Alma Mater Studiorum – Università di Bologna
in cotutela con Ghent University (Belgium)

DOTTORATO DI RICERCA IN

Scienze della Terra, della Vita e dell'Ambiente

Ciclo XXIX

Settore Concorsuale: 05/C1 Ecologia

Settore Scientifico Disciplinare: BIO/07 Ecologia

EFFECTS OF HUMAN PRESSURES ON ECOSYSTEM STRUCTURE, FUNCTIONING AND SERVICES IN SALT MARSHES

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Esame finale anno 2017

Effects of human pressures on ecosystem structure, functioning and services in salt marshes

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Doctoral Thesis

10 November 2017

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Abstract

Salt marsh ecosystems have been recognized as highly productive, diverse systems which provide key services to society, including coastal protection via erosion resistance and wave attenuation, nutrient cycling, carbon sequestration, and habitat provisioning. However, salt marsh ecosystems are facing an increasing number of pressures such as cultural eutrophication, erosion and coastal development, exacerbated by climate change impacts, which are leading to global losses of these valuable ecosystems. In this thesis, I aimed to assess the effects of human pressures on salt marsh status and functioning, focusing on the perennial *Spartina* spp. grasses across the Northern Adriatic Sea. Untangling these relationships is an important step towards informing management of these ecosystems for their continued provisioning of essential services. I approached this objective using a combination of field observational studies, manipulation of pressures on salt marshes, and wave mesocosm experiments:

1. I explored how variation in salt marsh vegetation structure across sites of the Northern Adriatic Sea mediates important ecosystem services such as habitat provisioning for diverse benthic macrobenthic invertebrate assemblages. Macrofaunal assemblages were related to sediment silt and organic matter content, surface water ammonium concentrations, and C:N ratios of *Spartina* spp. below-ground biomass. Our findings suggest that *Spartina* spp. indirectly influenced macrobenthos assemblages via organic enrichment of the sediments. The remarkably low macrobenthos diversity and abundance may be attributed to the cumulative impact of a wide range of anthropogenic stressors in this region, including high nutrient loads.
2. I investigated how lateral resistance to erosion in salt marshes is modified by the presence of *Spartina* spp. vegetation, sediment grain size, and the nutrient status of salt marshes across 230 km of the Italian Northern Adriatic coastline. *Spartina* spp. vegetation reduced erosion across our study sites, with a more pronounced effect in sandy soils, where erosion was reduced by 80% compared to 17% in silty soils. Erosion resistance was also enhanced by *Spartina* spp. root biomass. These results demonstrate that sediment properties are important influencers of erosion, and that vegetation also plays a direct and significant role in erosion resistance.
3. I investigated the effects of stressors, particularly high nutrient loads and heat waves on ecosystem structure and resistance to erosion in salt marshes. This study demonstrated

that: 1. Heat waves and fertilization can synergistically impact salt marsh structure by changing the vegetation composition, as we observed a rapid shift from *Spartina* spp. grasses to *Salicornia* spp. succulents in fertilized plots; 2. Heat waves, regardless of nutrient status, can trigger changes in sediment properties that reduce the ability of salt marshes to stabilize sediments; and 3. *Spartina* spp. below-ground biomass was still a significant factor in reducing erosion, despite being lower in both fertilized and unfertilized plots after the heat wave compared to the previous year.

4. I explored the effects of inundation by sea level rise on *Spartina* spp. root biomechanical properties, and resultant impacts on resistance to erosion, through a transplantation experiment. Inundation-induced mortality decreased above- and below-ground biomass over time, with complete shoot mortality in the sediment cores exposed to medium and high inundation levels. . Biomechanical properties measured at the end of the experiment (root tensile strength and % elongation) had a tendency to be lower in the treatments with higher inundation time. Overall, the best linear predictors of erosion were plant survival (indicated by % live shoot biomass) and % silt, which each decreased erosion.

The research in this thesis fills important knowledge gaps regarding the status of salt marshes and their functioning across lagoons of the Northern Adriatic Sea. A common thread in the studies presented here are the significance of biomass and sediment properties in influencing lateral resistance to erosion. Overall, these results help to consolidate the evidence of the important role of salt marsh vegetation in coastal protection, and demonstrates the conditions under which erosion resistance is most effective. This can aid in management considerations for where and how to best invest in salt marsh restoration and conservation efforts to preserve their important functioning and services to society.

Dedication

For Léa and Ignacio, and the joyful moments we shared through this journey

and will cherish in the future

Acknowledgements

As I close my PhD, I'm reminded of what my master's advisor once said: "The difference you make in life is in how you help others." I would like to thank my extraordinary team of advisors, whose knowledge and dedication to advancing ecology are inspiring. I have learned a great deal under the tutelage of Laura Airoldi and thank her for this opportunity, her insistence on high standards, and expertise on experimental design. Tjeerd Bouma was instrumental in providing many of the ideas in this research, and I am grateful for his sage advice not only on papers, but on life and career. Carl Van Colen followed every step of this research and was generous with his time and advice to make it better. Tom Moens not only provided expert advice; but his sympathetic ear and empathy helped me (and many other students) in times when I needed encouragement. My advising team has been wonderfully supportive throughout and after my pregnancy.

Moving between Italy, the Netherlands and Belgium and shuttling around vegetation and sediment cores will probably be one of the most complex logistical adventures of my lifetime (besides having a baby in between). Despite the complexity, it has been a transformative experience due to wonderful friends and colleagues. I would like to thank the members of the Airoldi lab, past and present, particularly Stef who bravely taught me how drive on manual and sampled with me in sweltering heat; Jo who provided fresh perspectives and advice; and Elena, Lorenzo, and Giulia whose hard work produced much data for this thesis. Robbie, Paolo, Eva, Massimo, Stef and Alice also provided great support throughout. Other UNIBO friends, Patricia, Vera and Augusto, brightened the long hours of research with their excellent company in and out of the lab, and we shared many highs, lows and unforgettable experiences in Italy.

At NIOZ, I was warmly welcomed by the De Keete residents and colleagues of the Estuarine and Delta Systems department. My deep gratitude to Jim for his extensive advice, ideas and support which were crucial for the erosion chapters. Special thanks go to Zhu, Loretta, Yan, Oli, Timur, Angelina, Maria, Simeon, James and the Chinese cooking crew, and many others for their good company, keeping me well-fed and accompanying me to the wave mescosms after hours. It was also a great privilege to work at the Marine Biology department at UGent. My fellow MARES cohorts, especially Eezin, Ines, Valentina, Sofia, and Christoph, and Michela and Francesca, have been my shoulder to lean on; we have laughed, cried and lived together. Thank you so much for the memories.

MARES (EU- 512002-1-2010-1-BE-EMJD) and the EU Erasmus doctoral programmes are a unique model for higher learning and cooperation between institutions, and I am grateful for the incredible opportunity to participate in this programme. Thanks to Tim Deprez, Pieter Blondeel and Veerle Rogge, who helped in so many ways promptly and professionally. This research was additionally supported by funding from the Italian government with the program TETRIS (Observing, modelling and TEsting synergies and TRade-offs for the adaptive management of multiple Impacts in coastal Systems, PRIN 2010-2011, MIUR).

My parents, Barbara and Don, have always encouraged me to take the adventurous, unexplored route, and they, my sisters, and friends from Canada have been cheering me on. My parents-in-law, Nuria and Jose, also took Léa under their wing for a month so I could concentrate on writing. My gratitude to you all.

How is one motivated to complete a PhD and move around multiple places with salt marsh samples and a baby girl? Certainly not without the unconditional love and never-ending support of one Ignacio Barbeito Sanchez. As a field volunteer, box mover, driver, lab mate, protector from aggressive goats in the field, encourager, and devoted father, he often put his career on hold so I could advance mine. And to our tiny one who is a giant source of pride and hope, our beautiful little Léa's joyous chatter and giggles gave me strength in the final stretch. I look forward to the day we can recount our PhD adventures to her and hope they will inspire her to pursue her dreams.

Veronica Lo

10 November 2017

CHAPTER 1

Introduction

Spartina spp. in Chioggia, Venice Lagoon (CHI)

1. Introduction

1.1 Ecosystem services provided by salt marshes in an era of global change

In this era of global change, current policy and research are focusing on the concept of ecosystem services and their contribution to enhancing resilience of socio-ecological systems to change. Ecosystem services are the benefits that people obtain from ecosystems, and include provisioning services, such as the production of food and water; regulating services, including climate and flood regulation; supporting services, such as nutrient cycling and crop pollination; and cultural services, such as educational and recreational benefits (Millennium Ecosystem Assessment, 2005). As estuarine and coastal ecosystems are situated at the interface between the coast, land and watersheds, they provide a multitude of cumulative benefits compared to coastal, land and watershed ecosystems considered separately (Barbier et al., 2011).

Vegetated coastal ecosystems such as salt marshes are one such example of ecosystems that provide critical ecosystem functions with multiple societal benefits. Salt marshes often form in sheltered environments where sediments can accumulate, and can be found on coast lines in temperate and high latitudes, in estuaries, shallow bays, barrier islands and spits (Fagherazzi et al., 2012). They depend on a regular supply of sediments to form, which are delivered to the marsh platform through flooding, and they (Fagherazzi et al., 2012). As highly productive ecosystems, salt marshes provide a variety of ecosystem services, including provisioning of nursery habitat for juvenile fish and shellfish, and habitat for aquatic organisms, birds and wildlife (Barbier et al., 2011; Beck et al., 2001). In recent years, salt marshes have gained much recognition for their regulating ecosystem services, including carbon sequestration and coastal protection through wave attenuation, flood-water retention and sediment stabilization/erosion resistance (Duarte et al., 2013). Increasing coastal hazards such as sea level rise and storm surges as a result of climate change have generated high interest in the role of coastal vegetation in ecosystem-based approaches to coastal defence. These include conservation measures such as marine protected areas; habitat restoration; and managed re-alignment where sea walls are opened up to re-connect coastal lands to the tidal system, thus re-flooding coastal lands and enabling marsh growth (Shepard et al., 2011; Spalding et al., 2014). Intact coastal habitats have been estimated to halve the number of people and total value of residential property that are most exposed to hazards (Arkema et al., 2013), thus, the economic and societal benefits of restoring and conserving coastal vegetated habitats is evident. Efforts to better manage and protect salt marsh ecosystems have been initiated at the local, national, regional and international levels; for example, the European Habitats Directive classifies salt marshes as sensitive habitat. In recent years, a number of international agreements have promoted the use of ecosystems in adapting to change. The Secretariat of the Convention on Biological Diversity (CBD) has called on governments to implement ecosystem-based approaches to climate change adaptation and disaster risk reduction, including through ecosystem restoration and conservation (Decision XII/20). The Ramsar Convention on Wetlands Resolution XII.13 on Wetlands and Disaster Risk Reduction and the Sendai Framework for Disaster Risk Reduction 2015-2030 are part of a growing body of agreements and policy frameworks that recognize the importance of healthy, functioning ecosystems to human well-being.

Role of *Spartina* spp. grasses in coastal protection

Stressful coastal environments which experience high ranges of salinity and inundation tend to be dominated by ecosystem engineers (Crain and Bertness, 2006), which are species that create, alter or maintain habitat, and influence community composition through non-trophic interactions (Jones et al. 1994). Perennial grasses of the *Spartina* genus are a prime example of ecosystem engineers and establish in the pioneer zone of salt marshes (Pennings and Bertness, 2001). As a perennial species, their dense below-ground structure remains intact year-round, providing continuous sediment stabilization (van Eerdt, 1985), compared to the above-ground structure which is lost during the winter (Bouma et al., 2014). Physiologically, *Spartina* spp. is well-adapted to regulation inundation, as its the root system has specialized aerenchyma (air-filled) tissue which enable its growth in anaerobic or anoxic conditions by the delivery of oxygen to roots (Tiner, 1999). As the physical structure of the *Spartina* spp. plant also modifies the environment and facilitates the presence of other organisms and communities, they are considered autogenic engineers (Bouma et al., 2009; Bruno et al., 2003; Crain and Bertness, 2006). By attenuating wave energy and water flow, *Spartina* spp. trap fine sediments, resulting in accretion and improved plant growth (Bouma et al., 2009). In terms of facilitation, shading by salt marsh vegetation such as *Spartina* spp. can increase macrofaunal species richness (Whitcraft and Levin, 2007), and sediment organic matter, which can be enhanced by salt marsh vegetation, has been linked to higher macrofaunal taxa densities (Talley and Levin, 1999); the dense below-ground matrix of roots and rhizomes can also play an inhibitory role by physical exclusion (van der Wal and Herman, 2011). In addition to autogenic engineers, salt marshes are habitat for allogenic engineers, which modify their environment by transforming living or nonliving material from one physical state to another, such as benthic fauna which rework sediments through burrow structures and burrow ventilation (Bouma et al. 2009). It is thought that the dynamics of facilitation and inhibition between autogenic engineers and allogenic engineers gives rise to patterns of patchiness and habitat diversity in salt marshes (Bouma et al. 2009).

The unique ecosystem engineering properties of *Spartina* spp. underpin their coastal protection function (Duarte et al., 2013). *Spartina* spp. vegetation indirectly influences erosion rates via modification of soil properties, including peat built-up and alteration of coastal bathymetry, and via sediment accretion, which in turn alters elevation and wave heights (Gedan et al. 2010). The density and flexibility of the salt marsh canopy also plays a role in decreasing flow velocity which enables sediment-trapping, wave attenuation and erosion resistance (Bouma et al., 2005; Heuner et al., 2015; Möller et al., 2014). Below-ground, *Spartina* spp. plays a direct role in sediment stabilization. The extensive matrix of roots and rhizomes increases the shear strength of sediments, enhancing cohesion and tensile strength (Micheli and Kirchner, 2002), helping to maintain shorelines and reduce erosion (Shepard et al., 2011).

The evolution of salt marshes is determined by complex relationships and feedbacks between ecological and geomorphological processes (Figure 1). Salt marshes are dynamic systems that undergo periods of seaward expansion and periods of shoreward (lateral) erosion due to tidal or wave-induced currents (Bouma et al., 2016; van de Koppel et al., 2005; Yapp et al., 1916). Sediment supply, depth and width of adjacent tidal flats, and wave action affect the balance between erosion and progradation (Mariotti and Fagherazzi, 2013). In the vertical dimension, the ability of salt marshes to keep pace with sea level rise by accretion is also dependent on sufficient sediment supply and the rate of landward expansion (Kirwan and Megonigal, 2013) (Figure 1). Historically, salt marshes have been able withstand sea level rise and maintain their position in the intertidal zone, as rates of sediment deposition and organic accretion have been similar to rates of sea level rise in most salt marshes (Fagherazzi et al., 2012). Under moderate scenarios of sea level rise, salt marshes can continue to persist due to feedbacks among inundation, plant growth, and organic matter accretion, but only given adequate sediment supply (Kirwan et al., 2010).

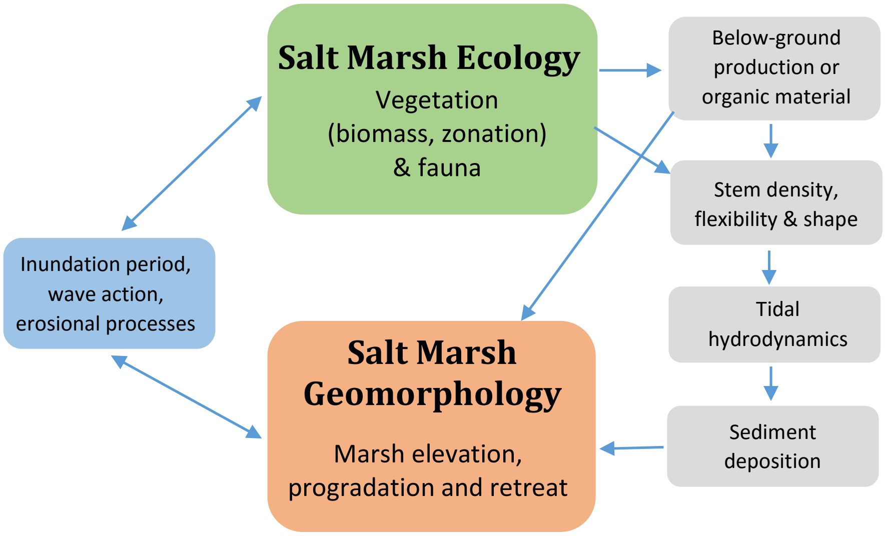

Figure 1. Simplified scheme of the interactions between ecological and geomorphological processes that govern salt marsh evolution (modified from Fagherazzi et al., 2004)

1.2 Multiple human pressures and consequences for salt marsh structure, functioning and services

Given their high value to society, coastal ecosystems are some of the most heavily used ecosystems globally with increasing populations (Lotze et al., 2006). Multiple human pressures are causing widespread and accelerating loss, with some estimates suggesting global losses of coastal wetlands and associated salt marshes ≥ 60-70 % (Airoldi and Beck, 2007; Lotze et al., 2006). High nutrient loads have been an additional suggested cause for collapse of salt marsh systems, as nutrient enrichment decreases investment in below-ground biomass and enhances microbial decomposition, thereby destabilizing soils (Deegan et al., 2012).

Such multiple and cumulative stressors have been shown to have antagonistic and synergistic impacts on ecosystems (Halpern et al. 2008, Crain et al. 2008). For example, the BP oil spill triggered geomorphic feedback mechanisms in Louisiana salt marshes, resulting in amplified erosion rates in a system already highly disturbed, limiting the recovery of salt marshes (Silliman et al., 2012). Synergistic interactions between trampling and restricting tidal flow was associated with changes in native plant cover, with higher cover of non-native plants in tidally restricted sites with trampled plots (Goldman Martone and Wasson, 2008).

Moreover, while providing protective functions from impacts of climate change, salt marshes are themselves vulnerable to the impacts of climate change, including relative sea level rise and erosion, which are exacerbating pressures on salt marshes (Shepard et al. 2011). Heat wave conditions (higher surface water temperature and reduced precipitation) were associated with replacement of *Spartina* spp. with shallow-rooted *Salicornia veneta* succulents, which have been demonstrated to be more vulnerable to inundation (Strain et al., 2017). Under projections of rapid sea-level rise, it is predicted that marshes will likely submerge near the end of the 21st century (Kirwan et al., 2010). Regardless of sea level rise scenarios, sediment starvation due to mining, dredging and extractive activities severely threatens the persistence of salt marshes (Airoldi and Beck, 2007; Fontolan et al., 2012). The adaptive capacity of salt marshes to respond to climate change impacts, for example, by migrating inland in response to higher inundation, is also restricted by physical structures in many coastal zones around the world as they tend to be heavily populated and urbanized, giving rise to a phenomenon known as “coastal squeeze” (Mills et al., 2016; Spalding et al., 2014).

The continued services provided by salt marshes will depend on their persistence in the face of these pressures combined with global change (Bouma et al., 2014). However, in many regions of the world, important baseline information is lacking on salt marsh structure and functioning, which is needed in order to prioritize management and conservation of salt marshes for their continued provisioning of ecosystem services. Given the growing evidence that salt marsh conservation and restoration can be effective in ecosystem-based approaches to adaptation (Shepard et al., 2011), and their rapid rate of decline globally, it is imperative to quantify their role in coastal protection, and identify which factors or combinations of factors enhance or limit this valuable ecosystem service (Bouma et al., 2014). While the interest in combating SLR has generated numerous studies on the ability of salt marshes to respond to changes in sea level salt marshes, there are comparably fewer studies on erosional processes that salt marshes are more vulnerable to (Fagherazzi et al., 2013; Kirwan et al., 2016), and the effect of sea level rise on salt marsh lateral erosion resistance is not well understood (Bouma et al., 2014).

1.3 Thesis Objectives and Outline

The **main objective** of this thesis is to assess the effects of human pressures on salt marsh status and functioning, focusing on *Spartina* spp., across the Northern Adriatic Sea.

The **specific objectives** of this thesis are to:

1. Determine variation in salt marsh vegetation structure across sites of the Northern Adriatic Sea and how it mediates important ecosystem services such as habitat provisioning for diverse benthic macrobenthic invertebrate assemblages;
2. Investigate factors affecting the erosion resistance of salt marshes, specifically the effect of vegetation, sediment properties and nutrient status on lateral erosion resistance;
3. Investigate the effects of stressors, particularly high nutrient loads and heat waves, on ecosystem structure and control of lateral resistance in saltmarshes
4. Analyze how inundation (as a proxy for relative sea level rise) and root biomechanical properties affects the susceptibility of salt marsh sediments to lateral erosion

Four main chapters (Chapters 2, 3, 4 and 5) constitute the main body of this thesis, with each one representing findings from observational studies or experiments and written in the format of a scientific article with the goal of publication in academic journals.

In **Chapter 2**, I investigate the status and diversity of *Spartina* spp.–dominated salt marshes along lagoons of the northern Adriatic coastline, relating it to diversity and abundance of macrobenthos communities and local sediment and nutrient conditions. Our results provide important baseline information to inform monitoring and management of these unique and productive ecosystems, and set the stage for further investigation into how salt marsh structure, functioning and ecosystem services are affected by human pressures.

The next chapters of this thesis focus on improving our understanding of control of erosional processes by salt marshes through a combination of observations, experimental manipulation of stressors, and quantification of erosion using wave mesocosms and novel photogrammetry techniques. In **Chapter 3**, I investigate factors affecting resistance to lateral erosion in salt marshes across 230 km of the Adriatic sea coastline. Specifically, I determine how lateral erosion varies in relation to the presence of *Spartina* spp. vegetation, sediment grain size, and the nutrient status of salt marshes. Resistance to lateral erosion was measured using wave mesocosms to subject vegetated and bare salt marsh sediment cores to waves. The results of this study have been published in the international journal *Marine Environmental Research* (Lo et al. 2017).[1]

The potential effects of high nutrient loads on erosion resistance of salt marshes is further explored in **Chapter 4**, where I describe a nitrogen enrichment field experiment in Grado Lagoon, I determined whether the presence or absence of *Spartina* spp., in combination with nutrient and sediment properties, affected erosion resistance of salt marsh sediment cores. In the second year of the experiment (2015), a heat wave affecting Italy and much of Europe induced high surface water temperatures and salinity, providing a unique opportunity to document how nutrient enrichment

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[1] Lo, V.B., Bouma, T.J., van Belzen, J., Van Colen, C., Airoldi, L., 2017. Interactive effects of vegetation and sediment properties on erosion of salt marshes in the Northern Adriatic Sea. *Marine Environmental Research* 131, 32–42. doi:10.1016/j.marenvres.2017.09.006

can trigger ecosystem changes in response to climactic disturbances such as heat waves, and to measure the functional consequences in terms of lateral erosion resistance.

In **Chapter 5,** I explore the effects of increased rapid inundation (as could occur as in a coastal squeeze context as a consequence of land-subsidence/ sea level rise) on lateral erosion resistance of salt marshes. While several studies have demonstrated impacts of sea level rise (SLR) on salt marsh structure, such as how SLR affects biomass and root to shoot ratios, there have been few empirical studies quantifying the impacts of SLR on functioning and related ecosystem services. I determined how erosion resistance of salt marshes is affected by sea level rise by experimentally transplanting *Spartina* spp. salt marsh sediment cores along a gradient of inundation, a proxy for relative SLR. After four months of inundation treatment, the sediment cores were extracted and subjected to wave treatment in wave mesocosms.

In **Chapter 6 (Conclusions and Future Perspectives),** I zoom out of the Adriatic coastline, synthesizing the findings of this thesis and seeing how this work as a whole fits into the global picture of current salt marsh knowledge by assessing knowledge gaps and providing recommendations for future research. This chapter also situates the research in the broader context of coastal zone conservation, management and policy in the face of multiple stressors and global change.

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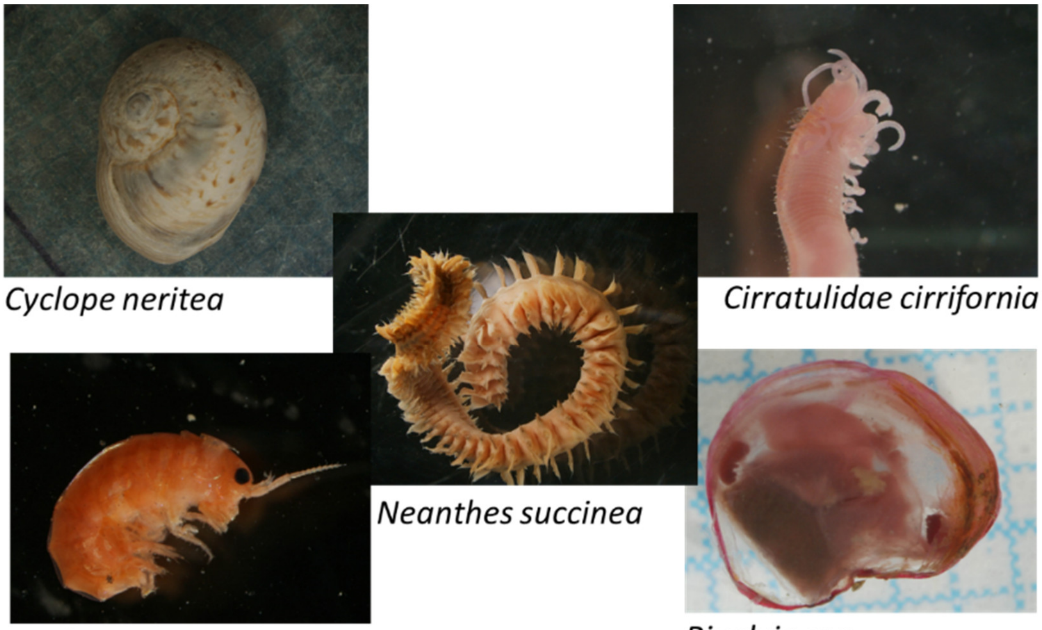
CHAPTER 2

The influence of salt marsh vegetation on benthic macrofaunal communities across lagoons of the Northern Adriatic coast



Cyclope neritea

Cirratulidae cirrifornia

Neanthes succinea

Orchestia spp.

Bivalvia spp.

Macrobenthic fauna in salt marshes of the Northern Adriatic Sea

2. The influence of salt marsh vegetation on benthic macrofaunal communities across lagoons of the Northern Adriatic coast

Abstract

Salt marshes are highly productive ecosystems that are recognized to provide multiple ecosystem services, but are under pressure from multiple stressors. We investigated the status and diversity of *Spartina* spp.–dominated salt marshes along lagoons of the northern Adriatic coastline (Italy), relating *Spartina* spp. vegetation status to diversity of associated salt marsh plant species, diversity and abundance of macrobenthos communities inside *Spartina* spp. patches, and abiotic sediment and nutrient conditions. *Spartina* spp. vegetation characteristics varied across sites. Within sites, there was local variation in root:shoot ratio, stem density and % cover. The presence of vegetation marginally enhanced organic matter and significantly decreased bulk density, although there was no effect of vegetation on grain size variables (% silt, %mud and median grain size). Macrofaunal assemblages were homogenously low in species richness, abundance, diversity and evenness across sites, and assemblages were related to sediment silt and organic matter content, surface water ammonium concentrations, and C:N ratios of *Spartina* spp. below-ground biomass, which together explained 43% of the total variability in assemblages. Our findings suggest that *Spartina* spp. indirectly influenced macrobenthos assemblages by modification of soil properties, i.e. via organic enrichment of the sediments. We also suggest that the remarkably low macrobenthos diversity and abundance may be attributed to the cumulative impact of a wide range of anthropogenic stressors including high nutrient loads.

2.1 Introduction

Vegetated coastal habitats such as salt marshes are among the most productive ecosystems in the world and provide ecosystem services of high value to society, including coastal protection, carbon sequestration, nutrient cycling, and habitat provisioning for a multitude of species (Gedan et al., 2009). The stressful environments in the coastal zone, with high fluctuations of salinity, temperature and hydrodynamic regimes, give rise to colonization by species that are highly tolerant of such changes or that can engineer or modify their environments to be more habitable (Crain and Bertness, 2006). Salt marsh vegetation such as the perennial *Spartina* spp. grasses are classic examples of such ecosystem engineers, in which biophysical feedbacks enable habitat modification and facilitate the presence of other organisms and communities (Bouma et al., 2009; Bruno et al., 2003; Crain and Bertness, 2006). By attenuating wave energy and water flow, *Spartina* spp. trap fine sediments, resulting in accretion and improved plant growth (Bouma et al., 2009). In turn, local sediment dynamics are modified to include higher amounts of fine sediment and organic content (Neira et al., 2006), supporting higher macrobenthic biomass (van der Wal and Herman 2012). Where salt marshes have been restored with *Spartina maritima*, the complexity (species richness and diversity) and abundance of the benthic macroinvertebrate community have been shown to have similar, or even higher values compared to natural marshes, although with different species composition (Curado et al., 2014).

Facilitation and inhibition of macrofaunal species by salt marshes can be conditional upon above- and below-ground structure and sediment properties (van der Wal and Herman 2012). For example, higher densities of macroinvertebrates have been associated with *Spartina* spp. culms (Rader, 1984), and shading by salt marsh vegetation can increase macrofaunal species richness (Whitcraft and Levin, 2007). Sediment organic matter, which can be enhanced by salt marsh vegetation, has been linked to higher macrofaunal taxa densities (Talley and Levin, 1999). Rather than facilitation, inhibition of species by salt marshes can occur – for example, root biomass has been demonstrated to mechanically exclude macroinvertebrates (Brusati and Grosholz, 2006; van der Wal and Herman, 2011; Van Wesenbeeck et al., 2007). Thus, salt marsh vegetation can play roles in both inhibition and facilitation of benthic communities. On the other hand, benthic macrofauna can also cause loss of salt marsh vegetation due to bioturbation and herbivory; e.g. *Spartina* establishment can be inhibited by low sediment stability induced by lugworms (Van Wesenbeeck et al., 2007), and ragworms consume microphytobenthos, filamentous algae, and seeds and seedlings of salt marsh plants (Paramor and Hughes 2005, Zhu et al. 2014). The balance between facilitation and inhibition of species by ecosystem engineers is a topic of research that needs more attention (Bouma et al. 2009).

While salt marsh ecosystems provide many essential ecosystem services to society, this provisioning is dependent on their continued stability and persistence (Bouma et al. 2014). Salt marsh ecosystems are facing an increasing number of pressures such as sea level rise due to climate change, erosion and coastal development, which have been shown to have deleterious effects on ecosystem functioning (Bricker et al 2008, Pinckney et al. 2001). Eutrophication has been an additional suggested cause for collapse of salt marsh systems, as nutrient enrichment decreases investment in below-ground biomass and enhances microbial decomposition, thereby destabilizing soils (Deegan et al., 2012a). In temperate estuaries, eutrophication has led to structural modifications of intertidal benthic assemblages, including declines in diversity and changes in trophic structure (Cardoso et al., 2004), and increases in opportunistic species such as polychaetes (Schückel and Kröncke, 2013). As a result of multiple pressures, coastal wetlands and associated salt marshes have suffered serious declines with some estimates suggesting global losses ≥ 60-70 % (Lotze et al. 2006, Airoldi & Beck 2007). In order to prioritize management and conservation of salt marshes for their continued provisioning of ecosystem services, it is critical to assess their structure and functioning.

The northern Adriatic coast of Italy features several lagoons with extensive salt marshes, but broad-scale studies on their current structure and functioning are limited. This area is heavily urbanized and influenced by anthropogenic pressures including port construction, dredging of sand and gravel, fish farms, gas extraction and tourism. Eutrophic phenomena are common (Penna et al., 2004). Erosion is occurring rapidly, with marsh retreating in some areas at a rate of approximately ten meters per year, caused by decreased sediment supply and wind influences (Fondazione Ca' Vendramin, 2010). As a result, unprecedented losses of salt marshes have been recorded over the last decade, e.g. in Venice and Grado-Marano lagoons (Fontolan et al., 2012; Lotze et al., 2011). Climate change is exacerbating these impacts. After a severe heat wave in 2003, large-scale diebacks of the native pioneer grass *Spartina maritima* occurred, which has been replaced by *Salicornia veneta*, an annual succulent (Belluco et al., 2006; Merloni, 2007). Such vegetation shifts can result in subsequent losses of structural complexity and hence potentially reduced provisioning of ecosystem services such as resistance to sea level rise (Strain et al. 2017) and to erosion (Lo et al., 2017). The 2003 heat wave also induced abrupt changes in salinity, ammonia, and dissolved oxygen, resulting in changes to benthic community structure and composition in Comacchio lagoon (Munari, 2011). To our knowledge this is the first study conducted on a broad scale linking salt marsh vegetation structure and environmental conditions to macrobenthic community assemblage and diversity in the Northern Adriatic Sea. Such baseline knowledge will be critical for management and conservation practices in the face of multiple pressures, given the significant role that salt marshes play in coastal defense and provisioning habitat and feeding grounds for important fish and shorebird species.

The objective of this paper is to determine how vegetation structure mediates benthic macroinvertebrate assemblage and diversity. We focus on *Spartina* spp. as it is the dominant grass species in the salt marshes of this region, and given its significance in provisioning of ecosystem services. We assess *Spartina* spp. characteristics including biomass, cover, and density; diversity and abundance of associated salt marsh vegetation species; and diversity and abundance of macrobenthic communities. We examine how these relationships are modulated by abiotic variables including sediment properties, surface water nutrient concentrations and nutrient loading as inferred by carbon to nitrogen ratios in the vegetation. These results will enable a clearer understanding of ecosystem engineering effects of *Spartina* spp. vegetation in this region. Such information will assist in management and conservation of these marshes for their continued ecosystem service provisioning, and could serve as the basis for potential indicators of marsh health.

2.2 Materials and Methods

Study Area

The Northern Adriatic Sea basin off the coast of Italy is the shallowest, northernmost region of the Mediterranean (Giani et al., 2012) and among the most productive with a high habitat diversity (Colombo, 1992). The area consists of lagoon-river delta systems with moderate exposure to wave action and a semi-diurnal micro-tidal regime with average tidal amplitude of about 50–130 cm (Russo et al. 2012). Average sea surface temperatures vary between 7 °C in winter and 27°C in summer, with highly variable average surface salinities between 30 and 38 psu (Russo et al., 2012). The lagoons in this region have a centuries-long tradition of being exploited for aquaculture (Ponti et al., 2007). Other major activities in the area include the petrochemical industry, tourism, fishing, seaport/port activities and shipping (S. Torresan, 2012), which are affecting the multitude of sensitive and protected habitats and species in the lagoons (Airoldi and Beck, 2007). Attempts to counteract coastal erosion have been made with limited success, including the construction of breakwaters and beach nourishments, which have resulted in changes to macrobenthic structure and functioning (Colosio et al. 2007). We sampled seven sites in coastal lagoons over a wide latitudinal gradient of *Spartina* spp. The sampling area spanned approximately 230 km of coastline covering the Comacchio, Caleri, Vallona, Venice and Grado-Marano lagoons, which span the provinces of Emilia-Romagna, Veneto and Friuli Venezia Giulia.

Spartina maritima, which is native to Europe, had been the only *Spartina* species recorded in the Northern Adriatic in the past; however, it is now largely being replaced by the hybrids *S. anglica* and *S. townsendii* (Wong, 2015). As it is difficult to distinguish these species morphologically until the flowering period, we will refer to our samples as *Spartina* spp.

Five of our sites are located in the Po River Delta, and two sites are located in the Grado-Marano lagoonal complex (Figure 1). The Po Delta lagoonal system is comprised of 8150 ha of lagoons, 8600 ha of banked fish farms, and over 1250 ha of wetlands (Fondazione Ca' Vendramin, 2010). Site 1 is located in a natural reserve in Sacca di Bellocchio (BEL, 44°37'47''N, 12°15'38''E), in the part of the Valli di Comacchio lagoonal ecosystem in the Parco Delta del Po dell'Emilia-Romagna. This shallow lagoon is eutrophic with inputs of nutrient-rich continental waters (Mistri et al. 2001). The adjacent salt pan is important for nesting and migrating birds (Munari, 2011). Site 2 is located in Vallona lagoon (VAL, 45°1'41 ''N, 12°23'7''E). The 11.5 km^2 lagoon was created unintentionally in the 1970's when previously drained farmland was re-flooded by seawater due to severe land subsidence. It is currently being used for private shellfish aquaculture and seasonal hunting (Wong et al. 2015). Site 3 is located in the municipality of Rosolina, in the southern portion of Caleri lagoon (ROS, 45°5'41''N, 12°19'37''E), within the Coastal Botanical Garden of Veneto. Sites 4 and 5 are located in Venice Lagoon, also part of the Parco Regionale Veneto del Delta del Po. The lagoon has an area of 550 km^2, with a mean water depth of 1.5 m (Silvestri, Defina, and Marani 2005). Human activities include dredging, building of jetties at the three inlets, and groundwater withdrawals, resulting in strong erosion in the southern and central parts of the lagoon. The lagoon has undergone subsidence of 25 cm in the last century, with respect to mean sea level, mainly attributed to groundwater pumping and some degree of eustacy (Tosi, Teatini, and Strozzi 2013). Salt marsh extent has been estimated to have decreased from 120 km^2 at the beginning of the century to 40 km^2 at present (Day et al. 2012).

Site 4 is located in the southern part of Venice Lagoon, in Chioggia Inlet (CHI, 45°14'ra53''N, 12°13'34''E). Site 5 (VEN) is located in the narrow fringing marshes in the San Felice Channel in the northern part of the lagoon, running north-east from the Porto di Lido inlet. Sites 6 and 7 are located in the Grado-Marano lagoon, in the northern part of the Adriatic Sea, spanning approximately 160 km^2 between the Isonzo and Tagliamento River deltas. The lagoon is connected to the sea by six tidal inlets, and supplied with freshwater by several rivers (further site description in Chapter 3). Site 6 is located in the tidal channel fringing marshes of Grado Lagoon (GRA, 45°42'12''N, 13°26'31''E), and site 7 is located approximately 1 km from a golf course (GOLF) (Figure 1).

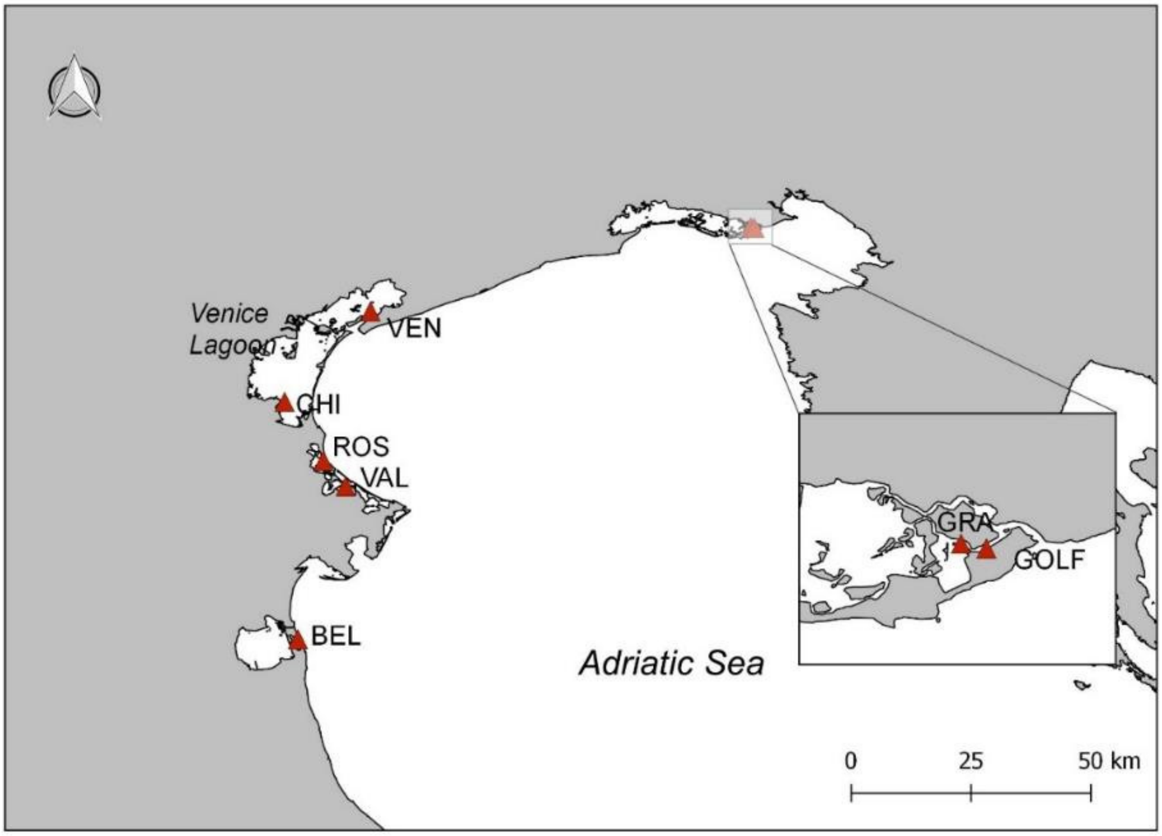


Figure 1: Location of sampling sites on the Northern Adriatic coast, Italy. Sampling sites: 1. Sacca di Bellocchio, Parco Delta del Po dell'Emilia-Romagna (BEL); 2. Vallona Lagoon (VAL); 3. Coastal Botanic Garden of Veneto, Parco Regionale Veneto del Delta del Po (ROS); 4. Chioggia Inlet, Venice Lagoon (CHI); 6. San Felice Channel, Venice Lagoon (VEN); and 7. Grado Lagoon (GRA).

Sampling Design

For each of the seven sites, we collected samples to analyze vegetation and environmental variables from each of four 20 m-long transects placed parallel to the shoreline, randomly placed over patches where *Spartina* spp. occurs, including where there were bare patches and other salt marsh vegetation. Sediments were sampled in bare and *Spartina* spp. vegetated patches within each transect. Sampling was completed in June and early July 2014. Unless otherwise specified, four replicates of *Spartina* spp. vegetation variables were taken per transect per site, while for environmental and macrobenthos data, 1 replicate per transect was sampled inside a vegetated patch.

Spartina spp. vegetation variables

Vegetation parameters measured were per cent cover of *Spartina* spp., density, and canopy height, measured within each of four 0.25-m^2 quadrats randomly placed along each 20-m transect. Canopy height of the vegetation was measured to the nearest 0.2 cm by recording the settling height of a Styrofoam disc (25 cm diameter, 75 g weight) that was dropped on top of the vegetation in the quadrat along a graduated pole (*cf.* (Stefanie Nolte, 2014). Four measurements of canopy height measured in each of the four corners of the quadrat were pooled to obtain one mean per quadrat.

Above and below-ground biomass were measured by randomly extracting sample cores in *Spartina*-vegetated areas along each transect (n=4 per transect). 10-cm diameter by 30-cm depth PVC coring devices were hammered into the sediment, and roots were cleanly sliced around the coring device with a knife before careful extraction of the sample. Samples were transferred to plastic bags and transported to the lab. Above and below-ground fractions were washed separately over a 1-mm mesh sieve to remove sediment from the plant material. The above-ground portion was cleanly sliced off at the sediment interface and frozen at -18^{0}C for subsequent analysis. After thawing, density and morphological analyses were performed: stems were separated into live and dead fractions and were counted to obtain live densities per sample core. Below-ground biomass was rinsed over a 1-mm mesh to obtain total biomass per sample core according to two different depth levels: 0-10 cm, and 10-20 cm. Each biomass component was dried to a constant weight at 60^{0}C and weighed to the nearest 0.01 g.

Salt marsh vegetation species were recorded along each of the 20-m long transects at each site. The percentage cover of plant species was calculated using the line-intercept method, where the intercept length of species was divided by the total length of the transect line * 100 (Cummings, J. and Smith, D., 2000). The Shannon-Weaver diversity index (H') was calculated per transect according to the percentage cover of species.

Environmental variables - sediments

Water and sediments were concurrently sampled with benthic fauna and vegetation diversity. Sediment samples were randomly taken with sterile modified syringes (2.5-cm diameter) with one replicate each taken in vegetated and unvegetated patches, and at depths of 0-10 cm and 10–20 cm, taken within each of the four randomly placed 20-m long transects. Sediment samples were dried at 60^0C until constant weight to determine bulk density, and dried samples were then analyzed for median grain size, per cent silt and per cent mud (silt defined as particle size >4 and <63 µm, and mud <63 µm, according to the Wentworth classification (Wentworth, 1922) using a Malvern laser particle sizer. A subsample was extracted for organic matter determination using standard loss-on-ignition techniques (Craft et al. 1991), with the samples heated to 450°C for six hours in a muffle furnace. Visible root fragments and macro-organic matter were removed from the samples prior to ignition.

Environmental variables - nutrients

Water samples were collected from the surface water column, with two samples taken from each of four randomly placed 20-m long transects. The samples were immediately filtered with 0.45-µm pore-size capsule filters into sterile polypropylene bottles, and placed on ice during transport to the laboratory, where they were then frozen at -20^0C until further analysis for nutrient concentrations. Nitrate, nitrite, phosphates, ammonium, and silicate concentrations were analyzed. To address the issue of values below limits of detection (LOD) (censored values), a variety of techniques are commonly used, including substitution with 0, LOD/2, LOD/sqrt2, or maximum likelihood estimates and extrapolation techniques (Croghan and Egeghy, 2003). For our samples below LOD(2 $\mu g \cdot L^{-1}$ for nitrites and nitrates, and 5 $\mu g \cdot L^{-1}$ for combined nitrite and nitrates), total concentrations were assigned a value of LOD/sqrt2 (Elias and Bernot, 2017), as substitution is considered adequate when <25% of the data are censored values, and as LOD/sqrt2 is suggested to be the best of the substitution methods (Croghan and Egeghy, 2003). The two replicates per transect were pooled to obtain a mean per transect.

In addition to surface water nutrient concentrations, nutrient availability/uptake was inferred by the ratio of total organic carbon to nitrogen (CN ratios) of the vegetation. CN ratios were obtained by collecting 5 young but fully grown *Spartina* spp. leaves from each of the quadrats. The leaves were dried at 70^0C, ground, and total organic C and N content was analyzed with an Element Analyzer N1500 (Carlo Erba) (4 replicates per site). CN ratios for below-ground biomass were obtained by extracting a subsample of dried biomass from each of the depths at which they were collected (0-10 cm, and 10-20 cm) (4 replicates per depth level and per site).

Macrobenthos

Macrobenthos samples were randomly collected in vegetated along each transect (n=4) with 10-cm diameter x 10-cm depth capped PVC core samplers. Our sampling methods were designed to capture macrobenthic infauna and not the epifauna (which would have required a different sampling design due to their surface motility). Due to sampling constraints, invertebrate samples at GOLF were not taken. Samples were gently rinsed over a 1-mm sieve on-site with seawater to remove sediments, then fixed with 4% formalin buffered with seawater, and stained with Rose

Bengal. In the laboratory, samples were washed in stages over a 1-mm sieve with deionized water to remove remaining sediment, vegetation and organic matter. Macroinvertebrates were then preserved in 70% ethanol, identified to the lowest possible taxonomic level and counted. Feeding traits were identified for each species, mainly based on the Biological Traits Information Catalogue (MarLIN, 2006) and the scientific expertise of the authors. Abundances were then classed according to feeding traits.

Data & Statistical Analyses

Trends in *Spartina* spp. vegetation characteristics across sites were visualized using principle components analysis (PCA), based on Euclidean distances of log-transformed and normalized data. Vegetation characteristics of *Spartina* spp. were analyzed within and across sites, as represented by the factors Site and Transect. Site was treated as a random factor with seven levels representing the sampling sites, and Transect was a random factor with 4 levels, nested within Site, n=4. Depth at 0-10 cm and 10-20 cm was an additional factor (fixed) for evaluating differences in below-ground biomass. Permutational multivariate ANOVA (PERMANOVA) (Anderson, 2001) was used to detect differences in univariate vegetation variables within and between sites, based on Euclidean distances of log-transformed data, using permutation tests with pseudo-*F* ratios. Tests that were significant were followed by *a posteriori* pairwise comparisons with the PERMANOVA *t* statistic. For salt marsh vegetation diversity, the Bray-Curtis distance measure based on log-transformed data was used to assess differences between sites with PERMANOVA.

Differences between environmental variables (sediment and nutrient properties) across sites were assessed using PERMANOVA, again specifying site as a random factor. Variables with more than one replicate per transect were pooled to obtain one average per transect. The effects of vegetation (with two levels, *Spartina* spp., or bare) and sampling depth (with two levels, 0-10 cm or

10-20 cm) (both fixed effects) on each of the sediment variables (% silt, % mud, median grain size, bulk density and organic matter) were assessed with PERMANOVA, again including site as a random effect. Likewise, the effects of sampling depth were also evaluated on C:N ratios of below-groudn biomass. PERMANOVAs were based on Euclidean distances of log-transformed data, and significant results were followed by pair-wise comparisons as above.

The structure of benthic assemblages at each site was assessed in terms of total mean number of species (i.e. richness), rarefied species richness (to compare diversity based on a standard number of individuals, calculated in Primer based on (Hurlbert, 1971)), Pielou's species evenness index and the Shannon-Weaver diversity index. Differences in community structure and diversity among sites were assessed with PERMANOVAs based on resemblance matrices with the Bray-Curtis distance measure on square root-transformed data. To visualize the taxa most responsible for patterns of multivariate dispersion, an ordination of benthic assemblages was constructed with principal coordinates analysis (PCO), based on Bray-Curtis distance measure on square root-transformed abundance data. To determine differences among sites for abundances based on feeding traits, one-way ANOVAs were performed in R (R Core Team, 2017).

The relationship between macrobenthic assemblages and vegetation, sediment and nutrient variables were then determined using distance-based linear modelling (DistLM), which generates *P* values by permutation (Anderson et al., 2008). The analysis was based on a resemblance matrix with the Euclidean distance measure on root-transformed and normalized data. The explanatory variables were first analyzed separately (marginal tests) to determine their relationship with macrobenthos assemblages, and then sequentially (sequential tests) with a forward step-wise selection procedure based on Akaike's information criterion (AIC). Significant collinear relationships were identified between predictor variables based on Pearson's r>0.7, and the redundant predictor variables that explained the least proportion of the variation in the model were omitted to avoid multi-collinearity. Non-significant predictor variables were also removed from the analysis.

PERMANOVAs were tested with 9999 permutations, and tests that were significant were followed by Permutational Analysis of Multivariate Dispersions, or homogeneity of multivariate dispersions (PERMDISP) to determine whether effects were influenced by heterogeneity of multivariate dispersions among sites (again using 9999 permutations). PERMDISP tests are powerful enough to detect small differences in dispersion that may not adversely affect significant PERMANOVA results (Anderson et al., 2008). In the case of significant PERMDISP tests, construction of ordination plots (by non-metric multidimensional scaling (MDS)) enabled the visual inspection of differences due to location or dispersion in multivariate space (Anderson et al., 2008). For all statistical tests, significance was tested at the α=0.05 level. Multivariate analyses were carried out with PRIMER (Plymouth Routines in Multivariate Ecological Research) v6.0 software with Permanova+ add-on software (Clarke and Gorley, 2006).

2.3 Results

Vegetation structure and diversity

Principal component analysis (PCA) showed mainly overlapping variation in vegetation biomass and morphological characteristics. CHI and VEN were grouped separately from VAL and BEL, mainly due to differences in root:shoot ratios. 56% of the variation in vegetation variables was explained by the two principal axes of the PCA analysis (30.3% for the principal axis and 26% for the second axis). The principal axis was mainly influenced by above-ground biomass (0.49), root-shoot ratio (-0.52), % cover (-0.32), and canopy height (0.38). Below-ground biomass (-0.56 for the shallow depth (0-10cm) and -0.30 for the deeper depth (10-20 cm)), stem density (-0.50), and leaf width (-0.41) contributed more to PC2 (numbers in parentheses are eigenvalues) (Figure 2).

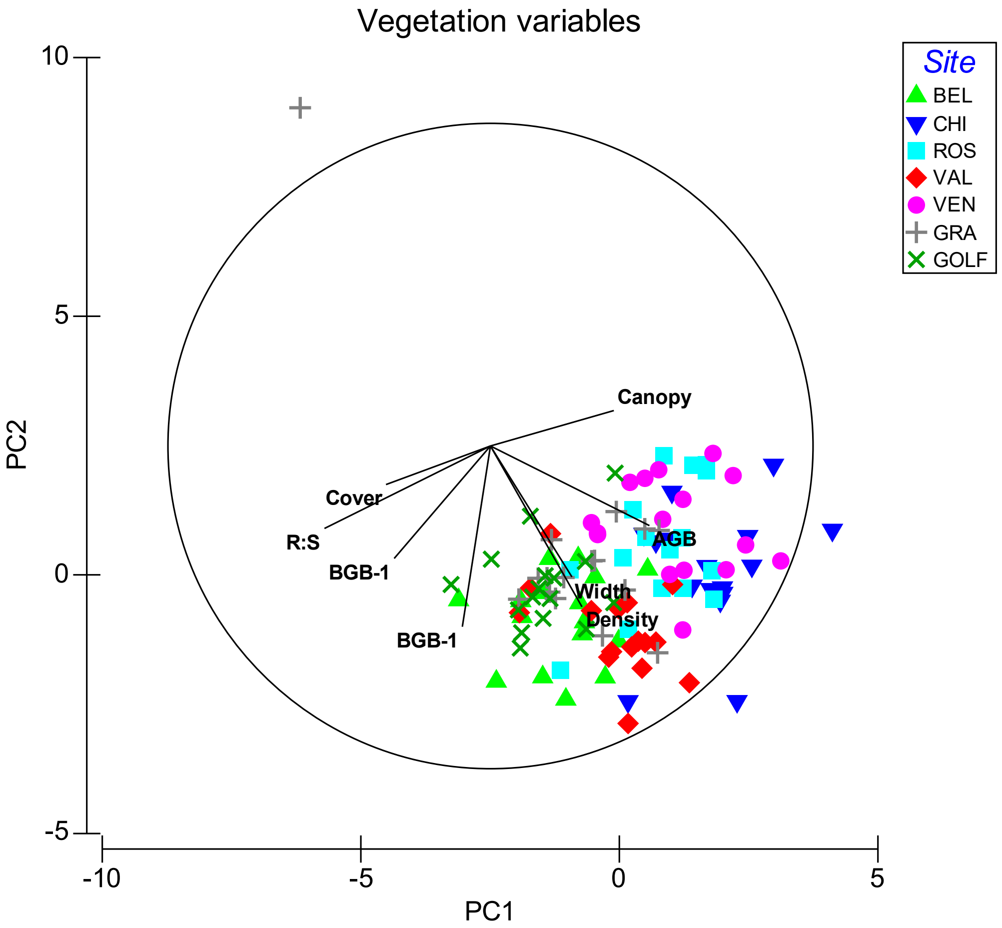


Figure 2: PCA of vegetation variables in the sampling sites based on log-transformed and normalized data, and using a Euclidean resemblance matrix. Biomass variables: AGB=above-ground biomass (g), BGB-1 and BGB-2 = live below-ground biomass (g) at depths of 0-10 cm and 10-20 cm, respectively, R:S =root to shoot ratio, Cover = per cent cover of vegetation in quadrats, Canopy=canopy height (cm), Width=leaf width (mm), Density=# of live stems per quadrat. Site labels as in Figure 1.

Spartina spp. above-ground biomass, canopy height and leaf width were fairly homogenous within sites (between transects). There were significant differences between transects for *Spartina* spp. root to shoot ratio in some sites (Pseudo-$F_{(21,84)}$=8.873, *p*(perm)=0.032), stem density (Pseudo-$F_{(21,84)}$=3.396, *p*(perm)=0.023), % cover (Pseudo-$F_{(21,84)}$=10.804, *p*(perm=0.0001), and below-ground biomass (Pseudo-$F_{(21,84)}$=2.116, *p*(perm)=0.005) (Table S1, Figure 3). There were significant differences between sites for each of the vegetation variables tested (Table S1). Mean below-ground biomass was higher at shallow (0-10 cm) compared to deeper (10-20cm) depths (Pseudo-$F_{(1,168)}$=131.60, *p*(perm)=0.0006). Pairwise tests indicated that VEN had lower mean below-ground biomass than BEL, VAL, GOLF; VAL had higher mean below-ground biomass compared to all other sites (pairwise comparisons, $p<0.05$ in all cases). BEL had lower mean above-ground biomass than CHI, ROS and VAL, and also lower canopy height compared to all other sites (pairwise comparisons, p(perm)<0.05 in all cases), but had higher mean stem densities than most sites (ROS, VAL, GRA and GOLF), (pairwise comparisons, p(perm)<0.05 in all cases)(Figure). GRA and GOLF, the two northern-most sites that were also geographically the closest (1 km away from each other), had similar ranges in values for the vegetation variables, and each had lower mean above-ground biomass than CHI, ROS and VAL (pairwise comparisons, p(perm)<-0.05 in all cases) (Table S1, Figure 3). PERMDISPs testing for homogeneity of variances among sites were significant for the variables of % cover, canopy height and leaf width (Table S1), and visual inspection of MDS plots revealed differences among sites both in location and dispersion.

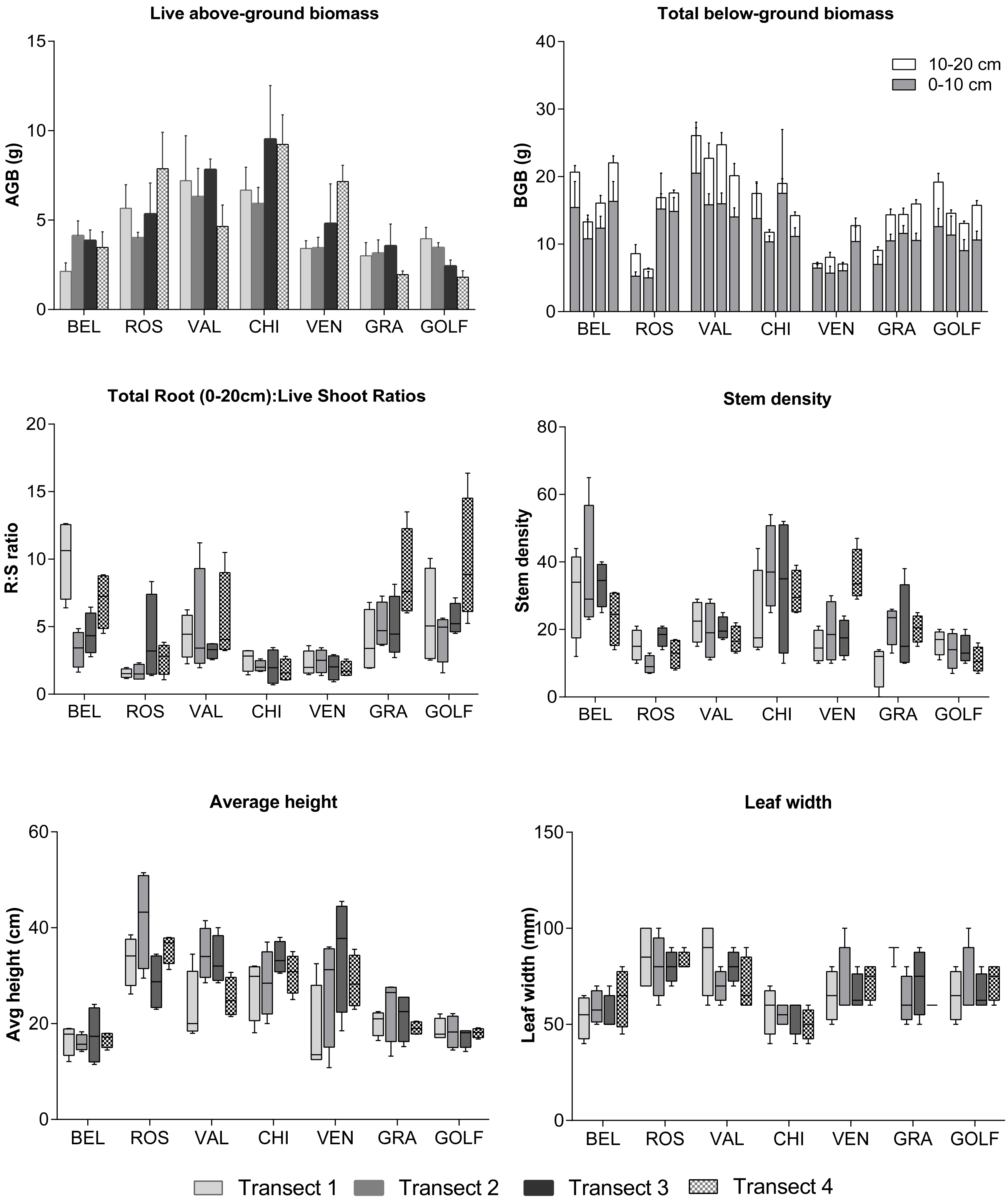


Figure 3: Vegetation variables for core samples - total live above-ground biomass (g) across sites and transects, total below-ground biomass (g) at 0-10 cm and 10-20 cm depths across sites (transects 1, 2, 3 and 4 shown from left to right under for site), root to shoot ratio, live stem density, canopy height (cm) and leaf width (mm), (*n*=4) for all variables. Error bars denote standard error of the mean. Site labels as in Figure 1.

The Shannon-Weaver diversity index for salt marsh vegetation was significantly different across sites (Table S1, Figure S1, Pseudo-$F_{(6,21)}$=3.56, p(perm)= p=0.003), with lower mean diversity in GOLF (0.44) compared to all other sites (ranging from 0.77-0.97) (Pairwise test, p(perm)<0.05 in all cases). The PERMDISP test was not significant for the main test (F = 2.498, P =0.339).

Abiotic environmental variables

Sediments

The effects of depth on sediment characteristics were not consistent across sites, as indicated by significant depth and site interactions for each sediment variable (Table S2). The effects of vegetation on sediment variables were also inconsistent across sites for several sediment variables: organic matter (Pseudo-$F_{(6,84)}$=2.34, P(perm)=0.036) and median grain size (Pseudo-$F_{(6,84)}$=204.38, P(perm)=0.0001), and the vegetation by site interaction was also marginally significant for % silt (Pseudo-$F_{(6,84)}$=2.13, P(perm)=0.053).

Overall, there were differences across sites for bulk density (Pseudo-$F_{(1,84)}$=8.79, P(perm)=0.013), and the presence of vegetation decreased overall mean bulk density values (Pseudo-$F_{(1,84)}$=12.56, P(perm)=0.013) and marginally enhanced organic matter (Pseudo-F=5.66$_{(1,84)}$, P(perm)=0.052, Table S2). There was no significant effect of vegetation on mean grain size variables (% silt, % mud and median grain sizw, Table S2). PERMDISP tests were significant for organic matter and grain size variables (Table S2), and MDS plots showed both differences in location and dispersal.

CHI, VEN, GRA and GOLF, the four northern-most sites, were characterized by higher overall mean % silt (ranging from 64-70%), % mud (ranging from 67-85%) and lower median grain size (ranging from 20-28 μm) than BEL, VAL and ROS, the three southern-most sites (17-22% silt, 19-25% mud, d_{50} 132-170 μm, Table 1). Mean bulk density values were lowest in the two northernmost sites of GRA and GOLF (mean 1.04±0.15 SE and 0.94 ±0 .19 SE g/cm^3, respectively) compared to the other sites which ranged from 1.16-1.28 g/cm^3. Mean organic matter followed the same inverse pattern, being highest in GRA and GOLF (mean 5.00 ± 1.07SE and 6.10 ± 1.87SE %, respectively) compared to ranges of 2.34-2.89 % across other sites. The exception was VEN, which had the lowest mean organic matter content of 0.04 ± 0.01 SE %.

Table 1: Mean sediment data for study sites (site labels as in Fig. 1, % Silt, % Mud, d_{50}=median grain size (μm), Bulk density in g/cm^3, organic matter=% loss on ignition. Data are averages and standard error of the mean of 16 replicates per site. Letters in subscript indicate significant pair-wise site differences for each sediment variable (p(perm)<0.05).

	BEL	**VAL**	**ROS**	**CHI**	**VEN**	**GRA**	**GOLF**
% Silt	19.97_{a} (14.50)	16.82_{b} (4.71)	22.32_{c} (17.29)	63.90_{abc} (7.12)	70.12_{abcd} (5.00)	67.86_{abc} (4.44)	66.07_{abcd} (5.38)
% Mud	24.78_{a} (19)	18.66_{b} (5.08)	25.30_{c} (19.67)	66.70_{abcd} (10.16)	82.49_{abc} (5.53)	85.12_{abcde} (3.15)	80.82_{abce} (6.23)
d_{50}	170.41_{a} (35.71)	132.45_{ab} (13.36)	169.40_{c} (70.28)	25.57_{abc} (12.17)	28.05_{abcd} (4.86)	20.00_{abcde} (3.47)	24.21_{abcde} (4.11)
Bulk Density	1.28_{a} (0.17)	1.19_{b} (0.15)	1.16_{c} (0.25)	1.18_{bd} (0.19)	1.20_{e} (0.12)	1.04_{abdef} (0.15)	0.94_{abcdef} (0.19)
Organic matter	2.34_{a} (1.07)	2.40_{b} (1.10)	2.78_{c} (2.78)	2.89_{d} (1.76)	0.04_{abcde} (0.01)	5.00_{abcdef} (1.07)	6.10_{abcdef} (1.87)

Nutrient characteristics

Surface water nutrient concentrations differed across sites (Table S3, Figure 4). Most notably, ROS had higher ammonium concentrations (mean 75.58 ± 14.21 SE $\mu g/L$) compared to VAL, VEN, GRA and GOLF (which ranged from 15.66 - 37.28 $\mu g/L$, p<0.05 for pairwise comparisons), and GOLF had the highest nitrate levels (mean 272.88 ± 1.98 SE $\mu g/L$), with higher nitrate concentrations than all other sites (which ranged from 5.3 - 55.96 $\mu g/L$, p<0.05 for pairwise comparisons). BEL had the highest level of phosphates (mean 58.18 ± 20.38 SE $\mu g/L$), and silicates (mean 1221.99 ± 302.44 SE $\mu g/L$), compared to all other sites (ranging from 1.41 – 9.02 $\mu g/L$ for phosphates, and 348.86 - 664.93 $\mu g/L$, *P*(perm)<0.05 for all pairwise comparisons) (Figure 4).

Leaf C:N ratios were significantly different across sites (Pseudo-$F_{(6,21)}$=6.985, P(perm)=0.0004)), and were higher in BEL (mean 23.61±1.31 SE) than all other sites except CHI (p<0.05 for all pairwise tests). GRA had the lowest mean leaf C:N ratio of 15.9 ±0.97 SE, which was significantly lower than VEN and VAL (P(perm)<0.05 for both pairwise tests, Figure 5). C:N ratios of below-ground biomass were also significant across sites(Pseudo-$F_{(6,21)}$=8.275, P(perm)=0.005), but there were different pairwise patterns of differences than with leaf C:N ratios. There were higher below-ground biomass C:N ratios in VEN (3.75) compare to all other sites except CHI (P(perm)<0.05 for all pairwise tests), and higher below-ground biomass C:N ratios in CHI (3.76) compared to ROS, VAL, GRA and GOLF (P(perm)<0.05 for all pairwise tests). VAL had the lowest mean below-ground biomass C:N ratios (2.50) compared to all other sites (Figure 5).

PERMDISP tests were significant for all nutrient variables except for leaf C:N ratios, indicating significant heterogeneity of multivariate dispersions among sites. Visual inspection of MDS plots constructed for each of the nutrient variables appeared to indicate that the degree of heterogeneity was small in comparison to the location effect.

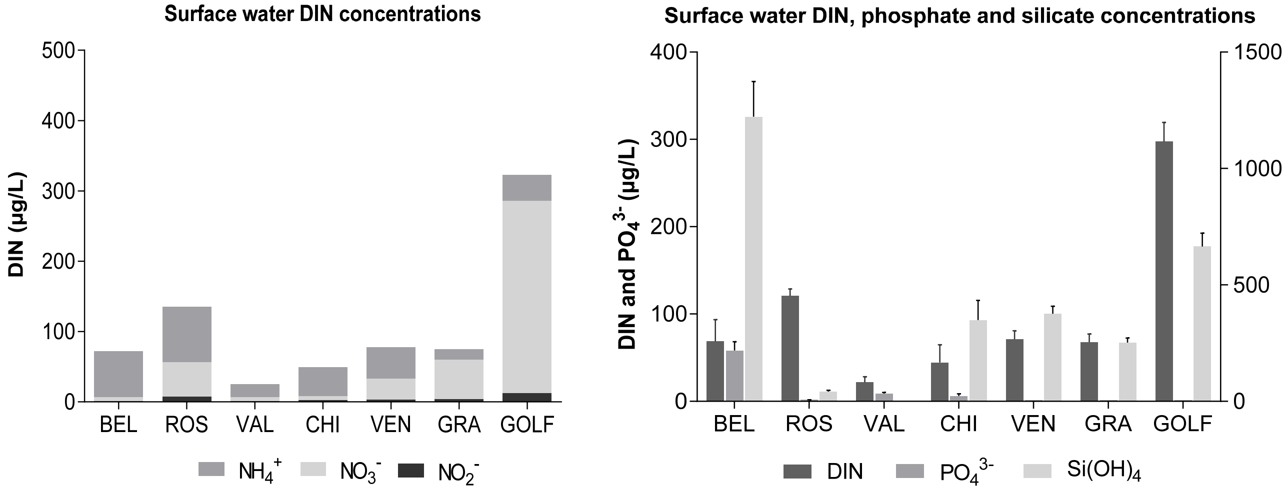


Figure 4: Surface water dissolved inorganic nitrogen (DIN) concentrations and DIN, phosphate and silicate concentrations ($\mu g \cdot L^{-1}$) across sites (n=4). Error bars denote standard error of the mean.

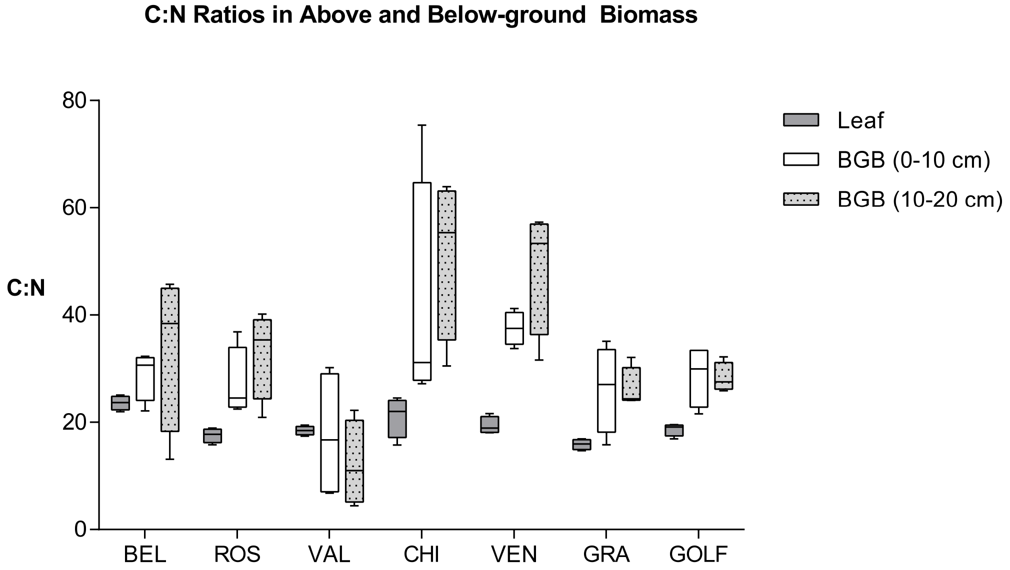


Figure 5: C:N ratios in above-ground biomass (leaf tissue) and below-ground biomass at 0-10 and 10-20 cm depths, across sites (*n*=4)

Macrobenthic assemblages

A total of 20 taxa were identified; these included 7 polychaete taxa that were mainly deposit feeders or omnivores, 1 oligochaete, 5 bivalves that were mainly suspension feeders, 3 gastropods and 4 arthropods (Table 2).

Table 2: Species list found in macrobenthic samples collected from the six sites. For feeding guild, SDF=surface deposit feeder, SSDF=sub-surface deposit feeder, OM=omnivorous, H=herbivore, SC=scavenger.

Class	Taxon	Feeding Guild	BEL	CHI	VAL	ROS	VEN	GRA
Polychaeta	*Neanthes succinea*	SDF	x	x		x		x
	Hediste diversicolor	OM			x			
	Capitella spp.	SSDF						x
	Syllidae							x
	Aphelochaeta marioni	SDF						x
	Perinereis cultrifera	OM					x	
	Terebellidae	SDF, SSDF, SF					x	
Clitellata	*Oligochaete*	SSDF	x	x		x	x	x
Bivalvia	*Abra tenuis*	SF, SDF		x				x
	Modiolus modiolus	SF		x	x			
	Bivalve				x			
	Cerastoderma spp.	SF				x		
	Tapes decussatus	SF					x	
Gastropoda	*Littorina spp.*	H	x	x				
	Gibbula cineraria	H		x				
	Bittium reticulatum	H					x	
Malacostraca	*Talitridae*	H	x			x		x
	Janiropsis brevicornis	H		x				
	Isopoda							x
Entognatha	*Collembola*	SC	x					

Species richness was generally low across sites, with site means ranging from 2 – 2.75, and rarefied richness was similarly low across sites, with site means ranging from 1.20 – 1.70. Site means for abundances per sample core ranged from 5 – 18.75, and site means for the Shannon-Weaver diversity index ranged from 0.29 – 0.97. No significant differences between sites were found for species richness, rarefied richness, abundance, or Shannon-Weaver diversity index (Figure 6, Table S4). Due to zero diversity in several samples, Pielou's evenness index could not be calculated and thus statistical analysis of patterns in evenness across sites was not possible.

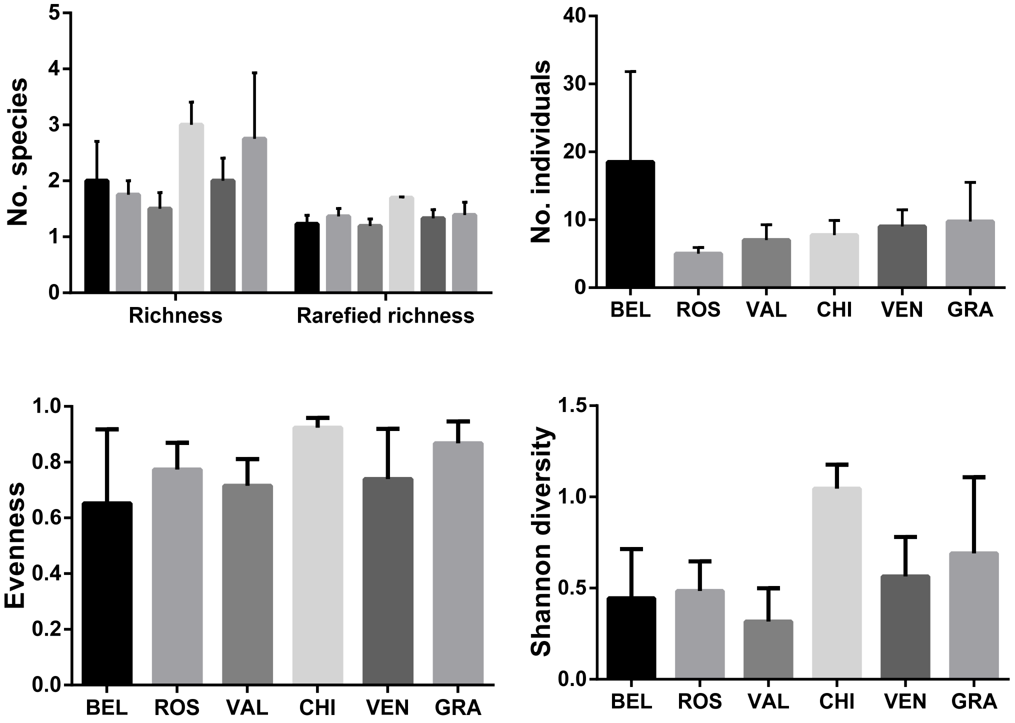


Figure 6: Species richness (pure and rarefied richness), abundance (No. individuals), Pielou's evenness index, and Shannon-Weaver diversity index per sample for each site (labels as in Figure 1). Data are means ± SE of 4 replicates.

The first two axes of the PCO explained 50.6% of total variance in macrobenthic assemblages (33.3% and 19.3%, respectively) (Figure 7). As with the PCO of vegetation variables (Figure 2), CHI and VEN were grouped separately from VAL and BEL (Figure 7). The dominant groups across the sites were polychaetes, crustaceans and molluscs (bivalves and gastropods). Of the polychaetes, *Neanthes succinea* was the most abundant taxon, and was present in all sites except VAL and VEN, while *Hediste diversicolor* (present only in VAL) and *Perinereis cultrifera* (present only in VEN) were the second most abundant polychaete taxa. The hexapod *Collembola* was the most abundant taxon, and was only present in BEL. The majority of gastropods were found in CHI and VEN.

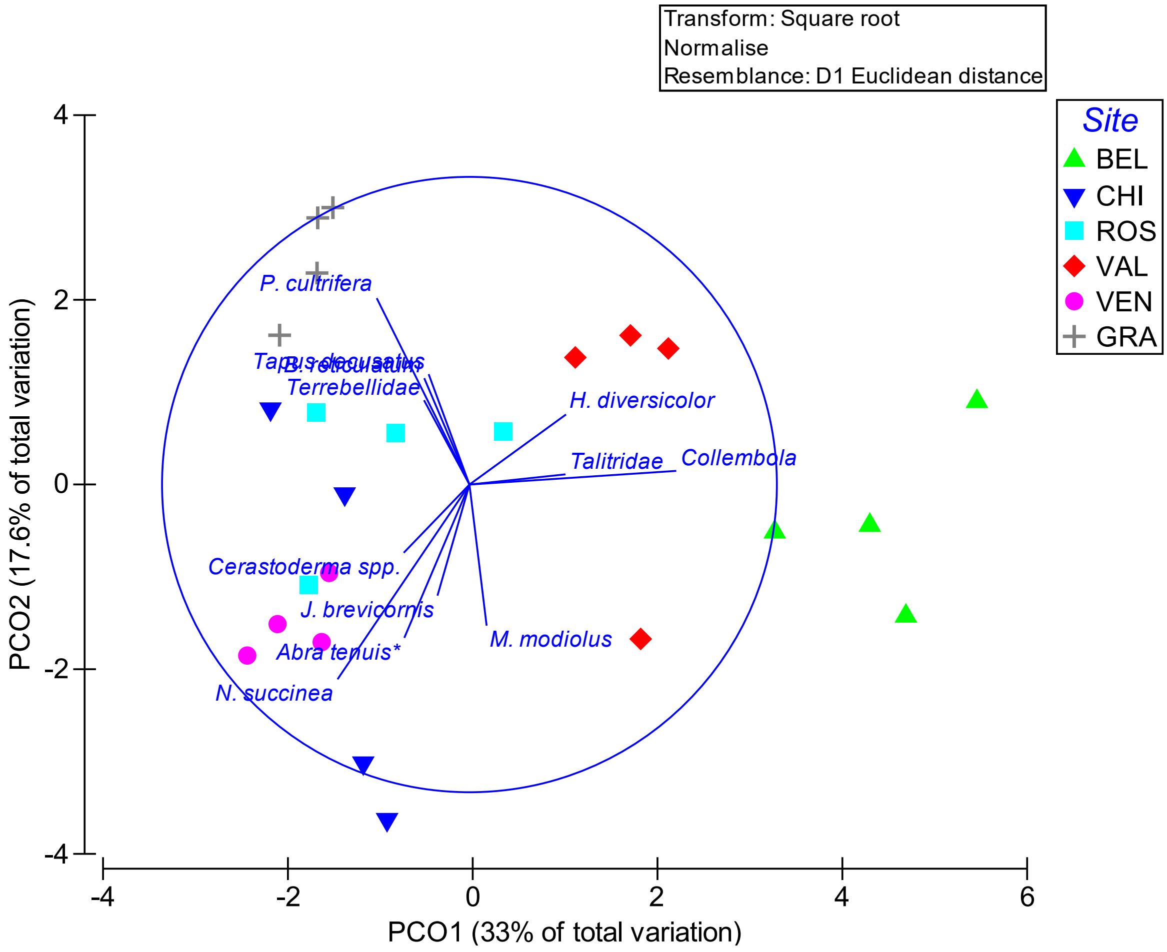


Figure 6. Plot of the first 2 principal coordinate axes (PCO) showing ordination of benthic assemblages in each site (captions as in Fig. 1). Analyses were based on Bray–Curtis dissimilarities after root transformation of the abundance of all 20 taxa (listed in Table 5). Vectors indicate the taxa that best correlated with the ordination of the assemblages (Spearman correlation of square root-transformed abundances with ordination axes r > 0.3). The direction of vectors indicates the correlation with different groups of sample points and the length is proportional to the value of correlation.

When abundances were classed according to feeding traits, varying compositions of these traits were evident across sites (Figure 8). BEL was dominated by the scavenger *Collembola* ($F_{(5, 18)}$ =7.015, p=0.006) and no other scavengers were present in the other sites. VAL and VEN had the highest abundances of omnivores (p<0.05 for all pairwise comparisons) mainly dominated by polychaetes (*H. diversicolor* in VAL and *P. cultrifera* in VEN). Mean abundances of surface deposit feeders also varied significantly across sites ($F_{(5, 18)}$ =11.01, p=0.0001), with the highest abundances in ROS, CHI and GRA (mainly dominated by the polychaete *N. succinea*). GRA also had the highest mean proportion of sub-surface deposit feeders (mainly of the polychaete *Capitella* spp.) although this was not significant. The abundances of herbivores did not differ significantly across sites.

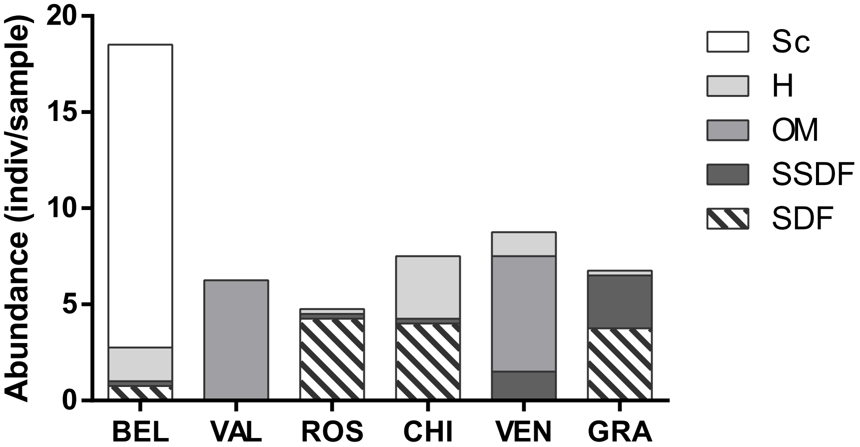


Figure 8: Macrofaunal abundances by classified by feeding trait. Data are mean abundances (number of individuals/sample) over 4 replicates. Feeding trait labels: Sc=scavenger, H=herbivore, OM=omnivore, SSDF=sub-surface deposit feeder, SDF=surface deposit feeder. Site labels as in Figure 1.

The DistLM analysis revealed that sediment silt and organic content were the most significant predictors of species assemblage composition ($p<0.05$ for both variables), explaining 12.1 and 18.3% of the total variation in marginal tests (Table 3). The C:N ratio of the below-ground biomass was also significant ($p=0.036$), explaining approximate 9.0% of the total variation in marginal tests (Table 3), however, when fitted sequentially after sediment variables and surface water ammonium concentrations, its significance was borderline($p=0.052$). Ammonium concentrations explained 7.53% of the variation and was significant in the sequential test ($p=0.032$). The explanatory variables explained 43.98% of the total variability in benthic assemblages.

Table 3: Results of DistLM for vegetation, sediment and nutrient variables (root-transformed, normalized data), and macrobenthic assemblages (based on Bray-Curtis dissimilarities on root-tranformed data). OM=organic matter, NH_4=surface water ammonium concentrations ($\mu g/L$), CN-BGB =C:N ratios of below-ground biomass. % variation, pseudo-*F* statistic, and *p* values are shown for variables fitted individually. % Cumulative variation and residual degrees of freedom (res. df) are additionally shown for variables fitted sequentially (where variability explained by each variable is conditional on the variables sequentially considered in the model). Significant *p*-values are in bold font.

Variable	**Fitted individually**							
	% Var	Pseudo-*F*	*P(perm)*	% Var	Pseudo-*F*	*P(perm)*	^{2}% Cumul. Var.	Res. *df*
% Silt	12.07	3.02	**0.007**					
OM	18.30	4.28	**0.0004**	18.37	5.547	**0.0001**	30.44	21
NH_4	6.78	1.600	0.131	7.53	2.428	**0.032**	37.98	20
CN-BGB	8.96	2.166	**0.036**	6.00	2.036	0.052	43.98	19

2.4 Discussion

Effect of vegetation, nutrient and sediment variables on macrobenthic assemblages

Macrofaunal associations with salt marsh vegetation have been mixed in the literature, with studies noting facilitation effects of vegetation and others noting exclusion, making the direction of the association challenging to predict in our study. Lower macrofaunal abundances could be expected where higher below-ground biomass was observed, as below-ground root structure can physically exclude benthic organisms (van der Wal and Herman, 2011), a mechanism that was also found to explain lower macrofaunal abundances in mangrove ecosystems (Leung, 2015). Other studies have found that salt marsh vegetation facilitates, rather than inhibits, macrofaunal abundance. Below-ground and dead aboveground biomass were associated with higher macrofaunal densities in subtropical salt marshes (Netto and Lana, 1997). Furthermore, greater complexity of macrofaunal assemblages (increased diversity and richness) have been found in salt marshes that have been restored with *Spartina maritima* compared to un-vegetated mudflats (Curado et al., 2014). Organic matter and bulk density in this restored marsh were the sediment variables that most influenced macroinvertebrate distribution (along with pH and redox potential) (Curado et al., 2014), and studies relating grain size to macrobenthos abundances in intertidal sediments have associated higher biomass with median grain size ~100 μm (Van der Wal 2008), or <150 μm with > 3.2% organic matter (Van Colen 2014).

Macrobenthos species richness, abundance, evenness and diversity were relatively homogenously low across all our lagoon sites, which had varying mean sediment grain sizes and significant differences between sites in below-ground biomass. The DistLM analysis revealed that sediment variables (% silt and organic matter) explained the most variation (30%) in species assemblages, while CN ratios of below-ground biomass and surface water ammonium concentrations explained 13%. While *Spartina* spp. vegetation variables and associated marsh vegetation diversity indices were not significant predictors for species assemblages, it is well established that as an ecosystem engineer, *Spartina* spp. vegetation enhances fine silt and organic content of sediments (Deegan et al., 2012a; Feagin et al., 2009). Given that vegetation enhanced organic sediment content which was a significant predictor of assemblages, this suggests that in our study, *Spartina* spp. vegetation had an indirect influence on assemblages by modifying soil properties.

Given that different salt marsh vegetation species have different macrofaunal community structures, we also expected that salt marsh vegetation diversity and resultant different plant structures would be a significant source of variation in macrobenthic assemblages. Talley and Levin (1999) reported lower macrofaunal density and assemblages of lower diversity in *Salicornia virginica* (an annual succulent) compared to *Spartina patens*, which also potentially points to the exclusion effect of *Spartina* spp. due to their dense root complex. Different *Spartina* species have also been found to be associated with different macrofaunal assemblages; e.g. Brusati and Grosholz (2006) found that a hybrid *Spartina* species modifies habitat structure more than native *S. foliosa* because of its greater biomass (taller stems, and greater plant biomass both above- and belowground), thereby excluding invertebrates such that the hybrid was associated with lower densities of benthic infauna. Based on the results of a recent broad-scale genetic survey conducted while this study was in progress, it is likely that some of our sites contained the native *Spartina maritima*, whereas other sites mainly had hybrid *Spartina* species (Wong, 2015). However, salt marsh vegetation diversity in our study sites was also not a significant source of variation in macrobenthic assemblages, either when considered marginally in the DistLM procedure, nor when considered sequentially in combination with other variables. Our findings correspond with results from van der wal (2012) who found no significant effects of plant cover or vegetation type on macrobenthic biomass, diversity, and community structure.

Relation of salt marsh macrofaunal structure to anthropogenic pressures

While several of the study sites were in designated parks or reserves, they have all been subjected to varying degrees of anthropogenic influences. Clam-farming is most intensive in Venice Lagoon (where sites CHI and VEN are located) (Boscolo Brusà et al., 2013; Melaku Canu et al., 2011), but also practiced privately in VAL (Wong et al., 2015), whereas commercial fishing is intensive in the Valli di Comacchio lagoonal system (location of BEL) (Ponti et al., 2010) but also practiced widely in the Grado-Marano lagoonal complex (where GRA, directly adjacent to a fish farm, and GOLF, directly adjacent to a golf course, are located)(Fontolan et al., 2012). GOLF had the highest mean nitrate concentrations, probably reflecting fertilizer run-off from the adjacent golf course, in addition to the ambient eutrophic conditions in Grado lagoon. BEL is situated in the Valli di Comacchio lagoonal complex which is subject to frequent dystrophic phenomena, particularly in the summer. BEL had the highest mean phosphate concentrations despite being located in a natural reserve; both BEL and GOLF had nutrient concentrations orders of magnitude above the others.

Studies of the effects of eutrophication on intertidal macrofauna have demonstrated mixed results (Ansari and Gill, 2013), suggesting that effects are context-specific. For example, in the Mondego estuary (Portugal), lower macrofaunal diversity was observed along a eutrophication gradient, with replacement of herbivores by detritivores, and an increase in polychaetes (Cardoso et al., 2004). In the Baltic Sea, higher abundance and biomass of intertidal macrofauna were recorded in response to increased food availability from enhanced sea grass abundance and biomass as a result of eutrophication (Boström et al., 2002). Within the *Spartina* spp. patch, the impacts of eutrophication might be expected to enhance macrofaunal densities, as higher nutrient loads have been shown to result in decreased investment in root growth compared to shoots (Deegan et al., 2012b; Turner et al., 2009), and *Spartina* spp. roots are known to physically exclude macrobenthic fauna (van der Wal and Herman, 2011). Below-ground biomass was significantly different across our sites and tended to be lower in sites with high nutrient loadings although this relationship was not always significant. While it was not a significant predictor of benthic assemblages in our study, indicators of nutrient loadings (surface water ammonium concentrations and C:N ratios of below-ground biomass) were significant influencers, suggesting a more direct role of nutrient loadings on assemblages rather than effects of root structure.

The low mean macrofaunal species diversity in VAL could be a result of marsh age and resultant influence on organic content, as marsh age has been demonstrated to influence macrofaunal species composition and faunal feeding modes (Levin et al., 1996). VAL was a more recently created marsh compared to the other sites, and had the lowest mean sediment organic matter in vegetated areas of the three sites characterized by sandy sediments. VAL was also dominated by *H. diversicolor*, a cosmopolitan polychaete known to be capable of exploiting patchy resources (Godbold et al., 2011) and tolerant to a wide range of disturbances and salinity, temperature and water quality (Rossi et al., 2007; Scaps, 2002; Zettler et al., 2013).

While comparisons of macrobenthic assemblages with those in other studies are difficult due to differences in sampling procedures, depths, habitats, and time, our results do correspond broadly to other studies of soft-sediment benthic communities from intertidal Italian transitional waters, in which surface deposit feeders were the dominant feeding guild, including the polychaete

N. succinea (Munari and Mistri, 2008). In our study, *N. succinea* was dominant across most sites and could be found in both sandy and fine sediments; another polychaete species was *H. diversicolor* which was unique to our samples in VAL, which had comparatively coarser sediments (d_{50}=132.45). Both polychaete species are known to occupy a wide range of sediment types, but highest abundances are typically found in organically enriched muddy sediments (Van Colen et al. 2014). For example, in a Belgian coastal lagoon, *H. diversicolor* was absent from sediments with 0.5% organic matter, but abundant in sediments with approximately 1 to 2% organic matter (Van Colen et al. 2014). This preference in sediment type may explain the absence of *N. succinea* and *H. diversicolor* from our sampling site in VEN, which, while fine-grained, had an almost negligible amount of organic matter (0.04% ± 0.15 (SE)) compared to the other sites, which ranged from approximately 2 to 6 % organic matter). While *N. succinea* and *H. diversicolor* were absent from our VEN samples, the polychaete *P. cultrifera* was abundant and unique in this site. These results are similar to Ghirardini et al. (1999), who found that *N. succinea* and *H. diversicolor* colonized Venice Lagoon in multiple areas with muddy sediments and high organic content, while *P. cultrifera* colonized sandy muds with low organic matter content and was characteristic of areas close to the inlets and main channels of the lagoon (Ghirardini et al., 1999). While *N. succinea*, *H. diversicolor*, and *P. cultrifera* have similar feeding behavior (i.e. all can deposit feed (MarLIN, 2006)), it is possible that *P. cultrifera* is more tolerant of sediments with absence of organic matter as a food source, such as the low-organic sediments found at our site in VEN. Moreover, commercial harvesting of *P. cultrifera* in Italy (as bait for recreational fishing or in aquaculture) occurs mainly in Venice Lagoon (Gambi et al., 1994) which could also explain its general presence at our site compared to its absence from the other lagoons we sampled.

2.5 Conclusions

We assessed *Spartina* spp. salt marsh vegetation structure and its influence on benthic macroinvertebrate community composition through this first broad-scale survey across lagoons of the Northern Adriatic Sea. Our analysis indicated that all *Spartina* spp. vegetation characteristics we measured varied across sites. Within sites, there was local variation in several *Spartina* spp. vegetation characteristics: root:shoot ratio, stem density and % cover. *Spartina* spp. vegetation modified sediments by decreasing bulk density and enhancing organic matter. Macrofaunal assemblages were dominated by polychaetes, crustaceans and molluscs and were influenced by sediment and nutrient variables (% silt, sediment organic matter, surface water ammonium concentrations, and C:N ratios of below-ground biomass), together which explained 43% of the total variability in benthic assemblages. Our findings suggest that *Spartina* spp. indirectly influenced macrobenthos assemblages by modification of soil properties, i.e. via organic enrichment of the sediments. Across sites, we found strikingly low richness, abundance, and diversity of macrobenthic fauna, with dominance by opportunistic deposit feeders, similar to other disturbed lagoons on the Adriatic coast.

Acknowledgements

This work was funded through a MARES Grant (www.mares-eu.org) and project TETRIS (PRIN 2010-2011, MIUR). MARES is a Joint Doctorate programme selected under Erasmus Mundus and coordinated by Ghent University (FPA 2011-0016). CVC acknowledges a postdoctoral fellowship provided by the Flemish Fund for Scientific Research (FWO-1.2.380.11.N.00). The authors are grateful to members of the Airoldi Lab (University of Bologna): Stefanie Broszeit, Lorenzo Gadoni, Giulia Finotti, Joanne Wong, Elena Piccioni, in addition to Ignacio Barbeito, for their extensive sampling assistance. We are grateful to members of the Research Group Marine Biology (UGent): Mohammed Alsebai for species identification, and Bart Beuselinck for sample processing. We also extend thanks to Loran Kleine Schaars (NIOZ) for help with species identification. We thank the Po Delta, Rosolina and Veneto Parks, Ferruccio Polo (Cooperativa Pescatori di Grado) Valle Artalina, and Golf Club Grado for access to the sampling sites.

2.6 References

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2.7 Supplementary Information

Table S1: Results of PERMANOVAs testing differences in *Spartina* spp. vegetation variables (log-transformed) between transects and sites, and PERMDISP tests. AGB=above-ground biomass (g), R:S=root to shoot ratio, density refers to stem density per 0.25 m^2 quadrat, Canopy=canopy height (cm), Width=leaf width, BGB=below-ground biomass evaluated at 2 different depth levels, *H'*=Shannon diversity index of salt marsh vegetation. Significant *P*-values in bold.

	Source	*Df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*	*Perms*
AGB	Site	6	14.092	2.349	7.538	**0.0003**	9951
	Transect(Site)	21	6.544	0.312	1.432	0.125	9914
	Res	84	18.282	0.218			
	Total	111	38.918				
	PERMDISP	*F*=0.505, *P*(perm)=0.812					
R:S	Site	6	21.471	3.579	8.873	**0.0001**	9945
	Transect(Site)	21	8.469	0.403	1.783	**0.032**	9918
	Res	84	19.003	0.226			
	Total	111	48.944				
	PERMDISP	*F*=1.393, *P*(perm)=0.282					
Density	Site	6	11.992	1.999	3.396	**0.011**	9956
	Transect(Site)	21	12.358	0.588	1.69	**0.023**	9891
	Res	84	29.25	0.348			
	Total	111	53.6				
	PERMDISP	*F*=1.474, *P*(perm)=0.595					
%Cover	Site	6	13.873	2.312	9.955	**0.0001**	9950
	Transect(Site)	21	4.877	0.232	2.794	**0.0002**	9918
	Res	84	6.982	0.083			
	Total	111	25.73				
	PERMDISP	*F*=10.804, *P*(perm)=**0.0001**					
Canopy	Site	6	7.053	1.175	12.073	**0.0001**	9955
	Transect(Site)	21	2.045	0.097	1.654	0.056	9927
	Res	84	4.944	0.059			
	Total	111	14.041				
	PERMDISP	*F*=6.994, *P*(perm)=**0.0001**					
Width	Site	6	4.001	0.667	2.281	**0.015**	9925
	Transect(Site)	21	6.138	0.292	0.663	0.982	9848
	Res	84	37.025	0.441			
	Total	111	47.164				
	PERMDISP	*F*=2.945, *P*(perm)=**0.001**					
BGB	Depth	1	60.318	60.318	131.69	**0.0006**	9906
	Site	6	3.064	3.064	8.351	**0.0002**	9955
	Transect(Site)	21	0.367	0.367	2.116	**0.005**	9901
	DepthxSite	6	0.458	0.458	2.332	**0.073**	9958
	DepthxTransect(Site)	21	0.196	0.196	1.133	0.312	9922
	Res	168	0.173	0.173			
	Total	223					
	PERMDISP	*F*=1.439, *P*(perm)=0.242					
H'	Site	6	2696.4	449.39	3.559	**0.003**	9906
	Res	21	2651.6	126.27			
	Total	27	5348				
	PERMDISP	*F*=2.137, *P*(perm)=0.322					

Table S2: Results of PERMANOVAs testing the effect of vegetation (*Spartina spp*., bare) and depth (0-10, 10-20 cm) on sediment characteristics (log-transformed), with site as a random effect, and PERMDISP tests. BD=bulk density, OM=organic matter, Silt=% silt, d50=median grain size, mud=% mud. Significant *P*-values in bold.

	Source	*Df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*	*Perms*
BD	**Veg**	**1**	**0.075**	**0.075**	**12.558**	**0.013**	9775
	Depth	1	0.047	0.047	3.414	0.118	9711
	Site	**6**	**0.286**	**0.048**	**8.788**	**0.0001**	9947
	VegxDepth	1	0.013	0.013	1.371	0.294	9857
	VegxSite	6	0.036	0.006	1.105	0.369	9942
	DepthxSite	**6**	**0.082**	**0.014**	**2.532**	**0.026**	9936
	VegxDepthxSite	6	0.057	0.009	1.742	0.121	9946
	Res	84	0.456	0.005			
	Total	111	1.052				
	PERMDISP	*F*=2.503 , *P*(perm)= 0.088					
OM	**Veg**	**1**	1.113	1.113	5.657	0.052	9529
	Depth	1	1.144	1.144	3.785	0.099	9706
	Site	**6**	35.653	5.942	70.815	**0.0001**	9936
	VegxDepth	1	0.007	0.007	0.076	0.796	9827
	VegxSite	**6**	1.181	0.197	2.345	**0.037**	9941
	DepthxSite	**6**	1.813	0.302	3.602	**0.003**	9944
	VegxDepthxSite	6	0.567	0.095	1.126	0.360	9942
	Res	84	7.048	0.084			
	Total	111	48.526				
	PERMDISP	*F*=14.058, *P*(perm)= **0.0001**					
Silt	Veg	1	0.858	0.858	2.923	0.139	9744
	Depth	1	0.344	0.344	0.541	0.507	9813
	Site	**6**	51.751	8.625	62.606	0.0001	9949
	VegxDepth	1	0.019	0.019	0.406	0.596	9627
	VegxSite	**6**	1.760	0.293	2.130	0.053	9953
	DepthxSite	**6**	3.821	0.637	4.623	0.0003	9958
	VegxDepthxSite	6	0.283	0.047	0.343	0.915	9947
	Res	84	11.573	0.138			
	Total	111	70.410				
	PERMDISP	*F*=32.898, *P*(perm)=**0.0001**					
d_{50}	Veg	1	0.261	0.261	1.397	0.393	9449
	Depth	1	0.204	0.204	0.974	0.402	9766
	Site	**6**	90.912	15.152	204.38	**0.0001**	9944
	VegxDepth	1	0.018	0.018	0.328	0.595	9797
	VegxSite	**6**	1.122	0.187	2.522	**0.026**	9946
	DepthxSite	**6**	1.258	0.210	2.828	**0.015**	9944
	VegxDepthxSite	6	0.333	0.056	0.749	0.611	9954
	Res	84	6.227	0.074			
	Total	111	100.340				
	PERMDISP	*F*=9.565, *P*(perm)=**0.0001**					
Mud	Veg	1	0.921	0.921	2.990	0.138	9719
	Depth	1	0.473	0.473	0.908	0.404	9814
	Site	**6**	56.039	9.340	60.471	**0.0001**	9943
	VegxDepth	1	0.000	0.000	0.067	0.807	9843
	VegxSite	6	1.849	0.308	1.995	0.076	9950
	DepthxSite	**6**	3.126	0.521	3.373	**0.004**	9942
	VegxDepthxSite	6	0.017	0.003	0.018	1.000	9953
	Res	84	12.974	0.154			
	Total	111	75.399				
	PERMDISP	*F*=36.689, *P*(perm)=**0.0001**					

Table S3: Results of PERMANOVAs testing differences in nutrient variables (log-transformed) among sites, and PERMDISP tests. Leaf CN=shoot C:N ratio, BGB CN=below-ground biomass C:N ratios. For surface water nutrient concentrations (μg/L): NO_2=nitrate, NO_3=nitrate, NH_4=ammonium, PO_4=phosphate, Si=silicate

	Source	*Df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*	*Perms*
Leaf CN	Site	6	0.377	0.063	6.985	**0.0004**	9948
	Res	21	0.189	0.009			
	Total	27	0.566				
	PERMDISP	*F*=1.870, *P*(perm)=0.507					
BGB CN	Site	6	8.529	1.422	9.072	**0.0001**	9955
	Depth	1	0.060	0.060	0.701	0.425	9707
	Site x Depth	6	0.510	0.085	0.543	0.782	9959
	Res	42	6.581	0.157			
	Total	55	15.68				
	PERMDISP	*F*=7.8837, *P*(perm)=**0.0001**					
NO_2	Site	6	15.203	2.534	45.049	**0.0001**	9949
	Res	21	1.181	0.056			
	Total	27	16.384				
	PERMDISP	*F*=8.275, *P*(perm)=**0.0015**					
NO_3	Site	6	53.525	8.921	68.581	**0.0001**	9955
	Res	21	2.732	0.131			
	Total	27	56.256				
	PERMDISP	*F*=5.622, *P*(perm)=**0.007**					
NH_4	Site	6	9.924	1.654	3.164	**0.024**	9941
	Res	21	10.977	0.523			
	Total	27	20.9				
	PERMDISP	*F*=10.373, *P*(perm)=**0.003**					
PO_4	Site	6	44.62	7.437	44.455	**0.0001**	9902
	Transect(Site)	21	3.513	0.167			
	Total	27	48.133				
	PERMDISP	*F*=16.509, *P*(perm)=**0.0001**					
Si	Site	6	6.365	1.061	16.027	**0.0002**	9942
	Res	21	1.390	0.066			
	Total	27	7.755				
	PERMDISP	*F*=4.426, *P*(perm)=**0.021**					

Table S4: Results of PERMANOVAs testing differences in macroinvertebrate abundance and diversity measures between sites: Richness, rarefied richness, Shannon-Weaver diversity index, abundance of individuals (root-transformed) and feeding trait proportions

	Source	*Df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*	*Perms*
Richness	Site	5	752.55	150.51	0.864	0.526	2476
	Residuals	18	3135	174.17			
	Total	23	3887.6				
Rarefied richness	Site	5	209.13	41.825	1.29	0.319	9950
	Residuals	18	583.6	32.422			
	Total	23	192.72				
Shannon-Weaver	Site	5	732.46	146.449	1.257	0.333	9950
	Residuals	18	2097.9	116.55			
	Total	23	2830.3				
Abundance	Site	5	630.65	126.13	0.298	0.955	9943
	Residuals	18	7626.8	423.71			
	Total	23	8257.5				
Feeding Traits	Site						
	Residuals						
	Total						

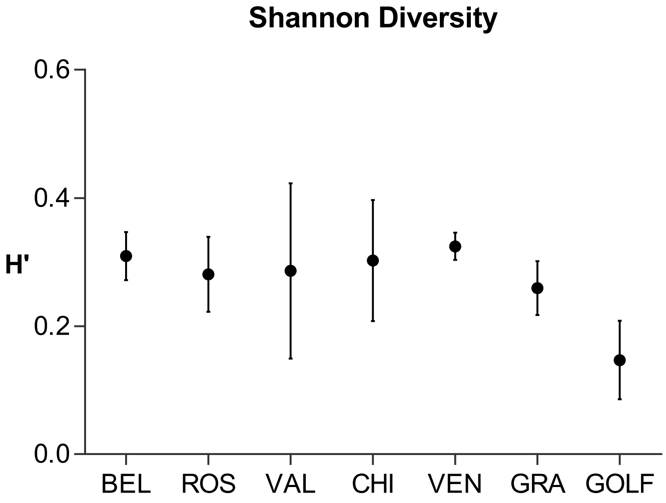

Figure S1: Shannon-Weaver diversity index estimated on percent salt marsh species vegetation cover per transect across sites (n=4). Site labels as in Figure 1.

CHAPTER 3

Interactive effects of vegetation and sediment properties on lateral erosion of salt marshes in the Northern Adriatic Sea

Spartina spp. at the Grado Golf Course (GOLF)

3. Interactive effects of vegetation and sediment properties on lateral erosion of salt marshes in the Northern Adriatic Sea[2]

Abstract

We investigated how lateral erosion control, measured by novel photogrammetry techniques, is modified by the presence of *Spartina* spp. vegetation, sediment grain size, and the nutrient status of salt marshes across 230 km of the Italian Northern Adriatic coastline. *Spartina* spp. vegetation reduced erosion across our study sites. The effect was more pronounced in sandy soils, where erosion was reduced by 80% compared to 17% in silty soils. Erosion resistance was also enhanced by *Spartina* spp. root biomass. In the absence of vegetation, erosion resistance was enhanced by silt content, with mean erosion 72% lower in silty *vs*. sandy soils. We found no relevant relationships with nutrient status, likely due to overall high nutrient concentrations and low C:N ratios across all sites. Our results contribute to quantifying coastal protection ecosystem services provided by salt marshes in both sandy and silty sediments.

3.1 Introduction

Vegetated coastal habitats such as salt marshes provide multiple ecosystem services, including habitat provisioning, nutrient cycling, climate regulation, and cultural services (Duarte et al., 2013; Gedan et al., 2009). The use of salt marshes in ecosystem-based approaches to flood defence is highly promising (Bouma et al., 2014; Narayan et al., 2016), as they have been demonstrated to attenuate waves even under storm-surge conditions (Möller et al., 2014a). There

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[2] Modified from Lo, V.B., Bouma, T.J., van Belzen, J., Van Colen, C., Airoldi, L., 2017. Interactive effects of vegetation and sediment properties on erosion of salt marshes in the Northern Adriatic Sea. Marine Environmental Research 131, 32–42. https://doi.org/10.1016/j.marenvres.2017.09.006

has thus been a focus in recent decades on the conservation and restoration of salt marshes to protect coasts from erosion, storm surges and sea level rise (Curado et al., 2013), and to prolong the life of traditional engineered structures such as sea walls and embankments (Spalding et al., 2014; Temmerman et al., 2013). The perennial saltmarsh grasses of the *Spartina* genus are of particular interest in coastal protection as they are pioneer species (Bouma et al., 2016b) with a global distribution (Duarte et al., 2013; Silliman, 2014). Their ecosystem engineering properties include trapping sediments from the water column, which enables accretion and establishment at the low marsh (Bouma et al., 2005).

Salt marshes are highly dynamic ecosystems with cyclic behavior, and the stability and size of salt marshes are governed by horizontal and vertical processes (Kirwan and Megonigal, 2013a). Horizontally, periods of seaward expansion alternate with periods of shoreward (lateral) erosion due to tidal or wave-induced currents (Bouma et al., 2016; van de Koppel et al., 2005; Yapp et al., 1916). Sediment supply, depth and width of adjacent tidal flats, and wave action affect the balance between erosion and progradation (Mariotti and Fagherazzi, 2013). Density and flexibility of the salt marsh vegetation canopy also play a role in decreasing flow velocity and enabling sediment-trapping, while attenuating waves and slowing erosion (Bouma et al., 2005; Heuner et al., 2015; Möller et al., 2014a). Vertically, the ability of salt marshes to keep pace with sea level rise by accretion is also dependent on sufficient sediment supply and the rate of landward expansion (Kirwan and Megonigal, 2013a). While research has mainly focused on vertical dynamics of salt marshes, especially in the context of sea level rise, there has been comparatively less research on the factors affecting the horizontal dynamics of salt marshes (Kirwan et al., 2016), which may be more important to marsh stability as marshes are more resistant to vertical change but less resistant in the lateral dimension (Fagherazzi et al., 2013). In order to use salt marshes for coastal defence, we need a greater understanding of these horizontal factors, including those controlling resistance to lateral erosion.

Previous studies have shown that below-ground vegetation can aid in inhibiting erosion by stabilizing soils via roots and rhizomes which enhances cohesion and tensile strength, resulting in higher soil shear strength (Micheli and Kirchner, 2002; Turner, 2011). Moreover, roots and rhizomes can provide a physical barrier between water and soil (Wolanski, 2007). It has been demonstrated that the loss of below-ground biomass can result in reduced soil strength or marsh erosion (Sheehan and Ellison, 2015; Silliman et al., 2016). Feagin et al. (2009) suggested that sediment properties can influence lateral erosion rates more than vegetation, with vegetation indirectly contributing to erosion resistance by incorporating detritus and fine-grained sediments into the soil matrix, rendering the soil less dense, less coarse and more cohesive (Feagin et al. 2009). Deegan et al. (2012) suggested that long-term nutrient addition to salt marshes could cause eventual collapse of the system, as increased availability of nutrients reduces relative investment in below-ground biomass and enhances microbial decomposition, thereby destabilizing soils. Wong et al. (2015) further demonstrated that saltmarsh vegetation can be more vulnerable to increased inundation in medium organic matter soils, particularly under high nutrient availability. Combining these findings suggests that a complex interplay between vegetation, sediment properties and anthropogenic-influenced nutrient levels can influence erosion resistance of salt marshes.

The role of salt marshes in coastal defence is particularly important in the Mediterranean Sea, where humans have been inhabiting and shaping the coastlines for millennia (Airoldi and Beck, 2007b). The coast has been dramatically transformed by altered sedimentation patterns, land reclamation, river diversions, embankments, and drainage, resulting in the loss of more than 70% of salt marshes in the region (Fontolan et al., 2012). Coastal defence with artificial structures such as groynes and breakwaters have been implemented on a large scale, leading to hardening of coastal areas and changes in sediment structure, and subsequent loss and alteration of native habitats and assemblages (Airoldi et al., 2005). The extensive construction of artificial infrastructures along these shorelines may limit land-ward rates of marsh expansion in response to sea-level rise, causing coastal squeeze (Doody, 2004). In addition to these development pressures, Mediterranean salt marshes are also impacted by climatic stressors including sea level rise, changed storminess, warm spells or heat waves resulting in their decline. Loss of salt marshes in the region has been caused by a reduction in sediment supply and land subsidence, exacerbated by sea level rise (Kirwan and Megonigal, 2013a), increases in extreme water elevations (Masina and Lamberti, 2013), and wind-induced waves (Day et al., 1998; Fontolan et al., 2012). Climate extremes, such as heat waves, have accelerated some of these losses, modifying the composition of the dominant vegetation (Strain et al., 2017). Moreover, the dense populations and intensive agriculture and farming along the coast and the plain of the Po river have enhanced nutrient levels along the coastline, and eutrophic conditions are common (de Wit and Bendoricchio, 2001; Lotze et al., 2011b), potentially increasing the sensitivity of salt marshes to erosion (Deegan et al., 2012b). Given the growing evidence that salt marsh conservation/restoration can be effective as nature-based coastal protection schemes (Shepard et al., 2011), and their rapid rate of decline in the Mediterranean and globally, it is imperative to quantify their role in slowing of coastal erosion rates, and identify which factors or combinations of factors enhance or limit this valuable ecosystem service (Bouma et al., 2014).

Our main objective was to quantify the role of salt marshes in slowing lateral coastal erosion rates in the Northern Adriatic Sea along a broad gradient of physical and anthropogenic factors. We concentrate our investigation on the role of salt marshes in decreasing erosion at the cliff edge, focusing on below-ground factors affecting erosion. Specifically, we investigated how erosion resistance may be modified by the presence of *Spartina* spp., the local sediment grain size, and the local nutrient status of the marsh, using C:N-ratios of above-ground biomass as a proxy for nutrient levels. We hypothesized that *Spartina* spp. below-ground biomass would significantly reduce lateral erosion, and that this effect would increase with increased density of *Spartina* spp. and in cohesive silty soils, while it would decrease with increased nutrient levels.

3.2 Materials and Methods

Study area

The Northern Adriatic Sea basin off the coast of Italy is the shallowest, northernmost region of the Mediterranean and among the most productive with high habitat diversity (Lotze et al., 2011b). The area consists of lagoon-river delta systems which host numerous transitional water bodies and is characterized by moderate exposure to wave action and a semi-diurnal micro-tidal regime with average tidal amplitudes of approximately 65 to 80 cm (Fontolan et al., 2012; Silvestri et al., 2005). Average sea surface temperatures in the region vary between 7 °C in winter and 27 °C in summer, with highly variable average surface salinities between 30 and 38 psu. Major activities in the region include the petrochemical industry, tourism, fishing, seaport/port activities and shipping (Torresan et al., 2012). Development pressures have resulted in overall reduced sediment supply in the region, and negative sediment budgets have been recorded in the lagoons of Venice and Grado-Marano due to subsidence, dredging and clam harvesting, such that the lagoons are experiencing accelerating erosion and unprecedented loss of salt marshes (Day et al., 1998; Fontolan et al., 2012; Sarretta et al., 2010; Torresan et al., 2012).

We sampled six sites in coastal lagoons along approximately 230 km of coastline along the regions of Emilia-Romagna, Veneto and Friuli Venezia Giulia (Figure 1), aiming to include a wide range of environmental (i.e., nutrient status) and physical (i.e., sediment type) conditions. From south to north, sites were located: (1) in the natural reserve Sacca di Bellocchio (hereafter BEL), in the the Valli di Comacchio lagoon ecosystem, Parco Delta del Po dell'Emilia-Romagna (44°37'47''N, 12°15'38''E) (further site description in Strain et al., 2017); (2) in the Vallona Lagoon (hereafter VAL), in an area that had previously been reclaimed for agriculture, and is currently being used for private aquaculture (45°1'42''N, 12°23'7''E) (further site description in Wong et al. 2015); (3) in the Coastal Botanic Garden of Veneto, municipality of Rosolina (hereafter ROS), in the southern portion of Caleri Lagoon, Parco Regionale Veneto del Delta del Po (45°5'42''N, 12°19'39''E); (4) in the Chioggia Inlet (hereafter CHI) in the southern part of Venice Lagoon (45°14'53''N, 12°13'34''E); (5) in Grado Lagoon (hereafter GRA), adjacent to an area privately used for aquaculture (45°42'13''N, 13°26'31''E), and (6) in another area in Grado Lagoon adjacent to a golf course (hereafter GOLF) (45°42'6''N, 13°27' 4''E) (Figure 1).

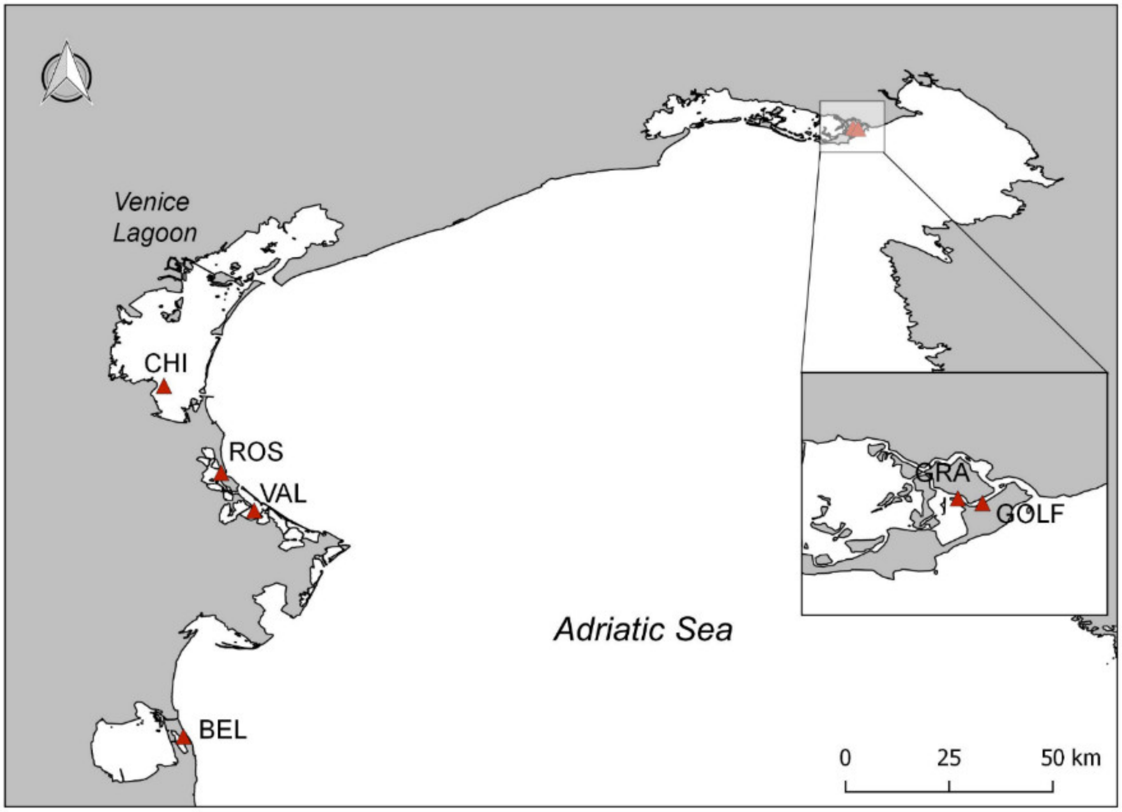

Figure 1: Location of sampling sites on the Northern Adriatic coast, Italy. Sampling sites: Sacca di Bellocchio, Parco Delta del Po dell'Emilia-Romagna (BEL); Vallona Lagoon (VAL); Coastal Botanic Garden of Veneto, Parco Regionale Veneto del Delta del Po (ROS); Chioggia Inlet, Venice Lagoon (CHI); Grado Lagoon (GRA) and Grado Golf Course (GOLF).

Salt marsh vegetation in these coastal lagoons is composed of perennial *Spartina* spp. cordgrass swards, annual glasswort swards, salt meadows, halophilous scrubs, and salt steppe associations. Species noted at the sampling locations within the *Spartina* spp. zone included

Salicornia spp, *Sarcocornia fruticose*, *Limonium narbonense*, *Juncus maritima*, *Inula crithmoides*, and *Aster tripolium*. *Spartina* spp. formed patchy to extensive meadows at all sites. Until recently, only *Spartina maritima,* the only *Spartina* species native in Europe (Strong and Ayres, 2013), was reported to occur in these lagoons. *Spartina townsendii* and *Spartina anglica* were first reported from Venice Lagoon in 2002 (Cazzin et al., 2009; Scarton et al., 2003), but their identification status and occurrence were not further explored. A recent broad-scale genetic study, conducted while this experiment was already in progress, showed that *S. martima* in the region has largely been replaced by *S. anglica* and *S. townsendii* (Wong, 2015). Genetic and chromosomal analyses indicate that these species can co-occur in mixed patches (Wong 2015), where they can be difficult to distinguish morphologically until the flowering period; therefore we will refer to them as *Spartina* spp.

Sediment properties

Sediment properties were characterised at the start of the work in June and July 2014, as we aimed to include sites with different soil characteristics in our study. At each study site we collected 8 sediment samples (2.5 cm in diameter and 8 cm long), 4 in vegetated and 4 in non-vegetated (hereafter “bare”) patches randomly placed within the *Spartina* spp. zone. The sediment samples were stored on ice during transport. In the laboratory, sediment samples were dried at 60^0C until constant weight to determine bulk density (g/cm^3). Grain size was determined using a Malvern Laser Particle Sizer, enabling the determination of total percent silt (particle size >4 and <63 µm, according to the Wentworth classification (Wentworth, 1922). A subsample was used to determine organic matter content with the loss-on-ignition technique (Craft et al., 1991), with the samples heated to 450 °C for six hours in a muffle furnace.

Sampling design and wave mesocosms set-up

Samples for the quantification of lateral erosion were collected in early October 2014, at the end of the *Spartina* spp. growing season. Ten cores, 5 in vegetated and 5 in bare patches, were randomly extracted at each of the 6 sites within the *Spartina* spp. zone using 30 cm length x 20 cm diameter PVC cylinders. The cylinders were lowered onto the marsh surface, and samples were carefully extracted by digging around the cylinders. A knife was used to cleanly cut roots at the bottom, and a plastic cap was placed at the bottom to prevent sediment loss. Sediment cores were carefully packed to prevent dislodgement and were immediately transported to wave mesocosm facilities at the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke, Netherlands, and stored in filtered seawater from the Scheldt estuary. Before placement in the wave mesocosms, the sediment cores were extruded from the PVC cylinders with a purpose-built extrusion device, which minimized compaction and loss of sediment. Samples were then placed into modified stainless steel cylinders bisected to expose them to waves. The sediment cores were then cleanly sliced at the bottom to a height of 15 cm, and the side facing the waves was trimmed flush to fit the steel cylinders. In this way, the core surface mimicked a cliff that is exposed to waves (Figure 2a).

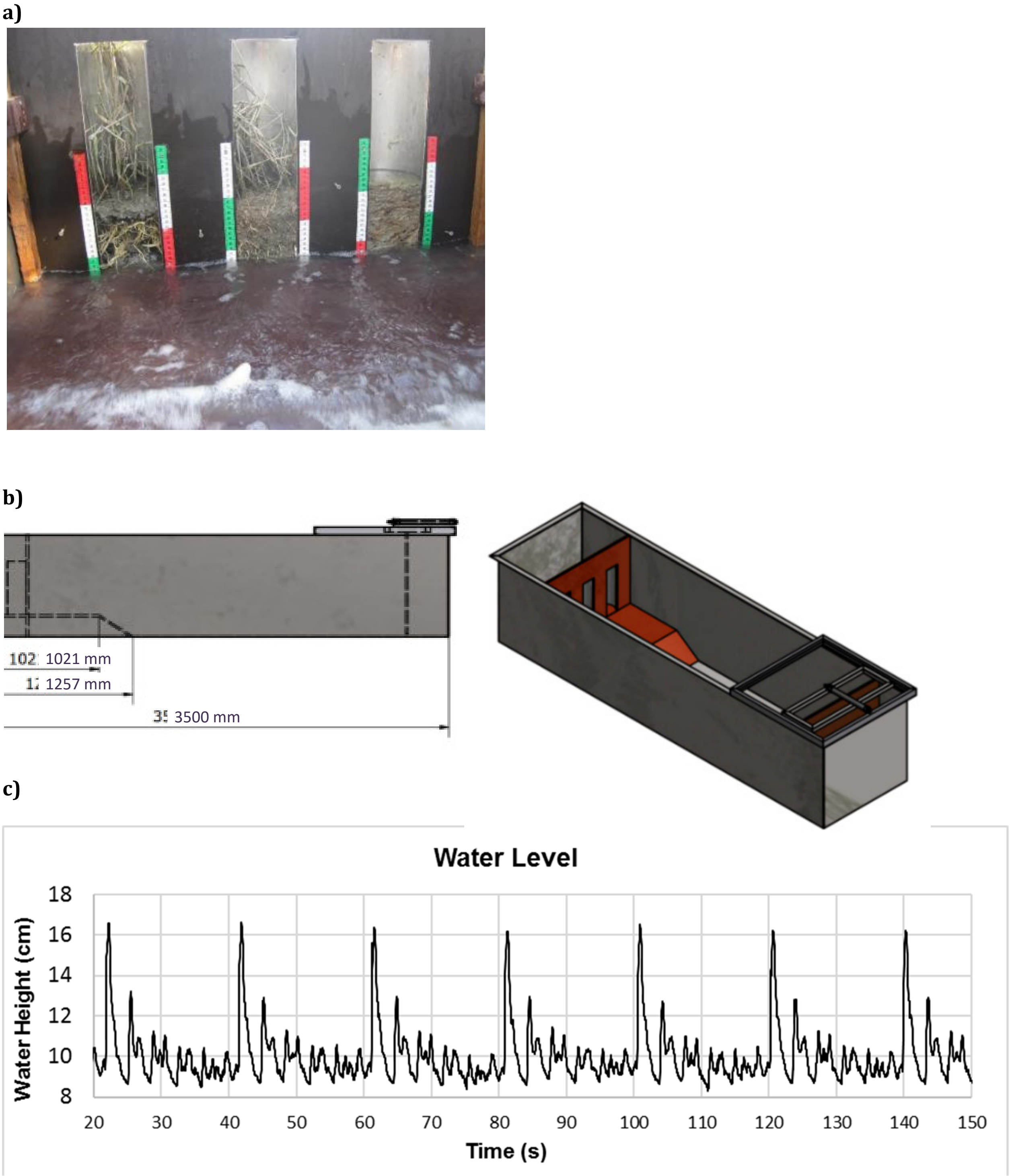

Figure 2: a) Schematic cross-sectional drawing of the wave mesocosms developed and built at NIOZ, dimensions shown in cm (for information contact tjeerd.bouma@nioz.nl; b) A picture of sliced sediment cores that were exposed to waves in the wave mesocosms; c). Water level measurements used to infer significant wave height (cm) over time (s) (only a segment of time is shown), measured with a pressure sensor at 1 cm distance from the marsh-water interface.

To obtain volume loss as a measure of erodibility, sediment cores were subjected for a 32-hour period to waves, generated by a piston-powered wave board placed in a mesocosm (La Nafie et al., 2012). The duration of wave treatment was based on initial tests that showed slow to constant erosion rates at 32 hours across different sediment characteristics. The wave mesocosms were 360 cm long × 90 cm high × 82 cm wide (Figure 2b), and were designed and built at NIOZ (for further information contact tjeerd.bouma@nioz.nl). The distance from the sediment cores to the wave board was 280 cm. There were four wave mesocosms, each holding 3 cores side by side, so that 12 cores could be subjected to wave conditions at the same time. To uniformly spread the potential variability from different storage times and wave mesocosm conditions across the tested factors, one replicate core for each site and vegetation state (vegetated *vs.* bare) was treated each time in a randomized block design. With 5 replicate cores per site and vegetation state, the experiment required a total of 5 wave mesocosm runs over a period of three weeks.

Sediment cores were placed on a platform elevated 17 cm from the bottom from which they exposed to wave treatment. To prevent a standing wave from developing, we combined a fast push with a slow retreat (La Nafie et al., 2012), which generates a semi-random wave spectrum (Figure 2c). This resulted in reflecting waves but they were small enough to prevent a standing wave from forming. The resulting wave pattern, while not mimicking wind-induced waves, do mimic hydrodynamic stress similar to boat wakes. Significant wave height at the water-sediment core interface was 5.34 cm, as measured with a Druck PTX 105 1830 pressure sensor placed in front of the sediment cores. 3976 recordings were collected at a sampling rate of 25 Hz, and pressure readings were converted to water level fluctuations (η). The waves intersected the portion of the soil below the surface to enable measurement of lateral erosion of the belowground material, as we do not address wave attenuation by stems/leaves above the surface in this study.

Estimates of lateral erosion

Eroded volume loss was estimated at each of 8 time steps over the 32-hour period. The time steps corresponded to the following elapsed time periods: t_0=beginning of wave treatment, t_1=15 minutes, t_2=1, t_3=4, t_4=8, t_5=16, t_6=24, and t_7=32 hours. Photogrammetry methods were applied to estimate erosion of the salt marsh sediments. At each time step a series of photographs (approximately 40 – 60 photos) of each sediment core sample were taken from several angles approximately one metre in front of the sample with a handheld compact camera (model Canon PowerShot D10) on automatic settings. To ensure consistent and high quality of photographs, use of flash was avoided. To compensate for low lighting conditions and minimize discrepancies between photos taken during day and night, portable fluorescent lights were fixed to the wave mesocosms to illuminate samples at night. The series of photographs for each sample at various time steps were translated into point clouds using the software VisualSFM (Wu, 2011; Wu et al., 2011). Dense reconstruction models were built with the CMVS/PMVS module within VisualSFM (Furukawa and Ponce, 2010). The point clouds were then converted into triangular meshes to build 3-dimensional models using MeshLab software (Cignoni et al., 2008). At this stage, anomalous points and possible errors could be detected for and corrected by re-calibrating dense reconstructions in VisualSFM. The volume loss between time intervals for each sediment core was calculated using MATLAB (The MathWorks, Inc., 2012). We validated this photogrammetry method using volumetric displacement with mudflat sediments from the Scheldt estuary. There was excellent agreement between the actual volume loss measured with the validation samples and the volume loss estimated with the photogrammetry method (Pearson correlation coefficient=0.99).

Above and below-ground biomass and C:N ratios

Spartina spp. stem densities were evaluated on vegetated cores. After erosion treatments in the wave mesocosms, above-ground biomass was clipped off the sediment cores, dried at 60^0C until constant weight, and weighed. The remaining sediment and the below-ground biomass were rinsed carefully over a 1 mm mesh, and the below-ground biomass was dried and weighed as above. sub-sample of the dried above-ground biomass was crushed with a mortar and pestle and analyzed for total organic C and N with an Element Analyzer N1500 (Carlo Erba). We acknowledge the potential under-estimation of biomass lost from the cores during the wave treatment. However, we minimized loss of above-ground vegetation by clipping it off non-invasively before it was completely eroded. Additionally, as we observed that root biomass in our samples tended to form a physical network that remained even when sediment was washed away, we assumed any loss of below-ground vegetation during wave treatment was minimal. C:N ratios were used as a proxy for nutrient levels in the salt marsh samples, as C:N-ratios in *Spartina* spp. leaf tissues have been shown in previous studies to reflect ambient nutrient concentrations in porewater (Tobias et al., 2014) and experimental nutrient enrichment treatments (Crain, 2007; Jiménez et al., 2011). The block design for wave treatment meant that potential variation in C:N results from different storage times would be spread out equally among all treatments.

Statistical analyses

Two-way ANOVAs were used to compare the main effects of vegetation status (*Spartina* spp. or bare), site, and their interaction on sediment properties (bulk density (g/cm^3), silt content (%), and organic matter content (%LOI). Pair-wise tests (Tukey's HSD) were used for a posteriori multiple comparisons of means among sites when significant site effects were found (α=0.05), in order to identify groups of sites with different sediment properties to be used in the following experimental test.

On the vegetated erosion sediment cores, we tested differences among sites in above-ground biomass, below-ground biomass, root to shoot ratio and stem density, and shoot C:N ratio, with one-way ANOVAs, specifying site as a main effect, followed by Tukey's HSD pair-wise tests when significant site effects were found.

To obtain estimates of erosion of the sediment cores, we fitted an exponential model to the experimental data of volume loss, to obtain maximum possible volume loss over time, in the form of $E=Q(1-e^{-kt})$, where E=volume eroded, Q=maximum volume loss, k=rate of volume loss, and t=time in hours. Fitted values of Q corresponded very closely to our values of volume loss estimated by photogrammetry, obtained at the end of the wave treatment at 32 hours (Pearson correlation coefficient=0.99). We then constructed a linear mixed effects model (Pinheiro and Bates, 2014) to describe the relationship between vegetation and sediment variables and the maximum volume loss, Q, across sites. As fixed effects, we entered the categorical variables vegetation (vegetated, bare), silt content (high and low silt content), and their interaction into the model. As random effects, we considered site and the sequence of wave treatment (block). The random block effect introduced negligible variation in model results, as demonstrated by comparing models with and without the block effect, so was subsequently dropped from the overall model. Variance partitioning was examined by employing marginal R^2 values (percent variation explained by fixed effects) and the conditional R^2 values (percent variation explained by both fixed and random effects) (Nakagawa and Schielzeth, 2013). Final model selection was based on the lowest Akaike's information criterion (AIC) and p-values (α=0.05) from stepwise modeling. The significance of fixed effects was tested using Type II Wald Chi-square tests.

On the vegetated sediment cores, we then modeled the effect of above-ground biomass, below-ground biomass, root to shoot ratio, stem density, and shoot C:N ratio on Q (maximum erosion). We again entered site as a random effect, and removed the block effect from the model as it introduced negligible variation. Model selection and p values were obtained as described above.

R open source software was used for all statistical analyses (R Core Team, 2017). Two-way and one-way ANOVAs and Tukey's HSD post-hoc tests for significant effects (α=0.05) were performed within R. The *nlme* (Pinheiro, J et al., 2017) package was used for the non-linear modeling. The *lme4* package (Bates et al., 2014) was used for the linear mixed modeling, and associated R^2 values were obtained with the *MuMin* package (Bartoń, 2016). Normality and homogeneity of the data were visually checked in the residual plots, and, when necessary, data were transformed to comply with assumptions of homogeneity of variance (see supplementary tables for details).

3.3 Results

Sediment properties and vegetation characteristics across sites

Silt content largely differed among sites (Figure 3, Tables S1 and S2, $F_{(5,36)}$=22.00, p<0.001). CHI, GRA and GOLF (hence referred to as silty sites) were characterized by predominantly silty soils in both vegetated and bare areas (average 71.69%, *SD*=10.95, *n*=24), while BEL, ROS and VAL (hence referred to as sandy sites) had a much lower mean silt content (17.83%, *SD*=10.05, *n*=24). The % silt was more variable in vegetated than in bare areas in the predominantly sandy sites. Vegetation status marginally affected the mean sediment % silt content, with higher mean % silt in vegetated *vs*. bare samples ($F_{(1,36)}$=3.32, *p*=0.076). The effect of vegetation on silt content was higher in the sandy *vs*. silty sites. Bulk density and organic matter were also variable among sites (Figure 3, Table S3, $F_{(5,36)}$=7.04, p<0.001 for bulk density, $F_{(5,36)}$=8.29, *p*<0.001 for organic matter). Bulk density ranged from 1.11 to 1.32 $g \cdot cm^{-3}$ in the sandy sites and 0.85-1.32 $g \cdot cm^{-3}$ in the silty sites, and organic matter ranged from 2.7 to 5.1 % LOI in the sandy sites and 1.5 to 7.0 in the silty sites (Table S3). Vegetation status did not significantly affect bulk density or organic matter (*p*>0.05).

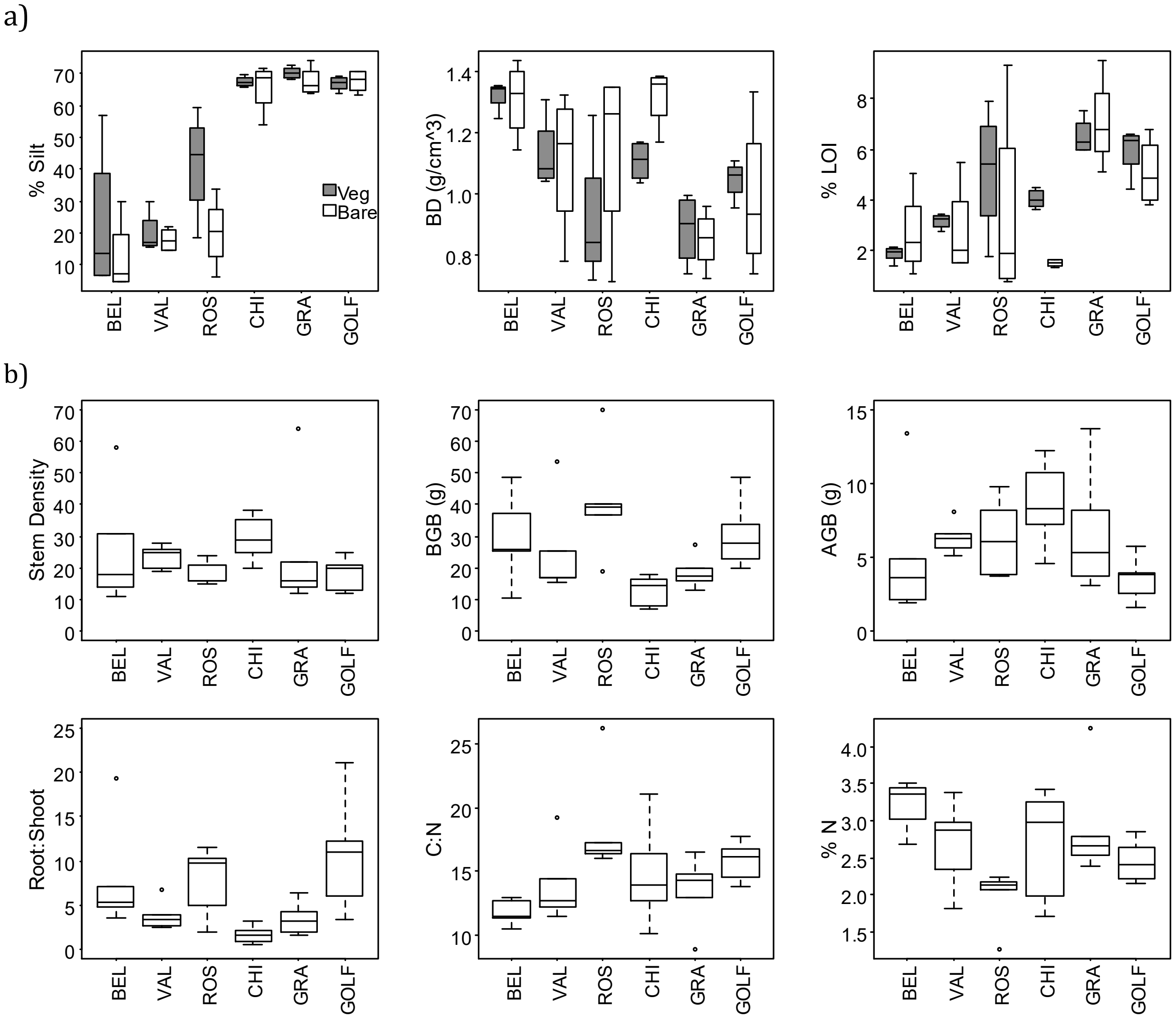

Figure 3: a) Boxplots describing sediment properties (% Silt, bulk density ($g\cdot cm^{-1}$), and organic matter (% LOI (loss on ignition)) at the 6 study sites (labels as in Fig. 1) across the Northern Adriatic coast, Italy, over 4 replicates. For each site we sampled both vegetated (shaded bars) and bare (un-shaded bars) areas. Horizontal lines are medians, boxes show the interquartile range (IQR), error bars show the full range excluding outliers, defined as 1.5 x IQR (plotted as points); and b) Boxplots describing vegetation properties evaluated on each of the vegetated sediment cores collected for erosion analysis, over 5 replicates (boxplot descriptions as above): Density of *Spartina* spp. vegetation (Stem Density), below-ground biomass (BGB in g), above-ground biomass (AGB in g), root to shoot ratio (RootShoot), C:N ratios in *Spartina* spp. shoots (C:N), and total percent nitrogen in *Spartina* spp. shoots (% N).

Vegetated sediment erosion cores did not differ in above-ground biomass or stem density between sites, whereas significant differences were found in below-ground biomass (Figure 3, Table S4, $F_{(5,24)}$=2.95, p<0.05). There was lower below-ground biomass in CHI compared to ROS (p<0.05, Table S5). A significant difference in root:shoot-ratio was also found among sites (Figure 3,

Table S4, $F_{(5,24)}$=5.15, p<0.01) with CHI having significantly lower root:shoot-ratio than ROS and GOLF (*p*<0.05 in both cases, Table S5). C:N-ratios of *Spartina* spp. shoots differed across sites (Figure 3, Table S4, $F_{(5,24)}$=2.95, p<0.05), with BEL having a significantly lower mean C:N-ratio of 11.82 than ROS (mean C:N-ratios of 18.48) (*p*<0.05 for both cases, Table S5).

Effect of vegetation and sediment properties on erosion

Sites had highly variable volume loss at 32 hours of wave treatment (Figure 4). In the silty sites, CHI, GRA and GOLF had mean volume losses of 1.2%, 11.3 and 8.9% respectively, for vegetated cores, and 5.7%, 8.1% and 57.5%, respectively, for bare cores. In the sandy sites, BEL, VAL and ROS had mean volume losses of 27.6%, 13.7% and 7.5%, respectively, for vegetated cores, and 79.3%, 90.6% and 83.2%, respectively, for bare cores. Overall, mean volume loss in vegetated cores was 16.26% (*n*=15, *SD*=25.21) for sandy cores and 7.11% (*n*=15, *SD*=5.90) for silty cores, and in bare cores, volume loss was 84.37% (*n*=15, *SD*=26.39) sandy cores and 23.44% (*n*=15, *SD*=33.32) in silty cores.

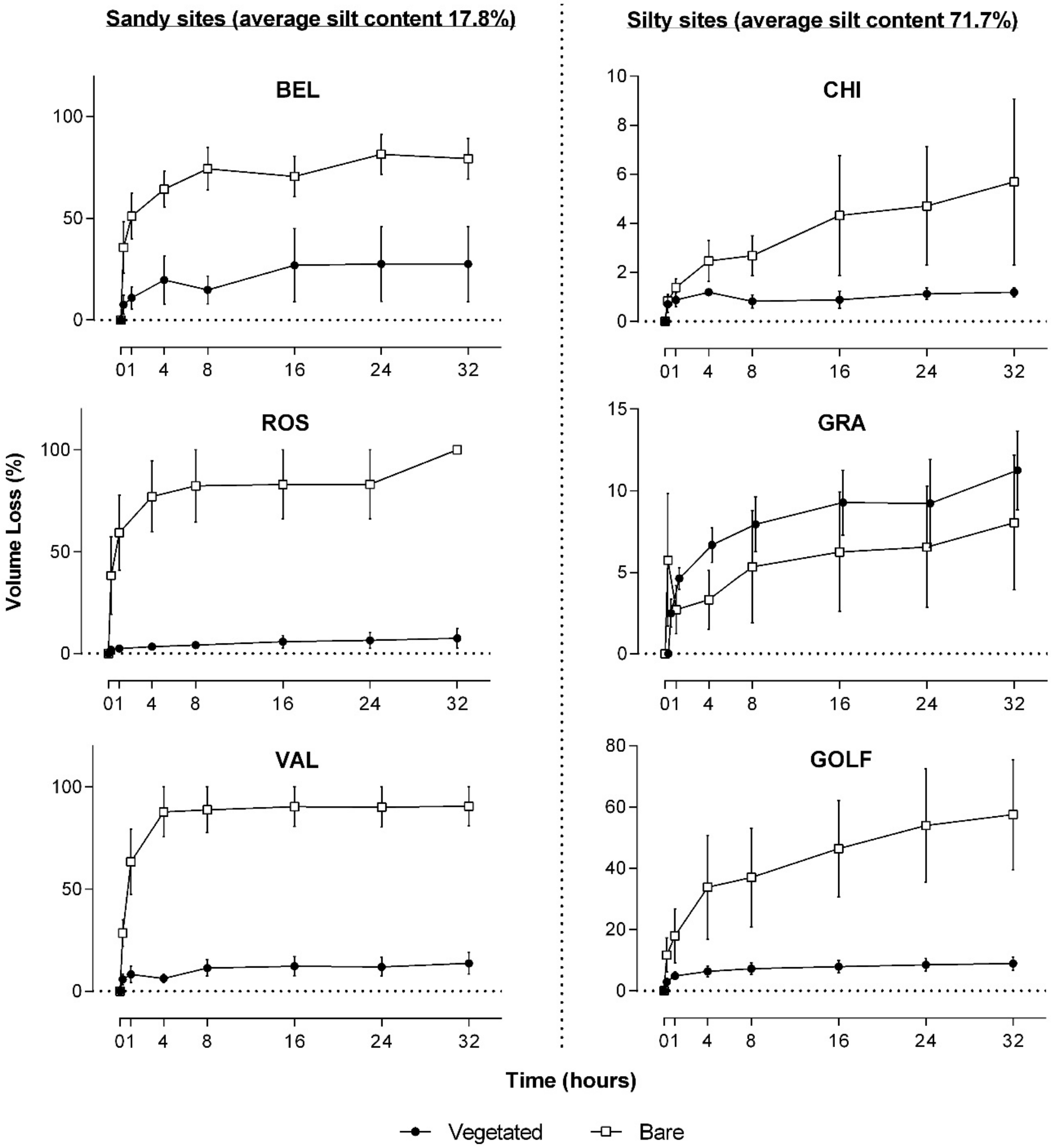

Figure 4*:* Volume loss (erosion) estimated over each time step, in sites with low silt fractions (shown in left column), and in sites with high silt fractions (shown in right column). Non-vegetated sediment cores (Bare) are marked for each site with open circles, and vegetated sediment cores (*Spartina* spp.) are marked with filled circles. Error bars denote standard error of the mean over 5 replicates.

The mixed effects linear model with the best fit yielded a highly significant effect of vegetation, grain size and their interaction on the total eroded volume (Table 1). Erosion was lower in vegetated *vs.* bare sediment cores ($\chi^2(1)=46.7$, $p<0.001$), and was also lower in cores with high silt *vs.* low silt content ($\chi^2(1)=23.8$, $p<0.001$). The difference in erosion between vegetated and bare cores was higher in sandy *vs.* silty soils ($\chi^2(1)=15.97$, $p<0.001$). The marginal R^2 value (variation related to fixed effects) was 0.59, and the conditional R^2 value (variation related to both fixed and random effects) was 0.62, indicating that most of the variation in erosion was explained by the fixed effects.

Table 1: a) Set of linear mixed effects models on erosion of vegetated and bare sediment cores, with site included as a random term in each model (*n*=60). The most supported model is marked in bold. Dependent variable codes: Veg = bare or vegetated, Silt = low silt or high silt. a_x = coefficients, -2ln*L*=-2log likelihood, AIC = Akaike's information criterion, DF = degrees of freedom (residuals). * = significant effects of model terms (p<0.05). Model p-values are in comparison with previous model in iteration. b) Linear mixed model on erosion of vegetated cores, with site included as a random term (*n*=30). BGB = below-ground biomass; other variable codes as above.

Model		**a_0**	**a_1**	**a_2**	**a_3**	**-2ln*L***	**AIC**	**DF**	**P**
a)	**Vegetated and bare sediment cores**								
	$a_0 + (a_1)$Veg*	52.82	-41.63			-287.4	582.9	56	
	$a_0 + (a_1)$Veg* + (a_2)Silt*	70.03	-41.63	-34.43		-283.4	575.3	55	<0.01
	$a_0 + (a_1)$Veg* + (a_2)Silt* + (a_3)Veg*Silt*	**82.20**	**-65.96**	**-58.76**	**48.66**	**-275.7**	**563.3**	**54**	**<0.01**
b)	**Vegetated cores**								
	$a_0 + (a_1)$BGB*	27.19	-0.61			-0.61	263.2	26	

The most parsimonious model exploring the effect of vegetation and nutrient variables on erosion incorporated only the amount of below-ground biomass as a significant predictor of erosion ($\chi^2(1)=7.54$, $p<0.01$)). Stem density, above-ground biomass, root:shoot ratios and C:N-ratios were not significant predictors of erosion, when considered alone or in any combination of variables. By plotting below-ground biomass as function of erosion, the effect of below-ground biomass on reducing erosion can be seen across all sites, except CHI, with the strongest effects seen in the sites with predominantly sandy soils (BEL, ROS and VAL), as indicated by the steeper slopes (Figure 5).

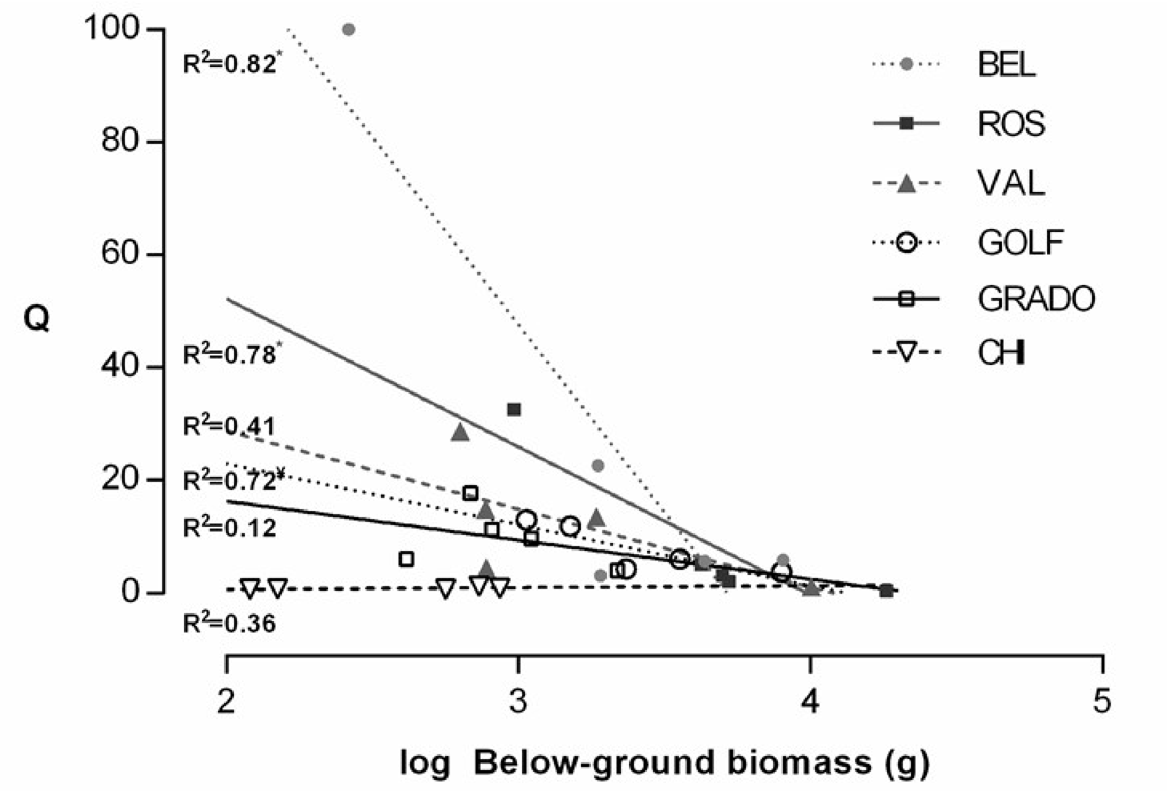

Figure 5: Scatterplot of maximum erosion, *Q* (% maximum erosion), *vs.* log-transformed below-ground biomass (g) for each site (site labels as in Fig. 1). Points in the scatterplots represent each of 5 replicates. Regression lines depict the linear relationship between *Q* and log-transformed below-ground biomass for each site, with R^2 values shown adjacent to each regression line (order of regression lines, top to bottom, follows site legend order). * = significant (p<0.05) and ¥ = marginally significant (p<0.1).

3.4 Discussion

Our wave mesocosm experiments showed that vegetated salt marsh sediment cores had higher resistance to lateral erosion compared to bare sediment cores. Vegetation reduced volume loss by 80% in sandy soils and 17% in silty soils, revealing the critical role of salt marshes in coastal defence. The below-ground component of vegetation was found to be the most significant predictor of erosion, while the contribution of the above-ground variables (above-ground biomass, stem density, shoot:root ratio) was marginal. Sediment properties also affected erosion rates in the absence of vegetation, with mean lateral erosion 72% lower in silty *vs.* sandy soils.

The relationship between erosion and below-ground biomass was stronger in sandy sites, but still evident in the silty sites, with the exception of CHI, where there was no clear effect of below-ground biomass on erosion. A broad-scale genetic survey of the Adriatic Coast that was completed after our erosion experiment revealed that the area from which we extracted erosion cores in CHI is most likely to be *S. maritima*, whereas the erosion cores in the other sites that we sampled were most likely to be a mix of non-native *Spartina* species (Wong et al. 2015). Our photographs of the experimental erosion cores did not allow us to unequivocally attribute the vegetation to the different species, and therefore we could not formally explore if there were any differences in erosion control among different *Spartina* species, but further research on this topic is certainly warranted. Morphological differences that have been found, for example, in above-ground biomass (Strong and Ayres, 2013), could alter the way in which wave energy is dissipated, as leaf stiffness, stem density and biomass of salt marsh vegetation have been demonstrated to influence wave attenuation (Bouma et al., 2005; Heuner et al., 2015). Differences in root morphology and architectural traits could also alter soil stabilization properties (Ghestem et al., 2014). Potential differences in the diversity and functional groups of faunal communities associated with different *Spartina* species may have consequences for sediment stability as well. It is well-documented that bio-stabilising inter-tidal organisms such as benthic microalgae can enhance cohesiveness, for example, via secretions of biofilms and thus alter the sediment critical erosion threshold; while bio-destabilizers such as bioturbating infauna can enhance erosion (Van Colen et al., 2012, 2014; Widdows and Brinsley, 2002). Thus, differences in bio-stabilizing and bio-destabilizing communities that may result from encroachment of non-native *Spartina* species have the potential to affect its ability to resist lateral erosion.

With regard to the role of sediments in lateral erosion, we report that erosion resistance in the bare cores were significantly enhanced by silt content, with mean erosion 71.5% lower in silty *vs*. sandy soils. This supports other studies, e.g., (Houwing, 1999) determined in an *in-situ* flume experiment that erosion in mudflats bordering salt marshes on the Dutch Wadden Sea coast decreased with higher mud content (which correlates with silt content). In another flume experiment, Ford et al. (2016) found that both the clay-silt fraction and salt marsh vegetation root biomass (Essex and Morecambe Bay, UK) were significant predictors of erosion in addition to other factors such as plant diversity, percent vegetation cover and carbon stock (Ford et al., 2016), although interactive effects of these factors were not tested. Our model results demonstrate significant interactive effects of vegetation status (vegetated *vs.* bare) and sediment silt content on erosion. We note the caveat that we measured sediment properties to a depth of 8 cm; however the erosion cores had a depth of 15 cm. Therefore, the sediment properties we sampled may not be representative of the entire erosion core, which is a potential source of error in our analysis. Nonetheless, we believe that the magnitude of difference in % silt between sites is large enough to allow for a general classification of sites as either sandy or silty.

In a similar wave mesocosm experiment, Feagin et al. (2009) concluded that sediment type was the most important factor controlling erosion, while vegetation affected erosion indirectly by modifying sediment properties. In the bare sediment core samples, we found significant effects of silt content on erosion. However, we did not find significant differences in bulk density or organic matter between vegetated and bare areas, and only marginal effects of vegetation on silt content. This suggests a more direct control of the vegetation on erosion rates rather than an indirect one mediated by changes in sediment characteristics as suggested by Feagin et al. (2009). As our results indicate root biomass to be most important in decreasing lateral erosion in salt marsh sediment cores with higher sand content, we expected an even stronger effect of root biomass in reducing erosion in the study by Feagin et al. (2009), where core samples had relatively higher sand content compared to our core samples (Feagin 2016, *pers. comm*. to V.B. Lo). We speculate that the difference in our findings may be due to the selection of the locations where the bare sediment cores were collected.

In our case, samples were collected from the pioneer zone, where pioneer vegetation species such as *Salicornia* spp. or *Spartina* spp. would not yet have influenced sediment characteristics, whereas Feagin et al. (2009) also sampled bare areas within the marsh interior (Galveston Island, Texas), where sediment characteristics may have been influenced by marsh formation over a longer period of time.

Contrary to our expectations, C:N ratios were not good predictors of erosion rates. Previous studies show that soil stability can decrease with higher nutrient loading rates as a result of increased soil metabolism and lower root and rhizome biomass (Turner et al., 2009; Turner, 2011). Deegan et al. (2012) found that nutrient addition led to an increase in above-ground biomass, decrease in below-ground biomass and increased microbial decomposition resulting in increased fine detrital organic matter. Finer-grained, less consolidated creek banks retained more water at the low tide, which increased pore water pressure and reduced frictional shear strength of soil, ultimately resulting in creek-bank collapse and subsequent conversion to non-vegetated mud (Deegan et al. 2012). A possible explanation of our result is that the study lagoons along the Adriatic Sea were all relatively nutrient-rich. The *Spartina* spp. tissue C:N-ratios in our sediment core samples ranged from 10-22, with significantly lower values found in BEL– the site where vegetated samples also experienced the highest mean amount of erosion, a pattern which persisted throughout the course of wave treatment (Fig. 5). The range of C:N-values in our sites is even lower than the range of C:N-values in *Spartina* spp. samples treated with fertilizer in Crain (2007) and Jiménez et al. (2011), who reported C:N-values of 25-30 and 20-25, respectively, while control samples in both studies had C:N-values ranging from 40-45.

GOLF had the highest erosion rates among the bare sediment cores with high silt content. In line with the above-mentioned studies, higher erosion in GOLF was expected to be due to high nitrate availability due to its proximity to a golf course. However, in contrast to our expectations, C:N-ratios were not found to be significantly lower in GOLF compared to other sites. The observed high erosion rate may be related to the higher sediment organic matter found in GOLF. Higher mean fine detrital organic matter (1-3 mm) was also found in nutrient-enriched salt marshes in Deegan et al. (2012), where higher observed erosion was attributed in part to accelerated decomposition in the presence of high nitrate levels, resulting in higher water retention and decreased structural integrity. However, in Feagin et al. (2009), fine, humic organic matter was found to reduce wave-induced erosion. Both studies found that erosion was enhanced by coarse organic material. This suggests that the role of organic matter in erosional processes is not just dependent on size fraction, but also on nutrient availability. The fact that vegetated sediment cores in GOLF experienced less erosion than the bare sediment cores suggest that even in silty soils with high ambient nutrient conditions, *Spartina* spp. vegetation still prevented erosion presumably through sediment binding.

3.5 Conclusions

Our study provides novel insight into the interactive effect of vegetation and sediment characteristics on the ability of salt marshes to resist lateral erosion. It also contributes to quantifying coastal ecosystem services provided by salt marsh vegetation, using novel photogrammetry methods, and highlights the critical role of the *Spartina* spp. root system in decreasing lateral erosion. Erosion was higher in bare *vs*. vegetated sediment cores, particularly in sites with low silt content, and below-ground biomass was negatively related to erosion. Although the effect of vegetation on erosion resistance was less prominent in siltier sediments, it was still significant. Given that multiple stressors are driving the loss of native *S. maritim*a vegetation in the Northern Adriatic Sea and its replacement by non-native *Spartina* spp. and *Salicornia* succulents (Strain et al 2017), further work should explore the potential effects of these habitat shifts on the system functional properties. Further work should also explore the potential of using these salt marshes in ecosystem-based approaches to coastal defence, and identifying the conservation and coastal management practices that would most help preserve their functioning and contribution to coastal defence under rapidly changing conditions.

Acknowledgements

This work was funded through a MARES Grant (www.mares-eu.org) and project TETRIS (PRIN 2010-2011, MIUR). MARES is a Joint Doctorate programme selected under Erasmus Mundus and coordinated by Ghent University (FPA 2011-0016). CVC acknowledges a postdoctoral fellowship provided by the Flemish Fund for Scientific Research (FWO-1.2.380.11.N.00). The authors are grateful to members of the Airoldi Lab (University of Bologna): Elena Piccioni, Joanne Wong, Stefanie Broszeit, Lorenzo Gadoni, Giulia Finotti; the Spatial Ecology department (NIOZ) including Zhu Zenchang and Jeroen van Dalen, Bart Beuselinck from the Research Group Marine Biology (UGent) and Ignacio Barbeito Sanchez for assistance at various stages of this research. We thank the Po Delta, Rosolina and Veneto Parks, Ferruccio Polo (Cooperativa Pescatori di Grado) Valle Artalina, and Golf Club Grado for access to the sampling sites. We also thank two anonymous reviewers whose thorough reviews and suggestions greatly improved this manuscript.

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3.7 Supplementary Information

Table S1: Results of two-way ANOVAs testing effect of site (six sites), veg (vegation: *Spartina* spp. or bare) and their interaction on sediment variables collected prior to the erosion experiment: % silt (log-transformed), bulk density ($g\cdot cm^{-3}$) and organic matter (% loss-on-ignition). Statistically significant effects (α=0.05) are shown in bold.

	Source of Variation	**SS**	**Df**	**MS**	**F-ratio**	**P**
% Silt	Site	22.031	5	4.406	21.984	**0.000**
	Veg	0.666	1	0.666	3.322	0.076
	Site*Veg	1.039	5	0.208	1.042	0.408
	Error	7.179	36	0.199		
Bulk Density	Site	1.006	5	0.201	7.042	**0.000**
	Veg	0.033	1	0.032	1.136	0.294
	Site*Veg	0.175	5	0.035	1.223	0.318
	Error	1.029	36	0.029		
Organic Matter	Site	123.620	5	24.724	8.291	**0.000**
	Veg	5.660	1	5.664	1.899	0.179
	Site*Veg	16.150	5	3.229	1.083	0.386
	Error	107.350	36	2.982		

Table S2: Results of Tukey's HSD post-hoc tests, pair-wise comparisons of effect of site on sediment variables (% Silt, bulk density ($g\cdot cm^{-3}$) and organic matter (% loss-on-ignition). Site labels as in Fig. 1. Statistically significant effects (α=0.05) are shown in bold.

	% Silt	**Bulk density**	**Organic Matter**
Comparison	*p*	*P*	*p*
CHI-BEL	0.000	0.824	0.993
GOLF-BEL	**0.000**	**0.000**	**0.000**
GRA-BEL	**0.000**	**0.013**	**0.008**
ROS-BEL	**0.028**	**0.020**	0.210
VAL-BEL	0.488	0.212	0.969
GOLF-CHI	1.000	**0.003**	**0.001**
GRA-CHI	1.000	0.205	**0.034**
ROS-CHI	**0.003**	0.283	0.499
VAL-CHI	**0.000**	0.877	1.000
GRA-GOLF	1.000	0.511	0.696
ROS-GOLF	**0.002**	0.399	0.071
VAL-GOLF	**0.000**	0.053	**0.001**
ROS-GRA	**0.002**	1.000	0.725
VAL-GRA	**0.000**	0.815	0.057
VAL-ROS	0.685	0.896	0.637

Table S3: Mean sediment properties evaluated across six sites in vegetated and bare sediment cores (site labels as in Fig. 1). Data are averages over 4 replicates.

Site	Vegetated	% Silt	Bulk Density ($g \cdot cm^{-3}$)	Organic Matter (%LOI)
BEL	Yes	22.8	1.32	1.9
	No	13.7	1.31	2.7
VAL	Yes	19.8	1.13	3.2
	No	17.9	1.11	2.8
ROS	Yes	41.7	0.92	5.1
	No	20.1	1.15	3.5
CHI	Yes	67.5	1.11	4.4
	No	65.8	1.32	1.5
GRA	Yes	67.0	0.95	6.2
	No	67.7	0.98	5.4
GOLF	Yes	70.2	0.89	6.5
	No	67.5	0.85	7.0

Table S4: Results of one-way ANOVAs testing effect of site on vegetation and nutrient variables (data collected from experimental erosion sediment cores). BGB = *Spartina* spp. below-ground biomass, AGB=above-ground biomass, Root:Shoot=root to shoot ratio (log-transformed), stems =# stems per core (log-transformed), C:N=C:N-ratios of *Spartina* spp. shoots. Statistically significant effects (α=0.05) are shown in bold.

	Source of Variation	SS	Df	MS	F-ratio	P
BGB	Site	2425	5	484.9	2.946	**0.033**
	Error	3951	24	164.6		
AGB	Site	72.26	5	14.45	1.421	0.253
	Error	244.08	24	10.17		
Root:Shoot	Site	6.496	5	1.2992	5.152	**0.002**
	Error	6.052	24	0.2522		
Stems	Site	0.743	5	0.1486	0.854	0.525
	Error	4.175	24	0.1739		
C:N	Site	128.4	5	25.688	2.695	**0.045**
	Error	228.7	24	9.531		

Table S5: Results of Tukey's HSD post-hoc tests, pair-wise comparisons of effect of site on erosion core vegetation variables (BGB=*Spartina* spp. below-ground biomass (g), Root:shoot = root:shoot ratios, C:N = C:N ratios of shoots). Site labels as in Fig. 1. Statistically significant effects (α=0.05) are shown in bold.

	BGB	**Root:Shoot**	**C:N**
Comparison	*p*	*P*	*p*
CHI-BEL	0.347	0.020	0.627
GOLF-BEL	1.000	0.952	0.350
GRA-BEL	0.770	0.399	0.950
ROS-BEL	0.710	1.000	**0.025**
VAL-BEL	0.997	0.608	0.867
GOLF-CHI	0.275	**0.003**	0.997
GRA-CHI	0.977	0.637	0.981
ROS-CHI	**0.021**	**0.022**	0.458
VAL-CHI	0.613	0.425	0.998
GRA-GOLF	0.684	0.090	0.846
ROS-GOLF	0.794	0.943	0.743
VAL-GOLF	0.989	0.176	0.938
ROS-GRA	0.102	0.421	0.152
VAL-GRA	0.951	0.999	1.000
VAL-ROS	0.434	0.632	0.238

CHAPTER 4

Combined nutrient enrichment and heat waves trigger rapid ecosystem shifts in salt marshes and loss of critical services

Spartina spp. grasses (lower left) in a fertilized plot that shifted to *Salicornia veneta* succulents (centre) after a heat wave in 2015 (Grado Lagoon, Italy)

4. Combined nutrient enrichment and heat waves trigger rapid ecosystem shifts in salt marshes and loss of critical services

Abstract

The intensity and frequency of extreme climate events are on the rise, as seen by several rapid successions of heat waves in the past decade. Such extreme events can cause changes in ecosystem structure and functioning, but the combined effects of heat waves and anthropogenic drivers of change on ecosystem services are not well documented. A severe heat wave in 2015 in Europe provided a rare and unique opportunity to observe how nutrient enrichment can trigger changes in coastal ecosystems in response to climactic disturbances, and to measure the functional consequences in terms of ecosystem service provision. In a two-year-long fertilisation experiment conducted in a salt marsh in the Northern Adriatic Sea we observed a rapid shift from *Spartina* spp. perennial grasses to the shallow-rooted annual succulent *Salicornia veneta* following the heat wave in fertilized plots. We also observed a three-fold increase of sediment organic matter and a marked decrease of sediment bulk density across all treatments after the heat wave. We analysed the effects of this vegetation shift on lateral sediment erosion, using wave mesocosms. We found that following the heat wave erosion significantly increased across all treatments compared to the previous year. Vegetated fertilised plots still retained higher erosion control compared to unvegetated ones, due to remnant *Spartina* spp. belowground biomass. Our study demonstrates how climactic disturbances can synergistically interact with local stressors such as nutrient enrichment to trigger rapid community shifts in salt marsh ecosystems, and affect critical ecosystem services.

4.1 Introduction

Salt marsh ecosystems provide coastal defense ecosystem services, including wave attenuation, shoreline stabilization, and floodwater retention. These services are highly valuable particularly as coastal communities currently face increasing storminess, sea level rise, and frequency of storm surges (Duarte et al., 2013). Wave attenuation occurs via vegetation slowing down velocity of waves, tides and currents; shoreline stabilization occurs via elevation through sediment deposition and through sediment reinforcement by the below-ground roots and rhizomes; and floodwater attenuation occurs via the salt marsh storing flood waters and reducing flood peaks (Shepard et al., 2011). Salt marshes also play a key role in carbon sequestration and regulation of nutrient biogeochemical cycles (Brin et al., 2010; Sousa et al., 2017).

While salt marshes and other vegetated coastal ecosystems are important for coastal protection and nutrient regulation, they are also vulnerable to the impacts of climate change and other anthropogenic stressors. The ability of salt marshes to provide ecosystem services in the future will depend on their ecological stability and persistence in the face of global change (Bouma et al., 2014). Multiple stressors have profoundly changed coastal landscapes and are currently driving the loss of salt marshes worldwide (Airoldi and Beck, 2007). In the Northern Adriatic Sea, the combined effects of increased temperature and decreased precipitation are associated with the loss of native *Spartina* spp. grasses and its replacement by *Salicornia* succulents, which have been shown by experimental studies to be more vulnerable to increased inundation from sea level rise (Strain et al., 2017). Extreme events such as heat waves are increasing in intensity and frequency (Russo et al., 2015), and the potential interaction between such extreme events and other anthropogenic drivers of change need further study as they may synergistically impair the functioning of ecosystems such as salt marshes that can help to mitigate the impacts of climate change.

Nutrient enrichment is one example of a drive of change in salt marsh systems (Deegan et al. 2012). In the Adriatic Sea, in the Mediterranean, dense population and intensive agriculture and farming along the coast and the plain of the Po river have locally enhanced nutrients (de Wit and Bendoricchio, 2001; Giani et al., 2012), leading to frequent eutrophic phenomena (Penna et al., 2004). Salt marsh vegetation is typically nitrogen-limited (Valiela and Teal, 1974), particularly in restored or constructed marshes which lack organic matter, which is a limiting factor for nitrogen fixation (Boyer and Zedler, 1998; Langis et al., 1991). Nutrient manipulation studies have shown that above-ground vegetation respond positively to an increase in nutrient input (Boyer and Zedler, 1998). Furthermore, increases in foliar nitrogen content indicate that salt marshes can be effective nutrient sinks (McFarlin et al., 2008; Sousa et al., 2017, 2008), as salt marsh plants adsorb excess nutrients and organic matter as peat or have high rates of denitrification such that the system remains nitrogen-limited (Deegan et al., 2007; Verhoeven et al., 2006). However, while salt marshes can act as a nutrient sink, studies suggest that exceeding a threshold concentration of nutrients can impact salt marsh structure and functioning. Both short- and long-term experiments in *Spartina* spp. marshes report increases in above-ground biomass and parallel decreases in below-ground biomass with enhanced nutrient enrichment (Deegan et al., 2012; Nelson and Zavaleta, 2012; Turner et al., 2009; Valiela et al., 1976). Other changes in vegetation structure include alterations in community composition; for example, nitrogen and phosphourus treatments in species-rich oligohaline marshes favoured dominance by high aboveground producing plants (Crain, 2007). Levine et al. (1998) suggested that under conditions of nutrient limitation, competitive dominance may result from efficient competition for nutrients, modifying patterns of plant zonation and favoring species with less below-ground biomass. Altered competition dynamics as a result of fertilization prompted a shift in species cover with loss of *Spartina alterniflora* and an increase in *Distichlis spicata*, another perennial grass, in addition to a shift from the short form of *S. alterniflora* to taller forms, hence increasing above-ground biomass (Fox et al., 2012).

Enhanced nutrient input has demonstrated effects not only on salt marsh ecosystem structure, but also functioning, with fertilization resulting in loss of elevation and reduced sediment shear strength (the degree to which the sediments can resist shear force or sliding failures), which may lead to decreased resistance to erosion by wind, waves, or storms as nutrient loading increases (Turner, 2011; Turner et al., 2009). Long-term nutrient addition experiments have demonstrated deleterious effects on salt marsh ecosystem functioning, including changes in root to shoot ratio causing a reduction in geomorphic stability and triggering of feedback loops resulting in marsh degradation (Deegan et al., 2012).

Given the increased eutrophication of coastlines globally (Deegan et al., 2012), it is important to understand how this will affect the ability of salt marshes to resist lateral erosion. Previous studies indicate that sediment properties including bulk density, organic matter and grain size are important predictors of erosion resistance in salt marshes (Feagin et al., 2009). Interactive effects of sediment properties and *Spartina* spp. vegetation have also been found to affect erosion resistance (Lo et al. 2017). There have been relatively few studies on the effects of nutrient enrichment on coastal protection ecosystem services by saltmarshes. Studies that have addressed this have largely focused on the response of enrichment on the vertical dynamics of salt marshes, namely their ability to respond to increased inundation from sea level rise (e.g. Nelson and Zavaleta, 2012; Watson et al., 2014; Wong et al., 2015). There is a paucity of research on the horizontal processes affecting salt marshes, i.e. lateral erosion (Fagherazzi et al., 2013; Kirwan et al., 2016).

In 2015, a severe heat wave affected Europe (Ardilouze et al., 2017; Jezequel et al., 2016), and provided a rare and unique opportunity to observe whether nutrient enrichment interacts with climactic disturbances to alter structure and functioning of salt marsh ecosystems, and to measure consequences in terms of ecosystem service provision. Our objectives were thus to investigate the impacts of increased nutrient loadings and conditions associated with the heat wave on lateral erosion resistance of salt marsh ecosystems through a two-year nitrogen enrichment field experiment in the Northern Adriatic Sea (Italy). We focus on *Spartina* species, a C4 marsh grass, as they are pioneering vegetation and establish at the low marsh, and given their wave attenuation and sediment stabilization properties, are widely considered to be on the frontlines of coastal defense in the temperate areas of the world in which they grow. We measured salt marsh sediment erosion in different conditions using wave mesocosms to determine the effect of *Spartina* spp. vegetation (vegetated or bare sediment), nutrient loadings (fertilized or unfertilized), and heat wave conditions on lateral erosion. On the plots containing *Spartina* spp*.,* we measured the effects over two years of nutrient enrichment on vegetation biomass, coverage and density. We hypothesized that: a) nutrient enrichment enhances above-ground vegetation variables but decreases below-ground biomass and shoot:root ratios; b) *Spartina* spp. vegetation enhances erosion resistance compared to bare sediment; and c) the effects of nutrient enrichment on erosion would be greater over time (i.e. in the second year of the experiment) and would interact with the heat wave. We also expected that, given the higher temperatures induced by the heat wave in the second year, we would expect changes to erosion resistance by alteration of vegetation or sediment properties.

4.2 Materials and Methods

Study area

Our study area is located in the tidal channel fringing marshes near Primero Inlet in the easternmost portion of Grado Lagoon, Italy (45°42'12''N, 13°26'33''E), on the coast of the northern Adriatic Sea, in the province of Friuli Venezia Giulia (Figure 1). The site is located within the larger Marano and Grado lagoonal complex, which connects to the Adriatic Sea through several inlets that cross through its barrier islands. Tides are semi-diurnal with a mean range of 0.65 m and spring and neap tides of 1.05 and 0.22 m (Fontolan et al. 2012). The lagoon has had a history of reclamation since the 1900s resulting in extensive loss of wetlands, construction of levees to prevent flooding, dredging of shipping canals and marinas, and extensive fish farming carried out on embanked areas of the lagoon enclosed by sluice gates (Fontolan et al., 2012).

As in other parts of the Italian northern Adriatic coastline, Grado Lagoon has a negative sediment budget and is actively eroding (Torresan et al., 2012). Projections and vulnerability assessments show that the Grado and Marano lagoons, along with Venice lagoon, are particularly vulnerable to sea level rise even without considering the high subsidence rates from fluid extraction from the near surface and sediment compaction (Lambeck et al., 2011; Torresan et al., 2012). About 16% of the marshes have been lost between 1954 and 2006 due to drowning from subsidence, compaction and eustatism (change in sea level from expanded volume of sea); edge retreat by wind-induced waves, erosion by vessel-generated waves, and coastal dynamics and inlet migration (Fontolan et al., 2012). Despite significant anthropogenic influences, the remaining marshes in the Grado-Marano system have considerable conservation value for the region (Baucon and Felletti, 2013).

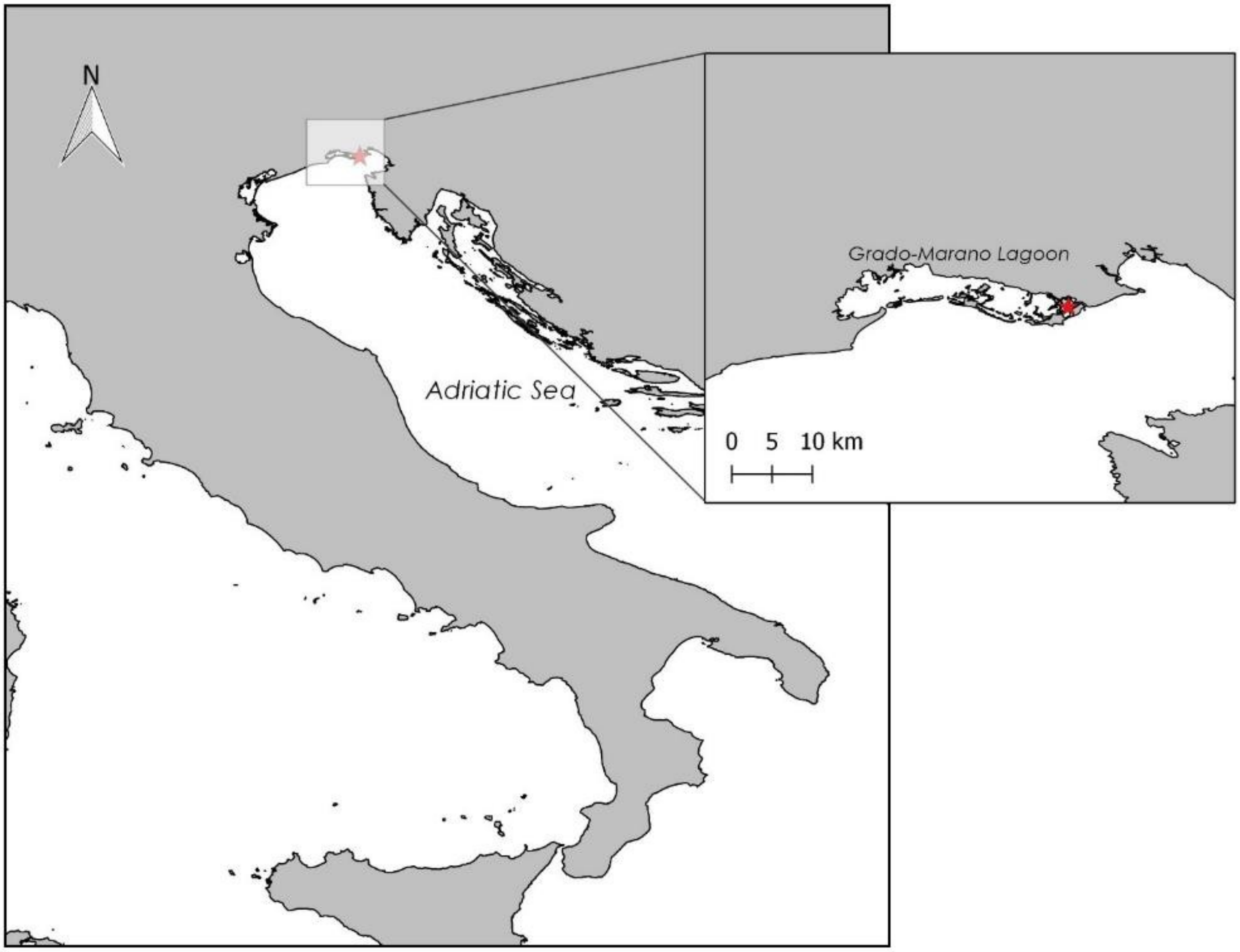

Figure 1: Location of study site (marked with a star) in Grado Lagoon, Italy

The severe heat wave in 2015 affected much of Western and Central Europe, resulting in record-breaking temperatures (Jezequel et al., 2016). In the Western Mediterranean region, the warmest July temperatures were recorded since the beginning of measurements in the region, with monthly anomalies up to 4°C in Spain, France and Italy (Jezequel et al., 2016), and the heat wave duration was particularly long in Northern Italy and Switzerland (Russo et al., 2015). This heat wave was accompanied by higher salinity and surface water temperatures recorded by provincial monitoring authorities near our sampling site, with particularly higher mean salinity and temperature values in July and August 2015 (Figure 2). Data were not available for within the lagoon, where probably higher temperatures and salinity would have been observed.

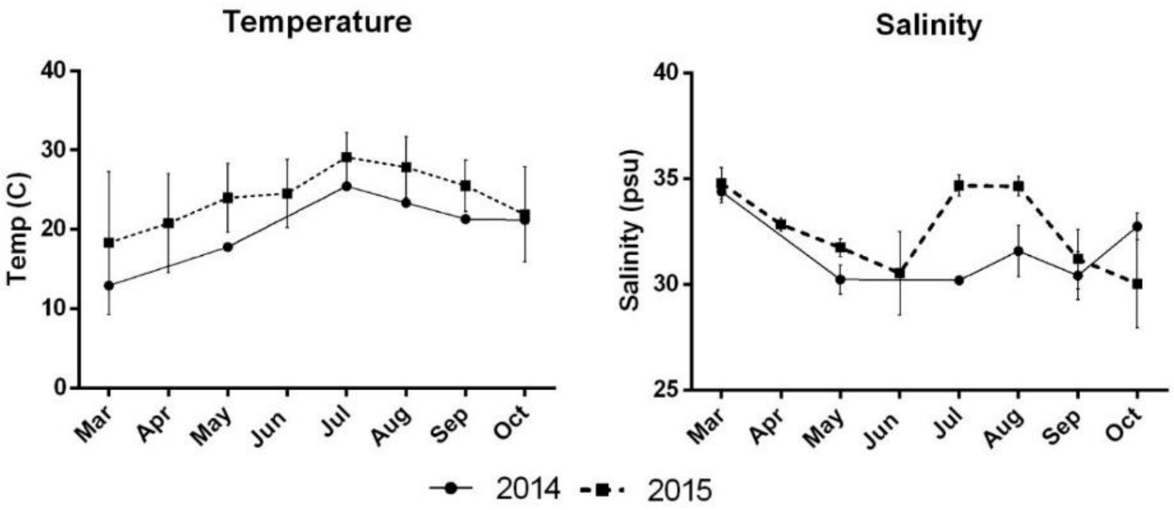

Figure 2: Mean spring/summer temperature and salinity in 2014 and 2015 measured in the three nearest monitoring stations to the sampling area in Grado Lagoon (data obtained from ARPA FVG). Error bars denote standard error of the mean. Data are means over three replicates, except for 2014 July values for temperature and salinity, which had only one replicate. In 2014 April and June, no values for temperature or salinity were available.

This section of the northern Adriatic coast had previously been dominated by *Spartina maritima* at the low marsh (Géhu, J.M. et al., 1984). However, a recent broad-scale genetic study, conducted while this experiment was already in progress, showed that *S. martima* in the region has largely been replaced by *S. anglica* and *S. townsendii* (Wong et al submitted). Genetic and chromosomal analyses indicate that these species can co-occur in mixed patches (Wong 2015), where they can be difficult to distinguish morphologically until the flowering period; therefore we will refer to them as *Spartina* spp. In the microtidal regime of Grado Lagoon, *Spartina* spp. occurred patches interspersed with bare sediment, with no clear zonation pattern.

Nutrient manipulation

We investigated how vegetation (*Spartina* spp. or bare) and nutrient enrichment affected erosion of sediments using a two-way fully factorial design. Five replicates of each of four treatments were randomly assigned to vegetated (with *Spartina* spp.) and bare patches of salt marsh, in plots of 0.25 m^2 placed at least 2 m apart: (1) vegetated, unfertilized; (2) vegetated, fertilized; (3) bare, unfertilized; and (4) bare, fertilized. All plots were marked with a small PVC pipe to identify different treatments. For the fertilized treatments, we applied 200 g of Scotts Standard inorganic slow release NPK fertilizer (15% nitrogen by weight). An additional five replicates of each treatment (20 plots in total) were demarcated for a follow-up experiment, with treatments continuing into the subsequent year. Fertilisation treatments began in June 2014. Porewater nutrients collected from the fertilized plots one month after fertilisation were significantly higher as compared to the unfertilized plots, demonstrating the effectiveness of the fertilisation treatment.

Vegetation monitoring

On the plots containing *Spartina* spp. vegetation, we determined % *Spartina* spp. cover beginning in June 2014, when the experiment was initiated, until October 2015. Given that *Salicornia veneta*, an annual C3 halophytic succulent, began to displace the *Spartina* spp. vegetation in the second year of the experiment, we also quantified % cover of *S. veneta* in the same manner as *Spartina* spp.[3]

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[3] Previous studies and surveys have recorded *Salicornia veneta* in Grado and Marano lagoons (Antisari et al., 2017; Cosolo et al., 2015; Minelli, 2009; Šajna et al., 2013). While there is some discussion about whether *S. veneta* should have a status as a separate species or be classified under the *Salicornia procumbens* subsp. *procumbens* (Antisari et al., 2017), until this is resolved we refer to the *Salicornia* species in our experiment as *Salicornia veneta*.

Spartina spp. above and below-ground biomass were also evaluated on vegetated sediment cores after wave treatment for each year of the experiment (see below). Above-ground biomass was clipped off the sediment cores, dried at 60^{0}C until constant weight, and weighed. The remaining sediment and the below-ground biomass were rinsed carefully over a 1 mm mesh, and the below-ground biomass was dried and weighed as above. Root:shoot ratios were calculated by dividing root biomass by shoot biomass.

Abiotic variables – C:N ratios, porewater nutrients and sediment variables

C:N ratios were used as a proxy for nutrient levels in the salt marsh samples, as C:N-ratios in *Spartina* spp. leaf tissues have been shown in previous studies to reflect ambient nutrient concentrations in porewater (Tobias et al., 2014), experimental nutrient enrichment treatments (Covin and Zedler, 1988; Crain, 2007; Jiménez et al., 2011), and indicators of nutrient uptake (Sousa et al., 2008). From each of the dried above-ground biomass samples originating from the sample cores subjected to erosion, a sub-sample was crushed with a mortar and pestle and approximately 10 – 15 mg from each sub-sample was extracted and analyzed for total organic C and N with an elemental analyzer (Carlo Erba N1500).

In October of each year, when sediment cores were taken from the experiment plots for wave treatment (described below), sediment porewater was sampled from each plot using rhizon samplers (Rhizosphere Research Products), and analyzed for nutrients. After extraction of porewater, samples were placed in sterile polyethylene containers (no further filtration was needed as rhizon pore size is 0.1 μm) and placed on ice, where they were stored at -18°C until analysis. Due to sampling constraints, porewater was only extracted from the vegetated plots in the first year, and in the second year porewater samples were taken from all plots (across all treatments). Nitrate, nitrite, orthophosphate (reactive phosphorous), ammonium, and orthosilicate (reactive silicate) concentrations were analyzed with an automated wet chemistry analyzer (Skalar San^{++}). In samples below limits of detection (2 $\mu g \cdot L^{-1}$ for nitrites and nitrates, and 5 $\mu g \cdot L^{-1}$ for combined nitrite and nitrates), total concentrations were assigned a value of half the limit of detection divided by the square root of 2 (as in Elias and Bernot, 2017).

Sediment samples were extracted from each experimental plot (2.5 cm in diameter and 8 cm long). The sediment samples were stored on ice during transport. In the laboratory, sub-samples of a known volume were dried at 60^{0}C until constant weight to determine bulk density ($g \cdot cm^{-3}$). Grain size properties were determined with a Malvern Laser Particle Sizer, including total per cent silt, mud and median grain size (silt=particle size >3.9 and <62.5 μm and mud=particle size <62.5 μm according to the Wentworth classification (Wentworth, 1922). A subsample was used to determine organic matter content with the loss-on-ignition technique (Craft et al., 1991), with the samples heated to 450 °C for six hours in a muffle furnace.

Wave treatment and quantification of erosion

Sediment cores for the quantification of lateral erosion were extracted with PVC cylinders in early October 2014, at the end of the *Spartina* spp. growing season, for the first year, and October 2015 in the second year. Sediment cores were carefully packed and transported to wave mesocosm facilities at the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke, Netherlands, where they were placed in filtered seawater (salinity ~ 30 ppt) from the Oosterschelde. Sediment cores were subjected to waves over the course of 24 hours in the wave mesocosm, following methods described in Lo *et al.* 2017. The wave mesocosm could subject three samples at a time to wave action. At least one replicate of a treatment (vegetated *vs.* bare) was exposed to waves within every two wave mesocosm cycles, in order to spread the potential variability from different storage times across the tested factors. With 5 replicate cores per treatment (for a total of 20 cores), the experiment required seven wave mesocosm cycles over a period of 9 days.

Eroded volume loss was estimated at each of 6 time steps over the 24-hour period, which had been determined to be a sufficient period of time to achieve a slow- to constant erosion rate in silty sediments (Lo *et al.* 2017). The time steps corresponded to the following elapsed time periods in hours: t_0=beginning of wave treatment, t_1=0.25, t_2=1, t_3=4, t_4=8, t_5=16, and t_6=24. Photogrammetry methods were applied to estimate erosion of the saltmarsh sediments (following methods described in Lo *et al.* 2017 and van Belzen *et al.* in prep).

Data & Statistical Analyses

Effect of fertilization on vegetation, sediment and nutrient variables and erosion

To obtain maximum possible volume loss over time, we fitted an exponential model to the experimental data of volume loss, in the form of $E=Q(1-e^{-kt})$, where E=volume eroded, Q=maximum volume loss, k=rate of volume loss, and t=time in hours, as in Lo *et al.* 2017. Values of Q corresponded closely to our values of erosion obtained at the end of wave treatment (Pearson's correlation=0.98 for both 2014 and 2015).

One-way ANOVAs were used to determine the effect of fertilization on vegetation variables (above- and below-ground biomass, root:shoot ratios) evaluated on the vegetated sediment cores that were exposed to wave treatment in the wave mesocosms. Two-way ANOVAs, with the factors of nutrient (fertilized, unfertilized) and vegetation (*Spartina* spp. or bare sediment) were also used to determine differences between treatments for sediment properties (% silt, bulk density and organic matter). Normality and homogeneity of the data were visually checked in the residual plots, and, when necessary, data were transformed to comply with assumptions of homogeneity of variance.

We constructed a linear mixed effects model to investigate the effects of the vegetation, sediment and nutrient predictor variables, combining the samples from both years of the experiment, and specifying year as a random effect. Given the large number of explanatory variables relative to the sample size, collinearity between variables was tested using Pearson's correlation coefficient, and highly collinear variables were removed. The model was parameterized in a step-wise approach using this reduced set of predictor variables initially. This was followed by one by-one elimination of non-significant variables ($p > 0.05$) starting with the predictor of least significance (highest *p*-value) first. After elimination of a single non-significant predictor variable, the model was re-parameterized and the process repeated until all predictor variables were significant at the 5% level.

ANOVAs and step-wise modeling were performed in the R environment (R Core Team, 2017), exponential models were constructed with the *nlme* package within R (Pinheiro et al., 2017), and the mixed-effects modeling was performed with the *lme4* package (Bates et al., 2014).

4.3 Results

Changes to abiotic environmental variables

Porewater nutrient concentrations obtained from the plots indicated that fertilization treatments were effective and significantly elevated nutrient concentrations (Figures S1, S2, Table S1). The effectiveness of fertilization was further confirmed with mean C:N ratios measurements in above-ground biomass, which were significantly lower in fertilized plots (Figure S3).

Sediment properties (% silt, bulk density and organic matter) did not differ significantly across treatments in either year ($p > 0.05$ in all cases). However, bulk density decreased approximately three-fold after the heat wave in the second year, with treatment means ranging from 0.49 - 0.59 g/cm^3 after the heat wave compared to 1.60 – 1.90 g/cm^3 before the heat wave.

These changes to bulk density paralleled a three-fold increase in organic matter, ranging from 13.49 – 15.99 % after the heat wave compared to 4.38 – 5.72% before the heat wave (Figure 3). Bulk density and organic matter values before the heat wave were comparable to values found in Lo et al. (2016).

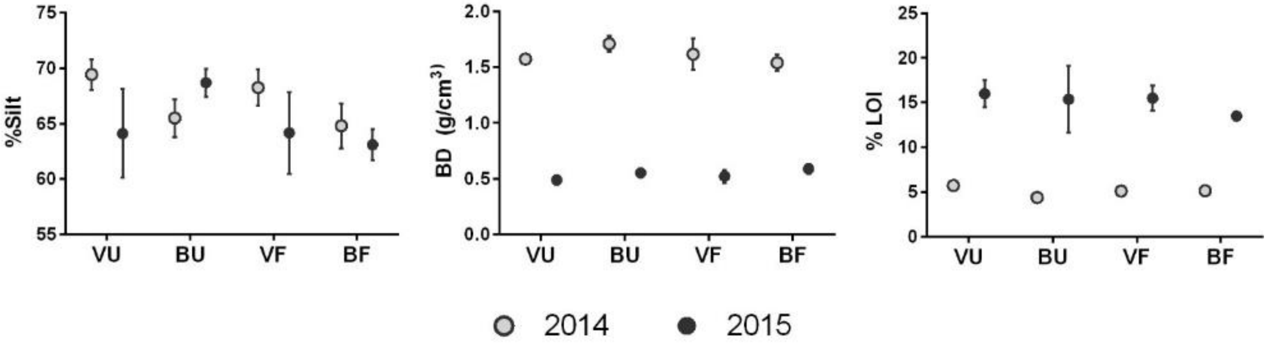

Figure 3: Mean sediment variables on sediment core samples exposed to wave treatment: % Silt, BD (bulk density (g/cm^3)) and organic matter (% Loss on Ignition (LOI)), across the four treatments. Data are means over 5 replicates, error bars denote standard error of the mean.

Rapid shift from *Spartina* spp. grasses to *Salicornia veneta* succulents

In the second year of the experiment (2015), following the heat wave, we observed that all of the *Spartina*-vegetated, fertilized plots had shifted from *Spartina* spp. to *S. veneta* cover, in some cases with *Spartina* spp. being entirely replaced in the plot by *S. veneta* (Figure 4). *S. veneta* also heavily colonized some of the bare fertilized plots (Figure 4). *S. veneta* was not observed in the vegetated, unfertilized plots. *S. veneta* % cover differed between treatments ($F_{(3, 16)}$=5.81, p=0.007) (Figure 4), with the highest % cover in vegetated, fertilized plots compared to vegetated, unfertilized plots (Tukey's HSD post-hoc test, p<0.001) and compared to bare, unfertilized plots (Tukey's HSD post-hoc test, p<0.05).

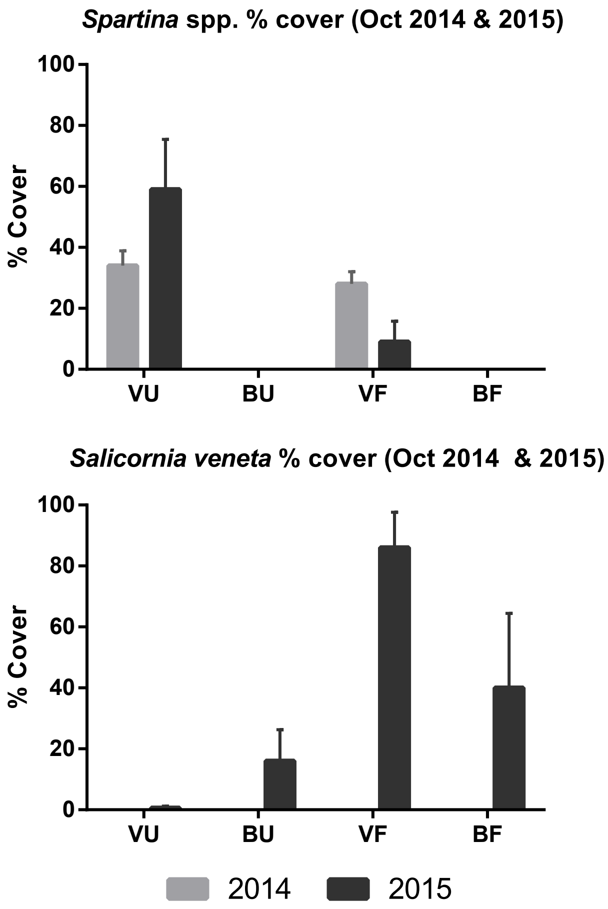

Figure 4: % Cover of *Spartina* spp. and *Salicornia veneta* measured in October 2014 and 2015 (at the conclusion of the experiment). Data are means over 5 replicates + 1 standard error. Grey bars=measurements taken in 2014, and dark grey bars=measurements taken in 2015. VU=vegetated, unfertilized; BU=bare, unfertilized, VF=vegetated, fertilized, BF=bare, fertilized.

For the *Spartina* spp. vegetation parameters measured in the sediment cores that were exposed to wave treatment, there were no significant differences between fertilized and unfertilized treatments in above-ground biomass, belowground biomass and root:shoot ratio for the first year of the experiment (p>0.05 in all cases) (Figure S4). Root:shoot ratios were thus marginally higher in the fertilized treatments compared (One-way ANOVA, $F_{(1,8)}$=3.64, p=0.09) (Table S2)). Overall, there was significantly lower *Spartina* density in the fertilized plots compared to non-fertilized plots after the heat wave due to the vegetation shift to *Salicornia* spp. ($F_{(1,8)}$ =60.2, p<0.0001) (Figure S4), and lower mean below-ground biomass in the second year of fertilization (mean 14.46 ±2.26 *SE* (g))compared to the first year (mean 28.53 ±3.16 *SE* (g)) (Figure S4, Table S2).

Erosion

Values of erosion in the first year of the experiment ranged from 18 – 23% erosion (Figure 5). In the second year of fertilization, after the heat wave, mean erosion increased across all treatments, ranging from 27 to 94% erosion. The largest increase in mean erosion was in the bare, fertilized plots (Figure 5).

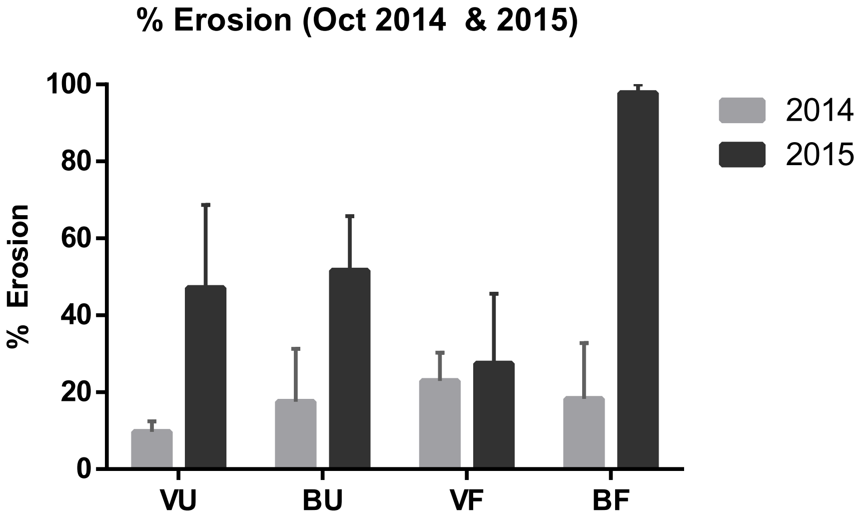

Figure 5: Modelled final % erosion values at 24 hours from sediment cores in Oct 2014 and Oct 2015. Data are means over 5 replicates + 1 Standard Error. VU=vegetated, unfertilized; BU=bare, unfertilized, VF=vegetated, fertilized, BF=bare, fertilized.

In the linear mixed effects model considering samples from both years combined (with year considered as a random effect), we considered a set of vegetation, sediment and nutrient explanatory variables after Pearson correlation analysis to remove significantly collinear variables. We considered for vegetation variables: *Spartin*a spp. below-ground biomass and % *S. veneta* cover (*Spartina* spp.cover was correlated to S. veneta cover); for sediment variables: bulk density (which was highly correlated to organic matter) and % silt; for nutrient levels: fertilization as a categorical variable (unfertilized, 1 year of fertilization, and 2 years of fertilization). In the final model, below-ground biomass, bulk density, and their interaction were the strongest predictors of erosion (Figure 6). Erosion decreased with increasing below-ground biomass ($\chi^2(1)=3.70$, $p=0.06$), and was also negatively correlated with bulk density ($\chi^2(1)=11.88$, $p=0.0015$. The effect of below-ground biomass on decreasing erosion was stronger in sediments with higher bulk density ($\chi^2(1)=9.86$, $p=0.003$) (Table S3).

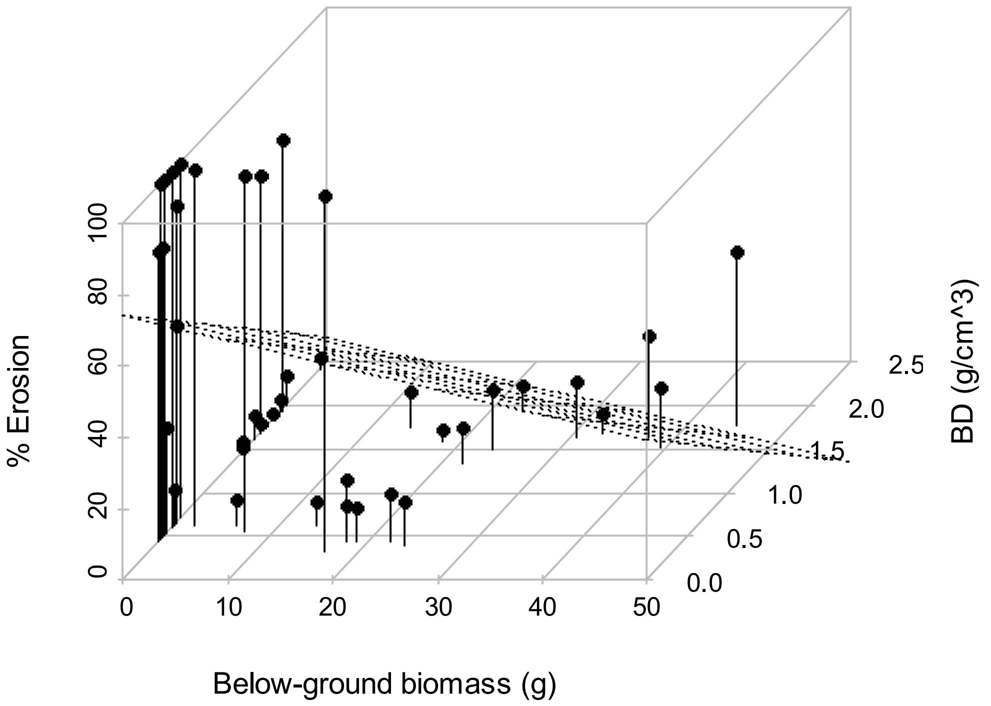

Figure 6: Relationship between % erosion, below-ground biomass, and bulk density (BD) on sample sediment cores from both years of the fertilization experiment (n=40). The 3-D plane represents the linear model % Erosion=74.5 – 0.7(below-ground biomass) -26.9(bulk density) ($F_{(2,37)}=7.28$, $p<0.001$), $R^2=0.28$

4.4 Discussion

Our two-year experiment offered us the opportunity to document the rapid shift from *Spartina* spp. perennial grasses to shallow-rooted annual succulents *Salicornia* veneta in the North

Adriatic saltmarshes following the 2015 heat wave, and to highlight that the shift was triggered by the combined effects of fertilization. Displacement of *Spartina* spp. by *Salicornia veneta* has also been recorded in the past decade in other marshes along the Adriatic sea, such as in Venice Lagoon (Belluco et al., 2006) and in Bellocchio (Comacchio) (Merloni et al. 2007), with a particularly rapid shift occurring in 2003, concurrent with a heat wave and drought (Strain et al., 2017). Experimental studies suggest that this rapid shift was triggered by interactive climatic stressors including increased temperatures and decreased rainfall associated with drought conditions, which *S. veneta* was demonstrated to be more tolerant of (Strain et al. 2017). The 2015 heat wave resulted in the second hottest summer in Europe since the 2003 heat wave (Jezequel et al., 2016), concurrent with increases in surface water temperature and salinity in our sampling area (Figure S3). The shift from *Spartina* spp. to *S. veneta* was induced by interactive climate stressors, as observed by Strain et al. (2017) in the case of Bellocchio. Our study documents the same vegetation shift in a different lagoon of the Northern Adriatic Sea, but as a result of climate stressors interacting with high nutrient loads. This enhanced the competitive advantage of *S. veneta* by alleviating nutrient limitation under potentially unfavourable environmental conditions (high temperatures and salinity caused by the heat wave) for *Spartina* spp. growth. Nutrient availability is thought to mediate salt marsh community structure by enhancing competitiveness of stress-tolerant species (Pennings et al., 2002). *Salicornia* spp. has been demonstrated to be a superior competitor for nitrogen with experimental fertilization (Covin and Zedler, 1988) and can tolerate higher soil salinity compared to *Spartina* spp., (Mahall and Park, 1976). Similarly, *Sarcocornia pacifica*, another C3 succulent closely related to *Salicornia* spp., outcompeted the marsh grass *D. spicata* in both fertilized and non-fertilized treatments, and it was suggested than an overall decrease in *D. spicata* cover was due to high ambient soil salinities as the experiment was conducted over two years of drought conditions (Ryan and Boyer, 2012), consistent with general observations made in a North American marsh (San Francisco Bay) that low marsh species are being replaced by halophytic species (Watson and Byrne, 2009). An additionally competitive advantage of *Salicornia* spp. is conferred by larger production of tidally dispersed speeds, which enables it to colonize disturbed patches, particularly where there is mortality of *Spartina* spp. (Dipper and Decd, 1998). The fine-haired seeds of *Salicornia* spp. aid in flotation and attachment to wrack (unlike *Spartina* spp. seeds which lack these hairs), facilitating their transport to other locations (Ellison, 1987), which may explain how *S. veneta* was able to colonize both the bare and *Spartina* spp. vegetated patches. Indeed, throughout the experiment, areas of wrack deposit were observed covering bare and *Spartina* spp. patches.

The 2015 heat wave in Italy induced a dramatic change in sediment properties across treatments, with a three-fold decrease in bulk density concurrent with an increase in organic matter. The overall increase in sediment organic matter in the second year could be due to enhanced microbial decomposition of detrital matter in the sediment, potentially from nutrient-induced enhanced algal growth, which have been demonstrated to proliferate in other fertilization experiments (e.g. Wasson et al. 2017), and which we observed in our experimental plots as well. The higher organic matter observed across treatments lends support to Deegan et al.'s (2012) observations than enhanced organic matter as a result of nutrient-induced increased decomposition can reduce the critical shear stress threshold of marsh sediments. Similarly, algal mats in salt marshes have been found to organic carbon content and water content which destabilized sediments (Defew et al., 2002). Bulk density and water content have been found to affect erosion in cohesive marsh sediments (such as in Grado Lagoon), with denser sediments decreasing erosion and higher water content enhancing erodibility (Winterwerp and van Kesteren, 2004). In tidal mudflats, higher bulk density as a result of evaporation during tidal emergence has been found to increase the sediment critical erosion threshold and thereby decrease erodibility (Fagherazzi et al., 2017).

An overall increase in erosion in the second year may also be related to a uniform decrease in silicate levels observed across all treatments after the second year of fertilization, possibly facilitated by the heat wave. These lower silicate levels may have limited the growth of biofilm-producing microalgae such as diatoms, which take up silicate for shell formation. The production of biofilms aids in sediment cohesion by increasing the critical erosion threshold in tidal flat sediments (Tolhurst et al., 2006). Eutrophication by excess phosphorous and nitrogen has been linked to decreases in silicate concentrations, altering the balance of nutrient ratios and limiting diatom growth, potentially in favour of non-siliceous organisms (Danielsson et al., 2008; Ferreira et al., 2011). While such effects of biofilms on sediment cohesivity are probably limited to the surface due to the nature of diatom migration in sediments, the reduction of surficial biofilms from benthic macroinvertebrate grazing pressure on diatoms has been found to enhance erosion rates overall in estuarine cohesive sediments (Kristensen et al., 2013).

As the decrease in bulk density and porewater silicates occurred across all plots regardless of whether they were vegetated, bare, or fertilized, we can conclude that the effects of the heat wave induced overall changes to the system that altered the critical shear stress of sediments as a result of enhanced microbial decomposition of organic matter, with a potential role of the reduction of biofilm-producing microphytobenthos such as diatoms. Bare, fertilized plots had the highest amount of erosion among all treatments, suggesting a possible interaction between fertilization and vegetation properties, as bare unfertilized plots, which also had no below-ground biomass, had erosion levels similar to the vegetated plots.

We expected that increasing *Salicornia veneta* cover would enhance erosion as *Salicornia* has a shallow root system (Waisel, 2012) compared to the extensive root and rhizome system of *Spartina* spp., which has been demonstrated to enhance resistance to erosion (Lo et al. 2017). However, interestingly the system remained resistant to erosion, probably as the below-ground

Spartina spp. biomass persisted until the end of this study also in the plots where the vegetation shift occurred. This persisting *Spartina spp.* below-ground structure promoted erosion resistance presumably through sediment binding or shielding sediments from wave action. In our system we observed that the shifts from *Spartina* spp. to *Salicornia veneta* can be rather persistent and took several years to recover from the 2003 shift that resulted from the heat wave. We speculate that over a longer time period, the remnant *Spartina* spp. below-ground biomass in the sediments would decompose, possibly leading to higher levels of erosion. Below-ground biomass has been found to significantly reduce erosion in our overall model of vegetated samples in this study, and similar results were obtained in a previous study comparing erosion across several lagoons of the Northern Adriatic Sea (Lo et al. 2017).

With increased sea level rise projected for the northern Adriatic coastline of Italy (Lambeck et al., 2011), a vegetation shift in salt marshes from *Spartina* spp. to *S. veneta* will have further negative implications for coastal protection ecosystem services. *S. veneta* was demonstrated in another lagoon of the Northern Adriatic sea (Bellocchio) to be more susceptible to drowning by sea level rise compared to *Spartina* spp. (Strain et al., 2017). In Bellocchio, sediments are coarser grained (approximately 23 % silt) compared to lagoons further north, including Venice Lagoon (approximately 68 % silt in Chioggia) (Lo et al. 2017), and the Grado-Marano lagoonal complex (60 - 70% silt) (Lo et al. 2017 and present paper (Figure 3b)). Sandier, less cohesive sediments have been shown to be more prone to lateral erosion in the absence of *Spartina* spp. below-ground biomass (Lo et al. 2017), thus the replacement of *Spartina* spp. by shallow-rooted *S. veneta* is especially problematic in sandy soils.

4.5 Conclusions

Our experimental study is among the first to empirically demonstrate how extreme climate events such as heat waves can interact with human pressures to alter not only structural traits of ecosystems such as species density and/or community composition, but also the functioning of ecosystems. Our study documented a rapid shift in salt marsh vegetation composition after a severe heat wave, from *Spartina* spp. C4 grasses to *Salicornia veneta* C3 succulents. We thus quantify a synergistic interaction between climactic disturbance (a heat wave in this case) and other anthropogenic drivers of change (nutrient enrichment), as this shift in vegetation only occurred in experimentally fertilized plots following the heat wave. Heat wave conditions also prompted an overall decrease in bulk density and increase in sediment organic matter, which by decreasing the critical erosion threshold of sediments, contributed to overall higher erosion in the second year of the study compared to the first year of the study. The presence of vegetation, specifically the remnant below-ground *Spartina* spp. biomass, significantly reduced erosion in fertilized plots. This confirms a previous erosion study of several sites across the Adriatic coastline (Lo et al.2017), providing strong evidence that below-ground biomass is an important factor in reducing lateral erosion.

Salt marsh ecosystems have been suffering degradation or losses for decades from multiple anthropogenic pressures such as land use change, and these losses are occurring more rapidly with the onset of climate-related extreme events. Intense development along coastlines around the world are preventing landward migration of salt marshes in response to coastal erosion, subsidence and sea level rise. An integrated approach to management and conservation of coastal vegetated areas is thus recommended. Such approaches should include enhanced conservation, monitoring, and management of nutrient pollution to ensure thresholds are not passed, especially in areas that are already highly eutrophic. As regional climate projections predict enhanced probabilities of heat waves in Europe (Russo et al., 2015), such multi-pronged approaches to management will be crucial to ensuring the persistence of salt marsh functioning and the regulating ecosystem services they provide, including erosion resistance and nutrient cycling.

Acknowledgements

This work was funded through a MARES Grant (www.mares-eu.org) and project TETRIS (PRIN 2010-2011, MIUR). MARES is a Joint Doctorate programme selected under Erasmus Mundus and coordinated by Ghent University (FPA 2011-0016). CVC acknowledges a postdoctoral fellowship provided by the Flemish Fund for Scientific Research (FWO-1.2.380.11.N.00). The authors are indebted to Elena Piccioni and Lorenzo Gadoni from the Airoldi Lab (University of Bologna) for their extensive field sampling assistance. We thank Angelina Smilinova and Heng Wang of the Department of Estuarine and Delta Systems (NIOZ) for assistance with photographs; Ignacio Barbeito Sanchez for sampling and assistance with wave mesocosms and photographs; and Bart Beuselinck from the Research Group Marine Biology (UGent) for nutrient and grain size analysis. We are also grateful to Ferruccio Polo from the Cooperativa Pescatori di Grado for generously granting continued access to the sampling site and use of his boat to transport samples.

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4.7 Supplementary Information

Table S1: Results of one-way ANOVAs testing effect of fertilization (2 levels: fertilized, unfertilized) in porewater. NH_4=porewater ammonium concentrations ($\mu g \cdot L^{-1}$) log-transformed. Statistically significant effects (α=0.05) are shown in bold.

	Source of Variation	SS	Df	MS	F-ratio	P
2014 NH_4	Fertilization	18.48	1	18.48	28.97	**0.0007**
	Error	5.1	8	0.64		
2015 NH_4	Fertilization	6.56	3	2.188	3.53	**0.039**
	Error	9.91	16	0.620		

Table S2: Results of one-way ANOVAs testing effect of fertilization (2 levels: fertilized, unfertilized) in vegetated plots. AGB=above-ground biomass, Root:shoot = root:shoot ratio of *Spartina* spp. from erosion cores, CN = CN ratio of Spartina spp. shoots from erosion cores. Statistically significant effects (α=0.05) are shown in bold, marginally significant effects are shown in italics.

	Source of Variation	SS	Df	MS	F-ratio	P
AGB 2015	Fertilization	25.61	1	25.61	9.321	**0.016**
	Error	21.98	8			
Root:shoot 2015	Fertilization	57.7	1	57.70	3.64	*0.093*
	Error	127.0	8	15.87		

Table S3: Best linear model of erosion in year 1 (2014) and year 2 (2015) of the experiment, vegetated cores (n=20) and non-vegetated cores (n=20). BGB=Spartina below-ground biomass (log-transformed), BD=bulk density (log-transformed), a_x = coefficients, DF = degrees of freedom (residuals)

Model	**a_0**	**a_1**	**a_2**	**a_3**	**DF**
$a_0 + (a_1)BGB + (a_2)BD + (a_3)BGBxBD$	99.94	-3.88	-45.89	2.25	34

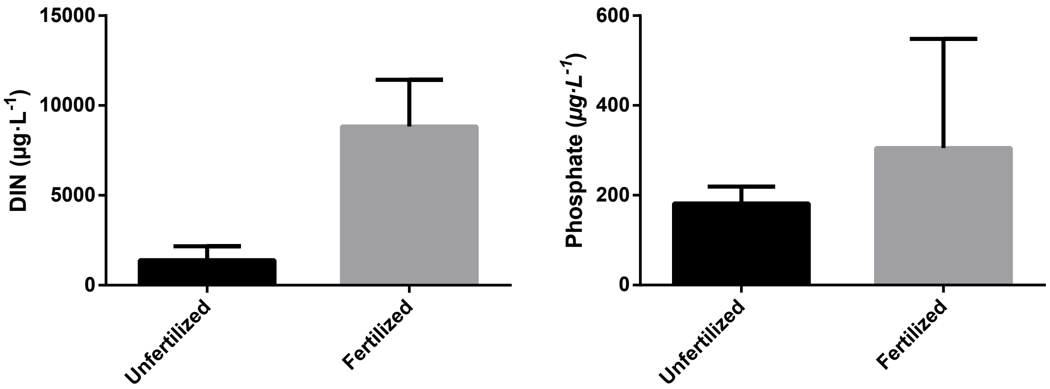

Figure S1: Porewater nutrient concentrations in unfertilized and fertilized plots after one month of treatment (June 2014). DIN=dissolved inorganic nitrogen, n=2 for unfertilized treatments and n=3 for fertilized treatments.

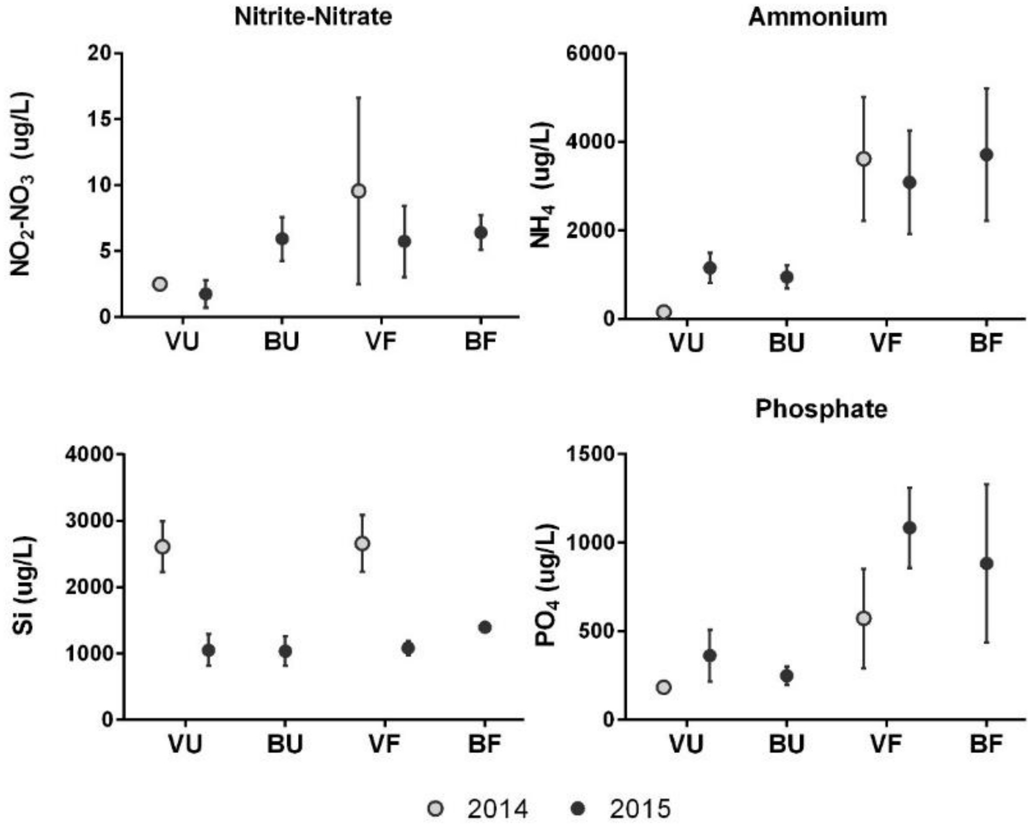

Figure S2: Porewater nutrient concentrations ($\mu g \cdot L^{-1}$) sampled from vegetated plots in year 1 (2014) and both vegetated and bare plots in year 2 (2015). Data shown are means over five replicates (with the exception of Si, where n=4 for VF treatment). Error bars denote standard error of the mean. VU=vegetated, unfertilized; BU=bare, unfertilized, VF=vegetated, fertilized, BF=bare, fertilized.

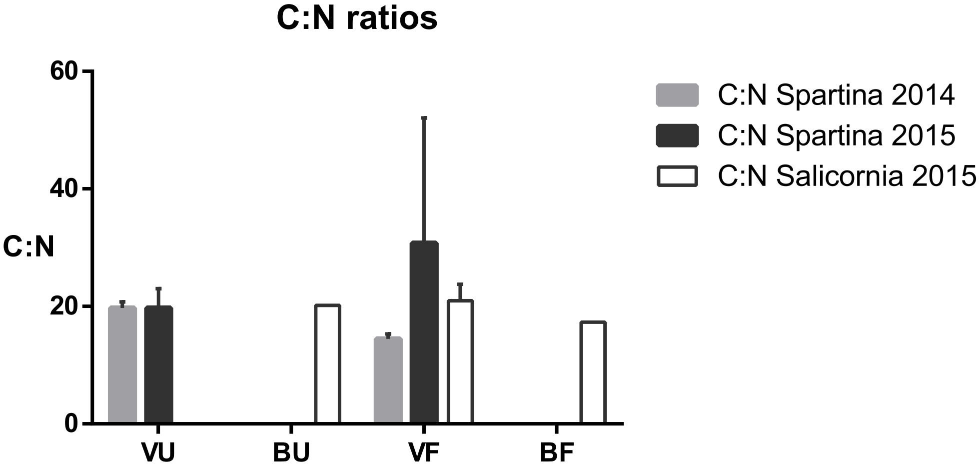

Figure S3: Mean C:N values in *Spartina* spp. above-ground biomass in 2014 and 2015, and C:N values in *Salicornia veneta* in 2015. For *Spartina* spp. above-ground biomass, *n*=5, with the exception of fertilized plots in 2015 in which only two replicates could be obtained due to loss of *Spartina* spp. from two plots. In 2015 *S. veneta* values, *n*=5 for vegetated, fertilized plots and *n*=1 for bare, unfertilized and bare, fertilized plots.

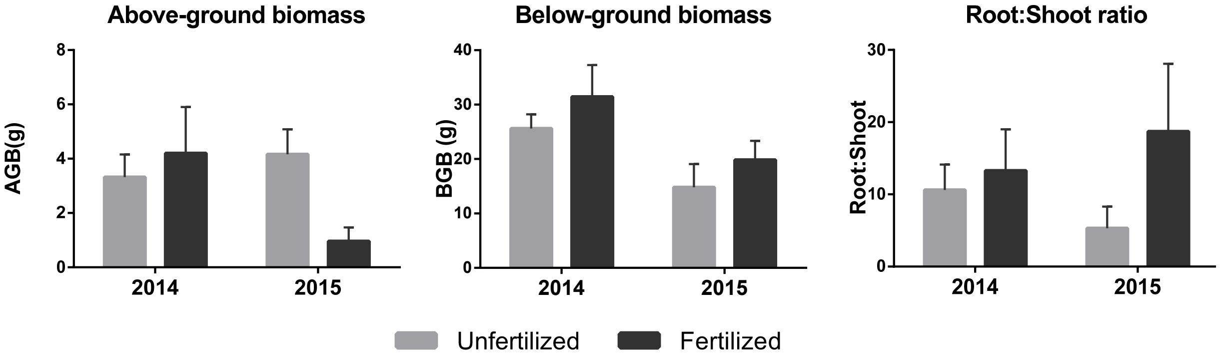

Figure S4: Mean vegetation variables on sediment core samples exposed to wave treatment: above-ground biomass (AGB) (g), below-ground biomass (BGB) (g), and root:shoot ratio, in fertilized and unfertilized plots, shown for the first and second years of the experiment (2014 and 2015, respectively). Data are means over 5 replicates, with the exception of Root:Shoot ratios in fertilized sediment cores in 2015 where n=3 as absence of Spartina spp. above-ground biomass prevented measurements of root:shoot ratio for 2 sediment cores. Error bars denote standard error of the mean.

CHAPTER 5

Effects of inundation on resistance to erosion in salt marshes

Spartina spp. patches in Bellocchio Lagoon (transplantation experiment conducted in the upper right section depicted in the photo)

5. Effects of inundation on resistance to erosion in salt marshes

Abstract

We determined how erosion resistance of salt marshes is affected by relative sea level rise (SLR) by experimentally transplanting *Spartina* spp. salt marsh sediment cores along a gradient of inundation, a proxy for relative SLR. After four months of inundation treatment, the sediment cores were extracted and subjected to wave treatment in wave mesocosms. Results demonstrate that sea-level rise induced mortality decreased above- and below-ground biomass over time, with complete shoot mortality in the sediment cores exposed to medium and high inundation levels. Mean sediment properties (bulk density, organic matter, per cent silt) did not change across different treatments. Biomechanical properties measured at the end of the experiment (root tensile strength and % elongation) did not differ significantly among inundation treatments, but had a tendency to be lower in the treatments with higher inundation time. Although not significant, mean erosion was higher in the medium and high-inundation sediment cores, compared to the sediment cores at average inundation level in the pioneer zone and control sediment cores. Overall, the best linear predictors of erosion were plant survival (as indicated by % live shoot biomass) and % silt, which each decreased erosion. Our results are among the first to provide empirical evidence that inundation-induced mortality of salt marshes has negative consequences for resistance to erosion.

5.1 Introduction

Coastal salt marsh vegetation provide numerous ecosystem services of high value to society, including habitat for a multitude of species, sediment stabilization, erosion control, carbon sequestration, nutrient cycling, recreation and tourism (Barbier et al., 2011). As ecosystem engineers, salt marsh grasses of the *Spartina* genus are capable of modifying the surrounding environment through multiple biophysical feedback mechanisms, including wave attenuation and sediment-trapping capabilities (van de Koppel et al., 2012). Regular tidal inundation provides the sediment supply needed in order for *Spartina* spp. to accrete and maintain marsh elevation (Fagherazzi, 2014; Kirwan and Megonigal, 2013), while their position in the tidal zone depends on the balance between periods of landward erosion and seaward expansion (Mariotti and Fagherazzi, 2013). While salt marshes have historically been used for coastal protection, in recent years there has been a particularly strong interest in salt marsh wave attenuation and erosion control properties (Duarte et al., 2013), given the climate-induced increase in intensity and frequency of extreme events, especially in highly populated coastal zones (IPCC, 2012). It is thus imperative to understand the limits and conditions under which salt marshes can provide such critical climate-regulating ecosystem services.

Historically, salt marshes have been able to maintain keep pace with sea level rise and maintain its position in the intertidal zone, as rates of sediment deposition and organic accretion have been similar to rates of sea level rise in most salt marshes (Fagherazzi et al., 2012). However, global change presents multiple challenges to the persistence of salt marshes and their ecosystem engineering capabilities. Rising sea levels as a result of climate change, in tandem with subsidence from liquid extraction or compaction, and other multiple stressors cause inundation stress, which can effectively drown salt marsh vegetation (Craft et al., 2009). The specialized aerenchyma (air-filled) tissue in *Spartina* spp. roots enable growth in anaerobic or anoxic conditions by the delivery of oxygen to roots (Tiner, 1999); however, prolonged waterlogging has been demonstrated to reduce oxygen flow to the root systems of *Spartina patens*, preventing complete aerobic respiration from occurring, even with extensive aerenchyma tissue (Burdick and Mendelsoon 1990). Mortality of the plant by increased inundation will in turn alter sediment shear strength, through the loss of *Spartina* spp. below-ground biomass which enhances the cohesion and tensile strength of marsh sediments (Micheli and Kirchner, 2002). Removal of vegetation has been demonstrated to decrease the sediment binding strength of root systems as they decay (Gray and Megahan, 1981) which may influence erosion control. Similarly, widespread mortality of *Spartina* spp. root matrix due to an oil spill resulted in a geomorphic response leading to erosion at the marsh edge (Silliman et al., 2012).

Studies examining the effects of inundation on salt marshes have demonstrated a reduction in vegetation biomass or increase in mortality. For example, inundation and salinity stress have been demonstrated across several coastal marsh species to reduce root and shoot biomass, with particularly negative effects on below-ground production (Janousek and Mayo, 2013), with lower above and below-ground biomass after one season of inundation mimicking a 10-cm rise in sea level (Wong et al., 2015), and complete mortality after two years of inundation mimicking a 30-cm rise in sea level in another study (Langley and Mozdzer, 2013). Nelson and Zavaleta (2012) found that inundation at a 10-cm rise in sea level did not significantly reduced vegetation biomass, but inundation at 30 cm induced complete mortality over time.

The interest in combating SLR has generated numerous studies on the ability of salt marshes to respond to changes in sea level. While it has been suggested that the vulnerability of salt marshes to SLR has been somewhat overestimated (Kirwan et al., 2016), in some coastal zones, particularly deltaic areas, altered sedimentation patterns and subsidence pose a greater risk to salt marshes than climate-related sea level rise (Ericson et al., 2006). The adaptive capacity of salt marshes to migrate inland in response to higher inundation levels will be restricted by physical structures such as dykes or port developments in many coastal zones around the world as they tend to be heavily populated and urbanized (Gedan et al., 2010). While several studies have demonstrated impacts to salt marsh structure through models or observations, there have been few empirical studies quantifying the impacts of SLR on functioning and related ecosystem services*; i.e.* how erosion resistance of salt marshes is affected by inundation. How does change in the vertical dimension (increased inundation) affect horizontal processes (lateral erosion), the latter of which salt marshes are thought to be more vulnerable (Fagherazzi et al., 2013; Kirwan et al., 2016)? We attempt to answer this by experimentally transplanting salt marsh sediment cores along a gradient of inundation, a proxy for relative sea level rise. Our objective was to determine whether SLR affects susceptibility of salt marsh sediments to erosion, focusing on the below-ground factors affecting lateral erosion at the cliff edge. We hypothesized that increasing inundation levels would: 1) decrease the amount of live root biomass and decrease the root biomass tensile strength that enables sediment binding; and 2) result in a decrease in the sediment stabilization properties of the *Spartina* root matrix, resulting in higher rates of lateral erosion when exposed to waves.

5.2 Methods

Study Area

Our study area is in the Po River Delta, along the Italian coastline of the Northern Adriatic Sea. Regional climate projections for Italy indicate that this portion of the coastline is particularly vulnerable to sea level rise (Lambeck et al., 2011). As a low-lying coastal area, the Po Delta is vulnerable to local sediment compaction, which, in combination with heavy industrial dredging and mining of sediments, and groundwater and hydrocarbon extraction, have accelerated subsidence of deltaic sediments (Ericson et al., 2006) resulting in coastal retreat of hundreds of meters (Cencini, 1998). This highly urbanized area is also susceptible to coastal flooding and erosion due to storm surges (Perini et al., 2016; Sekovski et al., 2015). Reduced riverine sediment supply, drainage, land reclamation, and coastal development have dramatically altered the coastline (Airoldi and Beck, 2007), resulting in unprecedented loss of salt marshes in some areas (Fontolan et al., 2012). Coastal erosion has led to the construction of hard defense structures, such as groynes, breakwaters, and artificial embankments, covering an estimated 60% of the coastline in Emilia Romagna (Perini et al., 2016). Unfortunately these coastal structures have had limited success and have even exacerbated erosion in some areas (Sekovski et al., 2015), and they furthermore prevent landward expansion of salt marshes.

Our experimental site is located in the natural reserve in Sacca di Bellocchio (hence referred to as “Bellocchio”), part of the Valli di Comacchio lagoonal ecosystem in the Parco Delta del Po dell’Emilia-Romagna (44°37'47''N, 12°15'38''E) (Figure 1). This shallow lagoon is eutrophic with inputs of nutrient-rich continental waters (Mistri et al. 2001). It has moderate exposure to wave action and a semi-diurnal micro-tidal regime with average tidal amplitude of about 50–130 cm (Russo et al. 2012). Average sea surface temperatures vary between 7 °C in winter and 27°C in summer, with highly variable average surface salinities between 30 and 38 psu (Russo et al., 2012). In Bellocchio, *Spartina* spp. grows at an elevation of 0.1 – 0.3 m above mean sea level (Wong et al. 2015) and we observed patches of *Spartina* spp. to be interspersed with patches of bare sediment and tufts of the sea rush *Juncus martimus*. The native *S. maritima* in this area has most likely been replaced by non-native hybrid *Spartina anglica* and *Spartina townsendii* species – however, these species are difficult to distinguish morphologically until the flowering period (Wong *et al.* submitted). We thus refer to our samples as *Spartina* spp. Despite being located in a natural reserve, this area is currently impacted by multiple stressors which have resulted in large-scale diebacks of *Spartina* spp. For example a severe heat wave in 2003, shifted the *Spartina* spp. vegetation to *Salicornia veneta s*ucculents (Merloni, 2007) which has been shown to have a lower tolerance to sea level rise (Strain et al., 2017).

Experimental Design

We conducted a field-based mesocosm inundation experiment, by transplanting *Spartina* spp. salt marsh sediment cores to three different inundation levels: (1) average inundation in the pioneer zone where Spartina spp. naturally occur (S0); (2) moderate inundation, representing a 20-30% increase in inundation time with sediment cores placed at 10 cm lower elevation relative to the pioneer zone (S10); and (3) high inundation, representing an 80-90% increase in inundation time, with sediment cores placed at 20 cm lower elevation relative to the pioneer zone (S20). 20 sediment cores for transplantations were extracted from the *Spartina* spp. vegetated pioneer zone by hammering in PVC pipes (15 cm in diameter and 20 cm in length), with holes drilled in the side to enable water flow. The sediment cores were carefully extracted and capped at the bottom with flexible perforated plastic caps to maintain the sediment type and volume from the pioneer zone while maintaining water infiltration. For the transplantations, we placed a transect parallel to the shoreline at each inundation treatment level (Figure S1). Along each transect, plots were randomly selected for placing the salt marsh sediment cores. At each plot, we dug holes to fit the diameter and length of the PVC cores, burying the sediment core until a depth of 22 cm to ensure that the top of the PVC pipe was level with the surface. The extracted sediment cores were then planted in the plots, and marked at an adjacent spot with a discretely marked bamboo stick for ease of identification.

For the average inundation treatment (S0), sediment cores were placed in bare areas with no vegetation (mimicking the bare areas that sediment cores for treatments S10 and S20 were placed in) to avoid confounding effects of existing vegetation in the pioneer zone on erosion. Inundation times had been determined from previous studies in the area (Strain et al., 2017; Wong et al., 2015) and were additionally confirmed with the use of dive loggers (ReefNet Sensus Ultra), which were attached to the bottom of one PVC pipe per each treatment).

In addition to the inundation treatments two procedural control treatments were established: For the first control (C1), one set of sediment cores were extracted from the *Spartina* spp. vegetated pioneer zone with PVC cores, and the cores were then planted back in the same place from where they were extracted, to determine if the physical act of transplantation affected erosion resistance. For the second control (C2), another set of sediment cores were extracted from the *Spartina* spp. vegetated pioneer zone and transplanted to a different vegetated area, to determine if erosion was affected by removal of the surrounding vegetation around the area. For each of the three treatment inundation levels and the two controls, we had 4 replicates, for a total of 20 samples.

Vegetation and sediment characteristics

Sediment cores were monitored over four months during the growing season (July to October 2015) to assess vegetation variables, before being extracted for erosion analysis in October. *Spartina* spp. stem density was counted, including per cent alive *vs*. dead stems, on each sediment core. Canopy height was also assessed on each sediment core by dropping a Styrofoam disc along a graduated pole on the vegetation (Nolte et al., 2014). Random height was measured by randomly selecting 4 stems from the core and measuring the stem height up to the culm – the 4 measurements were pooled to obtain a mean height per plot.

Sediment cores were extracted from each plot in October 2015 for wave treatment in wave mesocosms and erosion analysis (described below). Just before placement in the wave mesocosms, above-ground vegetation was clipped off the sediment core and assessed for density, including the number of live and dead stems, length, and dry weight. As our experiment was designed to address lateral cliff erosion, the waves intersected the sediment core below the sediment-water interface. Thus, the removal of above-ground vegetation would not affect erosion rates. It also enabled retaining the full amount of above-ground biomass which might otherwise be eroded away with sediments. After wave treatment, the below-ground biomass was successively sieved over meshes to remove sediment from the roots and rhizomes. A 1-mm mesh was used to retain fine roots, and a 2-mm mesh was used to retain coarse roots. Rhizomes were identified and weighed separately. The remaining biomass was further sorted by hand, using an arbitrary 1 mm root diameter cut-off value to distinguish between fine (<1 mm) and coarse (>1 mm) roots.

Sediment samples were taken from each core (2.5 cm in diameter and 3 cm deep). The sediment samples were stored on ice during transport. In the laboratory, samples were dried at 60^0C until constant weight to determine bulk density (g·cm^3). Grain size properties were determined with a Malvern Laser Particle Sizer, including total per cent silt, mud and median grain size (silt=particle size >3.9 and <62.5 μm and mud=particle size <62.5 μm according to the Wentworth classification (Wentworth, 1922)). Organic matter content was determined with the loss-on-ignition technique (Craft et al., 1991), with the samples heated to 450 °C for six hours in a muffle furnace.

Quantifying erosion resistance

Sediment cores were extracted near the end of the *Spartina* spp. growing season (mid-October 2015). The PVC cores were carefully removed from the surrounding marsh area and stored in lagoon water during transport to wave mesocosm facilities at the Royal Netherlands Institute for Sea Research (NIOZ). Sediment cores were then transferred to containers filled with in filtered seawater from the Scheldt estuary (ambient temperature, salinity ~ 30 ppt). The sediment cores were trimmed to 15 cm sediment depth before being placed in the wave mesocosms for wave treatment, using methods described in Lo et al. 2017. The wave mesocosm could hold three samples that could be simultaneously subjected to wave action. Photogrammetry methods were used to determine erosion after 24 hours of wave treatment (Lo et al., 2017). Erosion was monitored at the following time steps : T_0=0, T_1=0.25, T_2=1, T_3=4, T_5=8, T_6=24 hours. The time steps and overall length of wave treatment time were based on previous erosion measurements showing slow to constant erosion rate after 24 hours (Lo et al., 2017). To obtain maximum possible volume loss over time, we fitted an exponential model to the experimental data of volume loss over time, in the form of $E=Q(1-e^{-kt})$, where E=% erosion of the sediment core, Q=maximum erosion over time, k=rate of erosion, and t=time in hours, as in Lo et al. (2017).

Biomechanical measurements

We quantified biomechanical properties of the vegetation using a tensometer (Instron model 3342). We measured biomechanical properties on fine roots, as we predicted that this portion of biomass would show the most difference in tensile strength and % elongation with increasing inundation. Properties measured were: (1) Tensile strength, or force-to-tear strength (FTS, Newton (N)/mm^2), which is the maximum force per unit of cross-sectional area needed for breaking the plant tissue; (2) % elongation, or extensibility, which is the maximum length of the tissue (mm) that can be stretched or extended before it breaks from tensile stress. Fine roots were trimmed to 5 cm length, and the diameter was measured to the nearest 0.1 mm with a digital caliper. Biomechanical properties were then measured (as in La Nafie et al., 2012), where roots were individually clamped into 5Newton grips attached to the tensometer, placed 2.5 mm apart. The root was then extended at a velocity of 5 mm/min, and the extension of the root (mm) and the applied force (N) were recorded every 0.1 s until the roots broke. FTS was calculated by dividing the maximum force that the leaf can bear before breaking, by the cross-sectional area of the root, and % elongation was calculated by dividing the amount of extension of the root, or the amount of stretch (mm), at the breakage point by the total length of the root (*100 %).

Data and statistical analyses

We evaluated the effects of inundation on % live shoots over time by using repeated-measures ANOVA, with the Greenhouse-Geisser correction in case assumptions of sphericity were violated. The factors tested were date (4 repeated measures) and treatment (three inundation levels and two controls) as explanatory variables. The other vegetation variables measured over time, canopy height and random height, could not be analyzed statistically due to dead vegetation at the S20 and S10 inundation depths.

One-way ANOVAs were used to determine difference between treatments for vegetation variables evaluated on the sediment cores exposed to waves in the wave mesocosms (above- and below-ground biomass components, after four months of inundation treatment), followed by Tukey's HSD post-hoc tests. One-way ANOVAs were also used to determine differences between treatments for sediment properties (% silt, bulk density and organic matter), and for biomechanical properties of fine roots (FTS and % elongation). Normality and homogeneity of the data were visually checked in the residual plots, and, when necessary, data were transformed to comply with assumptions of homogeneity of variance. Forward step-wise linear modeling was used to determine the effect of vegetation (above-ground and below-ground biomass variables), sediment (% silt, bulk density, and organic matter) and biomechanical variables (FTS and % elongation) on erosion. While our study examines impacts of inundation below the sediment-water interface, above-ground variables were included in the model as they could be an indicator of plant decay that influences erosion (given that below-ground biomass experiences slower decay after plant mortality due to anoxic conditions in the sediment). There could also be other variables correlated with above-ground biomass (e.g. fauna) that influence erosion (and would not be affected by cutting off the vegetation before the wave treatment).

Repeated-measures ANOVAs were performed using GraphPad Prism Version 6.01 (GraphPad Software, 2012). ANOVAs and linear models were performed in the R environment (R Core Team, 2017), and the negative exponential models used to determine maximum erosion over time were constructed with the *nlme* package within R (Pinheiro, J et al., 2017).

5.3 Results

Effect of treatments on vegetation, sediment and root biomechanical properties

Mean *Spartina* spp. % live shoots was lower in each of the S20, S10, and S0 inundation treatments compared to the two controls (Tukey's HSD post-hoc test, $p<0.001$ in each case, Table S1). *Spartina* spp. % live shoots on the sediment cores decreased over time ($F_{(3, 48)}=347.4$, $p=0.0005$) and differed between treatments ($F_{(4, 15)}=19.33$, $p<.0001$) (Figure 2, Table S1). There was no significant difference in % live shoots between the two controls, nor between each of the three inundation treatments (Table S1). At the final monitoring date at the end of the experiment, mean % live shoots was 7.9±3.9 *SD* (%)in the S0 treatment; 33.8±10.8 *SD* (%) in the C1 control, and 35.6±13.6 *SD* % in the C2 control. No shoots survived in the S20 or S10 inundation treatments at the end of the experiment.

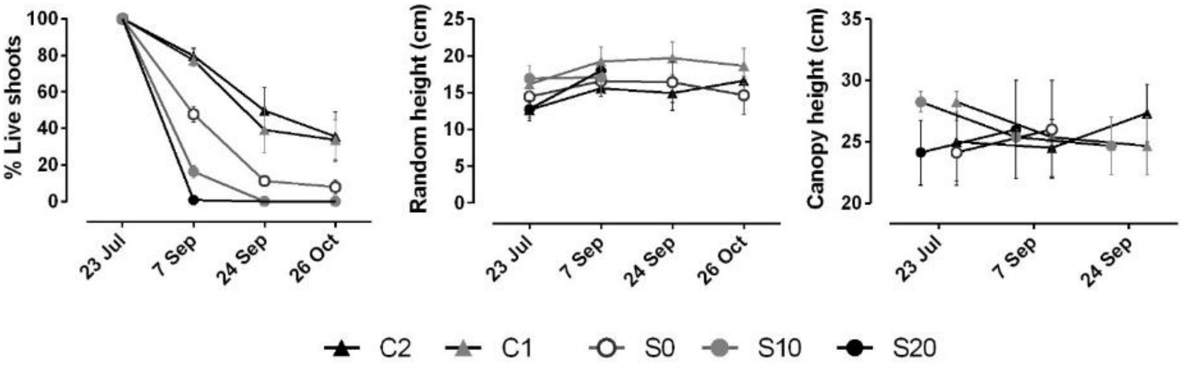

Figure 2: Mean values for *Spartina* spp. density % live shoots, random height (cm), and canopy height (cm) on sediment cores of different inundation treatments (S20, S10 and S0) and two controls (C1 and C2), measured over 4 monitoring dates. Error bars denote standard error of the mean. *n*=4 for each vegetation variable, with the exception of S20 inundation treatments, where n=1 for 7 Sep for random height given sparseness of surving shoots to make the measurements, and for the S0 inundation level, n=3 for 24 Sep and 26 Oct. For the S20 and S10 inundation treatments, low survivorship of shoots meant that random height and canopy height could not be measured on 24 Sep or 26 Oct.

Sediment properties (% silt, bulk density and organic matter) were not significantly different between the 5 treatments (Figure 3). Mean % silt ranged from 54 ±8(SE) to 64 ±7(SE) % across treatments, bulk density ranged from 0.40 ±0.02(SE) to 0.57 ±0.09(SE) g/cm^3, and organic matter ranged from 11.5±3.5(SE) to 14.6 ±2.7(SE) % LOI (Figure 3).

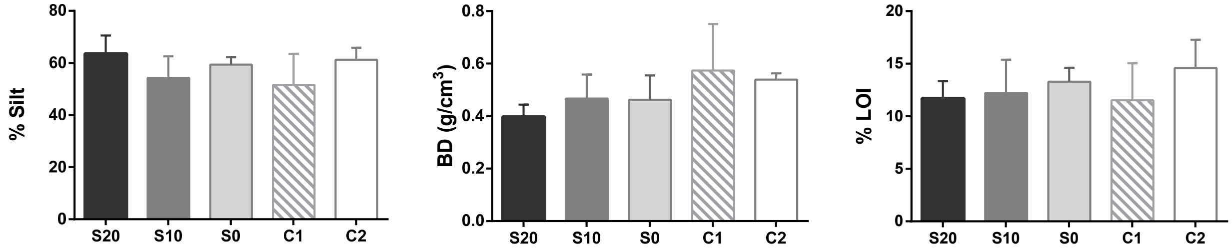

Figure 3: Mean values for sediment properties (% silt, bulk density (g/cm^3) and organic matter (% loss on ignition (LOI)) measured in sediment cores exposed to different inundation treatments. Data are means over four replicates.

Live above-ground biomass differed significantly between treatments ($F_{(4,15)}$=4.78, p=0.01), with lower live aboveground biomass in the S20 and S10 treatments (given that no shoots survived) compared to the C1 and C2 treatments (Tukey's HSD, p<0.05 for each pairwise comparison (Figure 4, Table S2). Dead above-ground biomass did not differ significantly between treatments (p=0.597). Total above-ground biomass (both live and dead fractions) was marginally significant ($F_{(6,13)}$=2.625, p=0.07). Total below-ground biomass also did not differ significantly between treatments (p=0.91), and each component of below-ground biomass (fine roots, coarse roots and rhizomes) did not differ significantly between treatments (p=0.537 (fine roots), p=0. 78 (coarse roots)). While the only significantly different biomass component among treatments was live above-ground biomass, there was a tendency for the treatments with higher inundation times (S10 and S20) to have lower total above-ground and below-ground biomass (Figure 4).

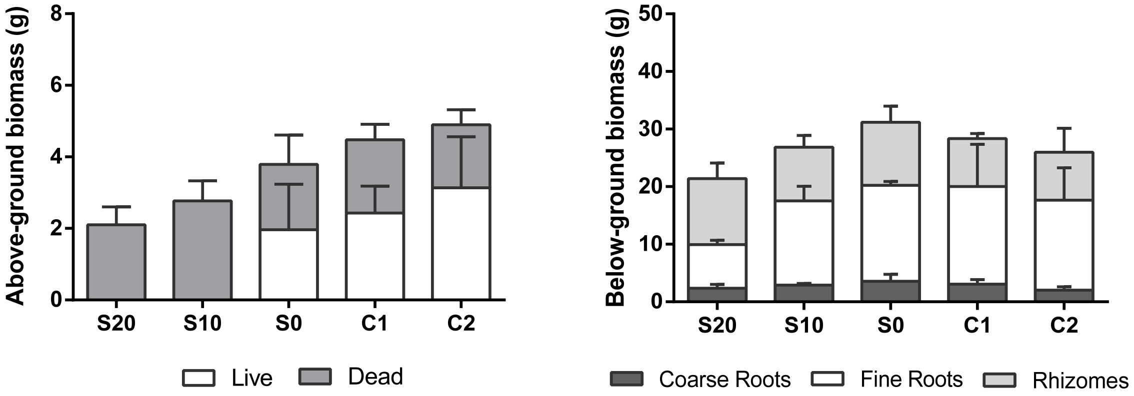

Figure 4: Mean values for above-ground biomass (live and dead) and below-ground biomass (coarse roots, fine roots, rhizomes) measured in sediment cores exposed to different inundation treatments.

There was no significant effect of treatment on mean tensile strength (FTS) of fine roots across treatments (p=0.916), although, as with the biomass, there was a tendency for the treatments with higher inundation levels (S10 and S20) to have lower FTS (i.e. weaker fine roots) (Figure 5). Similarly, there was no significant difference between treatments in maximum root elongation (p>0.05).

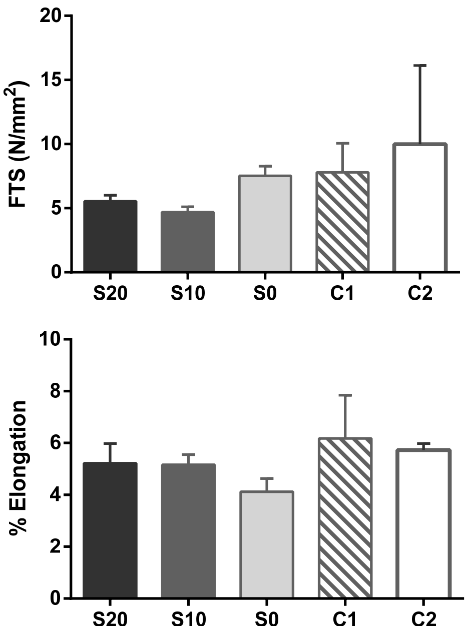

Figure 5: Tensile strength (FTS, N/mm^2) and maximum elongation (% elongation) of fine roots across inundation treatments. Data are means over 4 replicates, error bars denote standard error of the mean.

Effect of inundation treatments on erosion

Overall, mean erosion values were low across treatments, ranging from approximately 1 to 8 %. Mean % erosion of the sediment cores were highest in the treatments with higher inundation with 4.06 ± 1.3 (SE) % mean erosion in the S20 treatment and 4.8 ± 1.4 (SE) % mean erosion in the S10 treatment (Figure 6). The lowest mean erosion was in the S0 treatment, which had 2.0 ± 0.4 (SE) % erosion but erosion did not differ significantly across treatments ($p>0.05$).

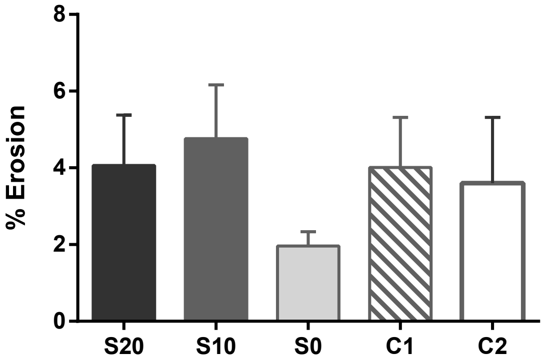

Figure 6: % Erosion of sediment cores across inundation treatments. Data are means over 4 replicates, error bars denote standard error of the mean.

The most significant linear predictors of erosion (n=20) were the amount of live shoot biomass and % silt ($F_{(2,17)}$=5.678, p=0.013, R^2=040) (Figure 7). Live shoot biomass and % silt each decreased erosion (Figure 7, Table S3). The other vegetation, sediment and biomechanical variables, considered individually or together, were not significant.

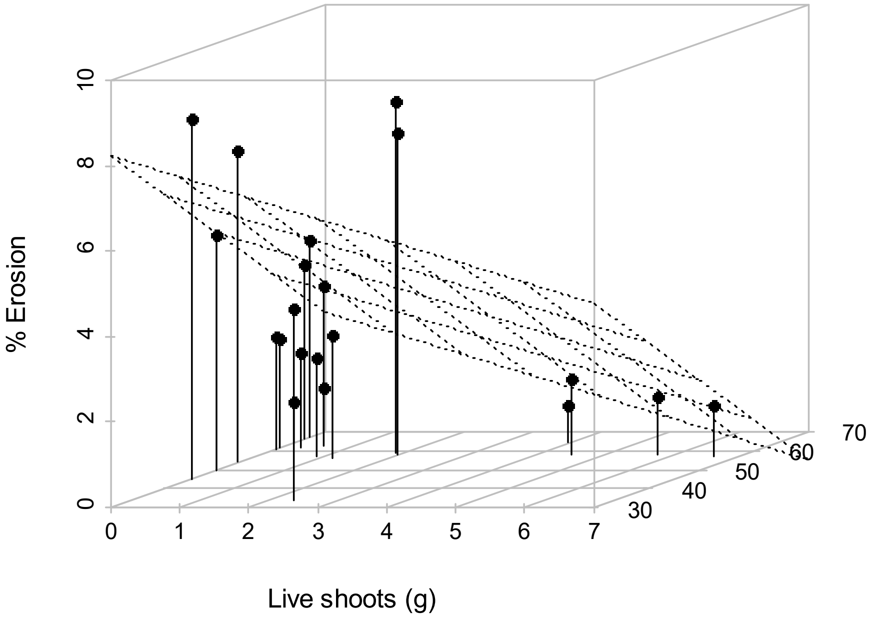

Figure 7: Relationship between % erosion, live shoot biomass (g), and % silt (BD) on sample sediment cores. The 3-D plane represents the linear model log(% Erosion)=12.31 – 0.49(live shoot biomass) -0.13(% silt), ($F_{(2,17)}$=3.953, p=0.034, adjusted R^2=0.23).

5.4 Discussion

Effects of inundation on vegetation survival and biomechanical properties

As expected, the proportion of live shoots significantly decreased over the duration of four months of treatment, such that no shoots survived at the end of the experiment in the two treatments mimicking a 20-30% inundation period (S10) and 80-90% inundation period (S20) (Figure 2). Mean plant survival, as indicated by % live shoots, was higher in the average inundation treatment (S0), but highest in the two controls (C1 and C2), which did not differ significantly from each other. The high mortality of the S10 and S20 treatments confirmed our hypothesis, and are generally in accordance with other salt marsh inundation experiments: Wong et al. (2015) found a marked decrease in shoot survival after a three-month inundation treatment (equivalent to our S10 treatment of 20% increased inundation time). Similarly, Nelson and Zavaleta's (2012) found complete mortality of *Sarcocornia pacifica* in the most extreme inundation treatment in the second year of treatment (Nelson and Zavaleta, 2012). Strain et al. (2017), on the other hand, found 60% shoot survival in *Spartina* spp. (in the same site as where this study was conducted) across inundation treatments, with 40% survival in treatments with inundation up to 50%. It is possible that inter-annual variation in climate conditions can explain the higher mortality in our experiment, as it was exposed to a severe heat wave in Europe in July 2015 (at the onset of the experiment) with higher surface water temperature and salinities (Lo et al. *in prep*). Local scale environmental variability may also have played a part in plant mortality, and explain the discrepancy between plant survival in the S0 and two control treatments, which we would have expected to have similar values for live shoots.

We expected that biomechanical variables would be significantly affected by inundation treatment, with higher inundation levels inducing mortality in biomass, corresponding with lower tensile strength (FTS) (i.e. weaker roots) and lower maximum % elongation values (extensibility). While we did not find a significant relationship between erosion and FTS or % elongation, we did observe a trend of lower mean FTS in the inundated treatments (S10 and S20) compared to the average inundation level (S0) and two controls (C1 and C2). This suggests a slower decay of *Spartina* spp. below-ground biomass in comparison to shoot decay which experienced complete mortality with inundation after four months of treatment. With increasing time and as decay of below-ground biomass progresses, we would expect a significant reduction in tensile strength and % elongation. Studies in seagrass species generally show lower tensile strength with exposure to stressors. For example, nutrient enrichment reduced the tensile strength of the seagrass *Zostera* *noltii* (La Nafie et al., 2012), and shading and nutrient enrichment resulted in weaker (lower tensile strength) and more elastic (greater % elongation) blades of a tropical seagrass species (La Nafie et al., 2013).

Influence of sediment, vegetation and root biomechanical variables on erosion

In our study, % silt was one of the significant predictors of erosion. This is in accordance with other salt marsh erosion studies, in which percentage of coarse sand (which is negatively correlated to % silt) was positively associated with erosion (Feagin et al., 2009) and sites with silty soils had lower erosion (Lo et al., 2017). The aforementioned study had characterized the study area as generally having sandy soils in the *Spartina* spp. zone, in which vegetation played a significant role in reducing erosion (Lo et al. 2017). However, a much higher percentage of silt was found in our experimental area which was adjacent to the previous experimental site, indicating high local and/or temporal variation in sediment characteristics. This high silt content might be the reason of the observed lower range of erosion across treatments.

An unexpected result was the difference in vegetation, biomechanical properties and lateral erosion between the average inundation (S0) treatment and the two controls. While not significant, the S0 sediment cores had lower mean erosion than the C1 and C2 controls. We speculate that these differences may be due to variation in below-ground biomass, which has been found to enhance erosion resistance. Below-ground biomass values, while not significant, were highest in the S0 treatment.

We expected that reduced tensile strength and elasticity of salt marsh below-ground biomass would affect erosion resistance. Root tensile strength is linearly associated with root density, and the stability of the salt marsh cliff is proportional to the number of roots intersecting the potential failure plane (van Eerdt, 1985). Sediment shear strength has been shown to increase in riverine systems with increasing root density (Grabowski, R.C., 2014). Thus, in response to inundation, the decay or death of the complex *Spartina* spp. root matrix would presumably decrease root density and tensile strength and in turn reduce overall sediment stabilization properties. Loss of below-ground biomass leading to increased erosion has occurred when there has been *Spartina* spp. mortality due to oil spills (Silliman et al., 2012) or when nutrient enrichment decreases relative investment in below-ground biomass in favour of above-ground production (Deegan et al., 2012; Turner et al., 2009). However, effects of tensile strength and % elongation on erosion were not significant, probably due to the length of the experiment (4 months) in which there was not sufficient time for the below-ground biomass to decay. Biomechanical properties of *Spartina* spp. are not only relevant to sediment stabilization, but also to wave attenuation, which is influenced by the stiffness of plant leaves, with dissipation of wave energy three-fold higher in vegetation with stiff leaves, such as *Spartina* spp. compared to those with flexible leaves, such as the seagrass *Zostera noltii* (Bouma et al., 2005). Thus, indirectly, loss of tensile strength (which is higher in stiffer *vs*. flexible vegetation) would also enhance erosion by reduction in wave attenuation.

Sea level rise in tandem with multiple stressors may act synergistically to reduce ecosystem functioning and provisioning of services. For example, Strain et al. (2017) demonstrated that conditions associated with heat waves, including reduced precipitation and higher temperatures, could induce a shift in vegetation from *Spartina* spp. grasses to *Salicornia* spp. succulents such as one observed after a severe heat wave in 2003. The same shift in salt marsh vegetation was seen after another severe heat wave in 2015 (Lo et al. in prep). Such vegetation shifts to shallow-rooted succulent species are predicted to reduce the ability of salt marshes to tolerate increased inundation from sea level rise, as unlike *Spartina* spp., *Salicornia* spp. have under-developed aerenchyma tissue, shallower and less porous roots, and the plants could become depleted of oxygen with increased inundation as (Strain et al. 2017). The same vegetation shift observed in another Northern Adriatic lagoon may also have consequences for erosion resistance, as *Spartina* spp. have a dense below-ground matrix of roots and rhizomes compared to the shallow-rooted *Salicornia* spp. (Chapter 4).

Our results are among the first to provide empirical evidence determining how changes to salt marsh vegetation induced by relative sea level rise will have consequences for resistance to erosion. Local-scale variability in silt content, while not significant between treatments, still had an effect on resistance to erosion. The twin pressures of sea level rise and lateral erosion affecting salt marshes pose challenges to the management of salt marsh ecosystems, and in combination with other human pressures have resulted in dramatic losses of salt marshes, (Airoldi and Beck, 2007; Fontolan et al., 2012). Restoration and conservation efforts, particularly those that enable landward expansion of salt marshes in response to these changes, will enhance efforts to adapt to climate change.

Acknowledgements

This work was funded through a MARES Grant (www.mares-eu.org) and project TETRIS (PRIN 2010-2011, MIUR). MARES is a Joint Doctorate programme selected under Erasmus Mundus and coordinated by Ghent University (FPA 2011-0016). CVC acknowledges a postdoctoral fellowship provided by the Flemish Fund for Scientific Research (FWO-1.2.380.11.N.00). The authors are grateful to Elena Piccioni, Ignacio Barbeito and Augusto Sepp Neves for assistance with experimental set-up and field sampling. We thank Maria Hayden Hughes and Daan Nieuwendijk for assistance in vegetation sorting and the wave mesocosms. We also thank the Po Delta Park and Spina Camping Village for access to the sampling site.

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5.6 Supplementary Information

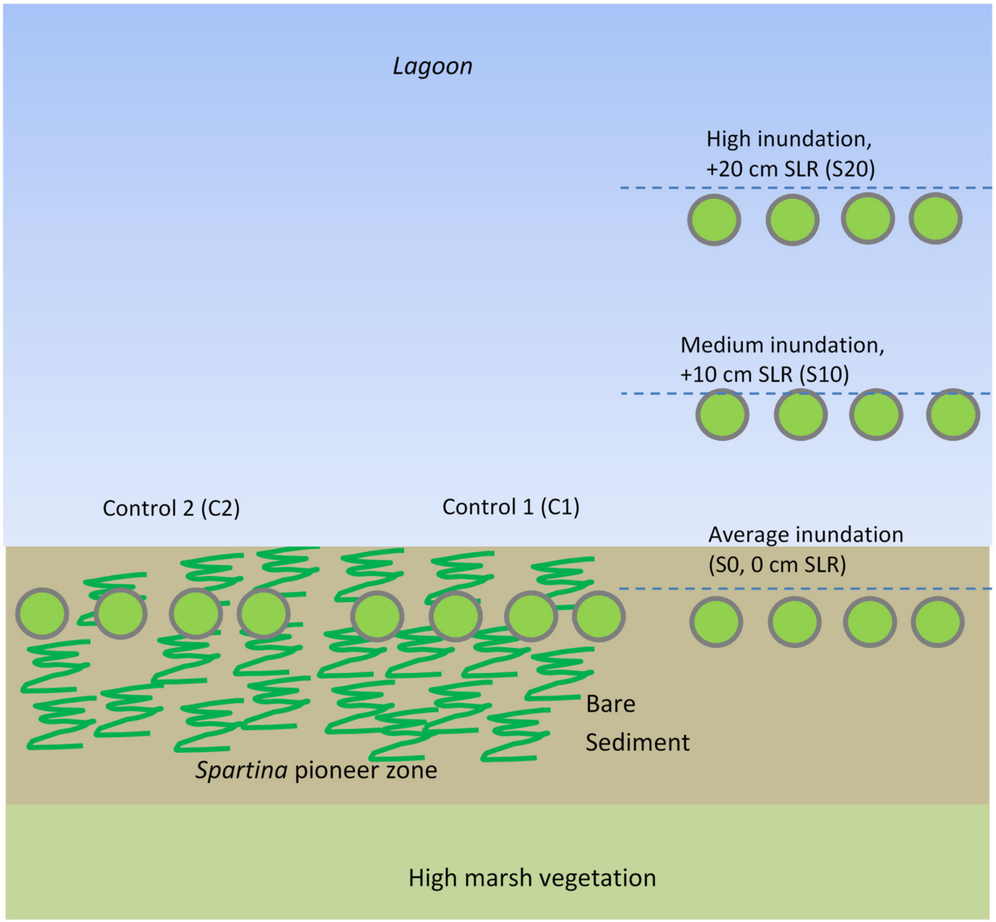

Figure S1: Schematic of experimental set-up with five different inundation treatments: average inundation at the pioneer zone where *Spartina* spp. naturally occur (S0), medium inundation (S10), high inundation (S20), and control 1 (C1) and control 2 (C2) (figure not drawn to scale).

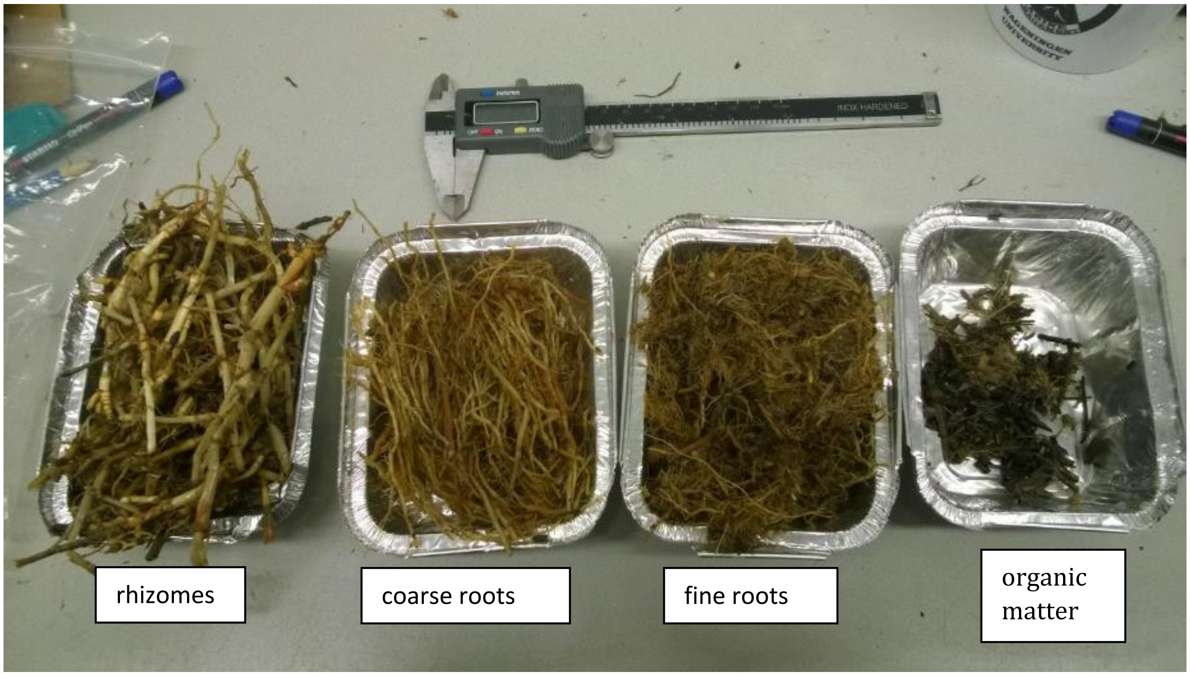

Figure S2: *Spartina* spp. rhizomes, coarse and fine roots, and unidentified organic matter

Table S1: Repeated-measures ANOVA summary table for effect of inundation treatment on percentage of live shoots over time

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	11097	12	924.8	F (12, 45) = 10.66	P < 0.0001
Time	90421	3	30140	F (3, 45) = 347.4	P < 0.0001
Treatment	22699	4	5675	F (4, 15) = 19.33	P < 0.0001
Subjects (matching)	4404	15	293.6	F (15, 45) = 3.384	P = 0.0008
Residual	3904	45	86.75		

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant
S20 vs. S10	-3.904	-22.61 to 14.80	No
S20 vs. S0	-16.55	-35.25 to 2.158	No
S20 vs. C1	-37.42	-56.12 to -18.71	Yes
S20 vs. C2	-41.09	-59.79 to -22.38	Yes
S10 vs. S0	-12.64	-31.35 to 6.063	No
S10 vs. C1	-33.51	-52.22 to -14.81	Yes
S10 vs. C2	-37.18	-55.89 to -18.47	Yes
S0 vs. C1	-20.87	-39.58 to -2.162	Yes
S0 vs. C2	-24.54	-43.24 to -5.831	Yes
C1 vs. C2	-3.669	-22.38 to 15.04	No

Table S2: ANOVA results for testing effect of inundation (5 levels) on log-transformed live shoot biomass (g) in sediment cores exposed to wave action.

Source of Variation	SS	Df	MS	F-ratio	P
Treatment	5.717	4	1.4293	4.783	**0.0109**
Residuals	4.482	15	0.2988		

Table S3: Best linear model on erosion of salt marsh sediment cores (log-transformed % erosion) of salt marsh sediment cores (n=20). Dependent ariable codes: LAGB = live above-ground biomass (g), Silt=sediment % silt. a_x = coefficients, DF = degrees of freedom (residuals)

Model	a_0	a_1	a_2	R^2	**DF**	**F**	**p**
$a_0 + (a_1)$LAGB+(a_2)Silt	3.617	-0.177	-0.039	0.400	17	5.678	0.013

CHAPTER 6

Conclusions and Future Perspectives

Grado Lagoon (GRA), Italy

6. Conclusions and future perspectives

6.1 Synthesis of key findings

The research conducted in this thesis fills important knowledge gaps regarding the functioning of salt marshes in lagoons of the Northern Adriatic Sea, and provides baseline knowledge on the status of salt marshes and some important ecosystem services (such as support for macrofaunal communities and control of erosion) along the Adriatic coastline. In Chapter 2, I assessed salt marsh structure and diversity across a variety of environmental conditions and related this to macrofaunal assemblage structure. The presence of vegetation marginally enhanced organic matter and significantly decreased bulk density. Macrofaunal assemblages were homogenously low in species richness, abundance, diversity and evenness across sites, and assemblages were related to sediment silt and organic matter content, surface water ammonium concentrations, and C:N ratios of *Spartina* spp. below-ground biomass, which together explained 43% of the total variability in assemblages. These findings suggest that *Spartina* spp. indirectly influenced macrobenthos assemblages by modification of soil properties, i.e. via organic enrichment of the sediments. The remarkably low macrobenthos diversity and abundance may be attributed to the cumulative impact of a wide range of anthropogenic stressors including high nutrient loads.

Chapter 3 addressed my objective to investigate factors affecting erosion resistance of salt marshes, specifically the effect of vegetation on erosion resistance under different sediment and nutrient conditions (Objective 2). Our study highlighted that the presence of vegetation reduced lateral erosion, and this effect was more pronounced in sandy soils. Below-ground biomass was the most significant vegetation variable associated with reducing erosion. One study previously suggested that sediment properties play a larger role in reducing lateral erosion, with vegetation cover and type not shown to directly reduce erosion (Feagin et al., 2009). thereby casting doubts on the role of vegetation in coastal protection (Gedan et al., 2011). My study showed different results from Feagin et al. (2009) in a direct and quantifiable manner: while sediment properties are indeed crucial influencers of erosion, we show that vegetation also plays a direct and significant role in erosion resistance.

Chapter 4 addressed the objective of investigating the effects of stressors, particularly high nutrient loads and heat waves, on ecosystem structure and lateral erosion resistance in salt marshes (Objective 3). The occurrence of a severe heat wave offered the opportunity to document potentially interactive or synergistic effects of heat wave conditions and fertilization on salt marsh structure and functioning. Following the heat wave, which resulted in higher surface water salinity and temperatures, we observed notable changes in sediment properties, with a three-fold increase in sediment organic matter and a parallel decrease in bulk density. These changes in the sediments occurred across both unfertilized and fertilized plots, and probably caused the measured overall higher erosion across treatments after the heat wave. Another significant change following the heat wave was that *Spartina* spp. was replaced by *Salicornia veneta* succulents. Differently from what was observed for sediment properties, this vegetation shift mostly occurred in fertilized plots. This important vegetation shift was expected to reduce erosion resistance of the sediments, but clear differences were not measured between treatments in erosion, probably because remaining *Spartina* spp. root biomass persisted in the system for the duration of the experiment. Overall this study demonstrated that: 1. Heat waves and fertilization can synergistically impact salt marsh structure by changing the vegetation composition; 2. Heat waves, regardless of nutrient status, can trigger changes in sediment properties that reduce the ability of salt marshes to stabilize sediments; and 3. *Spartina* spp. below-ground biomass was still a significant factor in reducing erosion, despite being lower in both fertilized and unfertilized plots after the heat wave compared to the previous year.

In Chapter 5, I explored the effects of inundation by sea level rise on *Spartina* spp. root biomechanical properties, and resultant impacts on resistance to erosion, through a four-month transplantation experiment (Objective 4). Inundation-induced mortality decreased above- and below-ground biomass over time, with complete shoot mortality in the sediment cores exposed to medium and high inundation levels. Biomechanical properties measured at the end of the experiment (root tensile strength and % elongation) did not differ significantly among inundation treatments, but had a tendency to be lower in the treatments with higher inundation time. Although not significant, mean erosion was higher in the medium and high-inundation sediment cores, compared to the sediment cores at average inundation level in the pioneer zone and control sediment cores. Overall, the best linear predictors of erosion were plant survival (indicated by % live shoot biomass) and % silt, which each decreased erosion. Our results are among the first to provide empirical evidence that inundation-induced mortality of salt marshes has negative consequences for resistance to erosion.

Our three wave mesocosm experiments (Chapters 3, 4 and 5) reveal the critical role of salt marshes in coastal defence. *Spartina* spp. below-ground biomass significantly reduced lateral erosion across sites with different sediment grain size (% silt), and under conditions simulating the pressures of eutrophication, heat waves, and sea level rise. Sediment properties were also a significant predictor of erosion in these studies. When testing erosion across sites with different grain size, erosion was reduced in sediments with increasing silt content (Chapter 3). The dramatic reduction in bulk density following a heat wave, linked to higher organic matter content, enhanced erosion in a site that previously had cohesive, fine-grained sediments (Chapter 4). % silt again was a significant predictor of erosion in our sea level rise experiment (Chapter 5). Thus, the overarching factors that determined erosion in our studies were biomass and sediment properties.

Our results help to consolidate the evidence of the important role of vegetation in coastal protection. Several studies have demonstrated the importance of the above-ground component of vegetation in wave attenuation, which is dependent on the amount of biomass, and the flexibility and density of shoots (Bouma et al., 2005). It has been shown that the above-ground portion of salt marsh vegetation can provide a protective function under storm surge and possibly more intense conditions (Möller et al., 2014). However, there are few studies quantifying the effect of the below-ground component of salt marshes on erosion resistance, a gap which we aimed to address. As we show that below-ground biomass is negatively correlated with erosion, the multiple benefits of salt marshes for coastal protection are evident, via both wave attenuation from the above-ground component and erosion resistance from the below-ground portion.

Our research also demonstrates the conditions under which erosion resistance is most effective, which can aid in management considerations for where and how to best invest in salt marshes restoration and conservation efforts. The effect of vegetation in reducing erosion was about four times higher in coarser sediments compared to silty sediments. Changes in sediment organic matter and bulk density due to a heat wave reduced the biomass and the ability of vegetation to stabilize sediments. Thus, salt marshes provide protection against erosion across multiple drivers of change, but the effect size will depend on sediment properties. As we observed in Chapter 3, sediment properties can also be affected by extreme climactic events such as heat waves and interact with stressors such as excess nutrients to cause a shift in vegetation with potentially negative implications for sediment stabilization. Thus, stressors to salt marshes such as excess nutrient loads must be monitored and controlled for continued provisioning of this critical ecosystem service.

6.2 Future research perspectives

Chapter 1 sheds light on the influence of salt marsh vegetation and possible influence of disturbance on salt marsh benthic assemblages. While there are numerous studies exploring the role of macrobenthos on sedimentary processes in salt marsh habitats, few directly incorporate measures to quantify resistance to erosion (but see Harris et al., 2016). Further studies in this regard will add to knowledge about factors affecting erosion of salt marshes.

Chapters 3, 4 and 5 evaluated the effects of vegetation, sediment properties, and stressors (high nutrient loads, heat waves and inundation) on resistance to erosion. Improvements can be made to the methodologies used to more accurately capture patterns of wave erosion over time. Wave mesocosms were combined with novel photogrammetry methods to estimate the volume loss over time as a proxy for lateral erosion methods. Photogrammetry methods have proven to be flexible approaches to estimating erosion. Any basic handheld camera will work with sufficient lighting conditions, and the proliferation of open source programs for post-processing and mesh construction make it cost-effective. However, our methods only capture a snapshot in time of erosional processes, not the long-term cyclic behaviour of waves punctuated by storm events. Additionally, the sampling is fairly destructive for sensitive marsh areas, is time- and labor-intensive, and can result in destruction of samples. Care is needed in handling the sediment cores to prevent compaction and other changes to sediment properties. If the physical size of the sample is too small, there is a higher chance of destroying the integrity of the sample such as through sediment compaction, mortality of biomass, loss of sediment, etc. throughout the different stages of sampling, transport, storage and placement in the wave mesocosms. Testing erosion across larger samples more representative of an intect section of the marsh platform will aid in improving the accuracy of wave mesocosm experiments.

Modeling work has suggested that long-term salt marsh deterioration is more influenced by average wave conditions rather than storm surges (Leonardi et al., 2016). Erosional processes thus need to be monitored over sufficient periods of time to discern trends. For this, the use of remote sensing techniques including satellite imagery and airborne laser scanners (ALS, common referred to as "lidar" (light detection and ranging)) can be used for long-term, coastal-scale monitoring (Moffett et al., 2015), and can thus be an important complement to wave mesocosm experiments. Advancement on these techniques are currently underway, such as using lidar to track patterns of erosion and accretion in marshes and expansion of *Spartina* spp. patches (Rosso et al., 2006), and the development of compact biomass lidar to track geomorphological features in salt marshes (Paynter et al., 2016).

In this thesis, a combination of field observational studies (chapters 2 and 3), manipulation of pressures on salt marshes (nutrient loads - chapter 4, inundation – chapter 5) and wave mesocosm experiments (chapters 3, 4 and 5) was employed to explore relationships between human pressures and critical functioning and services of salt marsh ecosystems. Observational studies across multiple sites have a high capacity for enhancing knowledge at larger spatial scales and for inclusion of realistic system complexity, but have lower capacity for creating and assessing currently unobservable conditions and disentangling multiple mechanisms and drivers (Isbell et al., 2017). Experimental manipulation of pressures, on the other hand, have the opposite problem – they have high capacity for determining specific drivers of change, but may not capture the complexity of the system (Isbell et al., 2017). By continuing to combine observational studies, including advancements in remote sensing, with experimental manipulation of stressors, we can enhance our understanding of the relationships between multiple pressures, functioning and services of salt marshes to inform better management strategies. This strategy will also contribute to establishing important baseline information for monitoring the evolution of these sensitive and valuable ecosystems in the face of global change.

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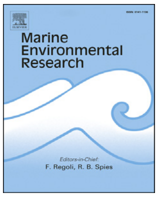
Annex I: Interactive effects of vegetation and sediment properties on resistance to erosion (publication)

Marine Environmental Research 131 (2017) 32–42

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev



Interactive effects of vegetation and sediment properties on erosion of salt marshes in the Northern Adriatic Sea

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ARTICLE INFO

Article history:
Received 4 June 2017
Received in revised form
8 September 2017
Accepted 9 September 2017
Available online 12 September 2017

Keywords:
Erosion
Saltmarsh
Spartina spp.
Eutrophication
Coastal defence
Coastal protection
Wave mesocosm
Coastal ecosystem services

ABSTRACT

We investigated how lateral erosion control, measured by novel photogrammetry techniques, is modified by the presence of *Spartina* spp. vegetation, sediment grain size, and the nutrient status of salt marshes across 230 km of the Italian Northern Adriatic coastline. *Spartina* spp. vegetation reduced erosion across our study sites. The effect was more pronounced in sandy soils, where erosion was reduced by 80% compared to 17% in silty soils. Erosion resistance was also enhanced by *Spartina* spp. root biomass. In the absence of vegetation, erosion resistance was enhanced by silt content, with mean erosion 72% lower in silty *vs.* sandy soils. We found no relevant relationships with nutrient status, likely due to overall high nutrient concentrations and low C:N ratios across all sites. Our results contribute to quantifying coastal protection ecosystem services provided by salt marshes in both sandy and silty sediments.

1. Introduction

Vegetated coastal habitats such as salt marshes provide multiple ecosystem services, including habitat provisioning, nutrient cycling, climate regulation, and cultural services (Duarte et al., 2013; Gedan et al., 2009). The use of salt marshes in ecosystem-based approaches to flood defence is highly promising (Bouma et al., 2014; Narayan et al., 2016), as they have been demonstrated to attenuate waves even under storm-surge conditions (Möller et al., 2014). There has thus been a focus in recent decades on the conservation and restoration of salt marshes to protect coasts from erosion, storm surges and sea level rise (Curado et al., 2013), and to prolong the life of traditional engineered structures such as sea walls and embankments (Spalding et al., 2014; Temmerman et al., 2013). The perennial saltmarsh grasses of the *Spartina* genus are of particular interest in coastal protection as they are pioneer species (Bouma et al., 2016) with a global distribution (Duarte et al., 2013; Silliman, 2014). Their ecosystem engineering properties include trapping sediments from the water column, which enables accretion and establishment at the low marsh (Bouma et al., 2005).

Salt marshes are highly dynamic ecosystems with cyclic behavior, and the stability and size of salt marshes are governed by horizontal and vertical processes (Kirwan and Megonigal, 2013). Horizontally, periods of seaward expansion alternate with periods of shoreward (lateral) erosion due to tidal or wave-induced currents (Bouma et al., 2016; van de Koppel et al., 2005; Yapp et al., 1916). Sediment supply, depth and width of adjacent tidal flats, and wave action affect the balance between erosion and progradation (Mariotti and Fagherazzi, 2013). Density and flexibility of the salt marsh vegetation canopy also play a role in decreasing flow velocity and enabling sediment-trapping, while attenuating waves and slowing erosion (Bouma et al., 2005; Heuner et al., 2015; Möller

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http://dx.doi.org/10.1016/j.marenvres.2017.09.006
0141-1136/

et al., 2014). Vertically, the ability of salt marshes to keep pace with sea level rise by accretion is also dependent on sufficient sediment supply and the rate of landward expansion (Kirwan and Megonigal, 2013). While research has mainly focused on vertical dynamics of salt marshes, especially in the context of sea level rise, there has been comparatively less research on the factors affecting the horizontal dynamics of salt marshes (Kirwan et al., 2016), which may be more important to marsh stability as marshes are more resistant to vertical change but less resistant in the lateral dimension (Fagherazzi et al., 2013). In order to use salt marshes for coastal defence, we need a greater understanding of these horizontal factors, including those controlling resistance to lateral erosion.

Previous studies have shown that below-ground vegetation can aid in inhibiting erosion by stabilizing soils via roots and rhizomes which enhances cohesion and tensile strength, resulting in higher soil shear strength (Micheli and Kirchner, 2002; Turner, 2011). Moreover, roots and rhizomes can provide a physical barrier between water and soil (Wolanski, 2007). It has been demonstrated that the loss of below-ground biomass can result in reduced soil strength or marsh erosion (Sheehan and Ellison, 2015; Silliman et al., 2016). Feagin et al. (2009) suggested that sediment properties can influence lateral erosion rates more than vegetation, with vegetation indirectly contributing to erosion resistance by incorporating detritus and fine-grained sediments into the soil matrix, rendering the soil less dense, less coarse and more cohesive. Deegan et al. (2012) suggested that long-term nutrient addition to salt marshes could cause eventual collapse of the system, as increased availability of nutrients reduces relative investment in below-ground biomass and enhances microbial decomposition, thereby destabilizing soils. Wong et al. (2015) further demonstrated that saltmarsh vegetation can be more vulnerable to increased inundation in medium organic matter soils, particularly under high nutrient availability. Combining these findings suggests that a complex interplay between vegetation, sediment properties and anthropogenic-influenced nutrient levels can influence erosion resistance of salt marshes.

The role of salt marshes in coastal defence is particularly important in the Mediterranean Sea, where humans have been inhabiting and shaping the coastlines for millennia (Airoldi and Beck, 2007). The coast has been dramatically transformed by altered sedimentation patterns, land reclamation, river diversions, embankments, and drainage, resulting in the loss of more than 70% of salt marshes in the region (Fontolan et al., 2012). Coastal defence with artificial structures such as groynes and breakwaters have been implemented on a large scale, leading to hardening of coastal areas and changes in sediment structure, and subsequent loss and alteration of native habitats and assemblages (Airoldi et al., 2005). The extensive construction of artificial infrastructures along these shorelines may limit land-ward rates of marsh expansion in response to sea-level rise, causing coastal squeeze (Doody, 2004). In addition to these development pressures, Mediterranean salt marshes are also impacted by climatic stressors including sea level rise, changed storminess, warm spells or heat waves (Airoldi and Beck, 2007; Christensen et al., 2013) resulting in their decline. Loss of salt marshes in the region has been caused by a reduction in sediment supply and land subsidence, exacerbated by sea level rise (Kirwan and Megonigal, 2013), increases in extreme water elevations (Masina and Lamberti, 2013), and wind-induced waves (Day et al., 1998; Fontolan et al., 2012). Climate extremes, such as heat waves, have accelerated some of these losses, modifying the composition of the dominant vegetation (Strain et al., 2017). Moreover, the dense populations and intensive agriculture and farming along the coast and the plain of the Po river have enhanced nutrient levels along the coastline, and eutrophic conditions are common (De Wit and Bendoricchio, 2001; Lotze et al., 2011), potentially increasing the sensitivity of salt marshes to erosion (Deegan et al., 2012). Given the growing evidence that salt marsh conservation/restoration can be effective as nature-based coastal protection schemes (Shepard et al., 2011), and their rapid rate of decline in the Mediterranean and globally, it is imperative to quantify their role in slowing of coastal erosion rates, and identify which factors or combinations of factors enhance or limit this valuable ecosystem service (Bouma et al., 2014).

Our main objective was to quantify the role of salt marshes in slowing lateral coastal erosion rates in the Northern Adriatic Sea along a broad gradient of physical and anthropogenic factors. We concentrate our investigation on the role of salt marshes in decreasing erosion at the cliff edge, focusing on below-ground factors affecting erosion. Specifically, we investigated how erosion resistance may be modified by the presence of *Spartina* spp., the local sediment grain size, and the local nutrient status of the marsh, using C:N-ratios of above-ground biomass as a proxy for nutrient levels. We hypothesized that *Spartina* spp. below-ground biomass would significantly reduce lateral erosion, and that this effect would increase with increased density of *Spartina* spp. and in cohesive silty soils, while it would decrease with increased nutrient levels.

2. Materials and methods

2.1. Study area

The Northern Adriatic Sea basin off the coast of Italy is the shallowest, northernmost region of the Mediterranean and among the most productive with high habitat diversity (Lotze et al., 2011). The area consists of lagoon-river delta systems which host numerous transitional water bodies and is characterized by moderate exposure to wave action and a semi-diurnal micro-tidal regime with average tidal amplitudes of approximately 65–80 cm (Fontolan et al., 2012; Silvestri et al., 2005). Average sea surface temperatures in the region vary between 7 °C in winter and 27 °C in summer, with highly variable average surface salinities between 30 and 38 psμ. Major activities in the region include the petrochemical industry, tourism, fishing, seaport/port activities and shipping (Torresan et al., 2012). Development pressures have resulted in overall reduced sediment supply in the region, and negative sediment budgets have been recorded in the lagoons of Venice and Grado-Marano due to subsidence, dredging and clam harvesting, such that the lagoons are experiencing accelerating erosion and unprecedented loss of salt marshes (Day et al., 1998; Fontolan et al., 2012; Sarretta et al., 2010; Torresan et al., 2012).

We sampled six sites in coastal lagoons along approximately 230 km of coastline along the regions of Emilia-Romagna, Veneto and Friuli Venezia Giulia (Fig. 1), aiming to include a wide range of environmental (i.e., nutrient status) and physical (i.e., sediment type) conditions. From south to north, sites were located: (1) in the natural reserve Sacca di Bellocchio (hereafter BEL), in the Valli di Comacchio lagoon ecosystem, Parco Delta del Po dell'Emilia-Romagna (44°37′47″N, 12°15′38″E) (further site description in Strain et al., 2017); (2) in the Vallona Lagoon (hereafter VAL), in an area that had previously been reclaimed for agriculture, and is currently being used for private aquaculture (45°1′42″N, 12°23′7″E) (further site description in Wong et al., 2015); (3) in the Coastal Botanic Garden of Veneto, municipality of Rosolina (hereafter ROS), in the southern portion of Caleri Lagoon, Parco Regionale Veneto del Delta del Po (45°5′42″N, 12°19′39″E); (4) in the Chioggia Inlet (hereafter CHI) in the southern part of Venice Lagoon (45°14′53″N, 12°13′34″E); (5) in Grado Lagoon (hereafter GRA), adjacent to an area privately used for aquaculture (45°42′13″N, 13°26′31″E), and (6) in another area in Grado Lagoon adjacent to a golf course

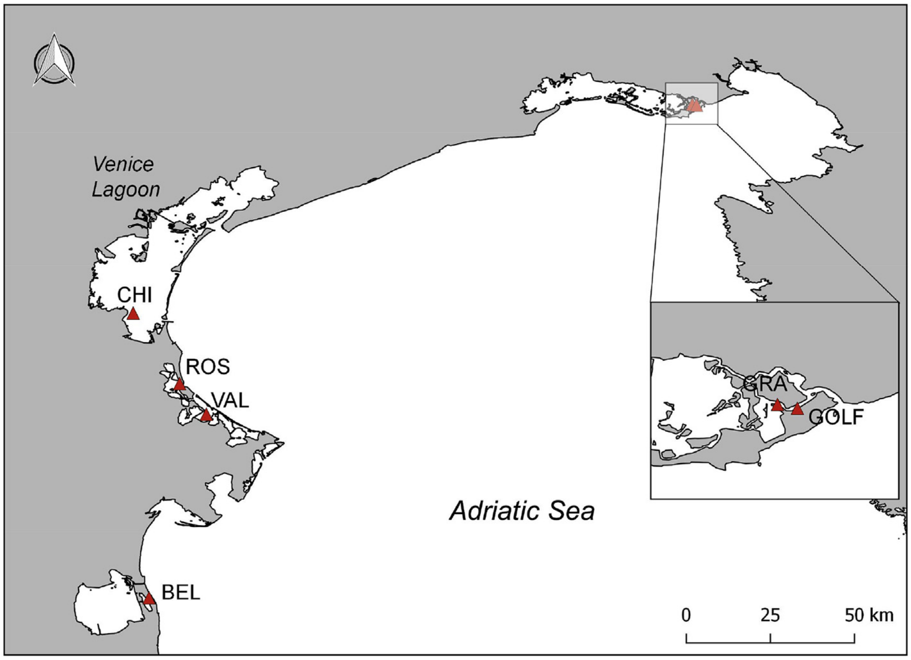

Fig. 1. Location of sampling sites on the Northern Adriatic coast, Italy. Sampling sites: Sacca di Bellocchio, Parco Delta del Po dell'Emilia-Romagna (BEL); Vallona Lagoon (VAL); Coastal Botanic Garden of Veneto, Parco Regionale Veneto del Delta del Po (ROS); Chioggia Inlet, Venice Lagoon (CHI); Grado Lagoon (GRA) and Grado Golf Course (GOLF).

(hereafter GOLF) (45°42′6″N, 13°27′ 4″E) (Fig. 1).

Salt marsh vegetation in these coastal lagoons is composed of perennial *Spartina* spp. cordgrass swards, annual glasswort swards, salt meadows, halophilous scrubs, and salt steppe associations. Species noted at the sampling locations within the *Spartina* spp. zone included *Salicornia* spp, *Sarcocornia fruticose*, *Limonium narbonense*, *Juncus maritima*, *Inula crithmoides*, and *Aster tripolium*. *Spartina* spp. formed patchy to extensive meadows at all sites. Until recently, only *Spartina maritima*, the only *Spartina* species native in Europe (Strong and Ayres, 2013), was reported to occur in these lagoons. *Spartina townsendii* and *Spartina anglica* were first reported from Venice Lagoon in 2002 (Cazzin et al., 2009; Scarton et al., 2003), but their identification status and occurrence were not further explored. A recent broad-scale genetic study, conducted while this experiment was already in progress, showed that *S. martima* in the region has largely been replaced by *S. anglica* and *S. townsendii* (Wong, 2015). Genetic and chromosomal analyses indicate that these species can co-occur in mixed patches (Wong, 2015), where they can be difficult to distinguish morphologically until the flowering period; therefore we will refer to them as *Spartina* spp.

2.2. Sediment properties

Sediment properties were characterized at the start of the work in June and July 2014, as we aimed to include sites with different soil characteristics in our study. At each study site we collected 8 sediment samples (2.5 cm in diameter and 8 cm long), 4 in vegetated and 4 in non-vegetated (hereafter "bare") patches randomly placed within the *Spartina* spp. zone. The sediment samples were stored on ice during transport. In the laboratory, sediment samples were dried at 60 °C until constant weight to determine bulk density (g/cm^3). Grain size was determined using a Malvern Laser Particle Sizer, enabling the determination of total percent silt particle size >4 and < 63 μm, according to the Wentworth classification (Wentworth, 1922). A subsample was used to determine organic matter content with the loss-on-ignition technique (Craft et al., 1991), with the samples heated to 450 °C for six hours in a muffle furnace.

2.3. Sampling design and wave mesocosms set-up

Samples for the quantification of lateral erosion were collected in early October 2014, at the end of the *Spartina* spp. growing season. Ten cores, 5 in vegetated and 5 in bare patches, were randomly extracted at each of the 6 sites within the *Spartina* spp. zone using 30 cm length x 20 cm diameter PVC cylinders. The cylinders were lowered onto the marsh surface, and samples were carefully extracted by digging around the cylinders. A knife was used to cleanly cut roots at the bottom, and a plastic cap was placed at the bottom to prevent sediment loss. Sediment cores were carefully packed to prevent dislodgement and were immediately transported to wave mesocosm facilities at the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke, Netherlands, and stored in filtered seawater from the Scheldt estuary. Before placement in the wave mesocosms, the sediment cores were extruded from the PVC cylinders with a purpose-built extrusion device, which minimized compaction and loss of sediment. Samples were then placed into modified stainless steel cylinders bisected to expose them to waves. The sediment cores were then cleanly sliced at the bottom to a height of 15 cm, and the side facing the waves was trimmed flush to fit the steel cylinders. In this way, the core surface mimicked a cliff that is exposed to waves (Fig. 2a).

To obtain volume loss as a measure of erodibility, sediment cores were subjected for a 32-h period to waves, generated by a piston-powered wave board placed in a mesocosm (La Nafie et al., 2012). The duration of wave treatment was based on initial tests that showed slow to constant erosion rates at 32 h across different sediment characteristics. The wave mesocosms were 360 cm long × 90 cm high × 82 cm wide (Fig. 2b), and were designed and built at NIOZ (for further information contact tjeerd.bouma@nioz.nl). The distance from the sediment cores to the wave board was

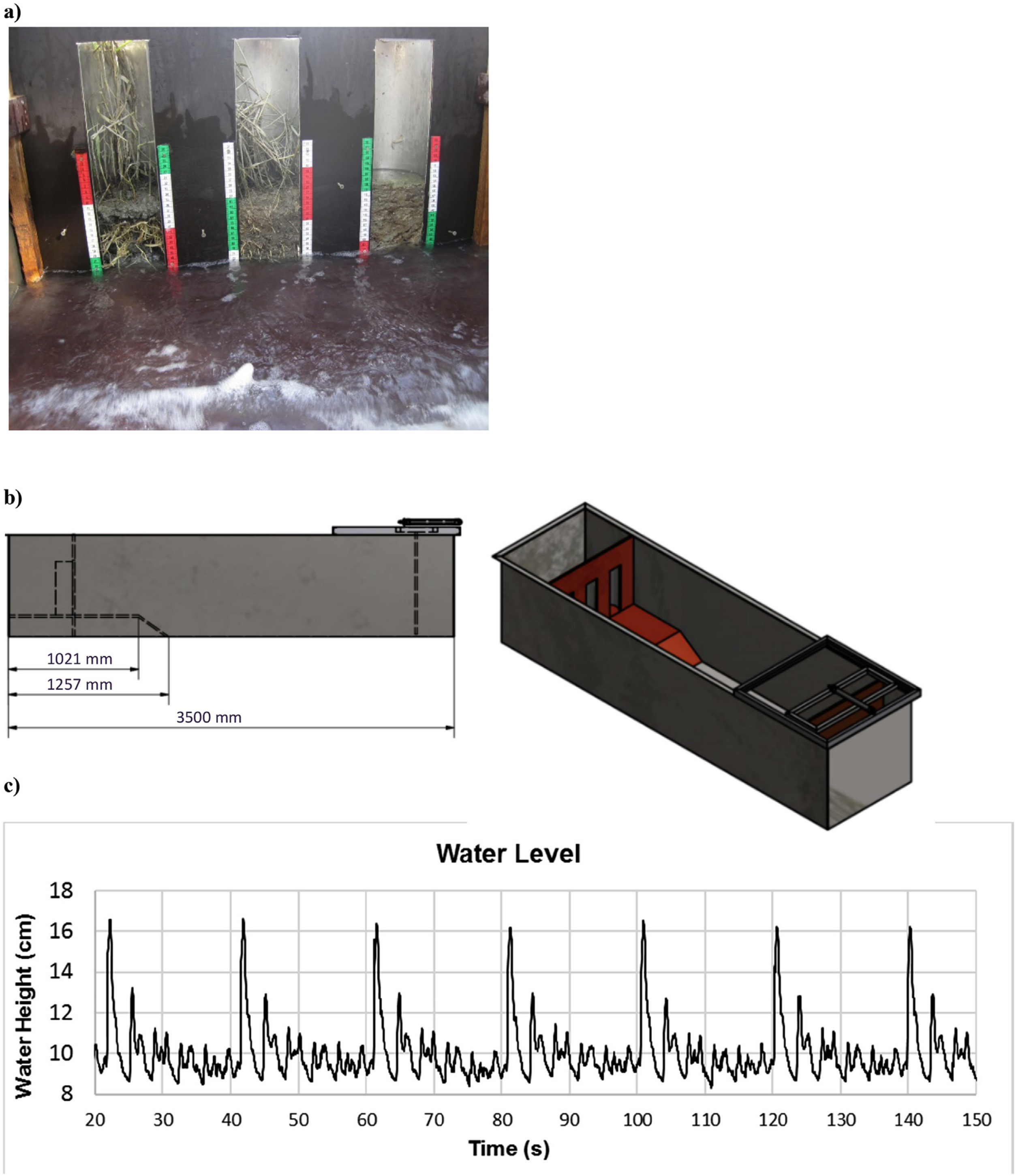

Fig. 2. a) Schematic cross-sectional drawing of the wave mesocosms developed and built at NIOZ, dimensions shown in cm (for information contact tjeerd.bouma@nioz.nl; b) A picture of sliced sediment cores that were exposed to waves in the wave mesocosms; c). Water level measurements used to infer significant wave height (cm) over time (s) (only a segment of time is shown), measured with a pressure sensor at 1 cm distance from the marsh-water interface.

280 cm. There were four wave mesocosms, each holding 3 cores side by side, so that 12 cores could be subjected to wave conditions at the same time. To uniformly spread the potential variability from different storage times and wave mesocosm conditions across the tested factors, one replicate core for each site and vegetation state (vegetated *vs.* bare) was treated each time in a randomized block design. With 5 replicate cores per site and vegetation state, the experiment required a total of 5 wave mesocosm runs over a period of three weeks.

Sediment cores were placed on a platform elevated 17 cm from the bottom from which they exposed to wave treatment. To prevent a standing wave from developing, we combined a fast push with a slow retreat (La Nafie et al., 2012), which generates a semi-random wave spectrum (Fig. 2c). This resulted in reflecting waves but they were small enough to prevent a standing wave from forming. The resulting wave pattern, while not mimicking wind-induced waves, do mimic hydrodynamic stress similar to boat wakes. Significant wave height at the water-sediment core interface was 5.34 cm, as measured with a Druck PTX 105 1830 pressure sensor placed in front of the sediment cores. 3976 recordings were collected at a sampling rate of 25 Hz, and pressure readings were converted to water level fluctuations (η). The waves intersected the portion of the soil below the surface to enable measurement of lateral erosion of the belowground material, as we do not address wave attenuation by stems/leaves above the surface in this study.

2.4. Estimates of lateral erosion

Eroded volume loss was estimated at each of 8 time steps over the 32-h period. The time steps corresponded to the following elapsed time periods: t_0 = beginning of wave treatment, $t_1 = 15$ min, $t_2 = 1$, $t_3 = 4$, $t_4 = 8$, $t_5 = 16$, $t_6 = 24$, and $t_7 = 32$ h. Photogrammetry methods were applied to estimate erosion of the salt marsh sediments. At each time step a series of photographs (approximately 40–60 photos) of each sediment core sample were taken from several angles with a handheld compact camera (model Canon PowerShot D10) on automatic settings. To ensure consistent and high quality of photographs, use of flash was avoided. To compensate for low lighting conditions and minimize discrepancies between photos taken during day and night, portable fluorescent lights were fixed to the wave mesocosms to illuminate samples at night. The series of photographs for each sample at various time steps were translated into point clouds and georeferenced using the software VisualSFM (Wu, 2011; Wu et al., 2011). Dense reconstruction models were built with the CMVS/PMVS module within VisualSFM (Furukawa and Ponce, 2010). The point clouds were then converted into triangular meshes to build 3-dimensional models using MeshLab software (Cignoni et al., 2008). At this stage, anomalous points and possible errors could be detected and corrected by re-performing dense reconstructions and geo-referencing in VisualSFM. Point cloud processing and rasterization was then performed within MATLAB (The MathWorks, Inc., 2012) to enable calculation of volume loss between time intervals for each sediment core. We validated this photogrammetry method using volumetric displacement with mudflat sediments from the Scheldt estuary. There was excellent agreement between the actual volume loss measured with the validation samples and the volume loss estimated with the photogrammetry method (Pearson correlation coefficient = 0.99).

2.5. Above and below-ground biomass and C:N ratios

Spartina spp. stem densities were evaluated on vegetated cores. After erosion treatments in the wave mesocosms, above-ground biomass was clipped off the sediment cores, dried at 60 °C until constant weight, and weighed. The remaining sediment and the below-ground biomass were rinsed carefully over a 1 mm mesh, and the below-ground biomass was dried and weighed as above. sub-sample of the dried above-ground biomass was crushed with a mortar and pestle and analyzed for total organic C and N with an Element Analyzer N1500 (Carlo Erba). We acknowledge the potential under-estimation of biomass lost from the cores during the wave treatment. However, we minimized loss of above-ground vegetation by clipping it off non-invasively before it was completely eroded. Additionally, as we observed that root biomass in our samples tended to form a physical network that remained even when sediment was washed away, we assumed any loss of below-ground vegetation during wave treatment was minimal. C:N ratios were used as a proxy for nutrient levels in the salt marsh samples, as C:N-ratios in *Spartina* spp. leaf tissues have been shown in previous studies to reflect ambient nutrient concentrations in porewater (Tobias et al., 2014) and experimental nutrient enrichment treatments (Crain, 2007; Jiménez et al., 2011). The block design for wave treatment meant that potential variation in C:N results from different storage times would be spread out equally among all treatments.

2.6. Statistical analyses

Two-way ANOVAs were used to compare the main effects of vegetation status (*Spartina* spp. or bare), site, and their interaction on sediment properties (bulk density (g/cm^3), silt content (%), and organic matter content (%LOI). Pair-wise tests (Tukey's HSD) were used for a posteriori multiple comparisons of means among sites when significant site effects were found ($\alpha = 0.05$), in order to identify groups of sites with different sediment properties to be used in the following experimental test.

On the vegetated erosion sediment cores, we tested differences among sites in above-ground biomass, below-ground biomass, root to shoot ratio and stem density, and shoot C:N ratio, with one-way ANOVAs, specifying site as a main effect, followed by Tukey's HSD pair-wise tests when significant site effects were found.

To obtain estimates of erosion of the sediment cores, we fitted an exponential model to the experimental data of volume loss, to obtain maximum possible volume loss over time, in the form of $E = Q(1\text{-}e^{-kt})$, where E = volume eroded, Q = maximum volume loss, k = rate of volume loss, and t = time in hours. Fitted values of Q corresponded very closely to our values of volume loss estimated by photogrammetry, obtained at the end of the wave treatment at 32 h (Pearson correlation coefficient = 0.99). We then constructed a linear mixed effects model (Pinheiro and Bates, 2000) to describe the relationship between vegetation and sediment variables and the maximum volume loss, Q, across sites. As fixed effects, we entered the categorical variables vegetation (vegetated, bare), silt content (high and low silt content), and their interaction into the model. As random effects, we considered site and the sequence of wave treatment (block). The random block effect introduced negligible variation in model results, as demonstrated by comparing models with and without the block effect, so was subsequently dropped from the overall model. Variance partitioning was examined by employing marginal R^2 values (percent variation explained by fixed effects) and the conditional R^2 values (percent variation explained by both fixed and random effects) (Nakagawa and Schielzeth, 2013). Final model selection was based on the lowest Akaike's information criterion (AIC) and p-values ($\alpha = 0.05$) from stepwise modeling. The significance of fixed effects was tested using Type II Wald Chi-square tests.

On the vegetated sediment cores, we then modeled the effect of above-ground biomass, below-ground biomass, root to shoot ratio, stem density, and shoot C:N ratio on Q (maximum erosion). We again entered site as a random effect, and removed the block effect from the model as it introduced negligible variation. Model selection and p values were obtained as described above.

R open source software was used for all statistical analyses (R Core Team, 2017). Two-way and one-way ANOVAs and Tukey's HSD post-hoc tests for significant effects ($\alpha = 0.05$) were performed within R. The *nlme* (Pinheiro et al., 2017) package was used for the non-linear modeling. The *lme4* package (Bates et al., 2014) was used for the linear mixed modeling, and associated R^2 values were obtained with the *MuMin* package (Bartoń, 2016). Normality and homogeneity of the data were visually checked in the residual plots, and, when necessary, data were transformed to comply with assumptions of homogeneity of variance (see supplementary tables for details).

3. Results

3.1. Sediment properties and vegetation characteristics across sites

Silt content largely differed among sites (Fig. 3, Tables S1 and S2, $F_{(5,36)} = 22.00$, $p < 0.001$). CHI, GRA and GOLF (hence referred to as silty sites) were characterized by predominantly silty soils in both vegetated and bare areas (average 71.69%, $SD = 10.95$, $n = 24$), while BEL, ROS and VAL (hence referred to as sandy sites) had a much lower mean silt content (17.83%, $SD = 10.05$, $n = 24$). The % silt was more variable in vegetated than in bare areas in the predominantly sandy sites. Vegetation status marginally affected the mean sediment % silt content, with higher mean % silt in vegetated *vs.* bare samples ($F_{(1,36)} = 3.32$, $p = 0.076$). The effect of vegetation on silt content was higher in the sandy *vs.* silty sites. Bulk density and organic matter were also variable among sites (Fig. 3, Table S3, $F_{(5,36)} = 7.04$, $p < 0.001$ for bulk density, $F_{(5,36)} = 8.29$, $p < 0.001$ for organic matter). Bulk density ranged from 1.11 to 1.32 g cm^{-3} in the sandy sites and 0.85–1.32 g cm^{-3} in the silty sites, and organic matter ranged from 2.7 to 5.1% LOI in the sandy sites and 1.5 to 7.0 in the silty sites (Table S3). Vegetation status did not significantly affect bulk density or organic matter ($p > 0.05$).

Vegetated sediment erosion cores did not differ in above-ground biomass or stem density between sites, whereas significant differences were found in below-ground biomass (Fig. 3, Table S4, $F_{(5,24)} = 2.95$, $p < 0.05$). There was lower below-ground biomass in CHI compared to ROS ($p < 0.05$, Table S5). A significant difference in root:shoot-ratio was also found among sites (Fig. 3, Table S4, $F_{(5,24)} = 5.15$, $p < 0.01$) with CHI having significantly lower root:shoot-ratio than ROS and GOLF ($p < 0.05$ in both cases, Table S5). C:N-ratios of *Spartina* spp. shoots differed across sites (Fig. 3, Table S4, $F_{(5,24)} = 2.95$, $p < 0.05$), with BEL having a significantly lower mean C:N-ratio of 11.82 than ROS (mean C:N-ratios of 18.48) ($p < 0.05$ for both cases, Table S5).

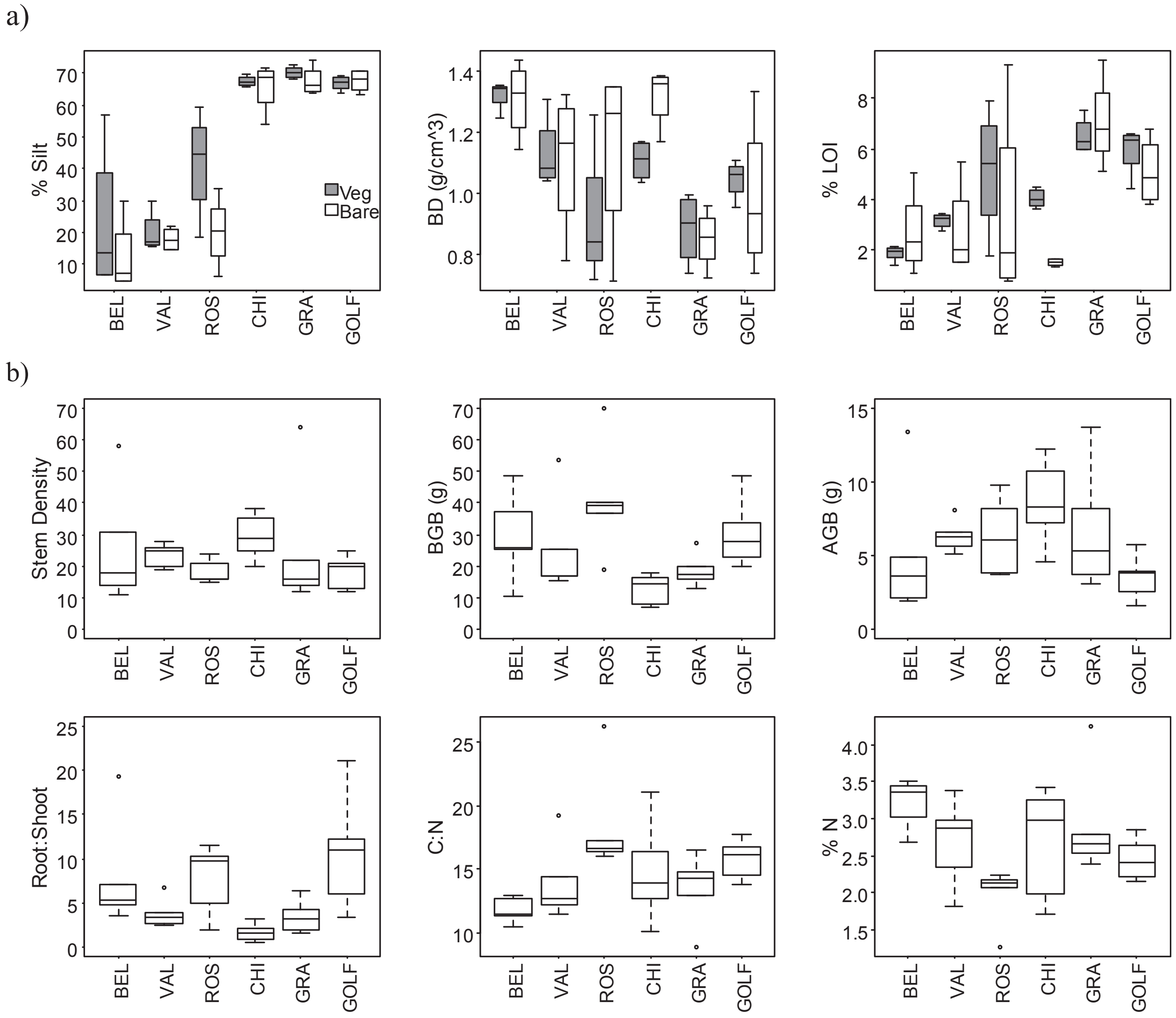

Fig. 3. a) Boxplots describing sediment properties (% Silt, bulk density (g·cm^{-1})), and organic matter (% LOI (loss on ignition)) at the 6 study sites (labels as in Fig. 1) across the Northern Adriatic coast, Italy, over 4 replicates. For each site we sampled both vegetated (shaded bars) and bare (un-shaded bars) areas. Horizontal lines are medians, boxes show the interquartile range (IQR), error bars show the full range excluding outliers, defined as 1.5 x IQR (plotted as points); and b) Boxplots describing vegetation properties evaluated on each of the vegetated sediment cores collected for erosion analysis, over 5 replicates (boxplot descriptions as above): Density of *Spartina* spp. vegetation (Stem Density), below-ground biomass (BGB in g), above-ground biomass (AGB in g), root to shoot ratio (RootShoot), C:N ratios in *Spartina* spp. shoots (C:N), and total percent nitrogen in *Spartina* spp. shoots (% N).

3.2. Effect of vegetation and sediment properties on erosion

Sites had highly variable volume loss at 32 h of wave treatment (Fig. 4). In the silty sites, CHI, GRA and GOLF had mean volume losses of 1.2%, 11.3 and 8.9% respectively, for vegetated cores, and 5.7%, 8.1% and 57.5%, respectively, for bare cores. In the sandy sites, BEL, VAL and ROS had mean volume losses of 27.6%, 13.7% and 7.5%, respectively, for vegetated cores, and 79.3%, 90.6% and 83.2%, respectively, for bare cores. Overall, mean volume loss in vegetated cores was 16.26% ($n = 15$, $SD = 25.21$) for sandy cores and 7.11% ($n = 15$, $SD = 5.90$) for silty cores, and in bare cores, volume loss was 84.37% ($n = 15$, $SD = 26.39$) sandy cores and 23.44% ($n = 15$, $SD = 33.32$) in silty cores.

The mixed effects linear model with the best fit yielded a highly significant effect of vegetation, grain size and their interaction on the total eroded volume (Table 1). Erosion was lower in vegetated *vs.* bare sediment cores ($\chi^2(1) = 46.7$, $p < 0.001$), and was also lower in cores with high silt *vs.* low silt content ($\chi^2(1) = 23.8$, $p < 0.001$). The difference in erosion between vegetated and bare cores was higher in sandy *vs.* silty soils ($\chi^2(1) = 15.97$, $p < 0.001$). The marginal R^2 value (variation related to fixed effects) was 0.59, and the conditional R^2 value (variation related to both fixed and random effects) was 0.62, indicating that most of the variation in erosion was explained by the fixed effects.

The most parsimonious model exploring the effect of vegetation and nutrient variables on erosion incorporated only the amount of below-ground biomass as a significant predictor of erosion ($\chi^2(1) = 7.54$, $p < 0.01$). Stem density, above-ground biomass, root:shoot ratios and C:N-ratios were not significant predictors of erosion, when considered alone or in any combination of variables. By plotting below-ground biomass as function of erosion, the effect of below-ground biomass on reducing erosion can be seen across all

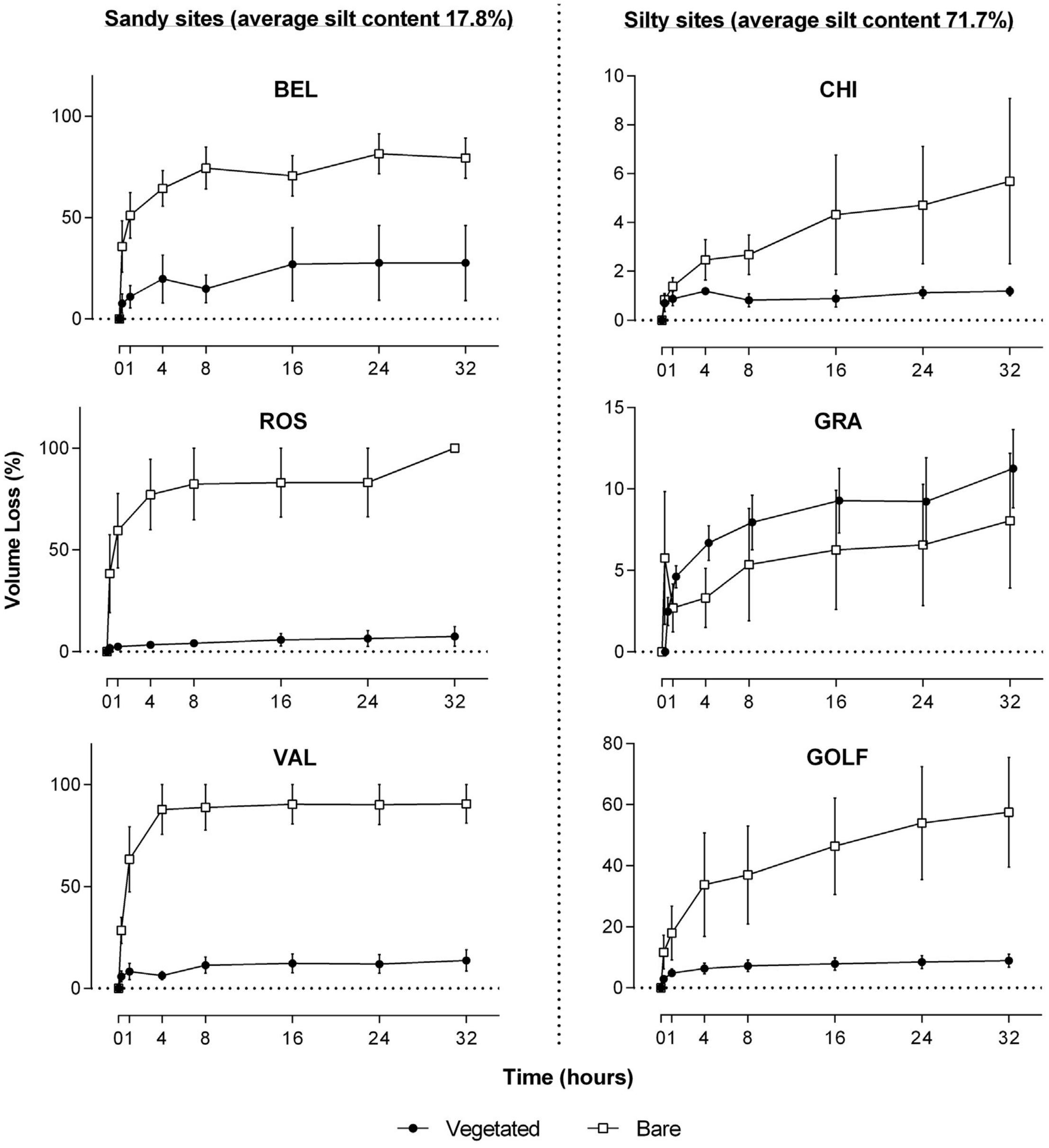

Fig. 4. Volume loss (erosion) estimated over each time step, in sites with low silt fractions (shown in left column), and in sites with high silt fractions (shown in right column). Non-vegetated sediment cores (Bare) are marked for each site with open circles, and vegetated sediment cores (*Spartina* spp.) are marked with filled circles. Error bars denote standard error of the mean over 5 replicates.

Table 1

a) Set of linear mixed effects models on erosion of vegetated and bare sediment cores, with site included as a random term in each model ($n = 60$). The most supported model is marked in bold. Dependent variable codes: Veg = bare or vegetated, Silt = low silt or high silt. a_x = coefficients, $-2\ln L = -2\log$ likelihood, AIC = Akaike's information criterion, DF = degrees of freedom (residuals). * = significant effects of model terms ($p < 0.05$). Model p-values are in comparison with previous model in iteration. b) Linear mixed model on erosion of vegetated cores, with site included as a random term ($n = 30$). BGB = below-ground biomass; other variable codes as above.

Model		a_0	a_1	a_2	a_3	$-2\ln L$	AIC	DF	P
a)	**Vegetated and bare sediment cores**								
	$a_0 + (a_1)$Veg*	52.82	−41.63			−287.4	582.9	56	
	$a_0 + (a_1)$Veg* + (a_2)Silt*	70.03	−41.63	−34.43		−283.4	575.3	55	<0.01
	$a_0 + (a_1)$Veg* + (a_2)Silt* + (a_3)Veg*Silt*	**82.20**	**−65.96**	**−58.76**	**48.66**	**−275.7**	**563.3**	**54**	**<0.01**
b)	**Vegetated cores**								
	$a_0 + (a_1)$BGB*	27.19	−0.61			−0.61	263.2	26	

sites, except CHI, with the strongest effects seen in the sites with predominantly sandy soils (BEL, ROS and VAL), as indicated by the steeper slopes (Fig. 5).

4. Discussion

Our wave mesocosm experiments showed that vegetated salt marsh sediment cores had higher resistance to lateral erosion compared to bare sediment cores. Vegetation reduced volume loss by 80% in sandy soils and 17% in silty soils, revealing the critical role of salt marshes in coastal defence. The below-ground component of vegetation was found to be the most significant predictor of erosion, while the contribution of the above-ground variables (above-ground biomass, stem density, shoot:root ratio) was marginal. Sediment properties also affected erosion rates in the absence of vegetation, with mean lateral erosion 72% lower in silty *vs.* sandy soils.

The relationship between erosion and below-ground biomass was stronger in sandy sites, but still evident in the silty sites, with the exception of CHI, where there was no clear effect of below-ground biomass on erosion. A broad-scale genetic survey of the Adriatic Coast that was completed after our erosion experiment revealed that the area from which we extracted erosion cores in CHI is most likely to be *S. maritima*, whereas the erosion cores in the other sites that we sampled were most likely to be a mix of non-native *Spartina* species (Wong, 2015). Our photographs of the experimental erosion cores did not allow us to unequivocally attribute the vegetation to the different species, and therefore we could not formally explore if there were any differences in erosion control among different *Spartina* species, but further research on this topic is certainly warranted. Morphological differences that have been found, for example, in above-ground biomass (Strong and Ayres, 2013), could alter the way in which wave energy is dissipated, as leaf stiffness, stem density and biomass of salt marsh vegetation have been demonstrated to influence wave attenuation (Bouma et al., 2005; Heuner et al., 2015). Differences in root morphology and architectural traits could also alter soil stabilization properties (Ghestem et al., 2014). Potential differences in the diversity and functional groups of faunal communities associated with different *Spartina* species may have consequences for sediment stability as well. It is well-documented that bio-stabilizing inter-tidal organisms such as benthic microalgae can enhance cohesiveness, for example, via secretions of biofilms and thus alter the sediment critical erosion threshold; while bio-destabilizers such as bioturbating infauna can enhance erosion (Van Colen et al., 2014, 2012; Widdows and Brinsley, 2002). Thus, differences in bio-stabilizing and bio-destabilizing communities that may result from encroachment of non-native *Spartina* species have the potential to affect its ability to resist lateral erosion.

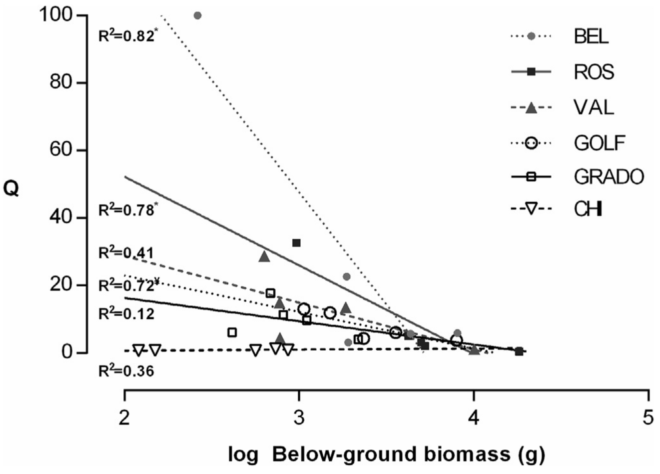

Fig. 5. Scatterplot of maximum erosion, Q (% maximum erosion), *vs.* log-transformed below-ground biomass (g) for each site (site labels as in Fig. 1). Points in the scatterplots represent each of 5 replicates. Regression lines depict the linear relationship between Q and log-transformed below-ground biomass for each site, with R^2 values shown adjacent to each regression line (order of regression lines, top to bottom, follows site legend order). * = significant ($p < 0.05$) and ¥ = marginally significant ($p < 0.1$).

With regard to the role of sediments in lateral erosion, we report that erosion resistance in the bare cores were significantly enhanced by silt content, with mean erosion 71.5% lower in silty *vs.* sandy soils. This supports other studies, e.g., Houwing (1999) determined in an *in-situ* flume experiment that erosion in mudflats bordering salt marshes on the Dutch Wadden Sea coast decreased with higher mud content (which correlates with silt content). In another flume experiment, Ford et al. (2016) found that both the clay-silt fraction and salt marsh vegetation root biomass (Essex and Morecambe Bay, UK) were significant predictors of erosion in addition to other factors such as plant diversity, percent vegetation cover and carbon stock, although interactive effects of these factors were not tested. Our model results demonstrate significant interactive effects of vegetation status (vegetated *vs.* bare) and sediment silt content on erosion. We note the caveat that we measured sediment properties to a depth of 8 cm; however the erosion cores had a depth of 15 cm. Therefore, the sediment properties we sampled may not be representative of the entire erosion core, which is a potential source of error in our analysis. Nonetheless, we believe that the magnitude of difference in % silt between sites is large enough to allow for a general classification of sites as either sandy or silty.

In a similar wave mesocosm experiment, Feagin et al. (2009) concluded that sediment type was the most important factor controlling erosion, while vegetation affected erosion indirectly by modifying sediment properties. In the bare sediment core samples, we found significant effects of silt content on erosion. However, we did not find significant differences in bulk density or organic matter between vegetated and bare areas, and only marginal effects of vegetation on silt content. This suggests a more direct control of the vegetation on erosion rates rather than an indirect one mediated by changes in sediment characteristics as suggested by Feagin et al. (2009). As our results indicate root biomass to be most important in decreasing lateral erosion in salt marsh sediment cores with higher sand content, we expected an even stronger effect of root biomass in reducing erosion in the study by Feagin et al. (2009), where core samples had relatively higher sand content compared to our core samples (R. Feagin, personal communication, June 19, 2016). We speculate that the difference in our findings may be due to the selection of the locations where the bare sediment cores were collected. In our case, samples were collected from the pioneer zone, where pioneer vegetation species such as *Salicornia* spp. or *Spartina* spp. would not yet have influenced sediment characteristics, whereas Feagin et al. (2009) also sampled bare areas within the marsh interior (Galveston Island, Texas), where sediment characteristics may have been influenced by marsh formation over a longer period of time.

Contrary to our expectations, C:N ratios were not good predictors of erosion rates. Previous studies show that soil stability can decrease with higher nutrient loading rates as a result of increased soil metabolism and lower root and rhizome biomass (Turner, 2011; Turner et al., 2009). Deegan et al. (2012) found that nutrient addition led to an increase in above-ground biomass, decrease in below-ground biomass and increased microbial decomposition resulting in increased fine detrital organic matter. Finer-grained, less consolidated creek banks retained more water at the low tide, which increased pore water pressure and reduced frictional shear strength of soil, ultimately resulting in creek-bank collapse and subsequent conversion to non-vegetated mud (Deegan et al., 2012). A possible explanation of our result is that the study lagoons along the Adriatic Sea were all relatively nutrient-rich. The *Spartina* spp. tissue C:N-ratios in our sediment core samples ranged from 10 to 22, with significantly lower values found in BEL— the site where vegetated samples also experienced the highest mean amount of erosion, a pattern which persisted throughout the course of wave treatment (Fig. 5). The range of C:N-values in our sites is even lower than the range of C:N-values in *Spartina* spp. samples treated with fertilizer in Crain (2007) and Jiménez et al. (2011), who reported C:N-values of 25–30 and 20–25, respectively, while control samples in both studies had C:N-values ranging from 40 to 45.

GOLF had the highest erosion rates among the bare sediment cores with high silt content. In line with the above-mentioned studies, higher erosion in GOLF was expected to be due to high nitrate availability due to its proximity to a golf course. However, in contrast to our expectations, C:N-ratios were not found to be significantly lower in GOLF compared to other sites. The observed high erosion rate may be related to the higher sediment organic matter found in GOLF. Higher mean fine detrital organic matter (1–3 mm) was also found in nutrient-enriched salt marshes in Deegan et al. (2012), where higher observed erosion was attributed in part to accelerated decomposition in the presence of high nitrate levels, resulting in higher water retention and decreased structural integrity. However, in Feagin et al. (2009), fine, humic organic matter was found to reduce wave-induced erosion. Both studies found that erosion was enhanced by coarse organic material. This suggests that the role of organic matter in erosional processes is not just dependent on size fraction, but also on nutrient availability. The fact that vegetated sediment cores in GOLF experienced less erosion than the bare sediment cores suggest that even in silty soils with high ambient nutrient conditions, *Spartina* spp. vegetation still prevented erosion presumably through sediment binding.

5. Conclusions

Our study provides novel insight into the interactive effect of vegetation and sediment characteristics on the ability of salt marshes to resist lateral erosion. It also contributes to quantifying coastal ecosystem services provided by salt marsh vegetation, using novel photogrammetry methods, and highlights the critical role of the *Spartina* spp. root system in decreasing lateral erosion. Erosion was higher in bare *vs*. vegetated sediment cores, particularly in sites with low silt content, and below-ground biomass was negatively related to erosion. Although the effect of vegetation on erosion resistance was less prominent in siltier sediments, it was still significant. Given that multiple stressors are driving the loss of native *S. maritima* vegetation in the Northern Adriatic Sea and its replacement by non-native *Spartina* spp. and *Salicornia* succulents (Strain et al., 2017), further research should explore the potential effects of these habitat shifts on the system functional properties. Further work should also explore the potential of using these salt marshes in ecosystem-based approaches to coastal defence, and identifying conservation and coastal management practices to help preserve their functioning and contribution to coastal defence under rapidly changing conditions.

Acknowledgements

This work was funded through a MARES Grant (EU- 512002-1-2010-1-BE-EMJD) (www.mares-eu.org) and project TETRIS (PRIN 2010-2011, MIUR). MARES is a Joint Doctorate programme selected under Erasmus Mundus and coordinated by Ghent University (FPA 2011-0016). CVC acknowledges a postdoctoral fellowship provided by the Flemish Fund for Scientific Research (FWO-1.2.380.11.N.00). The authors are grateful to members of the Airoldi Lab (University of Bologna): Elena Piccioni, Joanne Wong, Stefanie Broszeit, Lorenzo Gadoni, Giulia Finotti; the Spatial Ecology department (NIOZ) including Jeroen van Dalen, Heng Wang and Zhu Zhenchang; Bart Beuselinck from the Research Group Marine Biology (UGent) and Ignacio Barbeito Sanchez for assistance at various stages of this research. We thank the Po Delta, Rosolina and Veneto Parks, Ferruccio Polo (Cooperativa Pescatori di Grado) Valle Artalina, and Golf Club Grado for access to the sampling sites. We also thank two anonymous reviewers whose thorough reviews and suggestions greatly improved this manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.09.006.

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