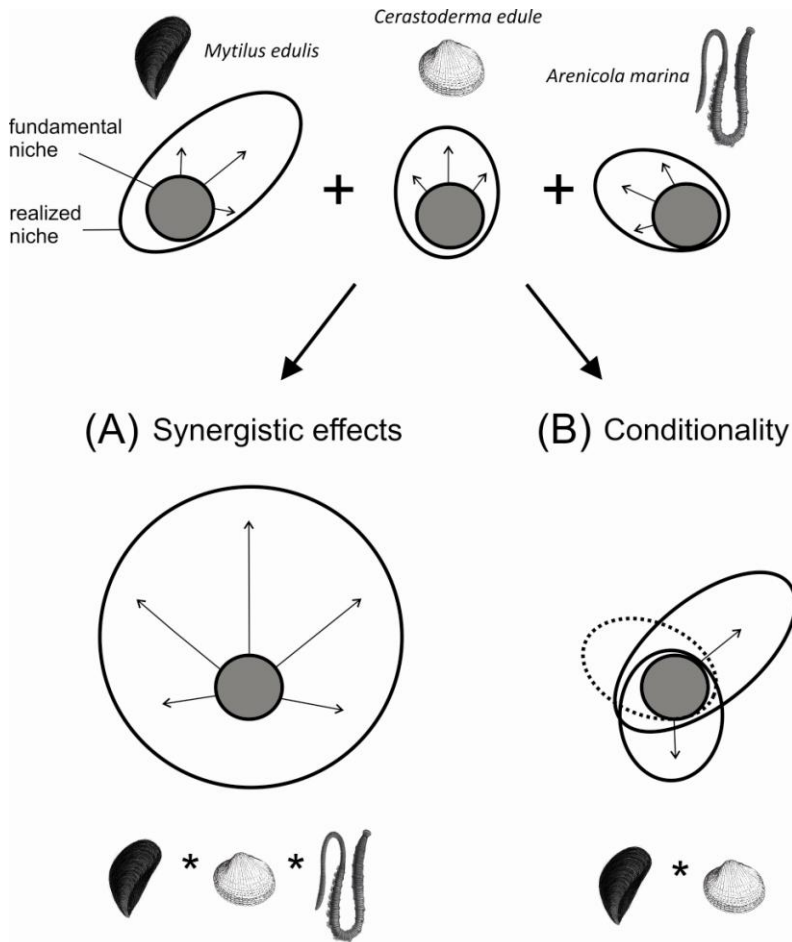
Fig.7.1. Conceptual model illustrating the effects of an engineering network. Through species-specific engineering, mussels, cockles and lugworms expand the ecological niche space of the species of the intertidal communities in different directions (Bruno et al 2003, this thesis). When these species co-occur, their engineering effects interact, so that the overall effect does not correspond to the sum of the individual effects. Rather, a network of engineers can generate (A) synergistic effects, i.e. the realized niche is larger than what can be predicted from the effects of single engineering species, and (B) context-dependent effects, i.e. the strength and direction of the effects of one engineering species depend on the presence of a second engineering species. For example, in the presence of mussel aggregations, engineering effects of lugworms are overwhelmed by engineering effects of mussels, while net effects of cockle engineering shift from sediment stabilization to bioturbation.

engineering networks rather than single ecosystem engineers contribute to the structuring of ecosystems. The existence of habitat-mediated interaction webs was already suggested by Jones and colleagues (1997) and Reise (2002), and some attempts have been made to incorporate ecosystem engineering networks into classical food web ecology (Olff et al. 2009, Kefi et al. 2012). Nevertheless, empirical evidence of such engineering webs was only limited to few case studies of interactions among two engineering species (Davidson and Lightfoot 2006, Baiser et al. 2008, Eklöf et al. 2011, Dijkstra et al. 2012). My work provides the first experimental evidence of a three-species engineering network and suggests that engineering networks might be a common phenomenon in natural ecosystems.

Furthermore, my work shows the high context-dependency of ecosystem engineering: the strength and direction of the effects of one ecosystem engineer might depend on the presence of a second ecosystem engineer, which typically has stronger impacts on the surroundings (Fig. 7.1B). For example, I found that cockles could promote microphytobenthos biomass at a local scale by increasing sediment stability (**Chapter 2**). However, these effects were observed only at bare sandy areas, where wave energy and sediment disturbance were high. Coastward of mussel reefs, cockles did not affect algal biomass, which was already enhanced by large-scale engineering effects of mussels on hydrodynamic and sediment properties. Similarly, engineering effects of cockles and lugworms on sediment parameters were often conditional to the vicinity of mussel reefs. Bioturbating effects of lugworms, such as enhanced sediment erosion and reduced sediment organic matter content, were detected mainly at the sandy sites unaffected by mussel beds (**Chapter 2** and **Chapter 3**). Also, cockles acted as sediment stabilizers at the sandy sites, where they promoted sediment accumulation (**Chapter 2** and **Chapter 3**), while they acted as bioturbators in the lee of mussel reefs, lowering the amount of organic matter in the sediments (**Chapter 3** and **Chapter 4**). These results suggest that combined effects of multiple ecosystem engineers are not necessarily predictable from the effects of the single species alone and emphasize the complex relationships occurring within engineering networks. The incorporation of such knowledge into current management strategies can improve the conservation and restoration of ecosystems that depend of networks of multiple engineering species.

Conclusion 2) Intertidal loops: positive feedbacks between bivalves, sediment stability and primary producers

My work shows that soft-bottom communities are regulated by multiple internal feedbacks between species and the environment which are tightly interlocked together and structure the community at both local and regional scale (Fig. 7.2).

Adult cockles can favor the recruitment of future generations by increasing sediment accumulation, suggesting that a positive feedback occurs between cockles and sediment stability (**Chapter 3**). This is in agreement with previous observations that coarser sediments attract lower densities of bivalve spat (Piersma et al. 2001, Beukema and Dekker 2005). However, such "cockle loop" might be much more complex, being connected to other abiotic-biotic loops. By affecting sediment properties, cockles facilitate microphytobenthos growth (**Chapter 2**), which is a source of food for suspension feeders (Sauriau and Kang 2000, Filgueira and Castro 2011), and can in turn benefit cockle survival. This gardening effect might occur not only at local scales, through the engineering of cockles, but also at large scales, within a "mussel loop". Indeed, mussel reefs increase microphytobenthos biomass over hundred meters from their reefs (**Chapter 2**), thus creating a source of food which is made available through sediment resuspension and transport by waves and currents (de Jonge and van Beusekom 1995). Furthermore, positive feedbacks can occur between sediment accumulation and microphytobenthos growth (van de Koppel et al. 2001). Low water content in locally elevated areas allows the persistence of extracellular polymeric substances (EPS), water-soluble colloidal carbohydrates excreted by diatoms which increase sediment cohesiveness, thereby stimulating diatom growth (Blanchard et al. 2000, Weerman et al. 2010). Finally, positive interactions between mussels and cockles might indicate an extra link between these intertidal loops. Mussel reefs can promote cockle recruitment in the lee of their reefs by slowing down current velocities (**Chapter 3** and **Chapter 5**), and cockle beds can provide hard substrata for the attachment of mussel spat (Ramón 1996), so that distributional patterns of mussel and cockle beds on soft-bottom intertidal areas might be correlated to a certain degree (**Chapter 5**). Hence, the tidal flats seem to be structured by a number of habitat-mediated positive feedbacks, which encompass different spatial scales (Fig. 7.2) and might be essential to guarantee

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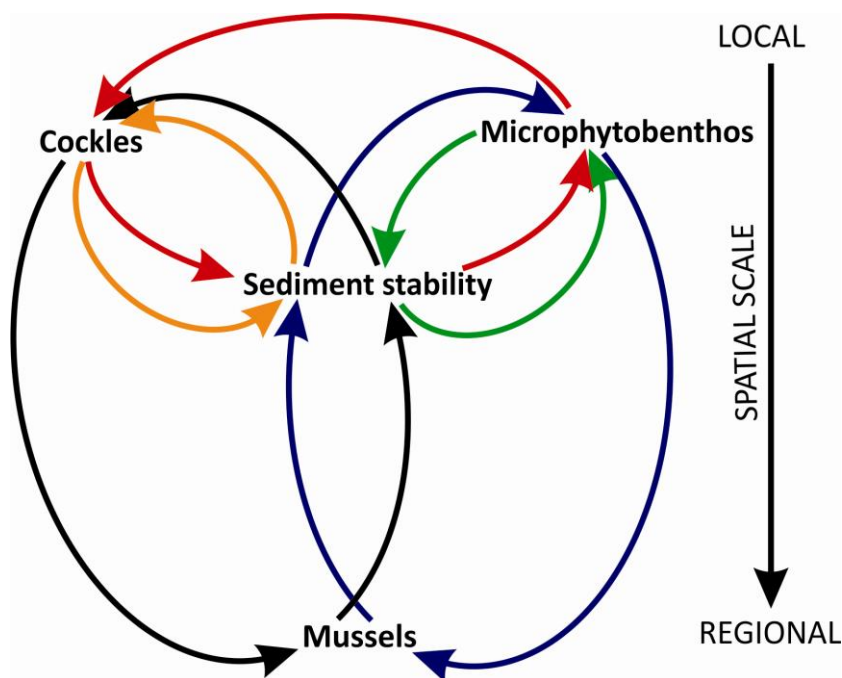
Fig. 7.2. Positive feedbacks between cockles, microphytobentos, mussels and sediment stability form several interconnected biotic-abiotic loops that encompass different spatial scales and structure the intertidal communities of the Wadden Sea.

high levels of benthic primary and secondary production (this thesis, van de Koppel et al. 2001).

The strong interplay between engineering species and environment leads to a high degree of spatial self-organization (Hui et al. 2004, Rietkerk and van de Koppel 2008). Indeed, regular spatial patterns in intertidal ecosystems are formed by mussels (van de Koppel et al. 2005), seagrass (van der Heide et al. 2010) and diatoms (Weerman et al. 2010) at a local scale, while large-scale distributional patterns of many benthic species covary with the presence of bivalve reefs (**Chapter 5** and **Chapter 6**). Spatial self-organization is an important emergent property of the ecosystem, enhancing the stability and productivity of the community (van de Koppel et al. 2005, van de Koppel et al. 2008, Weerman et al. 2010).

The occurrence of non-linear dynamics has important implications for the management and conservation of estuarine communities: overexploitation of ecosystem engineers and human-induced alteration of sediment conditions might remove important biological feedbacks, thereby leading to drastic and unexpected changes in the ecosystem functioning and very slow recovery dynamics (Nyström et al. 2012). This thesis shows that the tidal flats are complex systems characterized by high internal regulation and non-linear dynamics. Addressing such complexity will be an important future challenge to successfully understand and preserve soft-bottom intertidal communities.

Conclusion 3) Engineering effects of mussel reefs extend through space and trophic levels

I found that engineering effects of blue mussels can extend for several hundred meters from their reefs and affect multiple trophic levels at large spatial scales. Mussel beds therefore affect neighboring habitats and communities, and create long-range interactions between organisms that are typically sessile or sedentary.

In **Chapter 5** I show that reefs of blue mussels determined a peak in cockle densities at ~ 100 m coastward of the mussel reefs. This spatial pattern in cockle abundance was caused by the combination of facilitation of cockle recruitment, which prevailed at a distance from the mussel reefs, and inhibition of cockle growth and survival close to the reefs. Facilitative effects on cockle recruits were due to hydrodynamic stress alleviation, while negative effects were caused by the filter-feeding and biodeposition of mussels, which resulted in competition for food and deteriorated sediment conditions. Enhancement of cockle recruitment at a distance from mussel beds was also confirmed by fieldwork data reported in **Chapter 3**. This demonstrates that intertidal ecosystems may be structured by long-range interactions, and imply that effective protection of specific habitats should consider potential linkages to neighboring communities.

However, long-range engineering effects of mussels are not only limited to cockles. Microphytobenthos biomass can be enhanced at large spatial scales coastward of mussel reefs, where sediment disturbance is reduced (**Chapter 2**). Also, by creating spatial gradients of sediment organic matter and silt content, beds of mixed mussels and oysters (*Crassostrea gigas*) were found to influence

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the abundance of many benthic species (**Chapter 6**), which constitute an important source of food for several birds (Folmer et al. 2010). Macrobenthic species and birds that feed on them are thus dependent on large-scale sediment changes caused by the bivalve reefs rather than on the reefs themselves. All this evidence demonstrates that the influence of reef-building bivalves extends well beyond the boundaries of the actual reefs, affecting all levels of the intertidal trophic webs, from primary producers to higher predators. The extensive exploitation of beds of blue mussels, which in the 1990s largely contributed to the extirpation of mussel beds from the intertidal (Beukema and Cadee 1996, Eriksson et al. 2010), could therefore entail major consequences for the entire ecosystems of the tidal flats. The acknowledgment of long-range interactions is therefore crucial for effective conservation and restoration of the tidal flat ecosystem.

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Chapter 8

8

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SUMMARY

SUMMARY

Ecosystem engineers play a crucial role in both the terrestrial and marine realms. Through habitat-mediated interactions ecosystem engineers affect natural communities at various trophic levels and spatial scales, eventually determining ecosystem functioning. Despite increasing public awareness of their value, many ecosystem engineers are threatened worldwide. In the Wadden Sea, one of the world's largest intertidal soft-sediment ecosystems, engineering species such as the common blue mussel *Mytilus edulis* and the edible cockle *Cerastoderma edule* have declined dramatically in the last century, following human overexploitation (Eriksson et al. 2010, Kraan et al. 2011). These species might play an important role as sediment-stabilizers, preventing sediment erosion (Commito et al. 2005, Andersen et al. 2010), as opposed to bioturbators such as the polychaete *Arenicola marina* (lugworm), which promotes loss of fine particles and oxygen penetration in the sediments (Reise 2002, Volkenborn et al. 2007).

The general questions of this thesis were: **what are the effects of cockles, lugworms and mussels and their interactions on sediment stability, and how do these habitat changes affect the structure of soft-bottom communities and the function of the intertidal ecosystem?**

To answer these questions, I set up a large-scale field experiment on the tidal flats of Schiermonnikoog, where I manipulated a total of 2 400 m^2 by adding 64 000 lugworms and 800 000 cockles to different 5×5 m plots, both in the plumes of biodeposits created by mussel beds and in sandy areas without mussels.

Effects on microphytobenthos: facilitation or inhibition?

First of all, I investigated the engineering effects of cockles, lugworms and mussels on the microphytobenthos (**Chapter 2**). The microphytobenthos, which is mainly constituted by benthic diatoms, contributes significantly to the primary production of the tidal flats and can influence sediment dynamics by forming biofilms on the sediment surface (MacIntyre et al. 1996, Miller et al. 1996). My

large-scale experiments showed that while lugworms did not significantly affect algal growth, high density of cockles promoted algal biomass in sandy sediments where hydrodynamic stress was relatively high. By enhancing sediment stability the cockles prevented resuspension of algae and subsequent transport by waves and currents. The local effects of cockles were overruled by large-scale engineering of mussels coastward of their reefs, where enhanced microphytobenthos growth was likely due to hydrodynamic stress alleviation and biodeposition. Therefore, my results indicate that habitat-mediated interactions driven by both infaunal and reef-building bivalves play an important role for the primary production of soft-bottom intertidal areas.

The bivalve loop: sediment stabilization promotes cockle recruitment

Due to overfishing and habitat destruction, many bivalve populations have undergone dramatic reduction all over the world (Lotze et al. 2006). As many bivalves are ecosystem engineers that ameliorate their own environment (Bertness and Leonard 1997), I suggest that the lack of recovery can be partly caused by the loss of positive feedbacks from depleted populations (**Chapter 3**). To investigate whether positive feedbacks occur between cockle populations and engineered habitat, the abundance of cockle recruits was monitored on the experimental plots over one year. Cockles promoted the accumulation of sediment, thus enhancing sediment stability and their own recruitment in sandy areas with high wave and current energy, which was shown to be negatively correlated with cockle spat abundance. Coastward of mussel reefs, where hydrodynamic energy was reduced, cockle spat abundance was also high. This suggests that an increase in sediment stability due to engineering of cockles or mussel beds created a positive feedback on cockle recruitment, which might guarantee the persistence and survival of cockle populations in the Wadden Sea.

Engineering networks in the tidal flats structure the macrobenthic communities

In **Chapter 4** I explored interactive effects of cockles, lugworms and mussels on associated communities of macrofauna. While most research on ecosystem engineering focuses on the effects on single engineering species, my large-scale experiment offered the chance to investigate how multiple engineers interact to shape the surrounding community at a landscape level. By using Biological Trait Analysis (BTA), I show that through opposite effects on sediment organic matter content and bed level stability, cockles, lugworms and mussels together increased habitat heterogeneity, thereby leading to niche partitioning among different functional groups of the macrobenthic community. I suggest that the occurrence of such networks of engineers is widespread among natural ecosystems and its recognition can improve our general understanding of the importance of ecosystem engineering as well as conservation and restoration policies.

Engineering effects of mussel reefs extend through space and trophic levels

Finally, long-range engineering effects of mussel beds were studied in the field by means of field surveys and a small-scale experiment (**Chapters 5 and 6**). Transect data showed a peak in cockle density at 50-100 m coastward of three different mussel beds (**Chapter 5**). Using tagged cockles, I found that this striking pattern was caused by a scale-dependent effect of mussel beds: close to the reefs survival of cockles was hindered as the mussels depleted algal concentration in the water and caused deteriorated sediment conditions through biodeposition, while farther away reduction of water flow velocity caused by the reefs promoted cockle recruitment. In **Chapter 6**, I report on long-range engineering effects of mussel beds on higher trophic levels. Sediment parameters and abundance of feeding birds were measured in 119 station points within and around two reefs of mixed blue mussels and Pacific oysters. A Structural Equation Model (SEM) demonstrates that the abundance of many bird species was explained by a combination of distance from the reefs, sediment organic content, which decreased with the distance from the reefs, and food abundance, which was

positively correlated with sediment organic content. Removing the sampling points within the reefs did not change the model results, thus highlighting the importance and the spatial extent of engineering effects of the reefs on higher trophic levels.

Concluding remark

My thesis demonstrates that communities living in intertidal flats are governed and connected by strong internal interactions mediated by biogenic habitat transformation. To consider the Wadden Sea as an externally-driven system, solely regulated by environmental forcing and resource availability would clearly be an oversimplification. By changing sediment properties in different ways, multiple ecosystem engineers together create spatial heterogeneity, influence the structure and functioning of the intertidal communities and shape associated food-webs. This suggests that thoughtless exploitation of ecosystem engineers can seriously affect the functioning of the Wadden Sea ecosystem and disrupt positive feedbacks between habitat and engineers, thereby leading to deteriorated conditions that may be hard to reverse.

The tidal flats of Schiermonnikoog offered a perfect setting where to study the complexity and relevance of habitat-mediated interactions. I really hope that this beautiful ecosystem will continue to excite curiosity among ecologists and fascinate people as it did to me.

SAMENVATTING

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Ecosysteembouwers spelen een cruciale rol in zowel het terrestrische als marine domein. Door interacties met hun omgeving beïnvloeden zij de natuurlijke gemeenschappen op verschillende trofische niveaus en ruimtelijke schalen, en zijn daarmee uiteindelijk bepalend voor het functioneren van het ecosysteem. Ondanks een toenemend publiekelijk besef van hun waarde, worden wereldwijd vele ecosysteembouwers bedreigd. In de Waddenzee, een van werelds grootste zacht-sediment getijdegebied, zijn, als gevolg van overexploitatie door de mens, ecosysteembouwers zoals de gewone mossel *Mytilus edulis* en de eetbare kokkel *Cerastoderma edule* dramatisch afgenomen (Eriksson et al. 2010, Kraan et al. 2011). Deze soorten kunnen een belangrijke rol spelen als sediment stabilisatoren en daarmee erosie voorkomen (Commito et al. 2005, Andersen et al. 2010), dit in tegenstelling tot bioturbators zoals de borstelworm *Arenicola marina* (wadpier), die het verlies van fijne deeltjes en zuurstof doordringing in de sedimenten bevorderd (Reise 2002, Volkenborn et al. 2007).

De algemene vragen van dit proefschrift waren: **wat zijn de effecten van kokkels, wadpieren en mossels, en hun interacties, op de sediment stabiliteit, en hoe werken deze habitatveranderingen in op de structuur van de zachte-sediment gemeenschappen en de functie van het getijde ecosysteem?**

Om deze vragen te beantwoorden heb ik op de getijdeplaten van Schiermonnikoog een grootschalig veldexperiment opgezet, waarin ik in totaal 2.400 m^2 manipuleerde door 6.400 wadpieren en 800.000 kokkels toe te voegen aan percelen van 5x5 m in zowel de afzettingen gevormd door mosselbedden als in de zandige gebieden zonder mossels.

Effecten op microfytobenthos: faciliteren of remmen?

Allereerst heb ik de bouweffecten van kokkels, wadpieren en mossel op het microfytobenthos onderzocht (Hoofdstuk 2). Het microfytobenthos, dat voornamelijk bestaat uit benthische diatomeeën, draagt significant bij aan de

primaire productie van de getijdeplaten en kan de dynamiek van het sediment beïnvloeden door de vorming van biofilms op het sedimentoppervlak (MacIntyre et al. 1996, Miller et al. 1996). Mijn grootschalige experiment toonden aan dat hoge kokkeldichtheden de algen biomassa in de zandige sedimenten, waar de hydrodynamische stress relatief hoog is, bevorderden, terwijl de wadpieren de groei van de algen niet significant beïnvloedden. Door de verbetering van de sedimentstabiliteit voorkwamen de kokkels dat de algen resuspendeerden en door golven en stromingen getransporteerd werden. De lokale effecten van de kokkels werden tenietgedaan door grootschalig werk van mossels aan de landzijde van hun riffen. Daar werd de toegenomen groei van microfytobenthos waarschijnlijk veroorzaakt door het verlagen van de hydrodynamische stress en biologische afzettingen. Dus mijn resultaten geven aan dat zowel bodembewonende als rifbouwende tweekleppigen, via interacties met hun omgeving, een belangrijke rol spelen bij de primaire productie in getijdegebieden met zacht sediment.

De tweekleppigen lus: sediment stabilisering bevordert kokkel rekrutering

Als gevolg van overbevissing en habitat beschadiging zijn vele populaties van tweekleppigen een dramatische gereduceerd (Lotze et al. 2006). Aangezien vele tweekleppigen ecosysteembouwers zijn, die hun eigen omgeving kunnen verbeteren (Bertness and Leonard 1997), veronderstel ik dat het ontbreken van herstel voor een deel veroorzaakt wordt door het ontbreken van positieve terugkoppelingen van uitgeputte populaties (Hoofdstuk 3). Om te onderzoeken of deze terugkoppelingen tussen kokkelpopulaties en door hun gebouwde omgeving plaatsvinden, werd gedurende één jaar de rijkdom aan kokkel rekruten op de experiment percelen geobserveerd. Kokkels bevorderden de accumulatie van sediment en verbeterden hun eigen rekrutering op zandige plekken met hoge golf en stroom dynamiek, waarvan aangetoond was dat die negatief correleerden met de aantallen kokkellarven. Aan de landzijde van mosselbedden, waar de hydrodynamische druk minder groot was, waren de aantallen kokkellarven ook hoog. Dit suggereert dat een toename in stabiliteit van het sediment als gevolg van invloed van kokkels of mosselbedden een positieve terugkoppeling op de

kokkel rekrutering creëert, waarmee het voortbestaan en overleven van kokkelpopulaties in de Waddenzee gegarandeerd lijkt.

Bouwnetwerken in/op de getijdeplaten structureren de macrobenthische levensgemeenschappen

In **Hoofdstuk 4** onderzocht ik de interactieve effecten van kokkels, wadpieren en mossels op geassocieerde levensgemeenschappen van macrofauna. Terwijl het meeste onderzoek aan ecosysteembouwers zich richt op de effecten van een enkele bouwende soort, bood mijn grootschalig experiment de mogelijkheid om te onderzoeken hoe meerdere bouwers samenwerken die daarmee de omgevende levensgemeenschap op landschapsniveau vorm geven. Door gebruik te maken van de *Biological Trait Analysis* (BTA) toon ik aan dat door tegengestelde effecten op het gehalte organische stof in het sediment en op stabiliteit van de ondergrond, kokkels, wadpieren en mossels samen de heterogeniteit van de omgevingen deden toenemen, daarbij leidend naar niche-differentiatie onder de verschillende functionele groepen van de macrobenthische levensgemeenschap. Ik suggereer dat het voorkomen van dergelijke netwerken in natuurlijke systemen wijd verbreid is en door ze te er-/herkennen kan ons algemene begrip over het belang van ecosysteembouwers verbeteren, evenals strategieën beheer en herstel.

Bouweffecten van mosselbedden strekken zich uit door de tijd en trofische niveaus

Tenslotte werden de langdurige bouweffecten van mosselbedden in het veld bestudeerd door middel van veldopnamen en een kleinschalig experiment (Hoofdstukken 5 en 6). Transect-gegevens vertoonden een piek in de kokkeldichtheid op 50-100 m afstand aan de landzijde van drie mosselbedden (Hoofdstuk 5). Door gebruik te maken van gelabelde kokkels ontdekte ik dat dit opvallende patroon werd veroorzaakt door een schaal afhankelijk effect van mosselbedden: Dicht bij het bed werd het overleven van kokkels beperkt omdat de mossels de algen concentraties in het water uitputten en, door hun afzettingen, een verslechterd sediment veroorzaakten, terwijl verder weg de vermindering van de loopsnelheid van het water, veroorzaakt door de

mosselbedden, de kokkel rekrutering bevorderde. In Hoofdstuk 6 rapporteren wij over lange afstand bouweffecten van mosselbedden op hogere trofische niveaus. Sediment parameters en aantallen foeragerende vogels werden gemeten op 119 meetpunten in en rondom twee gemengde bedden met blauwe mossels en Pacifische oesters. Een *Structural Equation Model* (SEM) laat zien dat de aantallen van vele soorten vogels werd verklaard door een combinatie van afstand tot de bedden, het organische gehalte van het sediment (neemt af bij grotere afstand van de bedden) en de voedselrijkdom (is positief gecorreleerd met het organisch gehalte van het sediment). Als de meetpunten in de bedden uit het model werden verwijderd dan veranderden de resultaten van het model niet, daarmee aangevend het belang en ruimtelijke strekking van bouweffecten van mosselbedden op hogere trofische niveaus.

Afsluitende opmerkingen

Mijn proefschrift toont aan dat levensgemeenschappen, die op getijdeplaten leven, gestuurd en verbonden zijn door sterke interne interacties die gefaciliteerd worden biologisch gegenereerde habitat transformaties. Om de Waddenzee als een extern gestuurd systeem te beschouwen, enkel gestuurd door krachten uit de omgeving en beschikbaarheid van hulpbronnen, zou zonder meer een versimpeling zijn. Door de eigenschappen van het sediment op verschillende manieren te veranderen, creëren meerdere ecosysteembouwers samen ruimtelijke heterogeniteit, beïnvloeden de structuur en het functioneren van getijdelevensgemeenschappen, en profileren daarmee geassocieerde voedselketens. Dit betekent dat te lichtvaardig exploiteren van ecosysteembouwers, het functioneren van het Waddenzee ecosysteem ernstig kan verstoren en de positieve terugkoppelingen tussen habitat en bouwers kan ontregelen, daarbij afglijdend naar slechtere omstandigheden die moeilijk omkeerbaar zijn.

De getijdegebieden van Schiermonnikoog leverden een perfecte locatie om de complexiteit en het belang van habitat-gefaciliteerde interacties te bestuderen. Ik hoop van harte dat dit mooie ecosysteem de nieuwsgierigheid van ecologen zal blijven aanwakkeren en mensen blijven fascineren, net als het mij gedaan heeft.

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Mamma grazie, e grazie Stefano for being my family. Non vedo l'ora di bere una birretta con te!

Thank you Toni. Not sure I can find the words…Everything started on the rocks (ok, I know, maybe it was plastic ☺), and now goes much beyond that.
I love every single moment of my time next to you…

LIST OF PUBLICATIONS

Peer reviewed publications

Donadi, S., J. Westra, E. J. Weerman, T. van der Heide, E. M. van der Zee, J. van de Koppel, H. Olff, T. Piersma, H. W. van der Veer and B. K. Eriksson. Non-trophic interactions control benthic producers on intertidal flats. Ecosystems, DOI: 10.1007/s10021-013-9686-8.

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Van der Heide, T., J. S. Eklöf, E. H. van Nes, E. M. van der Zee, **S. Donadi**, E. J. Weerman, H. Olff and B. K. Eriksson. 2012. Ecosystem engineering interacts with herbivory to shape an intertidal landscape. Plos One 7: e42060.

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Eklöf, J. S., **S. Donadi**, T. van der Heide, E. M. van der Zee and B. K. Eriksson. Using traits to understand contrasting effects of ecosystem engineers on associated communities. Oikos.

Conference presentations

98th ESA Annual Meeting, Minneapolis, USA, 3-9 August 2013. Oral presentation: "Engineering networks structure coastal ecosystems".

NWO – ZKO Symposium, The Hague, The Netherlands, 7-8 November 2012. Oral presentation: "The importance of benthic bioengineers for the carrying capacity of the Wadden Sea".
Poster presentation: "Mussels and cockles facilitate microphytobenthos at different spatial scales".

50th ECSA Conference, Venice, Italy, 3-7 June 2012. Oral presentation: "Multiple ecosystem engineers facilitate microphytobenthos at different spatial scales".

Netherlands Annual Ecology Meeting (NAEM) 2011, Lunteren, The Netherlands, 8-9 February 2011. Oral presentation: "Scale-dependent effects of an ecosystem engineer determine the spatial distribution of bivalves in an intertidal ecosystem".

ASLO Aquatic Science Meeting, Puerto Rico, USA, 13-18 February 2011. Oral presentation: "Scale-dependent effects of an ecosystem engineer determine the spatial distribution of bivalves in an intertidal ecosystem".

Special courses

14-16 November 2012 "*Meta Analysis*", held by W. Viechtbauer, and organized by the Graduate School for Production Ecology and Resource Conservation , Wageningen University, The Netherlands.

16-20 January 2012 "*An introduction to Structural Equation Modeling (SEM) – a tool for studying complex systems*", held by J. B. Grace and J. Byrnes, and organized by the Department of Systems Ecology, Stockholm University, Sweden.

Awards received

Outstanding Student Presentation Award at ASLO Aquatic Science Meeting, Puerto Rico, USA, 13-18 February 2011

Fondazione Brovedani Prize as best student of the Department of Mathematical, Physical and Natural Sciences, University of Trieste, Italy, June 2008

BIOGRAPHY

Serena Donadi was born in San Donà di Piave, Italy, on October 20, 1982. After graduating from high school at the Science institute Galileo Galilei in San Donà di Piave she moved to Trieste (Italy), where she earned a Bachelor's degree in Natural Science (2005) and a Master's degree in Marine Biology (2008) with full grades. Over the whole course of her studies and upon completion of her Master's degree, she worked at the Marine Protected Area (MPA) of Miramare (Trieste, Italy) as marine biologist assistant, environmental educator and sea-watching guide. In 2006 she undertook a three-year research project financed by the Italian Ministry of Environment on the collection and analysis of bioacoustical data using echosounder for the evaluation of fish populations within the MPA of Miramare. In 2007 she was awarded a grant from the European Commission and spent three months in the MPA Penisola del Sinis (Sardinia, Italy) studying sea urchin populations (*Paracentrotus lividus*) as an ecological indicator for MPA management effectiveness.

Between March 2009 and May 2013, she carried out her PhD-project at the Department of Marine Benthic Ecology & Evolution of the University of Groningen (The Netherlands) under the supervision of Prof. Britas Klemens Eriksson and Prof. Jeanine Olsen. The project focused on the effects of ecosystem engineers on sediment stability and community structure of intertidal soft-bottom ecosystems (this thesis) and was financed by a grant from the ZKO program of the Netherlands Organization of Scientific Research (NWO). During her PhD-research and the intense fieldwork required, Serena supervised seven MSc students, gave several lectures and supervised four practical courses on marine biology at the University of Groningen.

In 2011 she joined the Zostera Experimental Network, a global collaborative network of scientists investigating how local resources and grazing affect the structure and functioning of eelgrass (*Zostera marina*) ecosystems. Within the ZEN project, she first conducted fieldwork in Bødo (Norway) in 2011, and then in Virginia (US) in 2012, under the supervision of Prof. Emmett Duffy and Dr. Pamela Reynolds.

Serena has been recently awarded a Junior Fellowship at the Hanse-Wissenschaftskolleg - Institute for Advanced Study in Delmenhorst (Germany) to

investigate the link between environmental factors and the functional diversity of intertidal communities. The project will be conducted between October 2013 and April 2014 in collaboration with Prof. Helmut Hillebrand from the Institut für Chemie und Biologie des Meeres (ICBM), University of Oldenburg (Germany).