THE EFFECTS OF BIOGEOCHEMICAL STRESSORS ON SEAGRASS ECOSYSTEMS

Laura Leone Govers
2014

Govers, Laura L. (2014): The effects of biogeochemical stressors on seagrass ecosystems. PhD thesis, Radboud University Nijmegen, 238p. With summaries in English, Dutch & French.

This PhD project was financially supported by Projectbureau Zeeweringen and Rijkswaterstaat Zeeland and part of the project 'Zeegrasmitigaties Oosterschelde'

Layout & Design: Lilian Admiraal, lilianadmiraal@gmail.com
Photos: Laura Govers
Printed by: Ipskamp Drukkers
Correspondence: lauralgovers@gmail.com, lauragovers.com

THE EFFECTS OF BIOGEOCHEMICAL STRESSORS ON SEAGRASS ECOSYSTEMS

Proefschrift
ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. Th.L.M. Engelen,
volgens besluit van het college van decanen

in het openbaar te verdedigen op vrijdag 12 december 2014

om 10.30 uur precies

door

Laura Leone Govers

geboren op 16 september 1985
te 's-Hertogenbosch

Promotor
Prof. Dr. Peter M.J. Herman

Copromotor
Dr. Tjeerd J. Bouma, NIOZ Yerseke
Dr. Marieke M. van Katwijk

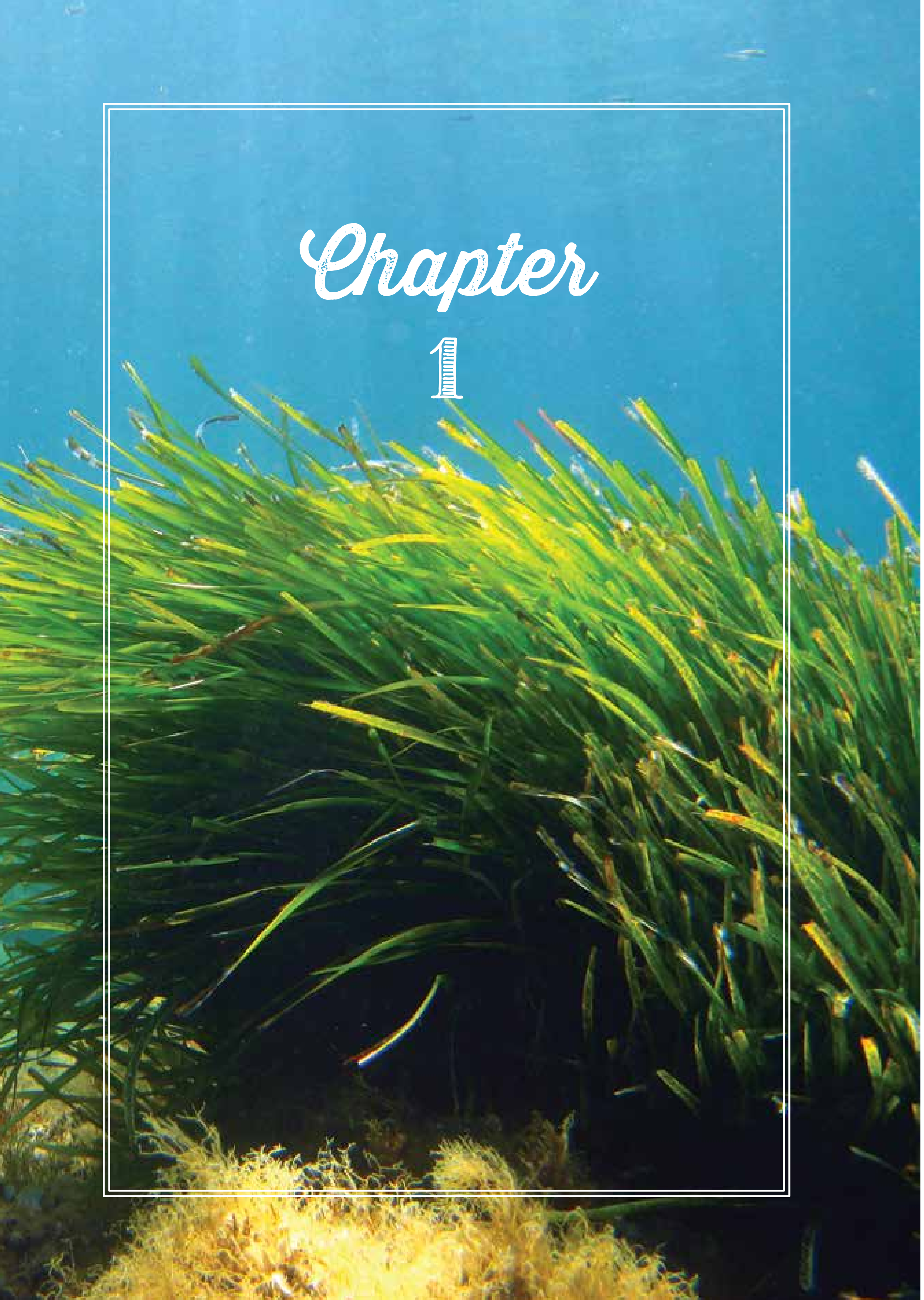
Manuscriptcommissie
Prof. dr. Jan G.M. Roelofs
Prof. dr. Jack B.M. Middelburg (Universiteit Utrecht)
Prof. dr. Marianne Holmer (Syddansk Universitet, Denmark)

Paranimfen
Marjolijn Christianen
Marloes Hendriks

“What we know is a drop, what we do not know is an ocean”

ISAAC NEWTON

CONTENTS



Chapter 1

INTRODUCTION

Seagrasses are aquatic angiosperms, the only flowering plants that are well adapted to live and reproduce in saline waters. Although they look somewhat similar to terrestrial grasses (Figure 1.1), they are not related to this group of plants, but evolved from freshwater macrophytes about 100 million years ago (den Hartog 1970, Les et al. 1997). The 12 genera of seagrass species belong to four different families: Zosteraceae, Posidoniaceae, Cymodoceae, and Hydrocharitaceae, forming an ecological rather than a taxonomical group (den Hartog and Kuo 2006). Like terrestrial grass species, seagrasses can form extensive meadows in shallow coastal waters all over the world (Green and Short 2003). The depth limit of seagrasses is determined by the water clarity, as they need light for photosynthesis, and the seagrass species *Posidonia oceanica* can be found up to 50 m depth in the clearest parts of the Mediterranean (Duarte 1991). In contrast, seagrasses in murky waters may grow to very shallow depths of only 1 m. Next to subtidal species, which grow constantly submerged, there are also seagrasses that occur in intertidal areas, where mainly desiccation stress and hydrodynamics determine their upper depth limits (Leuschner et al. 1998, Bjork et al. 1999).

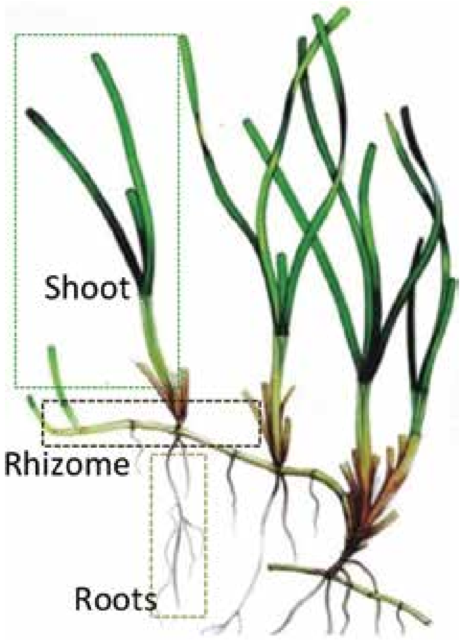


Figure 1.1 Impression of the structure of the tropical seagrass species *Cymodocea rotundata*. This plant consists of four clones, which are connected by a horizontal rhizome. Rhizomes are used for clonal expansion, but also for the storage of carbohydrate reserves. The roots take up nutrients from the sediment and anchor the plants. The shoots consist mainly of photosynthetic tissue, but are also used for the uptake of nutrients and carbon from the water column. Picture © Ruth Berry

The natural value of seagrass beds is often unknown or unrecognized compared to the attractive and colorful neighboring coral reefs, but the importance of seagrass ecosystems is comparable to that of coral reefs, mangroves and salt marshes in terms of ecosystem services and ecological richness. More importantly, these coastal ecosystems are often interconnected by means of migrating animals, nutrient fluxes, and organic carbon (Nagelkerken 2000, Cowen et al. 2006, Gillis et al. 2014). Seagrass ecosystems rank among the most productive and most valuable ecosystems on earth, in terms of value ($) per hectare (Costanza et al. 1997). This value can be attributed to the many ecosystem services that seagrass beds can provide: high rates of production and nutrient cycling (Duarte and Chiscano 1999), carbon sequestration (Fourqurean et al. 2012), coastal protection by attenuation of waves and currents and by stabilizing the sediment (Christianen et al. 2013), nursery habitat for commercial fish species (Nagelkerken 2000), and habitat and food for many endangered species (Valentine and Heck 1999, Christianen et al. 2012).

Seagrass beds under threat

Regrettably, seagrass beds have been declining rapidly over the past decades: with about 7% per year since 1990 – a rate of decline comparable to that of coral reefs and tropical rainforests (Orth et al. 2006, Waycott et al. 2009). The main reason for this decline is the increase of human activities in coastal areas (Short and Wyllie-Echeverria 1996). Nowadays, billions of people live in coastal areas all over the world (Cohen et al. 1997, Small and Nicholls 2003), and in a few decades, probably 50% of the entire human population will be living within 150 km from the shore (Cohen 2003). This development has lead to a steep increase of human activities in coastal areas such as dredging, aquaculture, sewage discharge, industrial activities, and deforestation (Cohen 2003, Mora 2008), which severely threaten coastal ecosystems, including seagrass beds.

These activities have resulted in eutrophication, high trace metal levels, habitat degradation, and increased water column sediment loads, which negatively affect seagrass ecosystems. In addition, also climate change may put pressure on global seagrass ecosystem functioning (Orth et al. 2006, Waycott et al. 2009). In theory, climate change may lead to circumstances favorable to seagrasses, as they evolved about 100 million years ago in times of higher CO_2 concentrations, temperatures and sea levels. However, current global change is much more rapid than ancient change rates and it may thus be hard for seagrasses to adapt to present changing environmental conditions (Orth et al. 2006), especially since an additional array of other stressors synergistically threaten seagrass ecosystems.
The most imminent threat to seagrass ecosystems is eutrophication (Burkholder et al. 2007). Coastal nutrient loads have strongly increased, due to agricultural run-off and untreated sewage discharge in bays, lagoons and estuaries (Short and Wyllie-Echeverria 1996, Burkholder et al. 2007). These high nutrient levels lead to increased turbidity and lower light-availability for seagrasses, as these favor fast growing plankton and macroalgae species at the expense of seagrasses (Figure 1.2) (Hauxwell et al. 2003, Kemp et al. 2005). Additionally, eutrophication may also directly affect seagrasses by promoting ammonium and sulfide toxicity (van Katwijk et al. 1997, Koch and Erskine 2001, Van der Heide et al. 2008).

Figure 1.2 Seagrasses (*Syringodium filiforme*) and (*Thalassia testudinum*) growing in a turbid, eutrophic bay on Curaçao, Netherlands Antilles.

Disappearance of seagrass beds also implies the loss of important ecosystem services that seagrasses provide, such as coastal protection (Christianen et al. 2013), fisheries (Gillanders 2006), and carbon sequestration (Fourqurean et al. 2012). Moreover, seagrass beds are strongly connected to other coastal ecosystems so degradation or disappearance of seagrasses may also affect ecosystem functioning and ecosystem services (e.g. fisheries) of nearby coral reefs, mangroves, and salt marshes. Therefore, stressors threatening seagrass beds and thus other coastal ecosystems should be recognized and halted to prevent further loss of coastal key-ecosystems and related ecosystem services. Fortunately, there is an increasing number of marine protected areas (MPAs) that include seagrass beds (Orth et al. 2006), and there have been worldwide initiatives on the restoration of seagrass meadows (Van Katwijk et al. submitted).

Seagrass dynamics and restoration

Seagrass beds form naturally dynamic landscapes which are maintained through asexual clonal expansion, sexual recruitment and the turnover of shoots (Duarte et al. 2006). These processes act over various spatial and time scales and unbalances in in these dynamic processes may result in changes on a meadow scale, such as patchy and heterogeneous landscapes, which may be more vulnerable to stressors than healthy seagrass beds (Chapter 2). Unbalanced seagrass dynamics may even result in catastrophic declines, due to altered disturbance-recovery dynamics (Chapter 4), which we discussed above (1.1) (Duarte et al. 2006). As seagrass beds have been disappearing on a global scale, many attempts have been made to restore seagrass beds to their original value. However, due to positive feedbacks in seagrass beds, seagrass restoration has been proven difficult (Van der Heide et al. 2007, van Katwijk et al. submitted). In the Netherlands, we have also been involved in large-scale mechanical seagrass transplants in the southwestern delta (Oosterschelde) (Box 1.1). In this thesis we answer some questions concerning these transplants: we investigate how important starch reserves are for the winter survival of transplanted seagrasses (Chapter 3) and we look further into the competition between *Zostera noltii* and the bioturbating lugworm *Arenicola marina* (Chapter 9) which has become more intense since lugworm numbers have been increasing in the Oosterschelde (Suykerbuyk et al. 2012).

BOX 1.1 SEAGRASS RESTORATION: A CASE STUDY FROM THE OOSTERSCHELDE

In the Dutch Oosterschelde Delta, Dike reinforcements have endangered the red-list seagrass species *Zostera noltii*, which was growing on the intertidal mudflats in the vicinity of the dikes. Therefore, large sods of *Zostera noltii* have been transplanted to several suitable locations within the Oosterschelde (Figure 1.3) (Giesen et al. 2012). These transplantations have been carried out in 2007, 2008, 2010, 2011, and 2012, and in total, about 3000 m^2 of dwarf eelgrass has been transplanted. As large-scale seagrass restoration is generally more successful than small-scale restoration (Van Katwijk et al. submitted), seagrass sods of 1.5x0.75 m were moved mechanically (Figure 1.4) and placed on a thick shell-layer to prevent lugworm bioturbation (Suykerbuyk et al. 2012).

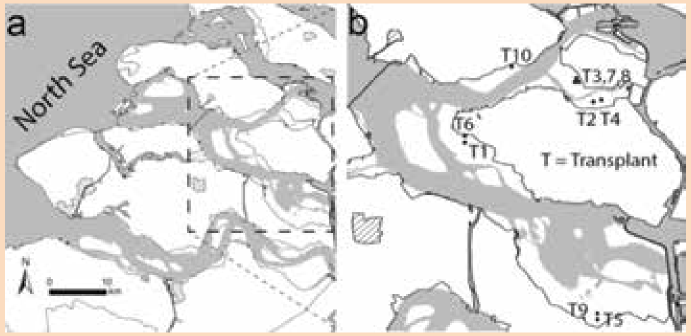


Figure 1.3 Map of the Dutch Delta (a), and the location of the transplant sites (b).

Seagrass transplants have been thoroughly monitored in the period 2007-2013 and many conditions have been measured, such as porewater biogeochemistry, seagrass densities, area, sediment conditions, seagrass tissue content, and environmental characteristics (e.g. number of lugworms, presence of macro algae). Unfortunately, apart from one greatly expanding seagrass transplant, most seagrass transplants have been decreasing in area since the moment of transplanting, and many have already disappeared (Suykerbuyk et al. submitted). This may be due to local effects such as sediment- and hydrodynamics, but it is also in line with the general decreasing trend of *Zostera noltii* in the Oosterschelde since the 1980s. This general decreasing trend may be a result of increased salinities, changed sediment dynamics and increased lugworm numbers since the implementation of the Delta Works, which is a large system of dams and dikes, that protects the Oosterschelde area and the province of Zeeland against flooding.

Figure 1.4 Photograph of mechanical large-scale seagrass transplantations

Feedbacks and seagrass ecosystem functioning

Seagrasses are considered as ecosystem engineers, which are species that "directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In doing so, they modify, maintain and recreate habitats" (Jones et al. 1994). Thus, seagrasses are able to manipulate their abiotic and biotic environment, which enables them to improve their own growing conditions with increasing density (Van der Heide et al. 2007, Van der Heide 2009). They can alleviate hydrodynamic stress by attenuating waves and currents (Fonseca and Cahalan 1992, Peterson et al. 2004), stabilize the sediment and entrap sediments and organic matter (Gacia et al. 2002, Carr et al. 2010). These processes reduce sediment resuspension and therefore improve the water clarity and subsequent light availability to seagrasses. Another important feedback mechanism in seagrass beds is density-dependent toxicity (Van der Heide et al. 2010), where e.g. ammonium toxicity (in the water layer) and sulfide toxicity (in the sediment) may be reduced with increasing seagrass densities as result of joint-detoxification, either by joint uptake of toxic compounds (reduced nitrogen) or by joint-prevention of intrusion (sulfide) by aeration of the sediment (Chapter 4).

A probable outcome of the presence of strong positive feedbacks in seagrass ecosystems is the existence of nonlinear dynamics or bistability (Scheffer et al. 2001, Scheffer and Carpenter 2003), which implies that systems may suddenly collapse when disturbances drive the system past a certain critical threshold (Scheffer et al. 2001, Folke et al. 2004). Such an unexpected collapse has also occurred in the Wadden Sea where extensive subtidal *Zostera marina* beds, suddenly and completely disappeared in the 1930, and have not recovered ever since (den Hartog and Polderman 1975, Giesen and van Katwijk 1990, Van der Heide et al. 2007).

Biogeochemical processes and nutrient cycling

Seagrasses live at the interface of the sediment and the water column. They may protrude into the water column from centimeters (Halophila spp.) up to meters (7m – *Zostera caulescens*) (Koch et al. 2007), and they may root in the sediment to up to a meter depth (*Posidonia oceanica*) (Mateo et al. 1997). Seagrasses not only produce organic matter, but also entrap organic matter, nutrients and fine sediment (Gacia and Duarte 2001, Gacia et al. 2002, Bos et al. 2007). This promotes very high rates of mineralization and nutrient cycling in seagrass beds, especially compared to unvegetated sediments (Marba et al. 2006). Seagrass beds may therefore be considered as biogeochemical hotspots (McClain et al. 2003, Layman et al. 2013). Decomposition of organic matter in seagrass sediments (both trapped allochtonous OM and senescent plant material) is an important source of nitrogen and phosphorus, which are the principal nutrients for seagrass growth (Pedersen and Borum 1992, Holmer et al. 2001). When organic matter input is enhanced by, for instance, eutrophication, increased decomposition rates may lead to such high nutrient availability which may yield either 1) direct toxic effects (Chapter 2) or 2) promote epiphyte blooms on the seagrasses (Burkholder et al. 2007). In contrast to a surplus of nutrients, seagrasses in oligotrophic areas may also be limited by either nitrogen or phosphorus availability (Bulthuis et al. 1992, Udy and Dennison 1997), which are the principal nutrients for seagrass growth (Touchette and Burkholder 2000). Nutrient limitation of seagrasses depends generally on the type of sediment (grain size and origin) and the distance to a nutrient source (Erftemeijer and Middelburg 1993, Fourqurean and Zieman 2002, van Katwijk et al. 2011). In literature, seagrasses growing on carbonate sediments are considered to be P-limited, while seagrasses growing on terrigenous sediments may be more N-limited (Erftemeijer and Middelburg 1993, Udy and Dennison 1997). As an adaptation to nutrient limitation, seagrasses are able to take up nutrient by both leaves and roots (Short 1987, Burkholder et al. 2007).

Sulfur cycling

Decomposition of organic matter mainly occurs anaerobically, as oxygen is rapidly depleted by the high microbial activity in the uppermost sediment layer (Holmer and Nielsen 1997). An alternative, but in seawater abundant, electron acceptor in the mineralization processes is sulfate (~28 mmol L^{-1}) (Schultz and Zabel 2006, Canfield and Farquhar 2009). Therefore, most organic matter is decomposed by sulfate reducing bacteria (Kristensen et al. 2000, Holmer et al. 2001). Sulfate reduction by sulfate reducing bacteria (Equation 1.1) takes place in the highly reduced organic sediments of seagrass beds, where organic matter is used as electron donor, and sulfate as electron acceptor (Jorgensen 1982). Dissolved free sulfides (H_2S, HS^-, S^{2-}) are produced in the process (Lamers et al. 2013).

$$SO_4^{2-} + 2\,CH_2O \rightarrow H_2S + 2\,HCO_3^- \qquad \textbf{Equation 1.1}$$

Sulfide is a potent phytotoxin, which affects plant physiology and energy balance through interference with cytochromes in the electron transfer chain (Bagarinao 1992, Erskine and Koch 2000). In addition, seagrass ecosystem functioning may be affected, as large-scale vegetation diebacks as a result of long-term exposure to high sulfide levels have been observed (Carlson et al. 1994, Koch and Erskine 2001, Borum et al. 2005). Seagrasses can naturally protect themselves against sulfide intrusion by radial oxygen loss in the rhizosphere (Pedersen et al. 1998), which can locally oxidize sulfides or prevent reduction of sulfates (Lamers et al. 2013). In addition, seagrasses can also form an iron hydroxide-rich zone in the rhizosphere, which may be used to trap sulfides (Deborde et al. 2008). However, when very high sulfide concentrations accumulate in the sediment or in periods of low radial oxygen loss, such as during the night, toxic sulfide may intrude the plants and cause seagrass mortality (Marba et al. 2006). External inputs of organic matter in seagrass beds by anthropogenic eutrophication may therefore not only affect seagrasses by reduced light conditions, but also by increased sulfide stress (Marba et al. 2006). Sulfide levels in the sediment may also be reduced by microbes living on the aerobic/anaerobic interface. Chemolithoautotrophic bacteria such as aerobic *Beggiatoa* or anaerobic *Thiobacillus* convert toxic sulfides to sulfur in an energy yielding process (Jorgensen 2006, Gosh and Dam 2009). Some marine animals, which live in environments rich in inorganic reduced sulfur compound (sulfides), harbor symbiotic sulfide-oxidizing bacteria in their tissues, which provide them with a source of energy (Seckbach 2004). Examples are the giant tubeworm *Riftia* that is common near sulfide-rich hydrothermal vents in the deep sea, and bivalves of the Lucinidae family (Cavanaugh 1983, Taylor and Glover 2006). In this thesis, we uncover an omnipresent sulfide-relieving mechanism in global seagrass beds involving Lucinid bivalves owing to a three-stage mutualism (chapter 5).

Trace metals and petrochemicals

Next to biogeochemical stressors, which result from eutrophication, chemical stressors such as trace metals and petrochemicals may affect seagrass health and ecosystem functioning (Ralph et al. 2007). Trace metals are naturally present in the marine environment as a result of weathering and erosion of rocks (Batley 1987, Prange and Dennison 2000), and many trace metals also function as micronutrients for seagrasses (Fe, Cu, Ni, Zn). However, trace metal pollution in coastal areas, originating from sewage, mining, industries, agriculture, and atmospheric deposition (Irvine and Birch 1998, Guzman and Garcia 2002), has lead to elevated trace metal concentrations in waters and sediments and introduced high levels of detrimental heavy metals (Cd, Pb, Hg) into the coastal environment (Costa et al. 2012). Trace metal pollution can be harmful to seagrasses, by negatively affecting metabolic activities and photosynthetic rates (Conroy et al. 1991, Ralph and Burchett 1998, Macfarlane and Burchett 2001), but more importantly, they may also accumulate in the seagrass dominated foodweb, with toxic effects to higher trophic levels (Prange and Dennison 2000). Seagrasses can therefore be used as first-level indicators for trace metal pollution in coastal ecosystems (Chapter 8).

Contamination of marine systems by petrochemicals is often concurring with trace metal pollution (Thorhaug et al. 1986). Petrochemical pollution originates mainly from oil industries (leakage, spills and refineries) and harbors (Lewis and Pryor 2013). Intertidal seagrasses may be threatened by smothering, which results in reduced growth rates and mortality, whereas leaf densities and flowering are affected in subtidal seagrasses (Dean et al. 1998, Peirano et al. 2005, Ralph et al. 2007). There is however a lack of data to support long-term effects of trace metal and petrochemical pollution on seagrasses and seagrass communities.

Species interactions in seagrass beds

Seagrass beds support a very diverse community of flora and fauna (Figure 1.5), which depend on seagrasses for food, shelter and nursery habitat (Valentine and Heck 1999, Nagelkerken 2000, Williams and Heck 2001). A large group of herbivores directly depends on seagrass beds as their most important food source; this includes mesograzers (amphipods, isopods, gastropods), large herbivores such as grazing geese and swans, and endangered mega herbivores such as dugongs and green turtles (Valentine and Heck 1999, Goecker et al. 2005, Heck and Valentine 2006). It has been suggested that the latter used to play a pivotal role in shaping tropical seagrass ecosystems, but as these mega herbivores have severely declined in numbers due to overexploitation, top-down effects have become less significant than before (Jackson 2001).

Furthermore, many other species living in seagrass beds do not directly rely on seagrasses, but are an important part of the food web (Bostrom et al. 2006). Apex predators, such as shark, rays, and sea otters may even protect seagrasses from the detrimental effects of eutrophication by consuming crabs that prey on seagrass epiphyte herbivores (Hughes et al. 2013). And shorebirds, such as the red knot, may affect the intertidal faunal community living in seagrass beds, by consuming large quantities of burrowing mollusks (van Gils et al. 2012). Next to epifauna, endobenthic fauna also plays an important part in the seagrass community. Lucinid bivalves, which live in-between the seagrass roots, even affect the sediment biogeochemistry (Chapter 5, Chapter 7) and are involved in a mutualistic relationship with seagrasses worldwide. Endobenthic fauna may also display contrasting characteristics compared to seagrasses; burrowing lugworms destabilize the sediment, while seagrasses stabilize the sediment (Chapter 8). These antagonistic characteristics may lead to an interesting competition between seagrasses and lugworm, of which they outcome may be context dependent. The interaction between bioturbation lugworms and seagrasses is further studied in Chapter 6.

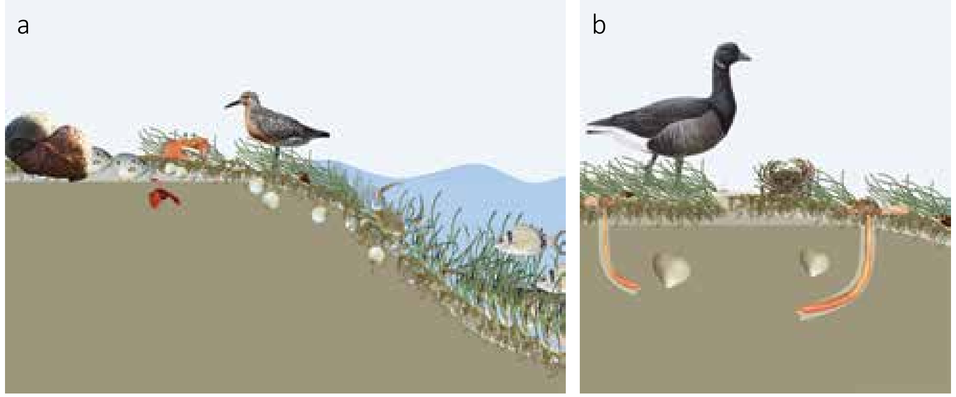


Figure 1.5 Impression of a (a) tropical (Mauritania) and (b) temperate (The Netherlands) intertidal seagrass community. Dominated by *Zostera noltii*. (a) Endobenthic fauna includes lucinid bivalves (*Loripes lucinalis*), *Anadara senilis* and *Heteromastus filiformis* and epibenthic fauna includes the giant predatory gastropod *Cymbium marmoratum*, fiddler crabs (*Uca* spp.) feeding on seagrass epiphytes, burrowing blue swimming crabs (*Callinectes marginatus*) and molluscivore waders such as the red knot (*Calidris canutus*). (b) The temperate *Zostera noltii* community features burying lugworms (*Arenicola marina*) and cockles (*Cerastoderma edule*) in the seagrass sediments, tiny shore crabs (*Carcinus maenas*) using the seagrass beds as a nursery areas, mudsnails (*Hydrobia ulvae*) feeding on epiphytes and brent geese (*Branta bernicla*) grazing in autumn. Proportions of animals and plants are not based on real measures.

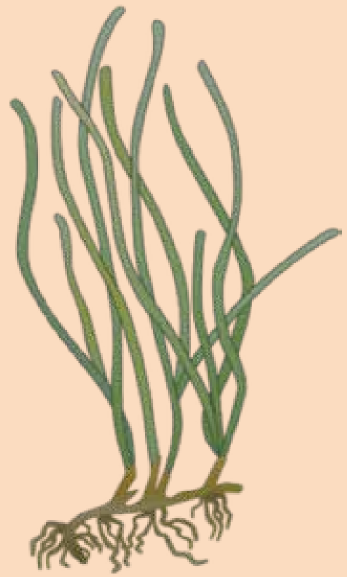
Lastly, seagrass beds also function as important nursery areas for many coral reef fish and shellfish species, which include a number of commercially and recreationally important species (Nagelkerken 2000, Heck et al. 2003, Delatorrecastro and Ronnback 2004).

Main objectives of this thesis

As described above, the main objectives of this thesis were to assess the effects of biogeochemical stressors, mainly sulfide, nutrients and trace metals on seagrass ecosystems. We thus studied seagrass dynamics and species interactions involving biogeochemical stressors. The questions and hypotheses addressed in this thesis are summarized in Table 1.1. In addition, all species studied in this thesis are introduced in box 1.2.

BOX 1.2 SPECIES IN THIS THESIS

Zostera noltii or dwarf eelgrass is the principal species in this thesis. This tiny *Zostera* species occurs along the coast of the northeastern Atlantic, from the Baltic Sea to the Banc d'Arguin in Mauritania (Green and Short 2003). This species can grow in the intertidal area up to the shallow (1-2 m) subtidal. Leaves from *Zostera noltii* can grow up to 20 cm in length and to 0.5-1.5 mm in width. *Zostera noltii* is a perennial species and can reproduce both clonally and with seeds (Moore and Short 2007). *Zostera noltii* areas have been declining in the last decades in several areas in Western Europe (Martin et al. 2010, Suykerbuyk et al. 2012).



© Ruth Berry

Thalassia testudinum or turtle grass is a widely distributed seagrass species, which can be found in shallow coastal zones in tropical and subtropical areas of the Western Atlantic (van Tussenbroek et al. 2006). Beds of *Thalassia testudinum* can be found in estuaries and coastal lagoons where it often occurs in the vicinity of mangroves and coral reefs. In most places, a strong interaction between these three tropical ecosystems is formed by both biotic and abiotic components (Nagelkerken 2010). The lush *Thalassia* beds in the Caribbean and along the southern coast of North America also serve as important nursery habitats for many coral reef fishes (Nagelkerken 2000), and as foraging habitat for the endangered green turtle (Williams and Heck 2001).

© Ruth Berry

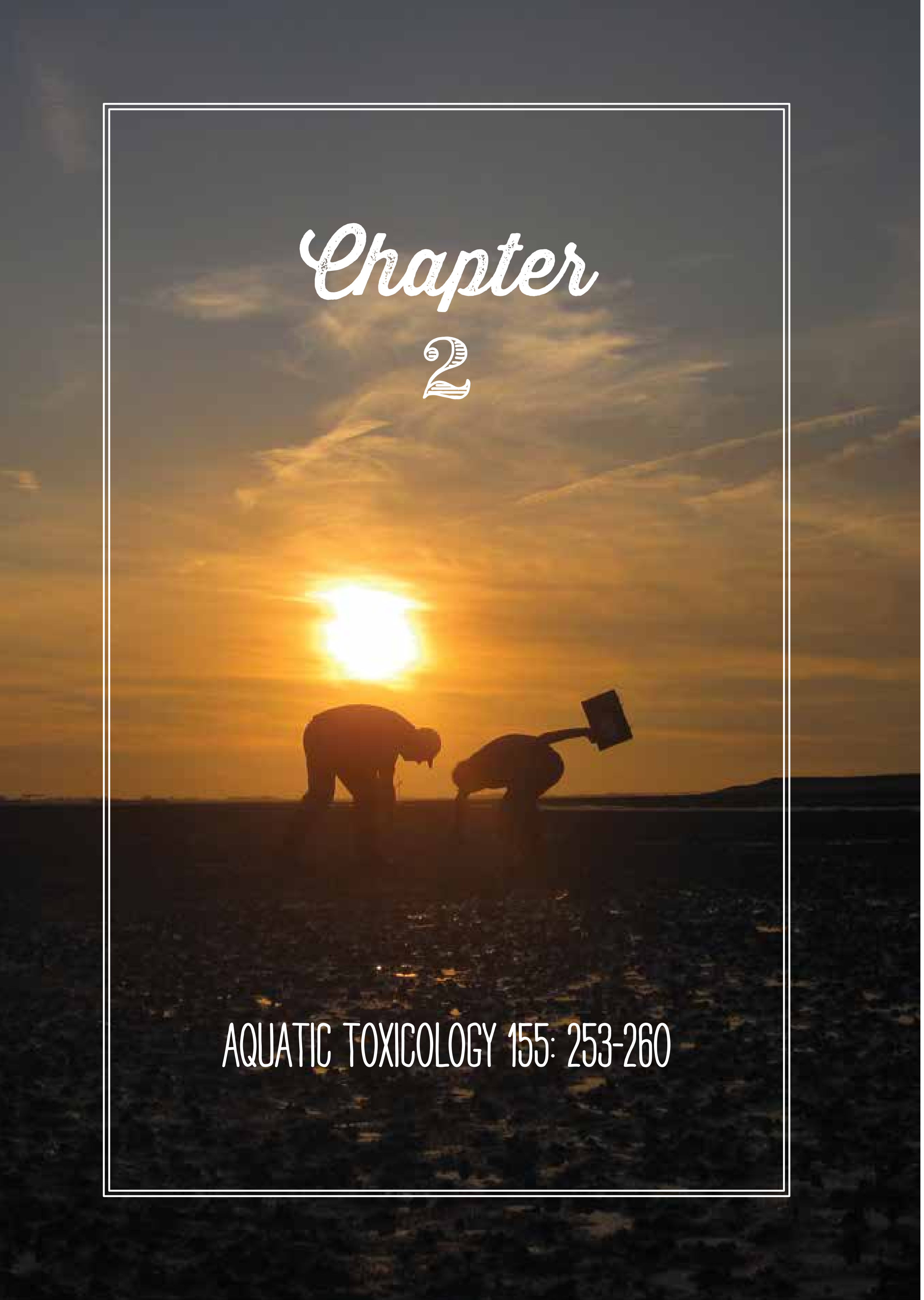
Syringodium filiforme or manatee grass is a tropical seagrass species, which can be found in coastal waters from the Gulf of Mexico to the Caribbean Sea (den Hartog and Kuo 2006). It usually occurs in waters between 1-3 meters depth, but can also occur in much deeper waters. *Syringodium filiforme* is an important food source for grazer such as parrotfish, manatees, sea urchins, and surgeonfish (Zieman et al. 1989).

Arenicola marina or lugworm is a large marine worm, which belongs to the phylum of Annelida. The lugworm can be found in intertidal areas all over North-Western Europe and can be recognized by the typical casts on top of the sediment. The lugworm is a burrowing, bioturbating animal, a deposit feeder, which reworks the sediment. Historically, *Arenicola marina* is found to co-occur with the seagrass species *Zostera noltii* (Jacobs et al. 1983), but has recently been found to hamper the recolonization of seagrasses in certain areas (Suykerbuyk et al. 2012).

Loripes lucinalis is a lucinid bivalve, which hosts endosymbiotic sulfide oxidizing bacteria in its gills. Bivalves from the genus of Lucinidae are known to be omnipresent is seagrass beds all over the world, where they form a mutualistic relationship with seagrasses (Chapter 7). *Loripes lucinalis* occurs from the French and British coast in the north, all the way to in Mauritania in the south, and the Black Sea in the East.

		QUESTION	HYPOTHESIS
Resilience & Restoration	CHAPTER 2	What are the biogeochemical constraints for seagrass patch survival and expansion?	High porewater sulfide concentrations may limit seagrass patch expansion and survival.
	CHAPTER 3	What are the bottlenecks for the winter survival of *Zostera noltii* in the Oosterschelde?	Low autumn starch reserves and low winter shoot densities may limit winter survival of *Zostera noltii*.
	CHAPTER 4	What are the effects of local environmental conditions and feedbacks on the resilience of seagrasses?	We expect lower seagrass recovery rates in low-density beds, especially with high sulfide concentrations, as feedbacks such as joint detoxification will be less efficient in lower seagrass densities.
Pollution	CHAPTER 5	What is the overall status of trace metals in seagrass beds worldwide and in the Caribbean in particular?	There has not yet been a complete literature review on trace metals in seagrass beds, we therefore provide a benchmark.
	CHAPTER 6	What is the nutrient status of the seagrass beds on Curaçao and Bonaire?	Eutrophication may threaten the important nursery habitats on Curaçao and Bonaire.
Biogeochemical species interactions	CHAPTER 7	How do seagrasses worldwide cope with sulfide stress in organic sediments?	A possible mutualistic interaction between seagrasses, lucinid bivalves and their symbiotic gill-bacteria may reduce the build-up of toxic sulfides.
	CHAPTER 8	What is the biogeochemical effect of lugworm activity on seagrasses?	We hypothesize that soil aeration by lugworm bioirrigation and soil reworking may relieve sulfide stress for seagrasses.
	CHAPTER 9	What happens with the three-stage seagrass-lucinid-gill-bacteria symbiosis in seagrass beds under desiccation stress?	A possible mutualism-breakdown could accelerate habitat degradation.

Table 1.1 Overview of questions and hypotheses in this thesis



Chapter 2

AQUATIC TOXICOLOGY 155: 253-260

TOXIC EFFECTS OF INCREASED SEDIMENT NUTRIENT AND ORGANIC MATTER LOADING ON THE SEAGRASS ZOSTERA NOLTII

Laura L Govers, Jan HF de Brouwer, Wouter Suykerbuyk, Tjeerd J Bouma, Leon PM Lamers, Alfons JP Smolders, Marieke M van Katwijk (2014)

Abstract

As a result of anthropogenic disturbances and natural stressors, seagrass beds are often patchy and heterogeneous. The effects of high loads of nutrients and organic matter in patch development and expansion in heterogeneous seagrass beds have, however, poorly been studied. We experimentally assessed the in situ effects of sediment quality on seagrass (Zostera noltii) patch dynamics by studying patch (0.35 m diameter) development and expansion for 4 sediment treatments: control, nutrient addition (NPK), organic matter addition (OM) and a combination (NPK+OM). OM addition strongly increased porewater sulfide concentrations whereas NPK increased porewater ammonium, nitrate and phosphate concentrations. As high nitrate concentrations suppressed sulfide production in NPK+OM, this treatment was biogeochemically comparable to NPK. Sulfide and ammonium concentrations differed within treatments, but over a 77 days period, seagrass patch survival and expansion were impaired by all additions compared to the control treatment. Expansion decreased at porewater ammonium concentrations >2000 µmol L^{-1}*. Mother patch biomass was not affected by high porewater ammonium concentrations as a result of its detoxification by higher seagrass densities. Sulfide concentrations >1000 µmol* L^{-1} *were toxic to both patch expansion and mother patch. We conclude that patch survival and expansion are constrained at high loads of nutrients or organic matter as a result of porewater ammonium or sulfide toxicity.*

INTRODUCTION

Seagrass beds are among the most productive and biodiverse ecosystems in the world (Hemminga and Duarte, 2000; Orth et al., 2006). Unfortunately, they are severely threatened by increasing human activities in coastal areas (Orth et al., 2006; Waycott et al., 2009). The most imminent problems for seagrass meadows are related to the large-scale eutrophication of coastal waters (Burkholder et al., 2007; Duarte, 2002; Orth et al., 2006; Waycott et al., 2009). Eutrophication results in light limitation for submerged species, as it promotes the growth of epiphytes and phytoplankton, but may also directly affect seagrass vitality through ammonium toxicity (Brun et al., 2002; Van der Heide et al., 2008; van Katwijk et al., 1997). High ammonium levels are known to be able to disrupt the uptake of essential cations such as potassium by higher plants, affect their pH regulation, and decrease overall growth rates (Britto and Kronzucker, 2002).

In addition, eutrophication related to land use change (including deforestation) often increases the organic matter input into coastal sediments, thereby not only increasing ammonium levels even further, but also fueling the production of sulfide (Burkholder et al., 2007; Van der Heide, 2009). Sulfide is a strongly phytotoxic compound, as it blocks the activity of cytochrome oxidase and other metal containing enzymes (Lamers et al., 2013). It is formed in anaerobic sediments rich in organic matter, where sulfate is used as an alternative terminal electron acceptor during the microbial breakdown of organic matter (Gray and Elliott, 2009; Holmer and Nielsen, 1997). Sulfide toxicity, due to the build-up of sulfides in the sediment, may lead to massive seagrass die-offs (Borum et al., 2005; Carlson et al., 1994; Terrados et al., 1999).

The deterioration of habitat quality, including eutrophication and sulfide toxicity, has led to the complete disappearance of seagrass beds in some regions (Borum et al., 2005; Burkholder et al., 2007). In other areas, it has created heterogeneous, patchy seagrass landscapes (Duarte and Sand-Jensen, 1990; Frederiksen, 2004; Pasqualini et al., 1999) which reflect processes of recovery from disturbances (Brun et al., 2003a; Duarte and Sand-Jensen, 1990; Pickett and White, 1985). A patch, defined as "a surface area that differs from its surrounding in nature or appearance" (Turner et al., 2001), may result from both sexual and asexual colonization (Bostrom et al., 2006; Duarte and Sand-Jensen, 1990), and from habitat fragmentation of seagrass beds. Patches expand through shoot expansion, mainly through horizontal elongation of rhizomes at the patch edges (Duarte et al., 2006a; Marba and Duarte, 1998; Olesen and Sand-Jensen, 1994; Vermaat, 2009). Their shoot densities increase exponentially (self-accelerating) with increasing patch age and size (Bostrom et al., 2006; Sintes et al., 2005; Vermaat, 2009; Vidondo et al., 1997).

Most studies have tended to focus on large-scale processes (Bell et al., 1999) in seagrass beds, rather than on ubiquitous but small-scale dynamics. Studies describing seagrass patch dynamics (Almela et al., 2008; Brun et al., 2003b; Campbell and Paling, 2003; Di Carlo and Kenworthy, 2008; Duarte and Sand-Jensen, 1990) focused on plant physiology rather than on the impact of environmental characteristics including local sediment biogeochemistry. Eutrophication and sulfide toxicity, two environmental stressors of seagrass meadows that have rapidly become common at a global scale, may not only contribute to the formation of patches, but can also be expected to affect patch expansion itself. As patchy seagrass meadows are expected to be more vulnerable to stressors than homogeneous fields due to their lower regrowth capacities and the higher impact of hydrodynamics (Bos and van Katwijk, 2007; Duarte and Sand-Jensen, 1990; Olesen and Sand-Jensen, 1994), it is important to know the effects of both important, potentially phytotoxic stressors on seagrass patches. To our knowledge, experimental manipulation of sediment quality to study the effects on seagrass patch expansion has never been carried out before. We therefore assessed the possible toxic effects of changes in biogeochemical sediment quality related to eutrophication (increased levels of nutrients and organic matter) on small-scale seagrass patches, using the fast growing *Zostera noltii* as a model species.

MATERIALS AND METHODS

Study Area

The experiment was conducted in an intertidal *Zostera noltii* bed on the mudflats of Viane (51°39' N, 4°01' E), the Oosterschelde, in the southwestern part of the Netherlands. The Oosterschelde delta has a surface area of 351 km^2 and a tidal amplitude of 2.5-3 m (Troost et al., 2009). Mean surface water temperature fluctuates between 0-22°C annually. Freshwater input into the former estuary has become highly limited after the implementation of major water works in the past, and salinity is generally around 30 (Nienhuis and Smaal, 1994).

Zostera noltii meadows cover around 75 ha of the mudflats in the Oosterschelde, growing mainly in sheltered areas near dikes. At the experimental site, *Zostera noltii* was growing within 30 meters of a dike, on a compact clay bank with a median grain size (D50) of 130 µm and % organic carbon of 0.72%. At the start of the experiment, the seagrass cover of the meadow was relatively homogeneous (2250±124 shoots m^{-1}) and biogeochemical characteristics of the meadow were also determined (sulfide=0.31±0.22, NH_4=13.14±2.09, NO_3=5.51±1.44, PO_4=8.95±0.84, µmol L^{-1}, n=21). Experimental seagrass patches, as well as the natural bed, were located on the mudflat at an average height of 54 cm above Amsterdam Ordnance Datum (NAP), resulting in exposition to the atmosphere for on average 7 hours during each low tide, depending on weather conditions. This intertidal mudflat was flooded and drained with each tidal cycle (semidiurnal).

Experimental design

Circular *Zostera noltii* sods of 35 cm in diameter and 7 cm depth were carefully collected from a homogeneously covered meadow, and transplanted to a bare stretch of the mudflat between the dike and the seagrass bed nearby. This area is known to be a suitable seagrass habitat, as seagrass was present and vital at this site until it was removed during dike reinforcement works in the previous growing season. The sods were transplanted into circular spots of 105 cm diameter and 7 cm depth. Before placing the sods, the different sediment treatments (details next section) were applied on the bare sediment. The sods, hereafter referred to as mother patches, were placed in the middle of the treated sediment spots and the remains were filled up with local sediment. Treatments were randomly assigned to the patches, using eight replicates (n=8) per treatment. The experiment was carried out for 77 days, from June till September 2009.

Sediment treatments: nutrient and organic matter addition

To study the separate and interacting effects of sediment eutrophication, we applied four different biogeochemical treatments: a control treatment (Control), an organic matter treatment (OM), a nutrient treatment (NPK) and a combined treatment of both nutrients and organic matter (NPK+OM). We fertilized NPK and NPK+OM plots with 1.11 kg m^{-2} Osmocote® slow release fertilizer (g:g:g ratio N:P:K 18:9:10) with a longevity of 8-9 months. The fertilizer was distributed evenly beneath the seagrass sods and the surrounding, bare sediment. Loading rates were around 57 mmol N m^{-2} day^{-1} and 13 mmol P m^{-2} day^{-1} (Christianen et al., 2012; Vanlent et al., 1995).

For the organic matter treatments (OM and NPK + OM), we added 2 g L^{-1} of organic matter (1 g starch + 1 g cellulose L^{-1} sediment), which equaled a loading rate of about 157 mmol C m^{-2} day^{-1} (for 47 days), under the seagrass sods, which was expected to stimulate sulfide production (Peralta et al., 2003; Ruiz-Halpern et al., 2008). All treatments were applied only once, at the start of the experiment. However, the NPK and NPK+OM treatments appeared to generate biogeochemically similar results, as high nitrate levels suppressed sulfide production. As a result, this unforeseen effect of combined NPK and OM addition did not result in an expected interaction treatment.

Data collection in the field

Sediment samples were collected at the start of the experiment for chemical analyses to characterize the local habitat. Patch heights were measured using a RTK-DGPS (Real Time Kinematic Global Positioning System) (Van der Heide et al., 2010a), but did not differ significantly among treatments. During the experiment, sediment porewater samples were collected anaerobically at t=0, 21, 49, 77 days, using 60 mL vacuumed syringes connected to ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), which

Table 2.1 Results of 2-way ANOVAs (NPK * OM) for all shown variables of *Zostera noltii* after 77 days. * 0.01≤P≤0.05. ** 0.001≤P≤0.01. *** P≤0.001. ns=not significant.

	NPK					**OM**					**NPK*OM**						
Parameter	**df**	**MS**	**F**	**P**		**df**	**MS**	**F**	**P**		**df**	**MS**	**F**	**P**		**Error**	**Transformation**
Total patch biomass (g DW m^{-2})	1	2298	2.14	0.155	ns	1	2782	2.59	0.119	ns	1	7808	7.27	0.012	*	32.8	Sqrt(max(x))-x
Total patch aboveground biomass (g DW m^{-2})	1	859	4.36	0.046	*	1	832	4.22	0.049	*	1	1399	7.10	0.013	*	14.0	Sqrt(max(x))-x
Total patch belowground biomass (g DW m^{-2})	1	347	0.86	0.36	ns	1	571	1.42	0.243	ns	1	2597	6.45	0.017	*	20.1	Sqrt(max(x))-x
Mother patch total biomass (g DW m^{-2})	1	1152	1.26	0.271	ns	1	1569	1.71	0.202	ns	1	6182	6.73	0.015	*	30.3	Sqrt(max(x))-x
Mother patch aboveground biomass (g DW m^{-2})	1	392	2.53	0.123	ns	1	436	2.81	0.11	ns	1	992	6.39	0.017	*	12.4	Sqrt(max(x))-x
Mother patch belowground biomass (g DW m^{-2})	1	196	0.54	0.467	ns	1	259	0.71	0.406	ns	1	2012	5.53	0.026	*	19.1	Sqrt(max(x))-x
Patch expansion total biomass (g DW m^{-2})	1	393	2.53	0.123	ns	1	436	2.81	0.105	ns	1	991	6.39	0.017	*	12.5	Sqrt(max(x))-x
Patch expansion aboveground biomass (g DW m^{-2})	1	196	0.54	0.569	ns	1	259	0.71	0.406	ns	1	2012	5.53	0.026	*	19.1	Sqrt(max(x))-x
Patch expansion belowground biomass (g DW m^{-2})	1	1152	1.26	0.272	ns	1	1569	1.71	0.202	ns	1	6182	6.74	0.0149	*	30.29	Sqrt(max(x))-x
Total N (%)	1	1.40	20.56	<0.001	***	1	0.06	0.805	0.387	ns	1	0.00	0.21	0.888	ns	0.27	
Total P (%)	1	0.09	2.97	0.116	ns	1	0.00	0.05	0.821	ns	1	0.01	0.31	0.591	ns	0.18	
Total C (%)	1	13.3	9.69	0.009	**	1	0.79	0.58	0.463	ns	1	4.21	3.01	0.106	ns	0.53	
Total S (%)	1	1.12	37.73	<0.001	***	1	0.02	0.738	0.410	ns	1	0.01	0.27	0.613	ns	0.17	
C:N (molar ratio)	1	4.87	8.15	0.015	*	1	0.92	1.55	0.237	ns	1	0.38	0.63	0.443	ns	0.77	
N:P (molar ratio)	1	52.8	12.78	0.005	**	1	0.38	0.09	0.767	ns	1	1.28	0.31	0.590	ns	2.03	

were placed in the top 7 cm of the sediment. For each experimental patch, two porewater samples were taken, one inside the mother patch and one just outside the patch, but within the sediment treatment area (potential expansion area). Samples were pooled for analysis. Of each anaerobic sample, 10 mL was used for sulfide analysis on the same day (see below), and 40 mL was frozen until further chemical analyses. After porewater extraction, shoot numbers within the transplanted patches and shoot numbers of the patch outgrowth (patch expansion) were monitored, by counting all shoots. After 77 days, the experiment was terminated and patch shoot density was determined. The patch expansion area (all seagrass biomass produced outside the 0.35 m diameter patch) was harvested for all patches and a small core (diameter 0.06 m) was taken inside the patch to determine patch biomass. Harvested samples were rinsed and split up into aboveground (leaves and sheath) and belowground (rhizomes and roots) biomass, and dried at 60°C until constant weight, and weighed (g DW).

Analysis of porewater and plant samples

Within 5 hours after sampling, total sulfide concentration in the porewater was measured in a mixture of 50% sample and 50% Sulfide Anti-Oxidation Buffer (SAOB) (Lamers et al., 1998), using an ion-specific silver-sulfide electrode. Porewater ammonium and ortho-phosphate concentrations were measured colorimetrically (Skalar and Seal autoanalyzer), using ammonium-molybdate and salicylate (Lamers et al., 1998). Nitrate was determined by sulphanilamide, after reduction of nitrate to nitrite in a cadmium column (Wood et al., 1967).

To assess seagrass nutrient status, dry plant material was used to determine %C and %N of both leaves and rhizomes, using an elemental analyzer (Type NA 1500 Carlo Erba Thermo Fisher Science, USA), coupled online via an interface (Finnigan Conflo III) to a mass spectrometer (Thermo Finnigan Delta Plus, USA). Total phosphorus in seagrass tissue was measured on an inductively-coupled-plasma emission spectrophotometer (ICP) (Spectroflame, Spectro Inc.), after digestion of dry plant material with nitric acid and H_2O_2 (Smolders et al., 2006). Sediment grain size distribution was determined on dried and sieved (1 mm mesh size) samples by laser diffraction on a Malvern (Master 2000) particle size analyzer.

Statistical analysis

Normal distribution of the data was tested on the residuals, and negatively skewed data were transformed using the square root of the maximum of x minus x (Field, 2005) to meet assumptions of the ANOVAs (Table 2.1). Biomass and elemental composition were analyzed by 2-way ANOVAs to assess the effects of NPK and OM, with a 95% confidence interval We used R 3.01 to perform 2-way ANOVAs. Porewater nutrient and sulfide data were tested using 3-way repeated measure ANOVAs, to analyze the effects of time (t0, t21, t49, t77), NPK (+/-) and OM (+/-) (Table 2.2). We used the Greenhouse-Geisser correction when sphericity

Table 2.2 Results of the 3-way (time*NPK*OM) repeated measures ANOVAs (t_0, t_{21}, t_{49}, t_{77}) for shoot densities (m^{-2}) and all relevant porewater nutrients. F values and significance levels are shown for all the main effects and interactions * 0.01≤P≤0.05. ** 0.001≤P≤0.01. *** P≤0.001. ns=not significant. df stands for adjusted degrees of freedom as a result of the Greenhouse-Geisser correction for violated sphericity.

	TIme				**Time*NPK**				**Time*OM**				**Time*NPK*OM**			
Parameter	**df**	**F**	**P**		**df**	**F**	**P**		**df**	**F**	**P**		**df**	**F**	**P**	
Shoots mother patch (shoots m^{-2})	1.4	5.62	0.009	**	1.1	1.88	0.226	ns	1.2	1.66	0.252	ns	1.1	7.42	0.034	*
Shoots patch expansion (shoots m^{-2})	1.1	5.88	0.041	*	1.1	2.09	0.189	ns	1.2	1.73	0.227	ns	1.1	0.73	0.435	ns
Sulfide (µmol L^{-1})	1.2	9.10	0.019	*	1.2	7.70	0.023	*	1.4	6.04	0.007	**	1.3	5.88	0.007	**
Ammonium (µmol L^{-1})	1.8	7.68	0.007	**	1.8	5.87	0.004	**	1.3	0.95	0.390	ns	1.6	0.66	0.501	ns
Nitrate (µmol L^{-1})	1.5	5.07	0.034	*	1.5	4.67	0.038	*	1.3	1.32	0.295	ns	1.6	1.21	0.322	ns
Phosphate (µmol L^{-1})	2.1	13.5	0.000	***	1.7	5.41	0.026	*	1.2	1.75	0.225	ns	3.0	0.70	0.562	ns

assumptions were violated. PASW-SPSS 18.0 was used for repeated measures ANOVAs. Exponential regressions were tested with linear models after log transformation the variables on the Y-axis (biomass mother patch and expansion) and untransformed variables on the X-axis (sulfide and ammonium). For sulfide-biomass relationships, only values from the control and OM treatments were taken into account, and for the ammonium-biomass relationships, only results from the control, NPK and NPK+OM treatments. This separate testing of treatments was done to distinguish between the effects of high porewater sulfide or high ammonium, which could each result in a low biomass. Outliers were detected with Dixon's Q-test and removed from the data (Dean and Dixon, 1951).

RESULTS

Porewater results

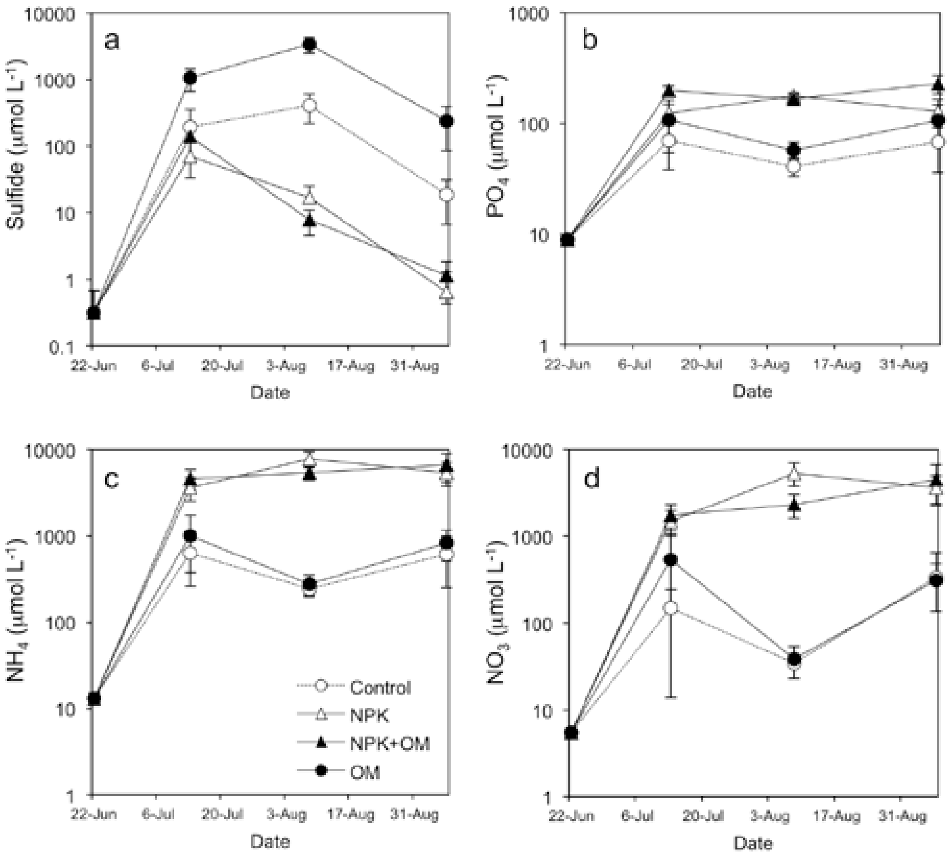


Figure 2.1 Porewater concentrations of sulfide (a), phosphate (b), ammonium (c) and nitrate (d) during the experiment. Error bars represent SEM (n=8).

Both the NPK and OM treatment successfully altered biogeochemical sediment characteristics, as expected (Figure 2.1). OM addition resulted in significantly elevated porewater sulfide levels (Figure 2.1a) up to concentrations of 3000 µmol L^{-1} in the OM treatment (repeated measures ANOVA, $F_{1.4}$=6.05, P=0.007). In contrast, porewater sulfide levels did not increase in the NPK+OM treatment. High amounts of nitrate were released in both NPK treatments, and sulfide production was almost completely suppressed in the NPK + OM treatment ($F_{1.3}$=5.88, P=0.007), because nitrate is a thermodynamically more favorable electron acceptor than sulfate (Lucassen et al., 2004). The NPK+OM treatment therefore resulted in biogeochemical properties, which were similar to the NPK treatment. The NPK treatment significantly decreased porewater sulfide concentrations relative to both the control and OM treatment (Figure 2.1, Table 2.2, $F_{7,71}$, P=0.023). Sulfide concentrations peaked at 3340 µmol L^{-1} on the 8th of August, but declined to 240 µmol L^{-1} by the 7th of September, suggesting that OM availability had been exhausted after the 8th of August (47 days). Furthermore, NPK addition resulted in significantly (Table 2.2) elevated porewater ammonium ($F_{1.8}$=5.87, P=0.004), nitrate ($F_{1.5}$=4.678, P=0.038) and phosphate concentrations ($F_{1.7}$=5.52, P=0.026) (Figure 2.1). Porewater ammonium levels in both nutrient treatments exceeded 4000 µmol L^{-1} (a more than 8-fold increase compared to the controls), and nitrate concentrations increased more than 50-fold compared to the controls up to >2000 µmol L^{-1}.

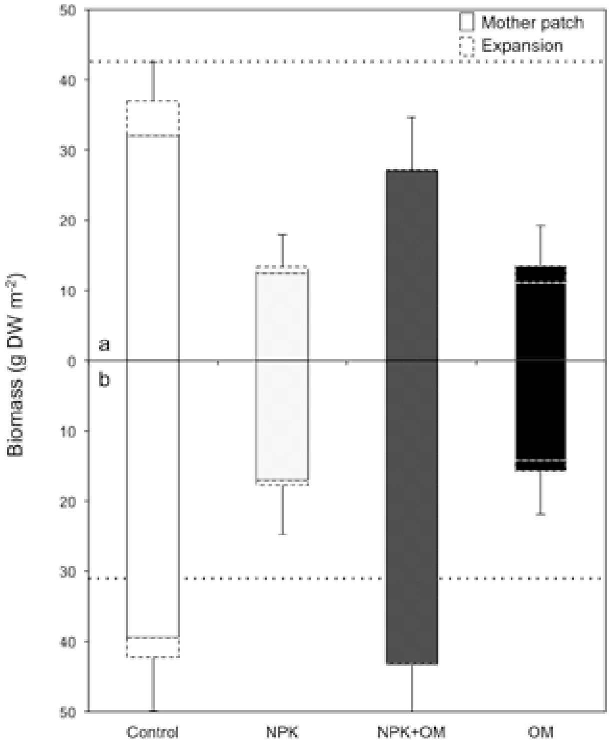


Figure 2.2 Aboveground (a) and belowground biomass (b) of patch expansion (dashed bars) and mother patch (straight line); dashed lines indicate initial aboveground and belowground biomasses. Error bars represent SEM of total aboveground and belowground biomass, n=8. >

Biomass response

We observed seagrass expansion (seagrass growth outside the 0.35 m diameter patches) in 100% of the control treatments (n=8). In contrast, 37.5% of the patches in the OM and the NPK+OM treatments and only 25% of the transplanted patches in the NPK treatments expanded. Aboveground biomass of the mother patches decreased in all treatments compared to the initial biomass of 43 g DW m^{-2}, without significant effects of NPK (2-way ANOVA, F_1=2.53, P=0.123) or OM (F_1=2.81, P=0.110). Shoot densities (shoots m^{-2}) in the mother patches decreased by approximately 75% in all addition treatments, whereas they did not change in time in the control treatment (Table 2.2, Figure 2.3). Belowground biomass of the mother patches increased in the control treatment, from an initial biomass of 31 g DW m^{-2} to a biomass of 40 g DW m^{-2} at the end of the experiment. In stark contrast, belowground biomass was reduced by almost 50% compared to the initial biomass in all other treatments (Table 2.1, Figure 2.2).

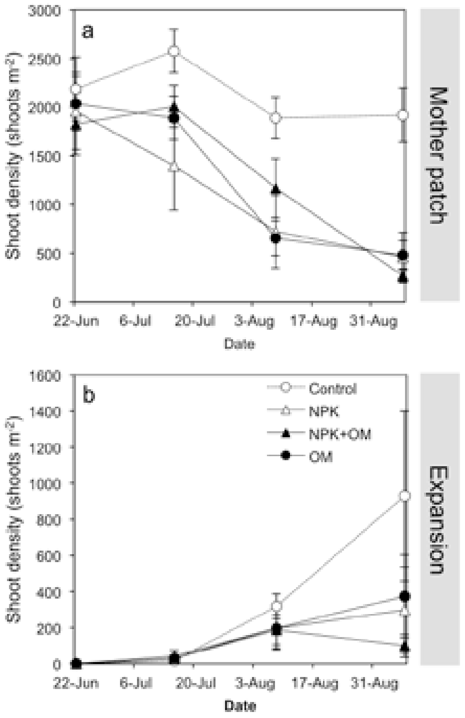


Figure 2.3 Shoot densities of the mother patch (a) and in the expansion area (b) in time; error bars represent SEM, n=8.

The aboveground biomass of the expansion did not differ among treatments (Table 2.1). In contrast, both NPK (F_1=4.36, P=0.046) and OM (F_1=4.22, P=0.049) addition negatively affected total patch aboveground biomass (Figure 2.2, Table 2.1). Even though all patches received standardized treatments, the effects of NPK or OM addition on biomass parameters (mother patch and expansion biomass) showed high variability (Figure 2.4), Some patches in the treatments (NPK, NPK+OM, OM) appeared to manifest less ammonium or sulfide stress (Figure 2.4) as reflected by higher biomasses in these units. We therefore tested whether this heterogeneity could be explained by differences in the concentrations in sulfide accumulated, and indeed found a very strong correlation between biomass and porewater sulfide concentrations, (R^2=0.69, P<0.001 for mother patch and R^2=0.70, P<0.001 for patch expansion). Sulfide levels above 1000 µmol L^{-1} completely prevented expansion of patches (Figure 2.4c) and resulted in their complete die-off (Figure 2.4a). Some of the patches already suffered from lower sulfide concentrations. Low patch expansion biomass was also strongly related to high porewater ammonium concentrations (Figure 2.4d, R^2=0.61, P<0.001), but we did not find effects on mother patch biomass (Figure 2.4b, R^2=0.20, P>0.05). We observed no patch expansion at ammonium concentrations >5000 µmol L^{-1}, but expansion was already strongly decreased by ammonium concentrations around 2000 µmol L^{-1} (Figure 2.4d). Contrastingly, some mother patches were able to tolerate ammonium concentrations >7000 µmol L^{-1}.

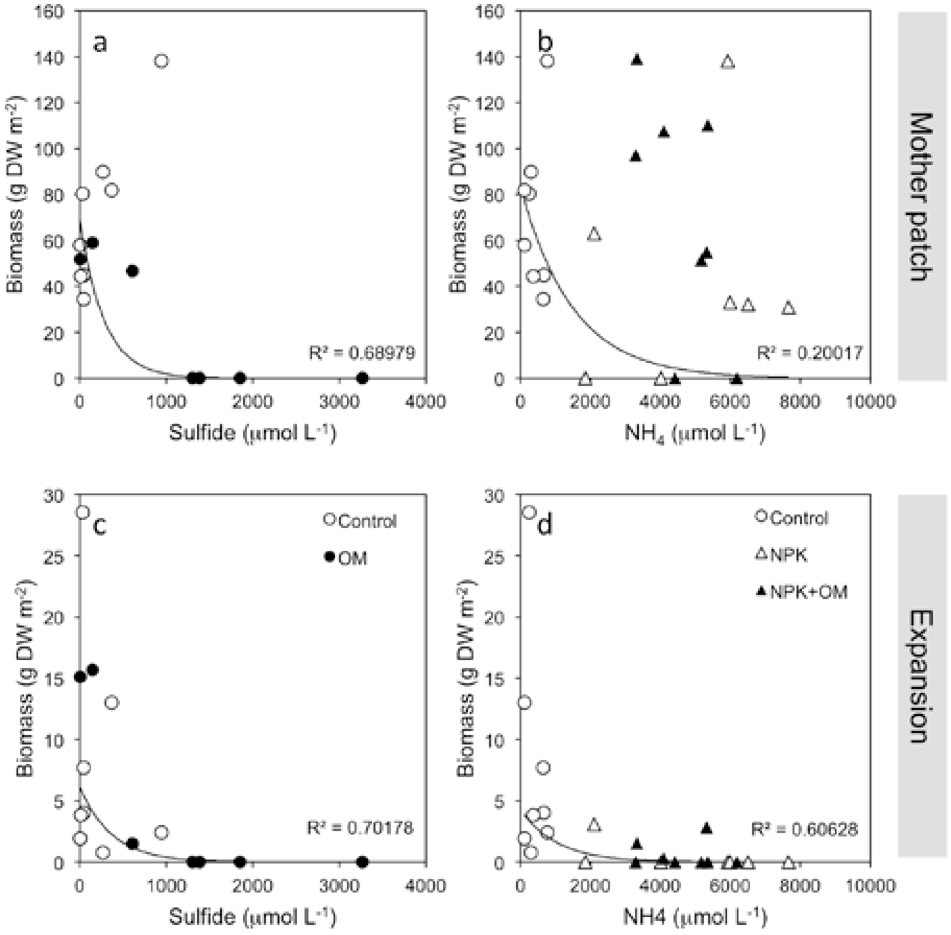


Figure 2.4 Scatterplots of mother patch biomass and weighted average porewater sulfide concentrations (a) and ammonium concentrations (b), and expansion biomass and weighted average porewater sulfide concentrations (c) and ammonium concentrations (d) with exponential regression lines and related R^2 values.

Plant elemental composition

Leaf nitrogen (%N) content of the seagrass leaves of the expansion increased by NPK addition (2-way ANOVA, F_1=20.56, P<0.001, Table 2.3). Leaf phosphorous content, in contrast, was not significantly affected by any treatment. *Zostera noltii* did not seem to be nutrient limited in our experiment, as average leaf nitrogen (3.8%) and phosphorous content (0.9%) were well above the values for absolute nutrient limitation (Duarte, 1990). Total sulfur content of the leaves (%S) was 40% lower in the NPK treatments (F_1=37.73, P<0.001,) than in the Control and OM only (OM) treatment (Table 2.3. 1.1% versus 1.7%).

Treatment	C:N:P	%C	%N	%P	%S
Control	121:10:01	39.3	3.83	0.89	1.77
NPK	161:15:01	40.3	4.40	0.65	1.11
NPK+OM	140:14:01	41.3	4.56	0.75	1.11
OM	114:10:01	38.0	3.94	0.87	1.66

Table 2.3 Elemental composition of the leaves. Molar C:N:P ratios and percentages (g^{-1}) of leaf tissue nutrients.

DISCUSSION

Limitations to seagrass patch survival and development have typically been attributed to physical and biological stressors in literature (Sintes et al., 2005; Townsend and Fonseca, 1998). In this study, we show that also biogeochemical toxins, such as high nutrient and sulfide concentrations, can be highly detrimental to seagrass patch survival and expansion. As the high nitrate levels in the NPK treatment strongly decreased (natural) sulfide production in the sediment, interacting effects of sulfide and ammonium toxicity could not be investigated, and we will therefore discuss the effects of both stressors separately.

Ammonium toxicity

This is the first study that actually shows a causal relationship for porewater ammonium and toxicity effects in seagrass. Previous studies mainly focused on the effects of high nitrate or ammonium concentrations in the surface water layer (Burkholder et al., 1992; Christianen et al., 2011; Van der Heide et al., 2008; van Katwijk et al., 1997). In a correlative study (Short, 1983b) already suggested that porewater nitrogen concentrations of 10 – 100 mmol m^{-2} were associated with decreasing eelgrass shoot densities. Ammonium concentrations >25 µmol L^{-1} in the water column are known to be potentially lethal to *Zostera* spp. (Brun et al., 2002; Van der Heide et al., 2008; van Katwijk et al., 1997). The porewater ammonium concentrations we found to completely restrict patch expansion, >2000- 4000 µmol L^{-1}, are more than a hundred times higher, although negative effects on biomass were already visible at 1000 µmol L^{-1}. We could therefore consider *Zostera noltii* a fairly persistent species for soil ammonium toxicity.

The high threshold values we found compared to surface water ammonium experiments indicates that *Zostera noltii* is much more resistant to sediment eutrophication than to water column eutrophication (Peralta et al., 2003). This is to be expected, as porewater nutrient concentrations are known to be at least ten times higher than ambient water column concentrations (Burkholder et al., 2007; Touchette and Burkholder, 2000). This difference could also be related to a higher affinity of leaves for ammonium as compared to roots (Thursby and Harlin, 1982). Also, sediment pH is usually lower than water column pH as a result of higher CO_2 concentrations (pH 7.5 vs. 8.2 on average), which might result in lower ammonium uptake rates in the roots (Toetz, 1973). Furthermore, most water column eutrophication experiments were conducted with *Zostera marina*, and *Z. noltii* may be more resistant to eutrophication of the water column than *Z. marina*, and vice versa, *Z. marina* may be less resistant to high porewater ammonium levels.

The negative effects of biogeochemical stressors seemed to be less pronounced for the mother patch than for patch expansion (Figure 2.4). This indicates that the lower shoot density and biomass in the expansion area makes the plants more vulnerable to toxicity, because toxicity cannot be alleviated by joint detoxification or by growth dilution (Van der Heide et al., 2010b). Both processes are strongly affected by population density, and result in denser vegetation being better able to cope with toxins and showing higher growth rates (Van der Heide et al., 2010b). This implies that sparse and patchy vegetation can be expected to be more vulnerable to ammonium toxicity than densely vegetated seagrass beds. At the same time, however, sparse patches may collect lower amounts of detritus leading to lower porewater ammonium and sulfide concentrations. Although even small patches of *Zostera noltii* seem to be persistent to high porewater ammonium, they were not able to expand or grow during these conditions in our experiment.

Another possible effect of direct nutrient toxicity may be an imbalanced carbon-nitrogen ratio due to increased carbon demand (Touchette et al., 2003). We did find a slightly significant effect of NPK addition on C:N ratios (11:1 vs. 12:1) in our experiment, which could imply that this treatment may have resulted in a carbon-nitrogen imbalance in the plants.

Our treatments resulted in very high porewater ammonium concentrations (up to 7000 µM), which is probably the result of the sediment properties (clay) at our experimental site. However, such high porewater ammonium concentrations (1000 up to 7000 µM) have been observed before in eutrophic coastal sediments (Fourqurean et al., 1992; Murray et al., 1978; Pages et al., 2012). And the high porewater ammonium concentrations in our experiment enabled us to investigate the effects of this wide range of ammonium concentrations, which has been observed in natural coastal sediments.

Sulfide toxicity

High sulfide concentrations of the porewater appeared to be another major constraint for seagrass patch expansion. Sulfide is known to be very toxic to seagrasses (Calleja et al., 2007; Koch and Erskine, 2001; Lamers et al., 2013; Marba et al., 2006; Terrados et al., 1999) and Van der Heide et al. (2012) showed a negative effect on *Zostera noltii* biomass production at porewater sulfide levels

<200 µmol L^{-1}. We found similar threshold values in our field experiment and also found that patch expansion was entirely blocked by sulfide concentrations >1000 µmol L^{-1}. At low sulfide levels, seagrass is usually able to cope with sulfide by oxidizing it in the root zone (Holmer and Bondgaard, 2001; Marba et al., 2006; Pedersen et al., 1998). However, if either sulfide levels become too high, as in our experiment, or when seagrass photosynthesis is constrained or biomass too low, sulfide intrudes into the plants (Garcia et al., 2013; Holmer and Kendrick, 2013). It then interferes with the cytochromes in the electron transport chain, resulting in a negative energy balance, which eventually causes seagrass mortality (Erskine and Koch, 2000; Holmer and Bondgaard, 2001).

Our results showed that lower sulfide stress is reflected in the sulfur content of *Zostera noltii* leaves and that sulfide intrusion and sulfur storage already takes place at sulfide concentrations >200 µmol L^{-1} (control). It is however remarkable that sulfur storage in the leaves of *Zostera noltii* did not differ between control (ambient sulfide levels) and OM treatments (elevated sulfide levels). This may be due to decreased vitality of the roots in the high-sulfide treatment (OM), which could severely decrease the uptake of sulfur by the roots.

Effects on patch development

Patch growth is a self-accelerating process, but it is usually slower than the potential growth rates, as physical and biological stressors interfere (Sintes et al., 2005; Townsend and Fonseca, 1998). Our study adds biogeochemical stressors to this process of patch development. We observed patch expansion in the control patches, but when we added biogeochemical stressors, patch growth was severely limited by ammonium or sulfide toxicity.

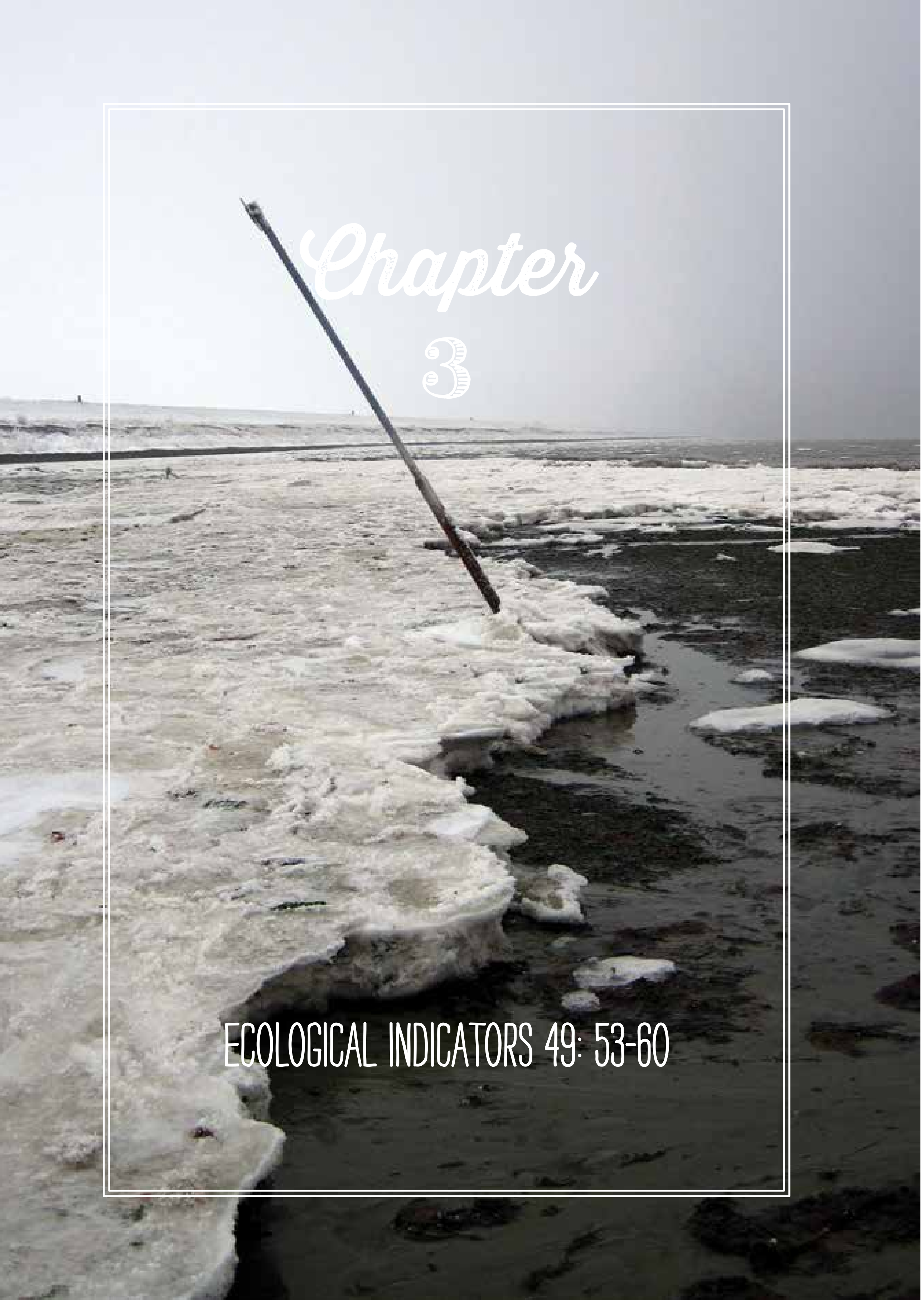
Ecological implications

Eutrophication is one of the major causes of seagrass decline worldwide (Duarte, 2002; Orth et al., 2006; Waycott et al., 2009). As it is known to promote high concentrations of ammonium and sulfide in the porewater (Hauxwell et al., 2001; Short, 1983a; Smolders and Roelofs, 1995), both factors represent important biogeochemical stressors and are expected to influence seagrass expansion processes at a global scale. Although the occurrence of patches is a natural phenomenon in seagrass meadows (Duarte et al., 2006a), the level of patchiness is strongly increasing as a result of various human activities (Bostrom et al., 2006).

Patches are very dynamic and normally ensure recovery of seagrass meadows (Duarte et al., 2006b). As we seek to preserve these constantly changing systems (preservation paradox; (Pickett and White, 1985)), it is vital to gain a better understanding of the effects of stressors on system dynamics. The present study showed, for the first time, that patch survival and expansion can be severely influenced by common biogeochemical stressors related to eutrophication, i.e. increased ammonium and sulfide levels, which not only led to thinning of the patches but also precluded patch expansion. Density-dependent alleviation of ammonium and sulfide toxicity will decrease due to the thinning, and subsequently accelerate the decline.

ACKNOWLEDGEMENTS

We like to acknowledge Marloes Hendriks, Madelon van de Kerk, Nel Govers, Camiel Doorenweerd, Jeroen Veeken, Eric Gerritsen, Bruce Schoelitz, Lucien Hanssen, Steef Hanssen and Jochem Vink, who were indispensable in the field and the laboratory. We are also grateful to Martin Versteeg, for his help in the field, to Jelle Eygensteyn, for his help with chemical analyses, and to Jim de Fouw for statistical advise. We also thank two anonymous reviewers for their contribution to the improvement of the manuscript. Rijkswaterstaat Zeeland and Projectbureau Zeeweringen funded this research.



Chapter 3

ECOLOGICAL INDICATORS 49: 53-60

RHIZOME STARCH AS INDICATOR FOR TEMPERATE SEAGRASS WINTER SURVIVAL

Laura L Govers, Wouter Suykerbuyk, Jacqueline HT Hoppenreijs, Kris Giesen, Tjeerd J Bouma, Marieke M van Katwijk (2014)

Abstract

Key-ecosystems such as seagrass beds are disappearing on a global scale. In order to counter-act local loss of seagrass beds, seagrass restoration projects have been carried out with varying degrees of success. As seagrass biomass peaks in summer, most restoration projects are monitored during this period, while wintering processes are largely ignored. We here attempted to elucidate some important bottlenecks for wintering survival in temperate areas of the intertidal model species Zostera noltii, using a three-year transplant-monitoring dataset and a field experiment. We found that next year's transplant success could not be predicted based on the preceding year's growth success, emphasizing the winter as a crucial period for survival of seagrass transplants. In addition, transplant success was neither determined by abiotic site characteristics. Low autumn rhizome starch concentrations in unsuccessful transplants, compared to successful transplants and natural beds, hinted at the importance of starch for winter survival. Hence, we tested the importance of starch, accumulated in autumn, versus the importance of the presence of sparse aboveground photosynthetic winter biomass for winter survival of seagrass transplants in a field experiment. We clipped the overwintering-leaves of three natural beds that naturally varied in their autumn rhizome starch concentrations. Decreased leaf densities in winter did not affect seagrass biomass in June, nor did this treatment affect rhizome starch concentration in June. Autumn rhizome starch reserves did however provide a good indication of next year's growth success, confirming the importance of starch reserves for winter survival. We thus conclude that autumn rhizome starch can be a good predictor of next year's growth success, whereas the preceding growing season shoot density and the presence of leaves during the winter were bad indicators of next year's growth success.

INTRODUCTION

Seagrass beds are important coastal ecosystems, as they are highly productive, sequester carbon, support high biodiversity, and can play a role in coastal protection by reducing wave energy and through sediment stabilization (Christianen et al., 2013; Duarte, 2002; Fourqurean et al., 2012; Hendriks et al., 2008). However, seagrass beds are currently disappearing on a global scale, mainly due to anthropogenic disturbances (Orth et al., 2006; Waycott et al., 2009). Therefore, many restoration projects have been carried out, with varying degrees of success (Fonseca et al., 2001), which cannot always be attributed to prevailing abiotic conditions (Orth et al., 2009; Suykerbuyk et al., submitted; Van Katwijk et al., 2009). As seagrass restoration projects are often costly, it is important to select suitable sites and to predict or identify bottlenecks that may affect these restoration attempts (Short et al., 2000; Short et al., 2002).

We expect winter processes to be important bottlenecks for transplant winter survival, based on the observation that transplant survival in winter is limited. Temperate and subtropical seagrasses typically display a seasonal biomass peak of high biomass during the growing season (summer), and a strongly decreased biomass during the winter (Duarte, 1989). Seagrass restoration sites are therefore typically monitored during the growing season, whereas wintering processes, which may also affect restoration success, are only occasionally studied (e.g. Marion and Orth, 2012; Vermaat and Verhagen, 1996). However, to date there are no quantitative analyses on the generality of this phenomenon or on what factors affect winter survival. So our overall objective is to identify parameters that have an indicative value for the winter survival of transplants.

Important processes that affect wintering seagrasses in temperate areas are the low temperatures and low light levels, which greatly decrease seagrass productivity. As photosynthesis is reduced, the carbon balance of seagrasses, which is determined by the carbon gain (photosynthesis) and carbon demand (respiration and growth), can become negative (Alcoverro et al., 2001; Alcoverro et al., 1999). During this stressful period, seagrasses become dependent on their carbohydrate reserves (Alcoverro et al., 1999; Brun et al., 2008; Zimmerman et al., 1995), which are thus important to seagrass winter survival.

For seagrasses, the most important non-soluble carbohydrate for long-term storage is starch (Pirc, 1989). Starch is mainly stored in the rhizomes, and produced in periods with a positive carbon balance (Olive et al., 2007; Zimmerman and Alberte, 1996). Wintering seagrasses are thus dependent on the production of starch reserves in the preceding growing season. The amount of starch needed for winter survival depends on both internal and external factors; respiration can be affected by temperature and light, but also by biomass and photosynthetic

activity (Alcoverro et al., 1999; Fourqurean and Zieman, 1991; Marsh et al., 1986; Vermaat and Verhagen, 1996). In addition, environmental stressors such as ammonium toxicity and sediment anoxia can increase carbon demand (Brun et al., 2008). Growth is another important process that contributes to carbon demand (Alcoverro et al., 1999). Although growth is marginal during the winter months (0.015 cm^2 cm^{-2} day^{-1} versus loss rates of -0.110 cm^2 cm^{-2} day^{-1} in *Zostera noltii*) (Vermaat and Verhagen, 1996), carbon demand for growth in early spring can deplete carbohydrate reserves.

In this study, we aimed to further elucidate mechanisms of seagrass winter survival with regard to seagrass transplants and to look for indicators of winter survival, as we noticed that many restoration efforts fail over winter, whereas other sites have been successful, without having a clear indication of distinctive abiotic differences among sites. Our goals were therefore: to test if and how next year's growth success depends on 1) shoot densities in the preceding growth season, 2) the presence of leaves in winter, and 3) the level of the starch reserves. To answer these questions, we combined monitoring data from large-scale transplants (Figure 3.1) with a winter leaf removal field experiment, using *Zostera noltii* as a model species.

MATERIALS AND METHODS

Location

We studied the winter survival of the natural *Zostera noltii* beds and transplanted *Zostera noltii* beds in the Oosterschelde Delta, The Netherlands (Figure 3.1a, 51°39′ N, 4°01′ E). The Oosterschelde has a surface area of 351 km^2, a tidal amplitude of 2.5-3 m (Troost et al., 2009) and freshwater input is highly limited,

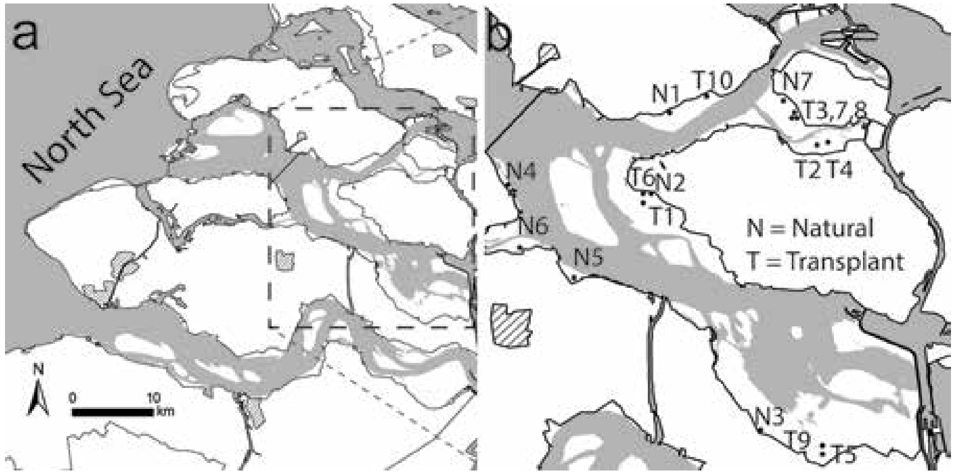


Figure 3.1 Maps of (a) the Dutch Delta, and (b) the locations of the transplant sites (T1-10) and natural seagrass beds (N1-7) in the Oosterschelde.

resulting in a salinity of 30 PSU (Nienhuis and Smaal, 1994). Intertidal *Zostera noltii* meadows cover around 75 ha of the mudflats in the Oosterschelde and are located on relatively stable sediments such as clay banks, saltmarsh remnants and natural shell layers (Suykerbuyk et al., 2012). Intertidal *Zostera noltii* in the Oosterschelde, which is sometimes referred to as *Zostera noltei*, are generally not nutrient limited (Govers et al., 2014a) and light availability (when submersed) is around 3-4.5 m secchi disc visibility in summer (Wetsteyn and Kromkamp, 1994).

Monitoring transplants and natural seagrass beds

As a part of a large-scale seagrass transplantation project, aiming to mitigate seagrass damage caused by dike reinforcements, seagrass sods were mechanically transplanted to 10x10 m or 15x15 m plots on several mudflats in the Oosterschelde (for details, see Suykerbuyk et al. (2012)) (Figure 3.1). Plots were monitored in spring and summer from spring 2009 – autumn 2011; we counted shoot densities and determined seagrass surface area plot^{-1} with a Real Time Kinematic differential GPS (RTK-dGPS, Trimble, USA). Samples for starch analysis were taken each spring (end of April / start of May) and each autumn (end of October, start of November). Simultaneously, samples for starch analysis were taken from a number of natural seagrass beds in the Oosterschelde. In addition several abiotic characteristics (sediment grain size, sediment organic C, leaf %C, leaf %N, porewater NH_4, NO_3, PO_4, and H_2S) were determined every summer (Table 3.1). For detailed methodological details on these measurements, please see Govers et al. (2014b).

To distinguish between successful and unsuccessful transplant sites, we compared shoot densities plot^{-1} in September with shoot densities plot^{-1} in the preceding September (year^{-1}). If shoot numbers were <15% of the shoot numbers in the preceding year, plots were marked as unsuccessful. For transplanted plots in the year of transplanting, we compared shoot densities plot^{-1} in June and September. If shoot numbers had decreased in this period (shoot numbers September<June), we marked those plots as unsuccessful. We classified a transplant site as successful if there were more successful than unsuccessful plots and vice versa. A single transplant site could thus be classified as successful in one year and unsuccessful in the next.

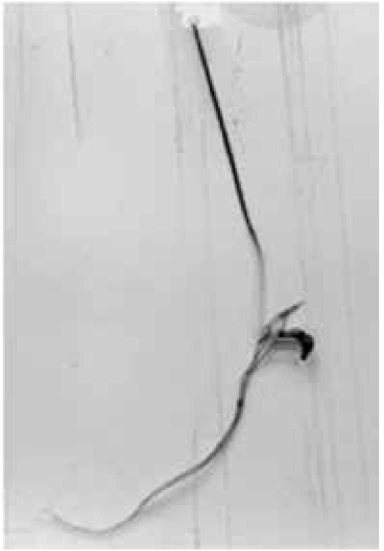


Figure 3.2 Photograph of wintering unit of *Zostera noltii* with one single shoot and an active meristem.

Leaf removal experiment set-up

Winter survival of *Zostera noltii* occurs by a short rhizome with a single shoot, which encloses an active meristem (Vermaat & Verhagen 1996) (Figure 3.2). To test the effect of leaf presence and autumn starch levels on seagrass winter survival, we selected three locations based on their differential starch concentration of rhizomes in November 2012. The average starch concentrations were 57.3 mg g^{-1} DW in Dortsman Noord (DMN – high starch, Figure 3.1b N2), 38.8 mg g^{-1} DW in Oostdijk (OD – medium starch, Figure 3.1b N3), and 18.8 mg g^{-1} DW in KATS (Kats – low starch, Figure 3.1b N4). Next to starch concentration, the length and biomass of the rhizome fragments (in November) also varied between sites at the start of the experiment: rhizome fragments were longer and heavier at OD (medium) and Kats (low) (14±0.44 mm, 3.7±0.07 mg DW and 14±0.40 mm, 4.7±0.08 mg DW respectively) than at DMN (high) (11±0.23 mm, 3.2±0.01 mg DW). A more detailed description of abiotic site characteristics is given in Table 3.2.

On each site, we selected five paired plots of 50x50 cm in November 2012, which were marked with bamboo poles and monitored every month in the period of November 2012 to June 2013. We monitored leaf photosynthetic performance (PAM), as stress indicator (Beer et al., 2001; Brun et al., 2008), shoot density m^{-2} and rhizome starch in each of these plots. In one of each paired plot, all visible aboveground biomass was removed monthly from November - January, which resulted in the leaf treatments + leaf and – leaf (n=5). Rhizomes were cut around the plot borders after each visit to prevent allocation of reserves into the plot. The experiment was terminated in June and the inner 30x30 cm square was harvested.

Processing leaf removal experiment samples

For the leaf PAM measurements, we collected fresh leaf material (~100 leaves) once a month on all three experimental sites in the period of November 2012 to May 2013. The material was randomly collected just next to the plots. Fresh leaf material was stored in a moist and dark cooler for transport to the lab where we measured photosynthetic performance (leaf fluorescence) on the day of collection. Photosynthetic performance was generally high (>0.7 Fv Fm^{-1}; for details, see results section) indicating that transport did not cause stress to the plants (Beer et al., 2001; Ralph and Burchett, 1998). Leaf fluorescence was measured with a Junior PAM (Walz Co, Effeltrich, Germany) on 20 rinsed and randomly selected leaves from each location (n=20). Plants were allowed to adapt to the dark for at least 1h before the saturating pulse was given (Beer et al., 1998; Christianen et al., 2011). Photosynthetic yield (maximum photochemical quantum yield of photosystem II) was calculated based on maximum photochemical efficiency of PSII ((Fm-F0)/Fm). Fm is the maximum phyto-fluorescence after dark-adaptation and F0 is the minimum fluorescence of the chlorophyll. Photosynthetic yield was

Table 3.1 Abiotic characteristic of the natural populations, successful transplants and unsuccessful transplants. Both mean values and standard errors (in italics) of all parameters are displayed. Parameters units are: sediment median grain size (µm), sediment organic C (%), leaf total nitrogen (%DW), leaf total carbon (%DW), porewater ammonium concentrations (µmol L^{-1}), porewater nitrate concentrations (µmol L^{-1}), porewater phosphate concentrations (µmol L^{-1}), and porewater sulfide concentrations (µmol L^{-1}). Hydrodynamic exposure was categorized into three categories: exposed, intermediated, and sheltered. Statistical differences are indicated by letters (a,b), and based on Tukey post-hoc tests.

Site	Grain size	Sediment C	Leaf %N	Leaf %C	NH_4	NO_3	PO_4	H_2S	Hydrodynamic Exposure
Natural populations	115^a *4.6*	0.44^a *0.06*	3.33^a *0.06*	36.1^a *0.4*	55^a *11*	2.0^a *0.8*	13.16^a *1.8*	1.49^a *1.0*	Exposed to sheltered
Successful transplants	133^a *7.9*	0.24^b *0.02*	3.28^a *0.08*	36.6^a *1.2*	88^a *17*	47.1^b *22.2*	17.97^a *2.6*	22.97^b *11*	Exposed to sheltered
Unsuccessful transplants	122^a *4.7*	0.26^b *0.03*	3.32^a *0.04*	36.3^a *0.9*	89^a *9*	26.7^b *3.6*	17.46^a *1.0*	26.65^b *12*	Exposed to sheltered

Table 3.2 Characterization of the leaf experiment sites. Abbreviations: DMN=Dortsman Noord, OD=Oostdijk, Kats=Kats. Sed.=sediment. Units are: starch (mg g^{-1} DW), sediment median grain size (D50, µm), sediment organic C (%), leaf total nitrogen (%DW), and porewater NH_4, NO_3, PO_4 and H_2S were measured in the porewater.

[1] Sampled and measured in November 2012
[2] Sampled and measured in September 2012
[3] Sampled and measured in September 2013

Location	Code map	Starch[1]	Starch characterization	Grain size	Sediment C	Leaf %N[1]	NH_4 [2]	NO_3 [2]	PO_4 [2]	H_2S[3]	Hydrodynamic exposure
DMN	N2	57.3	high	103	0.49	3.1	119.97	0.85	19.67	11.56	exposed
OD	N3	38.8	medium	78	0.44	2.8	22.75	0.00	10.55	23.48	sheltered
Kats	N4	18.8	low	117	NA	3.0	26.75	0.70	7.65	0.00	intermediate

measured on the middle part of each leaf (in the middle between the leaf tip and leaf base).

Simultaneous with the PAM measurements, we counted shoot numbers in the plots and sampled aboveground/belowground tissue in the vicinity of the plots (<5 m). These biomass samples were also transported to the lab, rinsed, and split up into aboveground and belowground biomass, frozen and subsequently freeze-dried, after which their dry weight was determined. Harvested samples were treated in the same way after termination of the experiment.

Starch analysis of transplants, natural beds & experiment

Freeze-dried rhizomes were ground for the starch measurements, and soluble sugars were extracted in ethanol. Subsequently, starch was extracted from the ethanol-insoluble fraction by hydrolysis in 5% HCL and boiled at 100°C for 30 min. Next, both soluble sugars and starch were measured by anthrone assay standardized to sucrose (Yemm and Folkes, 1954). All samples were measured in duplicate and a new duplicate calibration curve was prepared for every series of measurements.

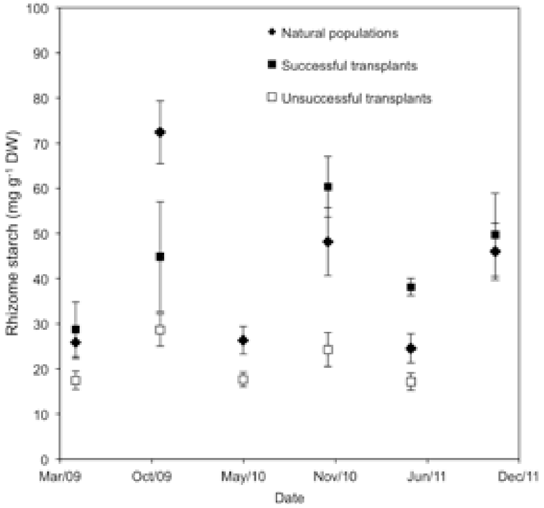


Figure 3.3 Rhizome starch concentration in spring and autumn in the period 2009-2011 of natural populations, successful transplants and unsuccessful transplants in the Oosterschelde. Displayed values are means and error bars represent standard errors (SE). >

Statistical analysis

Normal distribution of the data was tested on the data with the Shapiro Wilk test, and data were log-transformed prior to testing if assumptions were violated. Outliers were detected with Dixon's Q test (Dean and Dixon, 1951), and removed from the data. Abiotic differences between natural population sites, successful, and unsuccessful transplant sites were determined by one-way ANOVAs with a Tukey HSD post-hoc test. PAM yield, shoot density and rhizome starch were tested with a two factor (time, location) repeated measures ANOVA in IBM SPSS Statistics 21.0. Experimental effects and transplant effects were tested with linear mixed effect models using the lme function with a Tukey HSD post-hoc test and location or time as a random term. Data were tested in the R environment (version 2.15).

RESULTS

Transplants and natural beds

We found no correlation between the shoot densities in summer and the shoot densities in the following spring in the transplant plots (R^2=0.02, Figure S3.1). These results indicate that other processes than the preceding year's growth success play a role in predicting growth success in June. Subsequently, we compared rhizome starch concentration of transplanted seagrass patches with seagrass from the natural seagrass beds in the Oosterschelde (Figure 3.3b). Starch concentration varied seasonally, with the highest starch concentration in autumn and lowest starch concentration in spring. Starch concentration of all the unsuccessful transplant sites was always significantly lower than rhizome starch concentration from the successful transplants and the natural beds (Linear mixed model, $F_{2,160}$=23.63, P<0.001), which not differed mutually (Figure 3.3b). This difference was greater at the end of the growing season (autumn) than at the start, which suggests that seagrasses of both natural beds and successful transplants had more reserves to spend during the winter months than the unsuccessful transplants. In contrast, we did not find any significant differences in measured abiotic characteristics between successful and unsuccessful transplant sites (Table 3.1).

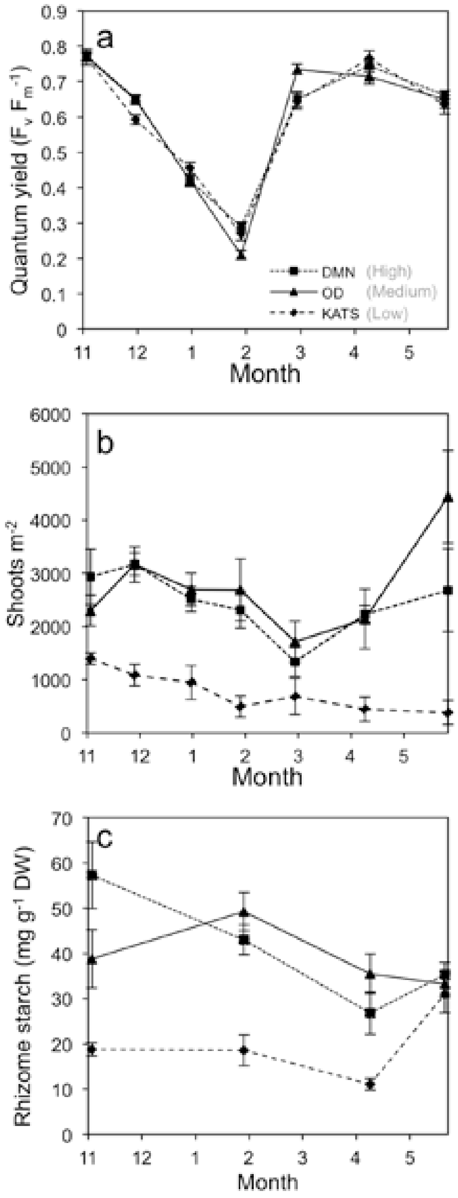


Figure 3.4 (a) PAM yield, (b) shoots m^{-2}, and (c) rhizome carbohydrate concentration of *Zostera noltii* on three different locations. Displayed values are means and error bars represent standard errors (SE).

Leaf removal experiment

Seagrass photosynthetic performance (maximum quantum yield) decreased dramatically in the winter (RPM ANOVA, $F_{3.6}$=282.25, P<0.001), with the lowest quantum yield in February 2013. In March (2013), photosynthetic performance strongly increased, back to growing season levels (Figure 3.4a). Photosynthetic performance did not vary between locations (F_2=0.54, P=0.562, n=20). In contrast, shoot density did vary between locations (F_2=21.23, P=0.001, n=5), and the lowest shoot densities were observed in KATS (low), with 2-3 times lower shoot densities than DMN (high) and OD (medium) (Figure 3.4b). Shoot density did not differ significantly between the latter two locations (Figure 3.4b). Shoot density also decreased during winter ($F_{1.78}$=7.496, P=0.019), but at least some aboveground biomass was present at all locations during the entire winter (<2000 shoots m^{-2} compared to >10,000 shoots m^{-2} in summer). Shoot density strongly increased after the winter at OD (medium) and DMN (high), but further decreased at KATS (low). The latter may reflect a lack of starch to enable regrowth. Rhizome starch concentration (Figure 3.4c) dropped during winter on all locations (F_2=11.054, P=0.001, n=5), and although DMN (high) and OD (medium) started off with different rhizome starch concentration in November 2012, there was no difference between the two locations during winter.

Rhizome starch however remained significantly lower at Kats (low) (F_2=43.77, P<0.001) than at the other two locations during the entire winter. Starch concentration increased again from May (2013) onwards. Although photosynthetic performance (Figure 3.4a) and shoot densities started to increase again around March (2013), in DMN (high) and OD (medium) (Figure 3.4b), rhizome starch concentration dropped from November (2012) onwards to the end of April (2013), and only started to increase again from May (2013) onwards.

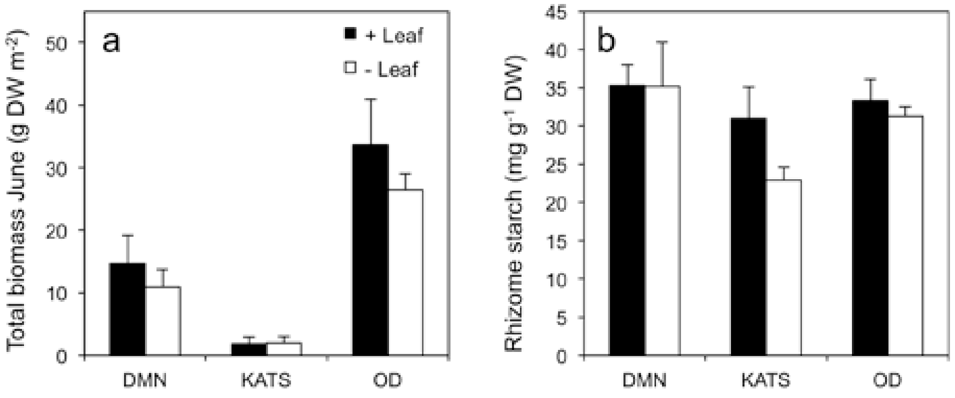


Figure 3.5 Effects of the leaf removal treatment (+leaf and -leaf) on (a) seagrass biomass, and (b) rhizome carbohydrate concentrations in June, at the end of the experiment on three locations: DMN (high), Kats (low), and OD (medium). Displayed values are means and error bars represent standard errors (SE)

Leaf removal treatments resulted in lower shoot densities at DMN (high) (Linear mixed model, $F_{1,53}=7.547$, $P=0.008$) and OD ($F_{1,53}=23.58$, $P<0.001$), where shoot densities dropped by 700-1500 shoots m^{-2} in the –leaf (leaf removal) treatments. The leaf removal treatment however, did not result in a significant decrease in shoot density at Kats (low) ($F_{1,53}=0.607$, $P=0.439$), where standing biomass was very low to begin with (Figure 3.4b). Although we successfully reduced shoot densities, we did not observe any effect of this treatment on seagrass biomass at the end of our experiment in June 2013, on any of the locations (Linear mixed model, $F_{1,26}=0.861$, $P=0.362$) (Figure 3.5a). Seagrass biomass differed significantly between all three locations (ANOVA, $F_{1,27}=31.68$, $P<0.001$), with biomass OD (medium) > DMN (high) > Kats (low) and Kats having a total biomass m^{-2} that was 10x lower than at OD (medium) (Figure 3.5a). Rhizome starch concentration did however not differ anymore between locations in June 2013 (ANOVA, $F_{2,26}=3.207$, $P=0.057$), despite the differences in autumn. Leaf removal treatment did not affect rhizome starch concentration in June 2013 (Linear mixed model, $F_{1,25}=1.81$, $P=0.191$) (Figure 3.5b).

$$y=67.69x-723.43$$ **Equation 3.1**

Rhizome starch concentration in November 2012 was positively related to shoot density in June 2013 ($R^2=0.51$, $P=0.006$) (Figure 3.6), showing the importance of a good start-off before winter for the success in next growing season. The relationship between November rhizome starch and shoot densities in the next June could be described according to the following equation (Figure 3.6).

In this equation (Equation 3.1), x describes rhizome starch levels in November (in mg g^{-1} DW) and y describes shoot densities (# m^{-2}) the following June.

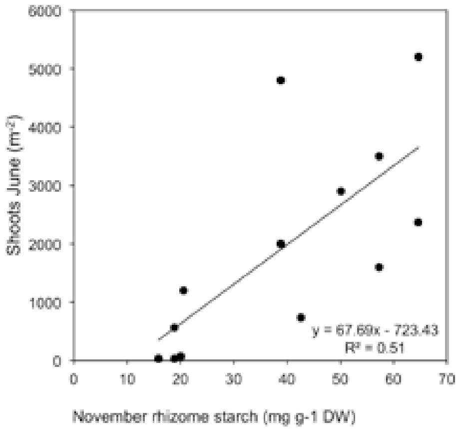


Figure 3.6 The relationship between rhizome carbohydrate concentration in autumn and next year's shoot density in June.

DISCUSSION

Seagrass restoration projects have had varying degrees of success, which sometimes remained unexplained by abiotic site characteristics, due to the high variability in some abiotic parameters and the lack of continuous measurements (Orth et al., 2009; Van Katwijk et al., 2009). We found that the winter period may be an important bottleneck for temperate seagrass transplants, but indicators to forecast transplant performance after winter were lacking. Autumn rhizome starch however, turned out to be an important indicator of growth success (shoot densities) in the following growing season.

Starch reserves and carbon balance

Rhizome starch reserves are important to wintering seagrasses, which experience decreased light availability, and our study shows that rhizome starch concentrations can be indicative of next year's growth success of transplants. Starch reserves reflect the carbon balance of wintering seagrasses. Decreasing starch concentrations indicate a negative carbon balance, while increasing starch concentrations reflect a positive carbon balance, such as we observed from May onwards.

Wintering in small, unbranched rhizome fragments (Vermaat and Verhagen, 1996) limits the total starch storage capacity (g $rhizome^{-1}$), but also reduces respiration costs of the belowground parts. In addition, biomass partitioning between aboveground and belowground parts is critical for the winter carbon balance, as belowground tissue is very important for starch storage and for anchorage. However, this tissue is also heterotrophic, relying on photosynthates from the shoots (Olive et al., 2007). High belowground to aboveground biomass ratios, such as we observed in Kats (low), may generate high respiration rates (Fourqurean and Zieman, 1991). As a result, such populations may even need higher carbohydrate reserves to survive the winter.

Based on our findings, we could predict a minimum starch level needed for winter survival of *Zostera noltii*. By solving equation 3.1 (y=0), a minimum of 10.69 mg g^{-1} starch is needed to yield more than 0 shoots m^{-2} in the next spring. However this number is just an indication as this result is based on observations during only one winter and starch demand may vary among winters due to varying wintering conditions. Furthermore, minimum starch levels for wintering will also vary among species (Silva et al., 2013), as carbon demand depends on plant respiration rates and species specific morphology (Ralph et al., 2007; Silva et al., 2013).

As autumn starch concentration is indicative of growth success in the next season, starch storage in the preceding growing season is very important to monitor in restoration projects. Starch storage at the end of the growing season depends on processes that determine carbon gain (light, temperature, shoot densities) (Ruiz and Romero, 2003), but also the internal carbon demand (Alcoverro et al., 2001). The internal carbon demand is affected by respiration and growth, which are in turn affected by factors such as the weather, sediment conditions, sediment dynamics, and hydrodynamics (Alcoverro et al., 2001; Burke et al., 1996; Herzka and Dunton, 1997). Starch storage at the end of the growing season may be positively affected by selecting suitable sites for transplants, which allows for sufficient photosynthetic gains and relatively low demand due to adverse growth conditions such as sufficient light and nutrient availability and beneficial sediment conditions (Ruiz and Romero, 2003).

Finally, autumn starch concentration may not be the only important determinant of seagrass winter survival. Other processes that may influence winter survival are for instance winter storms and resulting wave-driven sediment dynamics or erosion by ice scouring (Vermaat and Verhagen, 1996). The short roots of *Zostera noltii*, may give limited anchorage to persist these kind of physical disturbances (Cabaco and Santos, 2007; Han et al., 2012). Thus, populations with relative high starch reserves may remain sensitive to winter loss if the restoration site is too exposed or has too mobile sediment.

Cost-benefit analysis: winter leaves, a burden or an asset?

We found that photosynthetic performance of *Zostera noltii* leaves strongly decreased during the winter months, which indicates that plants are stressed (Brun et al., 2008; Howarth and Durako, 2012) by winter conditions (possibly low light levels and low temperatures), which did not vary among sites. In addition, Vermaat and Verhagen (1996) found highly decreased photosynthetic rates in January compared to July, but also stated that leaf photosynthesis is still important to support maintenance during winter. Next to maintenance, ongoing photosynthesis may be an important mechanism to protect belowground tissue and stored carbohydrates against anoxia, as produced oxygen may help to aerate the rhizosphere and prevent prolonged periods of anaerobic respiration (Alcoverro et al., 1999). Seagrasses are not only more vulnerable to intrusion of toxic sulfides during anaerobiosis (Lamers et al., 2013), but metabolites such as ethanol, amino acids, and organic acids may also drain carbohydrate reserves (Hemminga, 1998; Smith et al., 1984). In addition, maintaining a shoot during winter may also be beneficial when light levels and photosynthetic performance increase again in early spring. Instead of increasing carbon demand to grow new leaves, the old shoot can directly be used for photosynthesis.

Despite the above-described benefits of maintaining a leaf-bearing shoot during winter, we did not find any negative effect of our leaf removal treatment on seagrass spring biomass. We therefore conclude that the presence or absence of leaves in winter does not affect next year's growth success for the individual wintering shoots.

CONCLUSIONS AND PERSPECTIVES

Winter survival of temperate seagrasses is largely determined by plant carbohydrate reserves, and autumn starch concentrations can be used as indicators for the success of seagrass transplants in the next growing season. This knowledge may be very useful, since abiotic site characteristics seem to give a very limited indication of transplant success and winter survival. Additionally, high starch reserves also enable an earlier growth-start when the carbon balance is still negative, as was observed at our study sites. This early growth by branching may rapidly increase shoot numbers and thus photosynthetic rates and the resulting storage of starch. Due to this positive feedback, seagrasses on early growth sites may probably store more starch throughout the growing season and will have a better chance of survival in the next winter.

Indicators, such as autumn starch reserves may not only be used to predict transplant success, but also to determine the timing of transplanting, as to make sure that transplanted plants have enough reserves for recovery and regrowth (Zimmerman et al., 1995). From these seagrass wintering mechanisms, lessons may be learnt with respect to other processes that may drain carbohydrate reserves during stressful periods. Examples of such stressors are limited light availability due to eutrophication or dredging (Brun et al., 2008; Erftemeijer and Lewis, 2006; Ralph et al., 2007; Touchette and Burkholder, 2000), and the process of transplanting/moving seagrasses (Sheridan et al., 1998). Starch reserves may also be used to predict chances of survival in highly turbid waters (Burke et al., 1996). Events that may drain carbohydrate reserves, e.g. dredging and transplanting (Ruiz and Romero, 2003), could thus be more conveniently timed when the carbon balance of the involved seagrasses is taken into account. We would therefore recommend timing of such disturbances at the start of the growing season (late spring), when the carbon balance is already positive and seagrasses don't have to rely completely on their reserves. Additional high growth rates in this period may also help seagrasses to recover fast from disturbances such as dredging and transplanting. Hence, a better understanding of the carbon balance of wintering seagrasses may contribute to better protection and restoration of seagrasses.

ACKNOWLEDGEMENTS

We would like to acknowledge J. Eygensteyn of the department of general instruments, Radboud University Nijmegen, for the elemental analysis, and J. van Soelen and B. Sinke of the Netherlands Institute for Sea Research Yerseke, for their technical support. Furthermore, we would also like to acknowledge W. Giesen, P. Giesen, T. Driessen, M. Hendriks, T. Van der Heide, C. Faust, J. de Brouwer, N. Las, N. Govers, T. Pieck and JW. Wolters, for their contribution to transplant monitoring. We also thank Steef Hanssen for language editing and we finally also thank two anonymous reviewers for their valuable comments on an earlier version of the manuscript. This research was funded by Projectbureau Zeeweringen and Rijkswaterstaat Zeeland.

SUPPLEMENTARY DATA CHAPTER 3

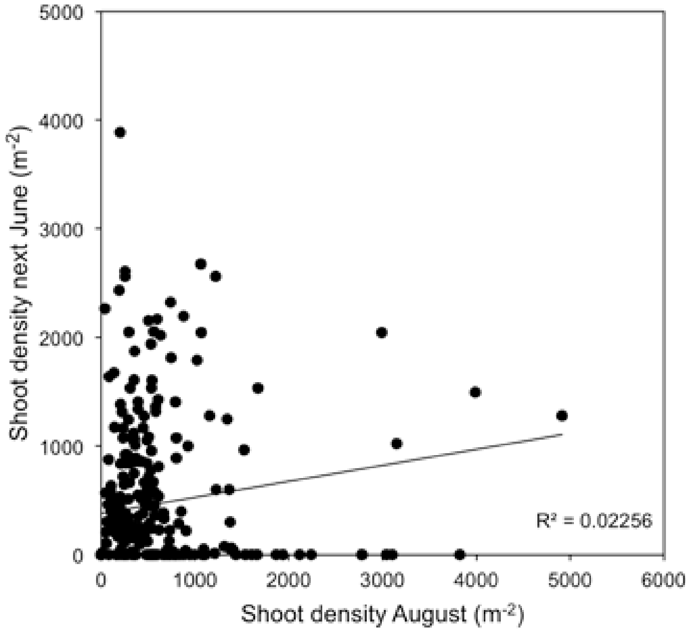
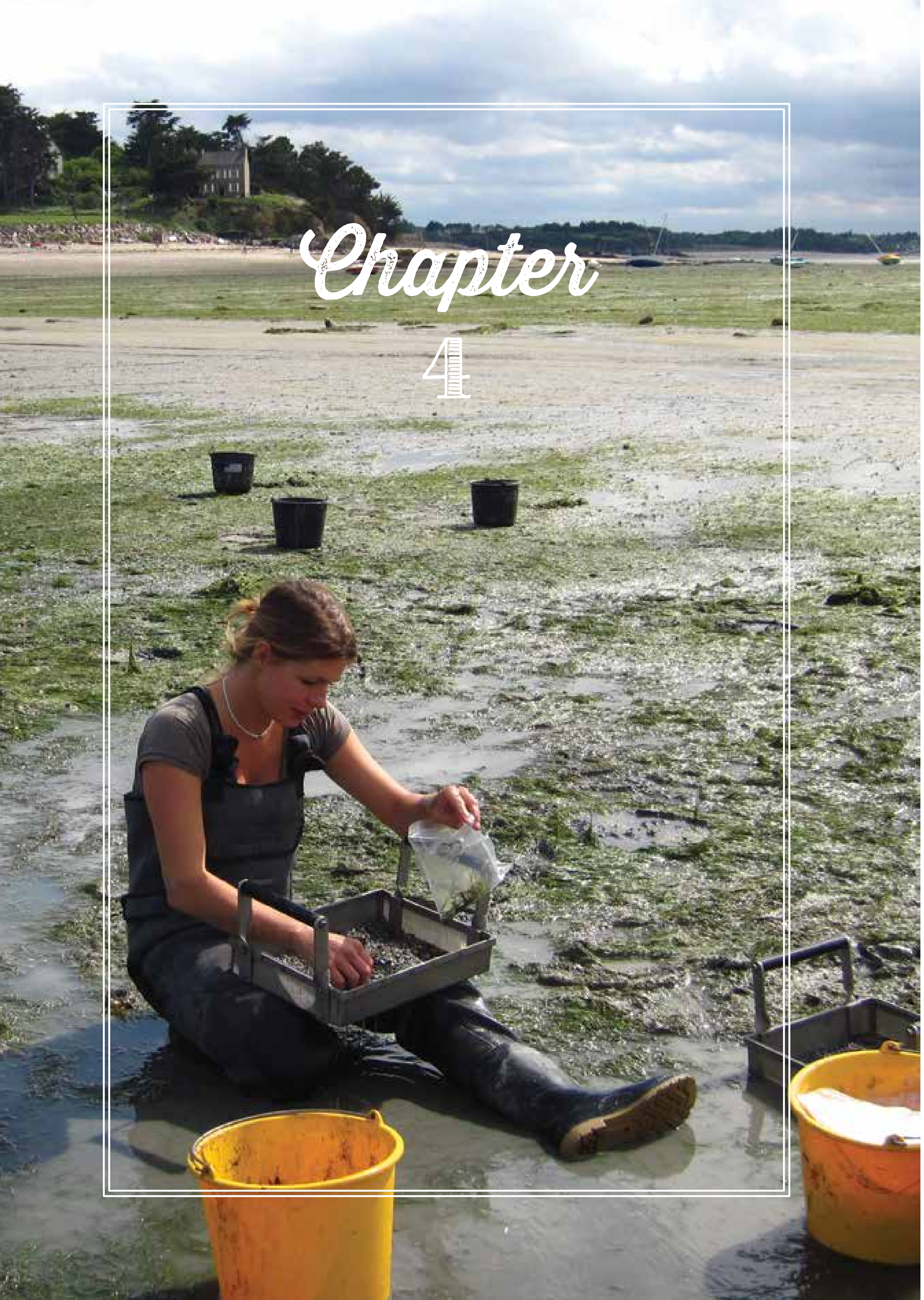


Figure S3.1 Correlation between shoot densities in summer and shoot densities in the following spring of the transplants in the first 36 months after transplanting.



FEEDBACKS AND LOCAL ENVIRONMENTAL SETTINGS AFFECT PERSISTENCE AND RECOVERY DYNAMICS OF A COASTAL ECOSYSTEM

Laura L. Govers, Tjeerd J. Bouma, Evelyn van der Ent, Wouter Suykerbuyk, Laurent Godet, Raghnild M. Asmus, Tjisse van der Heide, Marieke M. van Katwijk (manuscript in prep.)

Abstract

Human activities in coastal areas have altered background conditions and introduced new disturbance regimes, leading to the global degradation of coastal ecosystems. It is important to assess ecosystem resilience to disturbances to predict and thereby prevent disappearance of these ecosystems. To better understand resilience, it is essential to gain understanding of how local background conditions and feedbacks may interact with disturbances. We therefore studied two aspects of resilience: persistence to and recovery from stressors and small-scale disturbances. We questioned how 1) local sediment conditions, 2) enhanced stress through application of a standardized stressor (OM-addition), and 3) density-dependent feedbacks, determined local persistence (i.e., growth) and disturbance recovery (i.e., regrowth), using intertidal seagrass beds (Zostera noltii) as model systems. The study was carried out at three field sites across Europe, encompassing a gradient of sediment conditions, where we applied a small-scale disturbance (i.e., creating a gap). Persistence and recovery greatly differed between locations and revealed some clear general patterns. Recovery was locally decreased by high sulfide concentrations resulting from OM-addition. Persistence was much less affected by OM-addition, because dense seagrass stands alleviated sulfide toxicity (joint-detoxification). The interaction between the stress treatment (OM-addition) and local sediment conditions determined local porewater sulfide concentrations and subsequent effects on local persistence and recovery. As a result, joint-detoxification was conditional, namely less important in coarse-grained than in fine-grained sediments. Our results illustrate the complexity of assessing ecosystem resilience resulting from the intricate interplay between interacting local conditions, feedbacks and disturbances affecting persistence and recovery dynamics.

INTRODUCTION

Many valuable coastal ecosystems such as coral reefs, seagrass meadows, mangroves and salt marshes are globally declining as a result of human-induced stressors and disturbances such as eutrophication (Burkholder et al. 2007), increased trace metal levels (Eisler 1981), impoverished trophic relationships (Estes et al. 2011), and climate change (Hughes 1994, Alongi 2002, Orth et al. 2006, Waycott et al. 2009, Deegan et al. 2012). To counteract such losses, attempts have been made to restore these valuable habitats, but restoration of coastal ecosystems has proven to be notoriously difficult (Rinkevich 1995, van Katwijk and Hermus 2000, Zedler 2000, Van der Heide et al. 2007, van Katwijk et al. submitted). Therefore, managers and conservationists have been trying to predict and thereby prevent ecosystem collapses both by looking for early warning indicators and by trying to estimate the resilience of ecosystems to further stresses and disturbances (Elliott et al. 2007).

Quantifying ecosystem resilience has however proven to be challenging, which is also reflected by the ongoing debate on the definition of resilience (Peterson et al. 1998, Folke et al. 2004, Elliott et al. 2007). Resilience has been described as the capacity to deal with disturbances either as ‘a measure of persistence of systems and of their ability to absorb change and disturbance and still maintain the same relationships between populations or state variables’ (Holling 1973) or as ‘the time needed to return to the equilibrium state’ (Pimm 1984). So in a broad sense, resilience refers to both the capacity to persist stresses and to recovery from disturbance, implying that measuring resilience should focus on quantifying both continued growth following a stress and regrowth following disturbances. Quantifying both aspects may however be complicated by interacting effects between stress and disturbances.

Understanding interacting effects between stress and disturbances on resilience is highly important, as human activities have in many cases caused both enhanced disturbance regimes that may lead to ecosystem degradation (Orth et al., 2006; Waycott et al., 2009), and enhanced background stress levels (Nystrom and Folke, 2001). Little is known about the effects of background stress levels on the capacity to recover after disturbances in coastal ecosystems, but it is very likely that an increase in anthropogenic background stressors affects ecosystem resilience to disturbances. (Hughes et al. 2003). Even in the absence of anthropogenic stressors, local differences in natural environmental conditions such as sediment characteristics or hydrodynamic regimes, are likely to represent different stress levels that may also affect the resilience of coastal ecosystems to disturbances.

Foundation species, or ecosystem engineers, are key-components of most valuable ecosystems. Ecosystem engineers such as seagrasses, corals, mangroves and salt marsh plants alter their abiotic and biotic environment (Jones et al. 1994) resulting from positive feedback mechanisms (Van der Heide et al. 2011). A well known example of a positive feedback in vegetated coastal ecosystems, is the attenuation of waves and currents by seagrasses and salt marsh vegetation (Bouma et al. 2005). This results in stabilization of coastal sediments (Bos et al., 2007; Christianen et al., 2013) and the trapping small particles from the water column (Granata et al. 2001, Hendriks et al. 2010) which generally leads to an improved water clarity and thereby enhanced growth for seagrasses (Van der Heide et al. 2011). Similar positive feedback mechanisms exist for other coastal ecosystems (Allen 2000, Huisman et al. 2009, Wild et al. 2011, Altieri et al. 2013, Gillis et al. 2014), and typically require a minimum population density and/or coverage (Bouma et al. 2009). Therefore, it may be expected that stressors and disturbances have a bigger effect on ecosystem resilience when breaking self-facilitating positive feedbacks, e.g. by reducing density.

We thus questioned how 1) local environmental stress levels, 2) enhanced stress levels by application of a standardized stressor (organic matter addition to induce sulfide stress), and 3) density-dependent feedbacks, determined ecosystem resilience in terms of local persistence (i.e. growth) and disturbance recovery (i.e. regrowth), using intertidal seagrass beds (*Zostera noltii*) as model systems. Seagrasses are relevant model species as seagrass beds have been experiencing an accelerating loss due to anthropogenic stressors and disturbances (Waycott et al. 2009). Furthermore, *Zostera noltii* can easily been found on sites with contrasting local background stress levels (both natural and human-induced) and differing local densities, which may be expected to alter positive feedback strength. Moreover, additional stress can easily be applied in a standardized form, by organic matter addition (Govers et al.).

MATERIALS AND METHODS

Site description

The experiment was conducted in intertidal *Zostera noltii* beds in three countries in Western Europe: France, The Netherlands, and Germany (Figure 4.1). For the experiment, we selected a high-density and low-density bed on each location. The French site was located on the mudflats of Saint-Jacut-de-la-mer (hereafter referred to as St Jacut), on the north coast of Brittany (48°36 14 N, 2°11 41 W). This site was characterized by the highest sediment organic matter (OM) levels of all sites, and a very fine grain size (Table 4.1), due to aquaculture and limited circulation in the bay of Arguenon (Varcourt and Bonnot-Courtois 1992). The Dutch site was located on the mudflats of Viane, in the Oosterschelde Delta (51°61 88 N, 4°01 77 E), and was characterized by both intermediate sediment organic matter levels and grain size (Table 4.1). Finally, the German site was situated on the mudflats of Rantum, on the barrier island Sylt (hereafter referred to as Sylt) in the northern Wadden Sea (54°47 50 N, 8°17 43 E), and was characterized by coarse-grained sediment and low %OM (Table 4.1). Sediment Organic matter (%OM) was significantly higher in the high-density beds compared to the low-density beds on all locations (Linear mixed model, $F_{1,39}$=12.1, P=0.001) (Table 4.1). In contrast, median grain size (D50) was significantly lower in the high-density beds than in the low-density beds ($F_{1,44}$=5.6, P=0.022). Exposure time of the intertidal *Zostera noltii* beds during low tide was between 5-6 hours for all three sites. The experiment was conducted from late June to the end of August in the summer of 2011 and lasted for 52 days.

Figure 4.1 Map of Western Europe with the locations of the field sites: St Jacut in Brittany, France, Viane in the Oosterschelde, The Netherlands, and the island of Sylt, Germany.

Experimental set-up

On all three sites, we selected a high- and a low-density bed (Table 4.1), in the vicinity (max 100 m distance) of each other, to keep environmental characteristics as similar as possible. High-density and low-density sites were significantly different in terms of biomass on all locations (linear mixed model, $F_{1,32}$=29.3, P<0.001). In each bed, we selected 12 plots in a homogenously vegetated area, where we created circular 35 cm diameter gaps, in which we removed all seagrass biomass at t0. The removed biomass was collected and taken to the lab for further analysis. The gap was at the center of a larger 105 cm diameter circle (the experimental plot) along which we cut the rhizomes to a depth of 10 cm with a knife.

Table 4.1 Sediment and seagrass characteristics of the low and high-density bed at each location.

Location	Density	Sediment %OM	Sediment D50 (µm)	Biomass t52 (g DW m^{-2})
St Jacut (FR)	low	2.93	132	176
	high	3.77	119	266
Viane (NL)	low	1.26	198	17
	high	2.83	171	108
Sylt (DE)	low	0.46	325	44
	high	0.64	334	139

We randomly applied one of two stress-treatments to the plots (n=6): a control treatment (Control) or an organic matter addition treatment (+OM). For the OM-treatment, we produced OM-sticks consisting of labile organic matter (1:4 starch and sucrose) and water. 45 Sticks containing 20 g OM each were regularly distributed in the sediment (every 15x15 cm, to a depth of ~7 cm) in the large 105 cm circle. The labile OM was only reactive when it slowly dissolved from the sticks; we thus created a slow-release carbon source. To compensate for the physical disturbance of placing the sticks in the sediment, we applied a similar disturbance to the control plots using a knife. Next, we monitored the plots after 30 days and after 52 days.

Before the start of the experiment, we randomly selected 8 sites in each meadow (in both the high- and low-density beds), where we collected sediment samples and porewater samples. Porewater samples were collected anaerobically in the top 6 cm of the sediment with vacuumed 60 ml syringes, connected to Rhizon soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). At t30 and t52 days, we collected porewater samples in the experimental plots. Two samples were collected in each gap (hereafter referred to as gap) and subsequently pooled and two samples outside the gap, but inside the experimental plot (hereafter referred to as meadow), were collected in the same way. Seagrass biomass was sampled a t0 inside the gap as all biomass was removed from the gap. At t30, we sampled biomass outside the gap (in the meadow, within the experimental plot) with two small 6 cm diameter cores per plot, and at 52 we harvested all regrown biomass from the gap and again sampled two 6 cm diameter cores outside the gap.

Sample analysis

40 ml of each porewater sample was frozen after collection and transported to the lab for further analysis. The remainder of the samples was immediately used to measure total sulfide concentration in the porewater, in a mixture of 50% sample and 50% sulfide anti-oxidation buffer (SAOB) (Lamers et al. 1998), using an ion-specific silver-sulfide electrode. Porewater ammonium and ortho-phosphate concentrations were measured colorimetrically (Skalar and Seal autoanalyzer), using ammonium-molybdate and salicylate. Nitrate was determined by sulphanilamide, after reduction of nitrate to nitrite in a Cadmium column.

Seagrass biomass samples were rinsed and sorted into aboveground and belowground biomass and shoot numbers were counted. Subsequently, sorted seagrass was freeze-dried and weighed. Seagrass leaf nitrogen and carbon concentrations were determined on grinded freeze-dried plant material, using an elemental analyzer (Type NA 1500 Carlo Erba Thermo Fisher Science, USA), coupled online via an interface (Finnigan Conflo III, USA) to a mass spectrometer

(Thermo Finnigan Delta Plus, USA). Total phosphorus in seagrass tissue was measured on an inductively coupled plasma emission spectrophotometer (ICP) (Spectroflame, Spectro Inc., USA), after digestion of freeze-dried plant material with nitric acid and H_2O_2.

Sediment grain size distribution was determined on dried and sieved (1 mm mesh size) sediment samples by laser diffraction on a Malvern (Master 2000, UK) particle size analyzer. Sediment organic matter (%) was determined by weight loss on ignition (LOI) at 550°C.

Statistical analysis

We used two-way ANOVAs for data with two factors (density * treatment) and linear mixed models to test for the effects of joint detoxification (density * treatment * gap/meadow) with plot as a random factor (error term in aov). We used a stepwise multiple linear regression with a backward selection to test which measured biotic and abiotic parameters highly affected seagrass recovery and persistence biomass. For the backward stepwise multiple linear regression we first included all possible explaining parameters for recovery biomass (biomass meadow, sulfide gaps, sulfide meadow, NH_4 gap, NH_4 meadow, PO_4 gap, PO_4 meadow). Sulfide, NH_4 and PO_4 were included as the weighted average per plot (separately for gap and meadow). The final adjusted models were the most reduced models, which were not significantly worse ($P<0.05$) than the full model that included all factors, and model selection was based on the Akaike information criterion (AIC). Normality of the models was tested on the residuals, and data were log-transformed prior to analysis to meet the model assumptions of normally distributed data. Final models resulting from the stepwise multiple linear regressions were also tested for independence with the Durbin-Watson test and colinearity was checked using the variance inflation factor (vif) (Field et al. 2012). We used R 2.15 for all statistical analysis.

RESULTS

Comparison of persistence (growth) and recovery (regrowth)

Although we found no significant effect of density on recovery biomass in St Jacut (2-way ANOVA, $F_{1,20}=1.8$, $P=0.196$), we observed a significant decrease in recovery as a result of the OM- addition ($F_{1,20}=4.6$, $P=0.045$) (Figure 4.2a). Then, on the other two sites (Viane and Sylt, Figure 4.2b & c), we did not find a significant effects of OM-addition on recovery, but we did find a significant lower recovery biomass on the low-density sites (Viane: $F_{1,20}=24.6$, $P<0.001$, Sylt: $F_{1,20}=22.3$, $P<0.001$). Both St Jacut sites, the high-density site of Viane, and the low-density site of Sylt had comparable recovery biomass (2-4 g DW m^{-2}). However, we found almost 5x higher recovery biomass on the high-density site of Sylt than on the above sites (Figure 4.2c).

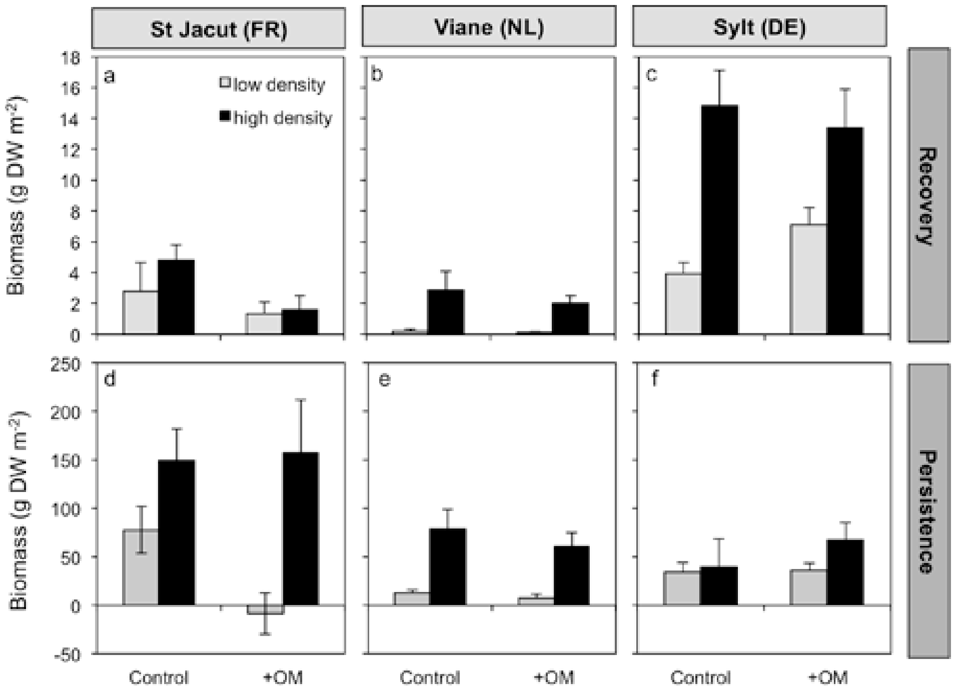


Figure 4.2 Absolute recovery biomass (regrowth biomass in the gap) of low- and high-density seagrass beds in (a) St Jacut, (b) Viane, and (c) Sylt and persistence (growth: biomass difference between t0 and t52) in (d) St Jacut, e) Viane, and f) Sylt. Displayed values are means and error bars represent standard errors, n=6.

We observed a different trend in the persistence (growth) biomass, which was the increase in seagrass biomass of the meadow between t52 and t0 (Figure 4.2). Persistence was highest by far on the high-density site of St Jacut (Figure 4.2d), namely an increase of ~150 g DW m^{-2} in 52 days, while we observed about 50% less growth (persistence) on St Jacut low-density and Viane high-density sites (Figure 4.2d-f). The lowest persistence was observed on the Viane low-density site (<10 g DW m^{-2} in 52 days). In both St Jacut and Viane, the low-density sites were significantly less persistent than the high-density sites ($F_{1,20}=11.2$, $P=0.003$, $F_{1,20}=70.0$, $P<0.001$ for St Jacut and Viane respectively) (Figure 4.2b & c). Additionally, persistence in Viane considerably decreased as a result of the OM-addition ($F_{1,20}=5.5$, $P=0.030$), while persistence was not significantly affected by OM-addition on the other two sites, even though some plots decreased in biomass during the experiment, which resulted in negative persistence (or growth, Figure 4.2d, low-density +OM).

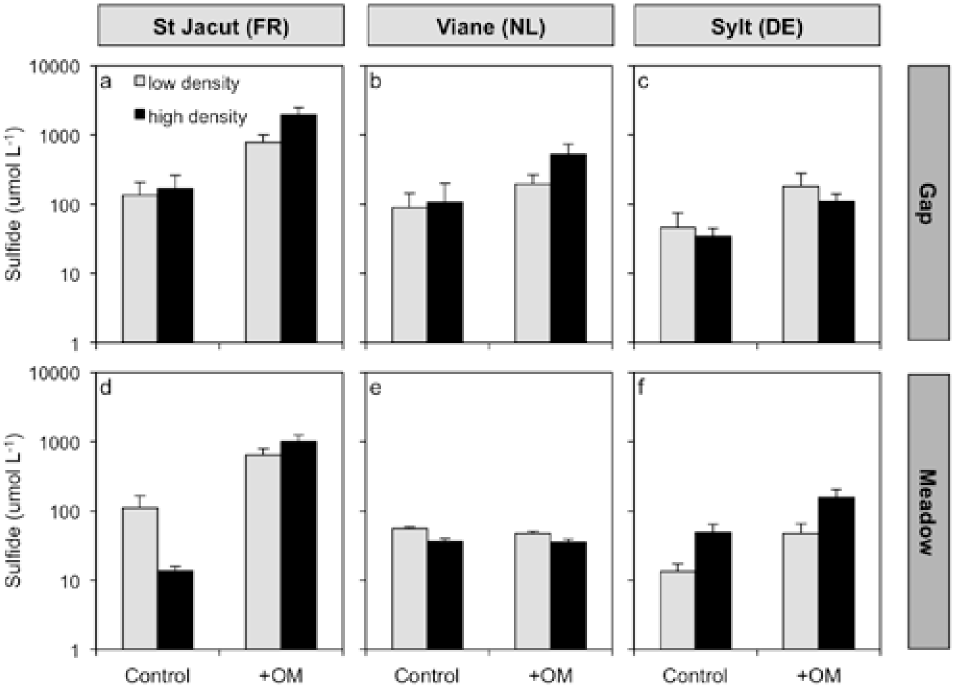


Figure 4.3 Porewater sulfide concentrations (weighted averages) in gap (a-c) and meadow (d-e) (outside the gap but inside the experimental circle) on three locations. And the effects of density and treatment on porewater sulfide concentrations. Displayed values are means and error bars represent standard errors, n=6.

Location and density effects on sulfide stress treatments

OM-addition successfully led to significantly elevated porewater sulfide concentrations in the gaps on all three locations (2-way ANOVAs, St Jacut $F_{1,20}$=27.2, P<0.001, Viane $F_{1,20}$=8.4, P=0.009, Sylt $F_{1,20}$=8.5, P=0.008) (Figure 4.3a-c). In St Jacut, the OM-treatment resulted in sulfide levels higher than 1000 µmol L^{-1}. However, we also detected rather high sulfide concentrations in the control treatments (200-300 µmol L^{-1}) of St Jacut. In Viane, OM-addition resulted in enhanced sulfide concentrations of 270 and 1000 µmol L^{-1} in the low- and high-density respectively. Finally, The lowest sulfide concentrations were measured in Sylt, where sulfide concentrations reached up to 350 µmol L^{-1} in the OM-treatments, but always kept below 50 µmol L^{-1} for the controls. We did not find an effect of density on gap sulfide concentrations.

Addition of OM also significantly increased porewater sulfide concentrations outside the gap, in the meadow (Figure 4.3d-f) on all locations but Viane (NL) (St Jacut $F_{1,20}$=74.3, P<0.001, Sylt $F_{1,20}$=10.2, P=0.005). OM-addition led to sulfide levels of 600-1000 µmol L^{-1} in St Jacut and 50-100 µmol L^{-1} in Sylt outside the gaps (meadow), whereas OM-addition did not affect porewater sulfide concentrations in Viane (35-50 µmol L^{-1}). In contrast to inside the gap, density did affect porewater sulfide concentrations in the meadow: Sulfide concentrations of the control treatments were lower in the high-density bed than in the low-density bed (Figure 4.3d, $F_{1,20}$=7.1, P=0.015) in St Jacut. In Viane, sulfide levels were lower in the high-density than in the low-density beds (Figure 4.3e, $F_{1,20}$=16.4, P<0.001), and contrastingly, we measured higher sulfide concentrations in the high-density bed than in the low-density bed in Sylt (Figure 4.3f, $F_{1,20}$=14.6, P=0.001).

As a proxy for joint detoxification, we calculated the difference in porewater sulfide concentrations between gaps and the surrounding meadow, assuming that all local conditions are more or less equal, except for vegetation density (Figure 4.4). We found a significant joint detoxification effect in St jacut (Figure 4.4a, Linear Mixed Model, $F_{1,39}$=4.7, P=0.037), where joint detoxification was as high as ~1000 µmol L^{-1} (50% reduction) in the high density OM-treatment. In Viane, we only found a significant joint-detoxification effect in the OM-treatment (Figure 4.4b, $F_{1,39}$=8.9, P=0.005) as higher seagrass densities outside the gaps reduced sulfide concentrations by 150-500 µmol L^{-1} (>90% reduction) tot non-lethal levels (<50 µmol L^{-1}). Additionally, we also observed joint-detoxification of about 30-130 µmol L^{-1} in Sylt, but surprisingly, only in the low-density beds (Figure 4.4c, $F_{1,39}$=5.5, P=0.024), as the difference in sulfide between gap and meadow was higher on the low-density beds than in the high-density beds.

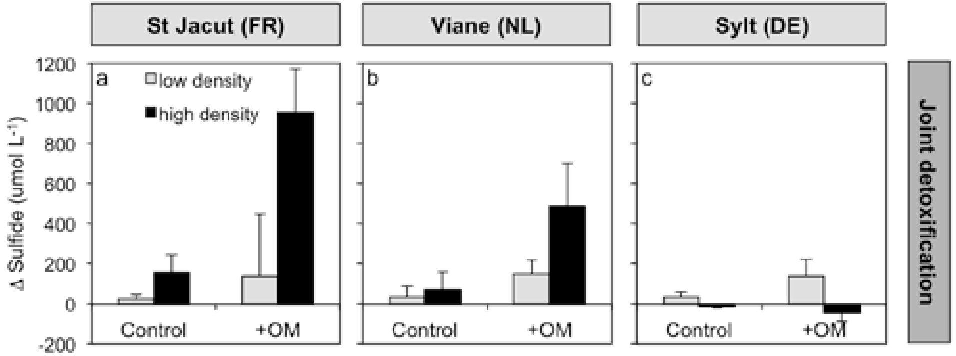


Figure 4.4 Joint detoxification effects on porewater sulfide concentrations (difference in sulfide concentrations between gap and surrounding meadow) of low- and high- density beds in (a) St Jacut, (b) Viane, and (c) Sylt and the effects of OM-addition on joint detoxification effects. Displayed values are means and error bars represent standard errors, n=6.

Identifying main drivers of seagrass persistence and recovery

Multiple linear regression analyses revealed which measured factors explained most of the variation in persistence and recovery biomass for all three locations (Table 4.2). In St Jacut, 35% of the variation in recovery biomass could be explained by sulfide (P=0.004) and PO_4 (not significant) concentrations in the gap. The same two factors, as well as the meadow biomass outside the gap explained 71% of the variation in recovery in Viane (P=0.003, P=0.054, and P<0.001 for gap PO_4, gap sulfide and meadow biomass respectively). In Sylt, 38% of the variation was explained by gap PO_4 concentrations (P=0.335) and meadow biomass (P=0.035). For persistence (i.e., growth), 33% of the variation in St Jacut was explained by sulfide concentrations in the meadow, sulfide in the gap, and PO_4 in the meadow (P=0.023, P=0.064, P=0.062 for sulfide gap, sulfide meadow and PO_4 meadow respectively). In Viane, porewater sulfide concentrations in the meadow alone explained 42% of the variation in persistence (P<0.001). Also in Sylt, porewater sulfide concentrations in the meadow were important and together with porewater PO_4 concentrations in the meadow explained 41% of the variation in persistence (growth) in Sylt.

Table 4.2 Results from the stepwise backwards multiple regression analysis of continuous biotic and abiotic variables on persistence and recovery biomass. Biomass is in g DW m^{-2} and PO_4 and sulfide concentrations are in µmol L^{-1}.

Recovery (regrowth biomass)						
Location		**δR²**	**B**	**SE B**	**P**	
St Jacut	**Adjusted model**	0.35	-0.30	2.50	0.901	
	PO_4 gap		2.42	1.38	0.094	
	Sulfide gap		-1.12	0.25	0.004	**
Viane	**Adjusted model**	0.71	-5.53	3.5	0.131	
	Biomass meadow		1.50	0.24	<0.001	***
	PO_4 gap		-5.73	1.66	0.003	**
	Sulfide gap		0.38	0.19	0.054	
Sylt	**Adjusted model**	0.38	-1.17	1.13	0.271	
	Biomass meadow		0.47	0.21	0.035	*
	PO_4 gap		-0.68	0.69	0.3354	

Persistence (growth biomass)						
Location		**δR²**	**B**	**SE B**	**P**	
St Jacut	**Adjusted model**	0.33	6.64	0.73	<0.001	***
	Sulfide gap		0.29	0.12	0.023	*
	Sulfide meadow		-0.18	0.09	0.064	
	PO_4 meadow		-0.90	0.45	0.062	
Viane	**Adjusted model**	0.42	13.51	2.51	<0.001	***
	Sulfide meadow		-2.67	0.67	<0.001	***
Sylt	**Adjusted model**	0.41	0.26	1.27	0.840	
	Sulfide meadow		0.29	0.11	0.015	*
	PO_4 meadow		1.67	0.70	0.027	*

Summarizing, variation in recovery was mostly explained by porewater sulfide concentrations in the gap (St Jacut, Viane) and meadow biomass (Viane, Sylt), whereas persistence variation was mostly explained by porewater sulfide concentrations in the meadow (Viane, Sylt) and PO_4 concentrations in the meadow (Sylt).

DISCUSSION

Human activities have enhanced background stress levels and disturbance regimes leading to the worldwide degradation of ecosystems (Alongi 2002, Hughes et al. 2003, Dudgeon et al. 2006, Waycott et al. 2009, Deegan et al. 2012). Little is known about the interaction between background stress levels and disturbances, although it is very likely that high background stress levels affect ecosystem resilience to disturbances (Nystrom and Folke, 2001). Furthermore, stressors and disturbances may have disproportionate effects on ecosystem resilience when breaking self-facilitating positive feedbacks that typify many foundation species. We therefore studied how local environmental conditions and potential positive feedbacks affect resilience of a vegetated coastal ecosystem (seagrass bed). We found distinct differences in local persistence and recovery among our three contrasting study sites as a result of interacting local background conditions and the applied stressor (OM): high sulfide levels negatively affected both persistence and recovery, although the level of this stressor was largely determined by local (sediment) conditions. In addition, sulfide concentrations were reduced outside the experimentally created gaps (meadow) compared to inside the gaps, which may be attributed to joint detoxification by higher seagrass densities outside the gap. This mechanism seemed particularly relevant to seagrass beds with sediment conditions that may promote high sulfide concentrations, or possess high seagrass densities (i.e. St Jacut). Hence, we found that persistence and recovery of a vegetated coastal ecosystem after disturbance were determined by interacting local background settings, the applied stressor, and a positive feedback.

Persistence and recovery

Ecosystems frequently experience both natural and human-induced disturbances, resulting in a heterogeneous landscape reflecting recovery processes (Pickett and White 1985, Brun et al. 2003). Gaps in vegetated coastal habitats, caused by disturbances such as burial, anchoring and dredging may quickly recover by clonal rhizomal growth of plants at the edges of the gaps (Rasheed 1999, Kenworthy et al. 2002, Ewanchuk and Bertness 2004, Macreadie et al. 2014). Recovery of the gaps may therefore depend on the surrounding shoot density or biomass, such as observed in our experiment (Figure 4.2, Table 4.2). Persistence and recovery differed greatly between the studied seagrass beds (Figure 4.2), which may be a result of different background conditions affecting persistence (i.e. growth) and interacting with recovery processes. We observed local differences in background sediment conditions (median grain size and soil organic matter content), which interacted with the applied stressor (OM-addition). These conditions may be the result of a biotic feedback loop, that is, higher seagrass densities trap more fine sediment and organic particles than lower densities (Hendriks et al. 2008). Alternatively, high sediment organic matter content may result from an external input of organic matter such as eutrophication or aquaculture (Cancemi et al. 2003, Burkholder et al. 2007). This leads to higher sediment organic matter loads, which promotes decomposition by sulfate reducing bacteria, a process which results in the production of toxic sulfides (Jørgensen 1984). Moreover, fine-grained sediments (Table 4.1, St Jacut, Viane) stimulate anaerobic conditions, which are ideal for sulfate reduction (Jorgensen 1982) and are characterized by reduced exchange rates between the porewater and surface water, promoting the build-up of toxic sulfides. Adding up to complexity, high seagrass densities may enhance joint detoxification of high sulfide concentrations as indicated by our study, see below. The observed differences between sites illustrate the importance of local stressors/background conditions when dealing with disturbances, as stressors may affect or even interact with disturbance events. Similar responses may be observed in other ecosystems, such as in coral reefs, where susceptibility of coral reefs to disturbances may also be site specific, and depending on background stress levels (Brown 1997).

Furthermore, when considering the response of a system after a disturbance and including persistence (i.e. local growth) and recovery (i.e. regrowth), we can distinguish between four different system responses (Figure 4.5c). Interestingly, three possible outcomes are represented by the contrasting responses of our experimental sites (Figure 4.5d). These possible outcomes, or 'strategies' are plotted in a scatterplot (Figure 4.5a) where the strength of the process is plotted on the 1:1 diagonal, with the most resilient sites in the upper right corner, whereas the dominance of the process (persistence vs. recovery) is plotted on the other diagonal. We can use this plot to scale-up from our small-scale disturbances to landscape-scale effects. A highly persistent and quickly recovering habitat could be considered resilient as such a system will be able to (quickly) return to it's equilibrium (the pre-disturbed state) after a disturbance. This conceptual model may also be applicable to other ecosystems such as salt marshes and coral reefs. For instance, a highly resilient coral reef will not only be able to persist prevailing background stressors, but will also be able to recover quickly from disturbances such as a tropical storm (Figure 4.5a) (Gunderson 2000, Folke et al. 2004). Contrastingly, cordgrass in salt marshes may be able to persist in large patches, whereas recovery/recruitment in drought-induced die-off areas may be limited (Angelini and Silliman 2012).

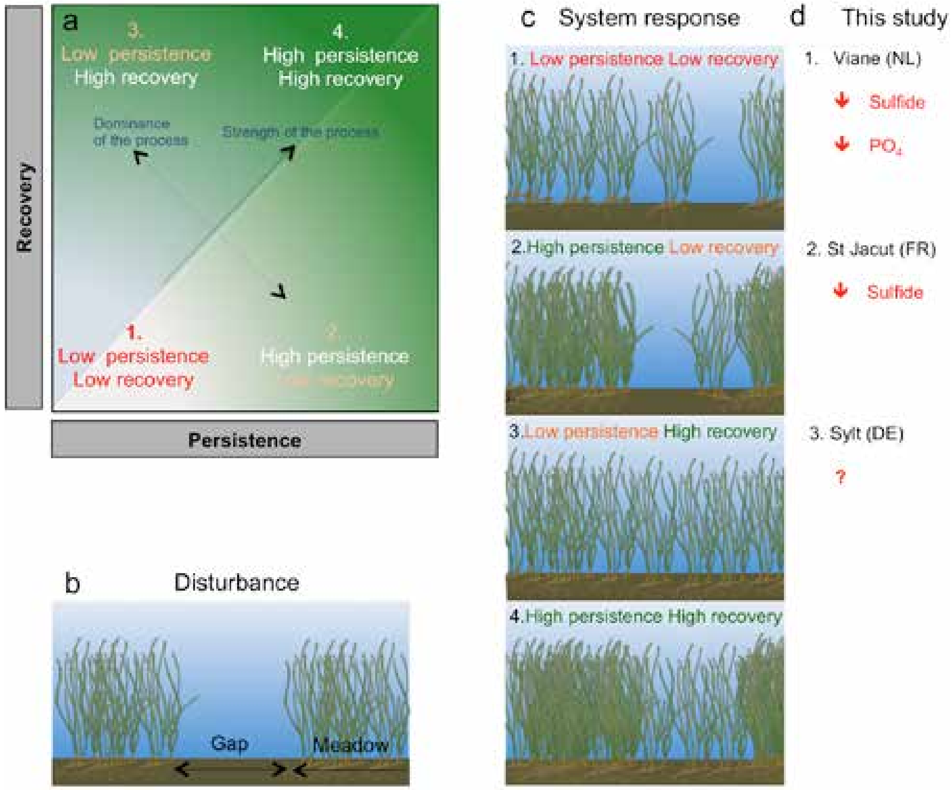


Figure 4.5 Conceptual idea of seagrass growth strategies after a small-scale disturbance. (a) Diagram based on the interplay between persistence and recovery determining resilience of a system. The dotted and striped arrows in the graph indicate the strength of the process (the stronger, the more resilient) and the dominant process (persistence vs. recovery). Additionally, (c) four different outcomes may result from a small-scale disturbance such as (b) the creation of a gap. Three out of four possible outcomes are represented (d) by our experimental sites. Factors that are negatively affecting persistence and recovery (Table 4.2) are indicated with downward arrows. We could not find a negative effect on persistence on Sylt (3).

Conditionality of density-dependent stress alleviation

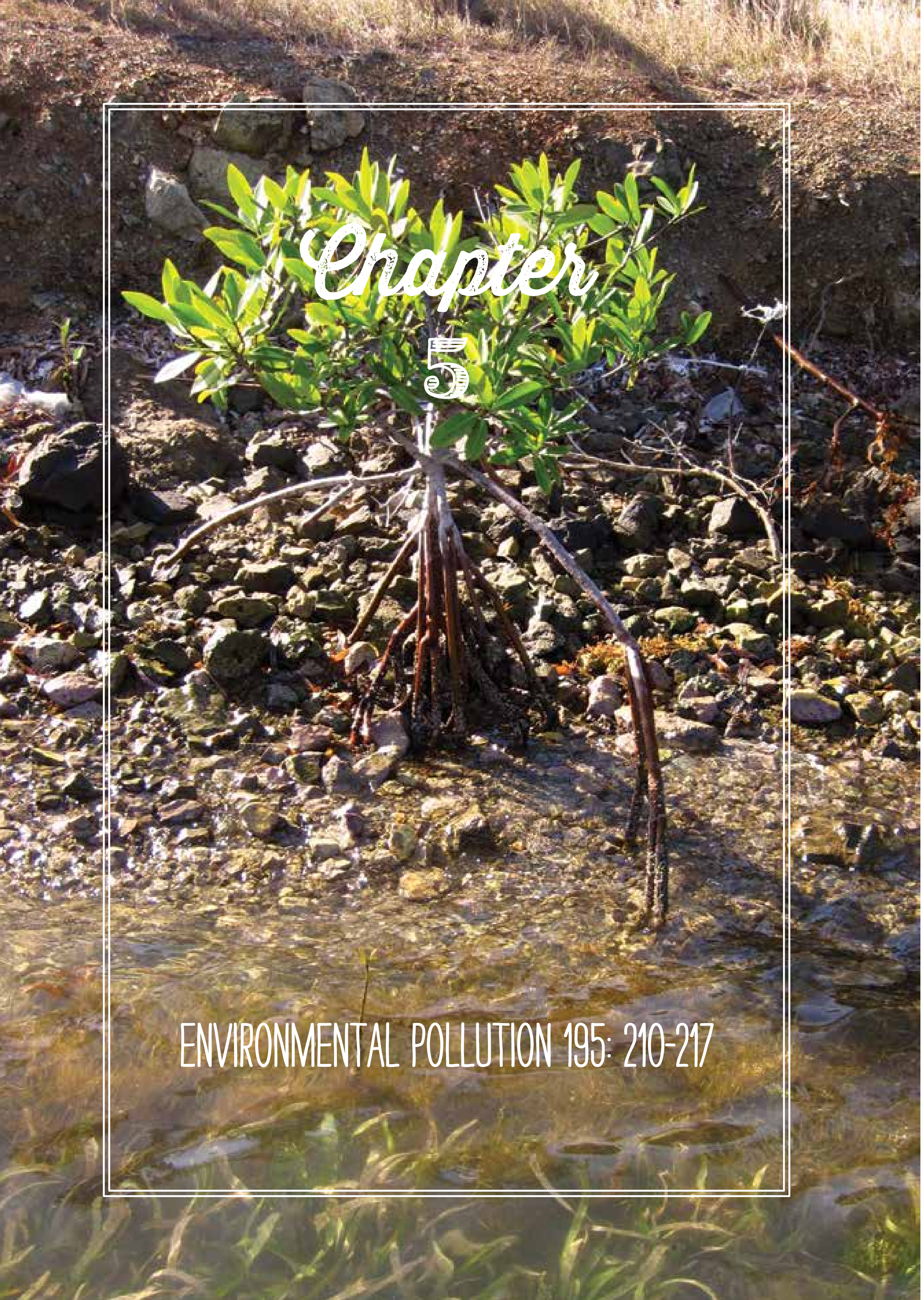
Sulfide is a potent soil phytotoxin (Lamers et al. 2013), known to generate toxic effects in freshwater macrophytes (Geurts et al. 2009), seagrasses (Koch et al. 2007, Holmer et al. 2011, Van der Heide et al. 2012), salt marshes (Koch et al. 1990, Lee 1999), and mangroves (McKee et al. 1988). Sulfide may negatively affect plant performance, and build-up of high sediment sulfide levels has even resulted in vegetation diebacks (Carlson et al. 1994, Terrados et al. 1999). High vegetation densities may counteract intrusion of toxic sulfide by loss of oxygen in the rhizosphere (Pedersen et al. 1998, Lee 1999), but low vegetation biomass may not be able to prevent toxic effects of sulfide. In line with this, we observed reduced porewater sulfide concentrations in the meadow (high ambient seagrass densities) compared to inside the created gaps (low density), which may be attributed to joint stress alleviation by high densities. Contrastingly to our expectations, we did not observe reduced sulfide concentrations on the high-density sites compared to the low-density sites as a result of entrapment of organic matter and fine sediments in dense vegetation. These conditions promote rather than reduce sulfide production. We did however observe joint-detoxification effects when locally comparing the densely vegetated meadow to the gaps. These observations imply that there is a positive feedback between the concentration of a toxicant and the population density or biomass; higher population biomass may lessen toxic effects, which could in turn stimulate growth (Van der Heide et al. 2010). Density-dependent toxicity has been described for a number of different systems and organisms (e.g freshwater algae, Pickhardt et al. (2002), yeast, Greig and Travisano (2008), salt marsh plants, Altieri et al. (2013)), including seagrasses (Van der Heide et al. 2008, Van der Heide et al. 2010, Govers et al.), and may also be applicable to other ecosystems. Population density may affect toxicity either by joint-detoxification, a process in which the toxicant is actively broken down by the exposed organism, or by growth-dilution. Growth-dilution is a mechanism involving non-degradable toxicants (such as trace metals) where the toxicant is stored in lower (non-lethal) concentrations, spread over a higher density/biomass (Van der Heide et al. 2010). However, we showed that joint-detoxification is conditional, and depending on the level of the toxicant (Figure 4.4c), which in turn results from and interacts with local background conditions (sediment characteristics).

CONCLUSIONS AND IMPLICATIONS

We are the first to show that local environmental settings, such as sediment conditions, and feedbacks may (interactively) affect persistence and recovery of a coastal ecosystem (seagrass bed). We found distinct differences between sites in persistence and recovery after small-scale disturbances (creation of a gap) (Figure 4.3), which we could relate to background conditions interacting with an applied stressor (OM addition to induce sulfide stress) and joint-detoxification of sulfides by high vegetation biomass. Our results indicate that it is important to identify possible feedbacks in ecosystems that affect resilience and to map local conditions to assess possible interactions between stressors and disturbances. We thus found that prevailing background conditions (sediment characteristics) may disturb system recovery from local disturbances (i.e. St Jacut, Viane). Similar mechanisms may be present in other vegetated ecosystems, where recovery of vegetation may also be hampered by high local sediment sulfide concentrations by a lack of stress amelioration by high densities (joint-detoxification) (Altieri et al. 2013). Non-rooting ecosystems such as corals may be typified by other interactions between background stressors and disturbances. For instance, coral reefs with high fishing pressure may be more vulnerable to eutrophication pulses due to a lack of herbivorous fish consuming resulting macroalgal blooms (Hoey and Bellwood 2011). This again illustrates the complexity of assessing ecosystem resilience for conservation and management, and we stress the importance of including feedbacks and interactions between stressors and disturbances in the assessment of ecosystem resilience.

ACKNOWLEDGEMENTS

We would like to acknowledge Nel Govers, Jim de Fouw, Jannes Heusinkveld, Timon Pieck, Nick Roskam, Kris Giesen, and Dominik Kneer for their logistic support in the field. We would also like to acknowledge Jelle Eygensteyn of the department of general instruments, Radboud University Nijmegen, and the lab of the Royal Netherlands Institute for Sea Research Yerseke (NIOZ Yerseke) for laboratory analysis. Finally, we also acknowledge Jêrome Fournier, for his information on the seagrass site in St Jacut.



Chapter 5

ENVIRONMENTAL POLLUTION 195: 210-217

SEAGRASSES AS INDICATORS FOR COASTAL TRACE METAL POLLUTION: A GLOBAL META-ANALYSIS SERVING AS A BENCHMARK, AND A CARIBBEAN CASE STUDY

Laura L Govers, Leon PM Lamers, Tjeerd J Bouma, Jelle Eygensteyn, Jan HF de Brouwer, A Jan Hendriks, Chantal M Huijbers, Marieke M van Katwijk (2014)

Abstract

Seagrass beds are highly productive coastal ecosystems providing a large array of ecosystem services including fisheries and carbon sequestration. As seagrasses are known to be highly sensitive to anthropogenic forcing, we evaluated the use of trace metal concentrations in seagrasses as bioindicators for trace metal pollution of coastal regions at both global and local scale. We carried out a meta-analysis based on literature data to provide a global benchmark list for trace metal accumulation in seagrasses, which was lacking in literature. We subsequently carried out a case study at the Caribbean islands of Curaçao and Bonaire to test for local-scale differences in trace metal concentrations in seagrasses, and internal metal allocation. The benchmark and local study show that trace metal concentrations in seagrass leaves, regardless of the species, can vary over a 100 to 1000-fold range, and are related to the level of anthropogenic pressure, making seagrasses highly valuable indicators.

INTRODUCTION

Billions of people live in coastal areas all over the world and it is expected that, in a couple of decades, even more than 50% of the expanding human population will be living within 150 km from the shore (Cohen, 2003; Cohen et al., 1997; Small and Nicholls, 2003). This leads to a steep increase in anthropogenic activities in coastal areas such as dredging, aquaculture, industrial activities and pollution, sewage discharge, and deforestation (Cohen, 2003; Mora, 2008). These activities severely threaten coastal ecosystems including coral reefs (Mumby et al., 2006; Mumby et al., 2007), mangroves (Valiela et al., 2001) and seagrass beds (Waycott et al., 2009), which not only provide a large suite of ecosystem services (Costanza et al., 1997), but are also strongly interconnected by fluxes of nutrients and dissolved organic matter, and by animal migration (Cowen et al., 2006; Nagelkerken, 2000).

To get insight into the extent and the spatial variation of anthropogenic pressure on coastal ecosystems and to locate sources of pollution, there is a strong need for good indicators. Bioindicators, including a variety of organisms such as clams, plants, copepods and microorganisms, can be used to identify anthropogenic disturbances and preferentially provide early warning signals for pollution or degradation (Linton and Warner, 2003). As they accumulate pollutants, these organisms also reflect low intensity, but chronic impacts, in contrast to physical or chemical parameters which often only present a snapshot of environmental conditions (Linton and Warner, 2003). Additionally, bioindicators can provide information on multiple spatial scales, as most ecosystems are heterogeneous, and are able to differentiate between natural variation and anthropogenic disturbance (Markert et al., 1999; Martínez-Crego et al., 2008).

Seagrasses are known to be good bioindicators (Lee et al., 2004; Orth et al., 2006) as they are widespread and sensitive to environmental changes (Bhattacharya et al., 2003; Ferrat et al., 2003; Udy and Dennison, 1997; Walker and McComb, 1992), and are able to integrate ecological conditions and processes over various timescales from weeks to years (Gonzalez-Correa et al., 2005; Madden et al., 2009; Meehan and West, 2000). Seagrass bioindicators have predominantly been used in the Mediterranean and in Florida, where several complex indices have been developed based on seagrass characteristics ranging from the individual physiological level to the community level (Bennett et al., 2011; Lopez y Royo et al., 2011; Montefalcone, 2009; Moreno et al., 2001; Romero et al., 2007). However, most of the proposed indicator values are specific for areas and species, and can therefore not be used at a global scale.

Seagrasses have been shown to be indicative of trace metal pollution (Lafabrie et al., 2007; Lafabrie et al., 2008; Sundelin and Eriksson, 2001), and as this type of pollution is becoming a major threat to coastal ecosystems in rapidly developing countries (Li et al., 2007), there is a strong need for reliable trace metal bioindicators. To use seagrasses as such, they need to be highly responsive in order to be able to detect differences at both local and global scale. As they are primary producers providing stock food to a large variety of coastal herbivores, seagrasses can also be expected to be indicative of trace metal concentrations at higher trophic levels. Their concentrations may therefore also be used to detect possible threats to ecosystem services such as fisheries. However, as of today, literature does not provide a complete reference overview of trace metal levels in seagrasses on a global scale. We therefore conducted a meta-analysis to compile this benchmark of global trace metal concentrations in seagrass leaves.

In addition, we studied potential trace metal pollution in seagrasses on a local scale to test whether it was possible to detect local-scale differences in trace metal concentrations in seagrasses. We focused on two Caribbean islands: Curaçao and Bonaire. The variation of anthropogenic pressure at an island scale makes these islands very suitable for this study. Moreover, the Caribbean represents a typical tropical area in which coastal ecosystems, including seagrass beds, are suffering from anthropogenic impacts such as coastal development, tourism and growing industries and harbors (Phillips, 1992), oil drilling and accompanying spills, trace metal pollution (Thorhaug et al., 1985; Vera, 1992), and eutrophication (Burkholder et al., 2007; Short and Wyllie-Echeverria, 1996). However, the effects of pollution on seagrass meadows have been poorly studied in this area.

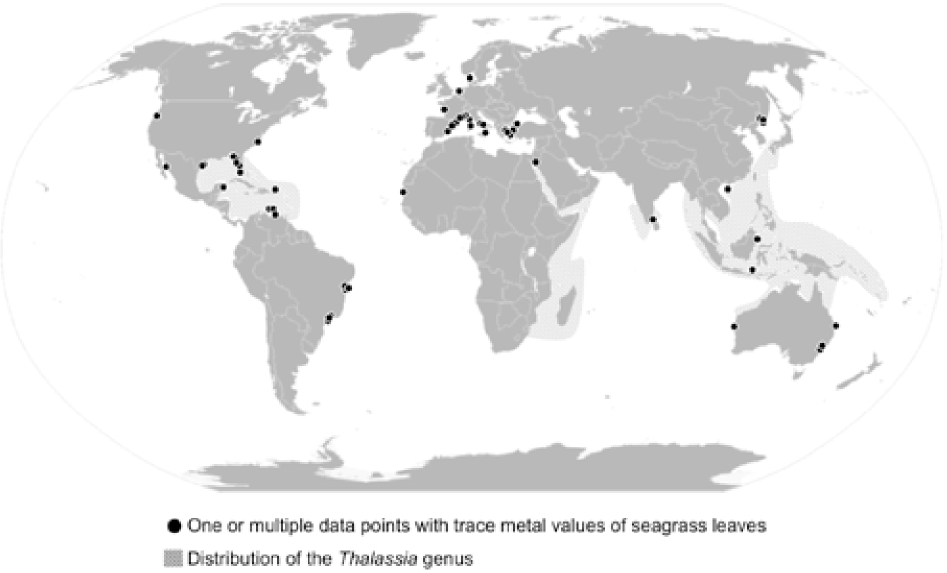


Figure 5.1 Overview of all seagrass locations included in the meta-analysis (Table 5.4) on trace metals in seagrass leaves, and the distribution of the tropical seagrass genus *Thalassia* (adapted from Green & Short 2003).

MATERIALS AND METHODS

Global meta-analysis of trace metal levels in seagrass

We compiled a benchmark database (Table S5.1) for trace metal levels (Co, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Zn) in seagrass leaves (μg g^{-1}) using Web of Science (ISI; search: seagrass AND metals), data from grey literature, and additional unpublished data of the authors on *Zostera noltii* (Mauritania and The Netherlands), *Amphibolis antarctica* (Australia) and Halodule uninervis (Indonesia). Belowground and whole plant data were excluded. Data were derived from either tables or figures in the selected papers, and each unique location as stated in the paper was used. The meta-analysis included data from 47 different studies on seagrass beds all over the world (Figure 5.1, Table S5.1). Replicate data points, including replicates from different seasons, were averaged per location, and data points were divided into polluted and unpolluted sites based on the description of the sites in the studies. Data included in our benchmark database spanned a 40-year period, from the 1970s up to 2011.

Table 5.1 General characteristics of the bays sampled. Bays varied in size and exposure to the open sea (related to the width of the bay mouth). The observed potential stressors are listed for each bay. The total number of sampling points refers to the number of replicates in each bay. Abbreviations of seagrass species: Tt=*Thalassia testudinum*, Sf=*Syringodium filiforme*, Hw=*Halodule wrightii* and Rm=*Ruppia maritima*. Only tissue of *Thalassia testudinum* was used for the trace metal analyses.

Bay	Island	Surface (km²)	Width bay mouth (m)	Seagrass species	Local disturbance	Total # sampling points	# Sampling points seagrass
Boka Ascension	Curaçao	0.05	200				
Outer bay				Tt, Sf, Hw	Pristine, turtle grazing	3	3
Inner bay				none	Heavily polluted, waste dumping	3	0
Lac Bay	Bonaire	7.5	1600	Tt, Sf, Hw, Rm	Pristine, light recreation	11	11
Piscadera Bay	Curaçao	0.75	90	Tt, Sf	Sewage discharge, boating	9	6
Santa Anna Bay	Curaçao	4	230	none	Heavy industry; oil refinery	8	0
Sint Joris Bay	Curaçao	2.5	240	Tt	Pristine, some waste dumping	6	5
Spanish Water Bay	Curaçao	3	90	Tt	Domestic sewage, boating	14	14

Local study

Samples were collected in January 2010 on the islands of Curaçao (12°04' N, 68°51' W) and Bonaire (12°15' N, 68°28' W) (Figure 5.2). We sampled six different inland bays, which included 50% of all the islands' bays with seagrass and 90% of the total seagrass area, varying in their levels of anthropogenic disturbance (Table 5.1). The bays are dominated by mangrove (*Rhizophora mangle*) communities along the shores, and by subtidal seagrass beds of turtle grass (*Thalassia testudinum*) and manatee grass (*Syringodium filiforme*). On the island of Curaçao, we sampled in Piscadera Bay, Spanish Water Bay, Boka Ascension, Santa Anna Bay and Sint Joris Bay, and on Bonaire, Lac Bay (Figure 5.2). Bays varied in size and morphology (Table 5.1), but they all experienced minimal wave stress and had a very limited tidal range of 30 cm (De Haan and Zaneveld, 1959). Sediment grain size of the bays ranged from coarse carbonate sediments on exposed sites (mean D50=600 μm) to finer grained, sandy sediments in the more sheltered areas (mean D50=240 μm) (Kuenen and Debrot, 1995). Boka Ascension was divided into inner and outer bay, as a large 10 cm deep shoal strongly limited water exchange between both parts.

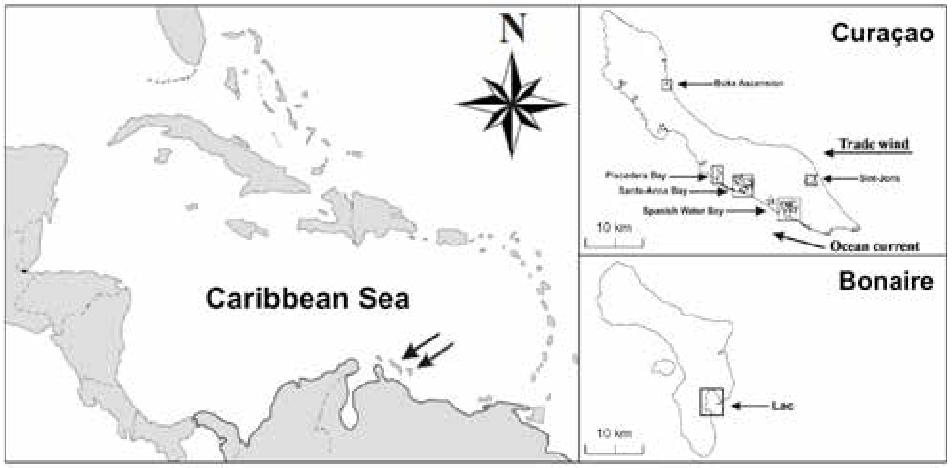


Figure 5.2 Map showing the locations of Curaçao and Bonaire in the Caribbean Sea, and maps of both islands with the locations of the sampled bays.

Seagrass and porewater sampling

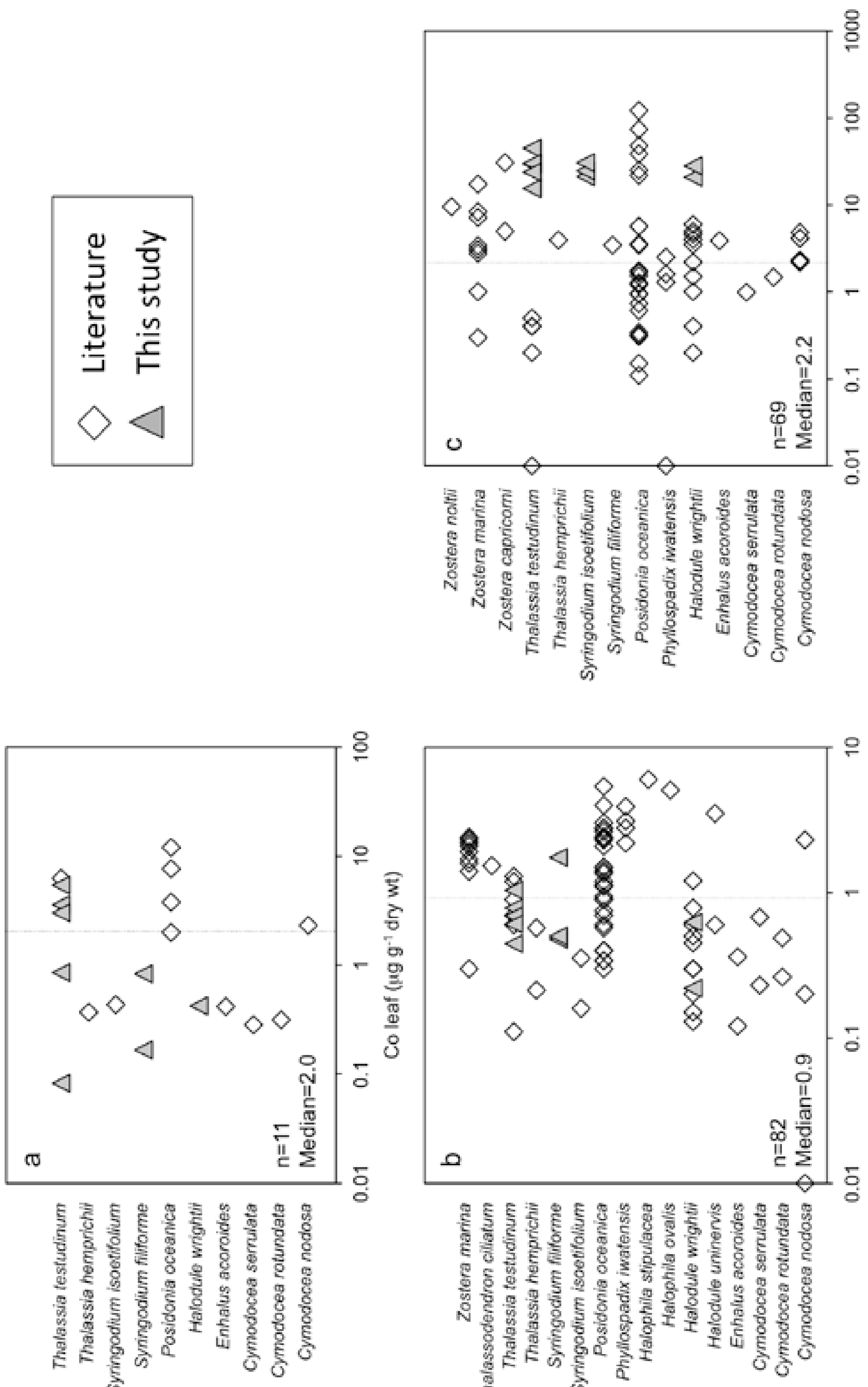
Although we sampled all seagrass species present in the bays, we mainly focused on *Thalassia testudinum* as this species and the very closely related *Thalassia hemprichii* are widely distributed among the tropics (Figure 5.1) (Green and Short, 2003; van Tussenbroek et al., 2006). Moreover, species of the genus *Thalassia* grow in shallow waters directly adjacent to coastal areas, are easy to recognize and are late successional species, which are able to accumulate trace metals (Fourqurean and Zieman, 2002).

Sampling sites were 20 m from the shore at most, and could be reached directly or by boat. Piscadera Bay is the most anthropogenically-impacted bay where seagrass is still present, although it only grows in the shallowest parts of the murky waters. In contrast, Lac Bay represents the most pristine seagrass system, as it is a well-protected nature reserve. For each bay, samples were collected in gradients from the pollution source to the bay inlet (Table 5.1). At sites including seagrass, both porewater and seagrass samples were taken in the seagrass bed; at sites lacking seagrass, porewater samples were taken from the bare sediment.

On each sampling site, two porewater samples were collected within 1 m to include the natural biogeochemical heterogeneity of the sediment, and pooled. Samples were collected anaerobically, using 60 mL vacuumed syringes connected to ceramic soil moisture samplers (pore size 0.15 µm;, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) placed in the top 7 cm of the soil (Govers et al., 2014). Surface water samples were collected similarly in the upper 5 cm of the water column to filter samples prior to laboratory analyses. After measuring their salinity and pH values, samples were frozen on the day of sampling and stored until further analytical analysis. On each sampling site, a pooled sample of at least 10 seagrass shoots with their belowground biomass was manually collected for each species at water depths between 0.5 and 2.5 m, while snorkeling.

Sample analysis

Seagrass samples were split up into roots, rhizomes, sheaths and leaves, and all epiphytes were carefully removed using a scalpel. Subsequently, the samples were dried at 60°C for 48 hours, weighed (g dry wt) and ground. Prior to elemental analyses, samples were digested in 5 mL pressure tubes, using 10 mg sample, 200 µL H_2O_2 and 700 µL HNO_3 in an autoclave for 30 min. at 121°C (ML autoclave, Tuttnauer, the Netherlands) and diluted with 9.1 water to 10 mL. Parallel analyses confirmed that the results of this method did not differ from those of another commonly used digestion method (Smolders et al., 2006) using 50 mg sample, 1 mL H_2O_2 and 2 mL HNO_3 in a digestion microwave (Ethos D, Milestone, Italy) diluted to 25 mL. Total concentrations of trace metals and other elements (Al, As, Ca, Cd, Co, Cr, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sr, Zn) in seagrass tissue (leaves and rhizomes) were measured by inductively coupled plasma emission spectrometry (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA, USA). Standard references (IPE-858, IPE-137; WEPAL, the Netherlands) were included in the analysis, and the average deviation amounted to 3.1%. Trace elements and trace metals in filtered, 3 times diluted porewater and surface water samples were measured using inductively coupled plasma emission spectrometry as described above.



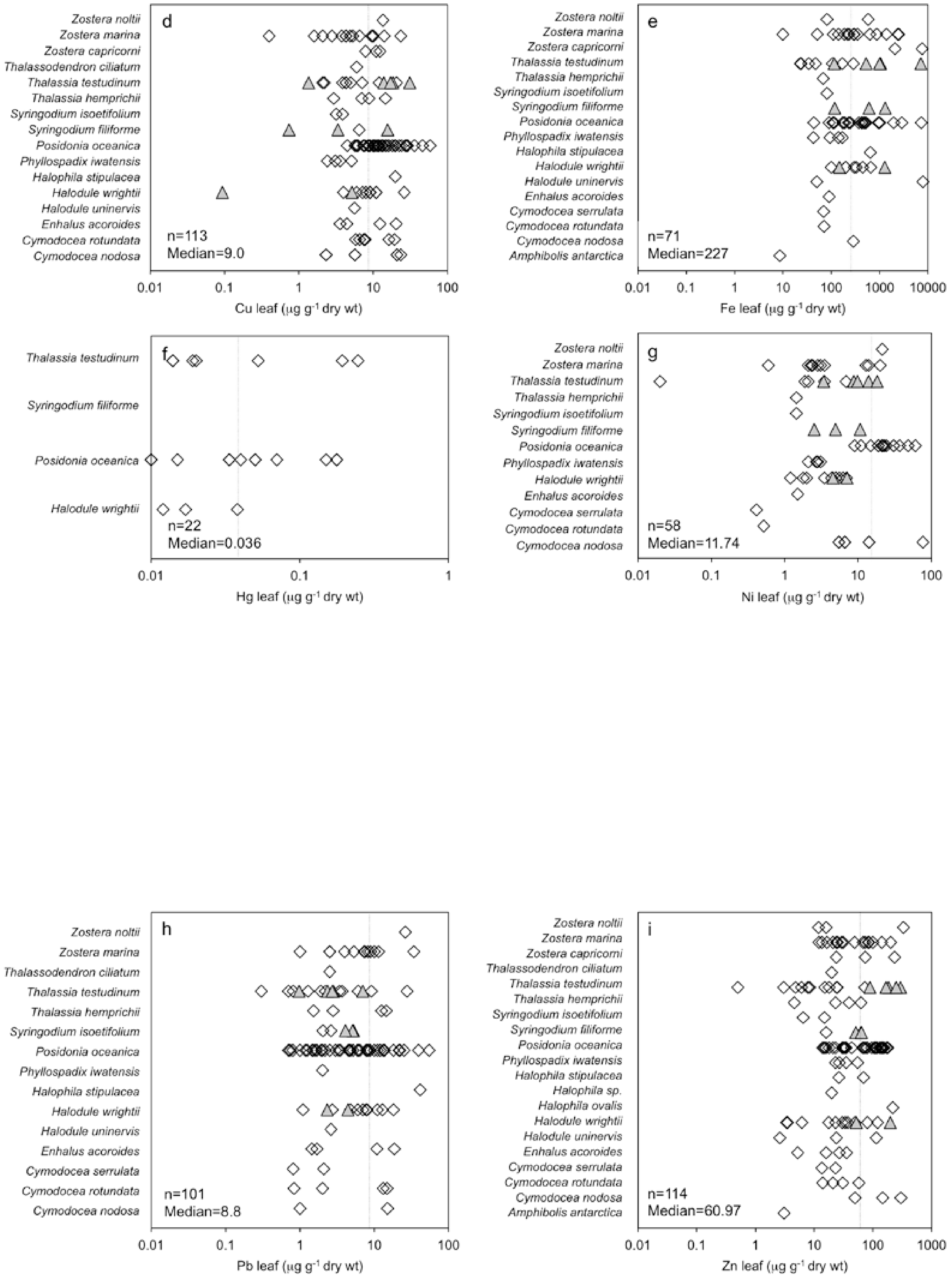


Figure 5.3 An overview of trace metal (Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Zn) concentrations in seagrass leaves from literature. The number of unique sample locations is indicated by n, displayed values are mean values as described in literature. The dotted line indicates the median value of all literature values. Grey triangles show mean trace metal concentrations measured in this study for 5 different bays (for number of replicates, see Table 5.1). Hg was not measured in our samples. Note that the logarithmic scales of the x-axes vary for the different metals.

Statistical analysis

Displayed values are means±standard error (SE). The number of replicates for each bay is displayed in Table 5.1. To compare the conditions among bays, we used a one-way ANOVA, with a homogeneity test prior to the analysis. If equal variances could not be assumed, data were log-transformed. We used a Tukey post-hoc test when the assumptions for the ANOVA were met, and a Games-Howell post-hoc test otherwise. Significant differences (P<0.05) are indicated by different letters. When comparing two different means (distance to residential areas in Spanish Water Bay), we used an independent t-test (see appendix). Correlations were tested using Pearson's (parametric), or Spearman's (non-parametric) correlation coefficient. All statistical tests were performed in IBM SPSS Statistics 19.0 and R 2.13.

RESULTS

Global benchmark study

To get insight into the trace metal concentration ranges at a global scale, as well as to put our own results into perspective, we plotted our data together with the median values of leaf metal values found in literature (Figure 5.3) for locations all over the world (Figure 5.1). Globally, trace metal concentrations in seagrass leaves ranged from <0.03 $\mu g\ g^{-1}$ dry wt for Cd to >4000 $\mu g\ g^{-1}$ dry wt for Fe (Figure 5.3). For cadmium (Cd), cobalt (Co), copper (Cu) and lead (Pb), most of the levels measured in seagrass leaves of Curaçao and Bonaire were well below median benchmark values. For iron (Fe), nickel (Ni) and zinc (Zn), in contrast, values were mostly above median values of our global benchmark database, and chromium (Cr) values were well above median values (Figure 5.3).

In our meta-analysis, we found significantly higher mean trace metal concentrations in seagrass leaves of polluted sites than in unpolluted sites for Cu, Hg, Ni, Pb, and Zn (Welch's t-test, P<0.05 Table S5.2). Leaf trace metal concentrations of polluted sites were on average 2 times higher (81 vs. 45 $\mu g\ g^{-1}$ dry wt for Zn, 15 vs. 8 $\mu g\ g^{-1}$ dry wt for Ni, 15 vs. 9 $\mu g\ g^{-1}$ dry wt for Cu), 3 times higher (15 vs. 5 $\mu g\ g^{-1}$ dry wt for Pb) or 4 times higher (0.13 vs. 0.03 $\mu g\ g^{-1}$ dry wt for Hg) on polluted sites compared to unpolluted sites. This implies that seagrass leaves can indeed be used as first-level bioindicators for trace metal pollution.

Internal distribution of trace metals

To investigate the internal allocation of metals to the different plant parts, we measured trace metal concentrations in both leaves and rhizomes of *Thalassia testudinum*. All essential metals (Cu, Fe, Ni, Zn, Cr) displayed significant, positive correlations between leaves and rhizomes (linear regression, $R^2>0.15$, P<0.001; Figure 5.4). For the non-essential metals, however, we only found such relationship for Cd (linear regression, $R^2=0.17$, P<0.001; Figure 5.4). We found the strongest relationships (high R^2 values) for the essential metals that were present in the highest concentrations in the plants: Fe, Zn and Cu. Although data for most metals in the graph were near the 1:1 line (Figure 5.4), some metals (e.g. Fe) were mainly accumulated in belowground parts, while others (e.g. Zn) were more concentrated in leaves.

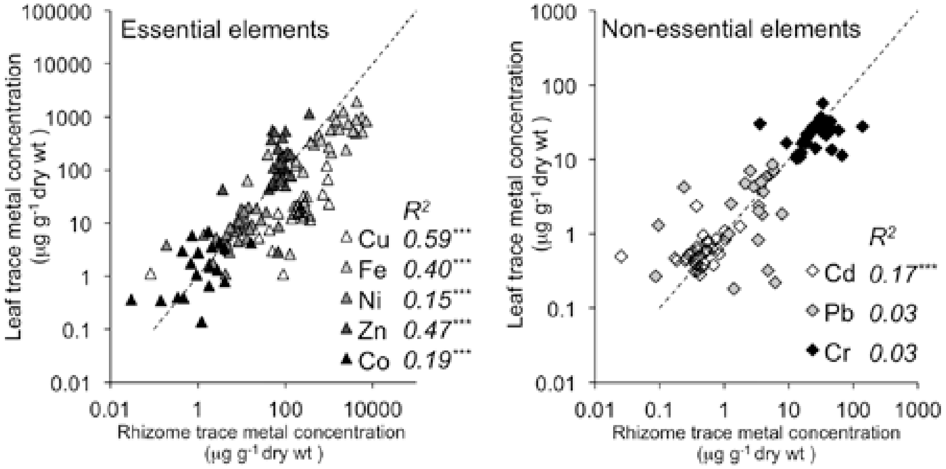


Figure 5.4 Correlations between leaf and rhizome metal concentrations of essential (Cu, Fe, Ni, Zn, Co) and nonessential (Cd, Hg, Pb, Cr) metals. The dotted line represents the 1:1 line. Note the logarithmic scale of the x-axis. ns=not significant, *=P<0.05, **=P<0.01 and ***=P<0.001.

Local trace metal concentrations in porewaters and leaves

In general, the polluted Piscadera Bay showed the highest leaf metal concentrations, while the protected Lac had the lowest levels for almost all measured metals (Table 5.2). In strong contrast, concentrations of trace metals in porewater and surface water samples (Table S5.3) did not show significant differences among the separate bays. In addition, we did not find any significant correlations between porewater and leaf concentrations of trace metals.

DISCUSSION

There is a strong need for bioindicators that can be used to assess the actual status and health of coastal ecosystems globally in relation to anthropogenic pressure, and to metal pollution in particular (Linton and Warner, 2003; van Katwijk et al., 2011). We therefore compelled the first global literature overview for seagrass metal levels, which is important as a benchmark for seagrass research related to metal pollution. In addition, we studied the use of seagrasses as bioindicators for trace metal pollution in the poorly studied Caribbean seagrass beds of two islands where various levels and sources of anthropogenic stressors were present. Based on our benchmark results, we could show that leaf concentrations of especially Cr, Fe, Ni and Zn were high at our case study sites.

Table 5.2 Trace metal concentrations in seagrass leaves (in mg g^{-1}, mean values per bay, for number of replicates see Table 5.1) of *Thalassia testudinum* in all studied bays. Significant differences ($P<0.05$) are indicated by different letters. Ranking is the mean pollution ranking of all 8 metals.

Bay	Cd	Co	Cr	Cu	Fe	Hg	Ni	Pb	Zn	Ranking
Boka Ascension Bay	0.93^{a}	3.10^{ab}	32.01^{ab}	10.27^{ab}	3725.63^{ac}	NA	9.49^{abc}	5.93^{a}	74.97^{a}	2
Lac Bay	0.44^{a}	0.08^{a}	29.50^{a}	1.36^{a}	115.48^{b}	NA	3.41^{a}	2.37^{b}	184.67^{a}	5
Piscadera Bay	0.79^{a}	3.57^{ab}	30.34^{a}	13.83^{ab}	1044.48^{a}	NA	18.13^{c}	2.83^{ab}	295.52^{a}	1
Sint Joris Bay	0.61^{a}	3.02^{b}	23.98^{ab}	17.21^{ab}	1003.03^{a}	NA	9.89^{b}	0.97^{b}	248.58^{a}	3
Spanish Water Bay	0.69^{a}	0.85^{ab}	15.57^{b}	31.02^{b}	523.94^{c}	NA	8.81^{b}	2.71^{b}	168.53^{a}	4

Global-scale benchmark

We have here compiled a review of, to our best knowledge, all available literature on trace metal concentrations in seagrass leaves. Seagrasses are known metal accumulators, and in our literature study, we found a wide, 100 – 1000-fold, range in concentrations for all individual metals (Figure 5.3), which underscores the suitability of seagrasses as sensitive bioindicators for the detection of trace metal pollution.

Literature data were, however, not equally divided among regions, and especially seagrass species occurring in the Mediterranean (e.g. *Posidonia oceanica*) have been very well studied. Some studies may show a bias for metal-polluted areas (Table S5.1, Table S5.2). Furthermore, for some metals such as Co and Hg, few data were available in the literature (Figure 5.2). Data on trace metal pollution in tropical areas, where pollution and degradation of coastal ecosystems is increasing at an alarming rate, is mostly lacking. This data gap could rapidly be filled by using ‘easy to pick’ seagrasses in the areas that have not been covered yet, for example plants of the widespread *Thalassia* genus (Figure 5.1).

Our global analysis of trace metal concentrations of polluted vs. unpolluted sites (Table S5.2) clearly shows that leaf trace metal values are significantly elevated (2-4 x higher) on polluted sites compared to unpolluted sites. This analysis proves that seagrass leaves can be used as first-level indicators for trace metal pollution of coastal areas.
Seagrass trace metal values are also known to vary seasonally, with lower trace metal values in the growing season than in the dormant season (Li and Huang, 2012; Schlacher-Hoenlinger and Schlacher, 1998). As most studies are conducted during the growing season (Table S5.1), our benchmark study may underestimate rather than overrate seagrass trace metal levels. Furthermore, seasonal differences were moderated in our data- set by averaging sample points from different seasons. It would however be very interesting to further investigate the importance of seasonality for the use of seagrasses as bioindicators for trace metal pollution.

Local study

We assessed trace metal concentrations in seagrasses as an indicator for their level of pollution in coastal areas and, more importantly, their biological availability. The bioavailability of metals and their mobility in the sediment is determined by their chemical speciation (Morillo et al., 2004), but seagrasses are also able take up trace metals from the water column (Batley, 1987; Bond et al., 1985). Similar to nutrient measurements in abiotic compartments, measurements of trace metals in the water only provide a snapshot of trace metal loads as they are subjected to large variations in concentrations and fluxes (Ralph et al., 2006), while seagrass trace metal concentrations display a longer-term trace metal accumulation and related stress (Lafabrie et al., 2007; Lafabrie et al., 2008; Sundelin and Eriksson, 2001).

Seagrass trace metal concentrations from the sampled bays indicated that Piscadera Bay, with a sewage outlet, was the most heavily polluted, whereas the conservation area Lac showed the lowest trace metal concentrations of all sampled bays, which is in accordance with our expectations. In contrast, porewater and surface water data did not provide this information, and were not correlated to plant concentrations, as expected.

The comparison of our own data to our worldwide benchmark list shows that levels of Cr appeared to be exceptionally high in all our study sites. Surprisingly, Cr levels appeared to be also high in the relatively undisturbed Lac, which suggests an external source of this metal. This is even more striking, because it is considered one of the least bioavailable metals in marine sediments (Morillo et al., 2004), which may imply that the Cr loads in our sampled bays are even higher than we might expect based on our results.

Although the concentrations of trace metals varied among sample points and between bays, it appeared to be very difficult to locate a point source for trace metals (Table S5.3 and Table S5.4) (Pergent-Martini and Pergent, 2000). Literature (Guzman and Garcia, 2002; Irvine and Birch, 1998) suggests that trace metal pollution in marine environments may originate from several processes and non-point-sources: runoff, flooding, mining, sewage, erosion, overuse of agrichemicals, industrial waste, atmospheric deposition, ports and refineries. We speculate that trace metals in the seagrass beds of Curacao originate from sewage (Piscadera Bay, Spanish Water Bay), ports and boating, but may also be of terrestrial origin, as high loads of terrigenous sediments are expected to have invaded the water due to deforestation and the construction of terrestrial drainage areas (Kuenen and Debrot, 1995). Furthermore, Curaçao is home to a large oil refinery and two of its bays (Caracas Bay and Bullen Bay, not sampled) have been subjected to frequent oil spills and accompanying pollution in the past (Nagelkerken and Debrot, 1995). This oil pollution may also have reached other, nearby bays.

Internal distribution

We compared metal concentrations in the different plant parts and found significant correlations between leaf and rhizome metal concentrations of the essential elements (Figure 5.3). As plants need these elements either directly as micronutrients (Cu, Fe, Ni, Zn) or as an essential element (Co) for the nitrogenase enzymes fueling N_2-fixation by associated microorganisms (Welsh, 2000), they possess mechanisms for active allocation for most of these metals (Adriano, 2001; Marschner, 1995). As the concentrations represent redistribution in addition to uptake, we cannot draw conclusions about specific uptake ratios for leaves and rhizomes. In addition, all metals, including the non-essential elements showed fairly similar metal concentrations for both the leaves and rhizomes (close to the 1:1 line), which underlines the use of seagrass leaf material as indicator for trace metals.

Seagrasses as indicators for bioaccumulation

Although we mainly focused on the use of trace metal concentrations in seagrasses as indicator for pollution, the accumulation of metals may also affect seagrass health. Many trace metals are naturally abundant in seagrass beds (Batley, 1987; Prange and Dennison, 2000), but high concentrations can become toxic to seagrass and also be indicative of toxicity to other coastal organisms (Macinnis-Ng and Ralph, 2002; Prange and Dennison, 2000; Ralph and Burchett, 1998). Even sublethal levels of trace metals may have large effects on seagrass dominated ecosystems, as they are persistent and may accumulate in the food web, with toxic effects at higher trophic levels (Ikem and Egiebor, 2005; Schüürmann and Markert, 1998). In addition, trace metal accumulation in plants is often associated with changes in photosynthetic rates (Conroy et al., 1991; Macfarlane and Burchett, 2001; Prange and Dennison, 2000) and inhibited metabolic activity (Ralph and Burchett, 1998). This may lead to decreased growth rates or even result in plant die-off (Clijsters and Van Assche, 1985).

Trace metal accumulation in seagrasses can be used as a first level measurement to assess the contamination of the specific marine environment (Prange and Dennison, 2000). Moreover, as accumulation magnifies in the food web for some trace elements, high trace metal levels in primary producers such as seagrasses may indicate serious trace metal pollution in the whole food web.

CONCLUSIONS AND RECOMMENDATIONS

Summarizing, we here present a global list for trace metal concentrations in seagrass leaves, which can be used in further studies to relate local trace metal pollution to a benchmark list. Moreover, we showed that seagrasses in general, and *Thalassia* spp. in particular, can be used as an easy to sample and widespread bioindicator species for trace metal pollution of especially Cu, Hg, Ni, Pb, and Zn. As little information is available on the effects of trace metal pollution on seagrass physiology and on the bioaccumulative effects of trace metals in seagrass-based foodwebs, we strongly recommend further research on these topics.

ACKNOWLEDGEMENTS

We would like to thank M. Grol, for her logistic support. We would also like to acknowledge the employees of Carmabi, Curaçao. STINAPA Bonaire extended the permit for the samples for Lac, and we would like to thank E. Beukenboom, R. de Leon and F. van Slobbe for making this arrangement. We would also like to thank R. Peters for sample processing and R. van Hintum for his help with some of the spatial analyses. We finally thank two anonymous reviewers for their valuable comments on an earlier version of the manuscript.

SUPPLEMENTARY DATA CHAPTER 5

Table S5.1 Studies used for the global meta-analysis of trace metal concentrations in seagrasses. Data were derived from 47 different studies. NA means not available.

Species	Country	Reference	Sampling time
Amphibolis antarctica	Australia	Personal unpublished data 2011	September 2011
Cymodocea nodosa	Greece	Catsiki and Panayotidis (1993)	Year round, 1984-1988
	Greece	Malea (1994)	December 1985, March, July, October 1986
	Greece	Malea et al. (1994)	December 1985, March, July, October 1986
	Greece	Nicolaidou and Nott (1998)	NA
	Spain	Marin-Guirao et al. (2005)	June 2001
Cymodocea rotundata	Indonesia	Nienhuis (1986)	October 1984
	India	Kannan et al. (2011)	March 2009
	China	Li and Huang (2012)	July 2009, January 2010
Cymodocea serrulata	Indonesia	Nienhuis (1986)	October 1984
	India	Kannan et al. (2011)	March 2009
Enhalus acoroides	Indonesia	Nienhuis (1986)	October 1984
	India	Kannan et al. (2011)	March 2009
	China	Li and Huang (2012)	July 2009, January 2010
Halodule uninervis	Jordan	Wahbeh (1984)	February-December 1982
	Indonesia	Nienhuis (1986)	October 1984
	Indonesia	Personal unpublished data 2008	June 2008
Halodule wrightii	Texas, USA	Pulich (1976)	NA
	Brazil	Amado Filho et al. (2004)	September 1997, August 1998
	Florida, USA	Lewis et al. (2007)	2001-2002
	Brazil	Amado Filho et al. (2008)	August 2000
	Bonaire	This study	January 2010
	Curaçao	This study	January 2010
Halophila ovalis	Jordan	Wahbeh (1984)	February-December 1982
Halophila sp.	Texas, USA	Pulich (1976)	NA
Halophila stipulacea	Jordan	Wahbeh (1984)	February-December 1982
	Greece	Malea and Haritonidis (1995)	NA
Phyllospadix iwatensis	Russia	Chernova et al. (2002)	August 1998
Posidonia oceanica	Greece	Catsiki and Panayotidis (1993)	Year round, 1984-1988
	Greece	Malea (1994)	December 1985, March, July, October 1986
	Corsica, France	Warnau et al. (1995)	March, June, November 1991, February 1992
	Corsica, France	Romeo et al. (1995)	October 1992, April 1993
	Italy	Warnau et al. (1995)	March, June, November 1991, February 1992
	France/Italy	Pergent-Martini (1998)	Spring 1993
	Italy	Schlacher-Hoenlinger and Schlacher (1998)	April 1993-July 1994
	Corsica, France	Pergent and Pergent-Martini (1999)	NA
	Italy	Pergent and Pergent-Martini (1999)	NA
	Sardinia, Italy	Caredda et al. (1999)	May-July 1996
	Sardinia, Italy	Baroli et al. (2001)	NA
	Sicily, Italy	Campanella et al. (2001)	May 1997
	Italy	Ancora et al. (2004)	November December 1997
	Sicily, Italy	Tranchina et al. (2005)	NA
	Corsica, France	Gosselin et al. (2006)	Various months, 1988-2004
	Corsica, France	Lafabrie et al. (2007)	Summer 2004, 2005
		Martínez-Crego et al. (2008)	October 2001
	Corsica, France	Lafabrie et al. (2008a)	Summer 2004, 2005
	Corsica, France	Lafabrie et al. (2008b)	Summer 2004, 2005
		Conti et al. (2010)	Summer 2004
	Corsica, France	Pergent et al. (2011)	October 2006
Syringodium filiforme	Texas, USA	Pulich (1976)	NA
	Bonaire	This study	January 2010
	Curaçao	This study	January 2010
Syringodium isoetifolium	Indonesia	Nienhuis (1986)	October 1984
	India	Kannan et al. (2011)	March 2009
Thalassia hemprichii	Indonesia	Nienhuis (1986)	October 1984
	India	Kannan et al. (2011)	March 2009
	China	Li and Huang (2012)	July 2009, January 2010
Thalassia testudinum	Texas, USA	Pulich (1976)	NA
	Puerto Rico	Schroeder and Thorhaug (1980)	Spring and winter 1971-1972
	Venezuela	Perez (1995)	NA
	Gulf of Mexico, USA	Fourqurean and Cai (2001)	Summer 1997

Species	Country	Reference	Sampling time
	Texas, USA	Whelan et al. (2005)	July, November 2002
	Florida, USA	Lewis et al. (2007)	2001-2002
	Mexico	Whelan et al. (2011)	May 2004, May 2005
	Bonaire	This study	January 2010
	Curaçao	This study	January 2010
Thalassodendron ciliatum	Indonesia	Nienhuis (1986)	October 1984
Zostera capricorni	Australia	Prange and Dennison (2000)	July, September 1998, January 1999
	Australia	Macinnis-Ng and Ralph (2004)	NA
Zostera marina	NC, USA	Drifmeyer et al. (1980)	June 1973-March 1977
	Denmark	(Brix and Lyngby, 1982, 1983)	September 1979-July 1980
	Turkey	Guven et al. (1993)	NA
	Russia	Chernova et al. (2002)	August 1998
	France	De Casabianca et al. (2004)	February 1994-March 1995
	Oregon, USA	Kaldy (2006)	March 2001-January 2003
	California, USA	Riosmena-Rodriguez et al. (2010)	November 2004, February, April 2005
Zostera noltii	The Netherlands	Personal unpublished data 2009	August 2009
	Mauritania	Personal unpublished data 2010	October 2010
	France	Dumon et al. (1994)	NA

Table S5.2 Leaf trace metal concentrations (μg g^{-1}, mean values) of unpolluted and polluted (as defined in papers) sites in the global meta-analysis. Statistical differences between mean leaf trace metal concentrations of unpolluted and polluted sites are indicated by: ns=not significant, *=P<0.05, **=P<0.01 and ***=P<0.001. NA; not available due to very limited data for Co on polluted sites.

Metal	Unpolluted	se	Polluted	se		Welch's t-test P values	df
Cd	1.41	0.18	1.14	0.34	ns	0.205	18.6
Co	NA		NA				
Cr	11.44	4.05	3.01	0.35	ns	0.636	45.7
Cu	9.12	0.99	15.20	1.82	***	0.000	56.9
Fe	766.25	263	618.44	185	*	0.047	42.8
Hg	0.03	0.01	0.13	0.04	*	0.036	2.8
Ni	7.97	1.79	15.03	3.82	**	0.004	43.4
Pb	5.16	0.61	14.93	2.94	***	0.000	33.3
Zn	44.64	5.22	80.57	16.44	*	0.012	38.6

Table S5.3 Trace metal concentrations (μg L^{-1}, mean values per bay) of porewater and surface water. Statistical differences are indicated by different letters. nd: not detectable; concentrations below detection limit (<0.1 μg L^{-1}).

Porewater	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
Boka Ascension inner	0.6^{ab}	2.5^{ab}	1.4^{a}	nd	246.9^{a}	20.8^{bc}	0.1^{a}	nd
Boka Ascension outer	0.9^{b}	0.8^{ab}	16.5^{a}	nd	189.7^{a}	24.4^{b}	0.5^{a}	nd
Lac Bay	0.2^{a}	0.6^{ab}	0.2^{a}	nd	23.0^{a}	10.3^{ac}	0.6^{a}	nd
Piscadera Bay	0.2^{a}	0.4^{a}	0.2^{a}	nd	110.6^{a}	11.3^{ab}	2.1^{a}	nd
Santa Anna Bay	0.3^{ab}	0.5^{ab}	2.6^{a}	nd	47.0^{a}	14.1^{ab}	1.7^{a}	nd
Sint-Joris Bay	0.3^{a}	0.3^{a}	2.0^{a}	nd	18.9^{a}	4.7^{a}	0.0^{a}	nd
Spanish Water Bay	0.4^{a}	1.1^{b}	9.4^{a}	nd	69.7^{a}	14.2^{ab}	0.0^{a}	nd

Surface water	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
Boka Ascension inner	0.2^{a}	0.2^{a}	4.4^{a}	nd	93.6^{a}	25.1^{b}	0.0^{a}	nd
Boka Ascension outer	0.0^{a}	0.0^{a}	3.9^{a}	nd	13.5^{a}	3.3^{a}	0.0^{a}	nd
Lac Bay	0.2^{a}	0.3^{a}	0.2^{a}	nd	6.1^{a}	5.9^{a}	0.3^{a}	nd
Piscadera Bay	0.3^{a}	0.3^{a}	0.4^{a}	nd	2.4^{a}	3.6^{a}	1.1^{a}	nd
Santa Anna Bay	0.4^{a}	0.6^{ab}	8.7^{a}	nd	50.8^{a}	9.4^{ab}	3.1^{a}	nd
Sint-Joris Bay	0.1^{a}	0.5^{ab}	15.5^{a}	nd	63.0^{a}	11.0^{ab}	0.0^{a}	nd
Spanish Water Bay	0.5^{a}	0.9^{b}	9.9^{a}	nd	43.1^{a}	8.7^{ab}	0.3^{a}	nd

Table S5.4 P-values for distance analyses of trace metal contents of *Thalassia testudinum* leaves and distances to the bay mouth. *=P<0.05; NA: not available due to the low number of data.

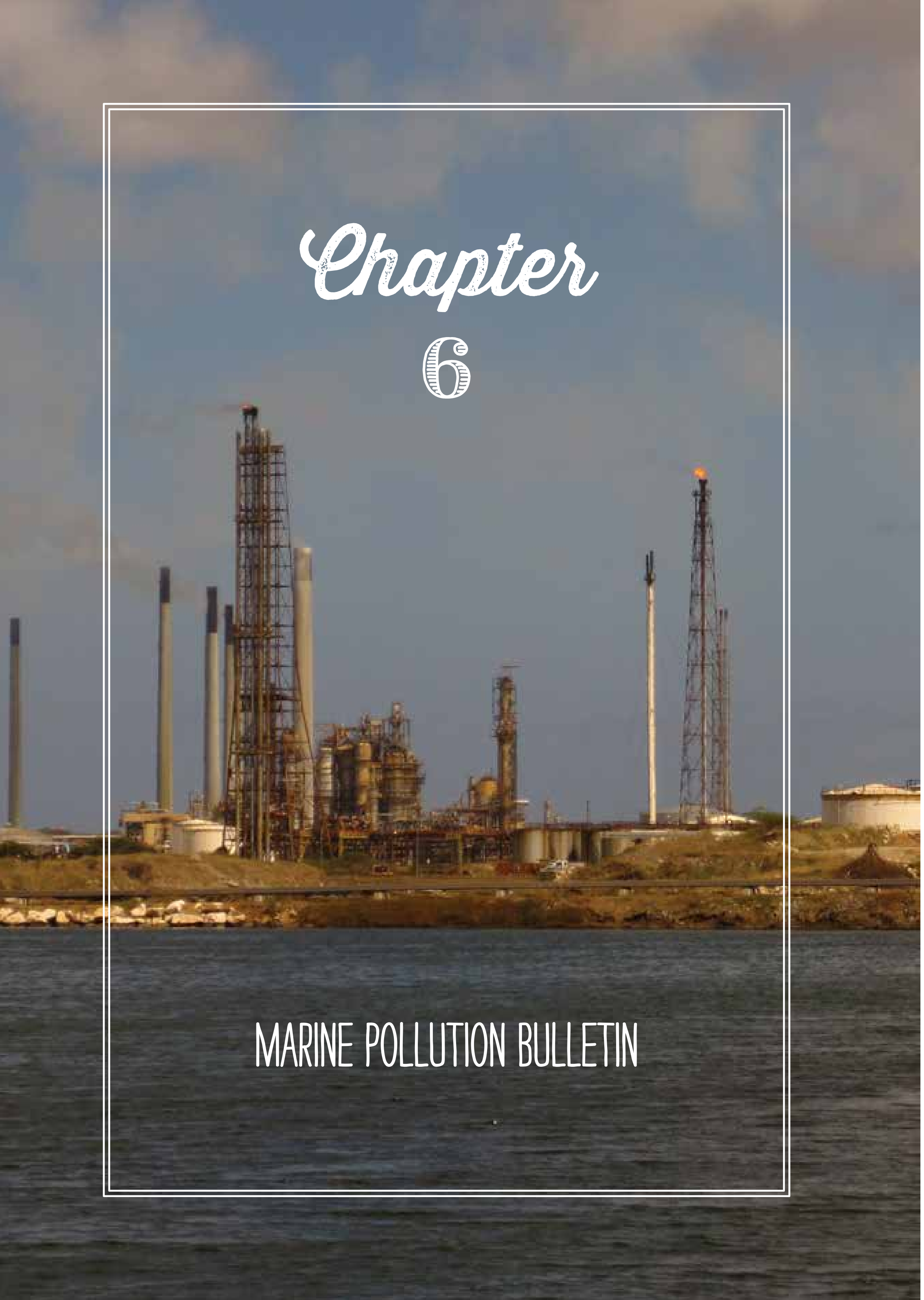
Bay	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
Boka Ascension Bay	NA	NA	NA	NA	NA	NA	NA	NA
Lac Bay	0.323	0.363	0.393	0.279	0.435	0.127	0.077	0.216
Piscadera Bay	0.328	0.312	0.298	0.313	0.228	0.404	0.051	0.288
Sint-Joris Bay	0.139	0.350	0.298	0.436	0.168	0.091	0.359	0.142
Spanish Water Bay	0.302	0.011^{*}	0.138	0.133	0.288	0.157	0.133	0.346

Table S5.5 P-values of point source independent t-tests in Spanish Water Bay. We tested for significant differences in seagrass leaf trace metal content of *Thalassia testudinum* near coastal residences (<200 m) and further away from coastal residences (>350 m). *=P<0.05.

Bay	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
Spanish Water Bay	0.260	0.775	0.071	0.090	0.430	0.594	0.719	0.604

Table S5.6 P-values of Pearson's correlation tests. We tested the correlations of distance to the sewage pipe in Piscadera bay and trace metal contents of seagrass leaves. We found a significant positive correlation between distance from the sewage pipe and leaf Ni content (R^2=0.54, P=0.048), which indicated that the source of Ni is probably located outside of the bay, or Ni availability was lower near the sewage pipe. *=P<0.05.

Species	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
Thalassia testudinum	0.239	0.223	0.338	0.224	0.139	0.315	0.140	0.199
Syringodium filiforme	0.275	0.418	0.097	0.400	0.164	0.048^{*}	0.170	0.234



Chapter
6

MARINE POLLUTION BULLETIN

EUTROPHICATION THREATENS CARIBBEAN SEAGRASSES – AN EXAMPLE FROM CURAÇAO AND BONAIRE

Laura L Govers, Leon PM Lamers, Tjeerd J Bouma, Jan HF de Brouwer, Marieke M van Katwijk (in press)

Abstract

Seagrass beds are globally declining due to human activities in coastal areas. We here aimed to identify threats from eutrophication to the valuable seagrass beds of Curaçao and Bonaire in the Caribbean, which function as nursery habitats for commercial fish species. We documented surface- and porewater nutrient concentrations, and seagrass nutrient concentrations in 6 bays varying in nutrient loads. Water measurements only provided a momentary snapshot, due to timing, tidal stage, etc., but Thalassia testudinum nutrient concentrations indicated long-term nutrient loads. Nutrient levels in most bays did not raise any concern, but high leaf %P values of Thalassia in Piscadera Bay (~0.31%) and Spanish Water Bay (~0.21%) showed that seagrasses may be threatened by eutrophication, due to emergency overflow of waste water and coastal housing. We thus showed that seagrasses may be threatened and measures should be taken to prevent loss of these important nursery areas due to eutrophication.

INTRODUCTION

Seagrass beds are key coastal ecosystems, which support high biodiversity and provide important ecosystem services such as carbon sequestration, fisheries, and coastal protection (Christianen et al., 2013; Fourqurean et al., 2012; Heck et al., 2003). However, seagrass meadows are rapidly declining all over the world, due to increasing anthropogenic activities in coastal areas (Waycott et al., 2009). Human pressures on coastal areas in the Caribbean are also increasing, and factors like booming tourism, growing industries, oil drillings and spills, trace metal pollution and eutrophication threaten coastal ecosystems (Phillips, 1992; Short and Wyllie-Echeverria, 1996; Thorhaug et al., 1985).

An important stressor that has not been well studied in the Caribbean is eutrophication (but see Gast et al. (1999)). Eutrophication can potentially lead to degradation or complete disappearance of seagrass beds due to epiphyte overgrowth and/or light limitation (Burkholder et al., 2007; Kuenen and Debrot, 1995). We therefore aimed to identify and to quantify the effects of eutrophication on the seagrass beds of the Caribbean islands of Curaçao and Bonaire. On these islands, the seagrass beds form essential nursery habitats for many commercially important fish species (Huijbers et al., 2013). However, seagrasses are present in bays with varying degrees of anthropogenic impacts (Debrot and Sybesma, 2000). Hence, we studied 1) the nutrient status of seagrasses in 6 bays on Curaçao and Bonaire, 2) the indicator value of seagrass leaf nutrient concentrations, and 3) possible threats of eutrophication in Curaçao and Bonaire bays.

MATERIALS AND METHODS

Samples were collected in January 2010 on Curaçao (12°04’ N, 68°51’ W) and Bonaire (12°15’ N, 68°28’ W), Netherlands Antilles. We sampled six different non-estuarine inland bays (50% of all bays with seagrass, which includes >90% of the total seagrass area) varying in anthropogenic disturbance levels (Table 6.1). The bays are dominated by mangrove (*Rhizophora mangle*) communities along the shores and by subtidal seagrass beds with turtle grass (*Thalassia testudinum*) and manatee grass (*Syringodium filiforme*). On Curaçao, we sampled Piscadera Bay, Spanish Water Bay, Boka Ascension Bay, Santa Anna Bay and Sint-Joris Bay, and on Bonaire Lac Bay (for more details, see Govers et al. (2014b)).

Sampling sites were maximally 10 m from the shore, and were reached either from the shore, or by boat. The bays were selected for their level of anthropogenic disturbance (Table 6.1). For each bay, samples were collected in gradients from the source of pollution to the bay mouth. At sites with seagrasses (Table 6.1), samples were taken in the seagrass bed; at sites without seagrass, porewater samples were taken from the bare sediment. More detailed information (date, tidal level, temperature, precipitation) can be found in the supplements (Table S6.1). The distances between sampling points and key-point sources (residential areas, sewage pipes) were measured using the ruler tool in Google Earth^{tm}.

At each sampling site, a minimum of 10 shoots with belowground biomass was manually sampled while snorkeling and pooled at depths between 0.5 and 2.5 m. All seagrass species present at a sampling site were collected. At each sampling site, two porewater samples were collected anaerobically, using 60 ml vacuum syringes connected to ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) placed in the top 7 cm of the soil. These duplicate porewater samples were subsequently pooled per sampling point. Surface water samples were collected similarly in the upper 5 cm of the water column. Samples were frozen on the day of sampling, for further analysis.

Seagrass samples were split up into roots, rhizomes, sheaths, and leaves, and all epiphytes were carefully removed using a scalpel. Subsequently, the samples were dried at 60°C for 48 hours, weighed (g DW) and ground. %C and %N of both leaves and rhizomes were determined with an elemental analyzer (Type NA 1500 Carlo Erba, Thermo Fisher Scientific Inc., USA), coupled online via an interface (Finnigan Conflo III) to a mass-spectrometer (Thermo Finnigan DeltaPlus, USA). Total concentration of phosphorous in seagrass tissue was measured with inductively-coupled-plasma emission spectrometry (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA, USA), after digestion with nitric acid, following Smolders et al. (2006) and as previously used for seagrass habitats by Govers et al. (2014a).

Porewater and surface water ammonium and ortho-phosphate concentrations were measured colorimetrically (Bran & Luebbe Autoanalyzer III, Seal Analytical, UK), using ammonium-molybdate and salicylate (Lamers et al., 1998). Nitrate was determined by sulphanilamide, after reduction of nitrate to nitrite in a Cadmium column and as previously used for tropical seagrass habitats by Christianen et al. (2012).

We additionally conducted a literature study to compare our *Thalassia* leaf nutrient concentrations to literature values for eutrophic and pristine *Thalassia* meadows (Table 6.4). References included in this table were found by using ISI Web of Science with the key words *Thalassia* AND nutrient*, *Thalassia testudinum* AND nutrient*, *Thalassia hemprichii* AND nutrient* or the same key words with either nitrogen or phosphorus instead of nutrient*.

Displayed values are means±standard error (SE), the number of replicates for each bay is indicated in Table 6.1. To compare conditions between bays, we used a one-way ANOVA with a Tukey HSD post-hoc test. Normality was tested prior to analysis with a Shapiro Wilk test and non-normal data were log-transformed prior to testing. To compare two different means (residential areas, literature values), we used an independent T-test. Correlations were tested with Pearson's correlation coefficient. Statistical tests were performed in IBM SPSS Statistics 19.0 and R 2.15.

Table 6.1 Characteristics of the sampled bays on the islands of Curaçao and Bonaire with the number of sampling points per bay. Seagrass species abbreviations: Tt=*Thalassia testudinum*, Sf=*Syringodium filiforme*, Hw=*Halodule wrightii*, and Rm=*Ruppia maritima*.

Bay	Island	Surface (km^2)	Width bay mouth (m)	Seagrass species	Local disturbance	Total # sampling points	# Sampling points seagrass
Boka Ascension Bay	Curaçao	0.05	200	Tt, Sf, Hw	Plastic pollution, turtle grazing	3	3
Lac Bay	Bonaire	7.5	1600	Tt, Sf, Hw, Rm	Protected, light recreation	11	11
Piscadera Bay	Curaçao	0.75	90	Tt, Sf	Sewage discharge, boating	9	6
Santa Anna Bay	Curaçao	4	230	none	Heavy industry; oil refinery	8	0
Sint Joris Bay	Curaçao	2.5	240	Tt	Some waste dumping	6	5
Spanish Water Bay	Curaçao	3	90	Tt, Sf, Hw, Rm	Domestic sewage, boating	14	14

RESULTS

Abiotic measurements such as surface water and porewater nutrient concentrations appeared to give a very limited indication of prevailing nutrient loads as we found no significant differences among bays (Table 6.2). However, surface water measurements indicated high nitrogen and phosphorus loads in St Anna Bay (13.64 and 1.83 µmol L^{-1} respectively) and Boka Ascension Bay (9.88 and 2.07 µmol L^{-1} respectively).

In contrast, tissue nutrient concentrations in *Thalassia testudinum* tissue reflected nutrient loads accumulated over a longer period (Table 6.3). Leaf %N concentrations were highest in Piscadera Bay (2.16±0.22 %N) and lowest in Sint-Joris Bay and Lac Bay (1.66±0.08 %N and 1.78±0.09 %N respectively). Leaf %P was also highest in Piscadera Bay, (0.25±0.03 %P), and lowest in Lac Bay (0.17±0.00 %P). We therefore identified Piscadera Bay as the bay with the highest nutrient loads, whereas Lac Bay and Sint Joris Bay had generally lower nutrient loads.
Mean leaf nutrient concentrations in Spanish Water Bay were not high compared to the other bays, possibly because of high mixing rates. However, when we looked at the effects of local eutrophication by coastal residential areas, we found a significance increase of >10% in *Thalassia* leaf %P in the vicinity (0-200

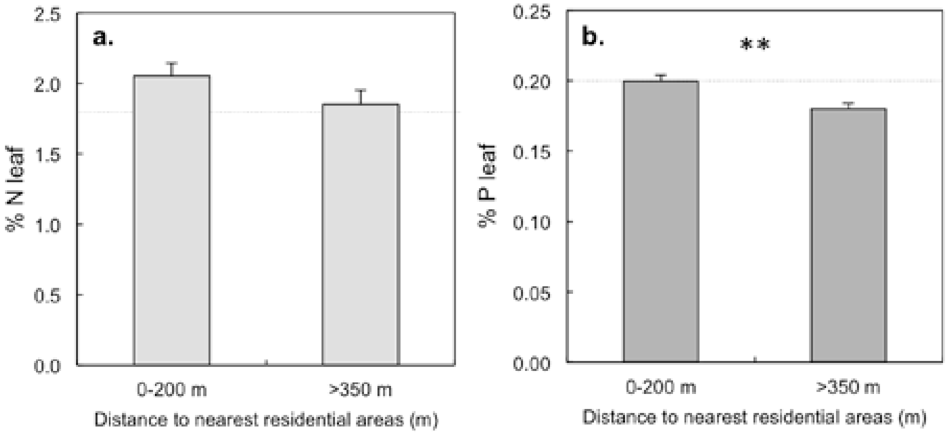


Figure 6.1 Local eutrophication effects of residential areas on (a) leaf %N values and (b) leaf %P values of *Thalassia testudinum* in Spanish Water Bay. Dotted lines indicate the absolute limitation levels (1.8 %N and 0.2 %P) for seagrasses, as stated by (Duarte, 1990). ** Indicates a significance level of P<0.01 (P=0.009). Displayed values are means and error bars present standard errors.

Table 6.2 Porewater and surface water total nitrogen (NH_4 + NO_3) and PO_4 concentrations in µmol L^{-1}. Displayed data are means (# replicates in Table 6.1), and significant differences (ANOVA) are indicated by letters (a, b, etc.). However, as all results did not differ significantly, everything is indicated by the letter a.

	Porewater		Surface water	
Bay	**Total N**	**PO_4**	**Total N**	**PO_4**
Boka Ascension Bay	18.30^{a}	4.72^{a}	9.88^{a}	2.07^{a}
Lac Bay	25.11^{a}	2.16^{a}	4.35^{a}	0.62^{a}
Piscadera Bay	8.05^{a}	1.49^{a}	6.95^{a}	0.83^{a}
Santa Anna Bay	26.40^{a}	2.54^{a}	13.64^{a}	1.83^{a}
Sint-Joris Bay	6.69^{a}	2.80^{a}	2.30^{a}	0.83^{a}
Spanish Water Bay	6.73^{a}	2.07^{a}	5.63^{a}	0.91^{a}

Table 6.3 Mean leaf nutrient concentrations of *Thalassia testudinum* in all sampled bays. Ratios are mol ratios. Significant differences between bays (ANOVA) are indicated by letters (a, b, etc.), as found by post-hoc comparisons. The same letters indicate non-significant differences between groups; different letters indicate significant differences between groups.

Bay	%C	%N	%P	C:N	N:P	C:P	C:N:P
Boka Ascension Bay	30.84^{a}	2.02^{a}	0.18^{ab}	18^{a}	25^{a}	451^{abc}	451:18:01
Lac Bay	34.05^{b}	1.78^{ab}	0.17^{a}	21^{bc}	24^{a}	524^{c}	524:21:01
Piscadera Bay	34.88^{b}	2.16^{a}	0.25^{c}	19^{ab}	19^{b}	366^{a}	366:19:01
Sint-Joris Bay	33.27^{ab}	1.66^{b}	0.18^{ab}	24^{c}	20^{b}	476^{bc}	476:24:01
Spanish Water Bay	33.93^{b}	1.96^{a}	0.19^{b}	20^{ab}	23^{ab}	461^{b}	461:20:01

m) of residential areas (T-test, P=0.009), compared to seagrass stands >350 m of residential areas (Figure 6.1b). In addition, leaf %N also appeared to increase slightly (~10%) near residential areas, but in contrast to %P, this increase was not significant (P=0.166) (Figure 6.1a). Increased nutrient availability near residential areas was also observed in the high macroalgal densities (Halimeda sp., see also (Kuenen and Debrot, 1995; Slijkerman et al., 2011)) in between *Thalassia testudinum* stands. In addition to the results of Spanish Water Bay, *Thalassia testudinum* tissue nutrient concentrations seemed to indicate increased nutrient loads near the emergency overflow pipe (<500 m) of Piscadera Bay, as *Thalassia* leaf %N (2.6%) and %P (0.31%) were strongly elevated (indicated by grey triangles) compared to leaf nutrient concentrations near the bay mouth (1.9 %N and 0.21 %P) (Figure 6.2) and compared to literature data for pristine *Thalassia* beds (Table 6.4). In contrast, *Syringodium filiforme* did not display such an increase in leaf nutrient concentrations (i.e., 1.2 %N and 0.18 %P nearest to the emergence overflow pipe and 1.4 %N and 0.19 %P nearest to the bay mouth). No seagrass was sampled within a distance of 460 m from the emergency overflow pipe; as no seagrass was present this close to the outlet. In Santa Anna Bay, no seagrass was found, so this bay could not be included in the seagrass nutrient analysis.

Based on our literature study (Table 6.4), we found that *Thalassia* leaf nutrient concentrations are generally between 1.68-3.02 %N and 0.14-0.7 %P for systems that were marked as eutrophic (or polluted), and between 0.91-2.4 %N and 0.073-0.18 %P for pristine *Thalassia* beds. Although overlapping, both *Thalassia* leaf %N and leaf %P values differed significantly between eutrophic and pristine sites (T-tests, P<0.001, P=0.007 for %N and %P respectively).

DISCUSSION

Seagrass nutrient concentrations reflected nutrient loads over a longer period, which confirms previous work (Ferdie and Fourqurean, 2004; Udy and Dennison, 1997; van Katwijk et al., 2011). In contrast, abiotic parameters (surface and porewater nutrient concentrations) gave a very limited indication of prevailing nutrients loads, which agrees with earlier observations that such measurements only provide a momentary snapshot (Short and McRoy, 1984), affected by timing, tidal stage, terrestrial runoff, freshwater input, and rapid uptake of nutrients by plankton, seagrasses and macroalgae (van Katwijk et al., 2011). However, mean surface water total nitrogen (>4 µmol L^{-1}) and phosphate (>0.6 µmol L^{-1}) levels from most bays were well above the values indicated as eutrophic reef waters by Gast et al. (1999) and other coastal Caribbean lagoons (Carruthers et al., 2005; Olsen and Valiela, 2010; Peterson et al., 2012). Our values thus seem to indicate that nutrient levels can be above the threshold values for eutrophication for corals (Gast et al., 1999), and also hint at possible negative effects of high nutrient levels for seagrasses. Especially St Anna Bay (or Harbor Bay), where no seagrass can be found, displayed high surface water nutrient levels, possibly, seagrasses have disappeared due to eutrophication in this heavily polluted bay (Gast et al., 1999). Yet, to gain a more complete picture of long-term nutrient loads in these bays, we mainly focused on the indicator value of seagrass tissue nutrient concentrations in this study.

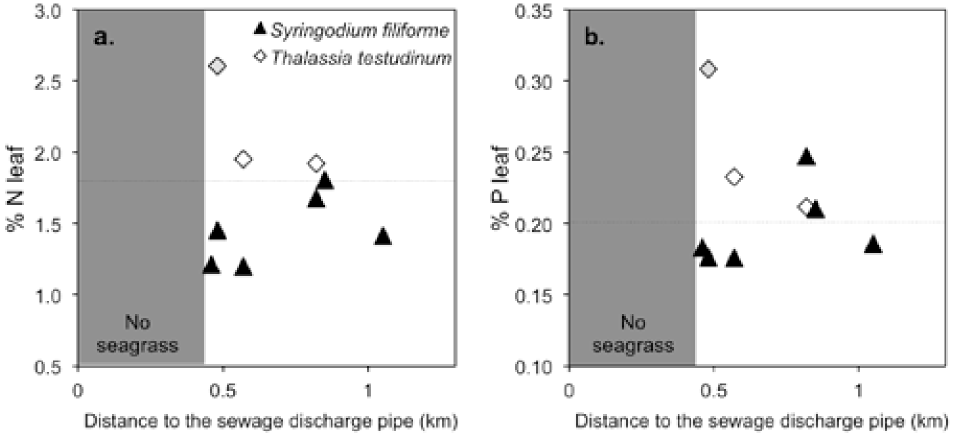


Figure 6.2 Distance analysis on the effects of a emergency overflow discharge pipe on (a) leaf %N and (b) leaf %P concentrations of *Thalassia testudinum* and *Syringodium filiforme* in Piscadera Bay. Dotted lines indicate the limitation levels (1.8 %N and 0.2 %P) for seagrasses, as stated by (Duarte, 1990). Displayed values are means and error bars present standard errors.

We found that *Thalassia* leaf nutrient concentrations for pristine seagrass beds are generally between 0.91-2.4 %N and 0.05-0.18 %P, whereas leaf nutrient concentrations found in nutrient enriched systems are between 1.87-3.02 %N and 0.14-0.70 %P (Table 6.4). When comparing these literature values with our data, Lac Bay, St Joris Bay, and Boka Ascension Bay fall within the limits of oligotrophic/pristine systems for both nitrogen and phosphorus. In addition, *Thalassia testudinum* leaf nitrogen concentrations also indicate limited nitrogen availability (<2.4 %N) in the other bays. However, relatively low nitrogen concentrations may also be the result of the terrigenous sediments in these bays, which may promote nitrogen limitation in seagrasses, due to higher phosphorus availability in terrigenous than in carbonate sediments (Erftemeijer and Middelburg, 1993; Touchette and Burkholder, 2000). Or possibly, sea turtle grazing, such as in Boka Ascension Bay, may promote nutrient export, thereby lowering bay nutrient loads and protecting seagrasses from eutrophication effects (Christianen et al., 2012). Total Phosphorus concentrations of both Piscadera Bay and Lac Bay may indicate excess phosphorus availability, based on comparison with literature data, which confirms the presence of point sources (coastal residencies and emergency overflow pipe) of eutrophication. Thus, our data, supported by our literature overview, show that *Thalassia* leaf nutrient concentrations (especially P) are commonly elevated in the vicinity of a nutrient source and can thus be used as bioindicator for nutrient pollution.

Table 6.4 Literature overview of *Thalassia* sp. leaf nitrogen (%N) and phosphorus (%P) concentrations from eutrophic and pristine areas. Values were derived from tables and figures of the indicated references. The last lines indicate the range of leaf nutrient concentrations for both eutrophic and pristine areas. NA stands for not available.

Study	Location	Species	%N	%P	Classification	Specifics	Reference
1	Florida, USA	*Thalassia testudinum*	2.5	0.51	eutrophic	Charlotte Habor	Fourqurean and Cai (2001)
2	Florida, USA	*Thalassia testudinum*	2.14	0.16	eutrophic	Near bird colony island	Fourqurean et al. (1992)
3	Panama	*Thalassia testudinum*	~ 2.4	0.26	eutrophic	Agricultural runoff/erosion	Carruthers et al. (2005)
10	Florida, USA	*Thalassia testudinum*	3.02	0.7	eutrophic	Polluted bay	Campbell et al. (2012)
11	Bahamas	*Thalassia testudinum*	1.87-3.02	0.17-0.11	eutrophic	Eutrophic (April/August)	Jensen et al. (1998)
13	Mexico	*Thalassia testudinum*	2.72	0.14	eutrophic	Near a city	Gallegos et al. (1993)
14	China	*Thalassia hemprichii*	2.4	0.24	eutrophic	Low intertidal	Zhang et al. (2014)
14	China	*Thalassia hemprichii*	2.8	0.28	eutrophic	High intertidal	Zhang et al. (2014)
15	Indonesia	*Thalassia hemprichii*	1.68	0.49	eutrophic	Close to river input	van Katwijk et al. (2011)
12	Florida, USA	*Thalassia testudinum*	2.33	0.43	eutrophic	PO_4 pollution	Rose and Dawes (1999)
13	Mexico	*Thalassia testudinum*	2.18	0.18	pristine	Reef, oligotrophic	Gallegos et al. (1993)
1	Florida, USA	*Thalassia testudinum*	2	0.1	pristine	Florida Bay	Fourqurean and Cai (2001)
2	Florida, USA	*Thalassia testudinum*	2.1	0.08	pristine	>120 m From bird colony island	Fourqurean et al. (1992)
4	Bahamas	*Thalassia testudinum*	0.91-1.14	NA	pristine	Tidal channel, mature leaves with epiphytes	Capone et al. (1979)
5	Barbados	*Thalassia testudinum*	2.29	0.157	pristine	NA	Patriquin (1972)
6	Mexico	*Thalassia testudinum*	2.25	0.13	pristine	Outer reef	Terrados et al. (2008)
7	Puerto Rico	*Thalassia testudinum*	2.4	0.14	pristine	Surrounded by agricultural/urban land, but decoupled from watershed	Olsen and Valiela (2010)
8	Bahamas	*Thalassia testudinum*	1.88	0.073	pristine	Undisturbed tidal creeks with fringing mangroves	Allgeier et al. (2011)
9	Jamaica	*Thalassia testudinum*	1.44	NA	pristine	No nitrogen rich upwelling	Peterson et al. (2012)
10	Florida, USA	*Thalassia testudinum*	2.14	0.08	pristine	Unpolluted bay	Campbell et al. (2012)
11	Bahamas	*Thalassia testudinum*	1.84-2.13	0.12-1.14	pristine	Oligotrophic (April/August)	Jensen et al. (1998)
15	Indonesia	*Thalassia hemprichii*	2.28	0.1	pristine	Far away from river input	van Katwijk et al. (2011)
16	Indonesia	*Thalassia hemprichii*	2.04	NA	pristine	Spermonde Archipelago	Vonk et al. (2008)
17	Indonesia	*Thalassia hemprichii*	1.9	0.14	pristine	Spermonde Archipelago	Erftemeijer and Middelburg (1993)
18	Indonesia	*Thalassia hemprichii*	1.99	0.18	pristine	Barang Lompo, 3rd leaf	Stapel and Hemminga (1997)
18	Indonesia	*Thalassia hemprichii*	1.99	0.16	pristine	Gusung Tallang, 3rd leaf	Stapel and Hemminga (1997)
		Eutrophic	**1.68-3.02**	**0.14-0.7**			
		Pristine	**0.91-2.4**	**0.073-0.18**			

We found a difference in nutrient accumulation in the leaves between a late and early successional seagrass species in the vicinity of a source of eutrophication (emergency overflow pipe, Piscadera Bay). In contrast to *Thalassia testudinum*, the fast growing *Syringodium filiforme* did not accumulate nutrients in the eutrophic bay, but seem to have used the extra nutrients for growth (pers. observations, Figure S6.1). Leaf nutrient concentrations of the late successional *Thalassia testudinum* were however >35% higher (0.31 %P, 2.6 %N) in vicinity of the overflow pipe than in plants near the bay mouth (0.21 %P, 1.9 %N). Christianen et al. (2011) found similar differences in leaf nutrient levels between an early successional and a late successional species, which might be explained by differences in growth strategy. An early successional species, such as *Syringodium filiforme* generally shows higher production rates (Barber and Behrens, 1985) and shoot turn-over rates (2.0 yr^{-1}; Gallegos et al. (1994)), and is able take up nutrients faster than late successional species (Duarte, 1991; Rollon et al., 1998). In contrast, the late successional species *Thalassia testudinum* is a slow growing species with low shoot turn-over rates (0.6 yr^{-1}; Gallegos et al. (1993)), which accumulates nutrients in the leaves (Carruthers et al., 2005; McGlathery et al., 1994). *Thalassia testudinum* proved also to be a good indicator of point sources of eutrophication in Spanish Water Bay, as leaf %P was elevated in the vicinity of residential areas up to concentrations >0.18% which may be indicative of eutrophication according to literature values (Table 6.4). However, additional to leaf nutrient concentrations (%N, %P), nitrogen isotopic ratios would have provided even more detailed information on anthropogenic nutrient input in the studied bays (Mutchler et al., 2007; Schubert et al., 2013).

CONCLUSIONS

We conclude that that leaf nutrient values of *Thalassia testudinum* may be used as bioindicator values for point sources of eutrophication. Additionally, we identified potential threats of eutrophication to seagrasses on Curaçao in Spanish water Bay (residential areas) and Piscadera Bay (emergency overflow discharge); nutrient levels in the other sampled bays did however not raise any concern yet. The seagrasses of Piscadera Bay have already retreated to the shallowest areas (<1m, pers. observations) of the murky waters and are under threat of complete disappearance with a further increase of nutrient loads. Moreover, *Thalassia testudinum* in Spanish Water Bay has been declining for some time (Kuenen and Debrot, 1995), and we are the first to suggest (leaf %P values) that this may be due to excess anthropogenic nutrient input by coastal residencies. This bay, with the largest seagrass area of Curaçao (Debrot et al., 1998; Kuenen and Debrot, 1995), highly contributes to coral reef fish populations by functioning as a nursery habitat (Huijbers et al., 2013). Possible disappearance of seagrasses due to eutrophication may therefore have serious consequences for the ecological and economical values of the coastal ecosystems of Curaçao. We have shown that some bays at Curaçao are subjected to excess nutrient inputs, which may already have contributed to seagrass loss and linked ecosystem services. We therefore hope that measures will be taken to prevent further loss of valuable seagrass beds due to eutrophication at Curaçao.

ACKNOWLEDGEMENTS

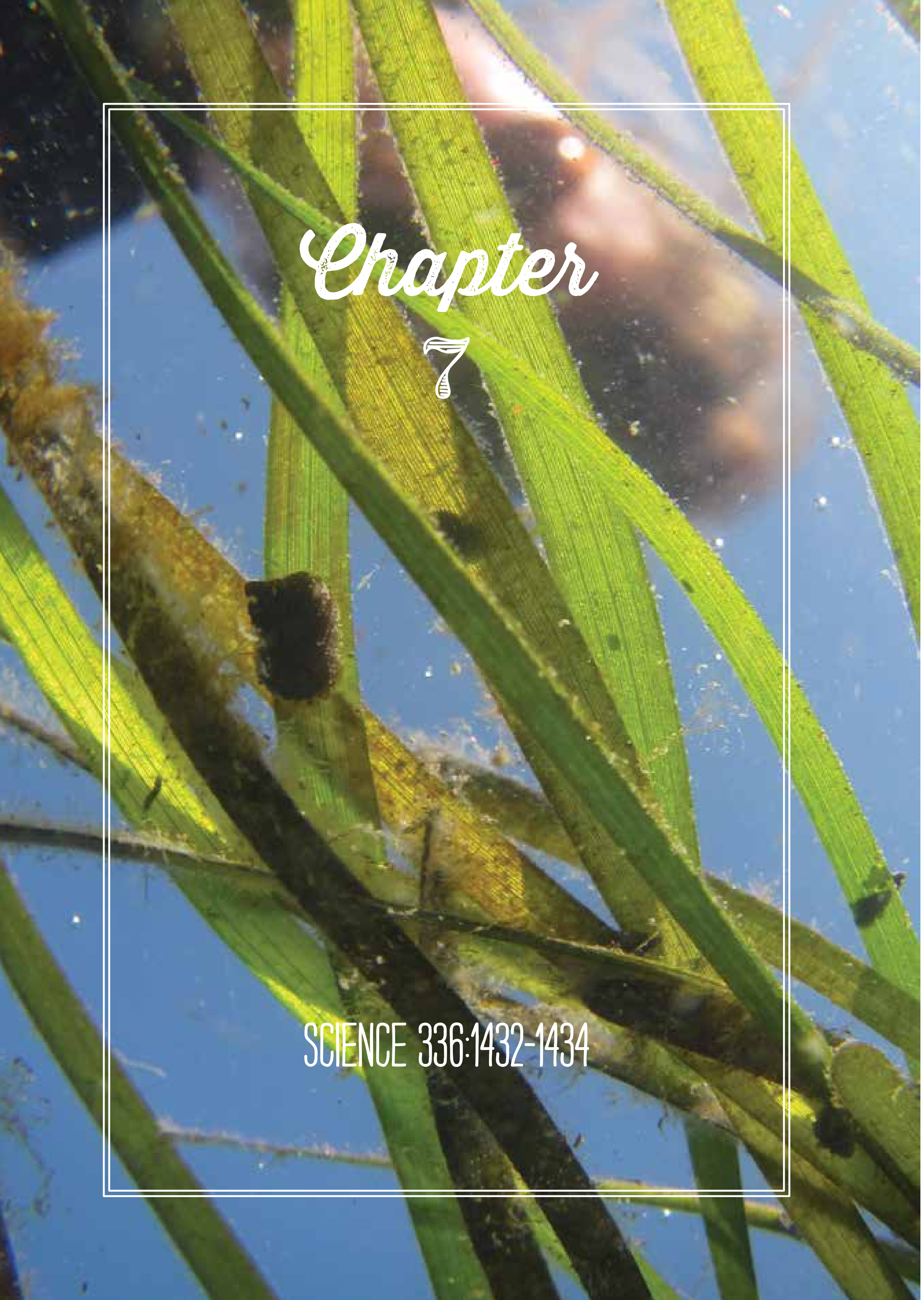
We would like to acknowledge Monique Grol, Chantal Huijbers for logistic support, Elsmarie Beukenboom, Ramon de Leon and Frank van Slobbe from STINAPA Bonaire for the sampling permit for Bonaire, and we would also like to thank Jelle Eygensteyn and Paul van de Ven for assistance with the lab analyses.

SUPPLEMENTARY DATA CHAPTER 6

Table S6.1 Details on sampling of porewater and surface water sampling. Samples were all taken in January 2010 between 10 am and 4 pm.Tidal stage reflects the tidal stage at Schottengat Baai/ Kralendijk during midday sampling (source: mr. tides). Tidal height is in cm. Tidal differences are limited and result from highly irregular mixed diurnal/semidiurnal tides. Temperature is the mean daily air temperature in °C and precipitation is total daily rainfall in mm (www.wunderground.com).

Bay	Date	Low tide	Height	High tide	Height	Temperature	Precipitation
Boka Ascension Bay	January 7, 2010	14:51	4.54	19:17	13.48	28	0.0
Lac Bay	January 18, 2010	23:44	32.00	14:13	50.00	28	0.0
	January 19, 2010	23:42	33.00	14:37	46.00	28	0.0
Piscadera Bay	January 12, 2010	19:48	-3.87	10:49	28.70	29	0.0
Santa Anna Bay	January 6, 2010	13:35	8.13	18:15	17.31	28	0.0
	January 16, 2010	22:47	-1.28	13:20	23.09	29	0.0
Sint-Joris Bay	January 5, 2010	11:59	11.08	17:05	21.46	29	0.0
Spanish Water Bay	January 8, 2010	15:57	1.26	7:56	32.00	28	0.0
	January 9, 2010	16:57	-1.25	8:36	32.00	28	0.4
	January 11, 2010	18:51	-3.67	10:04	30.00	28	0.0

Figure S6.1 Photo of *Syringodium filiforme* originating from Piscadera Bay on the right side and *S. filiforme* from Boka Ascension Bay on the left site.



A THREE-STAGE SYMBIOSIS FORMS THE FOUNDATION OF SEAGRASS ECOSYSTEMS

Tjisse van der Heide, Laura L. Govers, Jimmy de Fouw, Han Olff, Matthijs van der Geest, Marieke M. van Katwijk, Theunis Piersma, Johan van de Koppel, Brian R. Silliman, Alfons J. P. Smolders & Jan A. van Gils (2012)

Abstract

Seagrasses evolved from terrestrial plants into marine foundation species around 100 million years ago. Their ecological success, however, remains a mystery as natural organic matter accumulation within the beds should result in toxic sediment sulfide levels. Using a meta-analysis, a field study and a laboratory experiment, we reveal how an ancient three-stage symbiosis between seagrass, lucinid bivalves and their sulfide-oxidizing gill-bacteria reduces sulfide stress for seagrasses. We found that the bivalve-sulfide-oxidizer symbiosis reduced sulfide levels and enhanced seagrass production as measured by biomass. In turn, the bivalves and their endosymbionts profit from organic matter accumulation and radial oxygen release from the seagrass roots. These findings elucidate the long-term success of seagrasses in warm waters, and offer new prospects for seagrass ecosystem conservation.

Seagrass meadows are important ecological and thus economic components of coastal zones worldwide (Larkum et al. 2006, Waycott et al. 2009). In many areas, coral reefs and seagrass meadows are tightly linked habitats that form the basis for marine biodiversity (Nagelkerken 2009). Seagrasses serve as keystone habitat for migrating coral reef species, thousands of other animals including waterbirds, fish, dugongs, manatees and turtles, are important carbon and nutrient sinks, and are important to fisheries and coastline protection (Larkum et al. 2006, Nagelkerken 2009, Waycott et al. 2009). Dense seagrass meadows attenuate currents and waves and trap pelagic and benthic organic matter in the sediment (Larkum et al. 2006, van der Heide et al. 2007, van der Heide et al. 2011). Owing to a lack of oxygen in many coastal marine sediments, an important fraction of organic matter is decomposed by bacteria that use the abundant sulfate in seawater as an electron acceptor instead of oxygen, and produce toxic sulfide as a metabolic end product (Jorgensen 1982). Although seagrasses transport oxygen into their roots and the surrounding rhizosphere (radial oxygen release) (Larkum et al. 2006, Calleja et al. 2007), sulfide production outpaces oxygen release under warmer conditions, resulting in sulfide accumulation and seagrass mortality (Larkum et al. 2006, Calleja et al. 2007, Koch et al. 2007). Seagrass beds tend to accumulate organic matter and so it is expected that seagrass beds would build up toxic sulfides and hence have a limited productivity and diversity (Larkum et al. 2006). But this is not the observed case and the underlying reason for the long-term persistence of seagrass ecosystems is an enigma (Figure S7.1a).

We tested the hypothesis that a three-stage symbiosis between seagrasses, associated burrowing lucinid bivalves and their symbiotic gill-bacteria contribute to reducing the cyclic build-up of sulfide (Figure S7.1b-d). Paleo-records suggest that the Lucinidae and their endosymbiotic relation date back to the Silurian (Liljedahl 1991, Distel 1998, Taylor and Glover 2000), but that they increasingly diversified since the evolutionary emergence of seagrasses in the late Cretaceous (Stanley 1977, Larkum et al. 2006, Taylor et al. 2011). Seagrass communities later became widespread in the Eocene and lucinid remains frequently occur in association with their deposits since (Taylor et al. 2011, Vermeij 2011). Lucinids and their gill-inhabiting bacteria have a symbiosis in which the bivalves transport sulfide and oxygen to their gills (Figure S7.1d) where the bacteria oxidize sulfide for synthesizing sugars that fuel growth of both organisms (Cavanaugh 1983, Johnson et al. 1994, Anderson 1995, Reynolds et al. 2007, Childress and Girguis 2011). We hypothesized that seagrass meadows may provide an optimal habitat for these bivalves and their symbionts by indirectly stimulating sulfide production by high organic matter input, and by providing oxygen through radial oxygen release from the roots. In turn, lucinids remove sulfide, which could relieve any stress caused to seagrass growth by sulfide accumulation as organic matter is degraded (Figure S7.1a & b).

Indirect support for our hypothesis was provided by a worldwide meta-analysis of 84 studies describing the fauna of seagrass beds in 83 sites covering the entire climatic distribution of seagrasses, combined with a 110-point field survey that we conducted at Banc d'Arguin, Mauretania (Schanz et al. 2002). The meta-analysis reveals a relationship that covers 11 out of 12 seagrass genera (and *Ruppia* spp.) and at least 18 genera of Lucinidae (Figure 7.1 & Table S7.1). Only meadows of *Phyllospadix* spp., a seagrass genus that grows on bare rock, do not associate with Lucinidae. The association spans six out of seven continents, with bivalve densities ranging from 10 to over 1000 individuals per m^2. The bivalves were present in 97% of the tropical seagrass sites, 90% of the subtropical meadows and 56% of the temperate seagrass beds surveyed, indicating that the association may be dependent on temperature-related sulfide production (Koch et al. 2007). Furthermore, results from our field study showed a positive correlation between seagrasses and lucinids that explained 42% of their respective variation (Pearson's r=0.65; Figure S7.2).

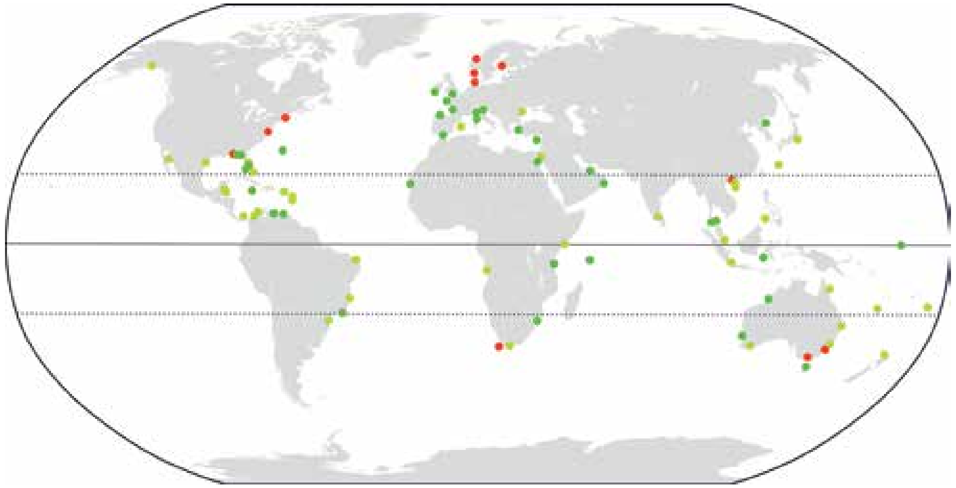


Figure 7.1 Presence (green; dark points are quantitative, light points are qualitative) and absence (red) of lucinids in seagrass ecosystems based on our meta-analysis. The bivalves were present in 97% (93% of the quantitative sites) of all tropical seagrass beds, 90% (83% of the quantitative sites) of the subtropical beds and 56% (50% of the quantitative sites) of the temperate seagrass meadows. The seagrass-lucinid association spans six out of seven continents, at least 18 genera of lucinids and 11 out of 12 seagrass genera (and *Ruppia* spp.). Only meadows of *Phyllospadix* spp., a seagrass genus that grows on bare rock, did not contain Lucinidae. The analyzed ecosystems generally contained high (~100 ind. m^{-2}) to extremely high densities (>1000 ind. m^{-2}) of lucinids (Table S7.1).

To experimentally test our hypothesis (Figure S7.1b), we investigated the effects of sulfide oxidation by the lucinid bivalve *Loripes lacteus* on the production of the seagrass species *Zostera noltii* and the potential reciprocal benefits for *Loripes* in a full factorial experiment under controlled conditions (Schanz et al. 2002). We set up *Zostera*, *Loripes*, *Zostera-Loripes* and bare sediment treatments in the top sections of 40 two-compartment columns (Figure S7.3), which were placed in a large seawater basin. The lower compartment of each column contained anaerobic seawater and an injection tube through which sulfide was added twice a week in half of the columns. The injected sulfide was allowed to diffuse into the top section through a porous membrane.

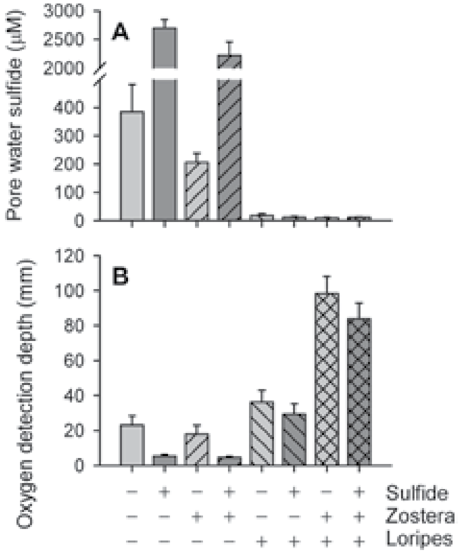


Figure 7.2 (a) Pore water sulfide concentrations and (b) oxygen detection depth after five weeks; error bars represent SEM (n=5). Oxygen detection depth decreased as sulfide was added (ANOVA: $F_{1,32}$=8.9, P<0.006). The presence of *Loripes* reduced sulfide levels (RM-ANOVA: $F_{1,32}$=268.8, P<0.001) and increased oxygen detection depth ($F_{1,32}$=125.0, P<0.001). Reduction of the sulfide concentration by *Zostera* alone was less, but still significant ($F_{1,32}$=6.8, P=0.014). That interactions occured between *Zostera* and *Loripes* was apparent in the oxygen measurements ($F_{1,32}$=48.3, P<0.001), but was also significant in the sulfide data ($F_{1,32}$=7.8, P=0.009). The interaction between *Loripes* and sulfide was significant for the sulfide measurements ($F_{1,32}$=102.7, P<0.001), but not for the oxygen data ($F_{1,32}$=0.3, P=0.578).

The presence of *Loripes*, and to a lesser extent of *Zostera* decreased sediment sulfide levels. After five weeks, pore water sulfide concentrations in the top sections of the sediment controls reached about 400 µmol L^{-1}, while the semi-weekly addition of sulfide caused levels to increase to nearly 2700 µmol L^{-1} (Figure 7.2a). The presence of *Zostera* decreased sulfide levels to around 200 µmol L^{-1} in the controls and 2200 µmol L^{-1} in the sulfide addition treatments. In contrast, sulfide levels remained low when *Loripes* was present (~15 µmol L^{-1}), even in the sulfide addition treatments. As expected, the oxygen detection depth was reduced when sulfide was added, but increased when only *Loripes*, but not *Zostera* was present, due to sulfide-oxidation and intake of surface water (Figure 7.2b). *Zostera* alone did not significantly affect sediment oxygen conditions. Strikingly, the joint presence of *Zostera* and *Loripes* enhanced oxygen detection depth beyond that of their separate effects.

Our experiment showed that *Zostera* production is facilitated by *Loripes*; both in the control and in the sulfide addition treatments. In the treatments without *Loripes*, sulfide addition reduced *Zostera* shoot biomass to 50% of the controls (Figure 7.3a). Reduced shoot biomass was accompanied by decreased root biomass (Figure 7.3b) and impaired phosphate uptake (Schanz et al. 2002). In contrast, the addition of *Loripes* increased *Zostera* shoot biomass 1.9-fold and root weight 1.5-fold seen in the sulfide addition treatments. In the treatments without additional sulfide, the presence of *Loripes* increased both shoot and root weight by 1.4-fold and 1.3-fold respectively.

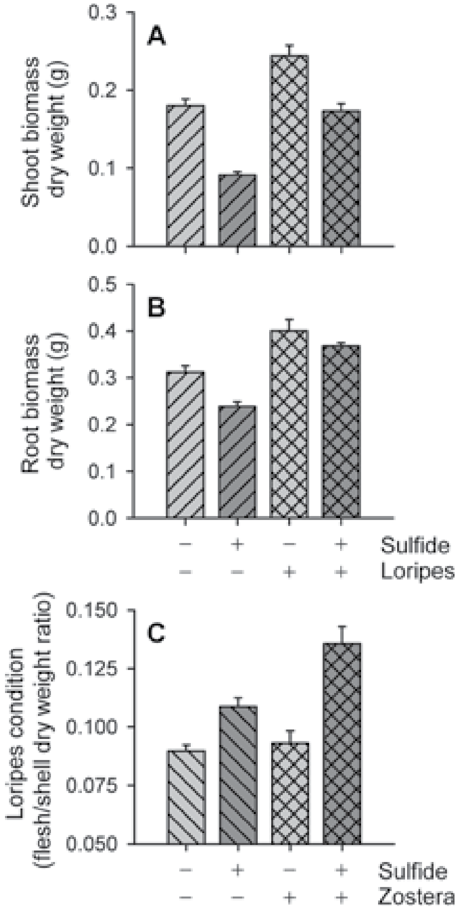


Figure 7.3 (a) *Zostera* shoot and (b) root dry weight biomass per column and (c) *Loripes* condition expressed as the dry weight flesh/shell ratio after five weeks; error bars represent SEM (n=5). *Zostera* biomass was reduced by sulfide addition (ANOVA: shoots $F_{1,16}$=72.6, P<0.001; roots $F_{1,16}$=12.0, P=0.003), whereas the presence of *Loripes* had a positive effect on both shoot ($F_{1,16}$=61.3, P<0.001) and root biomass ($F_{1,16}$=50.2, P<0.001). We found no significant effects on rhizome biomass. *Loripes* condition was positively affected by both sulfide addition (ANOVA: $F_{1,16}$=37.3, P<0.001) and *Zostera* presence ($F_{1,16}$=9.0, P=0.008). We also found a significant positive combined effect of the presence of *Zostera* and sulfide on *Loripes* condition ($F_{1,16}$=5.4, P=0.034).

Loripes condition, expressed as the flesh/shell dry weight ratio, was positively affected by sulfide addition (Figure 7.3c). Furthermore, the addition of *Zostera* did not affect *Loripes* in the units where no sulfide was added, but improved the bivalve's condition in the sulfide treatments. As hypothesized, the positive effect of *Zostera* on *Loripes* seems to result from radial oxygen release from the seagrass roots (Figure S7.1b). Although sulfide was almost completely removed in all *Loripes* treatments (Figure 7.2a), the bivalve was less able to profit from the addition of sulfide in the absence of *Zostera* (Figure 7.3c). This indicates that at least in the *Loripes* units without seagrass, sulfide was not completely oxidized by the symbiotic bacteria because of oxygen limitation.

Overall, our results confirm our hypothesis that a three-stage symbiosis between seagrass, lucinids and sulfide-oxidzing bacteria reduces sulfide stress in seagrass meadows. Even though radial oxygen release by *Zostera noltii* and of seagrasses in general is limited (Caffrey and Kemp 1991, Sand-Jensen et al. 2005), *Loripes* in our experiment clearly benefitted from the increased oxygen input in the sediment. In the field, the positive effects of seagrasses on lucinids are not confined to sediment oxygenation alone, but also by indirectly stimulating sulfide production and releasing dissolved organic molecules (Larkum et al. 2006, Reynolds et al. 2007). The positive effects of *Loripes* on *Zostera* in our experiment could not be explained by differences in nutrient availability (Schanz et al. 2002). Plants were not nutrient limited, but both *Zostera* and *Loripes* significantly lowered dissolved ammonium and phosphorus in the sediment pore water, whereas sulfide addition increased nutrient availability (Figure S7.4). We found that in our experiment, the negative effects of sulfide addition on *Zostera* biomass could not fully be prevented by *Loripes* addition (Figure 7.3a), despite the removal of almost all sulfide by *Loripes* after three days. As the observed experimental effects could not be attributed to differences in nutrient availability, this is most likely caused by the pulsed nature of our sulfide supply. This may have led to short periods of exposure of *Zostera* to toxic sulfide levels.

Coastal ecosystems, and seagrass meadows in particular, are currently declining at an alarming and increasing rate worldwide, leading to loss of biodiversity (Waycott et al. 2009). Extensive restoration efforts have had little success so far (<30%), despite their extremely high costs (±$100,000 per ha) (Fonseca et al. 2001). Similar to the function of mycorrhizae, pollinators or seed dispersers in terrestrial systems (van der Heijden et al. 1998, Bascompte and Jordano 2007, Bastolla et al. 2009), our findings indicate that restoration efforts should not only focus on environmental stressors like eutrophication, sediment run-off or high salinity as a cause of decline, but should also consider internal ecological interactions such as the presence and vigor of symbiotic or mutualistic relations. Breakdown of symbiotic interactions can affect ecosystem functioning, with bleaching events in coral reefs as a clear example (Carpenter et al. 2008). Similar to the well-known symbiosis between corals and their unicellular algal endosymbionts (Baker 2003), we conclude that symbioses, rather than one defining species forms the foundation of seagrass ecosystems.

ACKNOWLEDGMENTS

We thank G. Quaintenne and H. Blanchet for their help with the collection of *Loripes*; J. Eygensteyn and E. Pierson for technical assistance; and G.J. Vermeij, H. de Kroon, T.J. Bouma, E.J. Weerman and C. Smit for their comments on the manuscript. TvdH was financially supported by the “Waddenfonds” programme; MvdG and TP by the NWO-WOTRO Integrated Programme grant W.01.65.221.00 awarded to TP; and JdF and JvG by the NWO-VIDI grant 864.09.002 awarded to JvG. BS was supported by an NSF CAREER award, the Andrew Mellon Foundation and the Royal Netherlands Society Visiting Professorship. The authors declare no conflict of interest. A detailed description of all materials and methods, sources as well as supplementary information are available as Supporting Online Material.

SUPPLEMENTARY DATA CHAPTER 7

MATERIALS AND METHODS

Meta-analysis

To test the seagrass-lucinid association, we performed an extensive, worldwide meta-analysis that covered the entire climatic distribution of seagrasses. Criteria for including a study were: (1) seagrasses were present at the site, and (2) when Lucinidae were present, they were found inside the seagrass bed. In total, we analyzed 84 studies that sampled the fauna of seagrass beds in a total of 83 areas (temperature range=1 to 33°C, mean=22°C). Overall, 36 sites were from tropical areas, 31 from subtropical and 16 from temperate areas; quantitative data were available for 46 out of 83 sites. Apart from the geographical location of each site, and the seagrass and lucinid families found, we also report the annual seawater temperature range. These were obtained from freely available satellite imagery of the long-term monthly means (1971 – 2000) of the sea surface temperature (NOAA/OAR/ESRL/PSD 2011).

Field study

We conducted a field survey at Banc d’Arguin (Mauritania) to test the strength of the relation between seagrass biomass and lucinid density. Banc d’Arguin consists of about 500 km^2 of intertidal flat dominated by mixed meadows of *Zostera noltii*, *Halodule wrightii* and *Cymodocea nodosa* that are inhabited by the lucinid bivalve *Loripes lacteus* (Wolff et al. 1993). In total, we sampled 110 stations across seven intertidal flats. *Loripes* was sampled up to a depth of 20 cm using a cylindrical 15 cm diameter PVC core sampler and seagrass was sampled with a 7 cm diameter corer. Each sample was sieved over a 1 mm mesh sieve. Next, *Loripes* was counted and seagrass biomass was determined after drying for 24 h at 70°C. Prior to linear regression analysis, *Loripes* counts and seagrass dry weight from the cores were transformed with the Box-Cox procedure to achieve normality and homoscedasticity (Box and Cox 1964).

Laboratory experiment

Organisms and sediment for the experiment were collected in Arcachon Bay (southwest France) and transported at 15°C to the laboratory, where both species were separately acclimatized for three weeks in 100 L polyethylene tanks. *Zostera* units contained 15 cm of sediment and 20 cm of surface water; *Loripes* tanks contained 30 cm of sediment and 5 cm of surface water. We used artificial seawater (33-35 PSU Tropic Marin at 20°C) throughout the acclimatization period and during the experiment; pH was kept at 8.1 to 8.3 by CO_2 aeration. Light period was 16 h day^{-1}; intensity at the leaf surface was 300 µmol m^{-2} s^{-1}, similar to growing season conditions in the field (Isaksen and Finster 1996). During this three-week period, we did not observe any bivalve mortality, and seagrasses exhibited healthy vegetative growth.

Experimental setup

The lower 6 cm tall sections of 40 two-compartment PVC columns (diameter 8.4 cm) were filled with anaerobic seawater (Figure S7.3). These 330 mL sections contained an injection tube and were separated from their upper compartments through a porous 0.1 mm membrane. Sediment was passed through a 1 mm sieve and transferred to the upper 12 cm tall sections (surface area: 0.0055 m^2). Depending on the treatment, each unit then received either 1) *Loripes*, 2) *Zostera*, 3) both *Zostera* and *Loripes*, or 4) no further treatment. Nine *Loripes* specimens were added to each *Loripes* treatment (~1600 ind. m^{-2}; mean shell length ~9 mm) and 5 seagrass ramets with 2 or 3 shoots (12 shoots in total) were planted in each unit containing *Zostera* (~2200 sh. m^{-2}; ~0.12 g shoot, ~0.06 g rhizome and ~0.03 g DW root biomass per column). Each ramet contained one apical shoot to allow vegetative growth. Pilot experiments showed that this approach ensured consistent colonization of the units within the two-week adjustment period, with no detectable mortality of the plants. Densities of both species were well within reported ranges of densities in the field (up to 23000 sh. m^{-2} for *Zostera* and 3700 ind. m^{-2} for *Loripes*) (Vermaat and Verhagen 1996, Johnson et al. 2002, van der Geest et al. 2011).

A full factorial experiment was designed with eight treatments and five replicates per treatment. The columns were randomly placed in a 40 cm high 250 L polyethylene basin where water flow and oxygen saturation (measured with a 556 Multi Parameter Sampler, Yellow Springs Instruments) were maintained by two aquarium water pumps, and pH was kept constant (8.1-8.3) by CO_2 aeration. After setup, the units were allowed to adjust for two weeks. During this period, sulfide levels in the treatments containing *Loripes* stabilized at ~7 µmol L^{-1}, while sulfide in treatments without *Loripes* increased to ~233 µmol L^{-1}. Following the adjustment period, the experiment was performed for five weeks. Sulfide levels in the lower compartments of the sulfide addition treatments were increased twice a week by 3.3 ml injections of 100 mmol L^{-1} Na^2S solution with pH adjusted to sediment conditions (pH 7.5) with HCl, while control treatments were injected with anaerobic water. Before each injection, we used 5 cm Rhizon samplers to extract 3 ml of pore water from the main root zone (top 6 cm) of each upper compartment into vacuumized 30 mL flasks containing 3 ml Sulfide Anti-Oxidation Buffer (SAOB). After each sampling, columns were re-randomized in the basin to minimize possible differences in light levels and water flow velocities between units. Sulfide concentrations were determined immediately with an ion selective silver/sulfide electrode (Thermo Scientific (USA), Orion 9416 BN; reference electrode: Orion 900200). Oxygen detection depth was measured after five weeks with an oxygen-sensitive microelectrode (Microscale Measurements, 1 mm tip). Ammonium, nitrate and total dissolved phosphorus in the sediment pore water were also measured after five weeks. We used 5 cm Rhizon samplers to extract 10 ml of pore water from the main root zone (top 6 cm) of each upper compartment into vacuumized 30 mL flasks. Ammonium and nitrate concentrations were determined colorimetrically. Ammonium was measured with salicylate (Lamers et al. 1998) and nitrate was determined by sulfanilamide after reduction of nitrate to nitrite in a cadmium column (Wood et al. 1967). Dissolved phosphorus was measured on an Inductively Coupled Plasma emission spectrophotometer (ICP; Spectroflame, Spectro). Total nitrogen concentration in *Zostera* leaves was measured in freeze-dried tissues by a CNS analyzer (type NA1500; Carlo Erba Instruments, Milan, Italy) (Lamers et al. 1998). Total phosphorus was measured by ICP after digestion with nitric acid (Lamers et al. 1998). *Zostera* shoot, root and rhizome biomass and *Loripes* flesh were measured as dry weight after 24 h of freeze-drying. *Loripes* shell weight was measured after drying for 24 h at 70°C. *Loripes* condition was expressed as flesh/shell dry weight ratio, which is a commonly used size-and-age independent measure of fitness in bivalves (Lucas and Beninger 1985). Sulfur contents in the *Loripes* tissues were measured on ICP, following nitric acid digestion.

Statistical analyses

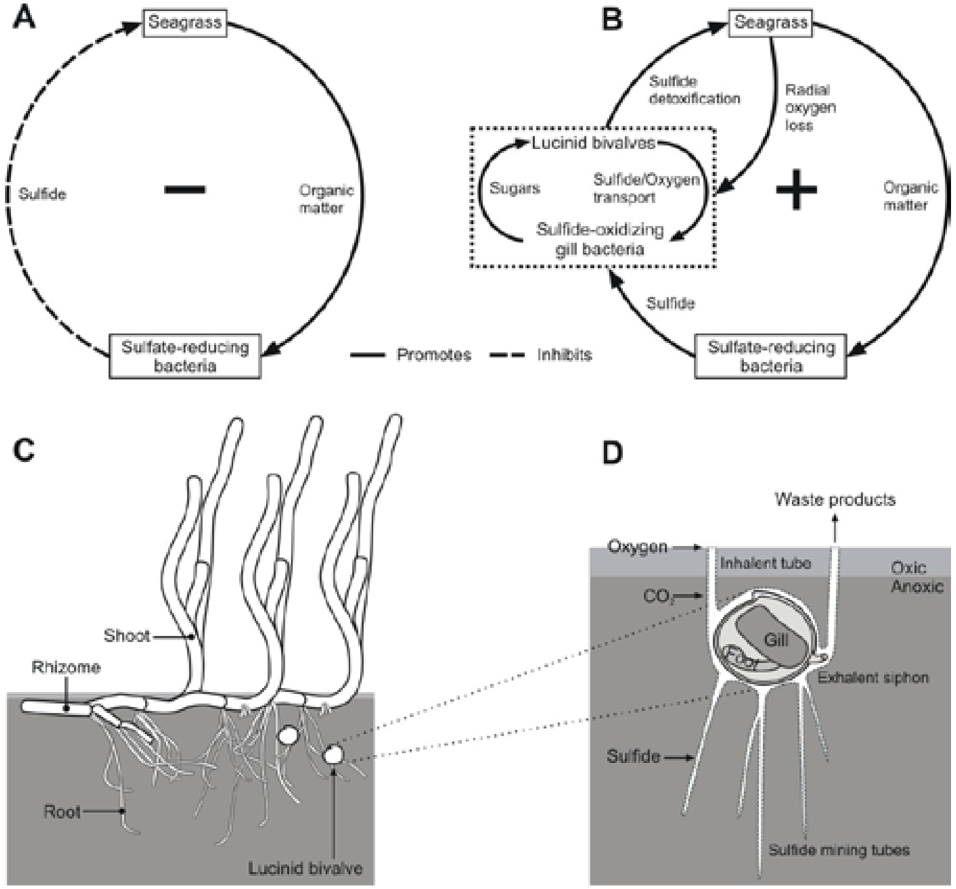
Data were tested for normality prior to analysis. Sulfide data were analyzed with Repeated-Measures three-factor ANOVA. All other variables were analyzed by two- or three-factor ANOVA. All relevant and/or significant effects and interactions are mentioned in the figure legends or supporting text. A complete overview of the statistical output for Figures 7.2, 7.3 and S7.4 is provided in Table S7.2.

SUPPORTING TEXT

Both *Zostera* and *Loripes* significantly lowered dissolved ammonium and phosphorus in the sediment pore water, while sulfide addition increased their availability (Figure S7.4). Nitrate concentrations were 0.8±0.9 µmol L^{-1} (mean±SD) on average with no significant differences between treatments. Mean leaf nitrogen and phosphorus content were 1.78±0.26 and 0.15±0.02% dry weight respectively, which is around reported median values from the field for both (1.8 and 0.2 %DW respectively) (Duarte 1990). None of the treatments had any significant effect on leaf nitrogen. Leaf phosphorus content was unaffected by *Loripes*, but decreased significantly in the sulfide addition and sulfide addition with *Loripes* treatments (from 0.17±0.01 to 0.13±0.01 %DW; ANOVA: $F_{1,16}=29.0$, $P<0.001$). Apparently, high sulfide levels impaired phosphorus uptake by *Zostera* in the sulfide addition treatment, leading to decreased leaf phosphorus content, despite high dissolved phosphorus availability in the pore water (Figure S7.4). Our pulsed sulfide addition also seemed to impair phosphorus uptake in the sulfide addition with *Loripes* treatment, which, by interacting with the reduced dissolved phosphorus pool may have limited growth of *Zostera* under our conditions (Figure 7.3).

Sulfide addition resulted in a significant increase in the relative (ANOVA: $F_{1,16}$=13.8, P=0.002) and absolute sulfur content (ANOVA: $F_{1,16}$=24.1, P<0.001) in the flesh of the bivalves. Relative sulfur content was 2.0±0.2% (g:g) in the control treatments and 3.0±0.9% in the sulfide addition treatments. The total amount of sulfur stored in *Loripes* tissues per unit was 1.3±0.2 mg in the control treatments and 3.0±1.1 mg in the sulfide addition treatments. These results suggest that the increased sulfide availability led to increased storage of sulfur in the tissues of the bivalves, for instance as sulfur granules in the gills (Anderson 1995). We found no significant effects of *Zostera* on *Loripes* sulfur content.

SUPPORTING FIGURES



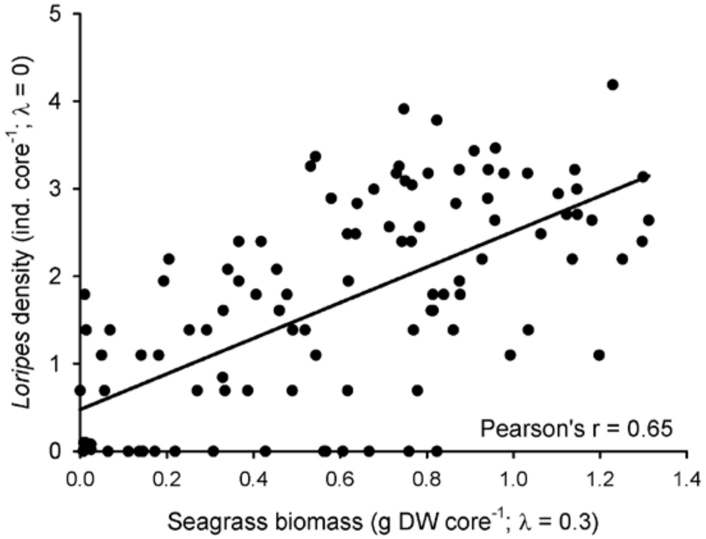


Figure S7.2 Positive correlation (Pearson's r=0.65) between seagrass biomass and *Loripes* density on Banc d'Arguin. *Loripes* counts and seagrass dry weight from the cores were transformed using the Box-Cox procedure prior to plotting and the regression analysis (see Materials & Methods).

< Figure S7.1 (a) Seagrasses generally create a negative feedback on their own growth through organic matter accumulation, which stimulates production of toxic sulfide by heterotrophic sulfate-reducing bacteria. (b) We propose in this study that the presence of lucinid bivalves and their sulfide-oxidizing gill-symbionts breaks the negative feedback, resulting in a network of positive interactions. (c) The bivalves are found in high abundances in the root zones of seagrass meadows in warmer, mild temperate to tropical regions where sulfide production rates are high. (d) They occur in the anoxic zone of the sediment and use their highly extensile foot to create tubes for sulfide mining, export of waste products and import of oxygen and CO_2 from the sediment pore water and surface water (Anderson 1995, Reynolds et al. 2007). Both sulfide and oxygen are transported to the gills where chemoautotrophic bacteria oxidize sulfide for synthesizing sugars that fuel growth of both the bacteria and the bivalve (Johnson et al. 1994, Anderson 1995, Reynolds et al. 2007, Childress and Girguis 2011).

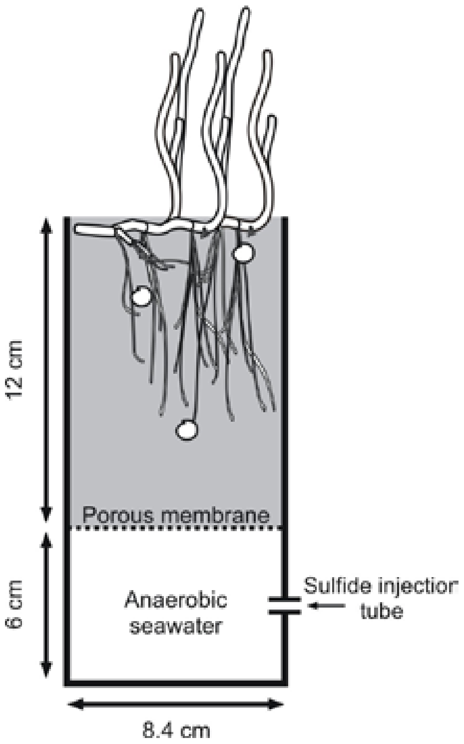


Figure S7.3 Schematic drawing of the setup of an experimental unit. The dimensions of the top section were chosen to fit the organisms and to resemble field conditions. The lower section was kept large enough to allow rapid mixing and upward diffusion. Sulfide was injected twice a week in the sulfide addition treatments and allowed to diffuse from the lower compartment into the upper section through a 0.1 mm porous membrane.

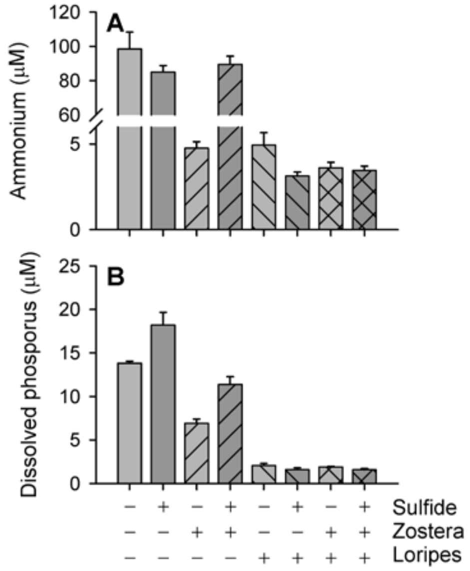


Figure S7.4 Pore water ammonium and dissolved phosphorus contents after five weeks; error bars represent SEM (n=5). Ammonium (a) was lowered significantly by *Zostera* (ANOVA: $F_{1,32}=59.7$, $P<0.001$) and *Loripes* ($F_{1,32}=505.9$, $P<0.001$), while sulfide addition caused an increase ($F_{1,32}=35.2$, $P<0.001$). We found significant interactions between all treatments (Z*L: $F_{1,32}=57.1$, $P<0.001$; Z*S: $F_{1,32}=73.3$, $P<0.001$; L*S: $F_{1,32}=39.3$, $P<0.001$; Z*L*S: $F_{1,32}=68.5$, $P<0.001$). The treatment effects on dissolved phosphorus (b) were similar to ammonium, with significant effects of *Zostera* ($F_{1,32}=58.2$, $P<0.001$), *Loripes* ($F_{1,32}=562.1$, $P<0.001$) and sulfide addition ($F_{1,32}=19.6$, $P<0.001$). We found significant interactions of *Zostera* and *Loripes* ($F_{1,32}=55.1$, $P<0.001$), and *Loripes* and sulfide addition ($F_{1,32}=28.2$, $P<0.001$).

SUPPORTING TABLES

Table S7.1 Lucinid bivalve densities found in seagrass beds. These data provide a basic indication of the association between seagrasses and lucinids worldwide.
Temp. depicts the mean annual temperature range based on the sea surface temperature (°C);
Clim. indicates type of climate (tropical, subtropical or temperate);
Lucinid density (spatial average): +=1-10; ++=11-100; +++=101-1000; ++++=>1000 ind/m^2
p=present (no abundance data); u=uncertain; 0=absent.

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
North America					
Alaska (Jewett et al. 1999, Dean and Jewett 2001)	5 – 13	Temp.	*Zostera*	Lucinidae	p
Boston Harbor (Leschen et al. 2009)	3 – 18	Temp.	*Zostera*		0
Chesapeake Bay (Orth 1973)	1 – 23	Temp.	*Zostera*		0
Apalachee Bay, Florida (Lewis and Stoner 1981)	18 – 29	Subtr.	*Syringodium, Thalassia*	*Codakia*	+
Biscayne Bay, Florida (Moore et al. 1968)	24 – 30	Subtr.	*Halodule, Syringodium, Thalassia*	*Anodontia, Codakia, Lucina*	++/+++
Florida Bay, Florida (Reynolds et al. 2007)	24 – 30	Subtr.	*Halodule, Syringodium, Thalassia*	*Anodontia, Codakia, Lucinesca*	++/+++
Indian River lag., Florida (Mikkelsen et al. 1995)	23 – 29	Subtr.	*Thalassia*	*Lucina*	p
St. Joseph's Bay, Florida (Fisher and Hand 1984)	18 – 29	Subtr.	*Thalassia*	*Lucina*	++/+++
Pensacola Bay, Florida (Stoner et al. 1983)	18 – 29	Subtr.	*Halodule*		0
Redfish Bay, Texas (Center for Coastal Studies 1996)	19 – 29	Subtr.	*Halodule, Thalassia*	*Anodontia, Lucina, Phacoides*	p
Gulf of California, Mexico (Torra Cosio and Bourillón 2000)	19 – 30	Subtr.	*Zostera, Halodule, Ruppia*	*Codakia, Divalinga*	p
Bahia de Chetumal, Mexico (Quesada et al. 2004)	27 – 29	Trop.	*Syringodium, Thalassia*	*Codakia, Lucina*	p
Turneffe Islands, Belize, Mexico (Hauser et al. 2007)	27 – 29	Trop.	*Thalassia*	*Codakia, Parvilucina*	p
Bocas del Toro, Panama (Continental Shelf Associates 1995)	27 – 29	Trop.	*Halodule, Syringodium, Thalassia*	*Codakia, Diplodonta Lucina, Phacoides*	p
Bahama's (Brissac 2009)	24 – 29	Trop.	*Thalassia*	*Codakia*	p
Jamaica (Jackson 1972, Greenway 1995)	27 – 29	Trop.	*Thalassia*	*Anodontia, Codakia, Ctena, Divaricella, Lucina, Parvilucina*	+++/++++

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
St Croix, Virgin Islands (Ferguson and Miller 2007)	26 – 29	Trop.	*Halodule, Syringodium, Thalassia*	*Codakia, Divalinga, Lucina, Parvilucina*	p
Guadeloupe (Gros et al. 2003)	26 – 29	Trop.	*Thalassia*	*Anodontia, Codakia*	p
Martinique (Brissac 2009)	26 – 29	Trop.	*Thalassia*	*Lucina*	p
Bermuda (Aurelia 1969, Schweimanns and Felbeck 1985)	19 – 28	Subtr.	*Thalassia*	*Codakia, Ctena*	++/+++
South America					
Bahia de Neguange, Columbia (Diaz 2003)	26 – 29	Trop.	*Thalassia, Syringodium*	*Codakia, Lucina, Anodontia*	p
Santiago de Tolú, Columbia (Otero Otero and Romani Lobo 2009)	27 – 29	Trop.	*Thalassia*	*Lucina*	p
Morrocoy, Venezuela (Bitter-Soto 1999)	26 – 28	Trop.	*Thalassia*	*Codakia*	+
Mochima Bay, Venezuela (Díaz and Liñero-Arana 2004)	25 – 28	Trop.	*Thalassia*	*Codakia*	+++
Parracho de Maracajaú, Brazil (Martinez 2008)	26 – 28	Trop.	*Halophila, Halodule*	*Codakia, Divaricella*	p
Abrolhos Bank, Bahia Brazil (Dutra et al. 2005)	25 – 28	Trop.	*Halophila, Halodule*	*Codakia, Ctena, Parvilucina*	p
Ilha do Japonês, Brazil (Marques and Creed 2000, Creed and Kinupp 2011)	23 – 27	Trop.	*Halodule*	*Codakia, Divaricella*	++++
Ilha do Mel, Paranaguá, Brazil (Couto and Savian 1998)	18 – 26	Trop.	*Halodule*	*Lucina*	p
Europe					
Western Atlantic, Norway (Fredriksen et al. 2010)	6 – 13	Temp.	*Zostera*		0
Skagerrak, Atlantic, Norway (Fredriksen et al. 2010)	4 – 17	Temp.	*Zostera*		0
Baltic, Finland (Bostrom and Bonsdorff 1997)	1 – 16	Temp.	*Zostera*		0
Sylt, Wadden Sea (Reise 1985)	4 – 18	Temp.	*Zostera*		0
South England (Dando et al. 1986)	8 – 17	Temp.	*Zostera*	*Lucinoma*	+
South Ireland (Dale et al. 2007)	9 – 17	Temp.	*Zostera*	*Lucinoma*	+++
Brittany, France (Monnat 1970, Hily and Bouteille 1999)	10 – 17	Temp.	*Zostera*	*Loripes, Lucinoma, Lucinella*	+++/++++
Arcachon, France (Blanchet et al. 2004)	12 – 21	Temp.	*Zostera*	*Loripes*	++
Eo estuary, Atlantic coast, Spain (de Paz et al. 2008)	13 – 19	Temp.	*Zostera*	*Loripes*	++/+++
Mediterranean, Spain (Rueda and Salas 2008)	15 – 23	Subtr.	*Zostera*	*Lucinella*	+++
Mallorca, Spain (Centeno 2008)	14 – 25	Subtr.	*Posidonia*	*Ctena, Loripes, Lucinella*	p
Corsica, France (Johnson et al. 2002)	13 – 24	Subtr.	*Cymodocea*	*Loripes*	+++/++++
Prelo Bay, Ligurian Sea (Harriague et al. 2006)	13 – 23	Subtr.	*Posidonia*	*Lucinella*	++/+++
Venice lag., Italy (Pranovi et al. 2000, Sfriso et al. 2001)	10 – 26	Subtr.	*Cymodocea, Zostera*	*Loripes*	+++/++++
Izmir Bay, Turkey (Cinar et al. 1998)	15 – 23	Subtr.	*Zostera*	*Loripes*	++
Cyprus (Argyrou et al. 1999)	17 – 28	Subtr.	*Posidonia*	*Loripes, Myrtea*	+
Black Sea, Romania (Nicolaev and Zaharia 2011)	6 – 24	Temp.	*Zostera*	*Loripes, Lucinella*	p
Africa					
Banc d'Arguin, Mauritania (van der Geest et al. 2011)	18 – 26	Subtr.	*Cymodocea, Halodule, Zostera*	*Loripes*	+++/++++
Baia da Corimba, Angola (Van-Dunem do Sacramento Neto dos Santos 2007)	22 – 29	Trop.	*Halodule*	*Loripes*	p
Kismayo, Somalia (Chelazzi and Vannini 1980)	25- 29	Trop.	*Halodule, Thalassia*	*Codakia, Lucina*	p
Zanzibar, Tanzania (Eklof et al. 2005)	25 – 29	Trop.	*Cymodocea, Thalassia, Enhalus, Thalassodendron*	Lucinidae	++/++++
Mahé, Seychelles (Taylor and Lewis 1970)	26 – 30	Trop.	*Thalassia*	*Anodontia, Codakia, Ctena,*	++
Inhaca, Mozambique (de Boer and Prins 2002)	23 – 27	Trop.	*Cymodocea, Halodule, Zostera*	*Anodontia, Cardiolucina, Loripes, Lucina, Pillucina*	++
Langebaan lag., South-Africa (Siebert and Branch 2005)	15 – 19	Subtr.	*Zostera*		0
Swartvlei estuary, South-Africa (Whitfield 1989)	17– 22	Subtr.	*Zostera*	*Loripes*	p

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
Asia/Pacific					
Jordan, Red Sea (Taylor et al. 2005)	21 – 28	Subtr.	*Halodule, Halophila*	*Rasta*	p
Egypt, Red Sea (Zuschin and Hohenegger 1998)	22 – 29	Subtr.	*Cymodocea, Halodule, Halophila*	*Cardiolucina, Divaricella, Pillucina, Wallucina*	++++
United Arab Emirates (Feulner and Hornby 2006)	21 – 33	Subtr.	*Halodule, Halophila*	*Anodontia, Pillucina*	++++
Oman (this study)	25 – 28	Trop.	*Halodule, Halophila*	*Pillucina*	++++
Palk Bay, India (Gophinadha-Pillai and Appukuttan 1980)	27 – 30	Trop.	*Cymodocea, Halodule, Syringodium, Thalassodendron*	*Codakia, Lucina*	p
Posyet Bay, Sea of Japan (Kharlamenko et al. 2001)	2 – 21	Temp.	*Zostera*	*Pillucina*	+++
Tokyo, Bay of Japan (Whanpetch 2011)	16 – 26	Subtr.	*Zostera*	*Luncinidae*	p
Okinawa, Japan (Yamaguchi 1999)	22 – 29	Subtr.	*Cymodocea, Enhalus, Halodule, Halophila, Thalassia*	*Codakia, Epicodakia*	p
Guangxi, China (Huang 2008)	20 – 29	Trop.	*Halodule, Halophila, Zostera*		0
Guangdong, China (Huang 2008)	21 – 29	Trop.	*Halodule, Halophila*	*Pillucina*	p
Hainan, China (Huang 2008)	22 – 29	Trop.	*Cymodocea, Enhalus, Halodule, Thalassia*	*Pillucina*	p
Tubbataha Reefs, Philipines (Yamaguchi 1999)	27 – 30	Trop.	*Halodule, Halophila, Thalassia*	*Epicodakia*	p
Kungkrabaen Bay, Thailand (Meyer et al. 2008)	28 – 30	Trop.	*Halodule*	*Anodontia, Indoaustriella, Pillucina*	++++
Had Chao Mai, Thailand (Nakaoka et al. 2002)	28 – 30	Trop.	*Cymodocea, Enhalus, Halodule, Halophila, Thalassia*	*Pillucina*	++++
Pulau Semakau, Singapore (Tan and Yeo 2010)	28 – 29	Trop.	*Cymodocea, Enhalus, Halodule, Halophila, Syringodium, Thalassia*	*Anodontia*	p
Bone Batang, Indonesia (Vonk et al. 2008)	28 – 30	Trop.	*Cymodocea, Enhalus, Halodule, Halophila, Thalassia,*	Lucinidae	+++
Banten Bay, Indonesia (Kuriandewa 2008)	28 – 30	Trop.	*Cymodocea, Enhalus, Halodule, Halophila, Syringodium, Thalassia*	*Anodontia, Codakia*	p
Tongapatu, Tonga (Yamaguchi 1999)	23 – 27	Trop.	*Halodule*	*Codakia, Epicodakia*	p
Tarawa Atoll (Paulay 2000)	28 – 29	Trop.	*Thalassia*	*Codakia, Wallucina*	++/+++
Oceania					
Roebuck Bay, Australia (Piersma et al. 2006)	25 – 30	Trop.	*Halodule, Halophila*	*Anodontia, Ctena, Divaricella*	+++
Lizard Island, Australia (Taylor and Glover 2008)	25 – 29	Trop.	*Halophila*	*Anodontia, Chaviana, Wallucina*	p
Moreton Bay, Australia (Taylor and Glover 2008)	21 – 26	Subtr.	*Cymodocea, Halodule, Halophila, Zostera*	*Anodontia, Pillucina*	p
Rottnest Island, Australia (Barnes and Hickman 1999)	19 – 23	Subtr.	*Posidonia*	*Wallucina*	+++/++++
South-West Australia (Hutchings et al. 1991)	16 – 20	Subtr.	*Amphibolis, Posidonia,*	*Anodontia*	p
New South-Wales, Australia (Gibbs et al. 1984)	19 – 24	Subtr.	*Halophila*	*Wallucina*	p
New South-Wales, Australia (McKinnon et al. 2009)	17 – 23	Subtr.	*Halophila, Zostera*		0
Western Port, Victoria, Australia (Watson et al. 1984, Edgar et al. 1994)	13 – 18	Temp.	*Halophila, Zostera*		0
Tasmania (Edgar et al. 1999a, Edgar et al. 1999b)	12 – 16	Temp.	*Heterozostera. Ruppia, Zostera*	*Wallucina*	++/+++
New Caledonia (Glover and Taylor 2007)	24 – 28	Subtr.	*Cymodocea, Halodule, Thalassia*	*Anodontia, Codakia, Ctena*	p
Slipper Island, New Zealand (Schwarz et al. 2006)	15 – 21	Subtr.	*Zostera*	*Divaricella*	p

Table S7.2 Overview of the statistical output from the analyses of the data presented in Figures 7.2, 7.3, and S7.4.

Treatment	df	F	P
Sulfide measurements (Figure 7.2a; repeated measures ANOVA)			
Zostera	1	6.8	0.014
Loripes	1	268.8	<0.001
Sulfide	1	109.7	<0.001
Zostera * *Loripes*	1	7.8	0.009
Zostera * Sulfide	1	2.2	0.150
Loripes * Sulfide	1	102.7	<0.001
Zostera * *Loripes* * Sulfide	1	2.4	0.127
Error	32		
Oxygen measurements (Figure 7.2b; ANOVA)			
Zostera	1	39.3	<0.001
Loripes	1	125.0	<0.001
Sulfide	1	8.9	0.006
Zostera * *Loripes*	1	48.3	<0.001
Zostera * Sulfide	1	0.0	0.862
Loripes * Sulfide	1	0.3	0.578
Zostera * *Loripes* * Sulfide	1	0.5	0.505
Error	32		
***Zostera* shoot biomass (Figure 7.3a; ANOVA)**			
Loripes	1	61.3	<0.001
Sulfide	1	72.6	<0.001
Loripes * Sulfide	1	0.9	0.348
Error	16		
***Zostera* root biomass (Figure 7.3b; ANOVA)**			
Loripes	1	50.2	<0.001
Sulfide	1	12.0	0.003
Loripes * Sulfide	1	1.7	0.211
Error	16		
***Loripes* fitness (Figure 7.3c; ANOVA)**			
Zostera	1	9.0	0.008
Sulfide	1	37.3	<0.001
Zostera * Sulfide	1	5.4	0.034
Error	16		
Ammonium (Figure S7.4a; ANOVA)			
Zostera	1	59.7	<0.001
Loripes	1	505.9	<0.001
Sulfide	1	35.2	<0.001
Zostera * *Loripes*	1	57.1	<0.001
Zostera * Sulfide	1	73.3	<0.001
Loripes * Sulfide	1	39.3	<0.001
Zostera * *Loripes* * Sulfide	1	68.5	<0.001
Error	32		
Phosphorus (Figure S7.4b; ANOVA)			
Zostera	1	58.2	<0.001
Loripes	1	562.1	<0.001
Sulfide	1	19.6	<0.001
Zostera * *Loripes*	1	55.1	<0.001
Zostera * Sulfide	1	0.0	0.888
Loripes * Sulfide	1	28.2	0.000
Zostera * *Loripes* * Sulfide	1	0.0	0.965
Error	32		

Chapter 8

OECOLOGIA 175: 677-685

SEAGRASSES ARE NEGATIVELY AFFECTED BY ORGANIC MATTER LOADING AND ARENICOLA MARINA ACTIVITY IN A LABORATORY EXPERIMENT

Laura L Govers, Timon Pieck, Tjeerd J Bouma, Wouter Suykerbuyk, Alfons JP Smolders, Marieke M van Katwijk (2014)

Abstract

When two ecosystem engineers share the same natural environment, the outcome of their interaction will be unclear if they have contrasting habitat-modifying effects (e.g. sediment stabilization vs. sediment destabilization). The outcome of the interaction may depend on local environmental conditions such as season or sediment type, which may affect the extent and type of habitat modification by the ecosystem engineers involved. We mechanistically studied the interaction between the sediment-stabilizing seagrass Zostera noltii and the bioturbating and sediment-destabilizing lugworm Arenicola marina, which sometimes co-occur for prolonged periods. We investigated (1) if the negative sediment destabilization effect of A. marina on Z. noltii might be counteracted by positive biogeochemical effects of bioirrigation (burrow flushing) by A. marina in sulfide-rich sediments, and (2) if previously observed nutrient release by A. marina bioirrigation could affect seagrasses. We tested the individual and combined effects of A. marina presence and high porewater sulfide concentrations (induced by organic matter addition) on seagrass biomass in a full-factorial lab experiment. Contrary to our expectations, we did not find an effect of A. marina on porewater sulfide concentrations. A. marina activities affected the seagrass physically as well as by pumping nutrients, mainly NH_4 *and* PO_4*, from the porewater to the surface water, which promoted epiphyte growth on seagrass leaves in our experimental set-up. We conclude that A. marina bioirrigation did not alleviate sulfide stress to seagrasses. Instead, we found synergistic negative effects of the presence of A. marina and high sediment sulfide levels on seagrass biomass.*

INTRODUCTION

Ecosystem engineers are “organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In doing so, they modify, maintain and create habitats” (Jones et al. 1994). The intertidal zone is a dynamic area, which is subject to many stressors such as waves and currents when submerged, but also to desiccation stress when exposed. A wide range of ecosystem engineers inhabit the dynamic intertidal flats (Passarelli et al. 2013), which may cause different and sometimes opposing habitat modifications (Bouma et al. 2009). In general terms, benthic engineering species living on the intertidal flats can be divided into epibenthic engineers, which live on top of the sediment, and endobenthic engineers, which mainly live within the sediment. Epibenthic ecosystem engineers, such as seagrasses and oyster reefs, typically modify the sedimentary habitat mainly by affecting hydrodynamics and sediment dynamics with their physical structures, which protrude into the water column (Bouma et al. 2005, Bos et al. 2007, Koch et al. 2009). In contrast, endobenthic engineers, such as several polychaete species, typically modify the sedimentary habitat by bioturbation (sediment reworking) and bioirrigation (burrow flushing) (Cadée 2001, Reise 2002, Meysman et al. 2006).

In recent decades, there has been a growing interest in the way in which ecosystem engineers can benefit other species (Bertness and Leonard 1997, Bruno et al. 2003), partly because such interactions can be highly relevant for coastal restoration projects (Crain and Bertness 2006, Van Katwijk et al. 2009). More recently, researchers have emphasized the importance of negative engineering interactions from an ecological and restoration perspective (Van Wesenbeeck et al. 2007, Suykerbuyk et al. 2012). This raises the questions how ecosystem engineers can interact, whether the engineering might have positive and negative effects at the same time, and how the outcomes depend on environmental conditions. In this study, we addressed this issue using sediment-stabilizing seagrasses (*Zostera noltii*) and bioturbating and bioirrigating lugworms (*Arenicola marina*) as model organisms.

Seagrasses are epibenthic ecosystem engineers that can improve their own growing conditions by stabilizing the sediment (Fonseca 1989, Bos and van Katwijk 2007, Christianen et al. 2013) and by attenuating waves and currents (Fonseca and Cahalan 1992, Peterson et al. 2004, Peralta et al. 2008), resulting in the accumulation of small sediment particles and suspended organic matter (Granata et al. 2001, Van der Heide et al. 2011). These ecosystem characteristics have also been identified for our model species *Zostera noltii* (Bouma et al. 2005, Brun et al. 2009). On the other hand, the entrapment of suspended organic matter and the production of organic matter by seagrasses often results in high sulfate reduction rates in the sediment, leading to the production of sulfides (Jørgensen 1982), which are toxic to the seagrasses (Borum et al. 2005, Calleja et al. 2007, Mascaró et al. 2009, Lamers et al. 2013). However, Van der Heide et al. (2012) showed that a common symbiosis between lucinid bivalves, their sulfide-oxidizing gill-symbionts and seagrass greatly reduces the sulfide stress to the seagrasses. Although this symbiosis is less prevalent in temperate systems (Van der Heide et al. 2012), it is conceivable that other benthic organisms than lucinids might play a role in alleviating sulfide stress to seagrasses in temperate areas.

The sediment-stabilizing seagrass beds are inhabited by numerous species of bioturbating animals, which rework and ventilate the sediment. The lugworm *Arenicola marina* is such a bioturbator, which is widely distributed in the North Atlantic (Cadée 1976, Flach and Beukeman 1994), and creates burrows and clearly visible casts that can reach a height of over 5 cm (pers. observations). These bioturbating animals physically destabilize the sediment by their sediment-reworking activities (Cadée 1976, Valdemarsen et al. 2011). In addition, they strongly affect local biogeochemistry (i) by modifying sediment texture, (ii) by dispersing solid particles, and (iii) by bioirrigation, which is the enhanced exchange of solutes between the porewater and the overlying water column (Banta et al. 1999, Meysman et al. 2006, Volkenborn and Reise 2006, Volkenborn et al. 2007, Wendelboe et al. 2013). Bioirrigation by *A. marina* has been observed to result in the release of ammonium from sandflats inhabited by *A. marina* to the water column in an open field-flume system (Asmus and Asmus 1998, Asmus et al. 1998) and *in situ* (Papaspyrou et al. 2007). Additionally, an increase in porewater nutrients has been observed in field experiments where *A. marina* was excluded (Volkenborn and Reise 2006, Volkenborn et al. 2007).

Interestingly, contrasting interactions have been observed between *Z. noltii* and *A. marina*. In some areas, the physical habitat modification by *A. marina* has been shown to hamper the growth of *Z. noltii* (Cadée 1976, Philippart 1994, Suykerbuyk et al. 2012). The activities of this worm can lead to seagrass plants being buried, which may completely suppress seagrass settlement in certain areas, and *Z. noltii* has been found to retreat to areas with compact sediments or natural shell layers, which are unsuitable for *A. marina* (Rijken 1979, Philippart 1994, Reise 2002). In other areas, however, dense seagrass meadows can sometimes reduce *A. marina* bioturbation as their thick rhizome mat restricts funnel formation (Philippart 1994, Valentine et al. 1994), and by shading the sediment, which hampers the growth of epiphytobenthos, an important food source for *A. marina* (Rijken 1979). In some locations, *Zostera* meadows co-occur with *A. marina* (Jacobs et al. (1983), pers. observations). Several authors (Philippart 1994, Suykerbuyk et al. 2012) suggested that seagrass coverage may even be positively correlated with the density of juvenile *A. marina*, which do not cause such unfavorable sediment-destabilizing effects as adults, and may benefit the seagrass by aerating the sediment and increasing nutrient availability.

Ecosystem engineering by *A. marina* might thus potentially have positive as well as negative effects on the seagrass *Z. noltii*, and it is still unclear how the effect may depend on the environmental conditions. The physical effects of bioturbation on seagrasses have been relatively well studied (Philippart 1994, Reise and Kohlus 2007, Suykerbuyk et al. 2012), but its biogeochemical effects on seagrasses still remain to be elucidated. Hence, we investigated how the combination of altered biogeochemistry and physical disturbance by *A. marina* bioturbation could affect the seagrass *Z. noltii* in sediments with contrasting organic matter contents. We hypothesized that *A. marina* could have positive effects on *Z. noltii* growth by alleviating sulfide toxicity in organically enriched sediments through sediment aeration and pumping to the overlying water column. However, the same flushing could lead to an increased release of nutrients to the water column, which might potentially cause toxic effects (NH_4) or epiphyte blooms (NH_4 and PO_4).

MATERIALS AND METHODS

Experimental design

To test the individual and interacting effects of *A. marina* bioturbation & bioirrigation and sulfide toxicity (organic matter addition) on *Z. noltii* biomass, we designed a full factorial experiment, in which aquaria were provided with organic matter (OM), *A. marina* (Ar), seagrass (Sg), or a combination of these treatments, resulting in a total of 8 different treatments: Control, OM, Ar, Sg, OMAr, OMSg, SgAr and OMSgAr. For the OM treatment, 2 g L^{-1} organic matter (1 g starch + 1 g cellulose L^{-1} sediment) was mixed into the sediment to stimulate sulfide production (Peralta et al. 2003, Govers et al. 2014), before the start of the experiment. We used 5 replicates per treatment, resulting in 40 experimental units, which were randomly placed in a water bath. During the experiment, the synthetic seawater was completely refreshed twice a week.

The experiment was conducted in 24 L glass aquaria (l x w x h=20x20x60 cm), which were filled with a 30 cm sediment layer (12 L) and a 25-30 cm water layer, which was prepared from deionized water and Tropic Marin© synthetic sea salt; salinity levels were comparable to levels measured in the field (27-29 psu). An aquarium pump and aquarium bubbler in each aquarium aerated the surface water. Aquaria were placed in a large water bath at 20°C. Light intensity was set at 300 µmol m^{-2} s^{-1}, with a day-night cycle of 14-10 hours, which is comparable to light conditions in the field (Isaksen and Finster 1996). The experiment lasted for 28 days and all treatments were run simultaneously.

Origins of sediment and biological materials

Sediment, seagrass (*Z. noltii*) and lugworms (*A. marina*) were obtained from the mudflats of the Oosterschelde area (51°39’ N, 4°01’ E), The Netherlands. Sandy sediment, with a median grain size (D50) of 180 µm and 0.6% organic matter, was collected on a single day at the exposed Dortsman mudflats (Suykerbuyk et al. 2012), transported to the laboratory in Nijmegen (The Netherlands), sieved (5 mm) to remove coarse material and macrobenthos and subsequently mixed to homogenize the sediment. This meant that mud snails (*Hydrobia ulvae*), which were smaller than 5 mm, were present (289±25 m^{-2}) in the experiment (Figure 8.4), providing a source of epiphyte grazing. We found no treatment effect on Hydrobia spp. densities (P=0.288) and the observed densities were not very high compared to the field densities of >4000 m^{-2} (personal observations) and 20,000 m^{-2} (Grilo et al. 2012). *Z. noltii* was collected in the field at the start of the growing season, and was immediately planted in the prepared sediment in the lab, at densities of 50 shoots per aquarium (1250 m^{-2}), which represents the average density at the start of the growing season (Vermaat and Verhagen 1996). *A. marina* were obtained from a professional collector (‘t Zeepiertje, Yerseke). On the day of collection, adult *A. marina* with a length of 20-25 cm were put in the aquaria, at densities of two individuals per aquarium (50 m^{-2}), which is similar to high adult *A. marina* densities in the field (Suykerbuyk et al. 2012). The *A. marina* were retrieved alive after the termination of the experiment

Sample collection and analysis

During the experiment, sediment porewater samples were collected on seven occasions (t=0, 3, 7, 14, 19, 24, 28 days), using vacuumed flasks connected to soil moisture samplers (Rhizons, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). 10 mL of porewater was collected at each sampling occasion for sulfide analysis, and 20 mL was sampled at the end of the experiment for both sulfide and nutrient analysis. The rhizons were placed horizontally, in the central part of all aquaria, at a depth of 5 cm in the sediment, which is the average rooting depth of *Z. noltii*. Similar porewater samples were simultaneously taken at depths of 10 cm and 20 cm. These porewater samples were immediately used to measure sulfide concentrations in a mixture of 50% sample and 50% Sulfide Anti-Oxidation Buffer (SAOB) (Lamers et al. 1998) using a calibrated ion-specific silver-sulfide electrode. At the end of the experiment (t=28 days), we used the same method to collect porewater samples for nutrient concentration measurements (20 mL), which were frozen and later analyzed in the lab. Surface water samples were collected through rhizons completely submerged in the water layer, in order to filter the water samples prior to analysis. Porewater and surface water ammonium and ortho-phosphate concentrations were measured colorimetrically (Skalar and Seal autoanalyzer), using ammonium-molybdate and salicylate. Nitrate was determined by sulfanilamide, after reduction of nitrate to nitrite in a cadmium column (Wood et al. 1967). All nutrients were measured at the analytical lab of the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke, The Netherlands.

Seagrass was harvested at the end of the experiment (t=28 days), after which epiphytes were carefully removed and plants were divided into leaves, sheaths, rhizomes and roots. Total dry weight (g DW) was determined after drying the material for 48 hours at 60°C.

Statistical analysis

All results are summarized as means±SE. Prior to analysis, extreme outliers were omitted based on Dixons' Q-test (Dean and Dixon 1951), and normality of the data was tested with a Shapiro Wilk test and QQ-plots. Non-normally distributed data were log-transformed prior to analysis. We used a three-factor repeated measure ANOVA to compare the effects of the treatments on porewater sulfide concentrations. All other data were tested using a 3-way (nutrients) or 2-way (biomass) ANOVA. Relevant statistical results are presented in the figure legends and in the results section. Differences with $P<0.05$ were considered significant. All statistical tests were performed with IBM SPSS Statistics 20.0 and R 2.15.

RESULTS

We successfully induced sulfide production in the sediment by adding organic matter (OM), which enabled us to study the effects of *A. marina* on porewater sulfide concentrations (Figure 8.1a). We obtained similar results from the sediment sulfide measurements at depths of 10 cm and 20 cm, so these results are not

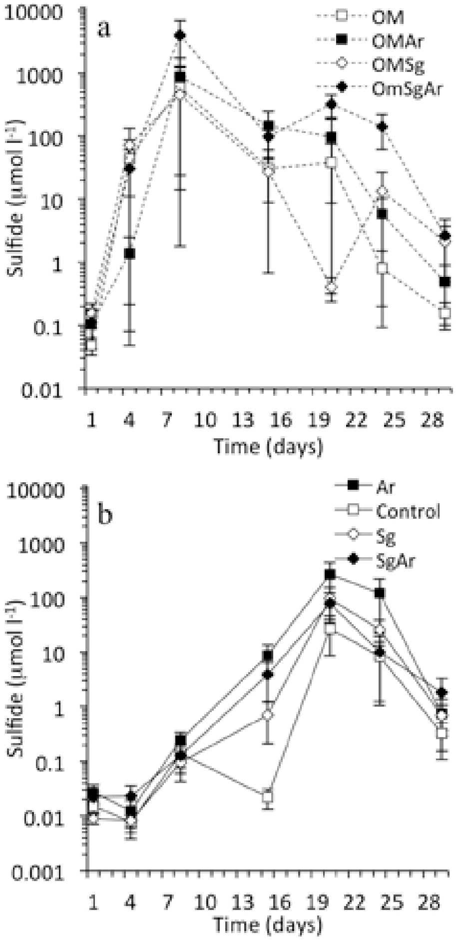


Figure 8.1 Porewater sulfide concentrations of treatments (a) with organic matter addition and (b) without organic matter addition. Added organic matter (OM, dashed lines) interacted with both *Arenicola marina* (Ar) and seagrass (*Zostera noltii*) (Sg) to increase sulfide concentrations in the porewater (OM x Ar P=0.009, OM x Sg P=0.049). A highly significant interaction between organic matter addition, *A. marina* and seagrass (OMSgAr) led to the highest porewater sulfide concentrations (P<0.001) (a). The presence of *A. marina* (Ar) did not significantly affect porewater sulfide concentrations (black symbols, P=0.075). Error bars represent SE (n=5). Note that the y-axis is displayed on a logarithmic scale.

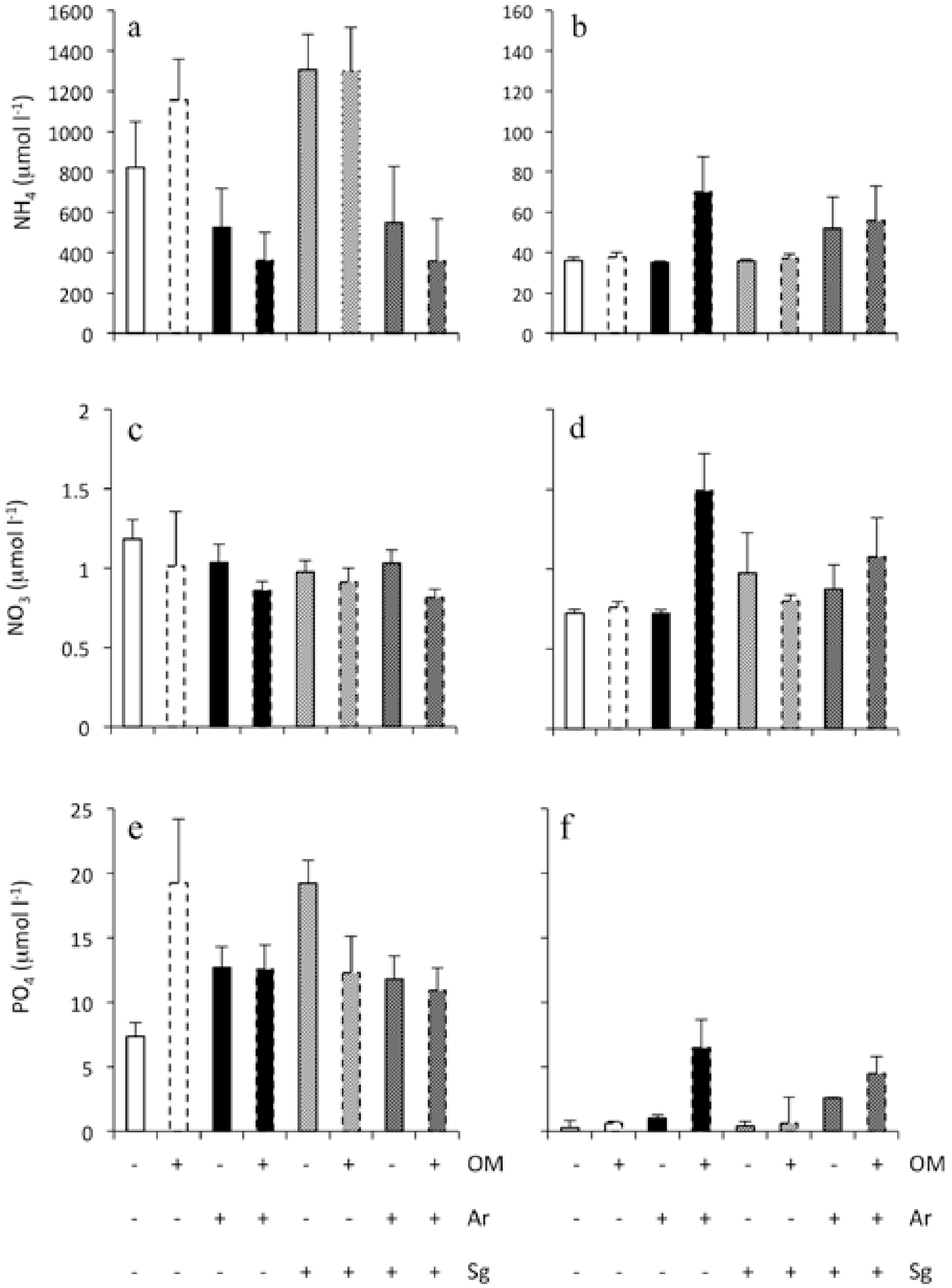


Figure 8.2 (a) Porewater ammonium (NH_4) concentrations were significantly (P<0.001) lowered by the presence of *A. marina* (Ar), while (b) surface water NH_4 concentrations increased significantly (P=0.032) as a result of bioturbation and bioirrigation by *A. marina*. The presence of seagrass (Sg) or the addition of organic matter (OM) affected neither porewater ammonium nor surface water ammonium concentrations. (c) Porewater nitrate (NO_3) concentrations were not affected by any of the treatments, whereas (d) surface water NO_3 was significantly elevated in the OMAr treatment (P=0.019). (e) Porewater phosphate (PO_4) concentrations were not affected by any of the treatments, but (f) surface water PO_4 concentrations were highly elevated in the Ar treatments (P<0.001). Error bars represent SE (n=5).

presented separately. We observed a sulfide peak in the porewater in the OM treatments on day 7, with sulfide levels of 450-800 µmol L^{-1} in the OM, OMAr and OMSg treatments, and >4000 µmol L^{-1} in the OMSgAr treatment. Sulfide levels in the OM treatments (Figure 8.1a) decreased after the observed sulfide peak to levels similar to those in the other treatments (10-100 µmol L^{-1}, Figure 8.1b).

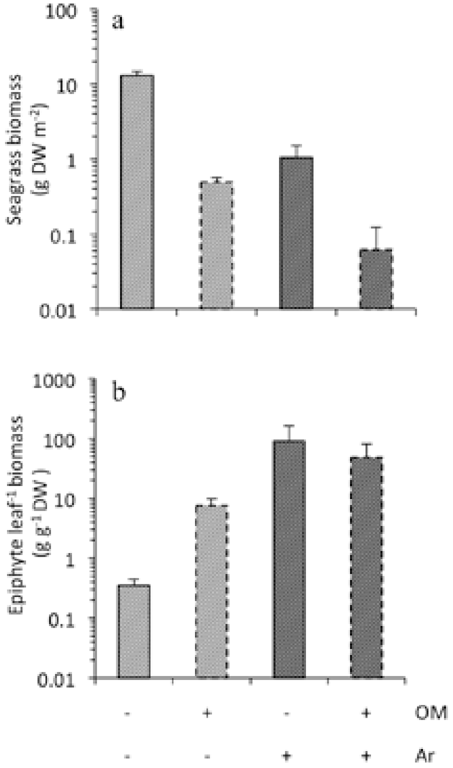


Figure 8.3 (a) Total seagrass biomass and (b) epiphyte biomass per leaf in the seagrass (Sg) treatments. *A. marina* activity (Ar) negatively affected seagrass biomass (P<0.001) and the addition of organic matter (OM) also had a significant negative effect on (a) seagrass biomass (P<0.001). There was also a significant negative interaction between OM and Ar (P<0.015). *A. marina* activity significantly increased the quantities of (b) epiphytes on seagrass leaves (P<0.001), and organic matter addition also significantly increased epiphyte biomass on the leaves (P<0.001). Error bars represent SE (n=5). Note that the y-axis is displayed on a logarithmic scale

On day 21, a small sulfide peak was observed (25-250 µmol L^{-1}) due to degradation of the organic matter (0.6%), which was naturally present in the sediment. Contrary to our hypothesis, *A. marina* had not decreased porewater sulfide concentrations at any monitoring moment during our experiment. Similarly, the presence of *Z. noltii* did not decrease porewater sulfide concentrations. This was also contrary to our expectations, as seagrasses are known to leak oxygen from their roots, which can decrease sulfate reduction rates in the sediment. After the strong initial increase, especially in the OM treatments, porewater sulfide concentrations showed a decrease, very probably due to diffusion or outgassing of (hydrogen) sulfides from the sediment to the water column and subsequently to the air. Porewater NH_4 concentrations were significantly lowered in the presence of *A. marina* (P<0.001, Figure 8.2a), whereas surface water NH_4 concentrations were significantly increased in the presence of *A. marina* (P<0.05, Figure 8.2b). Nevertheless, porewater NH_4 concentrations were still more than ten times higher than surface water concentrations. Porewater NO_3 (Figure 8.2c) and PO_4 (Figure 8.2e) levels were not affected by any of the treatments, but surface water NO_3 concentrations were significantly elevated through an interaction between organic matter and *A. marina* (P<0.05, Figure 8.2d), and the presence of *A. marina* significantly increased the surface water PO_4 (P<0.001, Figure 8.2f).

Total seagrass biomass m^{-2} was more than twelve times higher in the seagrass control treatment (Sg) than in the other treatments (Figure 8.3a). Both *A. marina* (Ar) and organic matter (OM) had strong negative effects on seagrass biomass (P<0.001 and P<0.001, respectively). The interaction of both stressors (OMAr) even led to a synergistic negative effect (P<0.001), as almost all of the seagrass died in this treatment, resulting in an extremely low (<0.1 g DW m^{-2}) seagrass biomass. Additionally, the seagrass leaves in the control treatments all had a healthy green color throughout the experiment, whereas the leaves in all other treatments gradually died off as a result of epiphyte overgrowth. As a consequence of the elevated surface water nutrient levels, epiphyte biomass per leaf (Figure 8.3b) increased strongly in the *A. marina* treatments (P<0.001), reaching values of up to 100 times more epiphyte biomass than leaf biomass. OM addition also led to significantly more epiphytes per leaf (P=0.036), but this was the result of the absolute decrease in leaf biomass rather than of the absolute increase in epiphyte biomass (as there were no significant effects of OM addition on absolute epiphyte biomass, results not shown). Epiphyte biomass was extremely high in the *A. marina* treatments, which meant that the leaves were completely overgrown in these treatments (Figure 8.4).

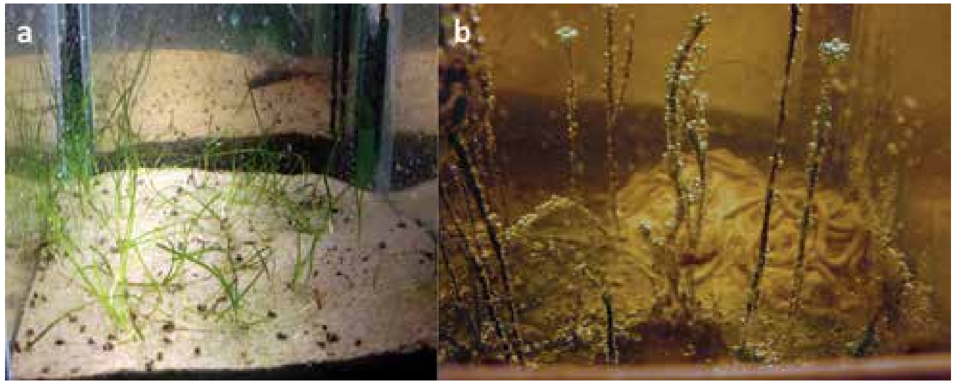


Figure 8.4 Photographs of (a) healthy *Z. noltii* in the seagrass (Sg) treatment and (b) epiphyte-overgrown *Z. noltii* in the *A. marina* (Ar) treatment

DISCUSSION

We falsified our hypothesis that oxygenation of the sediment by *A. marina* bioturbation and bioirrigation would potentially alleviate sulfide toxicity for seagrass in temperate systems. In our experiment, *A. marina* had no net beneficial effect on seagrass growth through reducing porewater sulfide concentrations. Secondly, we showed that the sediment-stabilizing seagrass and the sediment-reworking *A. marina* are not only physically contrasting ecosystem engineers (as is well known from the literature; e.g. Suykerbuyk et al. (2012)), but that they also display contrasting biogeochemical behavior. Whereas seagrasses are known to be important nutrient sinks (Romero et al. 2006), *A. marina* increased nutrient fluxes from the sediment to the surface water in our experiment, as was also observed in the field by Asmus and Asmus (1998) on bare mudflats. In our experiment, in the presence of seagrass, these fluxes promoted the growth of epiphytes on the seagrass leaves. In addition, the synergistic negative effects of organic matter addition (sulfide stress) and the presence of *A. marina* (physical and biogeochemical disturbance) led to the almost complete disappearance of seagrass biomass.

Sulfide biogeochemistry

Bioturbation is known to alter sediment biogeochemistry by increasing oxygen penetration into the sediment (Banta et al. 1999, Kristensen 2000, Timmermann et al. 2006), but also by increasing or changing the input of organic matter through sediment reworking (Hines and Jones 1985, Hansen et al. 1996, Kristensen 2000, Kristensen et al. 2012). Bioturbation is therefore expected to have two contrasting effects on sulfate reduction rates in the sediment; increased organic matter input by sediment reworking stimulates sulfate reduction (Hines and Jones 1985, Hansen et al. 1996, Holmer and Nielsen 1997, Valdemarsen et al. 2010), while on the other hand, sulfate reduction rates may also be reduced by the increased oxygen input that results from bioirrigation (Banta et al. 1999, Nielsen et al. 2003). Our nutrient measurements show that active bioirrigation by *A. marina* took place, as we found decreased porewater nutrient concentrations and increased surface water nutrient concentrations in the *A. marina* treatments. However, contrary to our expectations, *A. marina* did not decrease porewater sulfide concentrations. This indicates that *A. marina* may reduce sulfate reduction rates only locally, in a narrow zone of 5-15 mm surrounding the burrow (Nielsen et al. 2003). Additionally, the fact that we did not find decreasing sulfide concentrations in the *A. marina* treatments may be explained by *A. marina* respiration, which consumes a considerable proportion of the oxygen that is pumped into the burrow (Timmermann et al. 2006). Sediment sulfide concentrations became very high in the OMSgAr treatment (>4500 µmol L^{-1}), possibly because the combined effect of increased sulfide production and epiphyte growth resulted in seagrass roots dying off, which in turn stimulated sulfide production by providing easily degradable organic matter.

Nutrients, seagrass and epiphytes

As bioturbation is known to stimulate decomposition rates (Andersen and Kristensen 1992, Banta et al. 1999), one might expect an increase in the porewater nutrient concentrations (Meysman et al. 2006). However, in our experiment we found exactly the opposite in the *A. marina* treatments: porewater nutrient concentrations decreased while surface water nutrient concentrations increased. These results are in agreement with results found in open systems. Volkenborn et al. (2007) found that porewater NH_4 and o-PO_4 increased in plots where *A. marina* was excluded and Asmus and Asmus (1998) observed a release of ammonium from *A. marina* sandflats.

It is well known that the sediment-reworking activities of *A. marina* can hamper seagrass growth by burying or uprooting the plants (Cadée 1976, Philippart 1994, Valdemarsen et al. 2011, Suykerbuyk et al. 2012). However, our experiment included the biogeochemical effects of bioturbation, and showed that these could, in conjunction with physical disturbance, also negatively affect seagrass growth. We found a major decrease in seagrass biomass in all *A. marina* treatments, and even more so in the interactive OMSgAr treatment, which was probably due to the synergistic effects of sulfide toxicity and epiphyte bloom as a result of nutrient release. Soil sulfide toxicity may have triggered dieback of the root system, which in turn led to even higher sulfide production rates. Excessive epiphyte growth on seagrass leaves is known to reduce light intensity and decrease the uptake of carbon for photosynthesis, which may lead to reduced growth levels and eventually to seagrass leaves dying off (Sand-Jensen 1977, Tomasko and Lapointe 1991).

Several studies have also reported that *A. marina* bioturbation activities stimulate the growth of bacteria in the burrow system (Grossmann and Reichardt 1991, Ashforth et al. 2011), which is called ‘gardening’ (Hylleberg 1975). This gardening activity, which is an additional ecosystem engineering property of bioturbating organisms such as *A. marina*, might also extend to the epiphytobenthos, as *A. marina* might stimulate the growth of epibenthic algae by increasing the flow of nutrients to the surface, thus stimulating the growth of its own food source. We observed (but did not quantify) this in our *A. marina* treatments (Figure 8.4). This potential positive feedback in systems dominated by *A. marina* deserves further investigation.

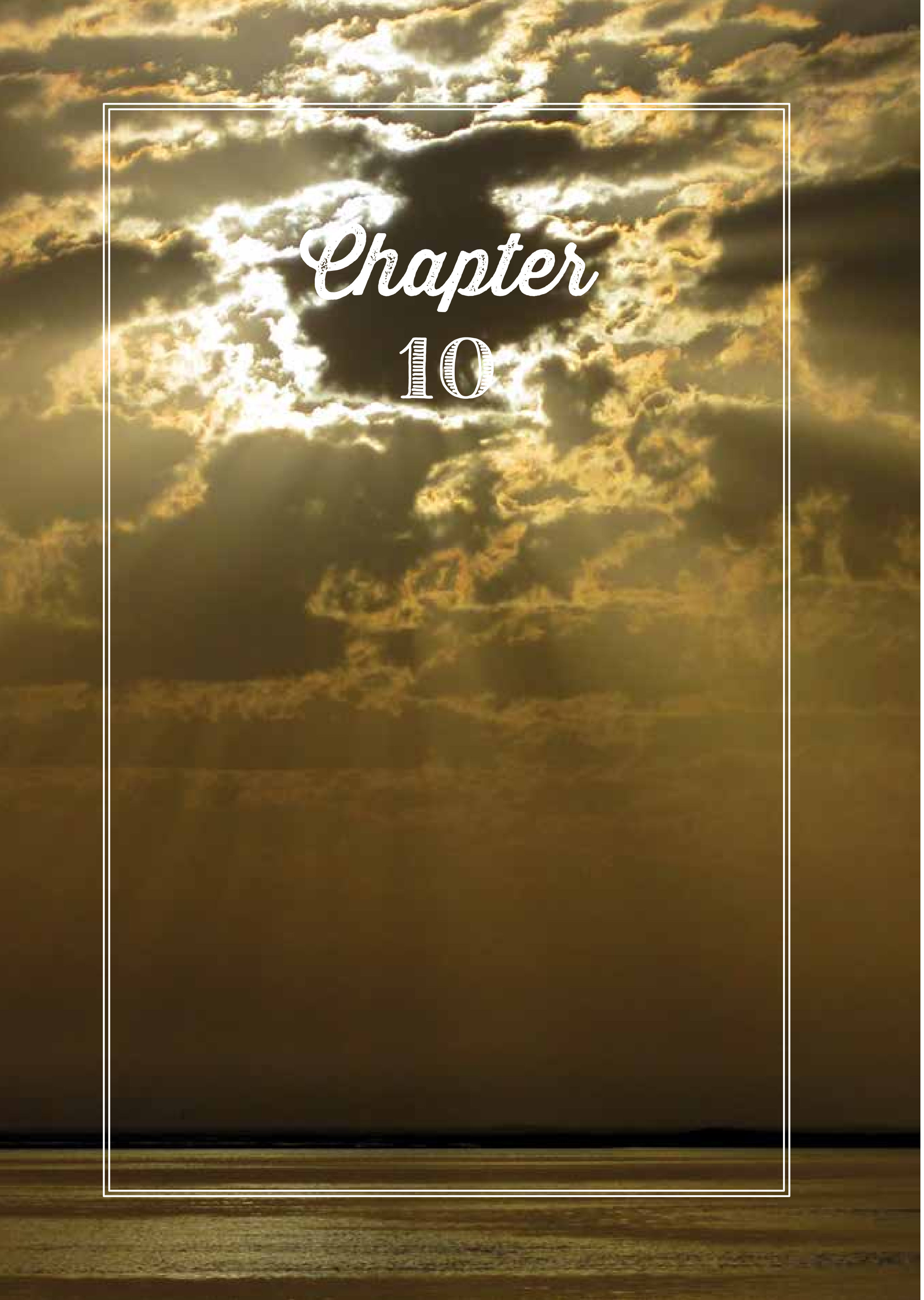
The experiment was conducted in aquaria with a refreshment rate of twice a week. Although this is much lower than the refreshment rate in natural systems, our findings suggest that increased surface water nutrient availability could severely affect seagrasses by promoting epiphyte growth in sheltered bays and estuaries with limited water movement. And even though nutrient loads and subsequent epiphyte loads may have been aggravated by our experimental set-up, we still expect similar effects, though less severe, to occur in field situations, as the nutrient efflux from the sediment passes through the canopy. In addition, in more exposed systems, with higher current velocities, *A. marina* can still increase the nutrient load of the system, as shown by Asmus et al. (1998) and Asmus and Asmus (1998), and nutrient release may even rise with increasing current velocities (Asmus et al. 1998), although these loads may not necessarily promote epiphyte blooms. Nonetheless, physical burial or smothering by *A. marina* may have more detrimental effects on seagrasses in the field than biogeochemical disturbance by bioturbation and bioirrigation. At the same time, however, increased epiphyte loading as a result of bioirrigation may render seagrasses more vulnerable to physical disturbance by sediment reworking. Hence, our study contributes to the unraveling of mechanisms that shape the complex interaction between seagrasses and bioturbating animals, which inhabit seagrass beds, rather than predicting the precise effect of bioirrigation by *A. marina* on seagrasses in a field situation.

CONCLUSIONS AND IMPLICATIONS

Contrary to our expectations, bioturbation and bioirrigation by *A. marina* did not alleviate sulfide stress to seagrasses in organic sediments in the present experiment. Instead, we found synergistic negative effects of stressors (i.e. *A. marina* activity and sulfide toxicity from organic loading, aggravated by *A. marina* activity). This knowledge adds to our understanding of the complex interaction between seagrasses and *A. marina*. Although the outcome of interactions in a particular field situation cannot be predicted from our findings, our study shows that (i) increasing *A. marina* numbers may represent an increased threat to seagrasses, and (ii) organic matter addition will further threaten seagrass beds. *A. marina* numbers in the Wadden Sea and the Dutch Delta have increased since the 1980s (Philippart 1994, Reise et al. 2008, Eriksson et al. 2010). This increase may have been caused by eutrophication, human exploitation, and/or climate change (Van Beusekom 2005, Reise et al. 2008). Our study shows that such increases in *A. marina* numbers, particularly in combination with organic matter loading, can seriously threaten seagrass beds. Therefore we expect mutual exclusion rather than co-occurrence of these two ecosystem engineers, even more so as each of the two, *A. marina* and seagrasses, displays self-facilitating positive feedbacks (Van Wesenbeeck et al. 2007, Van der Heide et al. 2011). The occasional co-occurrence of both species in the field is thus likely to be explained by disturbance or seasonal dynamics (Eklof et al. 2011), rather than being a long-term, stable outcome of biomechanical warfare or competition.

ACKNOWLEDGEMENTS

We are grateful to Niki de Lange for helping out during the experiment, to Germa Verheggen and Martin Versteeg for their technical support, to Jelle Eygensteyn for his help with the analyses, and to the people at the NIOZ Yerseke nutrient lab for their help with the nutrient analyses. We also thank three anonymous referees for their suggestions to improve the quality of our manuscript.



Chapter 10

SYNTHESIS

Seagrass beds are important and valuable coastal ecosystems that provide many ecosystem services (Orth et al. 2006). They trap nutrients, organic matter and sediment, thereby promoting local nutrient cycling (Marba et al. 2006), and are highly productive, which results in high carbon sequestration rates (Fourqurean et al. 2012). In addition, seagrass beds attenuate waves and currents (Fonseca and Cahalan 1992), and stabilize the sediment, thereby providing a form of coastal protection (Christianen et al. 2013); they also function as nursery areas for commercially important fish and shellfish species (Nagelkerken 2000), and promote high biodiversity by providing food and shelter for a large array of species (Heck and Valentine 2006, Larkum et al. 2006). Finally, seagrass beds are often connected to adjacent coastal ecosystems such as coral reefs, mangroves or saltmarshes, which promotes cross-habitat utilization of mobile species and the exchange of nutrients and carbon (Nagelkerken 2010).

Unfortunately, the valuable seagrass beds are threatened by increasing human activities in coastal areas (Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). Main threats to seagrasses are eutrophication, dredging, salinity changes, erosion of terrestrial habitats, resulting in increased sediment loads, industrial pollution, and altered food web composition as a result of overexploitation (Orth et al. 2006). Many of these stressors also interact, and multiple stressors may simultaneously affect seagrass ecosystem functioning, which complicates the assessment of seagrass health.

In this thesis, we focused on the effects of nutrients, high sulfide levels and trace metal pollution on seagrass dynamics, resilience and restoration, using intertidal *Zostera noltii* and subtidal *Thalassia testudinum* as model species. In addition, we highlighted the importance of species interactions for seagrass ecosystem functioning. This chapter integrates the most important findings of this thesis, describing the effects of biogeochemical stressors on seagrass dynamics and species interactions within seagrass beds.

Biogeochemical processes shape seagrass dynamics bottom-up

Seagrass dynamics, such as disturbance-recovery processes and patch dynamics, may be affected by top-down processes such as grazing (e.g. Christianen (2013)), but we here argue that biogeochemistry also plays an important role in shaping seagrass dynamics (Chapter 2, 4). Sulfide is a common toxicant to seagrasses, which is produced during anaerobic decomposition of organic matter by sulfate reducing bacteria (Jørgensen 1984). High porewater sulfide levels may not only disturb plant physiological processes, but also affect seagrass ecosystem functioning by causing large-scale diebacks (Carlson et al. 1994).

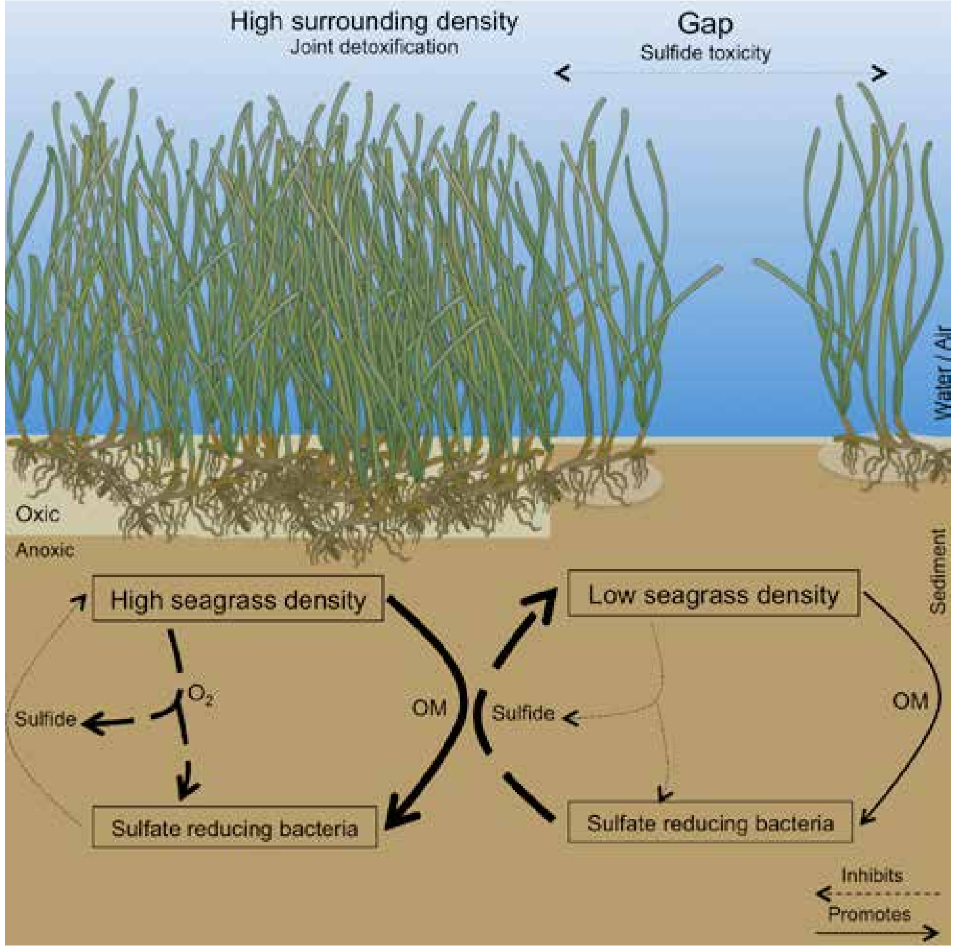


Figure 10.1 Graphic impression of joint detoxification of sulfide by high seagrass densities. High seagrass densities aerate the sediment by radial oxygen loss and thereby not only prevent the intrusion of toxic sulfides, but also oxidize sulfides present. This mechanism enables high seagrass growth in high densities despite high sediment sulfide production. In contrast, when a gap is created in a dense meadow, local joint-detoxification does not work anymore and sulfide levels in the gap may rise drastically compared to the surrounding bed, which complicates recovery (Chapter 4). Thickness of the arrows indicates the strength of the process.

We are the first to demonstrate empirically, that high sediment sulfide levels also affect medium-scale ecosystem processes such as patch dynamics and disturbance-recovery processes in seagrass beds. We found that seagrass patch survival and expansion are constrained by high porewater sulfide concentrations (Chapter 2). Especially patch expansion processes were affected, which hints at a higher vulnerability of low densities (expansion) to toxic substances than high densities (meadow), implying that toxicity may be a density-dependent process. We observed this density dependent toxicity for porewater ammonium in Chapter 2, and we are the first to show density-dependent toxicity of sulfide (Chapter 4). Density-dependent toxicity of sulfide, or ‘joint-detoxification’, is likely caused by high levels of radial oxygen loss in dense seagrass beds (Figure 10.1). Oxygenation of the rhizosphere can prevent intrusion of toxic sulfides, enabling seagrass growth even in high-sulfide sediments. We however showed that processes such as expansion and recovery, which are essential to seagrass meadow maintenance, are more vulnerable to sediment toxins (high ammonium and sulfide levels), due to a lack of the density-dependent feedbacks in bare sediments. This implies that seagrass beds suffering from high porewater sulfide and ammonium concentration, i.e. as a result of eutrophication, are less likely to expand or recover from disturbances, as bare zones may be biogeochemically impregnable areas. This may be especially problematic for patchy and heterogeneous seagrass beds, which are becoming more and more common due to increasing anthropogenic activities (Chapter 2). We thus illustrated that seagrass dynamics (patch expansion, gap recovery) may be shaped bottom-up due to the lack of toxin relieving density-dependent feedbacks (joint-detoxification) in bare areas.

Eutrophication threatens seagrasses: toxic effects & identification of sources

Nutrients are essential to seagrass growth and survival, but either a surplus or shortage of nutrients may constrain seagrass performance (Romero et al. 2006). Hence there is a precarious balance between nutrient excess and limitation. Excess nutrient availability is even one of the most serious threats to seagrasses worldwide (Burkholder et al. 2007). Elevated nutrient loads in the water layer can seriously affect seagrasses by either promoting direct toxicity (van Katwijk et al. 1997), or by decreasing light-availability by promoting plankton bloom in the water column. We demonstrated that next to toxic effects of high water column ammonium concentrations (Chapter 2), also high porewater ammonium concentrations might be toxic to seagrasses. Porewater ammonium toxicity may be important as nutrient levels may more easily increase in fine coastal sediments than in a constantly refreshed water column, due to limited exchange rates between pore- and surface water. We found that porewater ammonium concentrations only become toxic in very high concentrations (>2000 µmol L^{-1}) compared to surface water ammonium concentrations (<100 µmol L^{-1}), which may be explained by a difference in affinity for ammonium between leaves and roots. Moreover, high porewater ammonium concentrations as a result of high decomposition rates by sulfate reducing bacteria often occur simultaneously with high porewater sulfide levels. Co-occurrence of both toxins may increase plant stress and possibly lowers levels at which both stressors become toxic. It may thus be interesting to investigate the interaction between those two porewater toxins. Additionally, it would be interesting to compare the sensitivity of different seagrass species to high porewater ammonium levels, as has been done for surface water ammonium toxicity (Van der Heide et al. 2008, Christianen et al. 2011).

Extremely eutrophic seagrass beds are clearly recognizable as a result of seagrass die-offs due to limited light availability and direct (surface and porewater) toxic effects. However, to prevent seagrass loss caused by eutrophication, it is important to first identify imminent threats from high nutrient loads. A classical way to measure nutrient availability to seagrasses is by determining surface water and porewater nutrient concentrations (NH_4, NO_3, PO_4). However, we found that high nutrient loads are difficult to observe in the water column and in the porewater due to high exchange and flushing rates (Chapter 6), even in the immediacy of a sewage discharge pipe. Although poorly detectable, high nutrient pulses may still promote algal and epiphyte bloom (Figure 1.2), and yield subsequent negative effects to seagrasses. Fortunately, we found that high nutrient loads can be detected in the leaf tissue of late successional and thus slow-growing seagrass species such as *Thalassia* sp., a genus that can be found all over the tropics. *Thalassia* leaf nutrient concentrations (%N and %P) were elevated in the vicinity of the source of eutrophication (sewage discharge, coastal residencies). Using this information we could identify possible threats to the seagrass beds of Curaçao (Caribbean), which function as key nursery habitats for commercially important fish species.

Seagrasses as first-level indicators for eutrophication, trace metal pollution, and winter survival

We thus found that seagrasses are good bioindicators for nutrient pollution and may even be used to identify point sources of eutrophication. In addition to eutrophication, trace metal pollution may also threaten seagrass ecosystem functioning. Trace metal pollution in seagrass beds results from mining activities, sewage, agriculture and atmospheric deposition (Irvine and Birch 1998, Guzman and Garcia 2002). High trace metal levels may present a direct threat to seagrasses, by negatively affecting photosynthesis and metabolic rates (Ralph and Burchett 1998, Macfarlane and Burchett 2001), but trace metals also accumulate in leaves and rhizomes. We compiled a global benchmark for trace metal levels in seagrass leaves and conclude that seagrasses worldwide may be used as a first-level bioindicator for trace metal pollution in coastal ecosystems (Chapter 5). Seagrasses integrate ecological conditions over a longer timescale (Gonzalez-Correa et al. 2005, Madden et al. 2009) and trace metal levels in seagrass tissue therefore reflect long-term trace metal loading, which may not be measurable in the porewater or surface water (Chapter 5). More importantly, seagrasses are primary producers that provide stock food for a large variety of herbivores and are thus integrated in the coastal food web. Trace metal levels in seagrasses may therefore also be indicative of trace metal concentrations at higher trophic levels, which may be used to detect possible threats to ecosystem services such as fisheries. As most trace metals accumulate in the food web (Wang 2002), high trace metal levels in seagrass leaves may indicate serious trace metal pollution in the whole food web. We found such a relationship for lead (Pb) in the seagrass species *Thalassia testudinum* and the fish species Haemulon flavolineatum in the seagrass beds of Curaçao (Figure 10.2). Although this fish species does

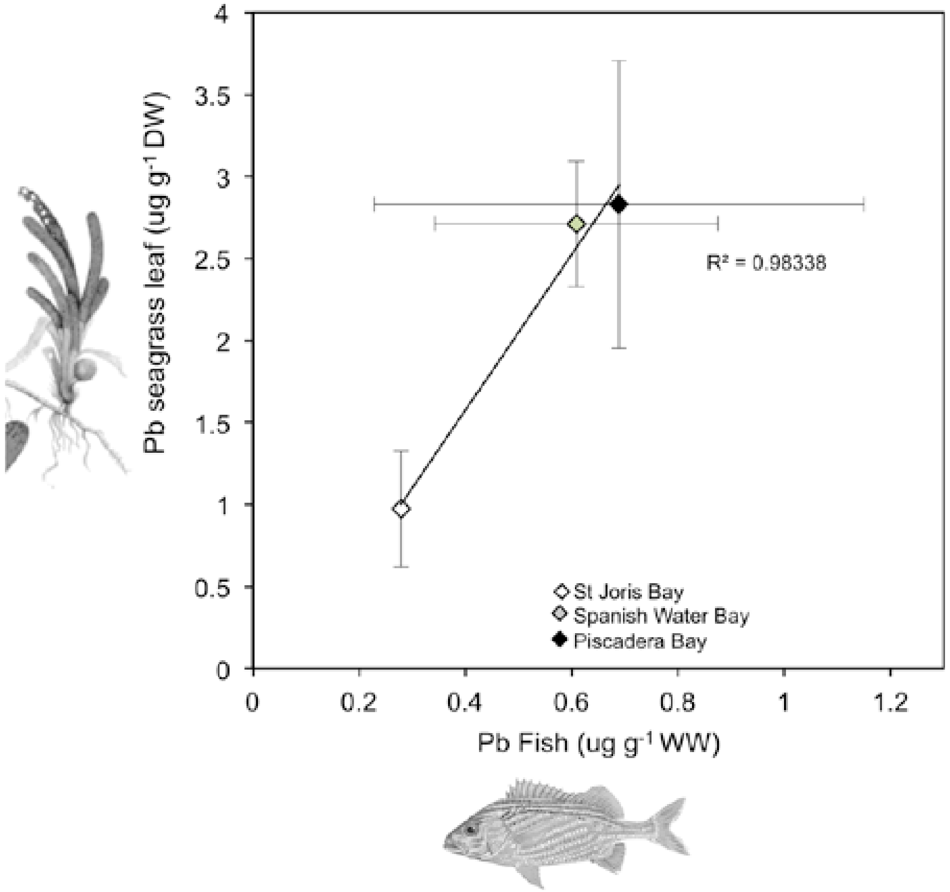


Figure 10.2 Relationship between seagrass leaf Pb and fish leaf Pb. R^2=0.983 and P=0.041. Displayed values are mean±standard error.

not consume seagrass directly, it generally feeds in seagrass beds as a juvenile and sub-adult fish (Verweij et al. 2006). We stress that it is very important to investigate such relationships, as these may even be used to examine if food safety standards for certain trace metals are exceeded. Seagrasses can thus be used as first-level indicators for coastal trace metal pollution, which may indicate threats to ecosystem health and ecosystem services such as fisheries.

Next to the indicator value of seagrasses for both nutrient and trace metal pollution, we also discovered that rhizome starch may predict the winter survival of intertidal temperate seagrasses (Chapter 3). Starch is an important carbohydrate reserve, which is stored in times of excess photosynthesis and used in times of reduced photosynthetic rates, such as the winter in temperate areas. It is especially important for seagrass monitoring and restoration projects to predict the winter survival of seagrasses, as this period is usually less intensely studied, but may present a bottleneck to seagrass survival (Suykerbuyk et al. in prep). We thus found that rhizome starch was indicative of winter survival of seagrasses, knowledge that may also be used for management and restoration purposes.

The importance of species interactions for seagrass ecosystem functioning

A vast array of different species depends directly or indirectly on seagrass ecosystems – either for food, shelter or nursery habitat (Valentine and Heck 1999, Nagelkerken 2000, Williams and Heck 2001). The importance of seagrass beds for grazers is quite well-known (Williams and Heck 2001, Heck and Valentine 2006), but the importance of seagrasses for benthic animals and vice versa is relatively poorly studied. Since seagrass beds are biogeochemical hotspots, it is to be expected that benthic infauna may also affect and be affected by biogeochemical processes in the sediment where they are living. We discovered that seagrasses are globally involved in a mutualistic relationship with lucinid bivalves and their sulfide-oxidizing gill symbionts through a biogeochemical feedback loop (Figure 10.3a, Chapter 7). As this mutualism forms the foundation of seagrass ecosystems, it may be called a keystone mutualism. Keystone mutualisms are also found in other coastal ecosystems such as coral reefs, mangroves, and salt marshes (Bertness 1984, Ellison et al. 1996, Hoegh-Guldberg et al. 2007), but were previously unknown to exist in seagrass beds. Rapid environmental change may disrupt keystone mutualisms and accelerate ecosystem degradation, which has been observed in plant-pollinator interactions, coral reefs and plant-mycorrhiza relationships (Hoegh-Guldberg et al. 2007, Kiers et al. 2010, Burkle et al. 2013). Therefore, it is important to understand and recognize keystone mutualisms such as the seagrass- lucinid bivalve- sulfide-oxidizing gill-symbionts symbiosis. In Chapter 9, we showed that the breakdown of this mutualism due to desiccation stress indeed accelerates habitat degradation in seagrass beds, thus strengthening the importance of this symbiosis.

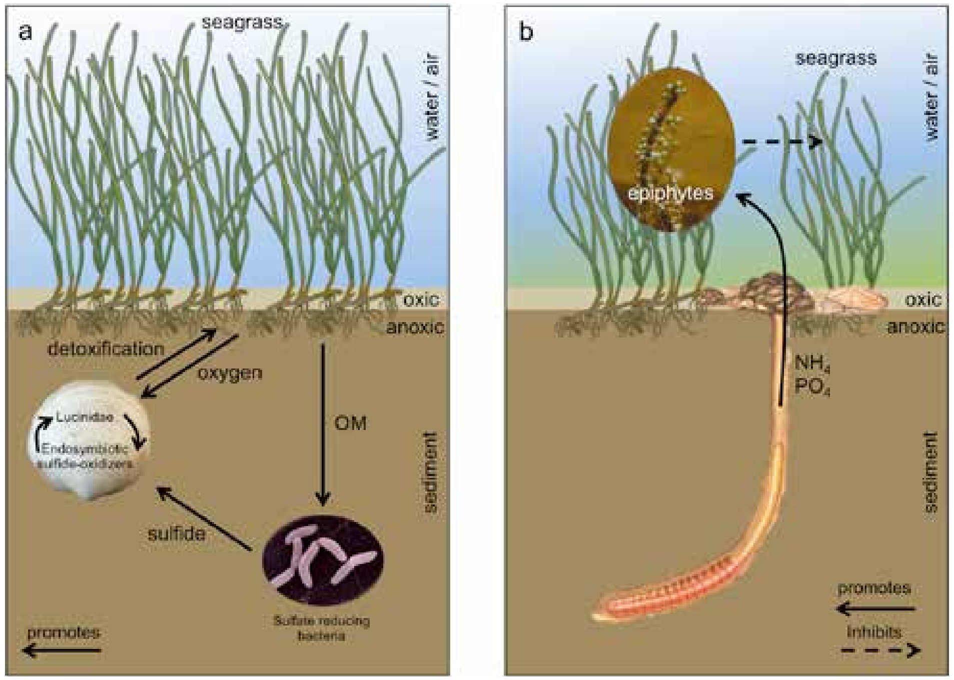


Figure 10.3 Contrasting effects of benthic animals on biogeochemical processes in seagrass beds. (a) The presence of Lucinid bivalves with their sulfide-oxidizing gill-symbionts facilitates seagrass growth in organic/sulfide-rich conditions by removing toxic sulfides from the sediment, while (b) lugworm (*Arenicola marina*) presence inhibits seagrass growth and survival by promoting epiphyte bloom on the seagrass leaves as a result of increased nutrient release by lugworm bioirrigation.

Many seagrasses in tropical (97%) and sub-tropical (90%) areas are associated with lucinid bivalves, but in temperate seagrass beds, this symbiosis seems less prevalent (56%) (Chapter 7). We therefore hypothesized that another common benthic associate of seagrasses, the lugworm *Arenicola marina*, might alleviate sulfide stress to temperate seagrasses by aerating the sediment through bioturbation and bioirrigation (Chapter 8). *Arenicola marina* activity did not, however, reduce porewater sulfide concentrations by flushing or aeration, but instead promoted harmful epiphyte bloom on seagrass leaves by increasing nutrient fluxes from the porewater to the surface water (Figure 10.3b). We thus showed fairly contrasting (positive sulfide relieving effect vs. negative epiphyte promoting effect), but strong effects of benthic animals on biogeochemical processes in seagrass beds.

The importance of the association between seagrasses and lucinid bivalves seems to decrease from tropical to temperate areas (97% vs. 56% presence of lucinids in seagrass beds, Chapter 7). Similarly, seagrass sensitivity to sulfide varies between species and between geographical ranges. Temperate *Zostera noltii* and *Zostera marina* seem fairly resistant to high sulfide levels (Chapter 2, Lamers et al.

BOX 10.1 SEAGRASS RESTORATION IN THE OOSTERSCHELDE – CONTEMPLATIONS

Seagrass restoration in the Oosterschelde (Box 1.1) has only been successful at two out of six tidal flats, one likely as a result of nearby natural bed expansion, and the other one likely as a result of transplanting (Suykerbuyk et al. in prep). Although large areas with high densities of *Zostera noltii* have been transplanted, survival of the transplants has been limited. We therefore identified several factors that could have affected transplant success.

Porewater biogeochemistry: high porewater sulfide levels are toxic to seagrasses and may strongly limit seagrass patch survival and expansion (Chapter 2). In addition, lucinid bivalves do not live in the Dutch Delta, sulfide levels are thus not reduced. However, we did not measure porewater sulfide concentrations >100 µmol L^{-1} in the transplants between 2007-2013 (Giesen et al. 2014). Hence, high sulfide levels have not affected transplant survival.

Nutrient limitation: seagrasses growing on terrigenous sediments may be nitrogen limited (Udy and Dennison 1997). We therefore measured seagrass nutrient content of *Zostera noltii* in the Oosterschelde and discovered that seagrass growth and survival in the Oosterschelde can not be attributed to nutrient limitation as we measured leaf %N of 3.8 %N and 0.9 %P in high summer (Chapter 2), which is high above the general limitation boundaries of 1.8 %N and 0.2 %P (Duarte 1990).

Hydrodynamics: High waves and high current velocities may decrease seagrass survival and expansion. We did however find successful transplants on both sheltered (Roelshoek) and exposed (Dortsman) sites. Hydrodynamics alone can therefore not be responsible for the lack of transplant success.

Bioturbation: Suykerbuyk et al. (2012) demonstrated that high adult lugworm densities decreased initial transplant survival, so lugworm excluding shell layers were implemented in all seagrass transplants from 2010 onwards. Furthermore, bioturbation may also have interacted with sediment dynamics (see below, Suykerbuyk et al. in prep).

Winter survival: We did not find any relationship between the preceding year's transplant success and next years growth success (Chapter 3) and therefore looked at wintering processes. We found that rhizome starch levels can be indicative of next year's growth success. Low rhizome starch concentrations may therefore be a bottleneck to transplant winter survival.

Macroalgae: Dense macroalgal layers may suffocate seagrasses and promote sulfide stress. We quantified macroalgae presence in the transplants, but seldom observed high densities of macroalgae (Giesen et al. 2012).

Grazing: Brent geese (Brenta bernicla) have been observed grazing on dwarf eelgrass in the Oosterschelde. Geese grazing pits have been observed in both natural seagrass beds and seagrass transplants in the Oosterschelde (Giesen et al. 2012). Although grazing may not have detrimental effects on healthy seagrass populations (Ganter 2000, van der Heide et al. 2012), vulnerable transplants may be negatively affected when heavily grazed.

Sediment dynamics: The implementation of the storm surge barrier in the Oosterschelde reduced the tidal regime and the supply of sediments from the North Sea into the Oosterschelde. These changes resulted in a loss of intertidal area and increased erosion of tidal flats, a process called 'sand hunger' (zandhonger). Due to this process, about ~50ha of intertidal habitat is lost annually, which is a catastrophe for foraging birds, but may also affect intertidal *Zostera noltii*. Dwarf eelgrass seems to have retreated to the areas with low sediment dynamics and low erosion rates (Suykerbuyk et al. In prep.). Local sediment dynamics may also have affected transplant survival.

Genetic diversity: High genetic diversity in seagrass beds can lead to increased resistance to disturbances such as grazing and heat stress (Williams 2001, Hughes and Stachowicz 2004, Reusch et al. 2005). Decreasing seagrass populations such as in the Oosterschelde may suffer from impoverished genotypic diversity, which may be reflected by low restoration success. We did however not investigate genotypic diversity of *Zostera noltii* in the Oosterschelde, but would recommend this for future research.

Concluding, the lack of restoration success in the Oosterschelde can probably be attributed to a complex interplay of many known and unknown factors.

2013), whereas some seagrass species such as *Posidonia oceanica* already show decreased performance at very low sulfide exposure (<100 µm L^{-1}) (Calleja et al. 2007). Temperate seagrass species seem less sensitive to sulfide toxicity than (sub) tropical species. Possibly, the highly common symbiosis between seagrasses and lucinids in the tropics and subtropics, combined with general oligotrophic conditions, may explain why seagrasses from those areas never experience, and thus are poorly adapted to, high sulfide concentrations.

Lessons for seagrass management and restoration

Seagrasses are important coastal ecosystems, but have been globally declining for decades (Orth et al. 2006, Waycott et al. 2009). To counteract and reduce these losses, many attempts to seagrass restoration have been undertaken, with various degrees of success (van Katwijk et al. submitted). Restoration success is related to the planting scale, with an increasing success rate with higher amounts of planted shoots, but also site characteristics, species, and planting techniques affect seagrass restoration performance (van Katwijk et al. submitted). This effect of planting scale on restoration success, may be attributed to the existence of positive feedback mechanisms in seagrass beds (Van der Heide 2009). A certain threshold size or density is needed to generate self-facilitation of seagrasses and increase restoration success (Van der Heide 2009, van Katwijk et al. submitted). However, these feedbacks are generally lacking in areas selected for restoration as a result of low or lacking seagrass densities. Restoration should thus be aimed at introducing high-enough seagrass densities to restore self-facilitating feedbacks. Furthermore, we showed (Chapter 2, 4) that especially processes important to successful restoration, such as expansion and recovery processes, are vulnerable to stressors (here: biogeochemical toxins) due to the lack of density-dependent stress-alleviating feedbacks (joint-detoxification). This again illustrates the importance of density thresholds for self-facilitating feedbacks, which should be taken into account by restoration projects, although important feedbacks should first be identified and studied.

Next to feedbacks, restoration success may also be affected by site characteristics such as background stress levels. Low, but continuous stress levels, such as elevated nutrient and sulfide levels (Chapter 2, 4, 6), trace metals (Chapter 6) and bioturbation pressure (Chapter 8) may interact with disturbance-recovery processes and thereby affect ecosystem resilience (Chapter 4) and restoration success. So in addition to identifying the presence (or lack) of feedbacks for management and restoration purposes, it is also important to identify background stress levels as they may interact with restoration attempts.

In this thesis, we reveal several tools for identifying background stress levels in seagrass beds such as high nutrient and trace metal levels (Chapter 5, 6). We found that high nutrient levels (%P and %N) in seagrass leaves are indicative of

point source eutrophication. In addition, high trace metal levels in the coastal environment are easily detected by studying heavy metal concentrations of seagrass leaves, which generally integrate metal loads over a longer time scale. Although trace metals by itself may not present a threat to seagrasses, seagrass trace metal levels may indicate threats to other trophic levels connected to the seagrass habitat. Additionally, we found that seagrasses in turn are also depending on co-occurring species, such as lucinid bivalves (Chapter 7, 9), which could be less resistant to metal pollution.

Overall, we have provided several important messages and tools for seagrass conservation, management and restoration in this thesis: 1) Meadow maintenance processes such as expansion and recovery may be constrained by the lack of density-dependent feedbacks, 2) background conditions and stress levels may interact with disturbances such as a restoration attempts, 3) Rhizome starch may be used to predict seagrass survival during/after low light level events such as winter, eutrophication induced algal blooms and dredging, 4) nutrient and trace metal pollution in coastal ecosystems may easily be detected by studying concentrations of seagrass leaves , 5) species interactions and keystone mutualisms should be taken into consideration for management and restoration of seagrass ecosystems, as the breakdown of keystone mutualisms may accelerate habitat degradation.

Concluding summary

Seagrass ecosystems are threatened by eutrophication (Chapter 2, 6) and trace metal pollution (Chapter 5), as a result of increased human activities in coastal areas. We showed that such threats to seagrass ecosystem functioning can be discovered by using seagrass nutrient and metal concentrations as bioindicators. The indicator function of seagrasses may also be used to gain insight in plant-level processes, as our findings illustrated that rhizome starch can be indicative of seagrass winter survival (Chapter 3). Next to promoting plankton bloom and subsequent reduction of light availability, eutrophication also promotes the input of organic matter in seagrass beds. This organic matter is mainly decomposed anaerobically, resulting in toxic sediment sulfide levels, which may affect seagrass meadow-scale dynamics (Chapter 2,4), due to the lack of density-dependent feedbacks in low seagrass densities. Seagrasses all over the world are coping with sulfide stress, but we discovered that a three-stage mutualism between seagrasses, lucinid bivalves and their sulfide-oxidizing gill bacteria globally enables growth of seagrasses in organic sediments (Chapter 7). The importance of this keystone mutualism is illustrated in Chapter 9, which showed that breakdown of this mutualism may lead to accelerated habitat degradation. Another benthic animal that frequently inhabits seagrass beds is the lugworm *Arenicola marina*. Seagrasses and lugworms are involved in a biomechanical warfare, as they display contrasting properties. In addition to the negative effects of lugworm bioturbation by burial, we found that lugworms may also negatively affect seagrasses by altering biogeochemistry; lugworm activity increases nutrient fluxes, promoting detrimental epiphyte bloom on seagrass leaves (Chapter 8). These examples (Chapter 7, 8, 9) emphasize the importance of including species interactions in seagrass ecosystem studies.

Based on our findings, I conclude that it is important to study all levels and aspects of biogeochemical stressors on seagrass ecosystems, from plant- to meadow-scale (Figure 10.4) and including species interactions, to gain a deeper understanding of ecosystem functioning, in order to provide useful tools for seagrass conservation, management, and restoration.

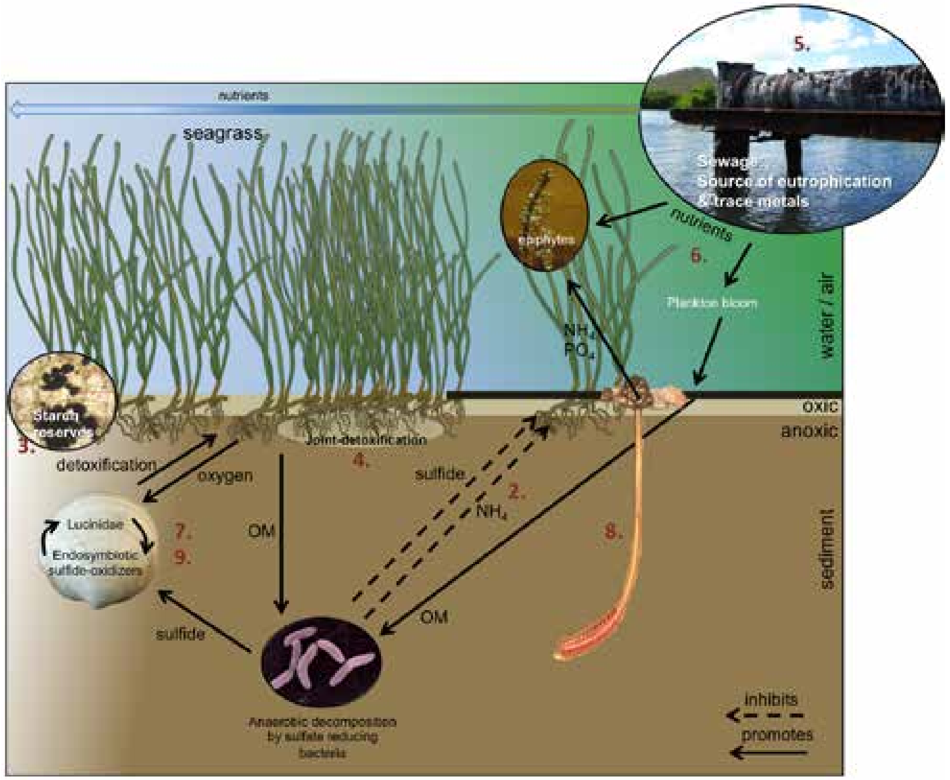


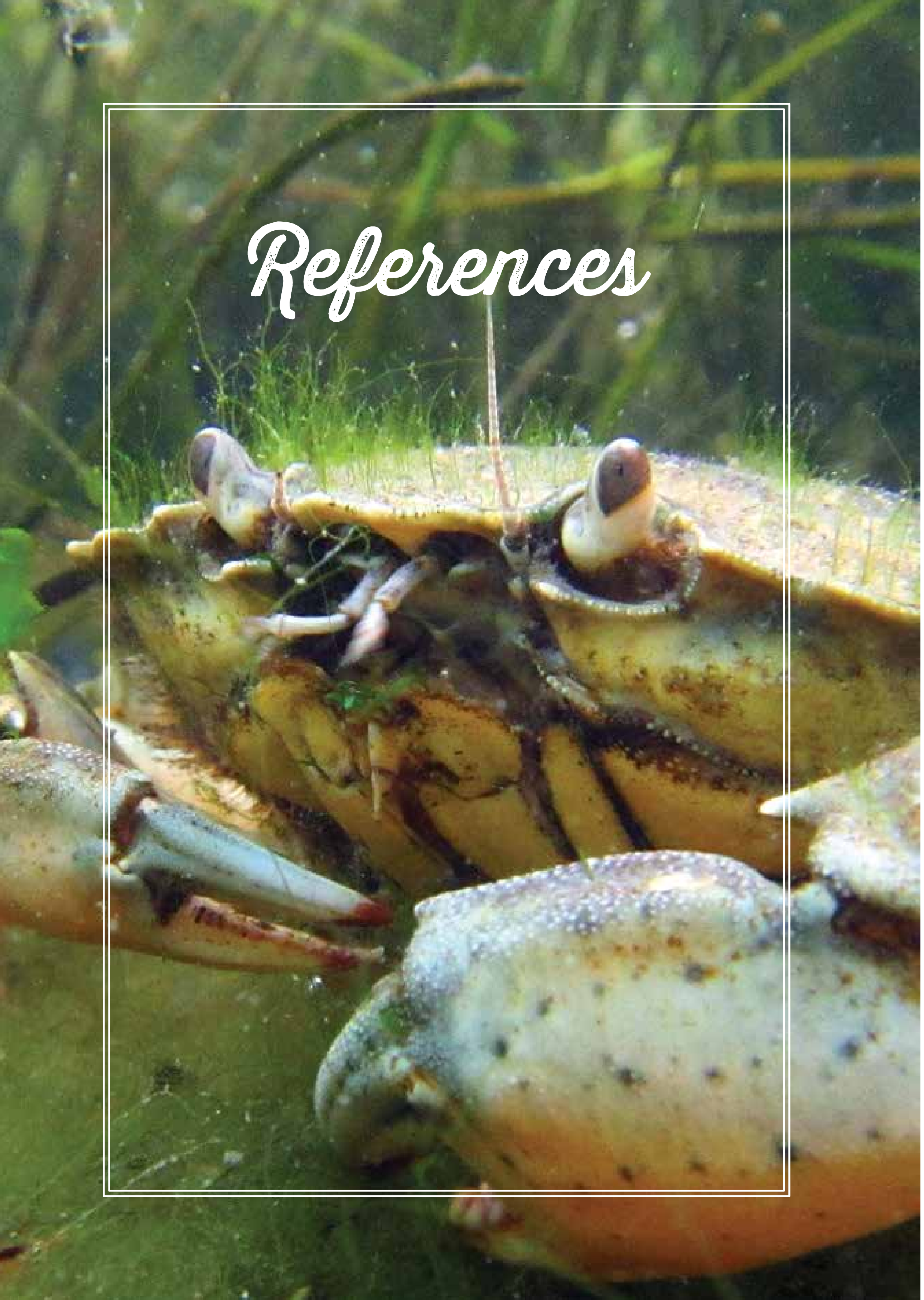
Figure 10.4 Graphical abstract of this thesis. Chapter numbers are indicated in red. Proportions of plants and animals are not based on real measures and species composition is not a realistic reproduction of a real-life seagrass ecosystem.

ACKNOWLEDGEMENTS

We would like acknowledge the following people for their contributions to this synthesis: Marieke van Katwijk, Tjeerd Bouma, Wouter Suykerbuyk, Wim Giesen, Dick de Jong, Tjisse van der Heide, , Jelle Eygensteyn, Rien van de Gaag, Leon Lamers, Jan de Brouwer, and Chantal Huijbers.

		QUESTION	ANSWER
Resilience & Restoration	CHAPTER 2	What are the biogeochemical constraints for seagrass patch survival and expansion?	High porewater sulfide and ammonium concentrations are toxic constraints to seagrass patch expansion and survival.
	CHAPTER 3	What are the bottlenecks for the winter survival of *Zostera noltii* in the Oosterschelde?	Autumn starch reserves indicate next year's growth success of *Zostera noltii*.
	CHAPTER 4	What are the effects of local environmental conditions and feedbacks on the resilience of seagrasses?	Local environmental conditions, such as sediment grain size and organic matter level, and feedbacks affect disturbance-recovery dynamics of intertidal seagrasses.
Pollution	CHAPTER 5	What is the overall status of trace metals in seagrass beds worldwide and in the Caribbean in particular?	Seagrasses are good bioindicators of trace metal pollution in coastal ecosystems worldwide. Additionally, some bays in the Caribbean are heavily polluted by trace metals, which may form a potential threat to higher trophic levels.
	CHAPTER 6	What is the nutrient status of the seagrass beds on Curaçao and Bonaire?	Eutrophication threatens important nursery habitats on Curaçao in Spanish Water Bay and Piscadera Bay.
Biogeochemical species interactions	CHAPTER 7	How do seagrasses worldwide cope with sulfide stress in organic sediments?	A common mutualistic interaction between seagrasses, lucinid bivalves and their symbiotic gill-bacteria forms the foundation of seagrass ecosystems by reducing sulfide stress.
	CHAPTER 8	What is the biogeochemical effect of lugworm activity on seagrasses?	Lugworms do not relieve sulfide stress to seagrasses, but instead stimulate nutrient fluxes from the porewater to the surface water, which in turn promote detrimental algal bloom on the leaves.
	CHAPTER 9	What happens with the three-stage seagrass-lucinid-gill-bacteria symbiosis in seagrass beds under desiccation stress?	A mutualism-breakdown due to desiccation stress accelerates habitat degradation in seagrass beds.

Table 10.1 Overview of questions and answers in this thesis



REFERENCES

A

Anderson, A. E. 1995. Metabolic responses to sulfur in lucinid bivalves. American Zoologist 35:121-131.

Adriano, D.C., 2001. Trace elements in terrestrial environments - Biogeochemistry, Bioavailability, and Risks of Metals, 2nd ed. Sheridan Books Inc., Ann Arbor, USA.

Ahmedou Salem, M. V., M. van der Geest, T. Piersma, Y. Saoud, and J. A. van Gils. 2014. Seasonal changes in mollusc abundance in a tropical intertidal ecosystem, Banc d'Arguin (Mauritania): Testing the 'depletion by shorebirds' hypothesis. Estuarine, Coastal and Shelf Science 136:26-34.

Alcoverro, T., Zimmerman, R.C., Kohrs, D.G., Alberte, R.S., 1999. Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. Marine Ecology Progress Series 187, 121-131.

Alcoverro, T., Manzanera, M., Romero, J., 2001. Annual metabolic carbon balance of the seagrass *Posidonia oceanica*: the importance of carbohydrate reserves. Marine Ecology Progress Series 211, 105-116.

Allen, J. R. L. 2000. Morphodynamics of Holocene salt marshes: a review sketch from the atlantic and southern North Sea coasts of Europe. Quartenary Science Reviews 19:1155-1231.

Allgeier, J.E., Rosemond, A.D., Layman, C.A., 2011. Variation in nutrient limitation and seagrass nutrient content in Bahamian tidal creek ecosystems. Journal of Experimental Marine Biology and Ecology 407, 330-336.

Almela, E.D., Marba, N., Alvarez, E., Santiago, R., Martinez, R., Duarte, C.M., 2008. Patch dynamics of the Mediterranean seagrass *Posidonia oceanica*: Implications for recolonisation process. Aquatic Botany 89, 397-403.

Alongi, D. M. 2002. Present state and future of the world's mangrove forests. Environmental Conservation 29:331-349.

Altieri, A. H., M. D. Bertness, T. C. Coverdale, E. E. Axelman, N. C. Herrmann, and P. L. Szathmary. 2013. Feebacks underlie the resilience of salt marshes and rapid reversal of consumer-driven die-off. Ecology 94.

Amado Filho, G.M., Creed, J.C., Andrade, L.R., Pfeiffer, W.C., 2004. Metal accumulation by *Halodule wrightii* populations. Aquatic Botany 80, 241-251.

Amado Filho, G.M., Salgado, L.T., Rebelo, M.F., Rezende, C.E., Karez, C.S., Pfeiffer, W.C., 2008. Heavy metals in benthic organisms from Todos os Santos Bay, Brazil. Brazilian Journal of Biology 68, 95-100.

Ancora, S., Bianchi, N., Butini, A., Buia, M.C., Gambi, M.C., Leonzio, C., 2004. *Posidonia oceanica* as a biomonitor of trace elements in the Gulf of Naples: Temporal trends by lepidochronology. Environmental Toxicology and Chemistry 23, 1093-1099.

Andersen, F. O. and E. Kristensen. 1992. The importance of benthic macrofauna in decomposition of microalgae in a coastal marine sediment. Limnology and Oceanography 37:1392-1403.

Angelini, C. and B. R. Silliman. 2012. Patch size-dependent community recovery after massive disturbance. Ecology 93:101-110.

Argyrou, M., A. Demetropoulos, and M. Hadjichristophorou. 1999. Expansion of the macroalga Caulerpa racemosa and changes in softbottom macrofaunal assemblages in Moni Bay, Cyprus. Oceanologica Acta 22:517-528.

Ashforth, E. J., P. J. W. Olive, and A. C. Ward. 2011. Phylogenetic characterisation of bacterial assemblages and the role of sulphur-cycle bacteria in an *Arenicola marina* bioturbated mesocosm. Marine Ecology Progress Series 439:19-30.

Asmus, H. and R. M. Asmus. 1998. The role of macrobenthic communities for sediment-water material exchange in the Sylt-Rømø tidal basin. Senckenbergiana maritima 29:111-119.

Asmus, R. M., M. H. Jensen, K. M. Jensen, E. Kristensen, H. Asmus, and A. Wille. 1998. The role of water movement and spatial scaling for measurement of dissolved inorganic nitrogen fluxes in intertidal sediments. Estuarine Coastal and Shelf Science 46:221-232.

Aurelia, M. 1969. The habitats of some subtidal pelecypods in Herrington Sound, Bermuda. Pages 39-52 in R. N. Ginsburg and Stevens, S. M., editors. Seminar on organism-sediment interelationship. Berm. Biol. Stn Spec. , Bermuda.

B

Bagarinao, T. 1992. Sulfide as an environmental factor and toxicant - Tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24:21-62.

Baker, A. C. 2003. Flexibility and specificity in coral-algal symbiosis: Diversity, ecology, and biogeography of Symbiodinium. Annual Review of Ecology Evolution and Systematics 34:661-689.

Ban, S. S., N. A. J. Graham, and S. R. Connolly. 2014. Evidence for multiple stressor interactions and effects on coral reefs. Global Change Biology 20:681-697.

Banta, G. T., M. Holmer, M. H. Jensen, and E. Kristensen. 1999. Effects of two polychaete worms: Nereis diversicolor and *Arenicola marina*, on aerobic and anaerobic decomposition in a sandy marine sediment Aquatic Microbial Ecology 19:189-204.

Barber, B.J., Behrens, P.J., 1985. Effects of elevated temperature on seasonal *in situ* leaf productivity of *Thalassia testudinum* Banks ex König and *Syringodium filiforme* Kützing. Aquatic Botany 22, 61-69.

Barnes, P. A. G. and C. S. Hickman. 1999. Lucinid bivalves and marine angiosperms: a search for causal relationships. Pages 215–238 in D. I. Walker and F. E. Wells, editors. The Seagrass Flora and Fauna of Rottnest Island, Western Australia. Western Australian Museum, Perth.

Baroli, M., Christin, A., Cosse, A., De Falco, G., Gazal, V., Pergent-Martini, C., 2001. Concentrations of trace metals (Cd, Cu, Fe, Pb) in *Posidonia oceanica* seagrass of Liscia Bay, Sardinia (Italy), in: Faranda, F.M., Guglielmo, L., Spezie, G. (Eds.), Mediterranean Ecosystems: structures and processes. Springer, New York.

Bascompte, J. and P. Jordano. 2007. Plant-animal mutualistic networks: The architecture of biodiversity. Annual Review of Ecology Evolution and Systematics 38:567-593.

Bascompte, J. 2009. Mutualistic networks. Frontiers in Ecology and the Environment 7:429-436.

Bascompte, J., and P. Jordano. 2014. Mutualistic networks. Princeton University Press.

Bastolla, U., M. A. Fortuna, A. Pascual-Garcia, A. Ferrera, B. Luque, and J. Bascompte. 2009. The architecture of mutualistic networks minimizes competition and increases biodiversity. Nature 458:1018-U1091.

Batley, G.E., 1987. Heavy metal speciation in water, sediments and biota from Lake Macquarie, New South Wales. Australian Journal of Marine and Freshwater Research 38, 591-606.

Beer, S., Vilenkin, B., Weil, A., Veste, M., Susel, L., Amram, E., 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. Marine Ecology Progress Series 174, 293-300Beer, S., Björk, M., Gademann, R., Ralph, P., 2001. Measurements of photosynthetic rates in seagrasses, in: Short, F.T., Coles, R. (Eds.), Global seagrass research methods. Elsevier, Amsterdam.

Bell, S.S., Robbins, B.D., Jensen, S.L., 1999. Gap Dynamics in a Seagrass Landscape. Ecosystems 2, 493-504.

Bennett, S., Roca, G., Romero, J., Alcoverro, T., 2011. Ecological status of seagrass ecosystems: An uncertainty analysis of the meadow classification based on the *Posidonia oceanica* multivariate index (POMI). Mar Pollut Bull 62, 1616-1621.

Bertness, M. D. 1984. Ribbed mussels and Spartina alterniflora production in a New England salt-marsh. Ecology 65:1794-1807.

Bertness, M. D. and G. H. Leonard. 1997. The role of positive interactions in communities: Lessons from the intertidal habitats. Ecology 78:1976-1989.

Bhattacharya, B., Sarkar, S.K., Das, R., 2003. Seasonal variations and inherent variability of selenium in marine biota of a tropical wetland exosystem: implications for bioindicator species. Ecological Indicators 2, 367-375.

Bitter-Soto, R. 1999. Benthic communities associated to *Thalassia testudinum* (Hydrocharitaceae) at three localities of Morrocoy National Park, Venezuela. Revista de Biología Tropical 47:443-452.

Bjork, M., J. Uku, A. Weil, and S. Beer. 1999. Photosynthetic tolerance to desiccation of tropical intertidal seagrasses. Marine Ecology Progress Series 191:121-126.

Blanchet, H., X. de Montaudouin, A. Lucas, and P. Chardy. 2004. Heterogeneity of macrozoobenthic assemblages within a *Zostera noltii* seagrass bed: diversity, abundance, biomass and structuring factors. Estuarine Coastal and Shelf Science 61:111-123.

Boese, B. L., K. E. Alayan, E. F. Gooch, and B. D. Robbins. 2003. Desiccation index: a measure of damage caused by adverse aerial exposure on intertidal eelgrass (*Zostera marina*) in an Oregon (USA) estuary. Aquatic Botany 76:329-337.

Bolker, B. M. 2008. Ecological Models and Data in R. Princeton University Press.

Bond, A.M., Bradbury, J.R., Hudson, A., Garnham, J.S., Hanna, P.J., Strother, S., 1985. Kinetic studies of lead (II) uptake by the seagrass *Zostera muelleri* in watr by radiotracing, Atomic Absorption Spectrometry and Electrochemical techniques. Marine Chemistry 16, 1-9

Borum, J., O. Pedersen, T. M. Greve, T. A. Frankovich, J. C. Zieman, J. W. Fourqurean, and C. J. Madden. 2005. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. Journal of Ecology 93:148-158.

Bos, A. and M. M. van Katwijk. 2007. Planting density, hydrodynamic exposure and mussel beds affect survival of transplanted intertidal seagrass. Marine Ecology Progress Series 336:121-129.

Bos, A., T. Bouma, G. Dekort, and M. Vankatwijk. 2007. Ecosystem engineering by annual intertidal seagrass beds: Sediment accretion and modification. Estuarine, Coastal and Shelf Science 74:344-348.

Bostrom, C. and E. Bonsdorff. 1997. Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L) beds in the northern Baltic Sea. Journal of Sea Research 37:153-166.

Bostrom, C., Jackson, E.L., Simenstad, C.A., 2006. Seagrass landscapes and their effects on associated fauna: A review. Estuarine Coastal and Shelf Science 68, 383-403.

Bouma, T. J., M. B. De Vries, E. Low, G. Peralta, C. Tanczos, J. Van de Koppel, and P. M. J. Herman. 2005. Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. Ecology 86:2187-2199.

Bouma, T. J., M. Friedrichs, B. K. van Wesenbeeck, S. Temmerman, G. Graf, and P. M. J. Herman. 2009. Density-dependent linkage of scale-dependent feedbacks: a flume study on the intertidal macrophyte Spartina anglica. Oikos 118:260-268.

Bouma, T. J., S. Olenin, K. Reise, and T. Ysebaert. 2009. Ecosystem engineering and biodiversity in coastal sediments: posing hypotheses. Helgoland Marine Research 63:95-106.

Box, G. E. P. and D. R. Cox. 1964. An analysis of transformations. Journal of the Royal Statistical Society, Series B 26:211-252.

Brissac, M. T. 2009. Nature, diversité et spécificité de l’association Lucinidae/bactéries sulfo-oxydantes. Universite Pierre et Marie Curie Paris.

Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. Journal of Plant Physiology 159, 567-684.

Brix, H., Lyngby, J.E., 1982. The distribution of cadmium, copper, lead and zinc in eelgrass *Zostera marina*. The Science of the total environment 24, 51-64.

Brix, H., Lyngby, J.E., 1983. The distribution of some metallic elements in eelgrass (*Zostera marina* L.) and sediment in the Limfjord, Denmark. Estuarine Coastal and Shelf Science 16, 455-467.

Bronstein, J. L., U. Dieckmann, and R. Ferrie`re. 2004. Evolutionary conservation biology. Cambridge University Press, Cambridge.

Brun, F.G., Hernandez, I., Vergara, J.J., Peralta, G., Perez-Llorens, J.L., 2002. Assessing the toxicity of ammonium pulses o the survival and growth of *Zostera noltii*. Marine Ecology Progress Series 225, 177-187.

Brun, F. G., J. L. Perez-Llorens, I. Hernandez, and J. J. Vergara. 2003. Patch distribution and within-patch dynamics of the seagrass *Zostera noltii* Hornem. In Los Torunos salt-marsh, Cadiz Bay, Natural Park, Spain. Botanica Marina 46:513-524.

Brun, F.G., Olivé, I., Malta, E., Vergara, J.J., Hernández, I., Pérez-Lloréns, J.L., 2008. Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency. Marine Ecology Progress Series 365, 67-75.

Brun, F. G., E. van Zetten, E. Cacabelos, and T. J. Bouma. 2009. Role of two contrasting ecosystem engineers (*Zostera noltii* and *Cymodocea nodosa*) on the food intake rate of *Cerastoderma edule*. Helgoland Marine Research 63:19-25.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18:119-125

Bulthuis, D. A., D. M. Axelrad, and M. J. Mickelson. 1992. Growth of the seagrass *Heterozostera tasmanica* limited by nitrogenin Port Philip Bay, Australia. Marine Ecology Progress Series 89:269-275.

Burke, M.K., Dennison, W.C., Moore, K.A., 1996. Non-structural carbohydrate reserves of eelgrass *Zostera marina*. Marine Ecology Progress Series 137, 195-201.

Burkholder, J.M., Mason, K.M., Glasgow, H.B.J., 1992. Water-column nitrate enrichment promotes decline on eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. Marine Ecology Progress Series 81, 163-178.

Burkholder, J., D. Tomasko, and B. Touchette. 2007. Seagrasses and eutrophication. Journal of Experimental Marine Biology and Ecology 350:46-72.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function. Science 339:1611-1615.

C

Cabaco, S., Santos, R., 2007. Effects of burial and erosion on the seagrass *Zostera noltii*. Journal of Experimental Marine Biology and Ecology 340, 204-212.

Cadée, G. C. 1976. Sediment reworking by *Arenicola marina* on tidal flats in the Dutch Wadden Sea. Netherlands Journal of Sea Research 10:440-460.

Cadée, G. C. 2001. Sediment dynamics by bioturbating organisms. Pages 127-236 in K. Reise, editor. Ecological comparisons of sedimentary shores. Springer, Berlin.

Caffrey, J. M. and W. M. Kemp. 1991. Seasonal and spatial patterns of oxygen production, respiration and root rhizome release in Potamogeton perfoliatus L. and *Zostera marina* L. Aquatic Botany 40:109-128.

Calleja, M.L., Marba, N., Duarte, C.M., 2007. The relationship between seagrass (*Posidonia oceanica*) decline and sulfide porewater concentration in carbonate sediments. Estuarine Coastal and Shelf Science 73, 583-588.

Campanella, L., Conti, M.E., Cubadda, F., Sucapane, C., 2001. Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean. Environmental Pollution 111, 117-126.

Campbell, M.L., Paling, E.I., 2003. Evaluating vegetative transplant success in Posidonia australis: a field trial with habitat enhancement. Marine Pollution Bulletin 46, 828-834.

Campbell, J.E., Yarbro, L.A., Fourqurean, J.W., 2012. Negative relationships between the nutrient and carbohydrate content of the seagrass *Thalassia testudinum*. Aquatic Botany 99, 56-60.

Cancemi, G., G. De Falco, and G. Pergent. 2003. Effects of organic matter input from a fish farming facility on a *Posidonia oceanica* meadow. Estuarine Coastal and Shelf Science 56:961-968.

Canfield, D. E. and J. Farquhar. 2009. Animal evolution, bioturbation, and the sulfate concentration of the oceans. Proceedings of the National Academy of Sciences 106:8123-8127.

Capone, D.G., Penhale, P.A., Oremland, R.S., Taylor, B.F., 1979. Relationship between productivity and N2 (C2H2) fixation in a *Thalassia testudinum* community. Limnology and Oceanography 24, 117-125.

Caredda, A.M., Cristini, A., Ferrara, C., Lobina, M.F., Baroli, M., 1999. Distribution of heavy metals in the Piscinas beach sediments (SW Sardinia, Italy). Environmental Geology 38, 91-100.

Carlson, P. R., L. A. Yarbro, and T. R. Barber. 1994. Relationship of sediment sulfide to mortality of *Thalassia testudinum* in Florida Bay. Bulletin of Marine Science 54:733-746.

Carpenter, K. E., M. Abrar, G. Aeby, R. B. Aronson, S. Banks, A. Bruckner, A. Chiriboga, J. Cortes, J. C. Delbeek, L. DeVantier, G. J. Edgar, A. J. Edwards, D. Fenner, H. M. Guzman, B. W. Hoeksema, G. Hodgson, O. Johan, W. Y. Licuanan, S. R. Livingstone, E. R. Lovell, J. A. Moore, D. O. Obura, D. Ochavillo, B. A. Polidoro, W. F. Precht, M. C. Quibilan, C. Reboton, Z. T. Richards, A. D. Rogers, J. Sanciangco, A. Sheppard, C. Sheppard, J. Smith, S. Stuart, E. Turak, J. E. N. Veron, C. Wallace, E. Weil, and E. Wood. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321:560-563.

Carr, J., P. D'Odorico, K. McGlathery, and P. Wiberg. 2010. Stability and bistability of seagrass ecosystems in shallow coastal lagoons: Role of feedbacks with sediment resuspension and light attenuation. Journal of Geophysical Research-Biogeosciences 115.

Carruthers, T.J.B., Barnes, P.A.G., Jacome, G.E., Fourqurean, J.W., 2005. Lagoon scale processes in a coastally influenced Caribbean system: Implications for the seagrass *Thalassia testudinum*. Caribbean Journal of Science 41, 441-455.

Catsiki, V.A., Panayotidis, P., 1993. Copper, chromium and nickel in tissues of the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* (potamogetonaceae) from Greek coastal areas. Chemosphere 26, 963-978.

Cavanaugh, C. M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. Nature 302:58-61.

Centeno, A. B. 2008. Ecología de Caulerpales: Fauna y Biomarcadores. Mallorca, Spain.

Center for Coastal Studies. 1996. Current status and historical trends of the estuarine living resources within the CCBNEP study area. Center for Coastal Studies, Texas A&M University, Corpus Christi.

Chelazzi, G. and M. Vannini. 1980. Zonation of intertidal molluscs on rocky shores of southern Somalia. Estuarine and Coastal Marine Science 10:569-583.

Chernova, E.N., Khristoforova, N.K., Vyshkvartsev, D.I., 2002. Heavy metals in seagrasses and algae of Pos'et Bay, Sea of Japan. Russian Journal Of Marine Biology 26, 387-392.

Childress, J. J. and P. R. Girguis. 2011. The metabolic demands of endosymbiotic chemoautotrophic metabolism on host physiological capacities. Journal of Experimental Biology 214:312-325.

Christianen, M.J.A., van der Heide, T., Bouma, T.J., Roelofs, J.G.M., van Katwijk, M.M., Lamers, L.P.M., 2011. Limited toxicity of NH(x) pulses on an early and late successional tropical seagrass species: Interactions with pH and light level. Aquatic Toxicology 104, 73-79.

Christianen, M.J.A., Govers, L.L., Kiswara, W., Roelofs, J.G.M., Bouma, T.J., Lamers, L.P.M., van Katwijk, M.M., 2012. Marine megaherbivore grazing may increase seagrass resilience to high nutrient loads. Journal of Ecology 100, 546-560

Christianen, M. J. A., J. Van Belzen, P. M. J. Herman, M. M. van Katwijk, L. P. M. Lamers, P. J. M. Van Leent, and T. J. Bouma. 2013. Low-canopy seagrass beds still provide important coastal protection services. Plos One 8.

Cinar, M. E., Z. Ergen, B. Ozturk, and F. Kirkim. 1998. Seasonal analysis of zoobenthos associated with a *Zostera marina* L. bed in Gulbahce Bay (Aegean Sea, Turkey). Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I 19:147-162.

Clijsters, H., Van Assche, F., 1985. Inhibition of photosynthesis by heavy metals. Photosynthesis Research 7, 31-40.

Cohen, J. E. 2003. Human population: the next half century. Science 302:1172-1175.

Cohen, J. E., C. Small, A. Mellinger, J. Gallup, and J. Sachs. 1997. Estimates of coastal populations. Science 278:1211-1212.

Conroy, B. A., P. Lake, N. Buchhorn, J. McDouall-Hill, and L. Hughes. 1991. Studies on the effects of heavy metals on seagrasses in Lake Macquarie.in J. H. Whitehead, R. W. Kidd, and H. A. Bridgeman, editors. Lake Macquarie: an environmental reappraisal. University of Newcastle, Newcastle.

Conti, M.E., Bocca, B., Iacobucci, M., Finoia, M.G., Mecozzi, M., Pino, A., Alimonti, A., 2010. Baseline Trace Metals in Seagrass, Algae, and Mollusks in a Southern Tyrrhenian Ecosystem (Linosa Island, Sicily). Archives of Environmental Contamination and Toxicology 58, 79-95.

Continental Shelf Associates, I. 1995. Synthesis of available biological, geological, chemical, socioeconomic, and cultural resource information for the South Florida area. Supplemental report: A comparison of seagrass beds in Panama and South Florida., New Orleans.

Costa, M. F., W. M. Landing, H. A. Kehrig, M. Barletta, C. D. Holmes, P. R. G. Barrocas, D. C. Evers, D. G. Buck, A. C. Vasconcellos, S. S. Hacon, J. C. Moreira, and O. Malm. 2012. Mercury in tropical and subtropical coastal environments. Environmental Research 119:88-100.

Costanza, R., R. dArge, R. deGroot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. Oneill, J. Paruelo, R. G. Raskin, P. Sutton, and M. vandenBelt. 1997. The value of the world's ecosystem services and natural capital. Nature 387:253-260.

Couto, E. C. G. and M. Savian. 1998. Caracterização sedimentológica da planície intertidal da parte sul do saco do limoeiro (Ilha do Mel - Paraná - Brasil). I. implicações ecológicas. Brazilian Archives of Biology and Technology 41:0.

Cowen, R.K., Paris, C.B., Srinivasan, A., 2006. Scaling of connectivity in marine populations. Science 311, 522-527.

Crain, C. M. and M. D. Bertness. 2006. Ecosystem engineering across environmental gradients: implications for conservation and management. Bioscience 56:211-218.

Creed, J. C. and M. Kinupp. 2011. Small-scale change in mollusk diversity along a depth gradient in a seagrass bed off Cabo Frio (Southeast Brazil). Brazilian Journal of Oceanography 59:267-276.

D

Dale, A. L., R. McAllen, and P. Whelan. 2007. Management considerations for subtidal *Zostera marina* beds in Ireland. Department of Zoology, Ecology & Plant Science University College Cork, Dublin, Ireland.

Dando, P. R., A. J. Southward, and E. C. Southward. 1986. Chemoautotrophic symbionts in the gills of the bivalve mollusc Lucinoma borealis and the sediment chemistry of its habitat. Proceedings of the Royal Society of London Series B-Biological Sciences 227:227-247.

De Boer W. F., H. H. T. Prins, Human exploitation and benthic community structure on a tropical intertidal flat. J. Sea Res. 48, 225 (2002).

De Casabianca, M.L., Tari, P.S., Gauchet, R., Raynaud, C., Rigollet, V., 2004. Relationships between heavy metal concentrations in sediments and eelgrass and environmental variables (*Zostera marina*, Thau lagoon, France). Vie Et Milieu-Life and Environment 54, 231-238.

De Haan, D., Zaneveld, J.S., 1959. Some notes on tides in Annabaai Harbour, Curaçao, Netherlands Antilles. Bulletin of Marine Science 9, 224-236.

De Paz, L., J. M. Neto, J. C. Marques, and A. J. Laborda. 2008. Response of intertidal macrobenthic communities to long term human induced changes in the Eo estuary (Asturias, Spain): Implications for environmental management. Marine Environmental Research 66:288-299.

Dean, R. B. and W. J. Dixon. 1951. Simplified statistics for small numbers of observations. Analytical Chemistry 23:636-638.

Dean, T. A., M. S. Stekoll, S. C. Jewett, R. O. Smith, and J. E. Hose. 1998. Eelgrass (*Zostera marina* L.) in Prince William Sound, Alaska: Effects of the Exxon Valdez oil spill. Marine Pollution Bulletin 36:201-210.

Dean, T. A. and S. C. Jewett. 2001. Habitat-specific recovery of shallow subtidal communities following the Exxon Valdez oil spill. Ecological Applications 11:1456-1471.

Deborde, J., G. Abril, A. Mouret, D. Jézéquel, G. Thouzeau, J. Clavier, G. Bachelet, and P. Anschutz. 2008. Effects of seasonal dynamics in a *Zostera noltii* meadow on phosphorus and iron cycles in a tidal mudflat (Arcachon Bay, France). Marine Ecology Progress Series 355:59-71.

Debrot, A.O., Kuenen, M.M.C.E., Dekker, K., 1998. Recent declines in the coral fauna of the Spaanse Water, Curaçao, Netherlands Antilles. Bulletin of Marine Science 63, 571-580.

Debrot, A.O., Sybesma, J., 2000. The Dutch Antilles, in: Sheppard, C.R.C. (Ed.), Seas at the millenium: an environmental evaluation. Elsevier, Amsterdam.

Deegan, L. A., D. S. Johnson, R. S. Warren, B. Peterson, J. W. Fleeger, S. Fagherazzi, and W. M. Wollheim. 2012. Coastal eutrophication as a driver of salt marsh loss. Nature 490:388-392.

Delatorrecastro, M. and P. Ronnback. 2004. Links between humans and seagrasses—an example from tropical East Africa. Ocean & Coastal Management 47:361-387.

Den Hartog, C. 1970. Seagrasses of the world. North-Holland publishing company, Amsterdam.

Den Hartog, C. and J. Kuo. 2006. Taxonomy and Biogeography of seagrasses.in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, Amsterdam.

Den Hartog, C. and P. J. G. Polderman. 1975. Changes in the seagrass populations of the Dutch Wadden Sea. Aquatic Botany 1.

Di Carlo, G., Kenworthy, W.J., 2008. Evaluation of aboveground and belowground biomass recovery in physically disturbed seagrass beds. Oecologia 158, 285-298.

Diaz J. M., 2003 in Las Praderas de Pastos Marinos en Colombia Estructura y Distribución de un Ecosistema Estratégico, J. M. Díaz Merlano, L. M. Barrios Suárez, D. I. Gómez-LópezDiaz, Eds. (Instituto de Investigaciones Marinas y Costeras "José Benito Vives De Andréis" Santa Marta, Columbia), vol. 10, pp. 159.

Diaz, J. M. 2003. Diversidad do moluscos en una pradera de *Thalassia testudinum* en el parque Nacional Natural Tayrona. Page 159 in J. M. Díaz Merlano, L. M. Barrios Suárez, and D. I. Gómez-LópezDiaz, editors. Las Praderas de Pastos Marinos en Colombia Estructura y Distribución de un Ecosistema Estratégico. Instituto de Investigaciones Marinas y Costeras "José Benito Vives De Andréis" Santa Marta, Columbia.

Díaz, O. D. and I. Liñero-Arana. 2004. Mollusks community associated to *Thalassia testudinum* (Bank et Köning 1805), in Mochima Bay, Venezuela. Acta Científica Venezolana 55:44-55.

Distel, D. L. 1998. Evolution of chemoautotrophic endosymbioses in bivalves - Bivalve-bacteria chemosymbioses are phylogenetically diverse but morphologically similar. Bioscience 48:277-286.

Dobson, A., D. Lodge, J. Alder, G. S. Cumming, J. Keymer, J. McGlade, H. Mooney, J. A. Rusak, O. Sala, V. Wolters, D. Wall, R. Winfree, and M. A. Xenopoulos. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. Ecology 87:1915-1924.

Drifmeyer, J.E., Thayer, G.W., Cross, F.A., Zieman, J.C., 1980. Cycling of manganese, iron, copper and zinc by eelgrass *Zostera marina*. American Journal of Botany 67, 1089-1096.

Duarte, C. M. 1989. Temporal biomass variability and reproduction biomass relationschip of seagrass communities. Marine Ecology Progress Series 51:269-276.

Duarte, C. M. 1990. Seagrass nutrient content. Marine Ecology-Progress Series 67:201-207.

Duarte, C.M., Sand-Jensen, K., 1990. Seagrass colonization - patch formation and patch growth in *Cymodocea nodosa*. Marine Ecology-Progress Series 65, 193-200.

Duarte, C. M. 1991. Seagrass depth limits. Aquatic Botany 40:363-377.

Duarte, C.M., 1991. Allometric scaling of seagrass form and productivity. Marine Ecology Progress Series 77, 289-300.

Duarte, C. M. and C. L. Chiscano. 1999. Seagrass biomass and production: a reassessment. Aquatic Botany 65:159-174.

Duarte, C.M., 2002. The future of seagrass meadows. Environmental Conservation 29, 192-206.

Duarte, C. M., J. W. Fourqurean, D. Krause-Jensen, and B. Olesen. 2006. Dynamics of seagrass stability and change.in A. W. D. Larkum, R. J. Orth, and A. C. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163-182.

Dumon, J.C., Lapaquellerie, Y., Latouche, C., 1994. Trace-elements of algae and marine phanerogams (*Zostera*) of the Arcachon marine lagoon. Vie Milieu 3/4, 167-183.

Dutra G. F., G. R. Allen, T. Werner, S. A. McKenna, A Rapid Marine Biodiversity Assessment of the Abrolhos Bank, Bahia, Brazil. Rapid Assesment Program, Bulletin of Biological Assesment (Washington, USA, 2005), vol. 38, pp. 160.

E

Edgar, G. J., C. Shaw, G. F. Watson, and L. S. Hammond. 1994. Comparisons of species richness, size-structure and production of benthos in vegetated and unvegetated habitats in Western Fort, Victoria. Journal of Experimental Marine Biology and Ecology 176:201-226.

Edgar, G. J., N. S. Barrett, and D. J. Gradon. 1999. A classification of Tasmanian estuaries and assessment of their conservation significance using ecological and physical attributes, population and land use. Published by the Marine Research Laboratories, University of Tasmania Tasmania.

Edgar, G. J., N. S. Barrett, and P. R. Last. 1999. The distribution of macroinvertebrates and fishes in Tasmanian estuaries. Journal of Biogeography 26:1169-1189.

Eisler, R. 1981. Trace metal concentrations in marine organisms. Pergamon Press, New York.

Eklof, J. S., M. de la Torre-Castro, L. Adelskold, N. S. Jiddawi, and N. Kautsky. 2005. Differences in macrofaunal and seagrass assemblages in seagrass beds with and without seaweed farms. Estuarine Coastal and Shelf Science 63:385-396.

Eklof, J. S., T. van der Heide, S. Donadi, E. M. van der Zee, R. O'Hara, and B. K. Eriksson. 2011. Habitat-Mediated Facilitation and Counteracting Ecosystem Engineering Interactively Influence Ecosystem Responses to Disturbance. Plos One 6.

Elliott, M., D. Burdon, K. L. Hemingway, and S. E. Apitz. 2007. Estuarine, coastal and marine ecosystem restoration: Confusing management and science – A revision of concepts. Estuarine, Coastal and Shelf Science 74:349-366.

Ellison, A. M., E. J. Farnsworth, and R. R. Twilley. 1996. Facultative mutualism between red mangroves and root-fouling sponges in Belizean mangal. Ecology 77:2431-2444.

Erftemeijer, P.L.A., Middelburg, J.J., 1993. Sediment-nutrient interactions in tropical seagrass beds - a comparison between a terrigenous and a carbonate sedimentary environment in South Sulawesi (Indonesia). Marine Ecology Progress Series 102, 187-198.

Erftemeijer, P.L.A., Lewis, R.R.R., 2006. Environmental impacts of dredging on seagrasses: a review. Marine Pollution Bulletin 52, 1553-1572.

Eriksson, B. K., T. van der Heide, J. van de Koppel, T. Piersma, H. W. van der Veer, and H. Olff. 2010. Major Changes in the Ecology of the Wadden Sea: Human Impacts, Ecosystem Engineering and Sediment Dynamics. Ecosystems 13:752-764.

Erskine, J.M., Koch, M.S., 2000. Sulfide effects on *Thalassia testudinum* carbon balance and adenylate energy charge. Aquatic Botany 67, 275-285.

Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. B. Shurin, A. R. E. Sinclair, M. E. Soule, R. Virtanen, and D. A. Wardle. 2011. Trophic downgrading of planet earth. Science 333:301-306.

Ewanchuk, P. J. and M. D. Bertness. 2004. Structure and organization of a north England salt marsh plant community. Journal of Ecology 92:72-85.

F

Ferdie, M., Fourqurean, J., 2004. Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. Limnology and Oceanography 49, 2082-2094.

Ferguson, C. A. and A. I. Miller. 2007. A sea change in Smuggler's Cove? Detection of decadal-scale compositional transitions in the subfossil record. Palaeogeography Palaeoclimatology Palaeoecology 254:418-429.

Ferrat, L., Pergent-Martini, C., Roméo, M., 2003. Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. Aquatic Toxicology 65, 187-204.

Feulner, G. R. and R. J. Hornby. 2006. Intertidal molluscs in UAE lagoons. Tribulus 16:17-23.

Field, A. P., J. Miles, and Z. Field. 2012. Discovering statistics using R. Sage, London.

Field, A.P., 2005. Discovering statistics using SPSS, 2nd edition ed. Sage Publications, London.

Fisher, M. R. and S. C. Hand. 1984. Chemoautotrophic symbionts in the bivalve Lucina floridana from seagrass beds. Biological Bulletin 167:445-459.

Flach, E. C. and J. J. Beukeman. 1994. Density-governing mechanisms in populations of the lugworm *Arenicola marina* on tidal flats. Marine Ecology Progress Series 115:139-149.

Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C. S. Holling. 2004. Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. Annual Review of Ecology, Evolution, and Systematics 35:557-581.

Folmer, E., M. Geest, E. Jansen, H. Olff, T. Michael Anderson, T. Piersma, and J. Gils. 2012. Seagrass–Sediment Feedback: An Exploration Using a Non-recursive Structural Equation Model. Ecosystems 15:1380-1393.

Fonseca, M. 1989. Sediment stabilization by Halophila decipiens in comparison to other seagrasses. Estuarine Coastal and Shelf Science 29:501-507.

Fonseca, M. S. and J. A. Cahalan. 1992. A preliminary evaluation of wave attenuation by 4 species of seagrass. Estuarine Coastal and Shelf Science 35:565-576.

Fonseca, M. S., W. J. Kenworthy, and G. W. Thayer. 1998. Guidelines for the Conservation and Restoration of Seagrasses in the United States and Adjacent Waters. US Dept. of Commerce, National Oceanic and Atmospheric Administration, Coastal Ocean Office.

Fonseca, M. S., W. J. Kenworthy, B. E. Julius, S. Shutler, and S. Fluke. 2001. Chapter 7: Seagrasses.in M. R. Perrow, editor. Handbook of Ecological Restoration. Cambridge University Press, Cambridge.

Fourqurean, J.W., Zieman, J.C., 1991. Photosynthesis, respiration and whole plant carbon budget of the seagrass *Thalassia testudinum* Marine Ecology Progress Series 69, 161-170.

Fourqurean, J., Zieman, J.C., Powell, G.V.N., 1992. Relationships between porewater nutrients and seagrasses in a subtropical carbonate environment. Marine Biology 114, 57-65.

Fourqurean, J.W., Zieman, J.C., Powell, G.V.N., 1992. Phosphorus limitation of primary production in Florida Bay - evidence from C-N-P ratios of the dominant seagrass *Thalassia testudinum*. Limnology and Oceanography 37, 162-171.

Fourqurean, J.W., Cai, Y., 2001. Arsenic and phosphorus in seagrass leaves from the Gulf of Mexico. Aquatic Botany 71, 247-258.

Fourqurean, J. W. and J. C. Zieman. 2002. Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida Keys USA. Biogeochemistry 61:229-245.

Fourqurean, J. W., C. M. Duarte, H. Kennedy, N. Marba, M. Holmer, M. Angel Mateo, E. T. Apostolaki, G. A. Kendrick, D. Krause-Jensen, K. J. MCGlathery, and O. Serrano. 2012. Seagrass ecosystens as a globally significant carbon stock. Nature Geoscience 5:505-509.

Frederiksen, M., 2004. Spatial and temporal variation in eelgrass (*Zostera marina*) landscapes: influence of physical setting. Aquatic Botany 78, 147-165.

Fredriksen, S., A. De Backer, C. Bostrom, and H. Christie. 2010. Infauna from *Zostera marina* L. meadows in Norway. Differences in vegetated and unvegetated areas. Marine Biology Research 6:189-200.

G

Gacia, E. and C. M. Duarte. 2001. Sediment retention by a mediterranean *Posidonia oceanica* meadow: The balance between deposition and resuspension. Estuarine Coastal and Shelf Science 52:505-514.

Gacia, E., C. M. Duarte, and J. J. Middelburg. 2002. Carbon and nutrient deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. Limnology and Oceanography 47:23-32.

Gallegos, M.E., Merino, M., Marba, N., Duarte, C.M., 1993. Biomass and dynamic of *Thalassia testudinum* in the Mexican Caribbean: elucidating rhizome growth. Marine Ecology Progress Series 95, 185-192.

Gallegos, M.E., Merino, M., Rodriguez, A., Marba, N., Duarte, C.M., 1994. Growth patterns and demography of pioneer Caribbean seagrasses *Halodule wrightii* and *Syringodium filiforme*. Marine Ecology Progress Series 109, 99-104.

Garcia, R., Holmer, M., Duarte, C.M., Marba, N., 2013. Global warming enhances sulphide stress in a key seagrass species (NW Mediterranean). Global Change Biology 19, 3629-3639.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, O. Afik, I. Bartomeus, F. Benjamin, V. Boreux, D. Cariveau, N. P. Chacoff, J. H. Dudenhöffer, B. M. Freitas, J. Ghazoul, S. Greenleaf, J. Hipólito, A. Holzschuh, B. Howlett, R. Isaacs, S. K. Javorek, C. M. Kennedy, K. M. Krewenka, S. Krishnan, Y. Mandelik, M. M. Mayfield, I. Motzke, T. Munyuli, B. A. Nault, M. Otieno, J. Petersen, G. Pisanty, S. G. Potts, R. Rader, T. H. Ricketts, M. Rundlöf, C. L. Seymour, C. Schüepp, H. Szentgyörgyi, H. Taki, T. Tscharntke, C. H. Vergara, B. F. Viana, T. C. Wanger, C. Westphal, N. Williams, and A. M. Klein. 2013. Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science 339:1608-1611.

Gast, G.J., Jonkers, P.J., Van Duyl, F.C., Bak, R.P.M., 1999. Bacteria, Flagellates and nutrients in island fringing coral reef waters: influence of the ocean, the reef and eutrophication. Bulletin of Marine Science 65, 523-538.

Geurts, J. J. M., J. M. Sarneel, B. J. C. Willers, J. G. M. Roelofs, J. T. A. Verhoeven, and L. P. M. Lamers. 2009. Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: A mesocosm experiment. Environmental Pollution 157:2072-2081.

Gibbs, P. J., G. B. Maguire, and L. C. Collett. 1984. The macrobenthic fauna of Halophila seagrass meadows in New South Wales. Wetlands (Australia) 4:23-32.

Giesen, W. B. J. T. and M. M. van Katwijk. 1990. Temperature, salinity, insolation and wasting disease of eelgrass (*Zostera marina* L.) in the Dutch Wadden Sea in the 1930s. Journal of Sea Research 25:395-404.

Giesen, W. B. J. T., K. Giesen, P. T. Giesen, L. L. Govers, W. Suykerbuyk, and M. M. van Katwijk. 2012. Zeegrasmitigaties Oosterschelde- proeven met verplaatsen van klein zeegras *Zostera noltii*i in de Oosterschelde: mitigatiemaatregel bij toekomstige dijkwerkzaamheden. Radboud Universiteit Nijmegen, Nijmegen.

Gillanders, B. M. 2006. Seagrasses, Fish, and Fisheries.in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. Seagrasses: biology, ecology and conservation. Springer, Dordrecht.

Gillis, L. G., T. J. Bouma, C. G. Jones, M. M. van Katwijk, I. Nagelkerken, C. J. L. Jeuken, P. M. J. Herman, and A. D. Ziegler. 2014. Potential for landscape-scale positive interactions among tropical marine ecosystems: a review. Marine Ecology Progress Series 503:289-303.

Glover, E. A. and J. D. Taylor. 2007. Diversity of chemosymbiotic bivalves on coral reefs: Lucinidae (Mollusca, Bivalvia) of New Caledonia and Lifou. Zoosystema 29:109-181.

Goecker, M. E., K. L. Heck, and J. F. Valentine. 2005. Effects of nitrogen concentrations in turtlegrass *Thalassia testudinum* on consumption by bucktooth parrotfish Sparisoma radians. Marine Ecology Progress Series 286:239-248.

Gonzalez-Correa, J.M., Bayle, J.T., Sanchez-Lizasa, J.L., Valle, C., Sanchez-Jerez, P., Ruiz, J.M., 2005. Recovery of deep *Posidonia oceanica* meadows degraded by trawling. Journal of Experimental Marine Biology and Ecology 320.

Gophinadha-Pillai, C. S. and K. K. Appukuttan. 1980. Distribution of molluscs in and around the coral reefs of the southeastern coast in India. Journal of the Bombay Natural History Society 77:26-48.

Gosh, W. and B. Dam. 2009. Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea. FEMS Microbiology Reviews 33:99-1043.

Gosselin, M., Bouquegneau, J.M., Lefebvre, F., Lepoint, G., Pergent, G., Pergent-Martini, C., Gobert, S., 2006. Trace metal concentrations in *Posidonia oceanica* of North Corsica (northwestern Mediterranean Sea) use as a biological monitor? BMC ecology 6, 12.

Govers, L.L., de Brouwer, J.H.F., Suykerbuyk, W., Bouma, T.J., Lamers, L.P.M., Smolders, A.J.P., van Katwijk, M.M., 2014. Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. Aquatic Toxicology 155, 253-260.

Govers, L.L., Lamers, L.P.M., Bouma, T.J., Eygensteyn, J., de Brouwer, J.H.F., Hendriks, A.J., Huijbers, C.M., van Katwijk, M.M., 2014. Seagrasses as indicators for coastal trace metal pollution: a global meta-analysis serving as a benchmark, and a Caribbean case study. Environmental Pollution 195, 210-217.

Govers, L.L., Pieck, T., Bouma, T.J., Suykerbuyk, W., Smolders, A., van Katwijk, M.M., 2014. Seagrasses are negatively affected by organic matter loading and *Arenicola marina* activity in a laboratory experiment. Oecologia 175, 677-685.

Granata, T. C., T. Serra, J. Colomer, X. Casamitjana, C. M. Duarte, and E. Gacia. 2001. Flow and particle distributions in a nearshore seagrass meadow before and after a storm. Marine Ecology-Progress Series 218:95-106.

Gray, J.S., Elliott, M., 2009. Ecology of marine sediments. Oxford University Press, New York.

Green, E. P. and F. T. Short. 2003. World Atlas of Seagrasses. University of California Press, Berkeley, California.

Greenway, M. 1995. Trophic relationship of macrofauna within a Jamaican seagrass meadow and the role of the echinoid Lytechinus variegatus (Lamarck). Bulletin of Marine Science 56:719-736.

Greig, D. and M. Travisano. 2008. Density-dependent effects on allelopathic interactions in yeast. Evolution 62:521-527.

Grilo, T. F., P. G. Cardoso, and M. A. Pardal. 2012. Implications of *Zostera noltii* recolonization on *Hydrobia ulvae* population structure success. Marine Environmental Research 73:78-84.

Gros, O., M. Liberge, and H. Felbeck. 2003. Interspecific infection of aposymbiotic juveniles of Codakia orbicularis by various tropical lucinid gill-endosymbionts. Marine Biology 142:57-66.

Grossmann, S. and W. Reichardt. 1991. Impact of *Arenicola marina* on bacteria in intertidal sediments. Marine Ecology Progress Series 77:85-93.

Gunderson, L. H. 2000. Ecological resilience- in theory and application. Annual Rev. Ecol. Syst. 31:425-439.

Guven, K.C., Saygi, N., Öztürk, B., 1993. Survey of metal contents of Bosphorus algae, *Zostera marina* and sediments. Botanica Marina 36, 175-178.

Guzman, H. M. and E. M. Garcia. 2002. Mercury levels in coral reefs along the Caribbean coast of Central America. Marine Pollution Bulletin 44:1451-1420.

H

Han, Q. Y., T. J. Bouma, F. G. Brun, W. Suykerbuyk, and M. M. van Katwijk. 2012. Resilience of *Zostera noltii* to burial or erosion disturbances. Marine Ecology Progress Series 449:133-143.

Hansen, J. C. R., and M. A. Reidenbach. 2012. Wave and tidally driven flows in eelgrass beds and their effect on sediment suspension. Marine Ecology-Progress Series 448:271-287.

Hansen, K., G. M. King, and E. Kristensen. 1996. Impact of the soft-shell clam Mya arenaria on sulfate reduction in an intertidal sediment. Aquatic Microbial Ecology 10:181-194.

Harriague, A. C., C. N. Bianchi, and G. Albertelli. 2006. Soft-bottom macrobenthic community composition and biomass in a *Posidonia oceanica* meadow in the Ligurian Sea (NW Mediterranean). Estuarine Coastal and Shelf Science 70:251-258.

Hauser, I., W. Oschmann, and E. Gischler. 2007. Modern bivalve shell assemblages on three atolls offshore Belize (Central America, Caribbean Sea). Facies 53:451-478.

Hauxwell, J., Cebrian, J., Furlong, C., Valiela, I., 2001. Macroalgal Canopies Contribute to Eelgrass (*Zostera marina*) Decline in Temperate Estuarine Ecosystems. Ecology 82, 1007-1022.

Hauxwell, J., J. Cébrian, and I. Valiela. 2003. Eelgrass (*Zostera marina* L.) loss in temperate estuaries: relationship to land-derived nitrogen loads and effects of light limitation imposed by algae. Marine Ecology Progress Series 247:59-73.

Heck, K. L., G. Hays, and R. J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology-Progress Series 253:123-136.

Heck, K. L. and J. Valentine. 2006. Plant–herbivore interactions in seagrass meadows. Journal of Experimental Marine Biology and Ecology 330:420-436.

Hemminga, M.A., 1998. The root/rhizome system of seagrasses: an asset and a burden. J. Sea Res. 39, 183-196.

Hemminga, M.A., Duarte, A.C., 2000. Seagrass Ecology. Cambridge University press, Cambridge.

Hendriks, I. E., T. Sintes, T. J. Bouma, and C. M. Duarte. 2008. Experimental assessment and modeling evaluation of the effects of the seagrass *Posidonia oceanica* on flow and particle trapping. Marine Ecology Progress Series 356:163-173.

Hendriks, I. E., T. J. Bouma, E. P. Morris, and C. M. Duarte. 2010. Effects of seagrasses and algae of the Caulerpa family on hydrodynamics and particle-trapping rates. Marine Biology 157:473-481.

Herzka, S.Z., Dunton, K.H., 1997. Seasonal photosynthetic patterns of the seagrass *Thalassia testudinum* in the western gulf of Mexico. Marine Ecology Progress Series 152, 103-117.

Hily, C. and M. Bouteille. 1999. Modifications of the specific diversity and feeding guilds in an intertidal sediment colonized by an eelgrass meadow (*Zostera marina*) (Brittany, France). Comptes Rendus De L'Academie Des Sciences- Serie III- Sciences De La Vie-Life Sciences 322:1121-1131.

Hines, M. E. and G. E. Jones. 1985. Microbial biogeochemistry and bioturbation in the sediments of Great Bay, New Hampshire. Estuarine Coastal and Shelf Science 20:729-742.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira, N. Knowlton, C. M. Eakin, R. Iglesias-Prieto, N. Muthiga, R. H. Bradbury, A. Dubi, and M. E. Hatziolos. 2007. Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742Hoegh-Guldberg, O. 2014. Coral reef sustainability through adaptation: glimmer of hope or persistent mirage? Current Opinion in Environmental Sustainability 7:127-133.

Hoey, A. S. and D. R. Bellwood. 2011. Suppression of herbivory by macroalgal density: a critical feedback on coral reefs? Ecol Lett 14:267-273.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual Rev. Ecol. Syst. 4:1-23.

Holmer, M. and S. L. Nielsen. 1997. Sediment sulfur dynamics related to biomass-density patterns in *Zostera marina* (eelgrass) beds. Marine Ecology-Progress Series 146:163-171.

Holmer, M., Bondgaard, E.J., 2001. Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. Aquatic Botany 70, 29-38.

Holmer, M., F. O. Andersen, S. L. Nielsen, and H. T. S. Boschker. 2001. The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments. Aquatic Botany 71:1-17.

Holmer, M., P. Wirachwong, and M. S. Thomsen. 2011. Negative effects of stress-resistant drift algae and high temperature on a small ephemeral seagrass species. Marine Biology 158:297-309.

Holmer, M., Kendrick, G., 2013. High sulfide intrusion in five temperate seagrass species growing under contrasting sediment conditions. Estuaries and Coasts 36, 116-126.

Honkoop, P. J. C., E. M. Berghuis, S. Holthuijsen, M. S. S. Lavaleye, and T. Piersma. 2008. Molluscan assemblages of seagrass-covered and bare intertidal flats on the Banc d'Arguin, Mauritania, in relation to characteristics of sediment and organic matter. Journal of Sea Research 60:235-243.

Howarth, J.F., Durako, M.J., 2012. Diurnal variation in chlorophyll fluorescence of *Thalassia testudinum* seedlings in response to controlled salinity and light conditions. Marine Biology 160, 591-605.

Huang, X. 2008. National report on seagrass in the South China Sea- China. South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China.

Hughes, T. P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265:1547-1551.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J. B. C. Jackson, J. Kleypas, J. M. Lough, P. Marshall, M. Nystrom, S. R. Palumbi, J. M. Pandolfi, B. Rosen, and J. Roughgarden. 2003. Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933.

Hughes, B. B., R. Eby, E. Van Dyke, T. M. Tinker, C. I. Marks, K. S. Johnson, and K. Wasson. 2013. Recovery of a top predator mediates negative eutrophic effects on seagrass. Proceedings of the National Academy of Sciences 110.

Huijbers, C.M., Nagelkerken, I., Debrot, A.O., Jongejans, E., 2013. Geographic coupling of juvenile and adult habitat shapes spatial population dynamics of a coral reef fish. Ecology 94, 1859-1870.

Huisman, T. J., F. Van Langevelde, and W. F. De Boer. 2009. Local positive feedback and the persistence and recovery of fringe Avicennia marina (Forssk.) vierh. mangroves. Wetlands Ecology and Management 17:601-611.

Hutchings P. A., Wells F. E., W. D. I., G. A. Kendrick, in The Flora and Fauna of the Albany Area, Western Australia, W. F. E., W. D. I., H. Kirkman, R. Lethbridge, Eds. (Western Australian Museum, Perth, 1991), pp. 611-633.

Hutchings, P. A., Wells F. E., W. D. I., and G. A. Kendrick. 1991. Seagrass, sediment and infauna – a comparison of Posidonia australis, Posidonia sinuosa and *Amphibolis antarctica* in Princess Royal Harbour, South–Western Australia. III. Consequences of seagrass loss. Pages 611-633 in W. F. E., W. D. I., H. Kirkman, and R. Lethbridge, editors. The Flora and Fauna of the Albany Area, Western Australia. Western Australian Museum, Perth.

Hylleberg, J. 1975. Influence of the lugworm *Arenicola marina* on porewater nutrient profiles of sandflat sediments. Marine Ecology Progress Series 62:241-248.

I

Ikem, A., Egiebor, N.O., 2005. Assessment of trace elements in canned fishes marketed in Georgia and Alabama. journal of Food Composition 18, 771-787.

Irvine, I. and G. F. Birch. 1998. Distribution of heavy metals in surficial sediments of Port Jackson, Sydney, New South Wales. Australian Journal of Earth Sciences 45:297-304.

Isaksen, M. F. and K. Finster. 1996. Sulphate reduction in the root zone of the seagrass *Zostera noltii* on the intertidal flats of a coastal lagoon (Arcachon, France). Marine Ecology-Progress Series 137:187-194.

J

Jackson, J. B. C. 1972. The ecology of the molluscs of *Thalassia* communities, Jamaica, West Indies. II. Molluscan population variability along an environmental stress gradient. Marine Biology 14:304-&.

Jackson, J. B. C. 2001. Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science 293:629-637.

Jacobs, R., H. H. Hegger, and A. Raswillems. 1983. Seasonal variations in the structure of a *Zostera* community in tidal flats in the SW Netherlands, with special reference to the benthic fauna. Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences 86:347-375.

Jensen, H.S., McGlathery, K.J., Marino, R., Howarth, R.W., 1998. Forms and availability of sediment phosphorus in carbonate sand of bermuda seagrass beds Limnology and Oceanography 43, 799-810.

Jewett, S. C., T. A. Dean, R. O. Smith, and A. Blanchard. 1999. 'Exxon Valdez' oil spill: impacts and recovery in the soft-bottom benthic community in and adjacent to eelgrass beds. Marine Ecology-Progress Series 185:59-83.

Johnson, M., M. Diouris, and M. Lepennec. 1994. Endosymbiotic bacterial contribution in the carbon nutrition of *Loripes lucinalis* (Mollusca: Bivalvia). Symbiosis 17:1-13.

Johnson, M. A., C. Fernandez, and G. Pergent. 2002. The ecological importance of an invertebrate chemoautotrophic symbiosis to phanerogam seagrass beds. Bulletin of Marine Science 71:1343-1351.

Johnson, N. C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185:631-647.

Jones, C. G., J. H. Lawton, and M. Shackak. 1994. Organisms as ecosystem engineers. Oikos 69:373-386.

Jorgensen, B. B. 1982. Mineralization of organic matter in the sea bed – The role of sulphate reduction. Nature 296:643-645.

Jorgensen, B. B. 2006. Bacteria and marine biogeochemistry. 2nd edition. Springer, Berlin Heidelberg New York.

K

Kaldy, J.E., 2006. Carbon, nitrogen, phosphorus and heavy metal budgets: How large is the eelgrass (*Zostera marina* L.) sink in a temperate estuary? Marine Pollution Bulletin 52, 342-353.

Kannan, R.R.R., Arumugam, R., Anantharaman, P., 2011. Chemometric Studies of Multielemental Composition of Few Seagrasses from Gulf of Mannar, India. Biological Trace Element Research 143, 1149-1158.

Kefi, S., E. L. Berlow, E. A. Wieters, S. A. Navarrete, O. L. Petchey, S. A. Wood, A. Boit, L. N. Joppa, K. D. Lafferty, R. J. Williams, N. D. Martinez, B. A. Menge, C. A. Blanchette, A. C. Iles, and U. Brose. 2012. More than a meal ... integrating non-feeding interactions into food webs. Ecology Letters 15:291-300.

Kemp, W. M., W. R. Boynton, J. E. Adolf, D. F. Boesch, W. C. Boicourt, G. Brush, J. C. Cornwell, T. R. Fisher, P. M. Glibert, J. D. Hagy, L. W. Harding, E. D. Houde, D. G. Kimmel, W. D. Miller, R. I. E. Newell, M. R. Roman, E. M. Smith, and J. C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology Progress Series 303:1-29.

Kenworthy, W. J., M. Fonseca, P. E. Withfield, and K. K. Hammerstrom. 2002. Analysis of seagrass recovery in experimental excavations and propeller-scar disturbances in the Florida Keys National Marine Sanctuary. Journal of Coastal Research 37:75-85.

Kharlamenko, V. I., S. I. Kiyashko, A. B. Imbs, and D. I. Vyshkvartzev. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology-Progress Series 220:103-117.

Kiers, E. T., T. M. Palmer, A. R. Ives, J. F. Bruno, and J. L. Bronstein. 2010. Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13:1459-1474.

Koch, E. M. 2001. Beyond light: Physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24:1-17.

Koch, M. S., I. A. Mendelssohn, and K. L. McKee. 1990. Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnology and Oceanography 35:399-408.

Koch, M.S., Erskine, J.M., 2001. Sulfide as a phytotoxin to the tropical seagrass *Thalassia testudinum*: interactions with light, salinity and temperature. Journal of Experimental Marine Biology and Ecology 266, 81-95.

Koch, E. W., J. D. Ackerman, J. Verduin, and M. van Keulen. 2007. Fluid dynamics in seagrass ecology- from molecules to ecosystems.in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. Seagrasses: Biology, Ecology, and Conservation. Springer, Amsterdam.

Koch, M. S., S. Schopmeyer, C. Kyhn-Hansen, and C. J. Madden. 2007. Synergistic effects of high temperature and sulfide on tropical seagrass. Journal of Experimental Marine Biology and Ecology 341:91-101.

Koch, E. W., E. B. Barbier, B. R. Silliman, D. J. Reed, G. M. Perillo, S. D. Hacker, E. F. Granek, J. H. Primavera, N. Muthiga, S. Polasky, B. S. Halpern, C. J. Kennedy, C. V. Kappel, and E. Wolanski. 2009. Non-linearity in ecosystem-services: temporal and spatial variability in coastal protection. Frontiers in Ecology and the Environment 7:29-37.

Kristensen, E. 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426:1-24.

Kristensen, E., F. O. Andersen, N. Holmboe, M. Holmer, and N. Thongtham. 2000. Carbon and nitrogen mineralization in sediments of the Bangrong mangrove area, Phuket, Thailand. Aquatic Microbial Ecology 22:199-213.

Kristensen, E., G. Penha-Lopes, M. Delefosse, T. Valdemarsen, C. O. Quintana, and G. T. Banta. 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Marine Ecology Progress Series 446:285-302.

Kuenen, M., Debrot, A.O., 1995. A quantitative study of the seagrass and algal meadows of the Spaanse Water, Curacao, the Netherlands Antilles. Aquatic Botany 51, 291-310.

Kuriandewa, T. E. 2008. National report on seagrass in the South China Sea - Indonesia. Puslit Oseanografi, Lipi, Jakarta, Indonesia.

L

Lafabrie, C., Pergent, G., Kantin, R., Pergent-Martini, C., Gonzalez, J.L., 2007. Trace metals assessment in water, sediment, mussel and seagrass species - Validation of the use of *Posidonia oceanica* as a metal biomonitor. Chemosphere 68, 2033-2039.

Lafabrie, C., Pergent-Martini, C., Pergent, G., 2008. Metal contamination of *Posidonia oceanica* meadows along the Corsican coastline (Mediterranean). Environmental Pollution 151, 262-268.

Lafabrie, C., Pergent-Martini, C., Pergent, G., 2008 First results on the study of metal contamination along the Corsican coastline using *Posidonia oceanica*. Marine Pollution Bulletin 57, 155-159.

Lamers, L. P. M., H. B. M. Tomassen, and J. G. M. Roelofs. 1998. Sulfate-induced entrophication and phytotoxicity in freshwater wetlands. Environmental Science & Technology 32:199-205.

Lamers, L. P., L. L. Govers, I. C. Janssen, J. J. Geurts, M. E. Van der Welle, M. M. Van Katwijk, T. Van der Heide, J. G. Roelofs, and A. J. Smolders. 2013. Sulfide as a soil phytotoxin - a review. Front Plant Science 4:268.

Larkum, A. W. D., R. J. Orth, and C. M. Duarte. 2006. Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands.

Layman, C. A., J. E. Allgeier, L. A. Yeager, and E. W. Stoner. 2013. Thresholds of ecosystem response to nutrient enrichment from fish aggregations. Ecology 94:530-536.

Les, D. H., M. A. Cleland, and M. Waycott. 1997. Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (seagrasses) and hydrophily. Systematic Botany 22:443-463.

Lee, R. W. 1999. Oxidation of sulfide by Spartina alterniflora roots. Limnology and Oceanography 44:1155-1159.

Lee, K.-S., Short, F.T., Burdick, D.M., 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. Aquatic Botany 78, 197-216.

Leschen, A. S., R. K. Kessler, and B. T. Estrella. 2009. Eelgrass restoration used as construction impact mitigation in Boston harbor, Massachusetts. Massachusetts Division of Marine Fisheries, New Bedford.

Leuschner, C., S. Landwehr, and U. Mehlig. 1998. Limitation of carbon assimilation of intertidal *Zostera noltii* and *Z. marina* by desiccation at low tide. Aquatic Botany 62:171-176.

Lewis, F. G. and A. W. Stoner. 1981. An examintation of methods for sampling macrobenthos in seagrass meadows. Bulletin of Marine Science 31:116-124.

Lewis, M.A., Dantin, D.D., Chancy, C.A., Abel, K.C., Lewis, C.G., 2007. Florida seagrass habitat evaluation: A comparative survey for chemical quality. Environmental Pollution 146, 206-218.

Lewis, M. and R. Pryor. 2013. Toxicities of oils, dispersants and dispersed oils to algae and aquatic plants: Review and database value to resource sustainability. Environmental Pollution 180:345-367.

Leyrer, J., T. Lok, M. Brugge, A. Dekinga, B. Spaans, J. A. van Gils, B. K. Sandercock, and T. Piersma. 2012. Small-scale demographic structure suggests preemptive behavior in a flocking shorebird. Behavioral Ecology 23:1226-1233.

Li, Q., Yu, Z., Chu, B., Zhang, N., Cai, S., Fang, J., 2007. Heavy metals in coastal wetland sediments of the Pearl River Estuary, China. Environmental Pollution 149, 158-164.

Li, L., Huang, X., 2012. Three tropical seagrasses as potential bio-indicators to trace metals in Xincun Bay, Hainan Island, South China. Chinese Journal of Oceanology and Limnology 30, 212-224.

Liljedahl, L. 1991. Contrasting feeding strategies in bivalves from the Silurian of Gotland. Palaeontology 34:219-235.

Linton, D.M., Warner, G.F., 2003. Biological indicators in the Caribbean coastal zone and their role in integrated coastal management. Ocean & Coastal Management 46, 261-276.

Lopez y Royo, C., Pergent, G., Alcoverro, T., Buia, M.C., Casazza, G., Martínez-Crego, B., Pérez, M., Silvestre, F., Romero, J., 2011. The seagrass *Posidonia oceanica* as indicator of coastal water quality: Experimental intercalibration of classification systems. Ecological Indicators 11, 557-563.

Loya, Sakai, Yamazato, Nakano, Sambali, and W. Van. 2001. Coral bleaching: the winners and the losers. Ecology Letters 4:122-131.

Lucas, A. and P. G. Beninger. 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44:187-200.

Lucassen, E.C.H.E.T., Smolders, A.J.P., van der Salm, A.L., Roelofs, J.G.M., 2004. High groundwater nitrate concentrations inhibit eutrophication of sulfate-rich freshwater wetlands. Biogeochemistry 67, 249-267.

M

Macfarlane, G. R. and M. D. Burchett. 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, Avicennia marina (Forsk.) Vierh. Marine Pollution Bulletin 42:233-240.

Macinnis-Ng, C.M.O., Ralph, P.J., 2002. Towards a more ecologically relevant assessment of the impact of heavy metals on the photosynthesis of the seagrass, *Zostera capricorni*. Marine Pollution Bulletin 45, 100-106.

Macinnis-Ng, C.M.O., Ralph, P.J., 2004. Variations in sensitivity to copper and zinc among three isolated populations of the seagrass, *Zostera capricorni*. Journal of Experimental Marine Biology and Ecology 302, 63-83.

Macreadie, P. I., P. H. York, and C. D. Sherman. 2014. Resilience of *Zostera muelleri* seagrass to small-scale disturbances: the relative importance of asexual versus sexual recovery. Ecol Evol 4:450-461.

Madden, C.J., Rudnick, D.T., McDonald, A.A., Cunniff, K.M., Fourqurean, J.W., 2009. Ecological indicators for assessing and communicating seagrass status and trends in Florida Bay. Ecological Indicators 9, S68-S82.

Malea, P., Haritonidis, S., Kevrekidis, T., 1994. Seasonal and local variations of heavy metal concentrations in the seagrass *Posidonia Oceanica* (L) Delile in the Antikyea Gulf, Greece. Science of the Total Environment 153, 225-235.

Malea, P., 1994. Seasonal variation and local distribution of metals in the seagrass Halpohila stipulacea (Forsk) Aschers in the Antikyra gulf, Greece. Environmental Pollution 85, 77-85.

Malea, P., Haritonidis, S., 1995. Local distribution and seasonal variation of Fe, Pb, Zn, Cu, Cd, Na, K, Ca, and Mg concentrations in the seagrass *Cymodocea nodosa* (Ucria) Aschers- in the Antikyra Gulf, Greece. Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I 16, 41-56.

Marba, N., Duarte, C.M., 1998. Rhizome elongation and seagrass clonal growth. Marine Ecology-Progress Series 174, 269-280.

Marba, N., Holmer, M., Gacia, E., Barron, C., 2006. Seagrass beds and coastal biogeochemistry, in: Larkum, A.W.D., Orth, R.J., Duarte, A.C. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands.

Marin-Guirao, L., Atucha, A.M., Barba, J.L., Lopez, E.M., Fernandez, A.J., 2005. Effects of mining wastes on a seagrass ecosystem: metal accumulation and bioavailability, seagrass dynamics and associated community structure. Mar Environ Res 60, 317-337.

Marion, S.R., Orth, R.J., 2012. Seedling establishment in eelgrass: seed burial efects on winter losses of developing seedlings. Marine Ecology Progress Series 448, 197-207.

Markert, B., Wappelhorst, O., Weckert, V., Herpin, U., Siewers, U., Friese, K., Breulmann, G., 1999. The use of bioindicators for monitoring the heavy-metal status of the environment. Journal of Radioanalytical and Nuclear Chemistry 240, 425-429.

Marques, L. V. and J. C. Creed. 2000. Biologia e ecologia das Fanérogamas marinhas do Brazil. Oecologia Brasiliensis 12:315-331.

Marschner, H., 1995. Mineral nutrition of higher plants, 2nd ed. Academic Press, London.

Marsh, J.A., Dennison, W.C., Alberte, R.C., 1986. Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). Journal of Experimental Marine Biology and Ecology 101, 257-267.

Martin, P., D. Sébastien, T. Gilles, A. Isabelle, X. de Montaudouin, É. Emery, N. Claire, and V. Christophe. 2010. Long-term evolution (1988–2008) of *Zostera* spp. meadows in Arcachon Bay (Bay of Biscay). Estuarine, Coastal and Shelf Science 87:357-366.

Martínez-Crego, B., Vergés, A., Alcoverro, T., Romero, J., 2008. Selection of multiple seagrass indicators for environmental biomonitoring. Marine Ecology Progress Series 361, 93-109.

Martinez, A. S. 2008. Distribuiç ao e abundância da malacofauna epibentônica no parracho de Maracajaú, RN, Brasil. Universidade federal do Rio Grande do norte centro de biociêcias, Natal, RN Brazil.

Mascaró, O., T. Valdemarsen, M. Holmer, M. Pérez, and J. Romero. 2009. Experimental manipulation of sediment organic content and water column aeration reduces *Zostera marina* (eelgrass) growth and survival. Journal of Experimental Marine Biology and Ecology 373:26-34.

Massa, S. I., S. Arnaud-Haond, G. A. Pearson, and E. A. Serrao. 2009. Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal). Hydrobiologia 619:195-201.

Mateo, M. A., J. Romero, M. Pérez, M. M. Littler, and D. S. Littler. 1997. Dynamics of millenary organic deposits resulting from growth of the Mediterranean seagrass *Posidonia oceanica*. Estuarine Coastal and Shelf Science 44:103-110.

McCann, K. 2007. Protecting biostructure. Nature 446:29-29.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, E. H. McDowell, and G. Pinay. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-312.

McGlathery, K.J., Marino, R., Howarth, R.W., 1994. Variable rates of phosphate uptake by shallow marine carbonate sediments: mechanisms and ecological significance. Biogeochemistry 25, 127-146.

McKee, K. L., I. A. Mendelssohn, and M. W. Hester. 1988. Reexamination of pore water sulfide concentrations and redox potentials near the aerial roots of *Rhizophora mangle* and Avicennia germinans. American Journal of Botany 75:1352-1359.

McKinnon, J. G., P. E. Gribben, A. R. Davis, D. F. Jolley, and J. T. Wright. 2009. Differences in soft-sediment macrobenthic assemblages invaded by Caulerpa taxifolia compared to uninvaded habitats. Marine Ecology-Progress Series 380:59-71.

Meehan, A.J., West, R.J., 2000. Recovery times for a damaged Posidonia australis bed in south eastern Australia. Aquatic Botany 67, 161-167.

Meyer, E., B. Nilkerd, E. A. Glover, and J. D. Taylor. 2008. Ecological importance of chemoautotrophic lucinid bivalves in a peri-mangrove commnity in eastern Thailand. Raffles Bulletin of Zoology 18:41-55.

Meysman, F. J., J. J. Middelburg, and C. H. Heip. 2006. Bioturbation: a fresh look at Darwin's last idea. Trends in Ecology & Evolution 21:688-695.

Mikkelsen, P. M., P. S. Mikkelsen, and D. J. Karlen. 1995. Molluscan biodiversity in the Indian river lagoon, Florida. Bulletin of Marine Science 57:94-127.

Monnat, J. Y. 1970. Introduction á l'étude de la reproduction chez Lucinoma borealis (Linné), Bivalvia, Lucinacea. Faculté de Science, Brest.

Montefalcone, M., 2009. Ecosystem health assessment using the Mediterranean seagrass *Posidonia oceanica*: A review. Ecological Indicators 9, 595-604.

Moore, H. B., L. T. Davies, T. H. Fraser, R. H. Gore, and N. R. Lopez. 1968. Some biomass figures from a tidal flat in Biscayne bay, Florida. Bulletin of Marine Science 18:261-&.

Moore, K. A. and F. T. Short. 2007. *Zostera*: Biology, Ecology and management.in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, Amsterdam.

Mora, C., 2008. A clear human footprint in the coral reefs of the Caribbean. Proc Biol Sci 275, 767-773.

Moreno, D., Aguilera, P.A., Castro, H., 2001. Assesment of the conservation status of seagrass (*Posidonia oceanica*) meadows: implications for monitoring strategy and the decision-making process. Biological Conservation 102, 325-332.

Morillo, J., Usero, J., Gracia, I., 2004. Heavy metal distribution in marine sediments from the southwest coast of Spain. Chemosphere 55, 431-442.

Mumby, P.J., Dahlgren, C.P., Harborne, A.R., Kappel, C.V., Micheli, F., Brumbaugh, D.R., Holmes, K.E., Mendes, J.M., Broad, K., Sanchirico, J.N., Buch, K., Box, S., Stoffle, R.W., Gill, A.B., 2006. Fishing, trophic cascades, and the process of grazing on coral reefs. Science 311, 98-101.

Mumby, P.J., Hastings, A., Edwards, H.J., 2007. Thresholds and the resilience of Caribbean coral reefs. Nature 450, 98-101.

Murray, J.W., Grundmanis, V., Smethie, W.M.J., 1978. Interstitial water chemistry in the sediments of Saanich Inlet. Geochimica Et Cosmochimica Acta 42, 1011-1026.

Mutchler, T., Dunton, K.H., Townsend-Small, A., Frederiksen, S., Rasser, M.K., 2007. Isotopic and elemental indicators of nutrient sources and status of coastal habitats in the Caribbean Sea, Yucatan Peninsula, Mexico. Estuarine, Coastal and Shelf Science 74, 449-457.

N

Nagelkerken, I., Debrot, A.O., 1995. Mollusc communities of tropical ruble shores of Curaçao: Long-term (7+ years) impacts of oil pollution. Marine Pollution Bulletin 9, 592-598.

Nagelkerken, I. 2000. Importance of Mangroves, Seagrass Beds and the Shallow Coral Reef as a Nursery for Important Coral Reef Fishes, Using a Visual Census Technique. Estuarine, Coastal and Shelf Science 51:31-44.

Nagelkerken, I. 2009. Ecological connectivity among tropical coastal ecosystems. Springer Science and Business Media, Dordrecht.

Nagelkerken, I. 2010. Ecological connectivity among tropical coastal ecosystems. Springer, Dordrecht.

Nakaoka, M., H. Mukai, and S. Chunhabundit. 2002. Impacts of dugong foraging on benthic animal communities in a Thailand seagrass bed. Ecological Research 17:625-638.

Nicolaev, S. and T. Zaharia. 2011. Report on the state of the marine and coastal environment in 2010. NIMRD, Constanta, Romania.

Nicolaidou, A., Nott, J.A., 1998. Metals in sediment, seagrass and gastropods near a nickel smelter in Greece: possible interactions. Marine Pollution Bulletin 36, 360-365.

Nielsen, O. I., E. Kristensen, and M. Holmer. 2003. Impact of *Arenicola marina* (Polychaeta) on sediment sulfur dynamics. Aquatic Microbial Ecology 33:95-105.

Nienhuis, P.H., Smaal, A.C., 1994. The Oosterschelde estuary, a case-study of a changing ecosystem- An introduction. Hydrobiologia 283, 1-14.

Nienhuis, P.H., 1986. Background levels of heavy metals in 9 tropical seagrass species in Indonesia. Marine Pollution Bulletin 17, 508-511.

NOAA/OAR/ESRL/PSD. 2011. Montlhly sea surface temperature means (NOAA_OI_SST_V2). NOAA/OAR/ESRL/PSD, Boulder, Colorado, USA.

O

Okuyama, T., and J. N. Holland. 2008. Network structural properties mediate the stability of mutualistic communities. Ecology Letters 11:208-216.

Olesen, B., Sand-Jensen, K., 1994. Patch dynamics of eelgrass *Zostera marina*. Marine Ecology-Progress Series 106, 147-156.

Olive, I., Brun, F., Vergara, J., Perezllorens, J., 2007. Effects of light and biomass partitioning on growth, photosynthesis and carbohydrate content of the seagrass *Zostera noltii* Hornem. Journal of Experimental Marine Biology and Ecology 345, 90-100.

Olsen, Y.S., Valiela, I., 2010. Effect of Sediment Nutrient Enrichment and Grazing on Turtle Grass *Thalassia testudinum* in Jobos Bay, Puerto Rico. Estuaries and Coasts 33, 769-783.

Orth, R. J. 1973. Benthic infauna of eelgrass, *Zostera marina*, beds. Chesapeake Bay Science 14:258–269.

Orth, R., K. Heck, and J. van Montfrans. 1984. Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries and Coasts 7:339-350.

Orth, R. J., T. J. B. Carruthers, W. C. Dennison, C. M. Duarte, J. W. Fourqurean, K. L. J. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, S. Olyarnik, F. T. Short, M. Waycott, and S. L. Williams. 2006. A Global Crisis for Seagrass Ecosystems. Bioscience 56:987-996.

Otero Otero, A. and J. Romani Lobo. 2009. Macroinvertebrados asociados a pastos marinos (*Thalassia testudinum*) en el golfo de Morrosquillo (Zone de Berrugas) Departamento de Sucre. Universidad de Sucre, Sincelejo, Columbia.

P

Pages, A., Teasdale, P.R., Robertson, D., Bennett, W.W., Schafer, J., Welsh, D.T., 2012. Representative measurement of two-dimensional reactive phosphate distributions and co-distributed iron(II) and sulfide in seagrass sediment porewaters. Chemosphere 85, 1256-1261.

Papaspyrou, S., E. Kristensen, and B. Christensen. 2007. *Arenicola marina* (Polychaeta) and organic matter mineralisation in sandy marine sediments: *In situ* and microcosm comparison. Estuarine, Coastal and Shelf Science 72:213-222.

Pasqualini, V., Pergent-Martini, C., Pergent, G., 1999. Environmental impact identification along the Corsican coast (Mediterranean sea) using image processing. Aquatic Botany 65, 311-320.

Passarelli, C., F. Olivier, D. M. Paterson, T. Meziane, and C. Hubas. 2013. Organisms as cooperative ecosystem engineers in intertidal flats. Journal of Sea Research.

Patriquin, D.G., 1972. The origin of nitrogen and phosphorus for growth of the marine angiosperm *Thalassia testudinum*. Marine Biology 15, 34-46.

Paulay. 2000. Benthic ecology and biota of Tarawa Atoll lagoon: Influence of equatorial upwelling, circulation and human harvest. Atoll Research Bulletin 487:1-41.

Pedersen, M. F. and J. Borum. 1992. Nitrogen dynamics of eelgrass *Zostera marina* during a late summer period of high growth and low nutrient availability. Marine Ecology Progress Series 80:65-73.

Pedersen, O., Borum, J., Duarte, C.M., Fortes, M.D., 1998. Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. Marine Ecology-Progress Series 169, 283-288.

Peirano, A., V. Damasso, M. Montefalcone, C. Morri, and C. N. Bianchi. 2005. Effects of climate, invasive species and anthropogenic impacts on the growth of the seagrass *Posidonia oceanica* (L.) Delile in Liguria (NW Mediterranean Sea). Marine Pollution Bulletin 50:817-822.

Peralta, G., Bouma, T.J., van Soelen, J., Perez-Llorens, J.L., Hernandez, I., 2003. On the use of sediment fertilization for seagrass restoration: a mesocosm study on *Zostera marina* L. Aquatic Botany 75, 95-110.

Peralta, G., L. A. van Duren, E. P. Morris, and T. J. Bouma. 2008. Consequences of shoot density and stiffness for ecosystem engineering by benthic macrophytes in flow dominated areas: a hydrodynamic flume study. Marine Ecology-Progress Series 368:103-115.

Perez, D., 1995. Mercurial pollution in the seagrass *Thalassia testudinum* (Banks ex Koning). Bulletin of Marine Science 56, 707-710.

Pergent-Martini, C., 1998. *Posidonia oceanica*: a biological indicator of past and present mercury contamination in the Mediterranean Sea Marine Environmental Research 45, 101-111.

Pergent-Martini, C., Pergent, G., 2000. Are marine phanerograms a valuable tool in the evaluation of marin etrace metal contamination: example of the Mediterranean sea? International Journal of Environmental Pollution 13, 126-147.

Pergent, G., Pergent-Martini, C., 1999. Mercury levels and fluxes in *Posidonia oceanica* meadows. Environmental Pollution 106, 33-37.

Pergent, G., Labbe, C., Lafabrie, C., Kantin, R., Pergent-Martini, C., 2011. Organic and inorganic human-induced contamination of *Posidonia oceanica* meadows. Ecological Engineering 37, 999-1002.

Peterson, G., C. R. Allen, and C. S. Holling. 1998. Ecological resilience, biodiversity and scale. Ecosystems 1:6-18.

Peterson, C. H., R. A. Luettich, F. Micheli, and G. A. Skilleter. 2004. Attenuation of water flow inside seagrass canopies of differing structure. Marine Ecology Progress Series 268:81-92.

Peterson, B.J., Stubler, A.D., Wall, C.C., Gobler, C.J., 2012. Nitrogen-rich groundwater intrusion affects productivity, but not herbivory, of the tropical seagrass *Thalassia testudinum*. Aquatic Biology 15, 1-9.

Philippart, C. J. M. 1994. Interactions between *Arenicola marina* and *Zostera noltii* on a tidal flat in the Wadden Sea. Marine Ecology-Progress Series 111:251-257.

Phillips, R.C., 1992. The seagrass ecosystem and resources in Latin America, in: Seeliger, U. (Ed.), Coastal Plant Communities of Latin America. Academic Press, San Diego, California.

Pickett, S. T. A. and P. S. White. 1985. The ecology of natural disturbance and patch dynamics. Academic Press, Inc., San Diego, California.

Pickhardt, P. C., C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. Proceedings of the National Academy of Sciences 99:4419-4423.

Pimm, S. L. 1984. The complexity and stability of ecosystems. Nature 307:321-326.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-Effects Models in Sand S-PLUS. Springer New York.

Pirc, H., 1989. Seasonal changes in soluble carbohydrates, starch, and energy content in Mediterranean seagrasses. Marine Ecology 10, 97-105.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25:345-353.

Prange, J. A. and W. C. Dennison. 2000. Physiological responses of five seagrass species to trace metals. Marine Pollution Bulletin 41:327-336.

Pranovi, F., D. Curiel, A. Rismondo, M. Marzocchi, and M. Scattolin. 2000. Variations of the macrobenthic community in a seagrass transplanted area of the Lagoon of Venice. Scientia Marina 64:303-310.

Pulich, W., 1976. Influence of sediment concentrations of trace-metals on Fe and Mn nutrition of tropical seagrasses. Plant Physiology 57, 82-82.

Q

Quesada, V. F., A. C. Jiménez, J. E. H. Díaz, E. E. Hoeflich, D. G. Carbonell, and T. C. Luhrs. 2004. Programa de manejo parque nacional Arrecifes de Xcalak. Mexico National Protected Areas Commission, Mexico City, Mexico.

R

Ralph, P. J. and M. D. Burchett. 1998. Photosynthetic response of Halophila ovalis to heavy metal stress. Environmental Pollution 103:91-101.

Ralph, P. J., D. Tomasko, K. Moore, S. Seddon, and C. M. O. Macinnis-Ng. 2007. Human impacts on seagrasses: Eutrophication, Sedimentation, and Contamination.in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, Amsterdam.

Ralph, P.J., Durako, M.J., Enriquez, S., Collier, C.J., Doblin, M.A., 2007. Impact of light limitation on seagrasses. Journal of Experimental Marine Biology and Ecology 350, 176-193.

Rasheed, M. A. 1999. Recovery of experimentally created gaps within a tropical *Zostera capricorni* (Aschers) seagrass meadow, Queensland Australia. Journal of Experimental Marine Biology and Ecology 235:183-200.

Reise, K. 1985. Tidal flat ecology: an experimental approach to species interactions. Springer-Verlag, New York.

Reise, K. 2002. Sediment mediated species interactions in coastal waters. Journal of Sea Research 48:127-141.

Reise, K. and J. Kohlus. 2007. Seagrass recovery in the Northern Wadden Sea? Helgoland Marine Research 62:77-84.

Reise, K., E. Herre, and M. Sturm. 2008. Mudflat biota since the 1930s: change beyond return? Helgoland Marine Research 62:13-22.

Reynolds, L. K., P. Berg, and J. C. Zieman. 2007. Lucinid clam influence on the biogeochemistry of the seagrass *Thalassia testudinum* sediments. Estuaries and Coasts 30:482-490.

Rijken, M. 1979. Food and food uptake in *Arenicola marina*. Netherlands Journal of Sea Research 13:406-421.

Rinkevich, B. 1995. Restoration strategies for coral reefs damaged by recreational activities: the use of sexual and asexual recruits. Restoration Ecology 3:241-251.

Riosmena-Rodriguez, R., Talavera-Saenz, A., Acosta-Vargas, B., Gardner, S.C., 2010. Heavy metals dynamics in seaweeds and seagrasses in Bahia Magdalena, BCS, M,xico. Journal of Applied Phycology 22, 283-291.

Rodriguez-Cabal, M. A., M. N. Barrios-Garcia, G. C. Amico, M. A. Aizen, and N. J. Sanders. 2013. Node-by-node disassembly of a mutualistic interaction web driven by species introductions. Proceedings of the National Academy of Sciences of the United States of America 110:16503-16507.

Rohacek, K., and M. Bartak. 1999. Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynthetica 37:339-363.

Rollon, R.N., Van Steveninck, E.D.D., van Vierssen, W., Fortes, M.D., 1998. Contrasting recolonization strategies in multi-species seagrass meadows. Marine Pollution Bulletin 37, 450-459.

Romeo, M., Gnassiabarelli, M., Juhel, T., Meinesz, A., 1995. Memorization of heavy metals by scales of the seagrass *Posidonia oceanica*, collected in the NW Meditarranean. Marine Ecology Progress Series 120, 211-218.

Romero, J., K. Lee, M. Perez, M. A. Mateo, and T. Alcoverro. 2006. Nutrient dynamics in seagrass ecosystems.in A. W. D. Larkum, R. J. Orth, and A. C. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands.

Romero, J., Martinez-Crego, B., Alcoverro, T., Perez, M., 2007. A multivariate index based on the seagrass *Posidonia oceanica* (POMI) to assess ecological status of coastal waters under the water framework directive (WFD). Mar Pollut Bull 55, 196-204.

Rose, C.D., Dawes, C.J., 1999. Effects of community structure on the seagrass *Thalassia testudinum*. Marine Ecology Progress Series 184, 83-95.

Rueda, J. L. and C. Salas. 2008. Molluscs associated with a subtidal *Zostera marina* L. bed in southern Spain: Linking seasonal changes of fauna and environmental variables. Estuarine Coastal and Shelf Science 79:157-167.

Ruiz, J.M., Romero, J., 2003. Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photsynthesis of the seagrass *Posidonia oceanica*. Marine Pollution Bulletin 46, 1523-1533.

Ruiz-Halpern, S., Macko, S.A., Fourqurean, J.W., 2008. The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment. Biogeochemistry 87, 113-126.

S

Sand-Jensen, K. 1977. Effects of epiphytes on eelgrass photo-synthesis. Aquatic Botany 3:55-63.

Sand-Jensen, K., O. Pedersen, T. Binzer, and J. Borum. 2005. Contrasting oxygen dynamics in the freshwater isoetid Lobelia dortmanna and the marine seagrass *Zostera marina*. Annals of Botany 96:613-623.

Schanz, A., P. Polte, and H. Asmus. 2002. Cascading effects of hydrodynamics on an epiphyte-grazer system in intertidal seagrass beds of the Wadden Sea. Marine Biology 141:287-297.

Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature 413:591-596.

Scheffer, M. and S. Carpenter. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends in Ecology & Evolution 8:275-279.

Schlacher-Hoenlinger, M.A., Schlacher, T.A., 1998. Accumulation, contamination, and seasonal variability of trace metals in the coastal zone - patterns in a seagrass meadow from the Medditerranean. Marine Biology 131, 401-410.

Schlacher-Hoenlinger, M.A., Schlacher, T.A., 1998. Differential accumulation patterns of heavy metals among the dominant macrophytes of a Mediterranean seagrass meadow. Chemosphere 37, 1511-1519.

Schroeder, P.B., Thorhaug, A., 1980. Trace metal cycling in tropical-subtropical estuaries dominated by the seagrass *Thalassia testudinum*. American Journal of Botany 67, 1075-1088.

Schubert, P.R., Karez, R., Reusch, T.B.H., Dierking, J., 2013. Isotopic signatures of eelgrass (*Zostera marina* L.) as bioindicator of anthropogenic nutrient input in the western Baltic Sea. Marine Pollution Bulletin 72, 64-70.

Schultz, H. D. and M. Zabel. 2006. Marine geochemistry. Springer-Verlag, Berlin.

Schüürmann, G., Markert, B., 1998. Ecotoxicology: Ecological Fundamentals, Chemical Exposure, and Biological Effects. Wiley and Spektrum Akademischer Verlag, New York and Heidelberg.

Schwarz, A. M., M. Morrison, I. Hawes, and H. J. 2006. Physical and biological characteristics of a rare marine habitat: sub-tidal seagrass beds of offshore islands. Science for Conservation 269:5-39.

Schweimanns, M. and H. Felbeck. 1985. Significance of the occurrence of chemoautotrophic bacterial endosymbionts in lucinid clams from Bermuda. Marine Ecology-Progress Series 24:113-120.

Seckbach, J. 2004. Symbiosis: mechanisms and model systems. Kluwer Academic Publishers, Dordrecht.

Sfriso, A., T. Birkemeyer, and P. F. Ghetti. 2001. Benthic macrofauna changes in areas of Venice lagoon populated by seagrasses or seaweeds. Marine Environmental Research 52:323-349.

Sheridan, P., McMahan, G., Hammerstrom, K., Pulich, W.J., 1998. Factors affecting restoration of *Halodule wrightii* to Galveston Bay, Texas. Restoration Ecology 6, 144-158.

Short, F.T., 1983. The response of interstitial ammonium in eelgrass (*Zostera marina* L.) beds to environmental pertubations. Journal of Experimental Marine Biology and Ecology 68, 195-208.

Short, F.T., 1983. The seagrass, *Zostera Marina* L.: Plant morphology and bed structure in relation to sediment ammonium in izembek lagoon, Alaska. Aquatic Botany 16, 149-161.

Short, F.T., McRoy, L.P., 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. . Botanica Marina 27, 547-555.

Short, F. T. 1987. Effects of sediment nutrients on seagrasses: literature review and mesocosm experiments. Aquatic Botany 27:41-57.

Short, F. T. and S. Wyllie-Echeverria. 1996. Natural and human-induced disturbances of seagrasses. Environmental Conservation 23:17-27.

Short, F.T., Burdick, D.M., Short, C.A., Davis, R.C., Morgan, P.A., 2000. Developing success criteria for restored eelgrass, salt marsh, and mud flat habitats. Ecological Engineering 15, 239-252.

Short, F.T., Davis, R.C., Kopp, B.S., Short, C.A., Burdick, D.M., 2002. Site-selection model for optimal transplantation of eelgrass *Zostera marina* in the northeastern US. Marine Ecology Progress Series 227, 253-267.

Siebert, T. and G. M. Branch. 2005. Interactions between *Zostera capensis* and *Callianassa kraussi*: influences on community composition of eelgrass beds and sandflats. African Journal of Marine Science 27:357-373.

Silva, J., Barrote, I., Costa, M.M., Albano, S., Santos, R., 2013. Physiological responses of *Zostera marina* and *Cymodocea nodosa* to light-limitation stress. PLoS One 8, e81058.

Sintes, T., Marba, N., Duarte, C.M., Kendrick, G.A., 2005. Nonlinear processes in seagrass colonisation explained by simple clonal growth rules. Oikos 108, 165-175.

Slijkerman, D.M.E., Peachey, R.B.J., Hausmann, P.S., Meesters, H.W.G., 2011. Eutrophication status of Lac, Bonaire, Durch Caribbean, inlcuding proposals for measures. IMARES Wageningen UR.

Small, C., Nicholls, R.J., 2003. Analysis of human settlement in coastal zones. Journal of Coastal Research 19, 584-599.

Smith, R.D., Dennison, W.C., Alberte, R.S., 1984. Role of seagrass photosynthesis in root aerobic processes. Plant Physiology 74, 1055-1058.

Smith, S. E., and D. J. Read. 1997 Mycorrhizal Symbiosis. Academic Press, San Diego, CA.

Smolders, A., Roelofs, J.G.M., 1995. Internal eutrophication, iron limitation and sulphide accumulation due to the inlet of rhiver Rhine water in peaty shallow waters in the Netherlands. Archiv Fur Hydrobiologie 133, 349-365.

Smolders, A.J.P., Moonen, M., Zwaga, K., Lucassen, E., Lamers, L.P.M., Roelofs, J.G.M., 2006. Changes in pore water chemistry of desiccating freshwater sediments with different sulphur contents. Geoderma 132, 372-383.

Stanley, S. M. 1977. Trends, rates and patterns in the evolution of the bivalvia.in A. Hallam, editor. Patterns of evolution as illustrated by the fossil record. Elsevier, Amsterdam, The Netherlands.

Stapel, J., Hemminga, M.A., 1997. Nutrient resorption from seagrass leaves. Marine Biology 128, 197-206.

Stewart, F. J., I. L. G. Newton, and C. M. Cavanaugh. 2005. Chemosynthetic endosymbioses: adaptations to oxic-anoxic interfaces. Trends in Microbiology 13:439-448.

Stoner, A. W., H. S. Greening, J. D. Ryan, and R. J. Livingston. 1983. Comparison of macrobenthos collected with cores and suction sampler in vegetated and unvegetated marine habitats. Estuaries 6:76-82.

Sundelin, B., Eriksson, A., 2001. Mobility and bioavailability of trace metals in sulfidic coastal sediments. Environmental Toxicology and Chemistry 20, 748-756.

Suykerbuyk, W., Bouma, T.J., van der Heide, T., Faust, C., Govers, L.L., Giesen, W.B.J.T., de Jong, D.J., van Katwijk, M.M., 2012. Suppressing antagonistic bioengineering feedbacks doubles restoration success. Ecological Applications 22, 1224-1231.

Suykerbuyk, W., Govers, L.L., Bouma, T.J., Giesen, W.B.J.T., de Jong, D.J., Van de Voort, R., Giesen, K., Giesen, P.T., van Katwijk, M.M., submitted. External forcing and stochastic processes determine seagrass transplantation success rather than positive feedbacks in a dynamic, intertidal environment.

T

Tan, S. K. and R. K. H. Yeo. 2010. The intertidal molluscs of Pulau Semakau: Preliminary results of “project Semakau” Nature in Singapore 3:287–296.

Taylor, J. D. and M. S. Lewis. 1970. The flora, fauna and sediments of the marine grass beds of Mahé, Seychelles. Journal of Natural History 4:199-&.

Taylor, J. D. and E. A. Glover. 2000. Functional anatomy, chemosymbiosis and evolution of the Lucinidae. Pages 207–225 in E. M. Harper, J. D. Taylor, and J. A. Crame, editors. The Evolutionary Biology of the Bivalvia. The Geological Society of London, London.

Taylor, J. D., E. A. Glover, M. Zuschin, P. C. Dworschak, and W. Waitzbauer. 2005. Another bivalve with dreadlocks: Living Rasta lamyi from Aqaba, Red Sea (Bivalvia : Lucinidae). Journal of Conchology 38:489-497.

Taylor, J. D. and E. A. Glover. 2006. Lucinidae (Bivalvia) - the most diverse group of chemosymbiotic molluscs. Zoological Journal of the Linnean Society 148:421-438.

Taylor, J. D. and E. A. Glover. 2008. Ancient chemosynthetic bivalves: Systematics of Solemyidae from eastern and southern Australia (Mollusca: Bivalvia). Pages 75-104 in D. P. J. F. and J. A. Phillips, editors. Proceedings of the 13th International Marine Biological Workshop - The Marine Fauna and Flora of Moreton Bay, Queensland. Queensland Museum, South Brisbane, Australia.

Taylor, J. D., E. A. Glover, L. Smith, P. Dyal, and S. T. Williams. 2011. Molecular phylogeny and classification of the chemosymbiotic bivalve family Lucinidae (Mollusca: Bivalvia). Zoological Journal of the Linnean Society 163:15-49.

Team, R. D. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Teillet, P. M., B. L. Markham, and R. R. Irish. 2006. Landsat cross-calibration based on near simultaneous imaging of common ground targets. Remote Sensing of Environment 102:264-270.

Terborgh, J., and J. A. Estes, editors. 2010. Trophic Cascades. Predators, prey, and the changing dynamics of nature. Island Press.

Terborgh, J., G. Nunez-Iturri, N. C. A. Pitman, F. H. C. Valverde, P. Alvarez, V. Swamy, E. G. Pringle, and C. E. T. Paine. 2008. Tree recruitment in an empty forest. Ecology 89:1757-1768.

Terrados, J., C. M. Duarte, L. Kamp-Nielsen, N. S. R. Agawin, E. Gacia, D. Lacap, M. D. Fortes, J. Borum, M. Lubanski, and T. Greve. 1999. Are seagrass growth and survival constrained by the reducing conditions of the sediment? Aquatic Botany 65:175-197.

Terrados, J., Ramírez-García, P., Hernández-Martínez, Ó., Pedraza, K., Quiroz, A., 2008. State of *Thalassia testudinum* Banks ex König meadows in the Veracruz Reef System, Veracruz, México. Aquatic Botany 88, 17-26.

Thorhaug, A., Miller, B., Jupp, B., Booker, F., 1985. Effects of a variety of impacts on seagrass restoration in Jamaica. Marine Pollution Bulletin 16, 355-360.

Thorhaug, A., J. Marcus, and F. Booker. 1986. Oil and dispersed oil on subtropical and tropical seagrasses in laboratory studies. Marine Pollution Bulletin 17:357-361.

Thursby, G.B., Harlin, M.M., 1982. Leaf-root interaction in the uptake of ammonia by *Zostera marina*. Marine Biology 72, 109-112.

Timmermann, K., G. T. Banta, and R. N. Glud. 2006. Linking *Arenicola marina* irrigation behavior to oxygen transport and dynamics in sandy sediments. Journal of Marine Research 64:915-938.

Toetz, D.W., 1973. The Kinetics of NH_4 uptake by Ceratophylum. Hydrobiologia 41, 275-290.

Tomasko, D. A. and B. E. Lapointe. 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. Marine Ecology Progress Series 75:9-17.

Torra Cosio, J. and L. Bourillón. 2000. Inventario y monitoreo del canal del infiernillo para el comanejo de los recursos marinos en el territorio. SNIB- CONABIO Mexico City.

Touchette, B. W. and J. M. Burkholder. 2000. Review of nitrogen and phosphorus metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology 250:133-167.

Touchette, B.W., Burkholder, J.M., 2000. Overview of the physiological ecology of carbon metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology 250, 169-205.

Touchette, B., Burkholder, J.M., Glasgow, H.B., 2003. Variations in eelgrass (*Zostera marina* L.) morphology and internal nutrient composition as influenced by increased temperature and watercolumn nitrate. Estuaries 26, 142-155.

Townsend, E.C., Fonseca, M.S., 1998. Bioturbation as a potential mechanism influencing spatial heterogeneity of North Carolina seagrass beds. Marine Ecology-Progress Series 169, 123-132.

Tranchina, L., Brai, M., D'Agostino, F., Bartolotta, A., Rizzo, G., 2005. Trace metals in "*Posidonia oceanica*" seagrass from south-eastern Sicily. Chemistry and Ecology 21, 109-118.

Troost, K., Gelderman, E., Kamermans, P., Smaal, A.C., Wolff, W.J., 2009. Effects of an increasing filter feeder stock on larval abundance in the Oosterschelde estuary (SW Netherlands). J. Sea Res. 61, 153-164.

Turner, M.G., Gardner, R.H., O'Neil, R.V., 2001. Landscape Ecology in Theory and Practise: Pattern and Process. Springer-Verlag, New York.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351-1363.

Tylianakis, J. M., E. Laliberte, A. Nielsen, and J. Bascompte. 2010. Conservation of species interaction networks. Biological Conservation 143:2270-2279.

U

Udy, J. W. and W. C. Dennison. 1997. Growth an physiological responses of three seagrass species to elevated nutrients in Moreton Bay, Australia. Journal of Experimental Marine Biology and Ecology 217:253-277.

V

Valdemarsen, T., E. Kristensen, and M. Holmer. 2010. Sulfur, carbon, and nitrogen cycling in faunated marine sediments impacted by repeated organic enrichment. Marine Ecology-Progress Series 400:37-53.

Valdemarsen, T., K. Wendelboe, J. T. Egelund, E. Kristensen, and M. R. Flindt. 2011. Burial of seeds and seedlings by the lugworm *Arenicola marina* hampers eelgrass (*Zostera marina*) recovery. Journal of Experimental Marine Biology and Ecology 410:45-52.

Valentine, J. F., K. L. Heck, P. Harper, and M. Beck. 1994. Effects of bioturbation in controlling turtlegrass (*Thalassia testudinum* Banks ex König) abundance: evidence from field enclosures and observations in the Northern Gulf of Mexico. Journal of Experimental Marine Biology and Ecology 178:181-192.

Valentine, J. F. and K. L. Heck. 1999. Seagrass herbivory: evidence for the continued grazing of marine grasses. Marine Ecology Progress Series 176:291-302.

Valiela, I., Bowen, J.L., York, J.K., 2001. Mangrove forests: one of the world's threatened major tropical environments. Bioscience 51, 807-815.

Van Beusekom, J. E. E. 2005. A historic perspective on Wadden Sea eutrophication. Helgoland Marine Research 59:45-54.

Van der Geest, M., J. A. van Gils, J. van der Meer, H. Olff, and T. Piersma. 2011. Suitability of calcein as an *in situ* growth marker in burrowing bivalves. Journal of Experimental Marine Biology and Ecology 399:1-7.

Van der Heide, T., E. H. Van Nes, G. W. Geerling, A. J. P. Smolders, T. J. Bouma, and M. M. Van Katwijk. 2007. Positive feedbacks in seagrass ecosystems: Implications for success in conservation and restoration. Ecosystems 10:1311-1322.

Van der Heide, T., A. Smolders, B. Rijkens, E. H. Van Nes, M. M. Van Katwijk, and J. Roelofs. 2008. Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. Oecologia 158:411-419.

Van der Heide, T., 2009. Stressors and feedbacks in temperate seagrass ecosystems, PhD thesis. Radboud University Nijmegen.

Van der Heide, T., Bouma, T.J., Van Nes, E.H., Van de Koppel, J., Scheffer, M., Roelofs, J.G.M., Van Katwijk, M.M., Smolders, A.J.P., 2010. Spatial self-organized patterning in seagrasses along a depth gradient of an intertidal ecosystem. Ecology 91, 362-369.

Van der Heide, T., E. H. Van Nes, M. M. Van Katwijk, M. Scheffer, A. J. Hendriks, and A. J. P. Smolders. 2010. Alternative Stable States Driven by Density-Dependent Toxicity. Ecosystems 13:841-850.

Van der Heide, T., E. H. van Nes, M. M. van Katwijk, H. Olff, and A. J. P. Smolders. 2011. Positive Feedbacks in Seagrass Ecosystems – Evidence from Large-Scale Empirical Data. PLoS ONE 6:e16504.

Van der Heide, T., Govers, L.L., de Fouw, J., Olff, H., van der Geest, M., van Katwijk, M.M., Piersma, T., van de Koppel, J., Silliman, B.R., Smolders, A.J.P., van Gils, J.A., 2012. A three-stage symbiosis forms the foundation of seagrass ecosystems. Science 336, 1432-1434.

Van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

Van Gils, J. A., M. van der Geest, E. J. Jansen, L. L. Govers, J. de Fouw, and T. Piersma. 2012. Trophic cascade induced by molluscivore predator alters pore-water biogeochemistry via competitive release of prey. Ecology 93:1143-1152.

Van Katwijk, M. M., L. H. T. Vergeer, G. H. W. Schmitz, and J. G. M. Roelofs. 1997. Ammonium toxicity in eelgrass *Zostera marina*. Marine Ecology Progress Series 157:159-173.

Van Katwijk, M. M. and D. C. R. Hermus. 2000. Habitat suitability of the Wadden Sea for restoration of *Zostera marina* beds. Helgoland Marine Research 54:117-128.

Van Katwijk, M. M., A. R. Bos, V. N. De Jonge, L. S. A. M. Hanssen, D. C. R. Hermus, and D. J. De Jong. 2009. Guidelines for seagrass restoration: Importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. Marine Pollution Bulletin 58:179-188.

Van Katwijk, M. M., M. E. W. van der Welle, E. C. H. E. T. Lucassen, J. A. Vonk, M. J. A. Christianen, W. Kiswara, I. I. al Hakim, A. Arifin, T. J. Bouma, J. G. M. Roelofs, and L. P. M. Lamers. 2011. Early warning indicators for river nutrient and sediment loads in tropical seagrass beds: A benchmark from a near-pristine archipelago in Indonesia. Marine Pollution Bulletin 62:1512-1520.

Van Katwijk, M.M., Vergeer, L.H.T., Schmitz, G.H.W., Roelofs, J.G.M., 1997. Ammonium toxicity in eelgrass *Zostera marina*. Marine Ecology Progress Series 157, 159-173.

Van Katwijk, M. M., A. Thorhaug, N. Marba, W. B. J. T. Giesen, C. Pickerell, I. H. J. Althuizen, E. Balestri, G. Bernard, M. L. Cambridge, A. Cunha, C. M. Duarte, C. Durance, Q. Han, S. Hosokawa, G. A. Kendrick, W. Kiswara, T. Komatsu, C. Lardicci, L. Kun-Seop, A. Meinesz, M. Nakaoka, E. I. Paling, A. M. A. Ransijn, J. J. Verduin, and R. J. Orth. submitted. Recovery of seagrass beds. Acceleration through restoration scale effects and economic incentives. submitted

Van Lent, F., P. H. Nienhuis, and J. M. Verschuure. 1991. Production and biomass of the seagrasses *Zostera noltii* Hornem. and Cymodecea nodosa (Ucria) Aschers. at the Banc d'Arguin (Mauritania, NW Africa): a preliminary approach. Aquatic Botany 41:353-367.

Van Lent, F., Verschuure, J.M., Vanveghel, M.L.J., 1995. Comparative study on populations of *Zostera marina* L (eelgrass)- *in situ* nitrogen enrichment and light manipulation. Journal of Experimental Marine Biology and Ecology 185, 55-76.

Van Tussenbroek, B.I., Vonk, J.A., Stapel, J., Erftemeijer, P.L.A., Middelburg, J.J., 2006. The biology of *Thalassia*: paradigms and recent advances in research in: Larkum, A.W.D., Orth, R.J., Duarte, A.C. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, the Netherlands.

Van Wesenbeeck, B. K., J. Van de Koppel, P. M. J. Herman, J. P. Bakker, and T. J. Bouma. 2007. Biomechanical warfare in ecology; negative interactions between species by habitat modification. Oikos 116:742-750.

Van-Dunem do Sacramento Neto dos Santos, C. I. 2007. Comunidades de macroninvertebrados e peixes associades à pradaria marinha de Hadule wrightii (Ascherson, 1868) na Laguno do Mussulo, Angola. Universidade de Lisboa, Lisboa.

Varcourt, C. and C. Bonnot-Courtois. 1992. Dynamique sédimentaire dans les baies de Lancieux et de l'Arguenon (Côte-d'Amour). Norois 153:29-44.

Vera, B., 1992. Seagrasses of the Venezuelan coast: distribution and community components, in: Seeliger, U. (Ed.), Coastal Plant Communities of Latin America. Academic Press, San DIego, California, pp. 135-140.

Vermaat, J. E., J. A. J. Beijer, R. Gijlstra, M. J. M. Hootsmans, C. J. M. Philippart, N. W. Vandenbrink, and W. Vanvierssen. 1993. Leaf dynamics and standing stocks of internal *Zostera noltii* Hornem and *Cymodocea nodosa* (Ucria) Ascherson on Banc d' Arguin, Mauritania. Hydrobiologia 258:59-72.

Vermaat, J. E. and F. C. A. Verhagen. 1996. Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: coupling demographic and physiological patterns. Aquatic Botany 52:259-281.

Vermaat, J.E., 2009. Linking clonal growth patterns and ecophysiology allows the prediction of meadow-scale dynamics of seagrass beds. Perspectives in Plant Ecology Evolution and Systematics 11, 137-155.

Vermeij, G. J. 2011. Shifting sources of productivity in the coastal marine tropics during the Cenozoic era. Proceedings of the Royal Society B-Biological Sciences 278:2362-2368.

Vidondo, B., Duarte, C.M., Middelboe, A.L., Stefansen, K., Lutzen, T., Nielsen, S.L., 1997. Dynamics of a landscape mosaic: size and age distributions, growth and demography of seagrass *Cymodocea nodosa* patches. Marine Ecology-Progress Series 158, 131-138.

Volkenborn, N. and K. Reise. 2006. Lugworm exclusion experiment: Responses by deposit feeding worms to biogenic habitat transformations. Journal of Experimental Marine Biology and Ecology 330:169-179.

Volkenborn, N., S. I. C. Hedtkamp, J. E. E. van Beusekom, and K. Reise. 2007. Effects of bioturbation and bioirrigation by lugworms (*Arenicola marina*) on physical and chemical sediment properties and implications for intertidal habitat succession. Estuarine Coastal and Shelf Science 74:331-343.

Vonk, J.A., Christianen, M.J.A., Stapel, J., 2008. Redefining the trophic importance of seagrasses for faunal in tropical Indo-Pacific meadows. Estuarine Coastal and Shelf Science 79, 653-660.

W

Wahbeh, M.I., 1984. Levels of zinc, manganese, magnesium, iron and cadmium in the three species of seagrass from Agaba (Jordan). Aquatic Botany 20, 179-183.

Walker, D.I., McComb, A.J., 1992. Seagrass degradation in Australian coastal waters. Aquatic Botany 25, 191-195.

Warnau, M., Ledent, G., Temara, A., Bouquegneau, J.M., Jangoux, M., Dubois, P., 1995. Heavy metals in the *Posidonia oceanica* and Paracentrotus lividus from seagrass beds of the north western Mediterranean. Science of the Total Environment 171, 95-99.

Watson, G. F., A. I. Robertson, and M. J. Littlejohn. 1984. Invertebrate macrobenthos of the seagrass communities in Western Port, Victoria. Aquatic Botany 18:175-&.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences 106, 12377-12381.

Welsh, D.T., 2000. Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria interactions and significance to primary productivity. Ecology Letters 3, 58-72.

Wendelboe, K., J. T. Egelund, M. R. Flindt, and T. Valdemarsen. 2013. Impact of lugworms (*Arenicola marina*) on mobilization and transport of fine particles and organic matter in marine sediments. Journal of Sea Research 76:31-38.

Wernegreen, J. J. 2012. Mutualism meltdown in insects: bacteria constrain thermal adaptation. Current Opinion in Microbiology 15:255-262.

Wetsteyn, L., Kromkamp, J.C., 1994. Turbidity, nutrients and phytoplankton primary production in the Oosterchelde (The Netherlands) before, during, and asfter a large-scale coastal engineering project (1980-1990). Hydrobiologia 283, 61-78.

Whanpetch, N. 2011. Variability and consequences of seagrass vegetation effect on macrobenthic invertebrate communities. Chiba University, Chiba, Japan.

Whelan, T., Espinoza, J., Villarreal, X., CottaGoma, M., 2005. Trace metal partitioning in *Thalassia testudinum* and sediments in the Lower Laguna Madre, Texas. Environment International 31, 15-24.

Whelan, T., 3rd, Van Tussenbroek, B.I., Santos, M.G., 2011. Changes in trace metals in *Thalassia testudinum* after hurricane impacts. Mar Pollut Bull 62, 2797-2802.

Whitfield, A. K. 1989. The benthic community of the Southern Cape estuary - Structure and possible food sources. Transactions of the Royal Society of South Africa 47:159-179.

Wild, C., O. Hoegh-Guldberg, M. S. Naumann, M. F. Colombo-Pallotta, M. Ateweberhan, W. K. Fitt, R. Iglesias-Prieto, C. Palmer, J. C. Bythell, J. C. Ortiz, Y. Loya, and R. Van Woesik. 2011. Climate change impedes scleractinian corals as primary reef ecosystem engineers. Marine and Freshwater Research 62:205-215.

Williams, S. L. and K. L. Heck. 2001. Seagrass community ecology.in M. D. Bertness, editor. Marine community ecology. Sinauer Associates, Sunderland, Massachusetts.

Wilson, G. W. T., C. W. Rice, M. C. Rillig, A. Springer, and D. C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters 12:452-461.

Wood, E. D., Armstron.Fa, and F. A. Richards. 1967. Determination of nitrate in sea water by Cadmium-Copper reduction to nitrite. Journal of the Marine Biological Association of the United Kingdom 47:23-&.

Wooldridge, S. A. 2013. Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences 10:1647-1658.

Y

Yamaguchi, M. 1999. Edible mollusks from tropical seagrass-beds in the Indo-Pacific – past and present. Page 217 in M. B., editor. Asian Marine Biology. University Press, Hong Kong

Yemm, E.W., Folkes, B.F., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57, 508-514.

Z

Zedler, J. B. 2000. Progress in wetland restoration ecology. Trends in Ecology & Evolution 15:402-407.

Zhang, J., Huang, X., Jiang, Z., 2014. Physiological responses of the seagrass *Thalassia hemprichii* (Ehrenb.) Aschers as indicators of nutrient loading. Mar Pollut Bull 83, 508-515.

Zieman, J. C., J. W. Fourqurean, and R. L. Iverson. 1989. Distribution, abundance and productivity of seagrasses and macroalgae in Florida Bay. Bulletin of Marine Science 44:292-311.

Zimmerman, R.C., Reguzzoni, J.L., Alberte, R.S., 1995. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: Role of light availability on metabolism, growth and survival. Aquatic Botany 51, 67-86.

Zimmerman, R.C., Alberte, R.S., 1996. Effect of light/dark transition on carbon translocation in eelgrass *Zostera marina* seedlings. Marine Ecology Progress Series 136, 305-309.

Zuschin, M. and J. Hohenegger. 1998. Subtropical coral-reef associated sedimentary facies characterized by molluscs (Northern bay of Safaga, Red Sea, Egypt). Facies 38:229-254.

SUMMARY

Seagrasses are rooting and flowering plants that can form extensive meadows in shallow coastal zones all over the world. These meadows form key-ecosystems for a wide diversity of species, including some highly endangered species such as dugongs and sea turtles. Seagrass beds are among the most productive and valuable ecosystems on earth, comparable to coral reefs, salt marshes and mangroves. These often strongly inter-connected coastal ecosystems provide important ecosystem services such as carbon sequestration, nutrient cycling, coastal protection by wave attenuation and sediment stabilization, and form a nursery habitat for commercially important fish species.

Unfortunately, seagrass beds all over the world are threatened and rapidly disappearing as a result of human activities in coastal areas. Threats to seagrasses include increased nutrient loads of coastal waters, industrial pollution, increased sediment loads, dredging, and erosion. Many of these stressors may simultaneously affect seagrass beds, and in this thesis, we focused on the effects of some particular stressors: high sulfide levels, pollution by heavy metals, and the effects of high nutrient loads on seagrass ecosystems. We especially looked at the importance of species inhabiting seagrass beds and seagrass plant and meadow scale processes in relation to these stressors.

Biogeochemical processes and nutrients

Biogeochemical processes are generally chemical processes in the sediment, mainly involving nutrient cycling processes, often performed by soil bacteria. Biogeochemical processes occur in every soil, but in marine sediments, biogeochemistry is especially affected by the composition of the seawater. Seawater naturally contains a lot of sulfate, a component that can be converted to sulfide under low oxygen conditions. Sulfide is volatile and can be recognized by the characteristic rotten egg smell. This horrid smelling compound is toxic to seagrasses and has been known to cause large-scale diebacks of seagrass beds. We discovered that small-scale expanding seagrass patches, which may result from seagrass habitat degradation, are much more vulnerable to sulfide toxicity that high seagrass densities. This may be due to a process called density-dependent

toxicity, as high seagrass densities pump oxygen into the root zone, which forms a protective layer against toxic sulfides. Furthermore, not only sulfides are toxic to low seagrass densities, extremely high nutrient levels may also result in disturbed seagrass patch dynamics.

Although nutrients (mainly nitrogen – N – and phosphorus – P –) are essential to seagrass growth, a surplus of nutrients may have negative effects on seagrasses, not only by affecting growth of patches, but mostly by decreasing the light availability for seagrasses, which need sunlight for photosynthesis, by promoting algal bloom in the water column. It is thus important to identify high nutrient loads to prevent loss of valuable seagrass beds. The seagrass beds of Curaçao and Bonaire are valuable nursery habitats for many commercially important fish species, but may be threatened by high nutrient loads (eutrophication). Hence, we evaluated the nutrient status of these seagrass beds and discovered that although nutrient loads could not be measured in the water layer, seagrass leaf tissue of *Thalassia testudinum* accumulated nutrients (N and P) in the vicinity of a nutrient source. Using this information, we could identify possible threats to the seagrass beds of Curaçao and Bonaire.

Heavy metal pollution

In addition to high nutrient levels, heavy metals (or trace metals) such as mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), originating from mining activities, sewage or agriculture, may also threaten seagrass beds and their inhabitants. We found that seagrasses can indicate long-term high nutrient loads as they apparently integrate ecological conditions over a longer time-scale. We also tested this for heavy metal pollution in seagrasses worldwide, and found that seagrass tissue also reflects long-term heavy metal pollution, which may not be measurable in the water layer. This is important, as seagrasses form the base of the food chain, and heavy metals may accumulate in the higher levels of the food chain. Heavy metal accumulation in seagrasses may thus be used as a first-level indicator for coastal metal pollution, indicating potential threats to fisheries.

The importance of species interactions for seagrass beds

Many species depend directly or indirectly on seagrass beds, not only animals, but also algae, and even bacteria inhabit seagrass beds. Seagrass beds are ecosystems with high biogeochemical activity and some animals living in seagrass beds may affect the biogeochemical processes in the sediment. We discovered that seagrasses are globally involved in a specialized partnership – 'mutualism' – with clams from the Lucinidae family, where the clams do not only benefit from the presence of seagrasses, but seagrasses in turn do need the clams to survive. This extraordinary relationship is based on another mutualism as the lucinid clams have special bacteria, living in their gills, which consume the toxic sulfides

produced in seagrass sediments. This symbiotic partnership enables seagrasses to grow in areas where they would have died from sulfide stress if the clams had not been present. The clams in turn need the seagrasses as they pump oxygen into the soil, which they need to cope with toxic sulfides. This mutualism may be called a keystone mutualism, as it forms the foundation of seagrass ecosystems, on which many other species depend. Although this mutualism forms a strong foundation of seagrass ecosystems, we found that breakdown of this mutualism may accelerate ecosystem collapse. This is important to recognize, as many other important ecosystems depend on keystone mutualisms such as plant-pollinator interactions, coral-zooxanthellae relationships and plant-mycorrhiza symbioses.
The important seagrass-lucinid clam-gill bacteria symbiosis, which enables seagrasses to grow in toxic soils, is less common in temperate areas. However, as sulfide may still pose a threat to seagrasses in those areas, we thought that perhaps another interaction between seagrasses and the common lugworm *Arenicola marina*, which produces characteristic castings on beaches and mudflats, could relieve sulfide stress for seagrasses. As these worms move around sediment (bioturbation) to feed on organic particles and algae, they mix oxygen into the soil, potentially decreasing toxic sulfide levels. However, in our experiment, we did not find any positive effects of lugworm activity on seagrasses. Lugworms did not relieve sulfide stress, but instead buried seagrasses and additionally promoted algal bloom on seagrass leaves by pumping nutrients from the sediment to the water layer. Thus, there might still be an alternative mechanism that relieves sulfide stress to temperate seagrasses, but it has yet to be discovered.

Lessons for seagrass management and restoration

Seagrass beds are very important ecosystems, but have been globally declining for decades. Therefore, many attempts to restore seagrass beds have been undertaken, with varying degrees of success. Lack of restoration success may sometimes be attributed to too low planting densities, as seagrasses generally need high-enough densities to restore self-facilitating feedbacks. We found that especially processes important to restoration such as expansion and recovery are vulnerable to stressors such as sulfide and high nutrient concentrations, due to the lack of density-dependent stress-alleviating feedbacks. Next to feedbacks, background stress levels such as heavy metal pollution, high nutrient levels, sulfide and bioturbation pressure may also affect seagrass restoration success. It is thus important to identify those background stress levels before attempting to restore seagrass beds. Furthermore, we found that rhizome starch levels may be used to predict next year's transplant success, which was previously hard to predict based on the preceding summer's seagrass density. Finally, as we discovered that a keystone mutualism forms the foundation of seagrass ecosystems, we stress that this mutualism should be taken into account when restoring seagrass beds. All these discoveries may be used to improve seagrass management and restoration efforts.

Conclusion

We conclude that seagrass beds are threatened by multiple stressors such as high nutrient levels, heavy metal pollution and sulfide, as a result of human activities in coastal areas. These stressors may interact and synergistically affect seagrass health and functioning. It is therefore important to detect potential threats to seagrass ecosystems by using seagrass nutrient and heavy metal concentrations as bioindicators of long-term pollutant loads. We discovered that stressors such as sulfide and high nutrient levels may affect meadow-scale dynamic processes, due to the lack of self-facilitating feedbacks in low seagrass densities. However, we also found that seagrasses have a mechanism to protect themselves from sulfide stress: A keystone mutualism between seagrasses, lucinid clams and their gill bacteria, enables seagrasses to grow in sulfide-rich soils all over the world. This illustrates the importance of studying all levels and aspects of biogeochemical stressors on seagrass ecosystems, including species interactions, to gain a deeper understanding of ecosystem functioning, which may also provide useful tools for seagrass conservation, management, and restoration.

SAMENVATTING

Zeegrassen zijn wortelende, bloeiende planten die uitgestrekte velden kunnen vormen in ondiepe kustwateren over de hele wereld. Zeegrasvelden vormen sleutel-ecosystemen, voor een brede diversiteit aan soorten, waaronder bedreigde diersoorten zoals zeeschildpadden en zeekoeien. Zeegrasvelden behoren tot de meest productieve en waardevolle ecosystemen op aarde, vergelijkbaar met koraalriffen, mangroves en kwelders. Deze kustecosystemen zijn vaak sterk met elkaar verbonden en leveren belangrijke ecosysteemdiensten, zoals koolstofopslag, recycling van voedingsstoffen, kustbescherming door golfuitdoving en stabilisatie van de bodem, en ze dienen als kraamkamer voor commercieel belangrijke vissoorten. Helaas worden zeegrasvelden over de hele wereld bedreigd en het areaal aan zeegras gaat snel achteruit door toegenomen menselijke activiteiten in kustgebieden. Zeegrassen worden o.a. bedreigd door sterk verhoogde concentraties van meststoffen in kustwateren, industriële vervuiling, baggerwerkzaamheden en erosie. Veel van deze stressoren kunnen, apart of tegelijkertijd, een negatief effect hebben op zeegrassen en op de algen en dieren die afhankelijk zijn van zeegrasvelden.

In dit proefschrift ligt de nadruk op een aantal specifieke stressoren: hoge sulfide concentraties, vervuiling door zware metalen, en de effecten van verhoogde niveaus van meststoffen op zeegrasecosystemen. We hebben voornamelijk gekeken naar het belang van dieren in het zeegras, en naar zeegrasprocessen op plant- en veldschaal, in relatie tot de eerder genoemde stressoren.

Biogeochemische processen en voedingsstoffen

Biogeochemische processen over het algemeen chemische processen die in de bodem plaatsvinden. Ze worden vaak uitgevoerd door bodembacteriën. Biogeochemische processen vinden plaats in iedere bodem, maar in zoute (mariene) bodems worden deze processen sterk beïnvloed door de samenstelling van zeewater. Zeewater bevat van nature veel sulfaat, een stof die onder zuurstofloze omstandigheden in de bodem kan worden omgezet in sulfide. Sulfide is een vluchtige stof die gemakkelijk te herkennen is aan de typische 'rotte

eieren' lucht. Dit vies ruikende goedje is erg giftig voor zeegrassen. Een hoge sulfideproductie in de bodem heeft zelfs op sommige plekken geleid tot massale zeegrassterfte.
Wij hebben ontdekt dat kleinschalige, zich uitbreidende zeegrasveldjes, die het resultaat zijn van versnipperde aaneengesloten zeegrasvelden, veel gevoeliger zijn voor giftig sulfide dan hoge zeegrasdichtheden. Dit zou kunnen worden veroorzaakt door een proces dat 'dichtheidsafhankelijke toxiciteit' wordt genoemd. Hoge zeegrasdichtheden pompen namelijk veel zuurstof in de bodem. Dat vormt een natuurlijk beschermend laagje tegen giftig sulfide. Lage zeegrasdichtheden kunnen niet zoveel zuurstof de bodem inpompen, waardoor het sulfide door kan dringen in de wortels. Bovendien is niet alleen sulfide giftig voor lage zeegrasdichtheden, ook hoge concentraties meststoffen in de bodem kunnen kleinschalige zeegrasveldprocessen verstoren.

Meststoffen, oftewel voedingsstoffen (voornamelijk stikstof – N – en fosfor – P –) zijn essentieel voor de groei van zeegrassen. Echter, de aanwezigheid van teveel voedingsstoffen kan juist negatieve effecten hebben op zeegrassen. Deze negatieve effecten worden vooral veroorzaakt doordat een teveel aan voedingstoffen in het water die door het stimuleren van algenbloei de beschikbaarheid van licht, dat zeegrassen nodig hebben voor fotosynthese, negatief beïnvloeden. Ook kunnen te hoge concentraties voedingsstoffen rechtstreekse giftige effecten hebben op zeegrassen. Om verder verlies van waardevolle zeegrasvelden te voorkomen is het van groot belang hoge belasting van meststoffen in kustwateren op te sporen. De zeegrasvelden van Curaçao en Bonaire zijn belangrijke kraamkamers voor commercieel belangrijke vissoorten, maar worden mogelijk bedreigd door te hoge voedingsstofbelasting (eutrofiëring). Daarom hebben we de voedingsstof-status van deze zeegrasvelden geëvalueerd en ontdekt dat, hoewel hoge concentraties voedingsstoffen niet in het water konden worden gemeten, deze waardes wel terug te meten waren in het blad van de zeegrassoort *Thalassia testudinum*. Vooral in de nabijheid van een bron van voedingsstoffen, zoals een rioleringspijp of huizen, waren de concentraties voedingsstoffen in de planten sterk verhoogd. Gebruikmakend van deze informatie waren we in staat om mogelijke bedreigingen te identificeren voor zeegrassen op Curaçao en Bonaire.

Vervuiling door zware metalen

Naast verhoogde concentraties voedingsstoffen kan het voortbestaan van zeegrasvelden ook bedreigd worden door zware metalen zoals kwik (Hg), lood (Pb), cadmium (Cd), en chroom (Cr), afkomstig van mijnactiviteiten, rioolwater en landbouw.
Zeegrassen kunnen gebruikt worden als indicator voor lange-termijnbelasting door hoge concentraties voedingsstoffen. Wij hebben getest of dit ook geldt voor zware metalen, en vonden dat zware metalen concentraties in zeegrasweefsel (blad en wortelstok) wereldwijd ook lange-termijnbelasting door zware metalen weergeven. Dit is een belangrijk gegeven omdat zeegrassen de basis vormen van de voedselketen en zware metalen zich mogelijk opstapelen in de hogere niveaus van de voedselketen. Dit vormt een mogelijk risico voor mensen als het consumptievis betreft. Zware metalen concentratie in zeegras kan dus mogelijk gebruikt worden als een eerste indicatie voor zware metalen vervuiling in kustgebieden en de mogelijke gevaren hiervan voor de visserij.

Het belang van soorteninteracties voor zeegrasbedden

Veel soorten zijn direct of indirect afhankelijk van zeegrasbedden. Niet alleen diersoorten, maar ook algen en zelfs bacteriën bewonen zeegrasvelden. Zoals eerder genoemd zijn zeegrasvelden een omgeving met een hoge biogeochemische activiteit en sommige dieren die in het zeegras leven kunnen deze processen mogelijk beïnvloeden, al is hier nog in zeer beperkte mate onderzoek naar gedaan. Wij hebben ontdekt dat zeegrassen wereldwijd betrokken zijn in een gespecialiseerd partnerschap – 'mutualisme' – met tweekleppige schelpdieren uit de Lucinidae familie, waarbij de schelpdieren niet alleen profijt hebben van de aanwezigheid van zeegras, maar waarbij zeegrassen op hun beurt ook de schelpdieren nodig hebben om te overleven. Deze bijzondere relatie is gebaseerd op een andere symbiose; de schelpdieren hebben namelijk speciale bacteriën in hun kieuwen, die het giftige sulfide dat in zeegrasbodems wordt geproduceerd kunnen gebruiken voor hun energievoorziening. Dit mutualisme tussen zeegrassen, schelpdieren, en hun kieuwbacteriën, heeft er voor gezorgd dat zeegrassen kunnen groeien in een omgeving waar ze anders waren doodgegaan door sulfidestress. Deze symbiose kan een 'sleutelmutualisme' worden genoemd, omdat het de fundering vormt voor het functioneren van zeegrasecosystemen, waar ook veel andere soorten van afhankelijk zijn. We vonden echter dat, hoewel dit mutualisme een stevige basis vormt voor zeegrasecosystemen, de afbraak van dit mutualisme, door bijvoorbeeld een externe stressor, juist de degradatie van een ecosysteem versnelt. Dit is belangrijke informatie, omdat over de hele wereld ecosystemen afhankelijk zijn van soortgelijke sleutelmutualismes, die onder druk staan door o.a. klimaatsverandering en vervuiling. Voorbeelden van soortgelijke sleutelmutualismes zijn plant-bestuiver interacties, koraalriffen die afhankelijk zijn van hun symbiose met algjes (zooxanthellae) en plant-mycorrhiza interacties (bodemschimmels).
De belangrijke symbiose tussen zeegrassen, schelpdieren en kieuwbacteriën, die het voor zeegrassen mogelijk maakt om in sulfiderijke bodems te groeien, is echter minder algemeen in de gematigde streken. Omdat ook in deze streken sulfidestress kan voorkomen, dachten wij dat mogelijk een andere interactie tussen zeegras en bodemdieren verantwoordelijk zou kunnen zijn voor een verlaging van sulfidestress. Hierbij dachten wij aan de veelvoorkomende wadpier, *Arenicola marina*, die kan worden herkend aan de typische wadpierhoopjes die

op het strand en op het wad te vinden zijn. Deze wormen bewegen de bodem heen en weer (bioturbatie) omdat ze zich voeden met kleine organische deeltjes en algen in het zand. Deze beweging kan ervoor zorgen dat zuurstof in de bodem wordt gemixt waarmee mogelijk sulfide-stress voor zeegrassen wordt verminderd. Echter, in ons experiment vonden we geen enkel positief effect van wadpieren op zeegras. Wadpieren verlichtten sulfide stress voor zeegrassen niet, en hadden zelfs alleen maar negatieve effecten op zeegras. Het ging dood door begraving, en doordat wadpieren algenbloei op de zeegrasbladeren stimuleerden omdat ze voedingsstoffen uit het sediment naar de waterlaag pompten. Mogelijk is er dus nog een alternatief mechanisme wat zeegrassen in gematigde gebieden beschermt tegen sulfidestress, maar dat moet nog worden ontdekt.

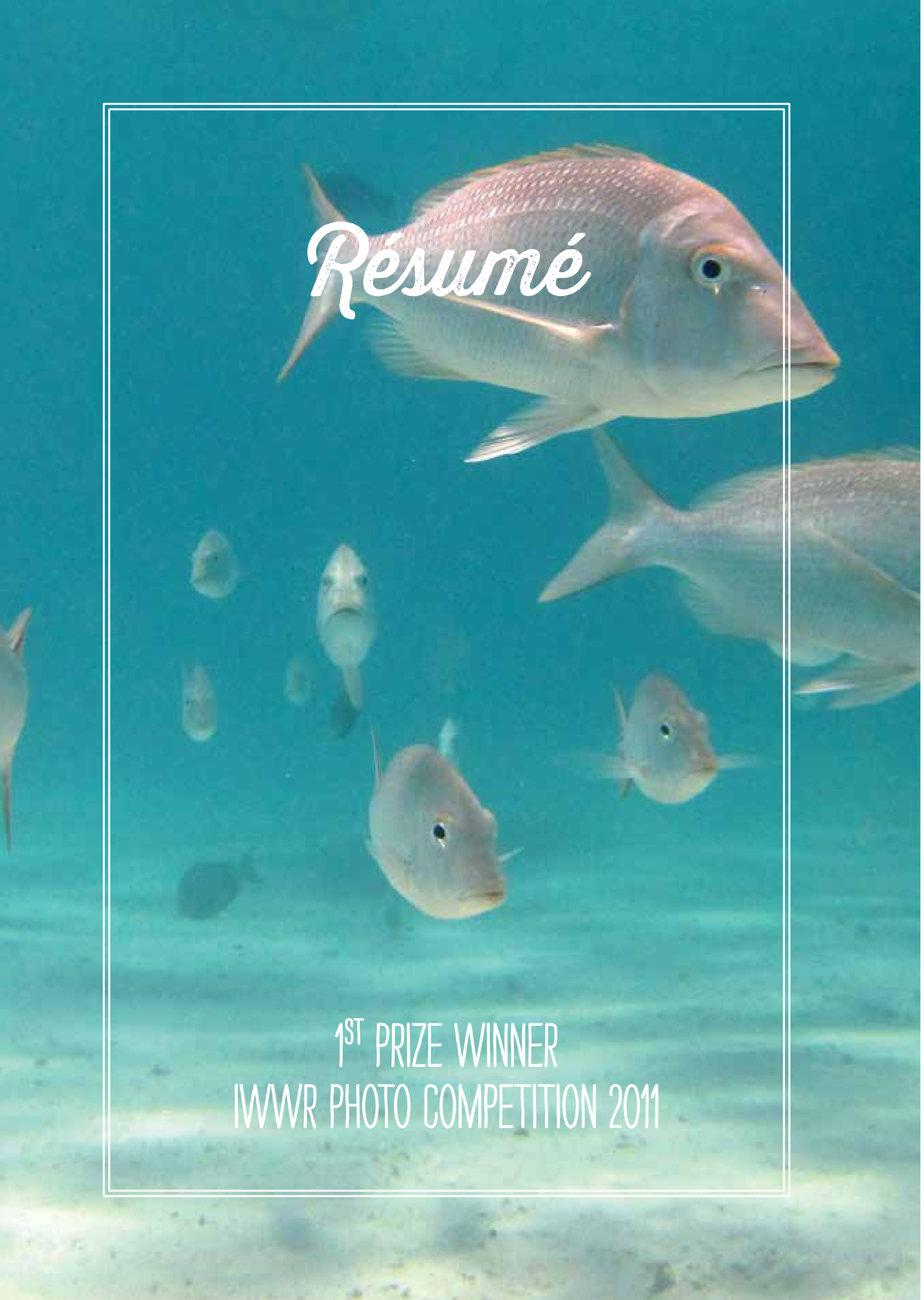
Lessen voor zeegrasmanagement en -herstel

Zeegrasvelden zijn belangrijke ecosystemen die al jaren een sterke achteruitgang vertonen. Daarom hebben er al veel pogingen plaatsgevonden om zeegrasvelden te herstellen, met verschillende gradaties van succes. Het gebrek aan restauratiesucces kan soms worden toegeschreven aan te lage plant-dichtheden. Zeegrassen hebben over het algemeen hoge dichtheden nodig om zelffaciliterende terugkoppelingsmechanismen, zoals het remmen van golven, in stand te houden. Wij vonden dat juist processen die van belang zijn voor herstel, zoals uitgroei en herstel, gevoelig zijn voor stressoren door het ontbreken van deze stressverlagende, zelffaciliterende terugkoppelingsmechanismen. Naast terugkoppelingsmechanismen zijn ook achtergrondstressniveaus, zoals concentraties van zware metalen, voedingsstoffen, sulfide en bioturbatiedruk van invloed op zeegrasrestauratiesucces. Dus is het belangrijk om deze achtergrondstressoren te identificeren, voordat restauratie daadwerkelijk plaatsvindt. Tevens hebben we ontdekt dat hoewel zeegrasrestauratiesucces na de winter moeilijk te voorspellen is op basis van zeegrasdichtheid in het voorgaande jaar,   het wel mogelijk is om een voorspelling te doen over zeegrasrestauratiesucces na de winter op basis van zetmeelniveaus in de wortelstokken van zeegras vóór de winter. Tot slot willen we benadrukken dat het sleutel-mutualisme tussen zeegrassen, schelpdieren en hun kieuwbacteriën, de basis vormt van zeegrasecosystemen en daarom ook in acht moet worden genomen bij zeegrasherstel. Al deze ontdekking kunnen worden gebruikt om zeegrasmanagement en herstel te verbeteren.

Conclusie

We concluderen dat zeegrasvelden wereldwijd worden bedreigd door veel verschillende stressoren zoals hoge concentraties voedingsstoffen, zware metalen vervuiling en sulfide, die het gevolg zijn van menselijke activiteiten in kustgebieden. Deze stressoren kunnen interacteren en gezamenlijk de gezondheid en het functioneren van zeegrasbedden negatief beïnvloeden.

Daarom is het belangrijk om mogelijke bedreigingen voor zeegrasvelden te identificeren door voedingsstof- en zware metalenconcentraties in zeegrasblad als indicatoren te gebruiken voor lange-termijn belasting door vervuiling. We hebben ontdekt dat stressoren zoals sulfide en hoge concentraties meststoffen kleinschalige zeegrasdynamiekprocessen kunnen beïnvloeden door het verstoren van zelffaciliterende positieve terugkoppelingsmechanismen. We hebben echter eveneens ontdekt dat zeegrassen zichzelf ook kunnen beschermen tegen een stressor als sulfide, door een samenwerkingsverband aan te gaan met schelpdieren van de Lucinidae familie en hun sulfide-consumerende kieuwbacteriën. Door het aangaan van dit mutualisme kunnen zeegrassen overal te wereld voorkomen in sulfide-rijke bodems. Deze ontdekkingen illustreren het belang van het bestuderen van alle niveaus en aspecten van biogeochemische stressoren in zeegrasecosystemen, inclusief zeegras-dierinteracties , om een groter begrip te vergaren van het functioneren van zeegrasecosystemen. Deze kennis kan vervolgens worden gebruikt voor het verbeteren van zeegrasbescherming, -management en-herstel.



1ST PRIZE WINNER
IWWR PHOTO COMPETITION 2011

RÉSUMÉ

Les herbiers de végétation aquatique sont composés de plantes marines (angiospermes marines) qui s'enracinent dans le sédiment et fleurissent pour former de vastes prairies dans les zones côtières peu profondes partout dans le monde. Ces prairies forment des écosystèmes clé ainsi que de véritables niches écologiques permettant le développement d'une grande diversité d'espèces, y compris certaines espèces très menacées comme les dugongs et les tortues marines. Les herbiers marins sont parmi les écosystèmes les plus productifs et les plus précieux sur terre, comparables aux récifs coralliens, aux marais salants ou aux mangroves. Ces écosystèmes côtiers souvent fortement interconnectés fournissent des services écosystémiques importants, tels que la séquestration du carbone, le cycle des nutriments, la protection du littoral par l'atténuation des vagues et la stabilisation des sédiments, et forment une zone d'alevinage pour les espèces de poissons à grande importance commerciale.

Malheureusement, les herbiers marins sont menacés à l'échelle mondiale et disparaissent rapidement en raison de l'intensification des activités humaines dans les zones côtières. Les menaces pour les herbiers peuvent provenir de l'augmentation des charges de nutriment dans les eaux côtières, de la pollution industrielle, de l'augmentation des charges de sédiments sur les côtes, du dragage et de l'érosion côtière. Bon nombre de ces facteurs de stress peuvent affecter simultanément les herbiers de végétation aquatique. Dans cette thèse, nous nous sommes concentrés sur les effets de certains facteurs de stress: les niveaux élevés de sulfure, la pollution par les métaux lourds ainsi que les effets des forts rejets de nutriments sur les écosystèmes d'herbiers. Nous avons particulièrement examiné l'importance des espèces qui peuplent les herbiers ainsi que les mécanismes de réponse à ces facteurs de stress à l'échelle des herbiers.

Les processus biogéochimiques et les nutriments

Les processus biogéochimiques sont généralement définis comme des processus chimiques dans les sédiments, affectant principalement le cycle des nutriments, et souvent effectués par des bactéries présentes dans le sédiment. Ces processus biogéochimiques ont lieu dans tous les types de sols, cependant dans les sédiments marins, ils sont particulièrement affectés par la composition de l'eau de mer. L'eau de mer contient naturellement un grand nombre de sulfates, un composant qui peut être converti en sulfure dans des conditions de faible teneur en oxygène. Le sulfure est un composant volatile et peut être reconnu par son odeur d'œuf pourri caractéristique. Ce composé à l'odeur fétide est aussi toxique pour les plantes d'herbiers marins et a été reconnu comme l'un des facteurs pouvant provoquer le dépérissement à grande échelle des herbiers marins. Nous avons constaté que l'expansion des zones d'herbiers à faible densité, qui pourraient résulter de la dégradation progressive de ces herbiers, est bien plus vulnérable à la toxicité des sulfures que celle (l'expansion) des herbiers de plus forte densité. Cela peut être dû à un processus caractérisé par une dépendance « toxicité-densité », étant donné que de fortes densités de zostères peuvent permettre aux plantes de pomper plus d'oxygène dans la zone racinaire, et ainsi de former une couche protectrice contre les sulfures toxiques. En outre, non seulement les sulfures sont toxiques pour les herbiers de faible densité, mais les concentrations extrêmement élevés en nutriments peuvent également entraîner des perturbations en terme d'évolution spatiale des herbiers (dynamique de patchs).

Bien que les nutriments (principalement l'azote - N - et le phosphore - P -) sont essentiels à la croissance herbiers, un excès de nutriments peut avoir des effets négatifs sur les herbiers, non seulement en affectant la croissance des « patchs » d'herbiers, mais surtout par la diminution de la luminosité disponible pour ces herbiers, qui ont besoin de lumière pour la photosynthèse, en favorisant la prolifération d'algues dans la colonne d'eau. Il est donc important d'identifier les charges en éléments nutritifs pour prévenir la perte des herbiers. Les herbiers de Curaçao et de Bonaire sont des habitats d'alevinage rares pour de nombreuses espèces de poissons commercialement importants, mais peuvent être menacées par des charges élevées en nutriments (eutrophisation). Par conséquent, nous avons évalué l'état nutritionnel de ces herbiers et découvert que, bien que les charges en nutriments soient difficilement mesurables dans la colonne d'eau, les tissus végétaux provenant des feuilles de *Thalassia testudinum* peuvent accumuler des nutriments (N et P) à proximité d'une source de nutriments. En utilisant cette information, nous avons pu identifier d'éventuelles menaces pour les herbiers de Curaçao et Bonaire.

La pollution par les métaux lourds

En plus des niveaux élevées en nutriments, les métaux lourds (ou métaux traces) comme le mercure (Hg), le plomb (Pb), le cadmium (Cd), le chrome (Cr), originaires des activités minières, des rejets d'eaux usées ou de l'agriculture, peuvent aussi menacer les herbiers et leurs habitants. Nous avons constaté que les herbiers permettent d'indiquer l'existence de fortes charges en nutriments à long terme car ils semblent pouvoir intégrer les conditions écologiques sur une échelle de temps plus longue. Nous avons également testé cela pour la pollution par les métaux lourds dans des herbiers du monde entier, et nous avons constaté que les tissus des plantes reflètent également la pollution par les métaux lourds à long terme, pollution qui ne semble pas être mesurable dans la colonne d'eau. Ceci est important étant donné que les herbiers forment la base de la chaîne alimentaire, et que les métaux lourds peuvent s'accumuler dans les niveaux supérieurs de cette même chaîne alimentaire. L'accumulation de métaux lourds dans les herbiers peut donc être utilisée comme un indicateur de premier niveau de pollution côtière par les métaux lourds, signalant les potentielles menaces de pollution pour la pêche.

L'importance des interactions entre les espèces pour les herbiers

De nombreuses espèces dépendent directement ou indirectement des herbiers de végétation marine, non seulement les animaux, mais aussi certaines algues, et même des bactéries qui demeurent dans les herbiers. Les herbiers marins forment des écosystèmes ayant une activité biogéochimique intense et certaines espèces d'animaux qui vivent dans ces herbiers peuvent affecter les processus biogéochimiques des sédiments. Nous avons découvert que les herbiers sont globalement impliqués dans un partenariat spécialisé de «mutualisme» avec des bivalves de la famille Lucinidae, où les bivalves non seulement bénéficient de la présence d'herbiers, mais les herbiers à leur tour ont besoin de ces bivalves pour survivre. Cette relation extraordinaire est basée sur un autre mutualisme : les bivalves Lucinidae disposent de bactéries spéciales, vivant dans leurs branchies, et qui consomment les sulfures toxiques produites dans les sédiments des herbiers marins. Ce partenariat symbiotique permet aux herbiers de croître dans des zones où ils auraient dépéris en raison du stress des sulfures si les bivalves n'avaient pas été présents. Les bivalves ont à leur tour besoin des herbiers qui pompent l'oxygène dans le sol pour faire face aux sulfures toxiques. Ce mutualisme peut être appelé un « mutualisme clé de voûte », car il constitue le fondement des écosystèmes de prairies sous-marines, desquels dépendent de nombreuses autres espèces. Bien que ce mutualisme constitue une base solide des écosystèmes d'herbiers sous-marins, nous avons constaté que la rupture de ce mutualisme peut accélérer l'effondrement de ces écosystèmes. Il est important de discerner ce mutualisme, tout comme pour de nombreux autres écosystèmes qui dépendent de tels mutualismes clés comme les interactions plantes-pollinisateurs, les relations entre coraux et zooxanthelles ou encore les symbioses plantes-mycorhizes.

La symbiose entre herbier-bivalve - branchies-bactéries, qui permet aux herbiers de se développer dans des sols toxiques, est moins fréquente dans les régions tempérées. Cependant, comme le sulfure peut encore constituer une menace pour les herbiers dans ces régions, nous avons pensé que peut-être une autre interaction entre les herbiers et l'arénicole commun *Arenicola marina*, qui produit des figures caractéristiques (cônes ou buttes) sur les plages et les vasières, pourrait soulager le stress dû aux sulfures dans les herbiers. Comme ces vers de vase perturbent le sédiment (bioturbation) pour s'alimenter en particules organiques et en algues, de l'oxygène se mélange dans le sol, ce qui peut diminuer les taux de sulfure toxiques. Cependant, dans notre expérience, nous n'avons trouvé aucun effet positif dû à l'activité des arénicoles sur les herbiers. La présence d'*Arenicola marina* ne diminue pas le stress dû à la présence de sulfures, mais leur activité enfouis les plantes d'herbiers et favorise la prolifération d'algues sur les feuilles des plantes marines par pompage des nutriments depuis les sédiments vers la colonne d'eau. Un autre mécanisme diminuant le stress dû à la présence de sulfure pourrait exister dans les herbiers de zones tempérées mais reste à découvrir.

Leçons pour la gestion des herbiers et leur restauration

Les herbiers marins constituent d'importants écosystèmes mais leur distribution a considérablement diminué à l'échelle mondiale au cours des dernières décennies. Afin d'y remédier de nombreuses tentatives de restauration des herbiers marins ont été entreprises, avec plus ou moins de succès. Ce manque de succès peut parfois être attribué à des densités de plantation trop faibles, comme les herbiers ont généralement besoin de densités suffisamment élevées pour rétablir les processus rétroactifs d'auto-facilitation. Nous avons observé que les processus de restauration particulièrement important tels que l'expansion et la récupération des herbiers sont sensibles à des facteurs de stress comme par exemple de trop hautes concentrations en sulfure ou en nutriments, en raison de l'absence de processus rétroactifs et dépendant de la densité qui pourraient atténuer le stress. En dehors de ces processus rétroactifs, les niveaux de stress initialement présents tels que la pollution par les métaux lourds, les niveaux de nutriments élevés, la forte présence de sulfure et la pression des organismes bioturbateurs peuvent également affecter les chances de succès de restauration des herbiers. Il est donc important d'identifier les niveaux de stress présents avant de tenter de restaurer les herbiers. En outre, nous avons constaté que les teneurs en amidon contenu dans les rhizomes des plantes peuvent être utilisés pour prédire le succès de la transplantation de l'année suivante. Succès qui était auparavant difficile à prédire en se basant uniquement sur les mesures de densité de plantes de l'été précédent. Enfin, comme nous avons découvert qu'un mutualisme clé était à la base des écosystèmes de prairies sous-marines, nous soulignons que celui-ci devrait être pris en compte lors de la restauration des herbiers. Toutes ces découvertes peuvent être utilisées afin d'améliorer la gestion des herbiers ainsi que les efforts de restauration.

Conclusion

Nous concluons que les herbiers marins sont menacés par de multiples facteurs de stress tels que les niveaux élevés en nutriments, la pollution par les métaux lourds et le sulfure, tout ceci en raison de la présence d'activités humaines dans les zones côtières. Ces facteurs de stress peuvent interagir et affecter simultanément l'état de santé des herbiers et leur fonctionnement. Il est donc important de détecter les menaces potentielles sur les écosystèmes des herbiers marins en utilisant les valeurs de concentrations en nutriments et métaux lourds comme bio-indicateurs de pollution à long terme. Nous avons découvert que les facteurs de stress tels que les niveaux élevés en nutriments ou en sulfures, peuvent affecter les processus dynamiques à l'échelle des herbiers, en raison de l'absence de processus rétroactifs d'auto-facilitation dans les herbiers à faible densité de plantes. Cependant, nous avons également constaté que les plantes marines bénéficient d'un mécanisme de protection contre le stress dû à la présence de sulfures: un mutualisme clé de voûte entre les herbiers, les bivalves Lucinidae et leurs bactéries branchiales, et qui permet aux herbiers de pousser dans des sols riches en sulfures dans le monde entier. Cela illustre l'importance de l'étude des herbiers à tous les niveaux et pour tous les aspects de stress biogéochimiques, y compris les interactions entre les espèces, afin d'acquérir une meilleure compréhension du fonctionnement des écosystèmes, qui peuvent également fournir des outils indispensables pour la conservation des herbiers, leur gestion et leur restauration.

ACKNOWLEDGEMENTS / DANKWOORD

Het schrijven van een proefschrift en al het onderzoek dat voorafgaat aan het daadwerkelijke schrijfproces is een hele opgave. Daarom wil ik graag iedereen die actief en/of op de achtergrond bij mijn leven betrokken is geweest gedurende deze periode hartelijk bedanken. Zonder jullie steun en vertrouwen had ik deze klus niet kunnen klaren!

Allereest wil ik mijn dagelijkse begeleiders, Marieke en Tjeerd bedanken. Marieke, Jij hebt mijn interesse in en enthousiasme voor zeegrasecosystemen aangewakkerd. Eerst in Indonesië, toen ik daar nog als master student rondliep, en in eerste instantie niet perse gecharmeerd was van die kortgegraasde zeegrassprietjes, en later door mij te betrekken bij het zeegrasherstelproject in Zeeland. Ook heb je voor mij deuren geopend naar de ongerepte zeegrasvelden van Mauritanië en Australië. Door jouw grote vertrouwen in mijn kunnen en zelfstandigheid heb ik kunnen groeien in mijn wetenschappelijke doen en laten, en is dit proefschrift geworden tot wat het is. Mijn dank hiervoor! Tjeerd, ook jou heb ik in 2008 in Indonesië leren kennen en jouw wetenschappelijk enthousiasme en vele ideeën vielen me toen al op. Ik was dan ook erg blij dat ik in mijn promotietraject nauw met je kon blijven samenwerken. Dit heeft ertoe geleid dat we samen allerlei plannen en ideeën hebben kunnen verwerken in mooie experimenten (met soms wel erg veel replicas ;-)) met meestal goede resultaten, waarvan nu al veel gepubliceerd is. Bedankt voor je enthousiasme en altijd opbeurende en stimulerende woorden!

Tjisse, Leon en Fons, ook al waren jullie geen officiële begeleiders van mijn promotietraject, ik ben wel erg blij dat ik met jullie heb kunnen samen werken! En ook dat we samen de ervaringen van een mooie expeditie naar Shark Bay hebben kunnen delen. Tjisse, bedankt voor de fijne samenwerking! Ik heb ontzettend veel geleerd van je passie voor wetenschap, scherpe inzichten en veldervaring! Ik hoop dat je nog een hele generatie jonge onderzoekers zult inspireren zoals je mij hebt geïnspireerd! Ik heb ook erg genoten en veel geleerd van onze gezamenlijke veldtrips naar Frankrijk, Mauritanië, Australië en de VS. Op naar nog meer mooie gezamenlijke publicaties! Leon, ook jij ben een belangrijke bron van inspiratie geweest tijdens mijn promotietraject. Niet alleen je interesse in mijn persoonlijke wel en wee, maar ook je enorme kennis over biogeochemische processen in zout- en in sterk verdund zoutwater (of moet ik zeggen in sterk geconcentreerd zoet- en zoetwater ;). Ook veel dank voor je zorgvuldige commentaren op mijn manuscripten. Ik ben blij dat je nu eindelijke professor bent en ik hoop dat je genoeg tijd over blijft houden, naast alle managementtaken, om je af en toe te bemoeien met promovendi zoals ik, die veel profijt hebben van jouw inzichten en ervaring. Fons, ook jouw onmetelijke kennis en inzichten in biogeochemische processen hebben een belangrijke bijdrage gevormd voor dit proefschrift. Bedankt voor de altijd snelle reacties op manuscripten en mooie ideeën om nog meer uit de data te halen. Marieke, Tjeerd, Tjisse, Leon, en Fons, ik heb onze samenwerking altijd als heel prettig ervaren en ik hoop ook in de toekomst met jullie samen te blijven werken!

Naast begeleiders wil ik ook mijn promotor, Peter Herman, bedanken. Peter, bedankt voor het snelle lezen van al mijn manuscripten, waardoor ik mijn proefschrift binnen de tijd heb af kunnen ronden. Ook wil ik graag Jan Hendriks bedanken als afdelingshoofd van Milieukunde en voor de belangrijke bijdrage aan het zware-metalenmanuscript. Jan Roelofs, bedankt voor de fijne samenwerking met Aquatische Ecologie, ik heb me echt heel erg thuis gevoeld bij de afdeling en met veel plezier en goed resultaat gebruik gemaakt van de geweldige labs. Ook bedankt voor de gezelligheid en ik vond het erg leuk dat je me twee keer de gelegenheid hebt gegeven om de Ierlandcursus mee te organiseren.

Iemand die ook belangrijk is geweest voor mijn promotieproject is mijn zeegrasmaatje Wouter. Wouter, bedankt voor de fijne en gezellige samenwerking en gezelschap op het wad bij weer en wind en nacht en ontij. Urenlang hebben we samen wadpieren en zeegrasssprietjes geteld, zandzakken gevuld en gesjouwd, zeegras van hot naar her verplaatst, zeegras uitgezocht, en al mijmerend over onze zeegrasprojecten thee/koffie gedronken. Ik ben erg blij dat we elkaar hebben kunnen bijstaan in deze bewogen periode en dat onze samenwerking heeft geleid, en nog verder zal leiden tot, een aantal mooie publicaties. Ik hoop dat je snel een fijne baan zal vinden die bij je past. En mocht je het zeegras gaan missen, dan bel maar op, dan gaan we gezellig een keer zeegrasdichtheden schatten op Roelshoek ;-). Ook dank aan Kris, die als veldassistent betrokken was bij het zeegrasrestauratieproject in de Oosterschelde. Kris, bedankt voor je inzet en betrokkenheid bij mijn winteroverlevingsexperiment, en ik wens je veel geluk in de toekomst! Wim, ook jij bedankt voor de fijne samenwerking in Zeeland. Ik heb altijd genoten van onze gezamenlijke weken in de Krabbenkreek. Ook wil ik graag Tine bedanken voor haar geweldige kookkunsten waarmee ze onze drukke veldwerkweken op een prettige manier ondersteunde. Dick, jouw inzichten en ervaring waren ook van grote waarde voor het zeegrasmitigatieproject. Ik heb veel opgestoken van onze gesprekken en discussies over zeegras. Ik hoop van harte dat je erg zult genieten van je welverdiende pensioen, maar ook dat je nog regelmatige jouw waardevolle kennis en ervaring met een jongere generatie ecologen zal blijven delen. Ook wil ik graag Roy bedanken voor de geweldige interesse en steun die projectbureau Zeeweringen al die tijd heeft getoond voor het zeegrasmitigatieproject in de Oosterschelde. Deze vruchtbare samenwerking tussen de RU, projectbureau Zeeweringen, Rijkswaterstaat en de provincie Zeeland heeft het onderzoek in de proefschrift mogelijk gemaakt.

Veel verschillende mensen hebben zich ingezet bij de jaarlijkse zeegrasmonitoring in Zeeland, voor zo ver nog niet genoemd wil ik ook nog Marloes Hendriks, Nel Govers, Niels Las, Henriette Govers, Frans Reijnders, Timon Pieck, Jan-Willem Wolters, Jan de Brouwer en Paul Giesen bedanken voor hun inzet.

Mijn promotieonderzoek was lang niet zo goed gegaan als ik niet al veel ervaring had opgedaan tijdens langdurige veldwerkperiodes in Curaçao en Indonesië. Chantal, bedankt voor de leerzame tijd op dat mooie Caribische eiland. Jij hebt me de eerste skills bijgebracht in marien veldwerk en ook kennis laten maken met de lange dagen die veldbiologen doorgaans maken. Dat was eerst even slikken, maar ik ben blij dat ik die les vroeg heb geleerd en ik heb sindsdien volop genoten van alles wat die mooie, lange veldwerkdagen me hebben gebracht. Ik vind het leuk dat onze samenwerking ook nog geleid heeft tot een gezamenlijke publicatie (hoofdstuk 6).

Marjolijn, ik ben blij dat ik je heb leren kennen op dat afgelegen eiland voor de kust van Indonesisch Borneo. Eerst leerde ik je kennen als begeleider, en later werden we vrienden, en hebben we vele mooie veldwerktrips mogen maken samen. Ik koester nog steeds die bijzondere herinneringen aan het schildpadeiland Derawan, waar jij me de fijne kneepjes van het veldwerk bij hebt gebracht! Later hebben we zelfs op 5 continenten samen naar zeegras gekeken! Ik vond het ook erg leuk om weer met je rond te snorkelen tussen het zeegras tijdens onze expeditie naar Shark Bay in 2011. Ik ben erg blij met je vriendschap en met de betrokkenheid en interesse die je altijd hebt getoond in mij en mijn onderzoek. Ik vind het heel erg fijn dat je nu aan mijn zijde staat als paranimf en ik hoop dat we in de toekomst nog veel zullen blijven samenwerken! Ik hoop ook dat we nog veel mooie zeegrasplekjes kunnen ontdekken samen.

Voor mijn veldwerk ben ik op veel verschillende plaatsen geweest en heb ook veel mensen ontmoet die me hebben geholpen en gesteund aldaar.
Je voudrais remercier les autorités du Parc National du Banc d'Arguin (PNBA), surtout Lemhaba Ould Yarba et Antonio Auraujo, pour autoriser notre recherche sur l'interaction entre les coquilles et les herbiers sur les vasières intertidales du Banc d'Arguin. Mes amis mauritanien: Hassan, Mubarek, Sidi Ould Ely, Mohamed V. A. Salem, Amadou Abderahmane Sall, Mohamed Camera, je vous remercie

beaucoup pour votre compagnie, aide et connaissance du Banc d'Arguin. J'ai joui beaucoup la beauté et qualité du Banc d'Arguin et l'hospitalité mauritanienne, surtout du thé! J'espère que je peux retourner un jour pour continuer notre recherche sur les herbiers du vasières en pour boire encore du thé mauritanien!
I've also been to Shark Bay, Australia, thanks to the commitment and efforts of many Australian colleagues. Unfortunately, the manuscript resulting from our Australian research is not yet finished, and therefore could not be included in this doctoral thesis. However, we want to thank our Australian collaborators for their important roles in our research: Gary Kendrick, Di Walker, John Statton, Matthew Fraser, David Holley, Wayne Moroney, Steve, Jamie Morgan, and Jennifer Verduin, thank you for your collaboration! I hope that our fruitful collaboration will soon result in a nice publication.

I would also like to thank Raghnild Asmus and Dominik Kneer from AWI, Sylt (Germany), and Laurent Godet and Jêrome Fournier from the University of Nantes, with whom I had the pleasure of collaborating for the work in chapter 4. Finally, I want to thank Christine Angelini for our great collaboration, although not part of this thesis, but I enjoyed our work together in Rhode Island and on Sapelo. Christine, I learnt a great deal from you, thanks! I admire your enthusiasm, knowledge and great spirit and I sincerely hope that we may continue to collaborate in the future.

Ook heb ik het genoegen (en soms de frustratie) gehad om verschillende studenten te mogen begeleiden: Timon, Niki, Evelyn, Jacqueline en Lieke. Timon, ik heb altijd prettig met je samengewerkt, ook later tijdens de monitoring in Zeeland. Onze proef verliep af en toe met horten en stoten en ondanks de lichte sulfide vergiftiging die wij daar beiden aan hebben opgelopen, heeft het toch nog een mooie publicatie opgeleverd! Ik hoop van harte dat je nooit van je leven meer sulfide hoeft te ruiken! Evelyn, samen zijn we twee keer heel West-Europa doorgereden en hebben we vooral heel veel sulfide gemeten. Ik hoop dat je ondanks de lange veld- en autodagen toch hebt genoten van het onderzoek! Hopelijk leiden onze inspanningen nog tot een mooie publicatie! Jacqueline, bedankt voor je enthousiasme en toewijding. Je hebt meteen in de eerste periode van je stage al gemerkt dat veld- en lab onderzoek niet altijd loopt zoals je wilt. Fijn dat je je zo flexibel hebt opgesteld, waardoor onze inspanningen uiteindelijk toch nog tot een mooie dataset en publicatie hebben geleid.

Mijn dank gaat speciaal ook uit naar een aantal analisten die mij vaak hebben geholpen bij het meten van samples. Zonder jullie hulp had er niet veel in dit boekje gestaan! Jelle, bedankt voor je geweldige inzichten en toewijding waardoor we alles heel nauwkeurig hebben kunnen meten. Jij hebt mij veel geleerd over een breed scala aan meettechnieken en analyses, maar ook om niet zomaar te vertrouwen op de output van machines en altijd zelf kritisch te blijven nadenken. Paul, bedankt voor het meten van de eindeloze series ICP-monsters! Roy en Germa, bedankt voor jullie hulp bij analyses en het verwerken van de grote stapels monsters waar ik altijd mee aan kwam zetten. Martin, ook al loop je niet meer rond op de afdeling, jouw hulp tijdens mijn eerste veldseizoen kan ik me nog goed herinneren en ik heb je inzet erg gewaardeerd! Als laatste wil ik ook de analisten van het nutriëntenlab in Yerseke bedanken voor de honderden analyses van mijn soms 'vieze' samples.

Een proefschrift schrijven en onderzoek doen kan niet zonder een prettige werksfeer. Ik heb het geluk gehad dat ik de sfeer op en van veel verschillende afdelingen en instituten heb mogen proeven.
Allereerst wil ik mijn collega's bij Ecologie bedanken. Ik heb altijd met veel plezier (en nu nog steeds), op de afdeling rondgelopen. Mijn oude 'cubicle'-genoten, Marco, Marloes en Janneke, wat hebben we het gezellig gehad samen. Naast wetenschappelijke discussies ging het vooral ook vaak over de andere belangrijke dingen in het leven. Marloes, bedankt ook voor de fysieke bijdrage die je hebt geleverd aan mijn proefschrift! Ik heb ontzettend veel waardering voor jouw veldwerkinspanningen in mijn 1e en 2e veldwerkzomer. Ik weet ook dat je stiekem je hart op kon halen in de modder, mijn dank daarvoor ;-) . Ook heel erg bedankt voor je steun en luisterend oor in leuke en in minder leuke tijden. Ik heb ook erg genoten van ons campertripje in Australië! Je bent voor mij altijd een rots in de branding geweest en ik ben ook erg blij dat je mijn paranimf wilt zijn! Ik wens je ook veel succes en geluk op de HAS en ik hoop dat je daar je plekje zult vinden! Marco, buddy, bedankt voor je steun in voor- en tegenspoed! Ik heb genoten en veel geleerd van onze gesprekken over de wetenschap, het leven als wetenschapper en onze menselijke deugden en ondeugden. Ook kan ik je geweldige kookkunsten ontzettend waarderen, evenals je Zuid-Afrikaanse hartelijkheid en gastvrijheid. Ik hoop dat we in de toekomst nog vaak etentjes/eten zullen delen, wellicht onder het genot van een concert of festival. Ik wens je ook veel wetenschappelijke voorspoed toe, maar ik heb het gevoel dat dat wel goed zal komen! Janneke, door ons gedeelde dagelijks leven op de afdeling hebben we elkaar de laatste jaren behoorlijk goed leren kennen en af en toe ook lief en leed gedeeld. Bedankt voor je lieve luisterende oor en ik heb veel bewondering voor jouw doorzettingsvermogen en voor het feit dat je bij je eigen principes durft te blijven. Ik hoop dat we nog vaak onder het genot van een kop thee, een lekkere salade, of een wandeling de wereld en mannen kunnen bespreken! Buiten onze cubicle, maar niet uitgesloten van onze gezelligheid, wil ik ook Jan Willem en Maartje bedanken. Maartje, thanks voor je sprankelende gezelschap en je geweldige panterprint 'I-love-Laura-maar-niet-heus' outfit! Ook bedankt voor alle gesprekken die niet perse over wetenschap gingen, zoals ons gezamenlijke voorliefde voor fantasy boeken, blonde vikingmannen op tv, en muziek. We hebben het heerlijk naar onze zin gehad op Rock Werchter en Best Kept Secret (2x), waar we de laatste keer, ondanks mijn voorliefde voor 'stomme

Indie folkbandjes' toch samen keihard hebben staan springen bij Franz Ferdinand (en de voorpagina hebben gehaald van de Volkskrantbijlage!). Mijn dank daarvoor! Jan Willem, dank voor alle goede zorgen over mijn lab- en veldwerkveiligheid! Ook fijn dat jij ons 'soms-iets-te-hard-werkende' wetenschappers af en toe weer even met de beide benen op de grond zette. En natuurlijk ook bedankt voor je goede gezelschap en scherpe inbreng in (niet-)wetenschappelijke discussies.

Naast ons cubicle-clubje hebben ook de andere AIOs van de afdeling bijgedragen aan de prettige werksfeer: Marjolein, Sarah Faye, Nils, Marlous, Eva, Caspar H., Caspar C., Ivan M., Annieke, Natan, Valerie, Ralf, Bart, Herman, Yingying, Qian, Laurie, Isabella, Moni, Gijs, en José, bedankt! And also special thanks to all previous PhD-students from the department: Monique Grol, Monique van Kempen, Chantal, Ismael, Matthias, Marjolijn, Christian, Francesco, en Jeroen. Thanks to all current and previous PhD-students for the great personal and scientific atmosphere, lunch walks, bowling challenges and shared stories! I wish you all the best in your lives and scientific careers!

Naast de AIOs waren er ook vele anderen op de afdeling die mede verantwoordelijk waren voor de goede sfeer (in willekeurige volgorde); Eric, Eelke, Niels, Nathan, Raquel, Joop, Hannie, Peter, Annemiek, Dries, Roy, Germa, Ankie, Martin, Heidi, Marij, Ivan, Gerard van der Velde, Marten, Stef, Jan Kuper, José B., José A., Peter C., Wilco, Henk, Hans, bedankt voor de interessante wetenschappelijke discussies, gezelligheid tijdens de lunch, jullie bezoeken aan de Lunchbox meetings, de kerstborrels, en onze potluck diners/lunches en ontbijt! Het was prettig om met jullie op dezelfde afdeling te werken! Mocht ik nu nog iemand vergeten zijn, dan is dat niet bewust en wil ik diegenen ook nog hartelijk bedanken! Ook wil ik graag mijn kamergenoten van het afgelopen jaar bedanken. Chris en Leon, bedankt voor de gezelligheid en gesprekken over het leven als wetenschapper, maar ook voor de rust, waardoor ik relatief ongestoord mijn proefschrift af kon schrijven.
I was also involved with the department of Environmental Science, Jan, Rob Leuven, Rob Lenders, Marc, Ad, Rosalie, Gina, Aafke, Mara, Pieter, Thomas, Jon, Joris, Rick, Anne, Anastasia, Ligia, Lisette, Daan, Isabel, Alessandra, Laura Verbruggen, Laura Golsteijn, and Pim, thank you for good collegiality.

Next to my colleagues from Nijmegen, I also want to thank my colleagues from the department of Spatial Ecology from NIOZ Yerseke. It was great to discuss my research with those working on topics more related to my own. I am glad that I got to sample some of the good atmosphere of the SE department, my thanks to Jim, Hélène, Lucy, Francesco, Sil, Monique, and Yayu. Laura, it's great to be able to share my passion for seagrass with another seagrass 'geek'. I hope that your hard work will pay off and thanks for translating my summary in French! Jos, bedankt voor het verzorgen van mijn zeegrasproef, ik hoop dat je nu kunt genieten van je welverdiende pensioen.

Ik heb ook behoorlijk wat tijd doorgebracht met collega's van NIOZ Texel. Jan, bedankt voor je wetenschappelijke enthousiasme en voor de uitnodiging om in 2010 mee naar Banc d'Arguin te gaan! Ik heb geweldig genoten en ontzettend veel geleerd van dit ongerepte zeegrassysteem, en zal nooit mijn verwondering vergeten van mijn eerste blik op de BdA, daar waar de gele woestijn overgaat in een zee van groen leven. Matthijs, ook jij bedankt voor het delen van je enthousiasme en kennis van het BdA-systeem. Thomas, ik vond het erg leuk dat we in 2011 ook tijdens dezelfde expeditie in BdA waren, succes met je onderzoek! Els, ook jou wil ik ook bedanken voor de prettige samenwerking die we begonnen zijn in Mauritanië!

Naast alle werkzaamheden, vele (werk gerelateerde) reisjes en lange schrijfdagen, is het ook belangrijk om af en toe te ontspannen. Dankzij mijn sporten, vrienden en familie bleef ik met beide benen stevig op de grond staan.

Hardlopen, wandelen en wielrennen hebben mij tijdens het schrijven gezond gehouden, maar ik heb ook altijd genoten van de judotrainingen op het sportcentrum. Rien, bedankt voor de inspirerende trainingen en wijze levenslessen! Ook heb ik enorm genoten van de fysieke activiteit en goede sfeer bij het recreantenteam van basketbalclub Joy-M in Malden. Bedankt Hans, Marco, Elias, Gerard, Wilma, Marion, Emilia, Inge, Erik, Taco, Sevket, Thijs, Maris, Cees, Geert, Lilian voor de fijne potten basketbal! Ik hoop ook in de toekomst nog af en toe een balletje met jullie te gooien!

Ik wil ook graag mijn oude studievrienden bedanken: Jeroen, Rob, Ruud, Camiel, Bruce, Cindy, die mij vooral in het begin hebben gesteund en ook fysiek hebben meegeholpen, bedankt! Jan, ook al zijn we niet meer samen, ik wil je toch heel hartelijk bedanken voor je liefde en steun tijdens de eerste periode van mijn promotietraject. Bedankt dat je me zo vaak hebt meegeholpen in het veld tijdens die eerste twee zomers. Jouw hulp staat aan de basis van 3 publicaties uit dit proefschrift.

Lieve meiden uit Den Dungen (Maaskantje!) bedankt voor de heerlijke etentjes, stapavonden en weekendjes weg. Bedankt voor jullie liefde en vriendschap! Evelien, Lilian, Syfra, Karlijn, Ingrid, Karin, Irene, bedankt voor alles! Hoewel wel allemaal door de jaren heen steeds meer onze eigen weg zijn gegaan, zijn we toch nog steeds met elkaar verbonden door onze vriendschap. Dat vind ik heel bijzonder en hopelijk blijven we nog lang bevriend. Lil, jou ken ik al 25 jaar en we hebben intussen al veel dingen samen en van elkaar meegemaakt. Ik vind het geweldig dat jij de opmaak en design van mijn boekje op je hebt genomen! Ontzettend bedankt voor alle tijd en moeite die je hierin hebt gestopt met dit briljante resultaat ;-)!

Ook andere vrienden wil ik bedanken voor hun vriendschap en gezelschap: Madelon, we hadden elkaar een tijdje niet hadden gezien, maar ik vond het erg leuk om je op te zoeken in Gainesville, FL. Ik ben blij om te merken dat onze vriendschappelijke basis uit Curaçao en Indonesië standhoudt. Ik wens je heel veel succes met je interessante onderzoek! Alex en Tim, ik heb jullie op bijzondere wijze ontmoet tijdens onze gemeenschappelijke groepsreis naar Zambia, Malawi en Tanzania. Ik heb erg genoten van onze vele spelletjes filosofenkwartet en filosofische discussies. Alex, bedankt voor stimuleren van mijn interesse in filosofische wijsheden! Ik hoop ook dat we samen nog vele concerten en festivals zullen bezoeken en filosofenkwartet zullen blijven spelen! Jannes, bedankt voor je warme gezelschap tijdens alle geweldige (en soms ook vage) concerten en festivals die we samen hebben bezocht en voor je steun in leuke en minder leuke tijden! Op naar nog meer mooie muziekervaringen en hopelijk ook nog meer mooie natuurschaatstochten! Ik vind het ook erg leuk dat we nu, door het zeegrasproject in de Waddenzee, weer wat meer samen werken!

En zonder de geweldige steun van mijn familie was ik ook zo ver niet gekomen! Los van elkaar, hebben beider kanten van mijn familie altijd veel interesse, begrip en enthousiasme getoond voor mijn werk.
Opa en Oma bedankt, ik hoop van harte dat jullie bij mijn promotie kunnen zijn! Van jullie heb ik al van jongs af aan al voorliefde voor 'groen' en 'dieren' meegekregen. Dat heb ik altijd gekoesterd en heeft er uiteindelijk toe geleid dat ik met zeegras aan de slag ben gegaan. Mijn tantes, Gerry & Henriette en ooms, Frans & Joan, bedankt voor jullie interesse, medeleven en heerlijke kookkunsten! Henriette en Frans, bedankt ook voor de al eerder genoemde fysieke hulp in het veld in Zeeland. Ook wil ik graag mijn lieve neefjes (al zijn ze me allemaal boven het hoofd gegroeid) bedanken, Jurriën, Jelmer, Bob, en Niels, bedankt voor jullie interesse en voor de bijzondere kameraadschap die ik met jullie deel.

Ook de andere kant van mijn familie wil ik graag bedanken; Opa, ik hoop dat je het kunt waarderen dat je kleindochter het opzoeken van haar 'Zeeuwse roots' wel erg letterlijk heeft genomen, en zich uren door de Zeeuwse modder heeft geploeterd om tot dit proefschrift te komen. Ook bedank ik mijn ooms en tantes, Luuk & Hester, Chiel & Anneli, en Guus & Nicole voor onze prettige familieband en jullie oprechte belangstelling in mijn werk. Lieve neven en nichten, Maaike, Marloes, Olaf, Berend, en kleine Vita, bedankt voor de warme familieband die we samen delen! Die is me heel erg dierbaar! Linda, ook tot jou wil ik hier een klein dankwoord richten: ik heb veel bewondering en respect voor de manier waarop je het nu aanpakt, maar ik dank je ook voor de interesse die je altijd hebt getoond in mij en mijn onderzoek.

Romain en Matiz, lieve broertjes, dank je wel voor jullie enthousiasme en interesse in mijn werk! Ik vond het erg leuk dat jullie altijd weer enthousiast waren over foto's van nóg meer zeegras en onderwaterbeesten. Ik hoop dat jullie, net als ik, je dromen na zullen jagen (en dat hoeft geen zeegrasonderzoek te zijn ;-)! Dat is hard werken, maar geeft wel veel voldoening!
Erik, dank je wel voor al je steun, toewijding en vertrouwen in mij. Ik ben blij dat we onze band hebben kunnen herstellen in de afgelopen 10 jaar. Ook al zijn we het niet altijd over alles eens, ik weet dat je mijn vuur ook waardeert en accepteert (en dat is wederzijds). Dank ook voor je altijd luisterende oor en voor de aansporing om altijd mijn dromen waar te blijven maken.

Tessa, lieve zus, het is voor mij niet makkelijk dat je niet bij mijn verdediging kan zijn, maar je weet dat ik je keuze volledig accepteer en respecteer. Ik hoop dat je kunt genieten van mijn levenswerk van de afgelopen 5 jaar, waar ik veel tijd, energie, en passie in heb gestopt. Bedankt voor je oprechte interesse in mijn wetenschap ,en ik wens je een geweldige baan toe waar je hopelijk net zoveel passie en plezier in kwijt zal kunnen. Helaas zijn zowel de wetenschap als jouw werkzaamheden geen vetpot, maar ik hoop van harte dat we die gezamenlijke parachutesprong toch nog kunnen gaan maken ergens in de komende 3 jaar!

Lieve mam, jij staat aan de basis van dit proefschrift, want door het vertrouwen en de liefde die je me hebt geschonken heb je me een stevige basis meegegeven. Jij hebt mijn enthousiasme altijd gewaardeerd en me gestimuleerd om mijn dromen waar te maken. Bedankt voor je steun en inhoudelijke belangstelling in mijn werk en ik vond het ontzettend fijn dat je me ook in het veld uit de brand hebt geholpen! Zo heb je in Frankrijk, Sylt en Zeeland eindeloos zeegrassprietjes uitelkaar gehaald, scheuten geteld, sulfide gemeten en modder rondgesjouwd. Ik denk dat je je nu oprecht een amateur-ecoloog mag noemen!

Tot slot, Jim, jou wil ik bedanken voor je liefde en steun tijdens de totstandkoming van dit proefschrift waarbij je voor mij erg belangrijk was! Helaas was onze samenwerking en ons samenzijn niet altijd even makkelijk voor ons beiden, wat ik erg verdrietig vind. Ik heb veel bewondering voor je doorzettingsvermogen en gedrevenheid en ik hoop dan ook dat ook jij snel je proefschrift af zult ronden!

Dit dankwoord is intussen wel erg lang geworden, maar toont aan dat er heel veel mensen belangrijk zijn geweest voor mij en mijn werk in de afgelopen jaren!

Liefs,

Laura

LIST OF PUBLICATIONS

Published Papers

Govers LL, Pieck T, Bouma TJ, Suykerbuyk W, Smolders AJP & Van Katwijk MM (2014) Seagrasses are negatively affected by organic matter loading and *Arenicola marina* activity in a laboratory experiment. *Oecologia* 175:677-685

Govers LL, de Brouwer JHF, Suykerbuyk W, Bouma TJ, Lamers LPM, Smolders AJP & van Katwijk MM (2014) Toxic effects of sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. *Aquatic Toxicology* 155:253-260

Govers LL, Lamers LPM, Bouma TJ, De Brouwer JHF, Hendriks AJ, Huijbers CM, and Van Katwijk MM (2014) A global benchmark list for seagrasses as bioindicators for coastal trace metal loading and a Caribbean case study. *Environmental Pollution* 195:210-217

Govers LL, Lamers LPM, Bouma TJ, De Brouwer JHF & Van Katwijk MM (2014) Eutrophication threatens Caribbean seagrasses – a case study from Curaçao and Bonaire. *Marine Pollution Bulletin* in press

Govers LL, Suykerbuyk W, Hoppenreijs J, Giesen K, Bouma TJ, Van Katwijk MM (2014) Rhizome starch as indicator for temperate seagrass winter survival. *Ecological Indicators* 49: 53-60

Lamers LPM, **Govers LL**, Janssen ICJM, Geurts JPM, Van der Welle MEW, Van Katwijk MM, Van der Heide T, Roelofs JGM & Smolders AJP (2013) Sulfide as a soil phytotoxin – a review. *Frontiers in Plant Science* 4:268

Van der Heide T, **Govers LL**, De Fouw J, Olff H, Van der Geest M, Van Katwijk MM, Piersma T, Van de Koppel J, Silliman BR, Smolders AJP & Van Gils JA (2012) A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science* 336:1432-1434

Suykerbuyk W, Bouma TJ, Van der Heide T, Faust C, **Govers LL**, Giesen WBJT, de Jong DJ & van Katwijk MM (2012) Suppressing antagonistic bioengineering feedbacks doubles restoration success. *Ecological Applications* 22(4):1224-1231

Van Gils JA, Van der Geest M, Jansen EJ, **Govers LL**, De Fouw J & Piersma T (2012) Trophic cascade induced by molluscivore predator alters porewater biogeochemistry via competitive release of prey. *Ecology* 93(5): 1143-1152

Christianen MJA, **Govers LL**, Kiswara W, Roelofs JGM, Bouma TJ, Lamers LPM & van Katwijk MM (2012) Marine megaherbivore grazing may increase seagrass tolerance to high nutrient loads. *Journal of Ecology* 100: 546-560

Huijbers CM, Nagelkerken I, **Govers LL**, van de Kerk M, Oldenburger JJ & de Brouwer JHF (2011) Habitat type and schooling interactively determine refuge-seeking behaviour in a coral reef fish throughout ontogeny. *Marine Ecology Progress Series* 437:241-251

Submitted papers

Govers LL, Bouma TJ, Van der Ent E, Suykerbuyk W, Godet L, Asmus R, Van der Heide T, Van Katwijk MM (submitted) Feedbacks and local environmental settings affect seagrass persistence and resilience

Govers LL, Leon PM Lamers, Alfons JP Smolders, Peter MJ Herman, Evelyn van der Ent, Tjeerd J Bouma, Marieke M van Katwijk (submitted) First experimental evidence of priming in seagrass beds

Van der Zee EM, Angelini C, **Govers LL**, Olff H, Christianen MJA, Altieri AH, Van der Reijden KJ, Silliman BR, Van de Koppel J, Van der Geest M, Van Gils JA, Van der Veer HW, Piersma T, Van der Heide T (submitted) Non-trophic facilitation as a primary drivers of food webs

De Fouw J, **Govers LL**, Christianen MJA, Van der Rijden K, Van der Geest M, Piersma T, Smolders AJP, Olff H, Lamers LPM, Van Gils JA, Van der Heide T (submitted) Mutualism breakdown drives large-scale seagrass habitat degradation

Suykerbuyk W, **Govers LL**, Giesen WBJT, Giesen K, Bouma TJ, Van Katwijk MM (submitted) External forcing and stochastic processes rather than positive feedbacks determine seagrass transplantation success in a dynamic, intertidal environment

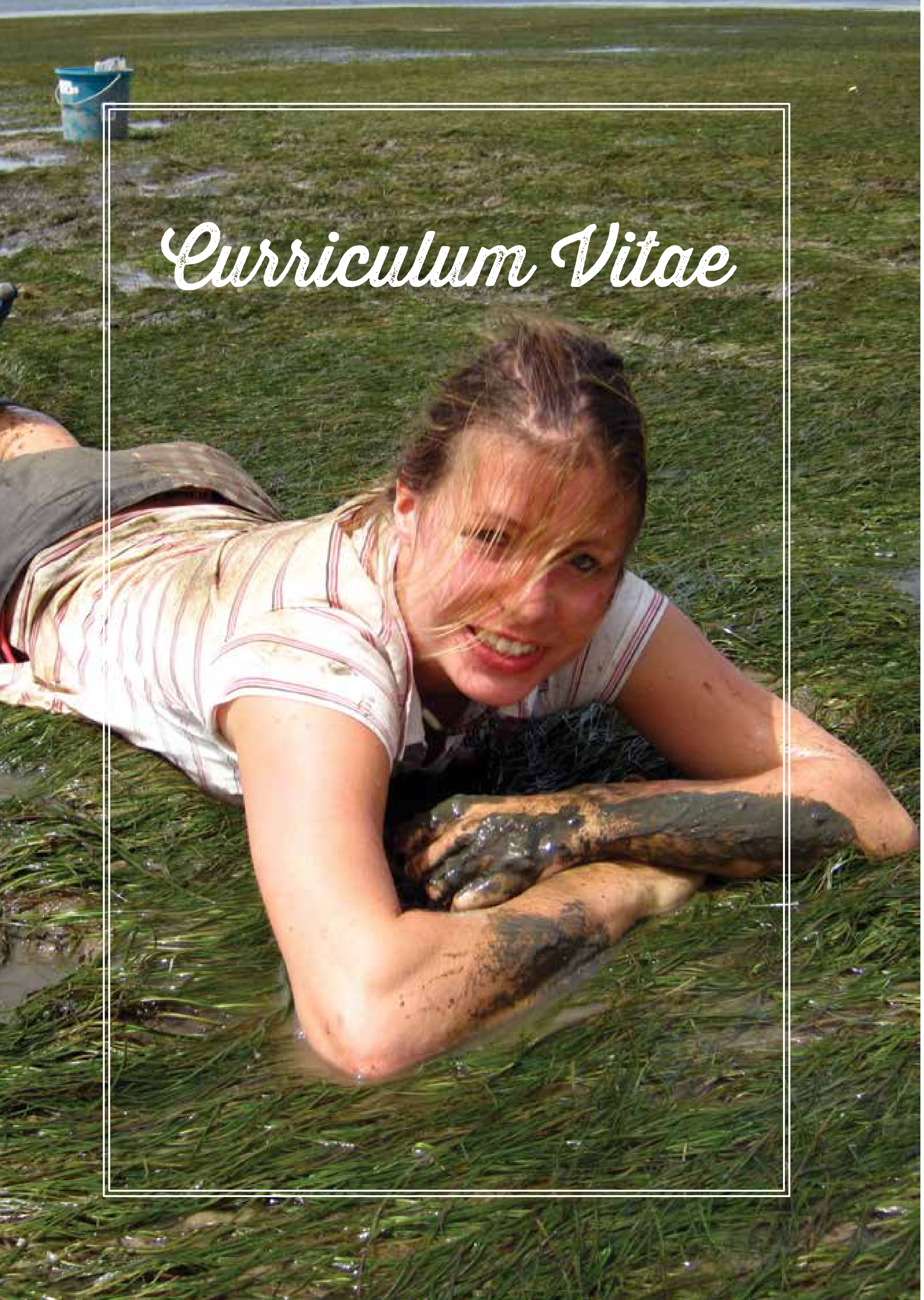
Suykerbuyk W, Van Oven P, **Govers LL**, Giesen K, Giesen WBJT, De Jong DJ, Bouma TJ, Van Katwijk MM (submitted) Living in the intertidal; desiccation reduces seagrass growth, but shading, high salinity or population of origin have no additional effect

Non peer-reviewed reports

Giesen WBJT, Giesen K, Giesen PT, **Govers LL**, Suykerbuyk W, Van Katwijk MM (2014) Zeegrasmitigaties Oosterschelde, proeven met verplaatsen van klein zeegras *Zostera noltii* in de Oosterschelde: mitigatiemaatregel bij dijkwerkzaamheden. Fasen 9-13.

Giesen WBJT, Giesen K, Giesen PT, **Govers LL**, Suykerbuyk W, Van Katwijk MM (2012) Zeegrasmitigaties Oosterschelde, proeven met verplaatsen van klein zeegras *Zostera noltii* in de Oosterschelde: mitigatiemaatregel bij dijkwerkzaamheden. Fasen 6-8.

Giesen WBJT, Giesen PT, **Govers LL**, Suykerbuyk W, Van Katwijk MM (2010) Zeegrasmitigaties Oosterschelde, proeven met verplaatsen van klein zeegras *Zostera noltii* in de Oosterschelde: mitigatiemaatregel bij dijkwerkzaamheden. Fasen 3-5.



Curriculum Vitae

CURRICULUM VITAE

Laura Leone Govers was born in ‘s–Hertogenbosch, the Netherlands, on September 16, 1985. The sea and its hidden treasures have always fascinated her, and she started snorkeling in the turbid waters of the North Sea when she was about 9 years old. This turned out to be quite disappointing due to poor visibility. However, these first encounters with the underwater world did not discourage her from loving the sea and its interesting inhabitants such as sea mammals and corals. So aged 11, she decided to become a marine biologist (instead of a sea mammal vet). This ambition has stuck to her ever since, and after graduating high school (Gymnasium Beekvliet, Sint-Michielsgestel) in 2003, the only logical choice was to start studying Biology at Radboud University Nijmegen. In her third year she followed a course on marine biology at the University of Amsterdam. This course, which included a field excursion to Mallorca, strengthened her interest in Marine biology and marine ecology in particular. After obtaining her BSc. degree in 2006, she started with the masters program ‘Limnology & Oceanography’ at the University of Amsterdam. After following courses in Amsterdam, she switched back to Nijmegen where she conducted her master’s research projects. After a first research topic on the food web structure of marine lakes in the indo-pacific region, she went to Curaçao for 5 months of fieldwork, investigating migration mechanisms of nursery fish species. As she could not get enough of tropical marine ecology, she went subsequently to a remote island in Indonesia (Derawan) to study top-down and bottom-up effects of sea turtle grazing in seagrass beds. After graduation in 2009 (*Cum Laude*), she became enthusiastically involved in seagrass research in the Netherlands and from June 2009 – June 2014 she carried out her PhD project, focusing on the effects of biogeochemical stressors in seagrass ecosystems, at the departments of Environmental Science and Aquatic Ecology and Environmental Biology of the Radboud University Nijmegen. This research was carried out in close collaboration with the Spatial Ecology group from NIOZ Yerseke, the Marine Ecology group from NIOZ Texel, and the Community and Conservation Ecology department from the University of Groningen. In this period, she supervised 5 students, was involved in 4 different BSc. and MSc. level courses, presented her research on international conferences in Brazil and the USA and participated in international expeditions to Mauritania (Banc d’Arguin), Australia (Shark Bay), and the USA (Rhode Island). She is currently doing postdoctoral research on the effects of *Phytophthora* (plant disease) on the seed germination of seagrasses, for a restoration project in the Wadden Sea.