Seed fate in space and time

Implications for regeneration of coastal wetlands

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2017

ISBN: 978-94-6295-564-6
Cover design: Proefschriftmaken.nl
Printed by: Proefschriftmaken.nl
Cover photograph copyright: Zhenchang Zhu
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Seed fate in space and time:
Implications for regeneration of coastal wetlands

Proefschrift

ter verkrijging van de graad van doctor

aan de Radboud Universiteit Nijmegen

op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,

volgens besluit van het college van decanen

in het openbaar te verdedigen op maandag

20 maart 2017

om 12.30 uur precies

door

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Chapter 1

1. General introduction

1.1 Seed fate

As the products of sexual reproduction, seeds are the means by which most vascular plants produce genetically diverse offspring and move across landscapes (Nathan and Muller-Landau 2000, Vander Wall et al. 2005). In recent years, 'seed fate' has become a term widely used to depict what happens between seed production and seedling establishment (Forget et al. 2005), including seed dispersal and post-dispersal outcomes (e.g. seed removal, predation and germination). Since natural selection act during all stages of a plant's life cycle (Harper, 1967; Janzen, 1969), a detailed knowledge of seed fates is critical to the understanding of the demography and evolution of plants.

Although scientists have long been interested in the ecology and function of seeds (Ridley 1930), it is only since the mid-1970s that studies of the dynamic role seeds play in plant demography has commenced (Vander Wall et al. 2005). Early interest primarily focused on pre-dispersal seed mortality, morphological adaptation for dispersal and the behaviour of animal dispersers (Vander Wall et al. 2005). Over the recent two decades, there has been remarkable growth of publications on the dispersal of seeds, with increasing recognition that the dispersal of seeds is often a complex, multi-step process that can involve more than one dispersal agent (Chambers and Macmahon 1994, Vander Wall and Longland 2004, Forget et al. 2005, Schupp et al. 2010).

Common dispersal modes include abiotic forces (e.g. wind and water flow), inadvertently or purposely by animals, and these varied means of dispersal are associated with adaptations of seed/fruit that facilitate dispersal (Chambers and Macmahon 1994, Vander Wall and Longland 2004). For many plant species, seeds are initially deposited on the ground surface, followed by secondary displacement to an alternate location, and eventually by seed burial that adds them to the soil seed bank (Chambers and Macmahon 1994, Vander Wall and

Longland 2004). Seeds are often transported primarily by one dispersal vector and secondarily by an alternative dispersal agent, with seed loss occurring at every step (Fig.1.1, Chambers and Macmahon 1994, Vander Wall and Longland 2004). However, most studies on seed dynamics did not fully account for the diverse fates and various pathways that a population of seeds might follow between production and germination (Vander Wall et al. 2005).

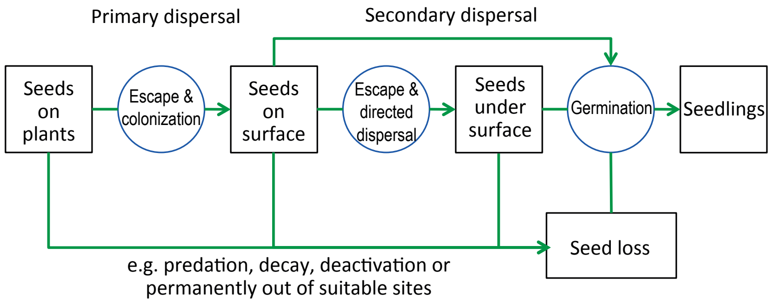

Fig.1.1 A generalized diagram (adapted from Vander Wall and Longland 2004) illustrating seed fate pathways from seed production until seedling emergence. Rectangles represent physical states, arrows represent a change in state, and circles represent the benefits resulting from dispersal.

Colonization by seeds controls the entry of plant species into ecosystems. Seed dispersal controls the migration ability of plant species in a changing world, and the fate of dispersed seeds determines the consequence of a dispersal/migration event. Therefore, a full understanding of seed fate in response to varying environmental conditions opens a window through which we can foresee the future of a plant, with far-reaching implications for population and community dynamics, as well as species migration/invasion and long-term persistence in a global change context (Levin et al. 2003, Levine and Murrell 2003, Ibáñez et al. 2006). Such information may be especially relevant for globally degraded ecosystems like coastal wetlands (Gedan et al. 2009, Barbier et al. 2011), given that their rejuvenation strongly depends on sexual reproduction sensitive to environmental changes (Friess et al. 2012, Balke et al. 2013, Hu et al. 2015).

1.2 Importance of coastal wetlands

Coastal wetlands e.g. salt marshes, seagrasses & mangroves (Fig.1.2) are rated as the most ecologically and socio-economically valuable systems on the globe (Costanza et al. 1997), due to numerous ecosystem services they provide such as carbon storage, biodiversity conservation, nursery function, water purification, flood and shoreline protection, and recreation (Zedler and Kercher 2005).

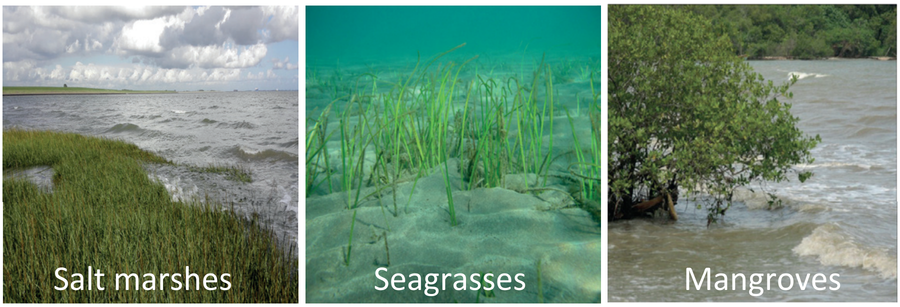

Fig.1.2 Photos of salt marsh[1,] seagrass[2] and mangrove[3]

Recently, there has been increasing recognition of the value of coastal vegetation in climate change mitigation and adaptation. Although they cover only 0.2% of the ocean surface, coastal vegetated systems contribute 50% of carbon burial in marine sediments (Duarte et al. 2005, McLeod et al. 2011). As ecosystem engineers (organisms that modify their habitat, *sensu* Jones et a., 1994), coastal vegetation can dissipate wave energy, stabilize sediment and increase the intertidal height, buffering the impacts of rising sea level and increasing storminess (Gedan et al. 2011, Duarte et al. 2013, Temmerman et al. 2013, Moller et al. 2014). This initiated a paradigm shift to a more ecosystem-based coastal protection using vegetated foreshores (Cheong et al. 2013, Duarte et al. 2013, Temmerman et al. 2013). This is especially significant in the face of environmental pressures, e.g. sea level rise and increasing storm frequencies, arising from climate change, alongside ever-increasing coastal population densities (Gedan et al. 2011, Duarte et al. 2013, Temmerman et al. 2013).

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[1] Photo taken by Zhenchang Zhu

[2] Source: http://fernandotuya.org/

[3] Source: www.conservationgateway.org

1.3 Problems of coastal wetlands in a changing globe

Due to the many ecosystem services, natural accessibility, and productivity, coastal wetlands are among the most heavily exploited natural systems across the world (Lotze et al. 2006, Worm et al. 2006, Halpern et al. 2008), and thereby have been suffering severe long-term habitat destruction resulting from human activities such as land reclamation, eutrophication, overharvesting, species introduction, commercial fisheries and aquaculture practices (Gedan et al. 2009, Barbier et al. 2011). Human disturbances and the resultant habitat decay of coastal wetlands cause reduction of biodiversity and ecosystem functions, poor water quality, increased biological invasions and decreased coastal protection from flooding and storm events (Cochard et al. 2008, Koch et al. 2009, Barbier et al. 2011). These threats emphasize the need for fundamental insights into how to manage and conserve existing coastal wetlands as well as in restoring and/or facilitating the creation of new ones.

Among coastal wetlands, salt marshes are the most abundant, fertile, and accessible coastal habitats on earth, yet suffering the most severe habitat deterioration (Gedan et al. 2009, Barbier et al. 2011). 50% of salt marshes are degraded worldwide, while it is 35% for mangroves and 29% for seagrasses (Valiela et al. 2001, Orth et al. 2006a, Barbier et al. 2011). Worse still, the loss of salt marsh habitat is expected to continue due to sea level rise and 'coastal squeeze'. The former may cause drowning and dieback of salt marshes (Gedan et al. 2009) if sediment accretion fails to keep pace with sea level rise because of sediment starvation (Cahoon 2006). Coastal squeeze ensues when marshes are squeezed out of the ecosystem when seawalls prevent marshes to migrate upward into the tidal range (Winn et al. 2003). Moreover, recent modeling studies indicate that sea level rise may also enhance the risk of lateral erosion (Mariotti and Fagherazzi 2010, Marani et al. 2011). The vulnerability of coastal marshes to environmental variations like sea level rise (Kirwan and Temmerman 2009, Kirwan and Megonigal 2013) and waves (Callaghan et al. 2010, Marani et al. 2011) raises the uncertainty over long-term ecosystem persistence in a changing climate. Such an uncertainty hampers the application of vegetated wetland ecosystems within coastal defense schemes (Bouma et al. 2014).

1.4 Importance of seedling establishment in coastal wetlands

Within the context of restoration/creation of coastal wetlands, a major issue that needs to be resolved is identifying and unraveling key processes governing seedling establishment (Bouma et al. 2009, Friess et al. 2012). The development of coastal wetlands like salt marshes often starts with pioneer species colonizing a bare intertidal flat through seedling establishment (Friess et al. 2012). Seeds also play a key role in the undesired fast spread of invasive coastal plants. The best-known example is cordgrass (*Spartina* spp.), whose rapid expansion through seedling recruitment (Fig.1.3) has been seen in some marshes of western US, Europe and China (Gray et al. 1991, Nehring and Hesse 2008, Strong and Ayres 2013).

Moreover, pioneer seedling establishment is also essential for the long-term dynamics of coastal wetlands. For instance, salt marshes are dynamic systems with cyclic alternations between a retreating phase of cliff erosion and an establishment phase by seedling recruitment (van de Koppel et al. 2005). Despite the critical role of sexual reproduction in vegetation initiation and long-term persistence in coastal wetlands, we still lack mechanistic insights into the processes that enable/disable seedling establishment in tidal flats (Bouma et al. 2009, Friess et al. 2012).

Fig.1.3 Establishing cordgrass seedlings on the mudflat; Photo taken by Zhenchang Zhu

1.5 Knowledge gap and research question

Seedling recruitment on tidal mudflats is often problematic (Bouma et al. 2009, Friess et al. 2012), despite being occasionally highly successful, as found for instance during the fast expansion of invasive cordgrass species (e.g. Gray et al. 1991, Ayres et al. 2008, Xiao et al. 2009), Seedling establishment can be limited by seed processes e.g. seed production, dispersal and survival, and/or seedling processes e.g. seedling emergence and survival (Friess et al. 2012). Previous studies suggest seedling establishment failure on tidal flats is mainly due to low seedling survival caused by physical stressors like salinity (Dethier and Hacker 2005, Engels et al. 2011) and disturbance events such as wave action and sediment erosion (Schwarz et al. 2011, Balke et al. 2013).

The recently developed window of opportunity (WoO) concept (Balke et al. 2011) stresses that seedling establishment success requires a period of free/low disturbance for the anchorage and survival of seedlings (Balke et al. 2014, Hu et al. 2015). However, opportunity only favors the 'prepared' seeds that arrive/are present at right location/position at the right time (Crooks et al. 2002, Greve et al. 2005, van Loon et al. 2007, Dixon et al. 2008), underlining the relevance of seed arrival and seed persistence in seedling establishment in coastal wetlands. Nevertheless, there is a lack of understanding of seed fate pathways including seed dispersal and post seed dispersal fate in coastal habitats.

In this thesis, I question to what extent, and in which way, seedling establishment in dynamic coastal habitats is limited by seed dispersal and post-dispersal fate. To address this research question, I employed cordgrass (*Spartina* spp.), one of the common coastal foundation marsh plants (Strong and Ayres 2009, Strong and Ayres 2013), as a model system (see 1.4, Fig.1.4). To achieve a more comprehensive understanding of seed fate of cordgrass, I studied the pathways from primary seed dispersal to seed-seedling transition (see 1.5, Fig.1.5). I aim at developing a more holistic knowledge on bottlenecks and thresholds to salt marsh pioneer establishment, which is an important step towards the conservation and sustainable management of valuable but threatened coastal vegetated ecosystems.

1.6 Model system

Over the last two centuries, a major change to many coastal ecosystems around the world has been the deliberate and accidental introduction of cordgrass species to saltmarsh and mudflat habitats as engineering species that stabilize the foreshores (Strong and Ayres 2009, Strong and Ayres 2013). Currently, such species are globally defining the shoreline of many temperate marshes, while still extending their global territory in some coastal habitats as invasive plants (Strong and Ayres 2009, Strong and Ayres 2013). Seed colonization plays a critical role in their natural (re-) establishment and range expansion, as well as the fast spread in many areas where the species has been willingly or accidentally been introduced (Gray et al. 1991, Ayres et al. 2008, Xiao et al. 2009).

Seedling establishment of cordgrass (Fig.1.4) occurs mainly in the pioneer zone and adjacent bare mudflats at suitable elevations, provided an annually built soil seed bank is present (Wolters and Bakker 2002, Xiao et al. 2009). Given the role of cordgrass as powerful ecosystem engineers globally and as invaders in various coastal regions (Strong and Ayres 2013), it is highly valuable to study seed fate pathways of cordgrass.

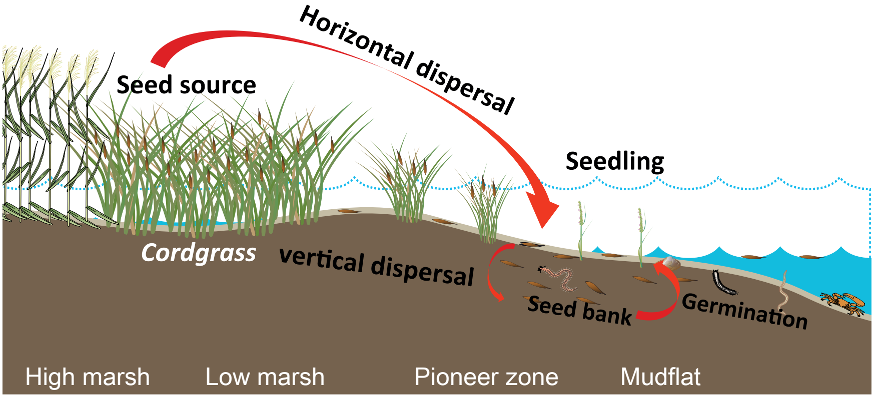

Fig.1.4 Schematized seed dispersal and seedling establishment processes of cordgrass

1.7 Thesis outline

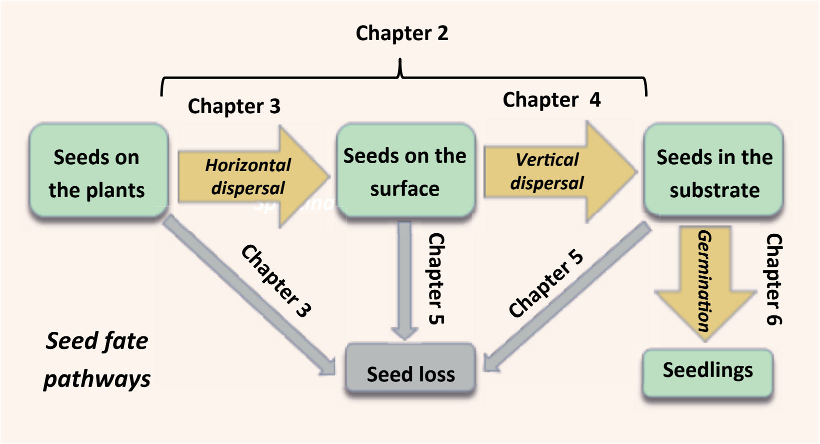

Fig. 1.5 Research diagram and relations with thesis chapters

In chapter 2, I identify the relative importance of seed delivery and seed persistence in limiting seed bank abundance on the tidal flats fronting a marsh, using field survey and manipulative experiments in the field.

In chapter 3, I investigate how maternal effects and their interactions with heterogeneity of dispersal agents (i.e. tide) shape effective primary seed dispersal (seaward transport of viable seeds), by linking seaward transport of viable seeds to maternal seed production patterns and tidal variability.

In chapter 4, I move to vertical seed transport, examining the importance of physical forcing and the interactions with soil bioturbators for seed burial and seed bank formation, using simplified but well-controlled flume experiments.

In chapter 5, I further quantitatively relate seed bank persistence on tidal flats to local wave disturbance and associated sediment disturbance, through *in situ* measurements and manipulative seed bank experiments along wave gradients. The importance of soil biota was also examined with a defaunation treatment.

In chapter 6, I eventually turn to seed-seedling transition by elucidating the impacts of a common seed-collecting animal in tidal flats on seed persistence, viability and seedling emergence, using a series of mesocosm experiments.

Chapter 2

2. Seed arrival and persistence at the tidal mudflat: identifying key processes for pioneer seedling establishment in salt marshes

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Published in 2014: *Marine Ecology Progress Series*: 513, 97-109

Abstract

Salt marshes are highly valuable ecosystems that provide numerous important ecosystem services. Given the global marsh decline, there is a pressing need to understand the natural bottlenecks and thresholds to their establishment and long-term ecological maintenance. Seed presence in the right place and time is a prerequisite for pioneer establishment. We performed field surveys and manipulative seed bank experiments on 2 mudflats with different levels of exposure in the Westerschelde, The Netherlands, to identify patterns of seed arrival and persistence and their role in determining seed bank abundance. Seed arrival decreased with distance from the seed-source area, i.e. the salt marsh, resulting in seeds being deposited on the mudflat close to the marsh edge. Once arrived, most of the surface seeds were washed away during subsequent tides, with retention of <20%. However, seed burial enhanced seed retention nonlinearly with increasing burial depth. Predation was not a major cause of seed loss. These results point to the importance of hydrodynamic and morphodynamic processes to both lateral seed dispersal and vertical seed burial on tidal flats and, by this, suggest that salt marsh establishment and expansion are closely linked to the physical dynamics of the entire estuarine system.

2.1 Introduction

Salt marshes are highly valued for their numerous ecosystem services such as carbon storage, biodiversity conservation, nursery function for fish, water purification, flood and shoreline protection, and recreation (Zedler and Kercher 2005). Unfortunately, these ecosystems have been suffering severe and long-term habitat losses due to increasing anthropogenic impact worldwide (reveiwed by Gedan et al. 2009). This loss is expected to continue due to sea level rise and 'coastal squeeze'. The former may cause drowning and dieback of salt marshes (reviewed by Gedan et al. 2009) if sediment accretion fails to keep pace with sea level rise because of sediment starvation(Cahoon et al. 2006). Coastal squeeze ensues when marshes are squeezed out of the ecosystem when seawalls prevent marshes from migrating upward into the tidal range as the sea level rises (Winn et al. 2003) Moreover, recent modeling studies indicate that sea level rise may also enhance the risk of marsh edge erosion (Mariotti and Fagherazzi 2010, Marani et al. 2011). Another worldwide problem facing salt marshes is posed by invasive species (e.g. *Spartina* spp.), whose strong spreading capacity endangers local biodiversity and ecosystem functioning (reviewed by Gedan et al. 2009). These threats to salt marshes emphasize the need for fundamental insights into how to manage and conserve existing marshes as well as in restoring and/or facilitating the creation of new salt marshes. Within this context, a major issue that needs to be resolved is identifying and unraveling key processes governing seedling establishment (Dethier and Hacker 2005, Bouma et al. 2009, Friess et al. 2012).

Salt marsh development starts with pioneer species colonizing a bare intertidal flat. Species of the genus *Spartina*, either native or invasive, are important salt marsh pioneers worldwide (Gedan et al. 2009). Colonization of mudflats by cordgrass (*Spartina* spp.) occurs initially by dispersal and settlement of propagules (e.g. seeds, seedlings and/or rhizome fragments) that are transported by tidal currents. Once established, these propagules can develop into discrete circular domed tussocks separated by open mudflat through clonal growth(Van Hulzen et al. 2007, Balke et al. 2012), which may eventually coalesce into continuous swards (Daehler and Strong 1994, Nehring and Hesse 2008).

Among the different possible propagules that can be dispersed, seeds often play a critical role in the dispersal and establishment of cordgrass species. For example, the long-distance spread of nascent populations of *Spartina alterniflora* along the US Pacific coast was primarily driven by seedling recruitment and to a lesser extent by the dispersal of vegetative fragments (Chapter 2, Sayce 1997, Ayres et al. 2004). Likewise, seedling recruitment during the spring was reported to be crucial in colonizing the tidal mudflat, achieving a rapid range expansion rate of the invasive *S. alterniflora* at the fast accreting saltmarshes in the Yangtze Estuary (Zhu et al. 2012). Similar scenarios also occurred in NW Europe, where *Spartina anglica* spread rapidly into the intertidal zones since the forming of a polyploid hybrid around 1900 (Gray et al. 1991, Nehring and Hesse 2008). Within NW Europe, *S.anglic* has been so widespread for such a long time, that in some countries it has become a target species for nature conservation and restoration in the Natura 2000 programme (Nehring and Hesse 2008).

In spite of considerable seed yield in salt marshes, success of seedling establishment of pioneer species on tidal flats is generally low due to ecological bottlenecks (e.g. fecundity, dispersal ability) and/or thresholds imposed by physical stressors (e.g. inudation, salinity, shear stress etc.) (Bouma et al. 2009, Friess et al. 2012). Seedling establishment can be limited by seed process (e.g. seed production, dispersal and survival) and /or seedling processes (e.g. seedling emergence & survival). Previous studies mostly concerned thresholds involved in seedling emergence and survival, which was found to relate with salinity (Dethier and Hacker 2005), inundation regime (Gray et al. 1991), hydrodynamic conditions (Houwing 2000, Hammond 2002, Schwarz et al. 2011), sediment stability (Ayres et al. 2004, Bouma et al. 2013) and bioturbation (van Wesenbeeck et al. 2007, Marion and Orth 2012). Regardless of all abiotic and biotic external conditions, pioneer seedling establishment is only possible if seeds are present at the right location, i.e. at the proper elevation in the intertidal zone (Crooks et al. 2002, van Loon et al. 2007, Dixon et al. 2008) and at the correct sediment depth (Harrison 1993, Greve et al. 2005) to enable germination, and at the right time (Balke et al. 2011). Yet there is still a lack of knowledge about the dynamics of seed dispersal and seed banks of pioneer species, as well as their consequences for pioneer seedling establishment in salt marshes. Most work on seed dispersal (e.g. Rand 2000, Chang et al. 2007, Chang et al. 2008) or seed bank (e.g. Bakker et al. 1996, Wolters and Bakker 2002) in salt marshes was done on species from the higher marsh.

Like in terrestrial ecosystems, seed dispersal in salt marshes involves two phases (Chambers and Macmahon 1994). First, seeds detach from their parent plant and arrive at the ground. After this initial deposition, they can be secondarily dispersed or remain at the site and gradually be incorporated into the local soil seed bank through seed burial (Wolters and Bakker 2002). The yearly formation of a soil seed bank on the mudflat is essential for the establishment of most salt marsh pioneer plants (e.g. cordgrass), since they only have transient seed banks (Thompson and Grime 1979) in which seeds persist for < 1 year (Wolters and Bakker 2002, Xiao et al. 2009). Thus seedling recruitment of such species relies mainly on the arrival of fresh seeds and their short-term persistence in the soil seed bank. Understanding to what extent and in which way pioneer seedling establishment is limited by seed dynamics, including seed arrival and seed bank persistence, may hold the key for effective and sustainable management decisions (e.g. extension of established marshes through seedling recruitment, seed-based invasive species control) and salt marsh restoration/ creation measures (e.g. seed-based techniques to facilitate seedling establishment).

In this study, we aim to develop a process-based understanding on the role of seed arrival and persistence in determining seed bank abundance on tidal flats. Through a combination of field surveys and manipulative experiments, we studied patterns of seed arrival and persistence of cordgrass *S. anglica* at the mudflat in front of a marsh. Here, seed arrival is measured as the deposition of seeds at the sediment surface. Seed persistence is defined as the retention of seeds in the sediments; the survival in terms of viability is beyond the scope of the current study. Specifically, we address the following questions: 1) how is seed arrival influenced by the distance to the seed source? 2) How does seed persistence depend on the burial depths of seeds in the sediment and local hydrodynamic conditions (e.g. wave exposure)? 3) Is there significant loss from the seed bank due to predation?

2.2 Materials and Methods

2.2.1 Study site

To test how local hydrodynamic conditions can affect seed persistence on the tidal flats, two salt marshes with contrasting wave exposure were selected, due to their position relative to the prevailing southwesterly winds (Callaghan et al. 2010): the relatively sheltered Paulinapolder (southern bank) and relatively wind-exposed Zuidgors (northern bank) in the Westerschelde, The Netherlands (51°N, 4°E) (Fig.2.1). The Westerschelde is tide-dominated and experiences a semi-diurnal tide; the spring tidal range varies from 4.4 m to 5.5 m (Baeyens et al. 1998). The pioneer vegetation consists mainly of *S. anglica*, which was introduced to the Westerschelde in 1925 (Nehring and Hesse 2008). At both field sites, *S. anglica* forms monocultures in the seaward part of the salt marshes.

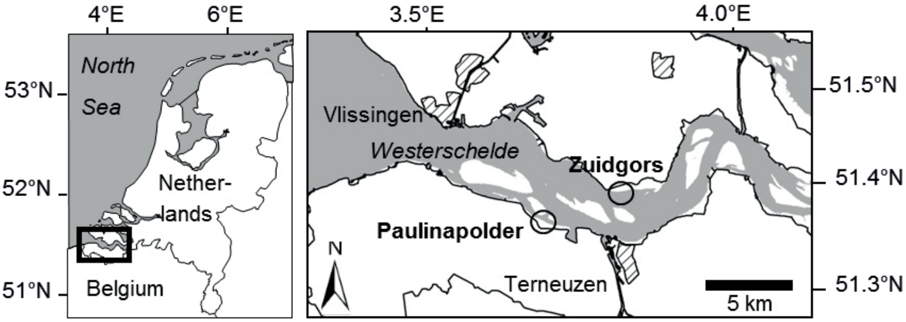

Fig.2.1 Location of Paulinapolder (relatively sheltered) and Zuidgors (wind-exposed) salt marshes in the Westerschelde (The Netherlands).

Our field survey and manipulative experiments were conducted in the pioneer zone of the marsh, where vegetation extension of pioneer species through seedling establishment can take place. This zone is characterized by scattered *S. anglica* tussocks on a predominantly bare mudflat that have suitable elevations for the seedling establishment of this species. *S. anglica* flowers from July to October and seeds ripen within 12 weeks. Seed release of *S. anglica* starts from autumn, extending to the winter and early spring of the following year (Huiskes et al. 1995). *S. anglica* germinate new seedlings in spring and may extend to summer as some seeds can remain viable in the seed bank till July, whereas no seeds survive at the end of the growing season(Groenendijk 1986). Based on this information, we ran our experiments from January to June to cover the period that seeds are dispersed and need to remain at the right location.

2.2.2 Survey of seed arrival and ambient soil seed bank

To assess seed arrival at the mudflat at both field sites, 15 AstroTurf® mats (artificial grass made from Polyethylene; Wolters et al. 2004), were deployed at 3 parallel transects located 5, 25 and 45 m in front of the marsh edge (Fig. 2.2a). These AstroTurf® mats (0.5 x 0.5 m each, Fig.2.2b) were deployed and recovered monthly from January to April 2012, with 5 replicates in each transect at an interval of ca.10 m. These mats were fixed to the ground surface with five PVC tubes perforated by steel bars to secure them from floating up.

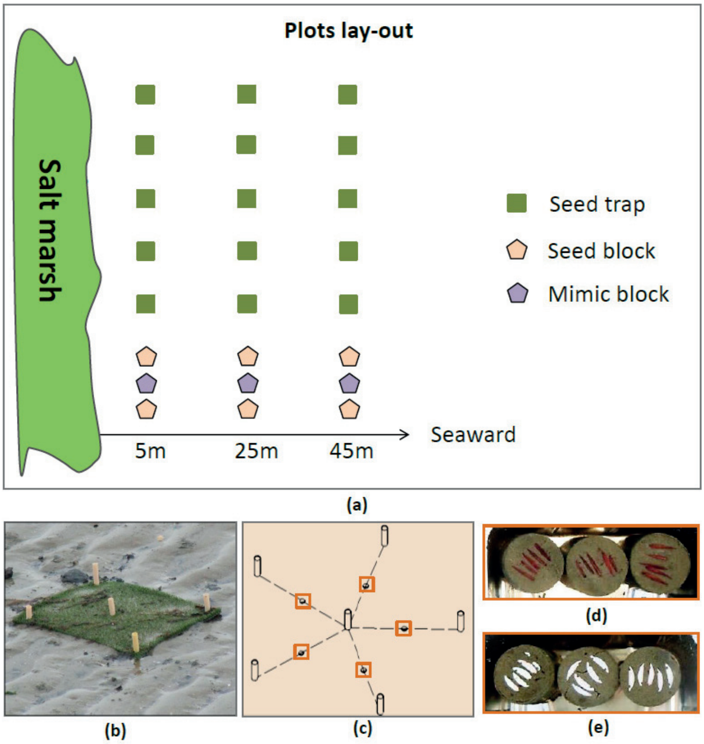

Fig.2.2 (a) Transects and experimental plots in front of the salt marsh, (b) Astroturf mat, (c) pentagon-shaped seed bank cores deployment, (d) seed bank cores with coloured (Bengal Rose) seeds, (e) seed bank cores with mimics.

To evaluate seed abundance (expressed as no. /m^2) of *S. anglica* in the ambient soil seed bank at the same locations, five sediment samples (0.25 x 0.5 x 0.05 m, Length x Width x Depth each) were excavated from the mudflat surrounding the Astroturf mats. Sampling was restricted to a depth of 50 mm because experiments showed that seeds germinating at a depth below 50 mm in the sediment have little chance to reach the sediment surface (T. J. Bouma et al., submitted). Each sampling point was between 0.5 to 2 m from each mat (Fig.2.2a), and was marked with PVC tubes after sampling, so as to avoid sampling the same spot twice. The survey of the seed bank was conducted monthly from January to June. Recovered mats and seed bank samples were rinsed and sieved in the lab through a 1mm sieve and *S. anglica* seeds were identified and counted.

2.2.3 Manipulative experiment on seed bank persistence

Manufacture of seed bank cores

To determine the pattern of seed persistence on the mudflat, a manipulated seed bank was established at the same locations as the ambient seed bank survey, by installing layered seed bank cores. These cores were made layer by layer with colored *S. anglica* seeds (Fig.2.2d) or seed mimics (Fig.2.2e) placed at different depths, simulating seeds that were just deposited on the surface or were buried to a certain depth. The application of non-digestible mimics in this experiment aimed to detect whether seed predation might occur by a common polychaete in European estuaries, *Hediste (*previously known as *Nereis) diversicolor,* as suggested by earlier studies (e.g. Emmerson 2000, Paramor and Hughes 2004). At our study sites, the density of this species at the tidal mudflats was found to range from several hundred to ca.2000 ind. m^{-2} in Paulinapolder (Van Colen et al. 2008) and 339.9 ± 78.7 ind. m^{-2} in Zuidgors (J. van Belzen et al., submitted). Mimics used were plastic chips with different colors, and of similar shape but slightly bigger size than *S. anglica* seeds.

Layered cores were created in 200ml syringes (Φ=36mm), using homogenized ambient sediment. 5 seeds or mimics were placed at the sediment surface (0 mm treatment) and at 5, 15 and 30 mm beneath the sediment surface (Fig.2.2d). To prevent seeds/mimics placed on the surface from dropping off during transporting, they were lightly pressed to the sediments. Seeds from different depth layers could be distinguished by giving them different color staining. Two types of normal ink (black or blue) and Rose Bengal (red) were employed to generate three colors. When a fourth layer was needed for the 5 mm depth, uncolored seeds were used. Each layer was frozen at a -20° before adding a new layer of sediment to minimize mixing. When all layers were ready, they were taken out of the syringes while still frozen, and kept in the freezer to facilitate placing them in the field.

Deployment and recovery

Seed bank cores were transported frozen in a foam box with dry ice. At each location, three pentagon-shaped blocks (Fig.2.2c), 3 meters apart, were set up, with the middle block for mimic cores and the other two for seed cores (Fig.2.2a). Within each block, five seed bank cores were installed. To enable accurate re-sampling the cores, they were placed through a hole (Φ=40 mm) drilled in the middle of a wooden template that in addition had both endpoints marked with holes that fitted PVC tubes inserted into the soil. The cores were deployed by first extracting a sediment core of the same depth from inside a slightly larger concentric tube, preventing the collapse of sediment into the evacuated space. Then the cores were placed with their surface level with the surrounding bottom. After 4 weeks, these cores were relocated using the same template and recovered to a depth of 100 mm, through PVC pipes (Φ=50 mm) which are larger than the original cores to ensure the whole core was re-sampled even if there is a slight imprecision in locating the re-sampling.

From January to June 2012, this experiment was repeated monthly in the field except in April. In January and February, seeds or mimics were placed at three sediment depths (0 mm, 15 mm and 30 mm), while a new depth of 5 mm was added in March and May. To detect whether any seeds had moved from the original position to the adjacent area, additional sediment samples were scooped from the surrounding 0.25 x 0.25 m area to a depth of 50 mm in March. Only seeds that were originally deposited at the sediment surface (i.e., 0 mm treatment) were found in these samples (Table 2.1), suggesting that the recovery of surface seeds could be slightly underestimated (ca. 2%) due to their movement within the close vicinity. Seeds displaced out of the 0.25 x 0.25 m area were regarded as 'lost'.

Table.2.1 Recovery of seeds/mimics in additional samples in March

Site	Location	Seeds	Mimics
Paulina	5 m	1	1
Paulina	25 m	0	0
Paulina	45 m	0	0
Zuidgors	5 m	0	1
Zuidgors	25 m	3	1
Zuidgors	45 m	1	0
Sum		5	3
Total deployed		300	150
Recovery		1.67%	2.00%

No seeds or mimics were visible on the surface of each plot when recovered. The recovered samples (depth = 100 mm) were transported to the lab, sliced every 5 mm and sieved through a 1 mm sieve to retrieve deployed seeds and mimics, which were classified as different layers according to their colors. Many seeds (but no mimics) had turned black when retrieved because of the anaerobic condition within the sediment. To identify the original color of those seeds, they were bathed in fresh water for at least 24h until their original colors re-appeared. The number of recovered seeds or mimics of each color was counted and recorded. Seed persistence in the seed bank was then calculated (Recovered/Total deployed).

2.2.4 Data analysis

To examine the effects of distance to the marsh edge ('Distance'), and timing ('Month') on seed arrival and seed bank abundance, analysis of covariance was implemented by using generalized linear models (GLMs). This was due to the non-normal errors in the count data of these two response variables. Each response variable was fitted by a GLM using family 'quasipoisson' because of overdispersion (Crawley 2007). To simplify each model, we first ran these models for each site separately with 'Distance' as the continuous variable and

‘Month’ as the categorical variable. In these models, both seed arrival and seed bank abundance showed a pattern of exponential decay with distance and the slopes did not differ with months. Thus ‘Month’ was not included in the further analysis to detect the differences 1) between seed arrival and seed bank abundance and 2) between sites. This was done by pooling data of both seed arrival and seed bank abundance from two sites together, followed by comparing their mean values and slopes within one GLM with ‘Distance’ as the continuous variable.

Pairwise Wilcoxon Rank Sum Test was adopted to test the difference in recovery between seeds and mimics. A linear correlation was used to examine the comparability of the material we used in mimicking *S. anglica* seeds with the actual seeds. GLMs were employed to test the effects of ‘Month’, ‘Distance’ and ‘Depth’ (i.e. vertical position in the sediment) on seed persistence in the manipulated seed bank. These analyses were conducted for each site separately, by specifying a GLM with ‘family’ = ‘binomial’ for the proportion data. When necessary, we refitted the model using ‘quasibinomial’ to account for the overdispersion (Crawley 2007). Pairwise Wilcoxon Rank Sum Tests were also applied to detect the differences between sites of seed persistence in each month.

All statistical analyses were run in R. Multiple comparisons between slopes or intercepts within each model were achieved through the ‘glht’ function in the package of ‘multcomp’. Each GLM started with the maximal model by fitting, for each level of the categorical factors, a line with separate slopes and intercepts; the minimum adequate model was ultimately generated by progressively removing non-significant terms (Crawley 2007). All tests were performed applying a significance level of $\alpha = 0.05$.

2.3 Results

2.3.1 Seed arrival at the mudflat

Deviance analysis of the minimum ‘quasipossion’ model showed that ‘distance’ had significant effect on seed arrival at the mudflat (Table.2.2). At both sheltered and exposed sites, the number of deposited seeds declined exponentially with increasing distance to the marsh edge (Fig.2.3a). Since ‘Distance’ did not have interactive effect with 'Month', the decay rate (slope) was consistent over time within each site. The wind-exposed site had a higher decay rate (steeper slope) than the relatively sheltered Paulina (p = 0.020). However, there was no significant difference of seed arrival between these two sites (p = 0.249).

Seed arrival was also significantly affected by 'Month' (Table.2.2) at both sites. At the relatively sheltered site, fewer seeds arrived at the mudflat in March than in January (p < 0.001) and February (p < 0.001), between which there was no significant difference (p=0.328). At the wind-exposed site, the same general trend of decrease in time was found. Here, seed deposition in January was significantly higher than in February (p < 0.001) and March (p < 0.001).

Table.2.2 Analysis of deviance table of the minimal adequate models for seed deposition, seed bank abundance, and seed persistence in the manipulated seed bank, respectively.

Response variable	***Site***	***Source***	***Df***	***Deviance***	***Resid. Df***	***Resid. Dev***	***Pr (>Chi)***
Seed deposition	Sheltered	Month	2	205.7	42	333.7	< 0.001 ***
		Distance	1	98.0	41	235.7	< 0.001 ***
	Exposed	Month	2	429.0	42	824.9	< 0.001 ***
		Distance	1	559.3	41	265.6	< 0.001 ***
Seed bank abundance	Sheltered	Distance	1	107.5	88	679.2	< 0.001 ***
	Exposed	Month	5	110.5	84	813.8	0.009 **
		Distance	1	274.7	83	539.2	< 0.001 ***
Seed persistence	Sheltered	Depth	1	1220.3	418	1126.0	< 0.001 ***
		Depth: Month	3	104.8	415	1021.2	0.002 **
	Exposed	Depth	1	1117.6	418	976.4	< 0.001 ***

Significance level: *** 0.001, ** 0.01, * 0.05

2.3.2 Seed bank abundance at the mudflat

At both sites, seed bank abundance in the top 50 mm of the ambient sediment fluctuated in time but the 'Month' effect was significant only at the wind-exposed site (Table.2.2). Generally, seed bank abundance paralleled the descending trend of seed arrival with the distance from the marsh edge (Fig.2.3). Differences between the slopes of seed arrival and seed bank abundance were not significant either at the relative sheltered site ($p = 0.250$) or wind-exposed site ($p = 0.946$). The number of seeds present in the seed bank at both sites was much smaller than the number deposited on the AstroTurf® mats during one month at the same sites (Fig.2.3). There was no significant difference of seed bank abundance between these two sites ($p = 0.067$).

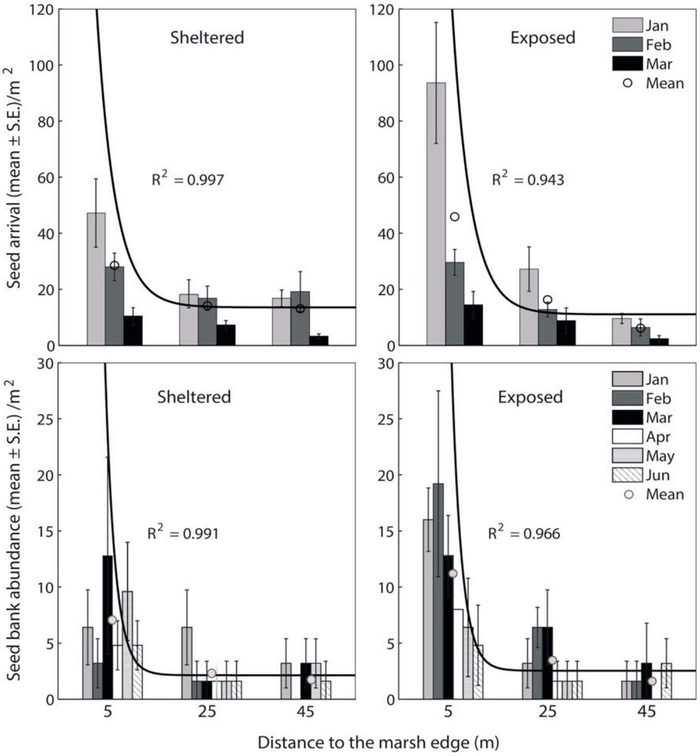

Fig.2.3 Seed arrival (top) from January to April and seed bank abundance (bottom) monthly surveyed from January to June at Paulinapolder (sheltered, left) and Zuidgors (exposed, Zuidgors). Both of them showed a trend of exponential decay as illustrated by the curves: y = a * exp (x) + b, fitted by the month-averaged seed deposition or seed bank abundance data.

2.3.3 Persistence of manipulated seed bank

There were no significant differences between the recovery of seeds and mimics for either relatively sheltered (Pariwise Wilcoxon Rank Sum Test, p = 0.11) or wind exposed sites (p = 0.24). Recovery of seeds and mimics were highly correlated with a correlation coefficient close to 1 (Fig.2.4). These results imply that seed loss at both sites was mainly due to physical disturbances (e.g. sediment erosion driven by hydrodynamics) rather than seed predation.

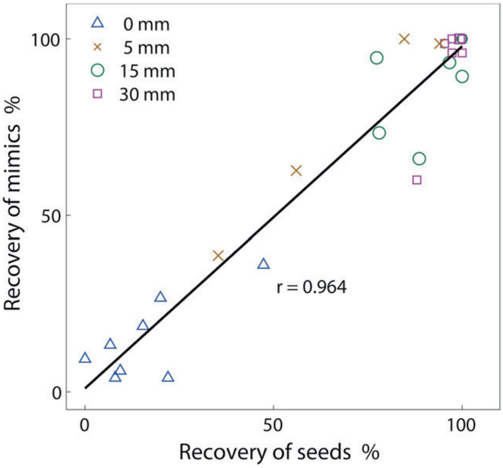

Fig.2.4 Correlation between the recovery of seeds vs. mimics, each data point represents the mean value at a given depth in each month at the site of Paulinapolder and Zuidgors

Seed persistence did not significantly change along the gradient of distance from the marsh edge at either the relatively sheltered or exposed sites (Table.2.2). In contrast, persistence of seeds was significantly affected by their vertical positions in the sediment at both sites (Fig.2.5). Overall, the retention of surface (i.e. 0 mm) seeds in the manipulated seed bank was low at both sheltered (mean ± SE, 11.5 ± 2.2 %, n=120) and wind exposed site (20.7 ± 3.0 %, n=120). Once buried, seed persistence was greatly improved even if buried as shallow as 5 mm (Sheltered site: 45.7 ± 5.6 %, Exposed site: 89.3 ± 3.0 %, n=60). Seed persistence increased with depth of burial, approximately following a power function (Fig.2.6). On average, over 90% of seeds remained in the seed bank at both sites, when placed at the depth of 15 mm and 30 mm.

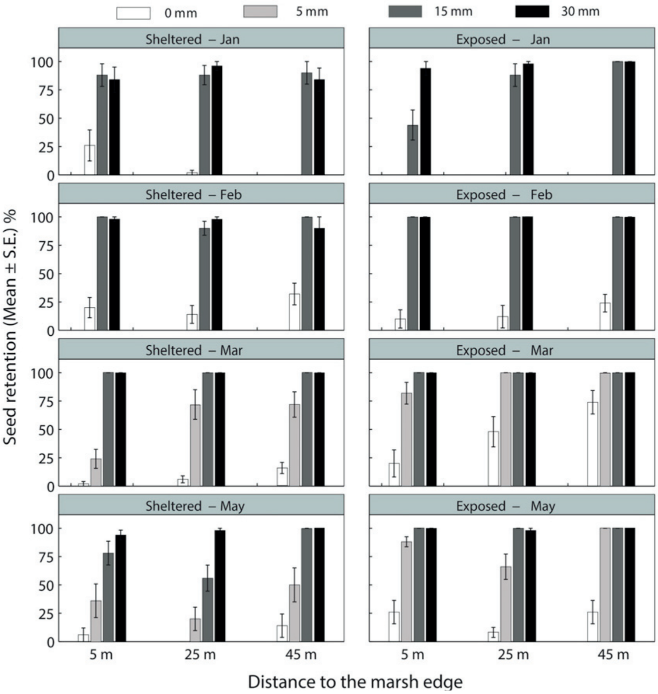

Fig.2.5 Persistence of the manipulated seed bank at Paulinapolder (sheltered) and Zuidgors (Exposed); Seeds were originally deployed at the depth of 0, 15, 30 mm in January and February, and a new layer at 5 mm was added in March and May

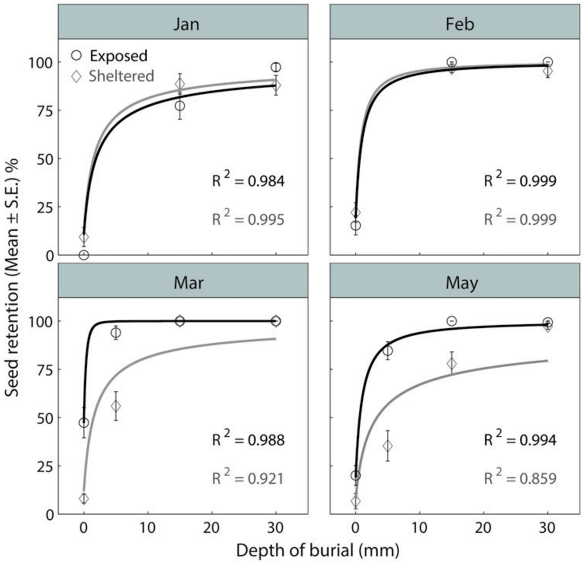

Fig.2.6 Persistence of the manipulated seed bank at Paulinapolder and Zuidgors; the equation for the fitted curves: y = 1 - (x+1.2) ^ a, (a<0)

There was no difference between seed persistence in the manipulated seed bank at relatively sheltered and wind exposed site in January (*p*=0.560) or February (*p*=0.580) site. Nonetheless, seed persistence was surprisingly higher in March (*p*< 0.001) and May (*p*< 0.001) at the wind exposed site than at the relatively sheltered site, albeit more exposed to the onshore winds in these two months (Fig.2.7).

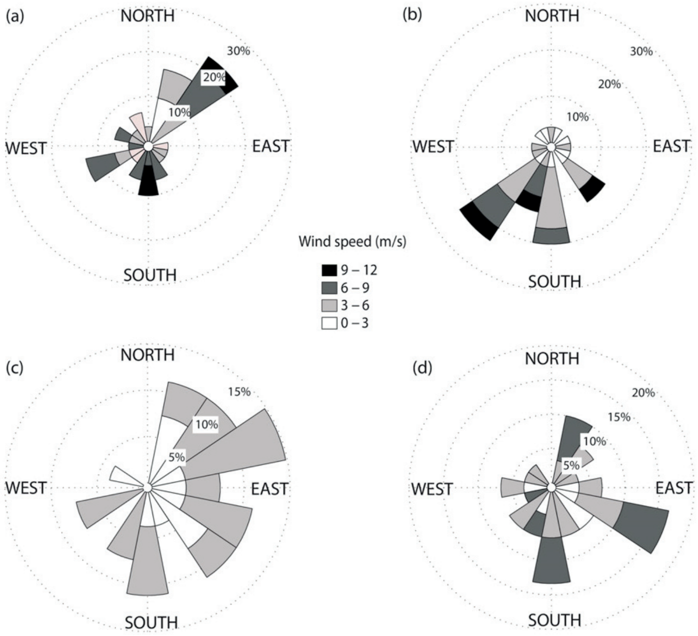

Fig.2.7 Wind roses for the experimental periods in (a) January, (b) February, (c) March, and (d) May. Data was acquired from the weather station of Vlissingen, the Netherlands (Fig.2.1)

2.4 Discussion

Successful seedling establishment in tidal flats requires windows of opportunity i.e. disturbance free period (Balke et al. 2011), due to thresholds imposed by the harsh environments they inhabit. However, opportunity only favors the 'prepared' seeds that arrive / are present in the right location at the right time, stressing the relevance of seed arrival and persistence processes in seedling establishment in intertidal ecosystems. The current study indicates that the existence of local source population and shallow seed burial are essential for generating an annual soil seed bank at the tidal mudflats.

Despite the potential for long-distance dispersal by the tidal currents(Koutstaal et al. 1987), salt marsh plants were found to mainly disperse their seeds locally (Rand 2000, Wolters et al. 2005). Medium/long-distance dispersal might occur but only under extreme weather conditions (Chang et al. 2007). Previous studies also showed that, at the local scale, seed deposition is inversely related to distance from the source population (Wolters et al. 2005). Our findings at two contrasting salt marshes with different wind exposure were consistent with this result. In both of our study sites, *S.anglica* seeds were found to deposit close to the marsh edge, and decreased exponentially with increasing distances, while the amount of deposited seeds was month specific and the decay rate was site specific. This might result from the heterogeneity in seed source patterns, seed release dynamics, hydrodynamic forces and their interactions, which were however out of the scope of this paper.

We identified the role of seed arrival and persistence for determining seed bank abundance at the adjacent tidal flat. Our results clearly indicated that the pattern of seed bank distribution at the adjacent tidal flats was set by initial seed arrival, while seed abundance in the seed bank was mainly constrained by subsequent persistence of arrived seeds, especially those on the surface that had a great chance (> 80% on average as found in our experiments) to be entrained and progressively transported to farther locations during subsequent tidal cycles. Thus seedling establishment potential at the mudflat can be limited by insufficient seed arrival when seed source is far away, whereas high seed loss can serve as a main bottleneck regardless of the distance to the seed source.

Biological events like seed predation by benthic animals were not responsible for seed loss from the manipulated seed bank in our experiments, since there was no difference of recovery but a high correlation between seeds and mimics. Given the size (ca. 15 mm in length) and the material of the seed mimics (non-digestible hard plastic) used in our experiments, the probability that seed mimics also suffered losses due to consumption by benthic animals was negligible. This implies that the loss of seeds was mainly due to physical disturbances (e.g. sediment erosion driven by hydrodynamics) rather than biological factors. This result contrasted with the lab experiment (Emmerson 2000) that suggested loss of *S. anglica* seeds was due to the ‘grazing’ by *H.*

diversicolor, one of the most frequently found benthic macro-invertebrates in European estuaries. This species was also reported to eat the seeds and seedlings of another salt marsh plant, *Salicornia europaea* (Paramor and Hughes 2004). It is possible that the herbivory by such species was a conditional outcome, e.g. *Spartina* seeds could only be consumed after germination or merely due to the absence of other food sources in their experiments. Yet this remains to be verified. Nonetheless, other effects of benthic animals like seed burial, either being directly transported or indirectly displaced through sediment mixing (Delefosse and Kristensen 2012) could play important roles in seed bank dynamics in salt marshes.

This study specifically highlights the importance of seed burial for seeds to persist at the right location at the right time. Our experiments demonstrated that generally seed burial enhanced seed retention nonlinearly with increasing burial depths. Once seeds moved down and escaped from the water-sediment interface, their persistence was greatly improved even if buried by only a thin layer of sediment. This result lent support to studies on other plants that inhabit tidal flats. Seagrass, as an example, benefits from seed burial which provides the seeds with a 'safe site'(Orth et al. 2006b).

However, deep burial can lead to the failure of the seed-seedling transition. A lab experiment (T.J. Bouma et al., subm.) on seedling emergence of *S. anglica* showed that seedling emergence linearly declined with increasing burial depths (regression line equation: $y=-1.12x+95.8$, $R^2 = 0.96$). In their experiment, > 80% of seeds buried in shallow depths (within 20 mm) were able to emerge seedlings, while > 80% of seeds buried deeper than 50 mm failed. A study on seagrass species *Zostera marina* found that seeds germinated from deeper than 50 mm cannot reach the sediment surface because of insufficient energy stored in hypocotyls (Greve et al. 2005). However it remains unclear how the survival of seed viability in the buried seed bank are influenced by abiotic and biotic factors in tidal habitats.

It seems that seed persistence in the sediments cannot be simply inferred from wind exposure. The more exposed site did not result in higher seed loss than the relatively sheltered site in our experiments. For instance, seeds buried at the depth of 5 mm had a seed loss of more than 50% in May at the relatively sheltered site, but only 10% at the wind exposed site (Fig.2.6). A previous study suggested that the percentage of seeds washed away from the buried seed bank was depending on the mobility of the top layer of the sediment (van Eerdt 1985). The effects from wave exposure might be confounded by other factors like sediment dynamics that can be influenced by abiotic factors (e.g. currents, waves, sediment properties) and biotic factors (e.g. bioturbation) that are variable in space and time.

In our experiments, short-term (one month) persistence of seeds in the buried seed bank was generally high. Nonetheless, the actual season-long (several months) seed persistence might be lower due to the dynamic nature of the tidal mudflats. Seeds that persisted for one month might be eroded when exposed to disturbance for a longer time. For example, an early study on the establishment of *S.anglica* on a tidal mudflat in the Oosterschelde, the Netherlands, showed that more than 90% of the planted seeds at the depth of 5 mm were lost after about two months (Groenendijk 1986). In another *in situ* experiment in the Oosterschelde, most seeds of *S. anglica* buried within 15 mm were gone from mudflats after 6 months (van Eerdt 1985). In contrast, seeds buried at the same depth (i.e. 15mm) for a shorter period (i.e. one month) in our experiments at both sites were relatively ‘safe’, with a retention of more than 90% on average (Fig.2.6).

Moreover, tidal flats are characterized by short stochastic events (e.g. storm surges) with a high disturbance magnitude, that alternate with periods of relatively low disturbance (normal hydrodynamic conditions) (Deloffre et al. 2006). Extreme hydrodynamic events may have disproportionately greater effects than normal tidal regimes on seed bank dynamics, because they can strongly influence sediment dynamics in tidal flats (e.g. Roman et al. 1997, Bartholdy and Aagaard 2001). One storm event may wipe out the majority of the buried seed bank by mobilizing a few cm of sediments. The same event might also do the opposite by transporting more seeds (e.g. Chang et al. 2007) to the tidal flats and burying them into the seed bank through sediment accretion, which was found to have a positive relationship with seed deposition (Goodson et al. 2003). Sediment dynamics driven by high-energy events can have considerable impact on seed bank dynamics at the tidal flats, especially when matching temporally with seed deposition. Thus one would expect large year-to-year variation in seedling establishment potential, since the timing of occurrence and severity of storms may vary a lot between years.

Our findings are highly relevant to the management aspect of restoration and/or creation of salt marshes, given that transplantation efforts can be enhanced by natural recruitment or seed based restoration techniques. For instance, seed mimics could be employed as an economical tool to help managers find out suitable locations, by indicating where restoration efforts are likely to succeed (Delefosse and Kristensen 2012, Marion and Orth 2012). Persistence of planted seeds can be improved if managers could manipulate seeding depths in the sediment. This study may also have implications for the control of invasive species e.g. *S. alterniflora* (Sayce 1997, Xiao et al. 2009) and *S. anglica* (Nehring and Hesse 2008). Consideration of seed arrival and seed persistence processes might help slow down the spreading of exotic species by measures which would prevent seed arrival at suitable locations, or hindering seed entry into the buried seed bank. However, it still remains a challenge to develop effective measures that are practical in the field.

In summary, the present study fills an important gap by providing insights into the patterns of seed arrival and seed persistence in salt marshes, which serve as key determinants for seed bank availability and thus colonization potential at the fronting tidal flats. It contributes to developing a more holistic understanding of bottlenecks and thresholds to salt marsh pioneer establishment, which is an important step towards conservation and sustainable management of this valuable and threatened ecosystem. This study also points at the importance of hydrodynamic and morphodynamic process on the tidal flat for the establishment potential of salt marshes, thus linking salt marsh establishment and extension to the physical dynamics of the entire estuarine system.

Chapter 3

3. Maternal effects and tidal pulsing interactively generate episodic effective seed dispersal of a coastal foundation species

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Submitted

Abstract

Understanding how effective seed dispersal (ESD) is shaped in space and time is essential to foresee plant reproductive success. As a maternally influenced plant character, both quantity and quality of seed dispersal components were previously found to be affected by the phenotypes and environments of their mother plants. Here we provide to our knowledge the first study examining how ESD can be shaped by the interactions of maternal effects and the heterogeneity of dispersal agents, using cordgrass, a hydrochorours coastal foundation plant, as model. We surveyed the variation of seed quantity and seed viability along the elevation gradient in two Dutch salt marshes, and determined the quantity and quality of the seeds delivered towards the fronting tidal flats under variable tidal flooding conditions, using floatable seed traps and germination tests.

The results showed that both seed production and seed viability generally increased with raised elevations of mother plants, whereas both quantity and viability of the seeds transported seaward mounted with elevated high water level of the tides. These relations jointly result in a pulsing seed dispersal pattern and episodic ESD, where spring tides and storm surges can deliver disproportionately higher amount of viable seeds. Present findings contribute to better predictions of the response of coastal foundation plants to climate change effects e.g. sea level rise, and also shed light on seed-based salt marsh restoration as well as the management and control of invasive cordgrass species.

3.1 Introduction

Seed dispersal is a key function of sexually reproducing plants, with far-reaching consequences for population and community dynamics, as well as species migration and persistence in a global change context (Levin et al. 2003, Levine and Murrell 2003, Ibáñez et al. 2006). The consequence of seed dispersal to reproductive success is determined by effective seed dispersal (ESD) involving both quantity and quality components of seed dispersal (Schupp 1993, Jordano and Schupp 2000, Schupp et al. 2010). Despite a growing body of seed dispersal studies and some research on seed dispersal effectiveness by animals (for recent reviews, see Schupp et al. 2010), overall it remains poorly understood how ESD is shaped in space and time.

It is widely recognized that maternal plants can have significant non-genetic impacts on offspring's traits as so-called 'maternal effects' (Badyaev and Uller 2009, Wolf and Wade 2009). Previous studies suggest that maternal effects can serve as a primary regulator of ESD. The quantity of dispersal and the distance that seeds travel were found to be affected by maternal traits such as plant height, branch orientation and spatial distribution of fruits that vary with environmental conditions (McCanny and Cavers 1989, Donohue 1998, Thomson et al. 2011). Maternal factors are also influential on diaspore quality. For instance, seed germinability can be markedly determined by the position of the inflorescence on the mother plants (Thomas et al. 1979, Gutterman 1994) or nitrogen availability of the maternal environment (Luzuriaga et al. 2006).

Maternal effects on seed dispersal may also act in conjunction with dispersal agents, either via traits that attract and reward animal dispersers (Willson and Whelan 1993, Bishop and Schemske 1998), or by vegetation structures that modulate physical forcing e.g. wind & water (Skarpaas et al. 2006, Chang et al. 2007, Chang et al. 2008). As physical dispersal agents such as e.g. wind speed and water level are typically variable in space and time, the location of a mother plant may determine the characteristics of dispersal agents her seeds experiences. Therefore, ESD pattern in a heterogeneous environment is likely to be shaped by the spatial interaction between the heterogeneity in maternal traits and the variability of dispersal agents.

Here, we focus our study on the spatially coupled effects of maternal factors and dispersal agents on ESD, using a tidal marsh system with 'hydrochory' as the main method of seed dispersal as model system (Huiskes et al. 1995, Chang et al. 2007). This study was performed on cordgrass (*Spartina spp.*), a world wide distributed salt marsh pioneer species, either native or invasive (Strong and Ayres 2009, Strong and Ayres 2013). As foundation species, cordgrass often form monocultures in the lower part of the marsh characterized by banded vegetation zonation along the elevation gradient (Pennings et al. 2005, Van der Wal et al. 2008, Gedan et al. 2009). Such species have long been noted for their plastic phenotype including zonal variation in seed production and seed viability (Marks and Truscott 1985, Mullins and Marks 1987, Xiao et al. 2009). This characteristic plus the spatio-temporal dynamics of the tide makes cordgrass marsh an excellent model system for exploring how maternal effects and the variability of dispersal vector may interactively regulate ESD. The study of ESD on this globally occurring coastal pioneer species is especially relevant in the context of climate change, given that coastal wetlands have been world widely degraded (Gedan et al. 2009, Barbier et al. 2011) and their rejuvenation strongly depend on sexual reproduction which is sensitive to environmental variations (Friess et al. 2012, Balke et al. 2013, Hu et al. 2015).

Specifically, we examined *i)* how the quantity and quality of cordgrass seeds are affected by the location of mother plants along the elevation gradient; and *ii)* how this shapes ESD by the interaction with tidal variability. Since seedling establishment of pioneer plants mainly occurs in the pioneer zone and the fronting tidal flats (Davis et al. 2004, Nehring and Hesse 2008, Deng et al. 2009), we only consider the seaward delivery of viable cordgrass seeds as effective seed dispersal in this study. Here, the number of viable seeds (seed quantity x seed viability) transported to the seaward side after one tide was employed as a proxy to measure ESD that cordgrass received from that tide. The knowledge on ESD gained here will contribute to understanding the potential response of coastal foundation plants to climate change effects e.g. rising sea level and increasing storminess that can modify hydrological conditions. Such information may also shed light on seed-based salt marsh restoration as well as the management and control of invasive cordgrass species that spread rapidly through seedling establishment (Strong and Ayres 2009, Strong and Ayres 2013).

3.2 Materials and methods

3.2.1 Study site

Field surveys on seed production and seed dispersal of cordgrass were conducted in two salt marshes, Paulinapolder (PA) and Ritthem (RI) in the Westerschelde, the Netherlands (Fig.3.1a). Both these Westerschelde field sites are tide-dominated and experience a semi-diurnal tide; the spring tidal range varies from 4.4 m to 5.5 m (Baeyens et al. 1998). The pioneer vegetation consists mainly of common cordgrass, *Spartina anglica* (Fig.3.1b), which was introduced in the Westerschelde in 1925 (Nehring and Hesse 2008), forming monocultures in the seaward part with elevations ranging from 60 to 200 cm NAP (Dutch Ordnance Level) of the marsh (Van der Wal et al. 2008). This species flowers from July to October and seeds ripen within 12 weeks. Seed release starts from autumn, extending through the winter and early spring of the following year (Huiskes et al. 1995).

3.2.2 Maternal effects on seed quantity and seed viability

To detect maternal effects on seed quantity and seed quality, we quantified seed production and seed viability along the elevation gradient. Field surveys on seed production were conducted in the beginning of November when most of seeds were still on the plants. This was done first in 2011 and then repeated in 2012 and 2013. In both marshes, we selected a ca. 20 m wide cross-marsh transect spanning from the upper to the lower limit of cordgrass zone. Along this transect, four sampling zones (Table.3.1) were established. Within each zone, we sampled five 1 x 1 m quadrates of comparable elevations measured with a dGPS rtk (Leica Geosystems). The flowering inflorescences within each quadrate were excised and transported to the lab in plastic bags. In 2011, a small proportion (PA: ca. 5%, RI: ca. 2%) of the spikelets from the plants growing in the upper part (PA: > 160 cm NAP; RI: > 140 cm NAP) of the marsh were infected by the fungus *Claviceps purpure*. In 2012 and 2013, less fungus infection was seen at both sites.

In the lab, the infected spikelets were counted and manually removed. The uninfected ones were released from the inflorescences and counted, after which seed production (no. of uninfected spikelets/m^2) was determined for each quadrate, respectively. The gathered seeds from each quadrate were separately placed into mesh storage bags submersed in seawater, labeled and stored in a 4 °C fridge for ca. 3 months, after which seed viability was determined by germination tests. To achieve this, a subsample of 100 seeds from each storage bag was put into a petri dish furnished with filter paper. All petri dishes were moved to a climate room with a constant temperature of 25 °C. The germination tests terminated when no more germinated seeds could be seen for one week. Seed viability for each sample was calculated by dividing the total number of germinated seeds by 100.

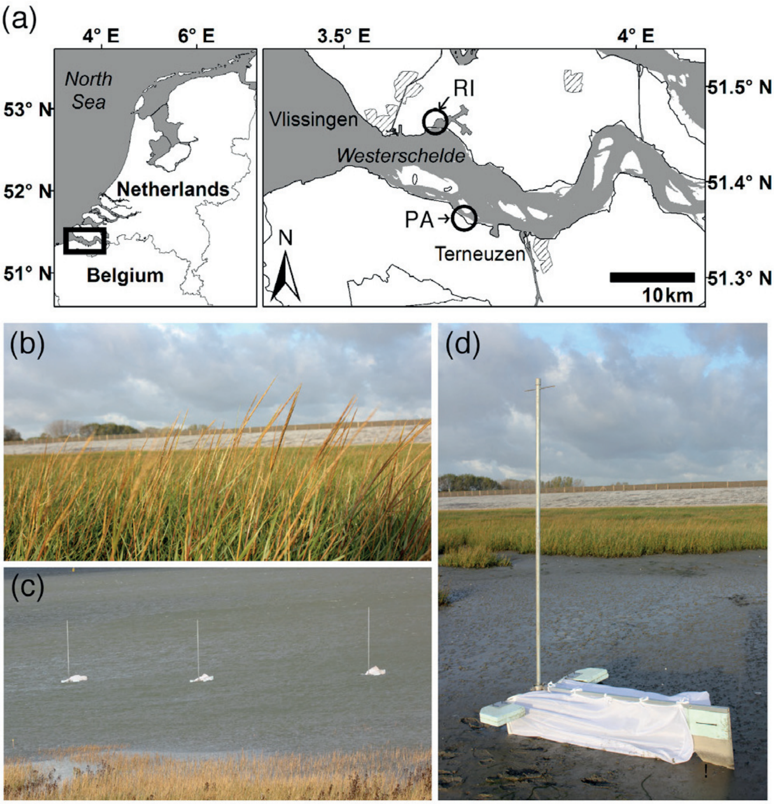

Fig.3.1 (a) Geographic location of the Westerschelde (top left), the study sites, Paulinapolder (PA) and Ritthem (RI) (top right); (b) flowering cordgrass; (c) & (d) floatable seed trapping nets deployed on the mudflat in front of the marsh.

Table.3.1 Elevations (cm NAP) of quadrates used for seed sampling in Paulinapolder (PA) and Ritthem (RI) in 2011, 2012 and 2013. NAP = Dutch Ordnance Level.

Site	Year	Zone1	Zone2	Zone3	Zone4
PA	2011	192	173	140	101
	2012	192	173	140	101
	2013	172	154	132	108
RI	2011	194	141	109	63
	2012	194	152	109	63
	2013	194	154	129	109

3.2.3 Seed release dynamics

To survey the seed release dynamics, we established 6 permanent plots (50 x 200 cm) in November 2013, in the higher (> 140 cm NAP) and lower part (< 140 cm NAP) of the cordgrass marsh, respectively. These plots were randomly selected and at least 5 m apart. The number of seeds remaining on the plants (no./m^2) was monthly determined by *i)* counting flowering inflorescence per m^2 and *ii)* quantifying the number of seeds per inflorescence. The former was done within each plot, whereas for the latter we took 10 inflorescences samples around each plot. This survey was conducted in both PA and RI and terminated in April 2014 when all the seeds were gone from the plants.

3.2.4 Seaward seed dispersal: quantity and viability

Surveys of seaward seed dispersal were conducted between November 2013 and February 2014, during which the majority of the seeds were dislodged from their mother plants (Fig.3.2a). To detect the effects of tidal variability on the quantity and quality of seeds delivered to the fronting tidal flats, we conducted the surveys 12 times under variable high water levels (Fig.3.2b), using floatable seed trapping nets (Fig.3.1c & d) adapted from the design in Huiskes et al. (1995). Such nets proved very effective in trapping cordgrass seeds that disperse via floating in the water column (Koutstaal et al. 1987, Huiskes et al. 1995). The field survey (Huiskes et al. 1995) using the floating net also showed that cordgrass seeds transported out of the marsh with ebb currents, while seeds that were transported into the marsh from elsewhere were comparatively neglectable.

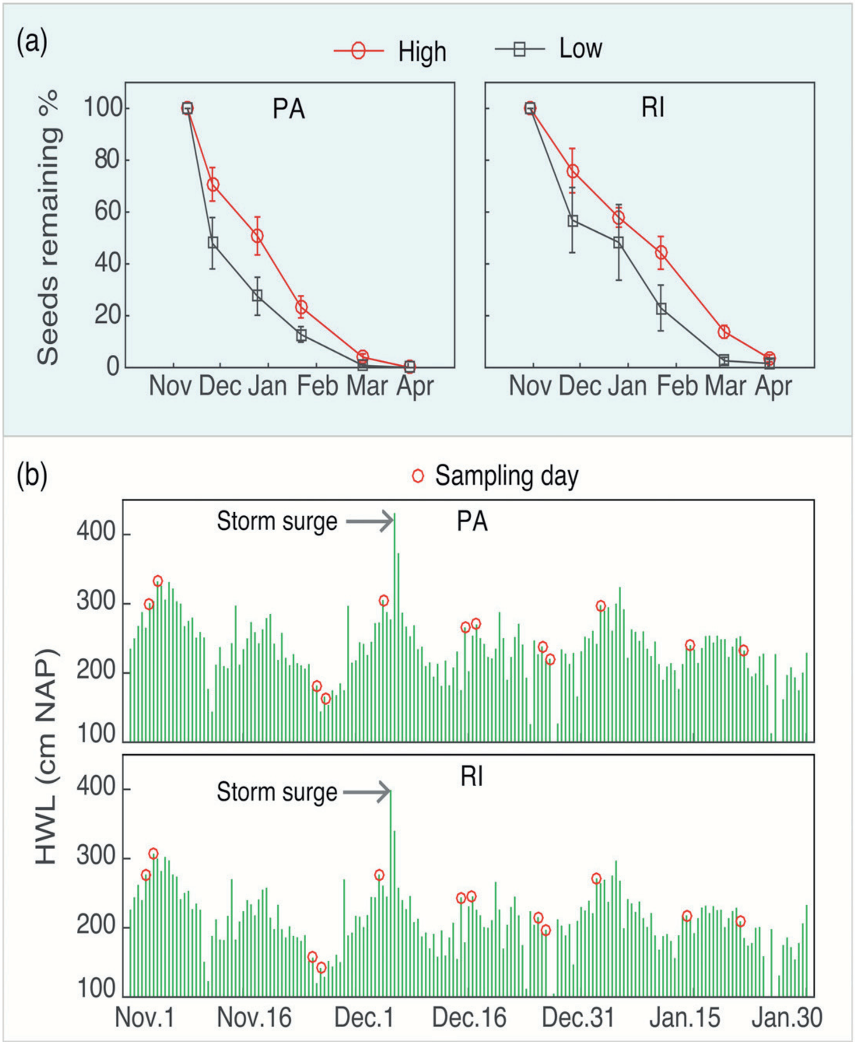

Fig.3.2 **(**a) Seed release dynamics (Pecentage of remaining seeds, Mean ± SE) in the higher (> 140 cm NAP) and lower part (< 140 cm NAP) of the cordgrass marsh at Paulinapolder (PA) and Ritthem (RI) during November 2013 and April 2014; (b) High water level (HWL) of the tides during November 2013 and February 2014, HWL of the sampling days are indicated in red circles. The data of HWL was from the nearby stations of Ternuzen and Vlissingen, for PA and RI (Fig.3.1a), respectively.

In both PA and RI, three nets (mesh size 100 µm), each fixed to a permanent steel pole (ca. 3 m above ground) through a steel ring, were deployed ca. 5m in front of the marsh edge and ca. 10 m apart (Fig.3.1c & d). The net had an opening of 68cm x 24 cm made out of steel rods and a length of 150 cm. Equipped with a rudder made of aluminum sheeting and three polystyrene floats (Fig.3.1d), the net can adjust its orientation with the current direction and move up and down the pole with the tides (Huiskes et al. 1995). For each survey, the nets were recovered on the next day (after two tides) and the number of captured cordgrass seeds by each net was counted and averaged for the three nets. Divided by two (tides), the number of seeds captured per net during one tide (SNT) was then calculated to measure seaward seed transport by each tide. For each survey, seeds captured from all three nets were pooled together and stored in the way as described in 2.2. After ca. 3 months, the viability of the seeds captured during each survey (SV) was determined by germination tests. When the total number was less than 800, all the seeds were tested, else we used a sub sample of ca. 800 seeds. To quantify seaward transport of viable seeds, we computed the number of viable seeds captured per net during one tide (VSNT) by multiplying SNT with SV, serving as a measure for ESD that cordgrass received from each tide.

3.2.5 Data analysis

ANCOVAs were applied to detect the effects of maternal location on seed quantity and seed viability, respectively, with 'Site' and 'Year' as category factors and 'Elevation' as covariate. For each response variable, we first conducted square root transformation to improve data normality, and then started ANCOVA with the maximum model by fitting, for each level of the categorical factors, a line with separate slopes and intercepts; the minimum adequate model was ultimately generated by progressively removing non-significant terms (Crawley 2007).

Linear regressions were employed to test the effects of tidal variability on the quantity and quality of seeds delivered seaward, respectively. Firstly the data of SNT and SV were square root transformed to improve normality. We then fit each of these variables with a linear model: $y = a*x + b$, where x is the high water level during each tide (HWL). Since the nets were recovered after two tides, the higher HWL of these two was used for each survey. The HWL data was obtained from two nearby gauge stations, Terneuzen and Vlissingen for PA and RI respectively. All the statistical analyses were done in R (http://www.R-project.org), applying a significance level of α = 0.05.

3.3 Results:

3.3.1 Maternal effects on seed production and seed viability

The ANCOVA results showed that both seed production and seed viability were significantly affected by maternal elevations, despite differences between sites and years (Table.3.2). In both sites, both seed production and seed viability generally increased with raised elevation of the mother plants (Fig.3.3).

Table.3.2 ANOVA table of the minimum adequate model for seed quantity and seed viability, respectively.

	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed quantity	Site	1	5217.4	5217.4	16.2	<0.001 ***
	Elevation	1	3134.3	3134.3	9.8	0.002 **
	Year	2	22987.1	11493.6	35.8	<0.001 ***
	Residuals	115	36955.7	321.4		
Seed viability	Site	1	0.163	0.163	34.3	<0.001 ***
	Elevation	1	0.400	0.400	84.3	<0.001 ***
	Year	2	0.791	0.395	83.4	<0.001 ***
	Site:Elevation	1	0.104	0.104	22.0	<0.001 ***
	Site:Year	2	0.662	0.331	69.9	<0.001 ***
	Elevation:Year	2	0.073	0.036	7.7	0.001 ***
	Site:Elevation:Year	2	0.062	0.031	6.5	0.002 **
	Residuals	108	0.512	0.005		

Significance level: * 0.05, ** 0.01, *** 0.001

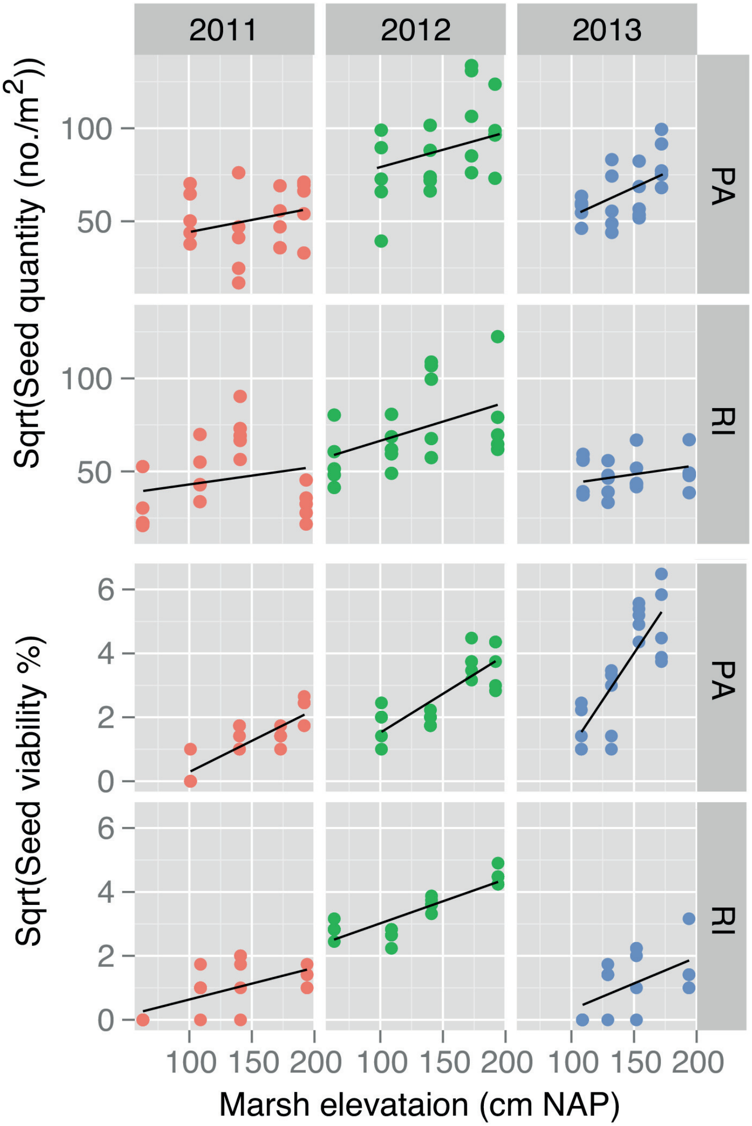

Fig.3.3 Seed production (top panel) and seed viability (bottom panel) along the elevation gradients in Paulinapolder (PA) and Ritthem (RI), surveyed in 2011, 2012 & 2013. The data of y-axes have been square root transformed.

3.3.2 Seaward seed dispersal: quantity, viability and ESD

The linear regressions indicated that, in both sites, both seed quantity i.e. the number of seeds captured per net per tide (SNT) and seed viability (SV) of those seeds were significantly related with HWL (Fig.3.4).

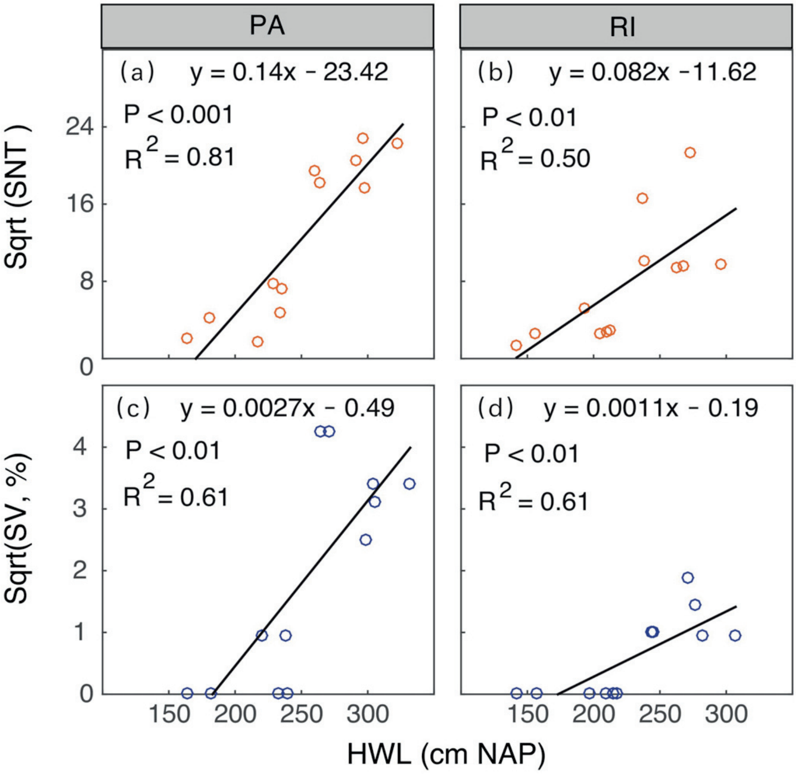

Fig.3.4 Linear regressions of quantity (SNT) and viability (SV) of the seaward transported seeds caputred per net after one tide in site Paulinapolder (PA) and Ritthem (RI) against High Water Level (HWL) of that tide. The data of y-axes were square root transformed.

Based on the regression equations for SNT (Fig.3.4 a & b) and time-series HWL data, we inferred the temporal dynamics of seaward seed transport during the main dispersal period (November-February) for each site, respectively. By multiplying such results with SV derived from the regression equations (Fig.3.4 c & d), we calculated the number of viable seeds captured per net during each tide (VSNT), from which we depicted the temporal pattern of ESD.

Taking site PA for an example, the modeled results revealed a pulsed pattern of seaward seed dispersal and episodic ESD, in which spring tides and storm surges deliver disproportionately higher numbers of viable seeds (Fig.3.5).

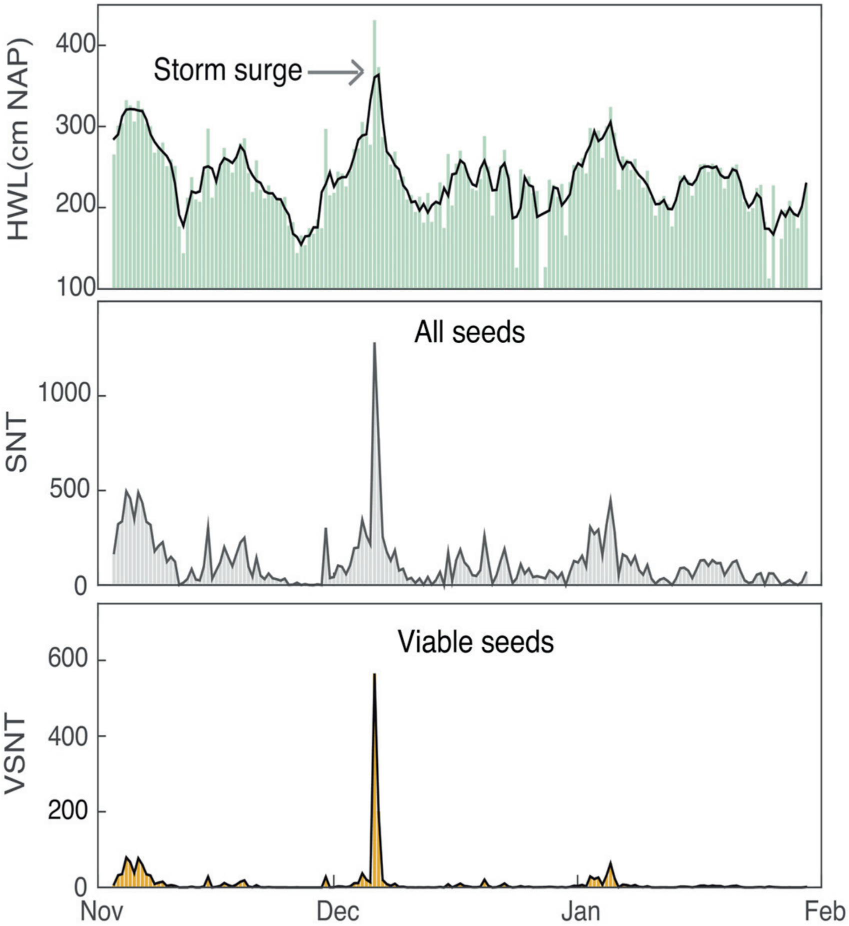

Fig.3.5 HWL (top panel) of each tide and the modeled results of seaward seed tranport in site Paulinapolder (PA) during the period between November 2013 and February 2014, measured as the number of total seeds (SNT) and viable seeds (VSNT) caputred per net after each tide.

3.4 Discussion:

Hydrochory has been increasingly valued for its importance in structuring aquatic plant communities (Huiskes et al. 1995, Jansson et al. 2005, Nilsson et al. 2010). Our study in a hydrochorours tidal system illustrates that the predominant dispersal agent, i.e. the tide, not only serves as an external forcing that determines the temporal rhythm of seed dispersal, but also interacts with maternal seed production and viability along the elevational inundation gradient. The former leads to a temporal pattern of pulsed seed dispersal, whereas the latter results in maternal heterogeneity of seed quantity and seed quality (viability), both of which were found to decrease with lowered elevations, i.e. increased inundation frequency. The coupling of these two outcomes generates an episodic pattern of effective seed dispersal (ESD).

Flooding pulses have proven vital for the dispersal of riparian plants with seed dispersal mainly occurs during high-flow periods when riparian zones are inundated, supporting the flood pulse theory (Boedeltje et al. 2004, Gurnell et al. 2006, Vogt et al. 2006). Our study of hydrochory in the less explored tidal system also stresses the relevance of tidal pulsing in the transport of aquatic seeds, by showing that spring tides and storm surges have a disproportionate contribution to seed dispersal. More importantly, our results additionally demonstrate that these high magnitude events deliver disproportionately higher numbers of *viable* seeds, which strengthens their contribution to ESD.

This study specifically highlights the importance of spring tides in seaward seed transport of the coastal foundation species cordgrass, which has seedling recruitment mainly in the pioneer zone and the adjacent mudflat (Davis et al. 2004, Nehring and Hesse 2008, Deng et al. 2009). In addition to a much larger ESD, spring tides may also favor the deposition of these viable seeds at the mudflat surface, because the spring tide provides a longer inundation period for seeds to become waterlogged, and may thus magnify the chance of seed settlement. At the same time, high water level during spring tides may also have adverse effect on plant propagation, by reducing seed bank persistence on the mudflat, when coinciding with strong disturbance events e.g. wind-driven waves that could cause higher seed removal (Chapter 5, Chang et al. 2007).

Coastal vegetated ecosystems like salt marshes are among the most ecologically and socio-economically valuable ecosystems (Costanza et al. 1997). Unfortunately these ecosystems have been globally degrading, which may be expected to continue in the face of climate change (Gedan et al. 2009). Given the worldwide distribution of cordgrass and their essential roles in building and maintaining coastal habitats (Strong and Ayres 2009, Strong and Ayres 2013), our findings suggest that the relations between ESD of cordgrass and tidal pulsing should be taken into account to predict the response of vegetated coastal ecosystems to sea level rise and increasing storminess. But such predictions are complicated, as effects can be conflicting. For example, the present study shows that on the one hand, increased inundation due to sea level rise and more frequent storm surges may pose negative impacts on seed production and seed germinability, whereas on the other hand these climate change effects may improve the probability of the transport of viable seeds. Assessing the net effect requires more extensive climate change scenario studies, preferably for areas with different tidal amplitudes. These scenario studies can then be combined with EDS analyses as provided in the present study.

Our study also suggests that seed-based restoration of coastal vegetated ecosystems such as salt marshes would benefit from selecting or regulating the hydrological conditions towards the delivery of sufficient good seeds to the potential recruitment sites. Similarly, our findings may also have implications for the control and management of invasive species, since hydrochory is also an import means through which non-native species colonize new areas (Nilsson et al. 2010). Hydrological regulations might be applicable in the control or management of the invasive cordgrass species, e.g. *Spartina alternifora and Spartina anglica*, whose fast spread through seed colonization has caused several big invasion events in Europe, US and China (Nehring and Hesse 2008, Strong and Ayres 2009, Strong and Ayres 2013). Yet the challenge would be to strike a balance between the conflicting roles of hydrological level on seed source quality and seed dispersal probabilities.

To our knowledge, this is the first example showing how intrinsic variation caused by maternal effects and extrinsic variability of dispersal vector can directly interact to affect effective seed dispersal. Although demonstrated for a tidal system, episodic effective seed dispersal may occur in other flood pulsing environment, e.g. rivers or streams where seasonal flooding pattern might coincide with zonal distribution of viable seeds. Given the ubiquity of maternal effects on seed production and seed dispersal in various ecosystems (e.g. Gutterman 1994, Donohue 1998, Thomson et al. 2011), interactions between maternal effects and vector variability could be applicable to other dispersal agents e.g. wind, or animals that vary both spatially and temporally (Schurr et al. 2008, Rawsthorne et al. 2011). A detailed understanding of how effective seed dispersal is shaped in space and time would offer the best estimation of the chances of plant reproductive success.

Chapter 4

4. Interactive effects between physical forces and ecosystem engineers on seed burial: a case study on cordgrass

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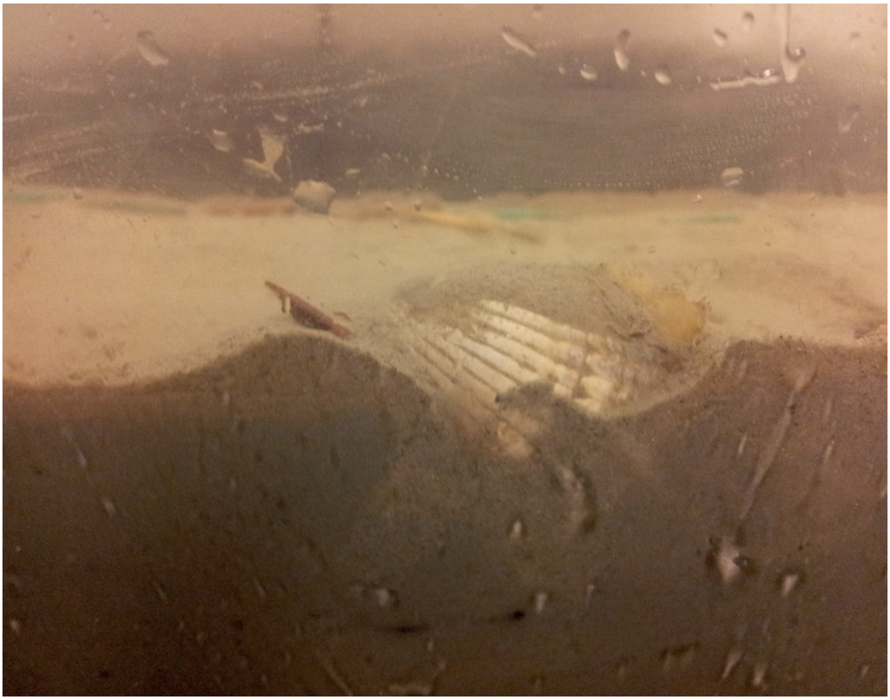
Published in 2016: *OIKOS*: 125, 98-106



Abstract

Seed burial (i.e. vertical seed dispersal) has been increasingly valued for its relevance for seed fate and plant recruitment. While ecosystem engineers were generally considered as the most important seed burial driver, the role of physical forces, e.g. wind or water flow, has been largely overlooked. Using tidal habitat as a model system, we investigated the effects of *i)* currents, *ii)* benthic animals with different engineering activities and *iii)* their interplay on seed burial of a common salt marsh pioneer plant, cordgrass *Spartina anglica*, through a combination of flume and mesocosm experiments. Our results reveal that in such system, water flow can be of equal or higher importance than ecosystem engineers for seed burial. For passive seed-burying engineers (PSE), their coupling with currents produced synergistic seed burial effect, while it was only additive for active seed-burying engineers (ASE). This paper extends current understanding on seed burial and seed bank formation by revealing the need to incorporate physical force into seed burial mechanisms. We provided the first empirical evidence that physical forces influence seed burial by synergistically interacting with ecosystem engineers, highlighting the role of biophysical interaction as an important driver for vertical seed movement.

4.1 Introduction

Seed dispersal is a critical process in the life history of flowering plants (Nathan and Muller-Landau 2000), often involving multiple steps (Vander Wall and Longland 2004). For many plant species, seeds are primarily deposited on the ground surface, followed by secondary displacement to an alternate location, and eventually by seed burial that adds them to the soil seed bank (Chambers and Macmahon 1994). Compared with horizontal transport (m ~ km), seed burial is only a tiny step (mm ~ cm) yet has large consequences for seed fate, plant recruitment and vegetation dynamics.

In terrestrial ecosystems, seed burial has long been shown to benefit seed survival by reducing exposure to biotic risks, e.g. predators or surface fungi (Chambers and Macmahon 1994, Gallery et al. 2007, Forey et al. 2011), and harsh physical conditions, e.g. fire, drought or frost (Cohen 1966, Forey et al. 2011). It could also improve seedling emergence by displacing seeds to soil microsites favorable for germination (Hanzawa et al. 1988, Wenny 2001, Forey et al. 2011). More recently, seed burial was demonstrated to be vital to seedling establishment of aquatic macrophytes e.g. seagrasses and marsh plants. A layer of sediment on top shields the seeds and emerging seedlings (Chapter 2, Marion and Orth 2012) from dislodgment by hydrodynamics and sediment erosion (Bouma et al. 2009, Balke et al. 2011, Infantes et al. 2011). In addition, seed burial is crucial for long-term population and community dynamics by fostering the formation of a persistent soil seed bank (Thompson 1987, Bakker et al. 1996). Despite the general recognition that seed burial is essential for a plant's reproductive success and long-term maintenance, there is insufficient knowledge on how seeds are translocated into the soil in natural ecosystems.

Although self-burial mechanisms exist for some species (e.g. Collins and Wein 1997), most plants rely on abiotic or biotic process to incorporate their seeds into the soil seed bank (Chambers and Macmahon 1994). Biotic seed burial i.e. vertical seed displacement by animal activity was generally considered to be the most universally important seed burial mechanism. Food caching animals e.g. ants, rodents and soil macro-invertebrates such as earthworms were well documented for their roles in seed burial of terrestrial plants (reviewed in

Chambers and Macmahon 1994, and Vander Wall and Longland 2004). In particular, mixing of soil layers (Bioturbation, Meysman et al. 2006) by earthworms as ecosystem engineers (Organisms that modify their abiotic environments, Jones et al.) was considered to be the major driving force for seed burial and seed bank formation in various terrestrial ecosystems (Milcu et al. 2006, Eisenhauer and Scheu 2008, Forey et al. 2011). Much less is known about seed burial of aquatic plants in general. In the wake of earthworms, several recent studies on seagrasses showed that marine worm (polychaete) engineers were also able to bury seagrass seeds either indirectly by sediment reworking or directly via active transport (Valdemarsen et al. 2011, Delefosse and Kristensen 2012, Blackburn and Orth 2013).

In spite of the clear importance of physical forces (e.g. wind, water movement) in driving horizontal seed movement (Koch et al. 2010, Nathan et al. 2011) and their ubiquity in nature, their role in seed burial has been largely overlooked. Only a few studies (Benvenuti 2007, Marthews et al. 2008) found that rainfall promoted the entry of seeds into the terrestrial soil matrix. Physical forces, interacting with ecosystem engineers, are known to drive soil/sediment dynamics (Meysman et al. 2006, Corenblit et al. 2011) and will most probably also affect seeds.

To improve our understanding of factors and mechanisms underlying seed burial processes, this paper examines the importance of physical forces and their interplay with ecosystem engineers for seed burial by using tidal flats as a model system. In such system, seedling recruitment of marine macrophytes (e.g. pioneer marsh plants & seagrasses) mainly takes places in the pioneer zone on the higher tidal flats (Chapter 2, Marion and Orth 2012), where sediment dynamics (e.g. erosion and accretion) is governed by hydrodynamics and sediment transport, tightly coupled with engineering activities of benthic animals (Herman et al. 1999, Widdows and Brinsley 2002). These characteristics make it a suitable model system to study seed burial induced by biophysical coupling.

Through a combination of flume and mesocosm experiments, we specifically address the following questions: 1) what is the relative importance of physical forces vs. ecosystem engineers for seed burial in this intertidal ecosystem? 2) Can physical forces affect seed burial through interacting with ecosystem engineers and how? 3) Does any interactive effect vary between species that differ in engineering modality?

4.2 Material and Methods

4.2.1 Target species

To address these questions, we focus on a common salt marsh pioneer plant, *Spartina anglica* (Nehring and Hesse 2008). Recruitment of this plant starts with dispersing seeds to adjacent tidal mudflats where successful seedling establishment requires the burial of seeds into the sediment matrix (Chapter 2) that are inhabited by a diverse benthic infauna community (Snelgrove 1998, Herman et al. 1999). Four common species in the Northwest European tidal flats were employed in our manipulative experiments. Their engineering modalities as a result of feeding and burrowing behaviors are as follows:

1) The lugworm, *Arenicola marina*, is a deep-burrowing subsurface deposit feeding polychaete. *A. marina* is an upward conveyor that feeds head-down in J-shaped burrows. It has a strong capacity for particle reworking by ingesting sediment at depth in a feeding funnel, and ejecting a characteristic 'worm-like' faecal cast at the surface (Kristensen 2001). This behavior enables *A. marina* to bury seagrass seeds to deep depths by covering the seeds with its massive faecal cast deposition (Delefosse and Kristensen 2012).

2) The ragworm, *Alitta (Nereis) virens*, lives in semi-permanent U- or Y-shaped burrows in the sediment, which may develop into a burrow gallery over time. *A. virens* behaves almost the same as its close relative, *Hediste (Nereis) diversicolor* (Kristensen 2001), which has been shown to actively search and move seeds to a shallow depth in their burrows (Delefosse and Kristensen 2012). Both species have been described as omnivores and detritivores feeding by swallowing surface sediment as well as plant and animal remains around the burrow opening (Kristensen 2001).

3) The cockle, *Cerastoderma edule,* is a motile, obligate suspension-feeding bivalve crawling through the top few cm of the sediment (Kamermans 1994). The physical structure of its shell protruding the sediment surface increases bottom roughness. Its movements destabilize the sediment surface. And by opening and sudden adduction of the valves, *C. edule* causes sediment to become resuspended (Montserrat et al. 2009).

4) The Baltic clam, *Macoma balthica*, is a facultative surface–deposit feeding bivalve (Kamermans 1994). In muddy sediments, *M. balthica* normally lives at shallow depths (1-5 cm). During the surface-deposit feeding, the animal uses its flexible inhalant siphon to manipulate and ingest the surface sediment around the siphon shaft in a radial fashion, changing the properties of the bed. The grazing of *M. balthica* also disrupts the sediment-armoring biofilm and increases susceptibility to erosion (Willows et al. 1998).

Generally, two types of seed burial were identified in our experiments. *A. virens* functioned as active seed burying engineer (ASE), while *M. balthica, C. edule* and *A. marina* influenced seed burial mainly through seed entrapment and sediment mixing, and are thus viewed as passive seed burying engineers (PSE).

4.2.2 Animal, seed and sediment preparation

C. edule, M. balthica and *A. marina* were collected on tidal flats of the Oosterschelde and Westerschelde, The Netherlands. *A. virens* was acquired from a bait shop (Topsy baits, http://www.topsybaits.nl), easing the collection of a large amount of such worms at the desired body size. Before use they were kept in aerated containers with sediment and filtered Oosterschelde water at a constant temperature of 18 °C and fed with an *Isochrysis galbana* algal solution.

S. anglica seeds (17.6 ± 1.4 mm length, 2.0 ± 0.1 mm width, Fig.4.1a) were collected from salt marshes in the Westerschelde, the Netherlands, where the sediment (45.0 μm d50 and 66.5 % mud content) for experimental use was scooped from the top layer (< 5 cm) of the mudflats. Prior to experiments, the sediment was sieved through a 1 mm mesh sieve to remove large particles, seeds and macrofauna and generate homogenized sediments. The meiofauna remain in the sediment but have a negligible effect on sediment reworking (Willows et al. 1998). To minimize the effect on sediment grain size, we sieved the sediments by manually pressing the sediments through the sieve, rather than washing it through with water, as the latter is likely to cause the loss of fine sediments.

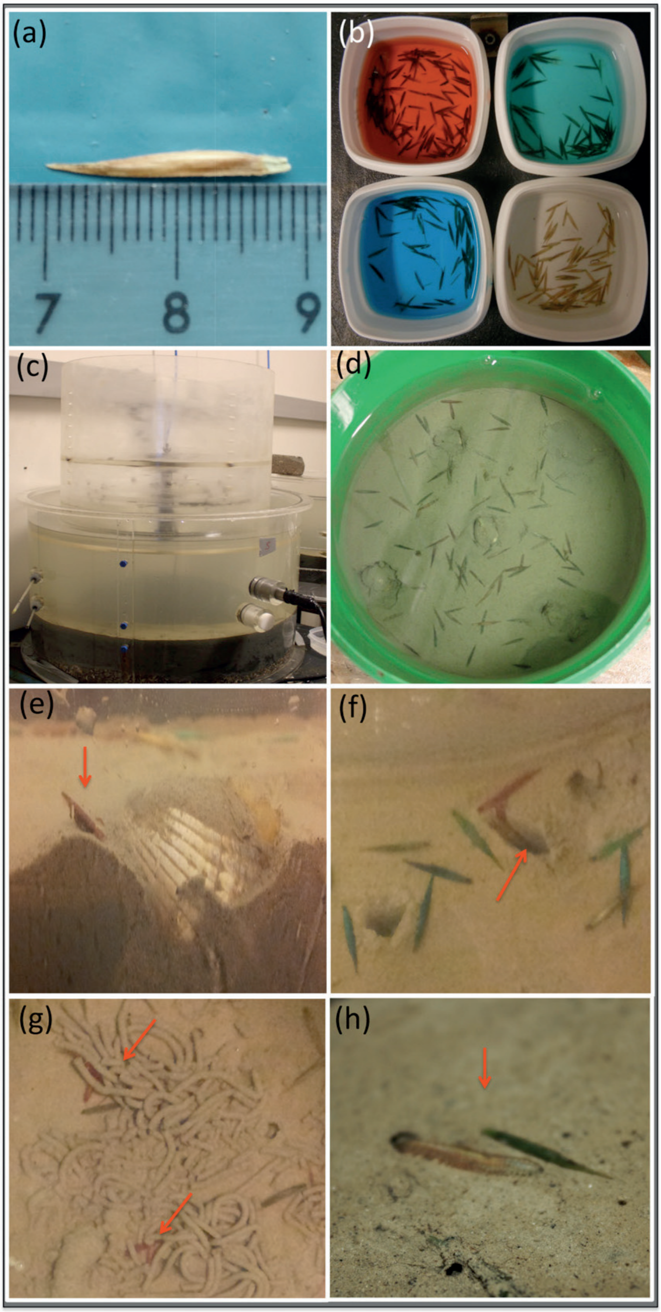


Fig.4.1. Spartina seed (a); Seeds of colour groups (b); Experiment units: annular flume (c) & bucket in the mesocosm (d); Seed entrapment by the protruding shell of C. edule (e), pits created by M. balthica (f), and fecal casts of A. marina (g); Active seed collecting behaviour of A. virens (h)

4.2.3 Flume experiments

Annular flume

To simulate currents, we used annular flumes (Fig.4.1c) of a variation of the design described by Widdows et al. 1998. They consist of two concentric cylinders of transparent acrylic material, creating a 10 cm wide channel, with a surface of ca. 0.15 m^2. Currents are created with a smooth, adjustable rotating disk, driven by a microprocessor-controlled engine. Prior to the experiment, the prepared sediment was put in the flume, mixed to a smooth mass and let consolidate for 3 day. The flumes were then filled with 31.4 L of filtered seawater.

Treatments

To investigate seed burial from purely physical process, we applied a 'currents only' treatment (PHY) without any animals present. To study the interactive effects between physical force and ecosystem engineers, treatments combining both currents and animals (BIO*PHY) were performed separately among different species. The effect of the animal in the absence of currents (BIO) was tested in mesocosm studies (see 4.2.4 Mesocosm experiments).

For making comparable results between species we adopted an equivalent total biomass (Ash Free Dry Weight, AFDW, mg/m2, Table.4.1). Dividing by the individual number, we calculated the individual body size (AFDW, mg) needed for each species, and this was then converted into body length with the conversion coefficients provided from the NIOZ - Yerseke Monitor Taskforce. For all the species, this total biomass is within the range found in the field (Ysebaert and Herman 2002). To examine if seed burial effect is related with total biomass of the ecosystem engineer, we additionally applied a low total biomass for *M. balthica*.

For each treatment, two flumes (replicates) were used. For each species, homogeneous-sized individuals were introduced into the flume and allowed time to burrow. The vast majority of the animals were found to bury themselves within a few minutes. Those that did not burrow were replaced with other individuals. After the animals acclimated for 2 days, 200 waterlogged seeds of 4 color groups (Fig.4.1b, red, green, blue and uncolored seeds, 50 each) were released into the water column and let settle down to the sediment surface. Seeds were color-coated with food dye (Bakery ingredient, www.steensma.com) to 1) enhance their visibility during observation, and 2) provide replicate measurements of seed burial.

Table.4.1 Treatments of PHY (currents only), BIO (animals only) and BIO * PHY (coupling between animals and currents); parameters related with animals were given for each treatment, respectively

Treatments		Total biomass (AFDW, mg)	no. of Ind.	Biomass density (AFDW, mg/m2)	Ind. body length (cm)	Abbr.
PHY	_	_	_	_	_	PHY
BIO*PHY	*M.balthica*	583	24	38.9	1.4	Mb*PHY_Low
	M. balthica	2428	100	161.9	1.4	Mb*PHY
	C.edule	2428	24	161.9	3	Ce*PHY
	A. marina	2428	24	161.9	6	Am*PHY
	N.virens	2428	24	161.9	10	Nv*PHY
BIO	*M. balthica*	809	33	161.9	1.4	Mb
	C.edule	809	8	161.9	3	Ce
	A. marina	809	8	161.9	6	Am
	N.virens	809	8	161.9	10	Nv

Protocol

Each flume experiment lasted for 4.5 days. To simulate two tidal cycles in the intertidal mudflats, there were 2 runs of currents each day from Day 1 to 4. Between these two runs, there were intervals of ca. 3 h to ensure the settlement of suspended sediment. There was only one run on Day 5 when the experiment ended and thereafter seed burial data were collected. We did not simulate fluctuating tidal height as occurs in the field, but adopted the typical current velocities observed in the pioneer zone of the marshes in the Scheldt estuary (Bouma et al. 2005), as the bed shear stress in the annular flume is determined by current velocity rather than water depth (Widdows et al. 1998, Willows et al. 1998). In each run, the current velocity was increased in a first step from 0 to 10 cm sec^{-1} and subsequently in steps of 5 cm sec^{-1} to a maximum velocity of 25 cm sec^{-1}. Each current step lasted 20 minutes. With increasing velocity, an increasing amount of sediment was eroded into the water column. Air was bubbled into the flume in between two runs.

Data collection

To quantify sediment erosion caused by currents and their coupling with ecosystem engineers, water turbidity was measured using an optical backscatter sensor (OBS 3+, Campbell scientific) every 30 seconds during each run. The OBS sensors were calibrated by gravimetric analysis and the data were converted in Suspended Sediment Concentration (SSC, g/L), Peak SSC served as a proxy to compare the capacity of sediment reworking between treatments.

Seeds were regarded as buried when they were no longer visible from the surface. Unburied seeds were removed, classified according to their color and counted. Seeds of green and blue colors were pooled as one group, with the rest two colors as the other group, due to the difficulties in distinguishing blue and green seeds after processing. Total burial (%) was measured as the number of buried seeds divided by the total number of deployed seeds. 40 sediment core samples were randomly taken by syringes (Diameter = 3.6 cm) to a depth of 10 cm, and then sliced every 1 cm to determine seed burial depth. The depth of each seed was recorded as the upper limit of its recovery depth range. Thereafter the proportion of seeds buried at each depth was estimated for each treatment.

4.2.4 Mesocosm experiments

To quantify seed burial effects of the different species under the 'no currents' condition (BIO), a mesocosm experiment was performed in plastic buckets (ca. 0.05 m^2 surface area, Fig.4.1d) with comparable seed density (20 for each color group) and animal biomass as the flume experiments (Table.4.1). After compaction of the sediment (depth ca. 15 cm), each bucket was filled by filtered seawater with bubbled air to a water level of 10 cm. Animals and seeds were introduced in the same way as in 2.3.2.

Since very few seeds were buried in this experiment (Fig.4.2), the top 1 cm sediment was carefully excavated using a spoon and sieved to detect the buried seeds. The buried seeds were all found within this layer with most of them merely covered by a thin sediment layer, except for the treatment with *A. virens.* To acquire more accurate seed burial depth by *A. virens* under 'no currents' condition, a supplementary experiment was conducted by using 5 PVC tubes

(Diameter = 20 cm, Height = 20 cm) with each 20 seeds and 3 worms introduced. These cores were immersed (10 cm above core surface) in a tank with air bubbled filtered seawater. On Day 5, the sediment cores were sliced every 1 cm, by applying a piston pushed up from the bottom of the tube to extrude the sediment in 1 cm increments. The slicing process terminated when all the buried seeds were retrieved from the sediment by rinsing in a 1 mm mesh sieve. The burial depths of the recovered seeds were recorded and the proportion of seeds buried at each depth was calculated.

4.2.5 Data analysis

Pairwise t test showed that seed color did not affect burial, since there was no significant difference of total burial between color groups ($p = 0.78$). One-Way ANOVAs were run to examine whether total burial ($n=4$, 2 color groups × 2 flumes/buckets) varied 1) between BIO, PHY and BIO*PHY treatments, and 2) between species in the BIO and BIO*PHY treatments, respectively.

To test whether there were interactive effects between currents and animals, one-way ANOVA was also employed to test the difference between total burial under BIO*PHY treatments with the sum of their separate effects i.e. BIO + PHY (adding the mean value of PHY to every single value of BIO);

To detect whether seed burial depth differed between treatments, we used one-way ANOVA to compare the proportion of seeds buried at the relatively shallow and deeper depths. Seed proportion ($n=2$, except $n=5$ for Av) at the range of 0-2 cm was compared for all the treatments, whereas comparisons at the range of > 2 cm were done for those that resulted in seed burial deeper than 2 cm.

Post Hoc pairwise comparisons were achieved through Tukey HSD tests. Prior to analysis, the data was arcsine transferred to improve data normality. To examine whether seed burial quantity is linked with sediment reworking ability, a linear correlation was carried out on the relationship between total burial and peak SSC. All the statistical analysis was done in R (http://www.R-project.org), applying a significance level of $\alpha = 0.05$.

4.3 Results

4.3.1 General observation

In the flume experiments, seeds started moving once current velocity reached 10 cm/s^{-1}; while moving near the bottom, it was observed that some seeds were trapped by protruding shells of *C. edule* (Fig.4.1e) and pits caused by *M. balthica* (Fig.4.1f). This was not observed for these two species at the absence of currents. During both flume and mesocosm experiments, some seeds were seen covered by the faecal casts *A. marina* produced (Fig.4.1g). Only *A. virens* was observed to deliberately grab and pull the seeds to their burrows (Fig.4.1h) regardless of the presence or absence of currents. When recovered, the buried seeds were frequently found inside the burrows of *A. virens*.

4.3.2 Total burial

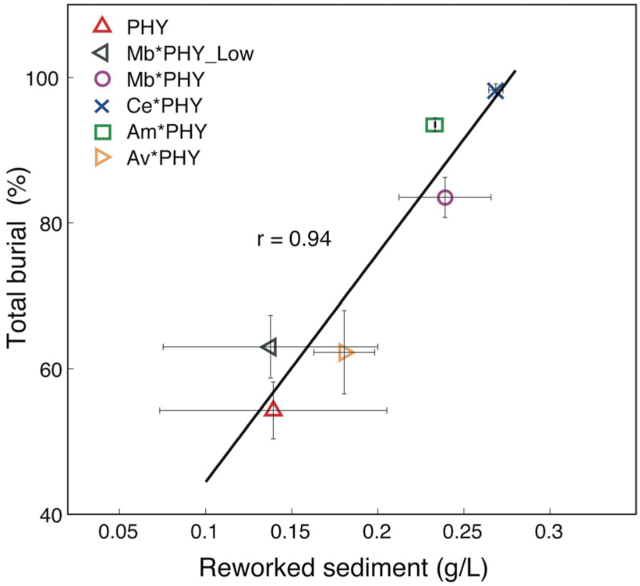


Fig.4.2. A linear correlation between total burial (Mean ± SE, n=4) and the amount of reworked sediment (Peak SSC, Mean ± SE, n=2) under purely physical process (PHY) and the coupling between currents and animals (the rest).

In the flume experiments, total burial showed a significant linear relationship ($p < 0.01$) with sediment reworking (Fig.4.2). With less sediment reworked, active seed burial engineer (ASE) *A. virens* (Av*PHY) buried much fewer seeds than the other species, whereas passive seed burial engineer (PSE) *C. edule* (Ce*PHY) had the greatest total burial meanwhile causing the highest amount of eroded sediment (Fig.4.2).

Table.4.2 Statistical results for pairwise comparisons (TukeyHSD test) of total burial between treatments; Definitions for all the abbreviations of treatments are given in Table.4.1; 'BIO + PHY' refers to the cumulative total burial of PHY and BIO (Mb, Ce, Am or Nv) treatments.

Comparisons				p
PHY vs BIO*PHY	PHY	vs	Mb*PHY_Low	0.701
	PHY	vs	Mb*PHY	< 0.001 ***
	PHY	vs	Ce*PHY	< 0.001 ***
	PHY	vs	Am*PHY	< 0.001 ***
	PHY	vs	Nv*PHY	0.756
PHY vs BIO	PHY	vs	Mb	< 0.001 ***
	PHY	vs	Ce	< 0.001 ***
	PHY	vs	Am	< 0.001 ***
	PHY	vs	Nv	< 0.001 ***
BIO*PHY vs BIO	Mb*PHY	vs	Mb	< 0.001 ***
	Ce*PHY	vs	Ce	< 0.001 ***
	Am*PHY	vs	Am	< 0.001 ***
	Nv*PHY	vs	Nv	< 0.001 ***
BIO*PHY vs BIO + PHY	Mb*PHY	vs	Mb + PHY	< 0.001 ***
	Ce*PHY	vs	Ce + PHY	< 0.001 ***
	Am*PHY	vs	Am + PHY	< 0.001 ***
	Nv*PHY	vs	Nv + PHY	0.762
High vs low density	Mb*PHY	vs	Mb*PHY_Low	0.008 **
Between species (BIO*PHY)	Mb*PHY	vs	Ce*PHY	< 0.001 ***
	Mb*PHY	vs	Am*PHY	0.13
	Mb*PHY	vs	Nv*PHY	0.006 **
	Ce*PHY	vs	Am*PHY	0.094
	Ce*PHY	vs	Nv*PHY	< 0.001 ***
	Am*PHY	vs	Nv*PHY	< 0.001 ***
Between species (BIO)	Mb	vs	Ce	0.996
	Mb	vs	Am	0.029 *
	Mb	vs	Nv	0.004 **
	Ce	vs	Am	0.043 *
	Ce	vs	Nv	0.006 **
	Am	vs	Nv	0.698

Significance level: * 0.05, ** 0.01, *** 0.001

Total burial significantly differed ($p < 0.001$) between treatments (Fig.4.3). For PHY treatment 54.3 ± 3.9 % (Mean ± SE) seeds were buried. This was much higher than that from any BIO treatment (Table.4.2), which was 3.8 ± 1.6 %, 4.3 ± 2.1 %, 13.1 ± 2.1 % and 16.3 ± 2.2 % for *M. balthica* (Mb), *C. edule* (Ce), *A. marina* (Am) and *A. virens* (Av) respectively. Among these treatments, total burial did not vary between the two worm species *A. marina* and *A. virens*, while both of them had a higher total burial than the two bivalve species *M. balthica* and *C. edule* (Table.4.2).

BIO*PHY treatments resulted in significantly higher (Table.4.2) total burial than that of PHY, except for ASE *A. virens* (Av*PHY) and low density *M. balthica* (Mb*PHY_Low). For *M. balthica,* a higher individual density (Mb*PHY) generated significantly higher total burial (Table.4.2). For all PSEs, their coupling with currents produced significant interactive effects that BIO*PHY treatments with PSEs lead to greater total burial than the sum of BIO and PHY, whereas in this respect there was no significant difference for ASE *A. virens* (Table.4.2).

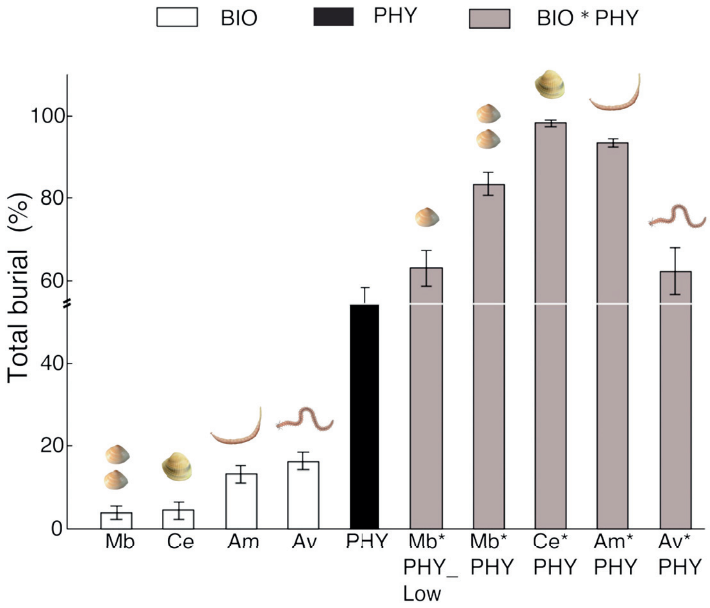


Fig.4.3.Proportions of seeds (Mean ± SE, n=4) that were buried for all the treatments of BIO (animals only), PHY (currents only) and BIO * PHY (coupling between animals and currents).

4.3.3 Seed burial depth

There was a general trend of decreasing seed proportion with burial depth, and for all the treatments more than two thirds of the buried seeds were buried into relatively shallow depths (0-2 cm, Fig.4.4).

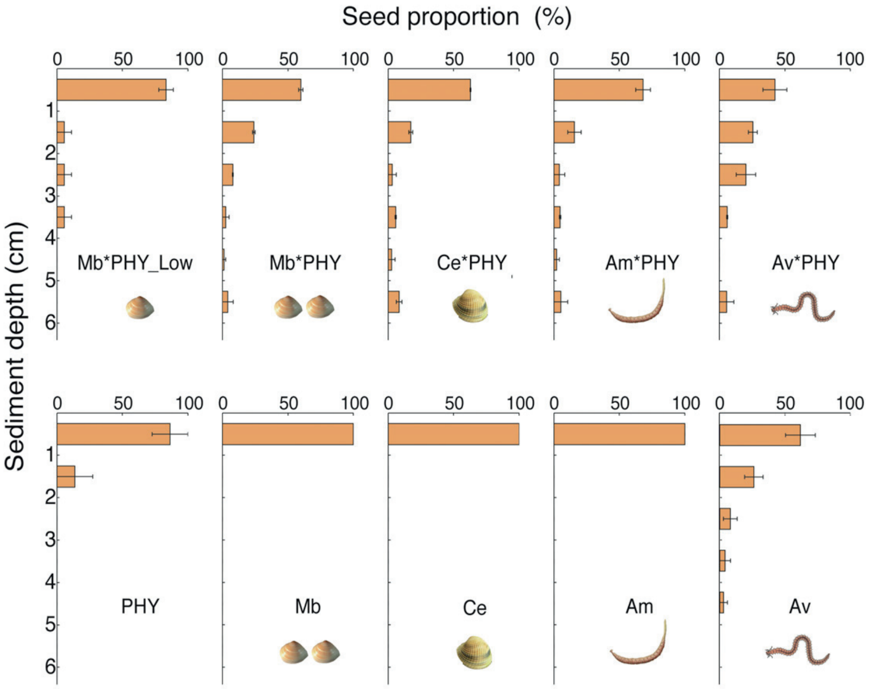


Fig.4.4 The vertical distribution of the buried seeds (Mean ± SE, n=2, except n=5 for Av) for each treatment, respectively.

Seed burial depth varied between treatments (Fig.4.4). Seeds were only buried to shallower depths (86.4 % at 1 cm and 13.6 % at 2cm) by the purely physical process (PHY). The range of burial depth became however much broader with some seeds buried deeper when coupled with animals. For all the BIO*PHY treatments, the maximum recovery depth reached 6 cm, except for the one of low density of *M. balthica* (Mb*PHY_Low) with a maximum depth of 4 cm. Without currents, seed burial was very limited for all PSEs (Mb, Ce & Am). The recovered seeds were only within the top 1 cm, most of which were found merely covered by a thin layer of sediment.

In contrast, in the absence of currents, ASE *A. virens* (Av) still buried the seeds to a broad range of 0-5 cm (Fig.4.4). Its coupling with currents (Av*PHY) did not reinforce seed burial effect either at deeper or shallower depths, compared with their separate effects. There was no significant difference ($p = 0.626$) in the proportion of seeds buried deeper than 2cm between Av and Av*PHY. Nor was there significant difference ($p = 0.456$) between Av*PHY and PHY in the proportion of seeds buried at shallower depths (0-2 cm).

4.4 Discussion

Traditionally, ecosystem engineers were credited as the most important seed burial driver for both terrestrial and aquatic plants (e.g. Chambers and Macmahon 1994, Blackburn and Orth 2013). Using tidal marsh as a model system, our study reveals that *i)* physical force can be at least as important as the effect of ecosystem engineers for seed burial, and *ii)* there are interactive effects between these two agents, serving as an important seed burial driver. Our flume experiments showed that purely physical force (i.e. currents) is able to generate widespread seed burial. However, more importantly, physical force and ecosystem engineers can act in synergy in seed burial, resulting in greater seed burial quantity and deeper burial depths.

4.4.1 Biophysical seed burial: towards a general concept

Our results indicated that the outcome of seed burial by biophysical coupling depended on the functional properties of the ecosystem engineers. The coupling of currents and active seed burial engineer (ASE) *A. virens* merely results in additive seed burial effects. This could be due to its low sediment reworking capacity and the nature of active seed displacement that operates independently of physical forces. Without currents, this species can still bury seeds through direct seed transport to their burrows. Such behaviour is analogous to other marine worms *Hediste diversicolor* & *Neanthes succinea* (Delefosse and Kristensen 2012, Blackburn and Orth 2013), and also earthworm *Lumbricus terrestris* (Milcu et al. 2006, Regnier et al. 2008). In contrast, passive seed burial engineers (PSE) and currents interactively produce large, synergistic effects with enhanced total burial and burial depth, regardless of the different modalities in which their bioturbation activities are performed (Fig.4.3 & Fig.4.4).

Two mechanisms can be involved in such biophysical synergy (Fig.4.5).

1) **Enhancement of seed entrapment**. In our study, some seeds were trapped by biogenic microtopography (e.g. protruding shells, pits and faecal casts, Fig.4.1) while moving along the bottom with currents. An early flume study showed that the presence of the polychaete *Clymenella torquata* greatly enhanced seed entrapment of seagrass seeds (Luckenbach and Orth 1999). Once seeds are trapped within burrows or depressions, they might be further displaced downward by animal movement or covered by infilling sediment, resulting in deep burial.

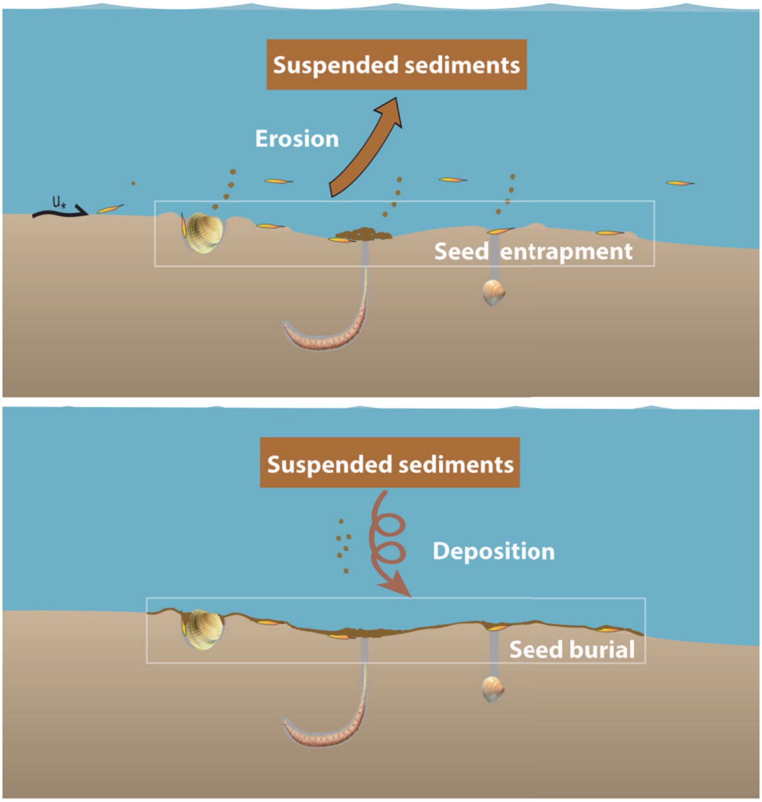


Fig.5 (a) Bottom roughness induced by PSEs (*C. edule, A. Marina & M. balthica*; left to right) promotes the entrapment of seeds (in yellow) moving along or settling to the sediment surface. Co-currently, the coupling between currents and bioturbation by PSEs reinforces erosion and resuspension of sediment particles (brown dots). (b) The increased sediment availability in the water column serves as a source for subsequent sediment deposition facilitating the burial of the entrapped seeds

2) **Promotion of sediment mixing**. Through destabilizing the substrate, ecosystem engineers promote erosion and spatial redistribution of sediments (this study, Widdows and Brinsley 2002, Montserrat et al. 2008, Kristensen et al. 2013). They can also aid sediment mixing via biodeposition, e.g. depositing particles as (pseudo) faeces or bioresuspension e.g. eject fluidized faecal pellets into the water column (Graf and Rosenberg 1997, Meysman et al. 2006). The eroded and resuspended sediments may later deposit and accumulate on the positions in which seeds were retained after settlement or entrapment.

Seed burial quantity from biophysical burial was found to differ between species due to species-specific variability on sediment reworking ability (Fig.4.2). The strength of such effect was also linked to biotic parameters e.g. population density as seen in our experiments with two different densities of *M. balthica*. Although not tested in the present study, it is likely that this also applies to other PSEs, since both sediment reworking (Willows et al. 1998) and seed retention (Luckenbach and Orth 1999) were found to relate with species abundance. A study (Delefosse and Kristensen 2012) on the purely biological seed burial effect of marine worms showed that seed burial quantity increased with animal abundance for PSEs, whereas the proportion of buried seeds was independent of individual density for the ASE *H. diversicolor* due to its selective and active search for seeds. Body size may also be important for seed burial since it influences organism metabolism rate (Max 1932) that serves as fueling processes for their engineering activities (Brown et al. 2004).

It must also be noted that seed burial rates estimated in our flume and mesocosm experiments should be treated in caution when extrapolated to the real world, since we did not include all the parameters (e.g. waves) that may affect seed/sediment transport in the field. Nevertheless, these simplified but well controlled lab experiments were effective to gain a mechanistic understanding of the interactive effects of physical forces and different types of ecosystem engineer on seed burial, as illustrated in this study.

4.4.2 Relevance beyond the intertidal

Biophysical seed burial discussed above is likely to be applicable to various ecosystems. For instance, we can think about macrophytes growing in aquatic systems with flowing water such as e.g. estuaries, coasts, riverbanks and streamsides. Seed burial effect can be habitat-specific due to the differences in physical settings e.g. magnitude, frequency and duration of the physical force (i.e. water flow), soil/sediment type. These variables not only determine the distribution of the benthic fauna (Cozzoli et al. 2013), but also primarily control soil/sediment erosion that are amplified by the activities from ecosystem engineers (Widdows and Brinsley 2002, Meysman et al. 2006, Corenblit et al. 2011). Seed burial effects may also differ between plants in response to variable seed properties and specific interactions with their environment. For instance, seed morphology (e.g. length, width and eccentricity) and seed mass affected seed entrapment in soils (Chambers et al. 1991). Seed buoyancy can affect seed retention by trapping structures in salt marshes (Chang et al. 2008). In addition, disturbance incidents such as waves or raindrops, might also affect seed burial due to their capacity to induce vertical movement of seeds and soil/sediment particles (Chang et al. 2008, Marthews et al. 2008).

Previous studies mainly focus on the importance of physical force in horizontal seed movement, e.g. the role of tidal currents and waves in driving the dispersal of seagrass seeds (Koch et al. 2010). Nonetheless, our study shows that the same physical force (i.e. currents) can cause both lateral seed transport (to move) and vertical translocation (to retain). The former increases the probability of encountering a microsite that favors seed entrapment. By interacting with ecosystem engineers, physical force facilitates the remobilization of the particles that can lead to seed burial. In one sense, to move is to find a better place to retain.

The importance of physical force for seed burial is not restricted to aquatic systems. In terrestrial systems, wind or precipitation induced overland flow can move seeds over the soil surface to microsites (e.g. burrows, mounds) caused by ecosystem engineers (Corenblit et al. 2011), and/or geophysical processes e.g. desiccation cracks (Burmeier et al. 2010). A wind tunnel experiment showed that seeds move frequently on smooth surfaces but were trapped on rougher surfaces (Johnson and Fryer 1992). On litter-covered surfaces, abiotic forces deposit seeds in the same locations as plant litter, after which animals often bury seeds in litter or under litter-covered surfaces (Chambers and Macmahon 1994). The potential couplings between physical forces such as wind or rainfall and terrestrial ecosystem engineers (e.g. ants, earthworms) on redistributing seeds and shaping soil dynamics, as well as their consequences for seed burial and soil seed bank formation would be interesting to investigate in future studies.

4.4.3 Conclusions

Overall, this study contributes to a better mechanistic understanding of seed burial and seed bank formation, by pointing out the need to incorporate physical force into the mechanisms that drive seed entry into the soil/sediment. To our knowledge, we presented the first empirical evidence that physical force can affect seed burial by synergistically interacting with ecosystem engineers, underscoring the significance of biophysical interaction as a driver for vertical seed movement. Although present work was illustrated for intertidal ecosystems, similar mechanisms may be expected to operate in other aquatic and also non-aquatic ecosystems. Hence an important challenge for the future will be to evaluate these mechanisms across ecosystem types.

Chapter 5

5. Climate-change intensified wave forcing risks the meltdown of coastal wetland seed banks

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Submitted

Abstract

Recently society shifts to a more ecosystem-based coastal protection in the face of climate change, incorporating soft defense structures like coastal wetlands. However, uncertainty arises due to a lack of understanding on how climate change may affect the resilience and sustainability of coastal vegetation. Using a space-for-time substitution strategy, we evaluate the potential impact of intensified physical disturbances under climate change on a key reproductive phase, seed bank persistence, by linking it to varying hydro-morphodynamics conditions along wave gradients. We conducted a large-scale field study in a NW European Delta, using a globally common coastal foundation plant cordgrass. The results indicated that 1) larger bed shear stress induced by stronger waves led to higher seed removal on the surface, 2) greater sediment dynamics eroded more buried seeds. Our results also revealed that benthos could affect seed bank persistence by tuning the magnitude of sediment disturbance. Our study suggests that increasing storminess and associated sediment variability under climate change, is likely to decline seed bank persistence on tidal flats, posing challenges for future seed colonization in coastal wetlands.

5.1 Introduction

Vegetated coastal ecosystems (e.g. salt marshes, seagrasses, mangroves) are among the most ecological and socio-economically valuable systems on the globe (Costanza et al. 1997). Over the last decades, there has been increasing recognition of the coastal defenses value of vegetation due to their strong ecosystem engineering capacities e.g. wave attenuation and sediment stabilization (Gedan et al. 2011, Temmerman et al. 2013, Moller et al. 2014). This initiated a paradigm shift to a more ecosystem-based flood protection using vegetated foreshores in the face of climate change (Cheong et al. 2013, Temmerman et al. 2013). Given a rising sea level and increasing storminess as predicted for many parts of the world (Donat et al. 2011, Young et al. 2011) and the vulnerability of coastal wetlands to environmental variations like sea level rise (Kirwan and Temmerman 2009, Kirwan and Megonigal 2013) and waves (Callaghan et al. 2010, Marani et al. 2011), it raises the uncertainty over a sustainable ecosystem-based coastal protection. The main knowledge gap hampering application of wetland ecosystems within coastal defense schemes is the uncertainty in the long-term ecosystem dynamics (Bouma et al. 2014).

Sexual recruitment of coastal foundation plants (e.g. salt marsh pioneer species) in tidal wetlands is a key process in the long-term persistence of ecosystems (van de Koppel et al. 2005, Friess et al. 2012). Unfortunately, such recruitment forms a bottleneck, as physical disturbances (e.g. waves and associated sediment dynamics) impose difficulties for both seedling survival (e.g. Bouma et al. 2009, Friess et al. 2012, Balke et al. 2013) and seed bank persistence (Chapter 2, Groenendijk 1986, Marion and Orth 2012). Although the minimal period that seed banks need to persist can be short, to cover the period between when seeds become available (fall) and seedlings start to emerge (spring), the persistence during this short period is critical.

Seed bank persistence on tidal flats is primarily constrained by high seed mobility on the surface (Chapter 2, Groenendijk 1986, Marion and Orth 2012), where wave action plays a critical role in remobilizing the initially deposited seeds (Chang et al. 2008, Koch et al. 2010). Seed banks in the coastal sediment matrix are vulnerable to hydrodynamic induced disturbance, as seed dislodgment due to erosion serves as a major source for seed bank degradation in tidal habitats (Chapter 2, Groenendijk 1986, Marion and Orth 2012). Therefore, climate-change intensified hydrodynamic disturbance and associated sediment erosion may be expected to reduce seed bank persistence of coastal foundation plants, with far-reaching consequences for long-term ecosystem stability and functions.

One of the keystone coastal plant engineers is cordgrass (*Spartina* spp.), which globally defines and stabilizes the shoreline of many temperate marshes, while still extending its global territory as an invader (Strong and Ayres 2009, Strong and Ayres 2013). Seed colonization plays a vital role in its natural (re-) establishment and range expansion, as well as the fast spread in many areas where the species has been willingly or accidentally been introduced (Gray et al. 1991, Ayres et al. 2008, Xiao et al. 2009). Seedling establishment of cordgrass occurs mainly in the pioneer zone and adjacent bare mudflats at suitable elevations at the presence of an annually built soil seed bank (Wolters and Bakker 2002, Xiao et al. 2009). Given the role of cordgrass in both enhancing coastal defense as powerful ecosystem engineers and threatening the biodiversity as invaders in various regions (Strong and Ayres 2013), it is highly valuable to study the potential effects of climate-induced shifts on the fate of its (annual) seed bank.

To evaluate the likely response of seed bank persistence to altered disturbance regimes under climate change, we employed a space-for-time substitution strategy, as utilized often in ecological studies regarding climate change (e.g. Banet and Trexler 2013, Blois et al. 2013, Lester et al. 2014). Specifically, we investigate the persistence of the cordgrass seed bank in tidal flats along wave gradients and examine whether increased physical disturbances decline seed bank persistence of cordgrass. Here we focus on the physical presence of seeds both on top of, and buried within sediment, whereas other components (e.g. viability) are beyond the scope of the present study. We hypothesize that 1) stronger wave disturbance leads to lower retention of surface seeds, thereby suppressing the formation of a buried seed bank, and 2) greater sediment dynamics erodes more seeds from the buried seed bank, lowering seed bank persistence. Testing these hypotheses by analyzing trends will deliver basic insights into forecasting how enhanced storminess might affect seed bank persistence.

To achieve this, we conducted a large-scale field study in the Scheldt estuary in NW Europe, where seed bank persistence of cordgrass in the tidal flats was linked to local hydrodynamics and resulting sediment dynamics by comparing multiple field sites with contrasting wave exposure. Sediment dynamics in tidal flats is not only governed by hydrodynamics and sediment transport, but is also tightly coupled with ecosystem engineering of benthos (Herman et al. 1999, Widdows and Brinsley 2002). Hence, at each site we also compared seed bank persistence in defaunated sediment with that in natural sediment to detect the importance and the effects of benthos.

5.2 Materials and Methods

5.2.1 Study site

The Scheldt estuary is a macrotidal estuary situated near the border between The Netherlands and Belgium (Fig.5.1). The mean tidal range increases from 3.8 m near the mouth of the estuary to >5.0 m upstream of the border (Baeyens et al. 1998). It was originally composed of two aligned and interconnected water bodies called Westerschelde and Oosterschelde. Due to land reclamation, the Oosterschelde was progressively separated from the Westerschelde. The pioneer salt marsh vegetation consists mainly of the perennial common cordgrass (*Spartina anglica*), which was introduced to the Scheldt estuary in the 1920s (Groenendijk 1986, Van der Wal et al. 2008).

The study area comprises seven salt marshes with different wave exposure due to their position relative to the prevailing southwesterly winds (Callaghan et al. 2010). Four of them are in the Westerschelde and the rest in the Oosterschelde (Table.5.1, Fig.5.1). All the experiments and measurements were conducted in adjacent tidal flats in front of a salt marsh, except for the more exposed site Dortsman (DO). This location potentially experiences strongest wave disturbances among all selected sites, as hinted by its coarsest sediment (Table.5.1) and the absence of vegetation. The elevation of the experimental area was ca. 90 cm above NAP (i.e., Dutch ordinance level, which is close to local mean sea level) for most field sites, while a higher elevation (175 cm NAP) was adopted in two Westerschelde sites, Zuidgors & Baarland where salt marshes extend less deep. At Zuidgors, we included an additional location with a lower elevation (ca. 90 cm NAP) for direct comparison between the higher and lower elevations. In total eight locations (Table.5.1) were selected for our field experiments and measurements implemented during April-July, 2013 (T0-T5, detailed schedule given in Table.5.2). We choose the period of April-July, to exclude the impacts of severe storms, to obtain conservative lower limit of the effect of climate change, and to facilitate the process of gaining a mechanistic insight in the underlying processes. At each location, the experimental area (ca. 30 x 5 m) consisted of two adjacent experimental zones (Zone S for surface seeds & Zone B for buried seeds, Fig.5.2).

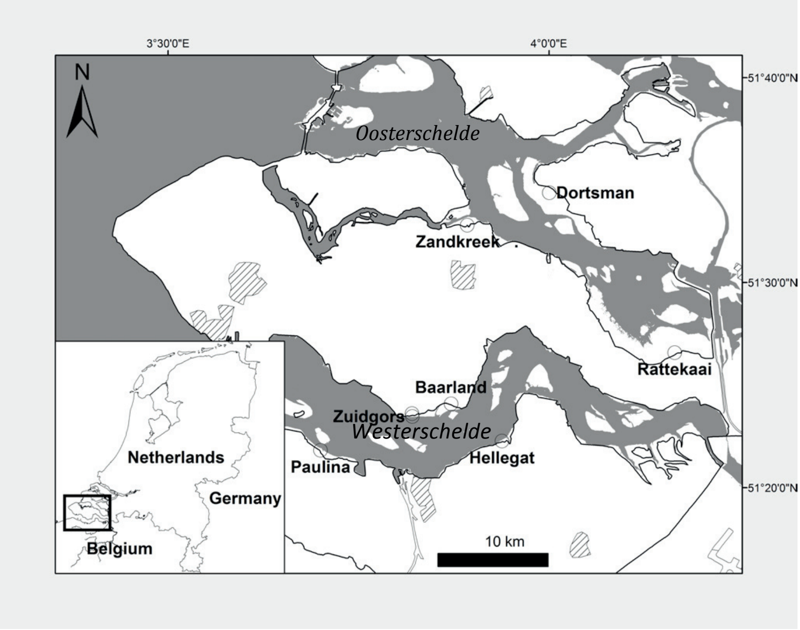


Fig.5.1 Geographic locations of selected study sites

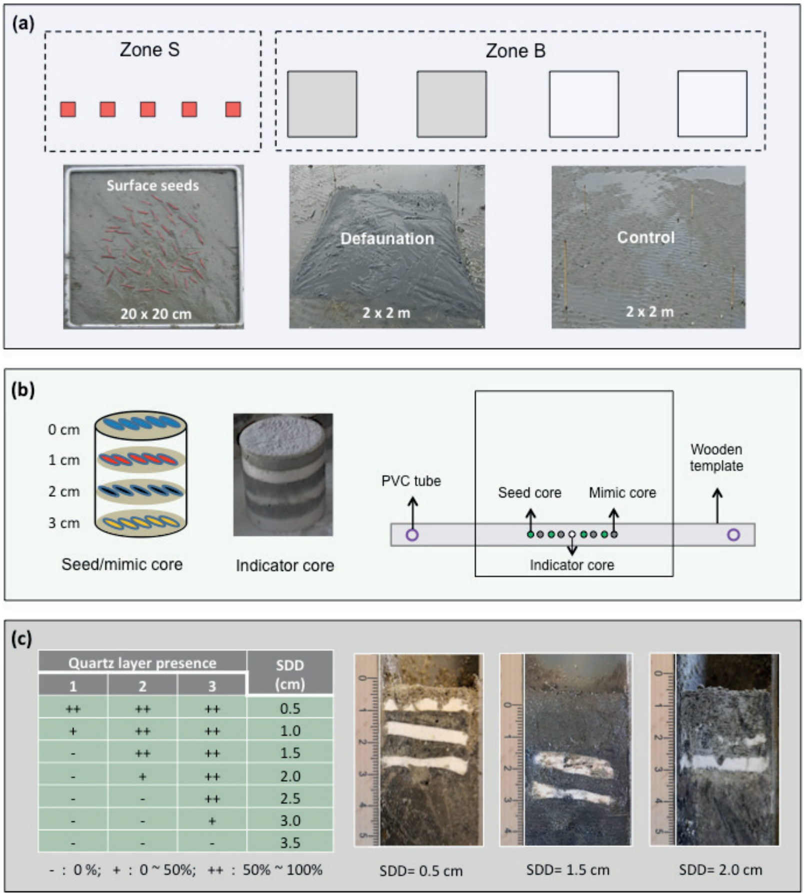
Table.5.1 Study locations and their properties

Location		Elevation (cm NAP)	Grain size (D50, μm)	Abbreviation
Oosterschelde	Dortsman	90	159.8	DO
	Rattekaai	92	98.1	RA
	Zandkreek	85	96.3	ZA
Westerschelde	Zuidgors	89	45.4	ZG_{LOW}
	Zuidgors	175	72.0	ZG_{HIGH}
	Baarland	175	23.6	BA
	Hellegat	102	109.6	HE
	Paulina	82	76.0	PA

5.2.2 Persistence of surface seeds in response to wave disturbance

To test the hypothesis that stronger wave disturbance leads to lower retention of surface seeds, we quantified the persistence of surface seeds at all locations by seed sowing and recovery experiment in Zone S during T0-T4 (Table.5.2). We repeated it three times with duration of 4 weeks (trial1: T0-T1; trial2: T1-T2) and 2 weeks (trial3: T3-T4), respectively. At each trial, five 20 x 20 cm quadrates were laid in a line at 1m intervals. Within each quadrate, 50 cordgrass seeds were randomly sowed on the sediment surface (Fig.5.2a). These quadrates were positioned through a 6 m-long string (marked every 1 m) with each end fixed at a PVC tube. The sowed seeds were dyed with Rose Bengal to be distinguished from the ambient seeds and were waterlogged to mimic the naturally settled cordgrass seeds. Cordgrass flowers from July to October and seeds ripen within 12 weeks. Seed release starts from autumn, extending to the winter and early spring of the following year (Huiskes et al. 1995). During this period, tides are generally higher, storms occur more frequently and there is more standing water present on the sediment surface during this period. Therefore, seeds are more likely to be waterlogged and will tend to remain at a microsite unless they are dislodged by wave action (Chang et al. 2008).

Upon recovery, sediment bulks (each 40 x 40 x 5 cm, Length x Width x Depth) were excavated after relocation of the quadrates by using the same string. There was no seed visible on the surface when recovered. Sampling the top 5 cm, ascertained the recovery of shallowly buried seeds. The recovered area was chosen to be twice as big as the seed-deployment area, to account for seeds that might have merely moved to the close vicinity. Seeds not recovered within this area were regarded as 'lost'. The retrieved sediment was transported back to the lab and sieved through a 1mm sieve, to quantify the remained seeds. Surface seed persistence (%) was calculated as recovered/deployed, which reflects the probability of seed removal due to secondary seed transport by hydrodynamics.



Quartz layer presence			SDD (cm)
1	2	3	
++	++	++	0.5
+	++	++	1.0
-	++	++	1.5
-	+	++	2.0
-	-	++	2.5
-	-	+	3.0
-	-	-	3.5

- : 0%; + : 0 ~ 50%; ++ : 50% ~ 100%

Fig.5.2 (a) Plot setup in the experiment zone; (b) seed bank core, indicator core and their deployment in the plots; (c) scoring rules and examples of SDD

Surface seed persistence (Mean, n=5) at each location was then linked to wave disturbance during the same period. Because the impact of waves on the sediment bed depends on water depth, we calculated the bed shear stress (BSS) induced by waves. BSS is a relevant proxy for the hydrodynamic energy in relation with sediment motion (Callaghan et al. 2010), making it a good proxy for the physical forcing that dislodge seeds on the surface. Both waves and currents can induce BSS. Here we however only focus on wave induced BSS (τ_{bw}) as this has been found to be more important than that induced by currents in the pioneer zone and tidal flats close to a marsh (Callaghan et al. 2010). τ_{bw} was quantified based on in situ wave parameters measured for each location throughout the experiments (see 5.2.4).

5.2.3 Seed bank persistence in response to sediment disturbance

To test the hypothesis that greater sediment dynamics erodes more seeds from the buried seed bank, we assessed the relationship between seed bank persistence and sediment disturbance through a manipulative seed bank experiment in all the selected locations during T1-T3 (Table.5.2).

Table.5.2 Time table for field measurements and experiments at each location

Location	T0	T1	T2	T3	T4	T5
DO	Apr.3	May.1	May.29	Jun.18	Jul. 2	Jul.8
RA	Apr.3	May.1	May.29	Jun.18	Jul. 2	Jul.8
ZA	Apr.3	May.1	May.29	Jun.18	Jul.4	Jul.9
ZG$_{LOW}$	Apr.4	May.2	May.30	Jun.19	Jul. 2	Jul.10
ZG$_{HIGH}$	Apr.4	May.2	May.30	Jun.19	Jul. 2	Jul.10
BA	Apr.4	May.2	May.30	Jun.19	Jul. 2	Jul.9
HE	Apr.5	May.3	May.31	Jun.20	Jul.4	Jul.11
PA	Apr.5	May.3	May.31	Jun.20	Jul.4	Jul.11

Preparation of seed bank cores & indicator cores

The manipulative seed bank was built by placing pre-prepared layered seed bank cores into the sediment. Seed mimics were used to detect if any biogenic seed loss (e.g. predation & decomposition) occurred. For each core, five seeds/mimics were placed at the surface and the depth of 1, 2 & 3 cm (Fig.5.2b). Layered seed/mimic cores were created in 200 ml top-truncated syringes (3.6 cm diameter) with homogenized ambient sediment and color-stained seeds/mimics, using the same methods and mimics (plastic chips of four colors) as described in Chapter 2. This material was proven to be highly effective in mimicking cordgrass seeds (Chapter 2). To prevent seed loss due to germination, cordgrass seeds were beforehand killed by repeated freeze-thaw process in a -20 °C freezer.

Layered indicator cores (Marion and Orth 2012) were employed to estimate sediment disturbance depth (SDD) by placing tracer particles at known depths. Such particles were intended to wash away when momentarily resuspended by wave disturbance, leaving a record of scouring depth (Marion and Orth 2012). In our experiment, Quartz Flour (manufactured silt, white, d50=100μm) served as tracer particles, which were deployed at 0.5cm intervals alternating with ambient sediments to make layered cores consisting of 3 tracer layers and 4 sediment layers (Fig.5.2c). Such cores were produced in the same syringes as the seed bank cores. Each layer was made by filling in homogenized tracer particles/ambient sediment to a thickness of 0.5cm and then sent to the – 20 °C freezer. While still frozen, a new layer was added to minimize mixing. When all the layers were ready, these cores were taken out of the syringes and preserved in the freezer until use.

Deployment of seed bank cores & indicator cores

In Zone B of each location, four permanent 2 x 2 m plots, ca. 3 m apart, were established and marked by PVC stakes (Fig.5.2a). Half of these plots were defaunated to detect the importance and the effects of the benthic animals on seed bank persistence. The defaunation was implemented in January 2013 by covering the plots with a thick rubber sheets that were buried to a depth of 30 cm along the plot edges (Van Colen et al. 2008). Removing the aboveground rubber materials, the defaunated plots were opened after 12 weeks, which was sufficient long to kill the benthic animals by hypoxia (Van Colen et al. 2008). Seed bank cores and indicator cores were established four weeks later at T3 (Table.5.2) when the sediment surface in defaunated plots was leveled by natural physical process. This defaunation method has been proven to greatly lower the abundance of benthic animals; recovery takes at least one summer (Beukema et al. 1999, Van Colen et al. 2008).

In each defaunation/control plot, 4 seed cores, 4 mimic cores and 1 indicator core were deployed. These cores were located in a line through a wooden template with 9 round holes (40 mm diameter), 15 cm apart. The indicator core was situated in the middle, while the seed cores and mimic cores were alternating each other on both sides at intervals of 15 cm (Fig.5.2b). All the cores were transported to the field in a foam box with dry ice to keep them frozen, and then placed with their surface level with the surrounding sediment in the method as described in Chapter 2. Both endpoints of the template possessed a hole that fitted a fixed PVC tube inserted into the soil, to allow accurate re-relocation upon retrieval.

Recovery of seed bank cores & indicator cores and data collection

After 7 weeks (T4, Table.5.2), all these cores were relocated using the same template and recovered to a depth of 100 mm, through PVC pipes which are larger in diameter (5 cm) than the original cores, to ascertain the whole core is re-sampled even if there is a slight imprecision in relocation. The recovered seed/mimic cores (Depth = 100 mm) were transported to the lab, sieved through a 1 mm sieve to retrieve deployed seeds and mimics, which were classified per layer according to their colors. Many seeds (but no mimics) turned black when retrieved because of the anaerobic condition within the sediment. To identify the stained color of those seeds, they were bathed in fresh water for at least 24h until their original stain re-appeared (Chapter 2)

The number of recovered seeds or mimics of each color was counted and recorded. Seed persistence (%) was then calculated as the recovered/total deployed. Higher seed persistence implies lower chance of seed dislodgment from the local seed bank by sediment disturbance.

Upon arrival in the lab, the retrieved indicator cores were stored in a -20 °C freezer until processing. While still frozen, they were longitudinally cross-sectioned using a band saw. The frozen smear left by the band saw was removed using a razor. Sediment disturbance depth (SDD) was estimated through a scoring system that examines the presence of Quartz Flour layers in the profile of the half core (scoring rules and examples given in Fig.5.2c). In total, 30 out of 32 indicator cores were scored, whereas two cores retrieved from one control plot at ZL and one defaunation plot at PA failed due to the damage during processing.

5.2.4 Measurements of physical conditions

Waves and wave-induced bottom shear stress

The wave forcing and tidal level of each location was measured during T0-T5 (Table.5.2), using pressure sensors (OSSI-010-003C; Ocean Sensor Systems, Inc.) deployed in the experimental zone. The pressure sensors were placed 5 cm above the tidal flat surface. The measuring interval and period were 15 minutes and 7 minutes, respectively. The wave analysis was based on pressure fluctuations, as measured with a frequency of 5 HZ. The recorded pressure readings were converted to water level fluctuations, which were then corrected by removing erroneous spikes, shifts, corrupted bursts and low frequency tidal components (c.f. Callaghan et al. 2010, Christianen et al. 2013). From the detrended data, wave parameters e.g. significant wave height (H_S) and peak wave period (Tp) were calculated based on the linear wave theory (Tucker and Pitt 2001).

The bed shear stress due to waves (τ_{bw}, Pa) is calculated as (van Rijn 1993):

$$\tau_{bw} = \frac{1}{4}\rho_w f_w \hat{U}_\delta^2$$

where ρ_w is the sea water density (kg/m3), $\hat{U}_\delta$ is near-bed orbital velocity, calculated as (van Rijn 1993)

$$\hat{U}_\delta = \frac{\pi H}{T\ \sinh(kh)}$$

in which H is wave height (m), T is wave period (s), k is wave number (m^{-1}), calculated by solving $(2\pi / T)^2 = gk\ \tanh(kh)$ (Holthuijsen 2007), and *h* is water depth (m). In practice, significant wave height H_s and peak wave period T_p are used as H and T in the formulae.

f_w is the wave friction coefficient (-) determined by hydraulic regime (Whitehouse et al. 2000):

$$f_w = \begin{cases} 2\mathrm{Re}_w^{-0.5} & , \mathrm{Re}_w \le 10^5 \text{ (laminar)} \\ 0.0521\mathrm{Re}_w^{-0.187} & , \mathrm{Re}_w > 10^5 \text{ (smooth turbulent)} \\ 0.237\ r^{-0.52} & , \text{(rough turbulent)} \end{cases}$$

where $\mathrm{Re}_w = \frac{\hat{U}_\delta \hat{A}_\delta}{\nu}$ and $r = \frac{\hat{A}_\delta}{k_s}$ are wave Reynolds number (-) and relative roughness (-), respectively. The peak orbital excursion $\hat{A}_\delta$ is expressed as $\hat{A}_\delta = \frac{H}{2\sinh(kh)}$, k_s is Nikuradse roughness given by $k_s = 2.5d_{50}$ where d_{50} is median grain size of the bed sediment (m), and ν is the kinematic viscosity of sea water (m2/s).

Breaking-wave check was carried out before τ_{bw} calculation, as the theory mentioned above is applied to non-breaking waves. Data points of $H_s > \gamma h$, where γ is wave-breaking index with the value of 0.78 (Kaminsky and Kraus 1993), were eliminated. In our cases, wave heights over water depths (H_s/h) were all less than 0.78, indicating local non-breaking condition.

Sediment dynamics

To determine the general accretion/erosion pattern of each location, the elevation in the experimental zone was monthly monitored using a 3D Laser scanner (RIEGL VZ-400). All the scans were georeferenced with the Riscan program that came with the scanner. The scans were clipped to the experimental zone. The resulting points were exported to a LAS-file that was later imported to

ArcGis10. For each 5 x 5 cm the maximum value is used to produce a raster. To partly fill the holes in the raster the maximum value for each 20 x 20 cm was also adopted. The two rasters were mosaiced, 5 cm on top. For each location, 20 random points from an undisturbed area (ca. 75 x 75 cm) in each control plot (zone B) and the undisturbed area in between plots were selected, respectively, to calculate the mean elevation at each time point. The scanning was conducted at T0, T1, T2 and T6 (Table.5.2). Due to the instrument failure, elevation values were not available at T2 for DO, RA & ZA.

5.2.5 Statistics

To examine the relationship between surface seed persistence and $\tau_{bw,,}$ we first conducted ANCOVAs with τ_{bw} as continuous variable and 'trial' as category factor. Prior to analysis, the response variable (surface seed persistence) was square root transformed to improve data normality. Since there was no significant difference (Pairwise Wilcoxon Rank Sum Test, p=0.33) between the persistence of seeds and mimics in the manipulated seed bank experiment, their data were pooled together in further analysis. Pairwise Wilcoxon Rank Sum Tests were then employed to detect the difference of seed bank persistence between the defaunated plots and control, conducted for surface seeds and buried seeds, respectively. To examine the relationship between seed bank persistence and SDD, linear correlations were conducted, separately for surface seeds and buried seeds. All the statistical analysis was done in R (http://www.R-project.org), applying a significance level of $\alpha = 0.05$.

5.3 Results

5.3.1 Overview of physical conditions at all locations

The selected locations exhibited variable wave conditions (Table.5.3). The mean significant wave height $H_{s,}$ during three trials covered a range of 0.03 – 0.12 m, and the range of the maximum H_s was 0.17 - 0.44 m. The range of near-bed orbital velocity $\hat{U}_\delta$ was 0.06 - 0.23 m/s, and the maximum value range from 0.24 to 0.60 m/s.

Sediment dynamics also varied with locations. DO, RA, ZA, HE and PA were relatively stable, while ZG_{HIGH}, ZG_{LOW} and BA were more dynamic (Fig.5.3).

Even within the same marsh, ZG$_{HIGH}$ (176 cm NAP) and ZG$_{LOW}$ (94 cm NAP) displayed remarkably distinct accretion/erosion patterns (Fig.5.3). ZG$_{HIGH}$ showed an overall trend of erosion, whereas ZG$_{LOW}$ continuously experienced fast sediment accretion with a cumulative height increase of 4.2 cm after ca. 3 months (T0-T5). Wave induced BSS was higher in ZG$_{HIGH}$ than in ZG$_{LOW}$ (Fig.5.4).

Table.5.3 Significant wave height *Hs* and near-bed orbital velocity $\hat{U}_\delta$ at each location during T0-T1, T1-T2 & T3-T4, respectively.

Location	H_s (m)						$\hat{U}_\delta$ (m/s)					
	T0-T1		T1-T2		T3-T4		T0-T1		T1-T2		T3-T4	
	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
DO	0.07	0.38	0.09	0.37	0.11	0.37	0.12	0.47	0.15	0.49	0.18	0.50
RA	0.07	0.33	0.10	0.38	0.11	0.33	0.12	0.41	0.14	0.43	0.12	0.37
ZA	0.04	0.32	0.07	0.32	0.04	0.17	0.10	0.47	0.14	0.59	0.07	0.36
ZG$_{LOW}$	0.09	0.40	0.10	0.44	0.12	0.39	0.15	0.61	0.15	0.60	0.17	0.59
ZG$_{HIGH}$	0.08	0.37	0.09	0.31	0.09	0.28	0.18	0.54	0.21	0.52	0.22	0.50
BA	0.03	0.20	0.03	0.17	0.04	0.21	0.06	0.24	0.07	0.31	0.09	0.32
HE	0.09	0.34	0.10	0.32	0.08	0.28	0.13	0.45	0.13	0.44	0.10	0.43
PA	0.07	0.25	0.07	0.24	0.06	0.31	0.11	0.37	0.10	0.35	0.10	0.48

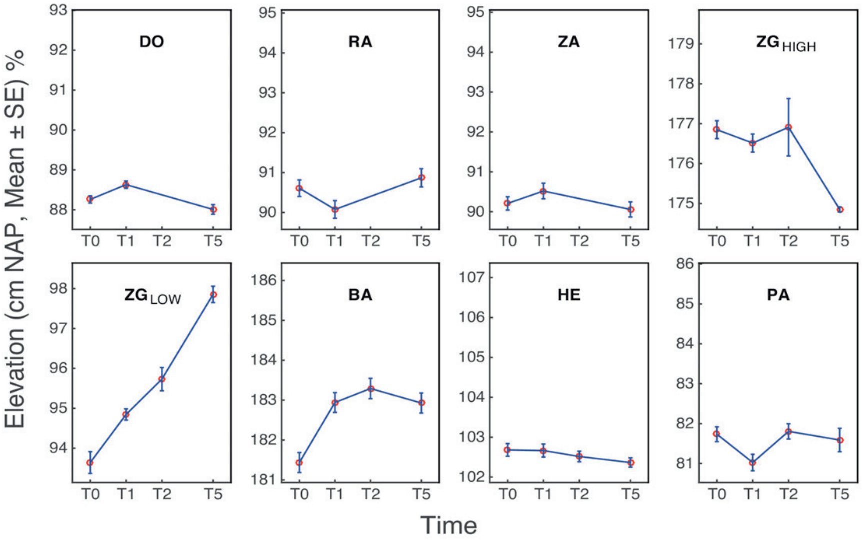


Fig.5.3 Elevation changes in all study locations during the experiment period; Definitions for all the abbreviations of locations are given in Table.5.2.

5.3.2 Relationship between surface seed persistence and wave induced BSS

Regarding surface seed persistence, statistics showed that there was no significant difference between trials (Table.5.4). Generally, surface seed persistence declined with rising τ_{bw} (Fig.5.4). This relationship was not significant when including data from all locations, whereas it became significant if the only fast accreting location ZG_{LOW} was removed from the analysis (Fig.5.4, Table.5.4). Among all locations, the highest surface seed persistence was observed at ZG_{LOW} during T1-T2, despite τ_{bw} at this location was higher than several other locations. High sediment accretion at ZG_{LOW} may enable the seeds a window of opportunity to get sufficiently buried to escape subsequent wave disturbance.

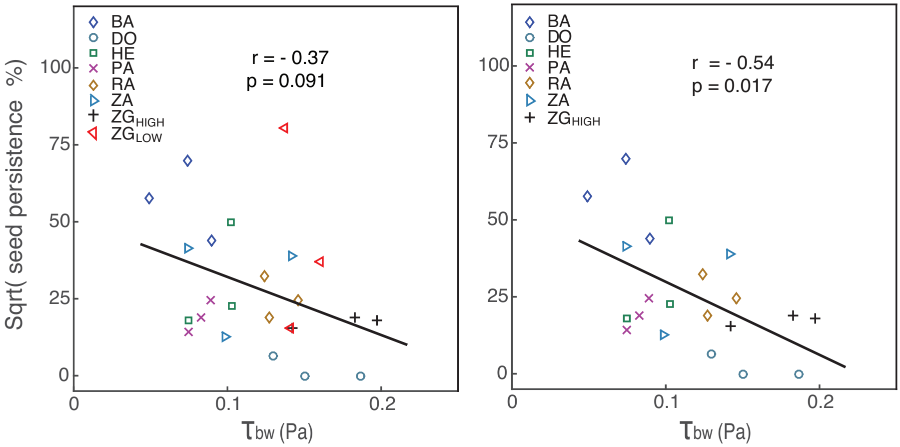


Fig.5.4 Correlation between seed persistence (square root transformed) on the surface and wave induced bed shear stress (tbw); Graph on the left included the data from all 8 locations, while the location 'ZG_{LOW}' that showed continuous sediment accretion (see Fig.5.3) was excluded in the right one.

Table 5.4 ANOVA table of the ANCOVA for surface seed persistence (response variable); ANCOVA was done using the data from a) all study locations and b) all locations except ZG_{LOW}, respectively.

Data	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
a) All locations	Trial	1	0.003	0.003	0.081	0.779
	τ_{bw}	1	0.129	0.129	3.156	0.091
	Trial:τ_{bw}	1	0.028	0.028	0.694	0.415
	Residuals	20	0.818	0.041		
b) ZG_{LOW} excluced	Trial	1	0.014	0.014	0.535	0.475
	τ_{bw}	1	0.184	0.184	6.971	0.017 *
	Trial:τ_{bw}	1	0.025	0.025	0.955	0.342
	Residuals	17	0.448	0.026		

Significance level: * 0.05

5.3.3 Relationship between buried seed persistence and sediment disturbance

Generally, seed persistence in the manipulated seed banks negatively correlated with sediment disturbance depth (SDD, Fig.5.5), while it was more so for the buried seeds (r=-0.64, p<0.001) than the surface seeds (r= -0.44, p=0.015). Further analysis revealed that seed persistence was enhanced non-linearly with depths and the response curve was affected by SDD (Fig.5.6). Higher SDD led to lower seed persistence and vice versa.

5.3.4 Effects from benthos

Pairwise Wilcoxon Rank Sum Tests showed that there was no significant difference (p=0.09) of surface seed persistence between the defaunated and control plots. In contrast, seed persistence of the buried seeds in the defaunated plots was significantly lower (p<0.001) than that in the control plots. This could be explained by the fact that the defaunation plots had greater SDD (p<0.001).

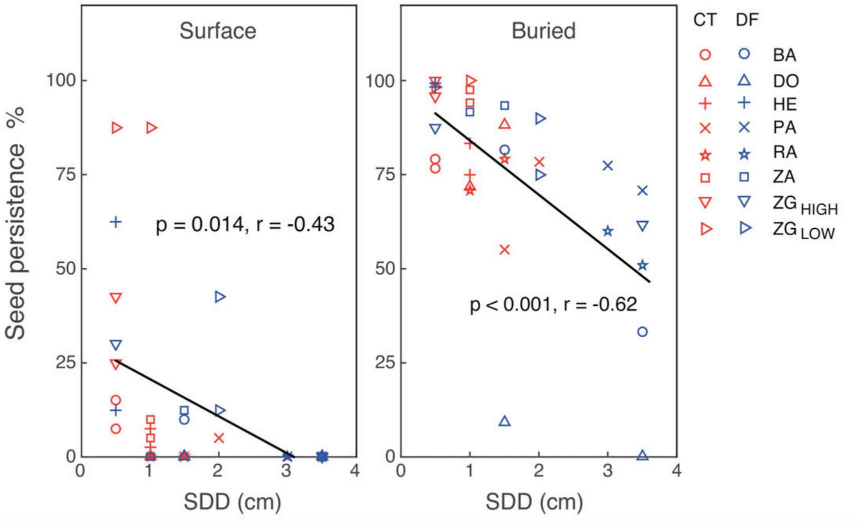


Fig.5.5 Correlation between persistence of surface/buried seeds and SDD; CT and DF denotes 'control' and 'defaunation', respectively; each symbol represents the data from a specific study location; Definitions for the abbreviations of all locations are given in Table.5.2.

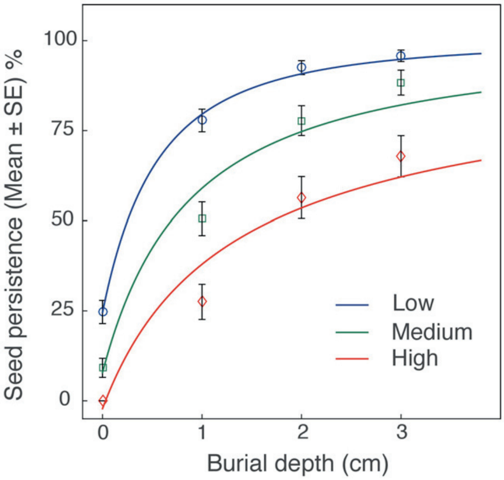


Fig.5.6 Effects of sediment disturbance (three SDD levels: low, medium and high) on the persistence of seeds from different burial depths; Low: SDD <=1 cm, Medium: 1< SDD <=2 cm, High: SDD > 2 cm; the response curves were fit with function: y = 1 - (x+a) ^ b (Chapter 2).

5.4 Discussion

5.4.1 Mechanistic relationship between seed bank persistence and physical disturbance in coastal habitats

To our knowledge, this study, for the first time, reveals the mechanistic relationships between seed bank persistence and the two major disturbance types in tidal flats i.e. wave disturbance and associated sediment disturbance. The variable wave and sediment dynamics conditions in our large scale experiments allowed us to quantify the response of seed bank persistence to varying magnitude of physical disturbances, thus enabling us to evaluate the potential effect of climate change on seed bank persistence with a space-for-time substitution strategy. By excluding the direct effects of winter storms, present observations provide the conservative lower limit of the effect of climate change. Overall, our findings clearly suggest that climate change may reduce seed bank persistence in coastal wetlands via intensifying wave forcing.

Using cordgrass as an example, this study demonstrated that surface seed retention decreased with increased strength of wave disturbance (i.e., wave induced BSS). This trend may occasionally be disrupted by well-timed and adequate seed burial caused by fast sediment accretion (e.g. see site ZG_{LOW}; this study), yet this is expected to be rare, given worldwide declines in sediment delivery to coasts (Walling and Fang 2003, Syvitski et al. 2009) along with amplified physical disturbances e.g. increasing storminess as predicted for many parts of the world (Donat et al. 2011, Young et al. 2011). In such scenarios, more frequent and stronger wave disturbance events that occurred in winter and early spring are likely to diminish seed retention on the tidal flat surface, suppressing the formation of a buried seed bank.

Seed retention in tidal flats can be enhanced by burial that results from physical process (e.g. sediment accretion), biological activities (active seed transport or bioturbation) and their interactions (Chapter 4, Delefosse and Kristensen 2012, Blackburn and Orth 2013). However, the buried seeds can still be turned over by sediment disturbance, depending on the burial depth (Groenendijk 1986, Marion and Orth 2012). The current study on cordgrass confirms and extends on this by showing that the quantity of seed loss from the buried seed bank was directly determined by the magnitude of hydrodynamics-induced sediment disturbance tuned also by benthos activities. Many benthic organisms (e.g. polychaetes, bivalves) are known to increase local sediment erodibility by bioturbating activities (Meysman et al. 2006), while some others (e.g. diatoms) can armor and stabilize the sediment (Stal 2003). In our large-scale field experiments, the reduction of benthos resulted in overall higher sediment disturbance that caused lower seed persistence. This finding suggest that sediment erodiblity and seed bank mobility may be nonlinearly enhanced by intensified wave disturbance, given that benthos abundance in intertidal flats tends to be lower under stronger physical forcing (e.g. Cozzoli et al. 2014).

Although wave disturbance might ease seed burial by promoting sediment mixing (Chapter 4), the present study showed that amplified wave disturbance and sediment variability led to overall increased seed removal in tidal flats. The eroded seeds may be transported seaward to distant places or washed ashore, with a net landward transport (Huiskes et al. 1995). The landward transported seeds can either be trapped by the standing vegetation (Chang et al. 2008) or added into driftline materials deposited near/on the dike (Wolters and Bakker 2002). Our results imply that, in coastal habitats with predicted intensifying storminess under global change, more frequent and stronger waves in winter and early spring is likely to lower seed bank formation and persistence in more exposed areas, i.e. the pioneer zone and tidal flats; In contrast, seeds would tend to settle down in more sheltered areas (e.g. within vegetation), where seedling establishment is however less meaningful due to the lack of niches.

5.4.2 Implications for coastal wetlands in the face of climate change

Coastal wetlands like salt marshes are dynamic systems with long-term cyclic alternations between a retreating phase of cliff erosion and an establishment phase by seedling recruitment (Allen 2000, e.g. Adam 2002, van de Koppel et al. 2005). Key to system resilience and persistence, seedling recruitment in coastal wetlands is however often problematic due to physical disturbances (Bouma et al. 2009, Friess et al. 2012), requiring the presence of windows of opportunity (WoO), i.e. disturbance free periods (Balke et al. 2011, Balke et al. 2014, Hu et al.

2015) to enable seedlings to reach a size threshold for survival. With amplified disturbance characteristics (frequency and magnitude) under climate change, seed colonization potential of coastal vegetated systems may decline due to worsened seed bank formation and seed bank persistence. Such effect combined with reduced WoO for seedling survival due to amplified physical disturbances can lead to a substantial reduction of regeneration capacity.

Consequently, an impaired regeneration capacity might affect long-term persistence by disabling the shift of available niches from a bare state to a vegetated state, thus 'locking' the system to the retreating phase. This will inevitably threaten long-term persistence of valuable coastal wetlands whose degradation has already been occurring world widely (Duke et al. 2007, Gedan et al. 2009, Waycott et al. 2009), thereby risking the loss of invaluable ecosystems services such as coastal defense values (Bouma et al. 2014). Uncertainty in the long-term ecosystem dynamics has been identified as the main bottleneck hampering application of wetland ecosystems within coastal defense schemes, which is further emphasized by the present study.

Therefore, ecosystem-based flood protection involving coastal vegetation should include measures to create more dissipative foreshores that allow for long-term marsh persistence (Cheong et al. 2013, Temmerman et al. 2013). Construction of low stone dams (Van Loon-Steensma and Slim 2012) that damp wave energy can be used, but will ultimately stop marsh dynamics and marsh rejuvenation, thereby reducing the nature value of the salt marsh. Disposing dredging materials (Temmerman et al. 2013) that can further increase the sediment supply to promote marsh accretion, might hence be a better solution. Such measure may be expected to benefit both seed bank persistence (this study) and seedling survival and thus marsh regeneration capacity. Moreover, measures that enhance wave dissipation in front of the marsh by reshaping the bathymetry of the tidal flat can strongly increase the overall width of a marsh (Hu et al. 2015).

Our findings suggest that climate change have the potential to disturb plant invasions in coastal wetlands via reducing the reproductive success. A weakened reproduction capacity due to changed physical settings may be good news for the management and control of invasive plants like cordgrass (the genus of

Spartina), since their fast spread in the infected areas was largely based on high success seedling recruitment (Gray et al. 1991, Xiao et al. 2009, Strong and Ayres 2013). Amplified physical disturbance induced by climate change may slow down its rapid expansion into undesired areas by seed colonization, which will depend on whether and to what extent the reproductive performance can be inhibited. On the downside however, native species that depend on seed dispersal are likely to suffer the same problem, so that the invasive may keep a competitive advantage. Overall it might be speculated that with increasing wave disturbance, opportunistic annual species with extremely high seed production may obtain an advantage over perennials with lower seed production, as typically observed following disturbances (e.g. Hulzen et al. 2006).

5.4.3 Concluding remarks

The sensitivity of the reproductive process to environmental change renders early plant regeneration a critical phase for predicting climate change induced effects on plant fitness, persistence and ecosystem functions (Holtmeier and Broll 2005, Walck et al. 2011, Mok et al. 2012). While direct climate change (e.g. variation in temperature and precipitation) effects on seed bank persistence has been extensively documented (Ooi et al. 2009, Ooi 2012, Long et al. 2014 and references there in), present study contributes to an improved understanding by showing that seed bank persistence in disturbance-prone environments can be strongly affected by climate influenced disturbance regimes.

Given the central role of sexual reproduction in plant demography and its sensitivity to environmental changes, we propose our findings should be incorporated into models for long-term biogeomorphological development of vegetated coastal ecosystems. The knowledge gained here provides a basis for more accurate predictions on how climatically driven environmental changes may alter the fitness, resilience and persistence of coastal foundation plants, with implications for the management of ecosystem-based coastal defense and biological invasions in coastal wetlands.

Chapter 6

6. Sprouting as a gardening strategy to obtain superior supplementary food: evidence from a seed-caching marine worm

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Published in 2016 as: *Ecology*: 97(12):3278-3284

Abstract

Only a handful of non-human animals are known to grow their own food by cultivating high-yield fungal or algal crops as staple food. Here we report an alternative strategy utilized by an omnivorous marine worm *Hediste diversicolor* to supplement its diet: gardening by sprouting seeds. In addition to having many other known feeding modes, we showed using video recordings and manipulative mesocosm experiments that this species can also behave like gardeners by deliberately burying cordgrass seeds in their burrows, which has been previously shown to reduce the loss of seeds to water. These seeds, however, are protected by the seed husk, and we used feeding experiments to show that they were not edible for *H. diversicolor* until they had sprouted or the seed husk had been artificially removed. Additionally, sprouts were shown to be highly nutritious, permitting higher growth rates in *H. diversicolor* than the low-quality basal food, detritus. We propose both a proximate cause (seed husk as a physical barrier) and ultimate cause (nutritional demand) for this peculiar feeding behavior. Our findings suggest that sprouting may be a common strategy used by seed-collecting animals to exploit nutrients from well-protected seeds.

6.1 Introduction

Agriculture, defined as the cultivation of high-yield staple foods, was a pivotal development in human history, yet it is not a human monopoly. Long before the arrival of human agriculture, at least three terrestrial invertebrate lineages had independently evolved the ability to grow fungi, including ants, termites and ambrosia beetles (Farrell et al. 2001, Aanen et al. 2002, Mueller et al. 2005). More recently, marine animals have also been documented to cultivate foods, including fungal-farming snails (Silliman and Newell 2003) and damselfish that grow algae (Hata and Kato 2006). Despite different cultivation strategies, all these animal farmers are able to achieve a sufficient supply of their staple food to sustain themselves from their high-yield fungal or algal crops.

Here we identify a novel, sprout-based, gardening strategy utilized by the common ragworm species *Hediste diversicolor* (Fig. 6.1a). This species is widely distributed along intertidal flats that often neighbor salt marsh pioneer plants like cordgrass in the NW Atlantic (Olivier et al. 1996, Paramor and Hughes 2007). Although *H. diversicolor* is an opportunistic omnivore that does not rely on a single food source, we present evidence that this species can also behave like a gardener, growing nutritious supplementary rather than high-yield staple food. They have been shown to deliberately bury cordgrass seeds into their burrows, thereby reducing seed loss to hydrodynamic processes (Chapter 2 & Marion and Orth 2012). While ragworms were thought to be seed predators (Emmerson 2000, Paramor and Hughes 2004), the direct consumption of intact cordgrass seeds had never been observed. In fact, only dehusked cordgrass seeds were found to be consumed (Emmerson 2000), suggesting that the husks protect seeds from consumption. In this paper, we present evidence that *H. diversicolor* can actually acquire nutrients from these well-protected seeds by sprouting them.

With highly concentrated nutrients stored to sustain subsequent plant growth and development, seeds are often an attractive food resource for animals across ecosystems (Hulme and Benkman 2002). Nevertheless, seeds may not always be directly consumed due to their big size, physical defenses (e.g. hard seed coat) and/or chemical protection (e.g. toxins) (Hulme and Benkman 2002, Vander

Wall 2010). By a process of natural conversion, sprouting can improve digestibility and nutritional quality, compared with non-sprouted seeds (Chavan and Kadam 1989). Sprouting for food (e.g. soy sprouts) has previously only been observed in human behavior. Sprouted foods, which have been part of East Asian diets for centuries, are currently becoming popular in the western world because of their high nutritional value, including high protein, amino acid and vitamin content (Chavan and Kadam 1989).

High-protein diets (e.g. alga) have been shown to enhance growth rates and reproductive success of *H. diversicolor* (Olivier et al. 1996, Nesto et al. 2012). However, the most abundant food source in tidal flats, i.e. sedimentary organic matter such as detritus, is of low quality due to low nitrogen content (Dauwe et al. 1999, Herman et al. 1999). This poor quality of the basal resource fosters omnivory as a general strategy in benthic communities (Herman et al. 1999, HilleRisLambers et al. 2006), as omnivory allows for feeding on both abundant, low quality food and less abundant, more nutritious food (Coll and Guershon 2002). To meet its nutritional requirements, *H. diversicolor* displays many adaptations to obtain a more nitrogen-rich diet, such as grazing of diatoms (e.g. Smith et al. 1996) and algae (e.g. Olivier et al. 1996), and predation of macrozoobenthos (e.g. Rönn et al. 1988). In this study, we examine the hypothesis that seed-caching behavior and sprout consumption can enable the omnivorous *H. diversicolor* to supplement its diet with high quality food, using manipulative mesocosm experiments.

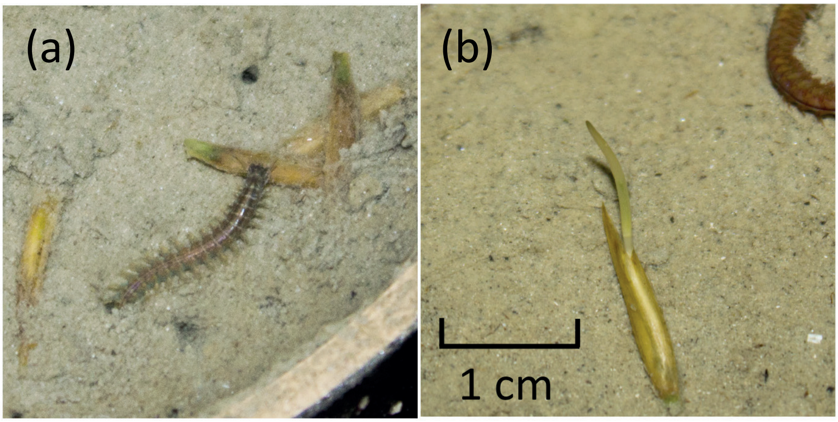

Fig.6.1 a) *H. diversicolor* collecting a cordgrass seed; b) sprouting cordgrass seed.

6.2 Materials and Methods:

6.2.1 Living and feeding habits of H. diversicolor

The common ragworm, *H. diversicolor*, is an opportunistic omnivore living in semi-permanent U- or Y-shaped burrows, with a life span of 1-3 years. The individual burrow is a well-defined territory and may develop into multi-branched structures over time. This species has a wide array of different feeding strategies: filter feeding, deposit feeding, scavenging and predation. For the last 3 strategies, the worm partially emerges from the burrow, feeding on the sediment around the burrow or taking a proboscis full of material (e.g. plant/animal remains) from the surface and retreating into the burrow. It can hunt for small invertebrates, seizing them with its strong jaws (reviewed in Scaps 2002). This species has also been suggested to stimulate bacterial growth (i.e. microbial gardening) in its burrow, where constant irrigation maintains aerobic conditions favorable for bacterial growth, as found in other marine worms, e.g. *Abarenicola vagabunda* (Hylleberg 1975, Scaps 2002). *H. diversicolor* can grasp soft plant materials like seagrass leaves, break them and pull them into its burrow (Hughes et al. 2000). More recently, *H. diversicolor* (Delefosse and Kristensen 2012) and its close relatives, *Alitta virens* (Chapter 4) and *Neanthes* s*uccinea* (Blackburn and Orth 2013), have been reported to collect and bury seeds of seagrass (Delefosse and Kristensen 2012), glasswort (Paramor and Hughes 2004) and cordgrass (Chapter 4 & Emmerson 2000). However, direct seed consumption has only been reported for the seagrass *Zostera noltii* (Hughes et al. 2000), which has small seeds of about 2 mm in length.

6.2.2 Food preference of Ragworm

To test the hypothesis that cordgrass seeds cannot be directly consumed by ragworm *H. diversicolor* unless they have sprouted, we conducted a feeding experiment in a climate room at a constant temperature of 18 °C. Six PVC cylinders (Ø = 12.5 cm, Height = 17cm) were filled with muddy sediment (Medium grain size = 45.0 μm) and let consolidate for 3 days. The sediment was collected in November 2013 from a mudflat in the Westerschelde (51°27'37.4"N, 3°58'28.8"E), the Netherlands, where *H. diversicolor* is observed in large densities. The sediment was defaunated using freeze-thaw method (repeated twice) in a –20°C freezer prior to use. Six individuals of *H. diversicolor* (5-10 cm length) collected from the same site were introduced to each cylinder. Each cylinder was submerged (5 cm above sediment surface) in a tank filled with air bubbled filtered seawater.

After the animals acclimated for 2 days, we randomly sowed both sterilized seeds and sprouting seeds (Fig.6.1b) on the mud surface of each cylinder. The number was 5 for the former, but 4 for the latter due to limited availability (< 30) at that time. Dead seeds were obtained by freezing them in a –20°C freezer for two weeks, and were stained with Rose Bengal. Color differences enable us to easily distinguish the dead seeds from the sprouting seeds, especially those that had likely lose their sprouts due to the consumption by ragworms. The sprouted seeds were obtained by germinating cordgrass seeds in a petri dish under the room temperature (16-20°C). The used seeds were collected in November 2012 from a marsh in the Westerschelde (51°27'37.4"N, 3°58'28.8"E), the Netherland, and had been stored in a 4 °C fridge till use.

This experiment lasted for three weeks, during which the foraging behavior of ragworms and the state of the food items was monitored daily by direct visual observation. In the end, recovery (%) of dead and sprouted seeds was determined to quantify which types of seeds were eaten more often. Due to sensitivity of the *H. diversicolor* to human presence, we additionally set up a microcosm equipped with a webcam system (Logitech® HD Webcam c615) to observe the feeding behavior of this animal. In this system, we introduced 5 worms to a transparent tray (20 x 12 x 12 cm) filled with the muddy sediment to a depth of 8cm, and sowed 5 seeds and 3 sprouting seeds on the surface after the acclimation (2 days) of the worms.

6.2.3 Seed redistribution and viability after handling

To detect if handling and burial by *H. diversicolor* affect seed viability, we conducted a mesocosm experiment using untreated cordgrass seeds harvested in a Westerschelde marsh in November 2013. In this experiment, we used cordgrass seeds harvested in a Westerschelde marsh in November 2013. Previous studies showed that only a very low percentage of cordgrass seeds could germinate immediately after ripening given optimum conditions; adding a cold stratification of a few months does greatly improve germinability (Hubbard 1970, Xiao et al. 2009), with the germination rate increasing as temperature increased from 7 to 25 °C (Nehring 2006). Therefore, this experiment was run in a green house in the winter (January 2014), where the temperature fluctuated between 3-12 °C, to minimize seed germination. In total, there were 2 worm-treatments, i) 'worm + surface seeds' (WS) and ii) 'worm + buried seeds' (WB), and 2 control treatments i) surface seeds (CS) and ii) buried seeds (CB), each with 8 replicates. This design allowed us to examine 1) the seed redistribution and 2) the effect on seed viability after handling and burial by *H. diversicolor*.

We first filled 32 PVC cylinders (Ø = 12.5 cm, Height = 17cm) with defaunated sediment for WS and CS, we randomly sowed 25 seeds on the sediment surface, while for WB and CB seeds were buried at 1 cm. We applied this depth as previous studies on ragworms revealed a shallow seed burial pattern with most seeds buried within the top 1 cm (Chapter 4, Delefosse and Kristensen 2012, Blackburn and Orth 2013). Cylinders for WS and WB were restocked with *H. diversicolor* (5-10cm, 6 no. /cylinder), while CS and CB remained defaunated (control). The worm density (488 no./m^2) used here is within the range found in the field (Scaps 2002).

All the cylinders were placed in a tank, inundated for 3 h per 12h twice a day with filtered seawater using an automated pumping system. Such inundation regime is typical for the pioneer zone of the Westerschelde marshes where *H. diversicolor* and cordgrass coexist (Van der Wal et al. 2008). Cylinders for the two controls were wrapped with nylon nets (1 mm square mesh) to prevent the exchange of worms between pots from the treatment groups by swimming during flooding period. The incubation lasted for 4 weeks, during which active seed transport by *H. diversicolor* was frequently observed. Seed germination was not seen in the any of the treatment groups. After incubation, we retrieved all remaining seeds from the sediment core in each cylinder. For the treatment groups, the sediment core was sliced every 1 cm to determine the vertical seed distribution. To achieve this, a piston was pushed up from the bottom to extrude the sediment in 1 cm increments. Each slice was rinsed in a 1 mm mesh sieve. For the control groups, we recovered all the seeds by sieving the entire sediment core. No worm was found during this process in the control groups, confirming that the wire-netting worked sufficiently in preventing the exchange of worm between experimental pots. The retrieved seeds from each cylinder were then put into a petri dish furnished with filter paper and kept moist. All petri dishes were moved to a climate room where seed viability was determined via a germination test at a constant temperature of 25 °C. The number of germinated seeds was counted every 4-6 days and the test terminated after 79 days when no more germinated seeds could be detected. Total germination (%) was calculated for each treatment/control.

6.2.4 Consequences of nutrition on ragworm growth

To elucidate potential benefits of gardening behavior by *H. diversicolor* by sprouting, we tested the hypothesis that sprouted seeds enables *H. diversicolor* to exploit the rich nutrients stored in cordgrass seeds, and further improves the nutritional qualities of their diet. We performed a mesocosm experiment in a climate room (18 °C), to compare the growth of *H. diversicolor* under four different diet types: *i)* muddy sediment + intact seeds (M+S), *ii)* muddy sediment + dehusked seeds (M+DS), *iii)* muddy sediment + sprouted seeds (M+SP), and *iv)* muddy sediment only (M). Except for M, the worms were fed every 3 days with 1 intact/dehusked/sprouted seed, respectively, 8 times in total. The intact seeds and dehusked seeds were sterilized by freezing them in a –20°C freezer for two weeks to prevent germination. The sprouted seeds were freshly germinated seeds with sprout length of 5-10 mm. For the 'muddy sediment only' diet, no food was added.

Plastic cups (Ø =9cm, Height=10cm) filled with muddy sediment were employed as experiment units, with one individual of *H. diversicolor* per cup. Altogether, 80 cups were used, giving 20 replicates per diet type. Muddy sediment (Medium grain size = 40.9 μm) and *H. diversicolor* were collected from a Westerschelde mudflat (51°27'37.4"N, 3°58'28.8"E) in April 2015. Prior to the experiment, the sediment was defaunated (the same method as used in 6.2.1) and homogenized (sieving) to minimize the variation in resource availability. The cups were placed randomly in two glass containers (i.e., 40 cups each), but not inundated to avoid the exchange of worms between beakers by swimming. Each time before feeding, we manually added filtered seawater to each beaker to keep sediment moist.

Prior to experiment, all the worms were weighed (fresh weight). To minimize the difference of initial weight between groups, we divided the worms into two weight classes (Table.6.1) and applied equivalent number (n=10) of worms from each weight class for each diet group. At the end of the experiment (Day 28), we retrieved the worm from each beaker by rinsing the sediment core in 1 mm mesh sieve. Some of the worms were found dead or missing from the cup (Table 6.1). The four missing worms were recovered on the bottom of the container, but were excluded from the data analysis, as it was not possible to determine when and from which cup these worms escaped. Where living worms were retrieved, the residual food items (intact, dehusked, or sprouted seed) were also recovered to quantify the consumption (% of the consumed/deployed) of each food item.

The final weight of the living worms was determined using an electronic balance (Table.6.1). To minimize variability in weight due to differences in gut and external water content, every worm was kept for 24 h in a separate cup with filtered seawater to empty the gut, and afterwards placed on a dry paper towel for 1 min to get rid of surplus water, prior to the measurement (both initial and final). The weight change (%) was calculated as: 100% * (Final -Initial)/Initial.

To compare the nutritional value of each diet, organic carbon content (OC) and total nitrogen content (TN) was determined for sprout, dehusked seed, seed husk and the muddy sediment. The carbon to nitrogen ratio (C:N) was then calculated as OC/TN. For the preparation of the sprout sample, the sprouts (5-10 mm) were excised from 150 sprouting seeds and pooled together. 150 non-sprouting seeds were dehusked and combined to produce the samples for dehusked seed and seed husk, respectively. The sediment sample was made through taking a sediment core (Depth = 2 cm) with a 100 ml syringe. All the samples were first freeze-dried, weighted. For determination of OC and TN, the samples were acidified to remove carbonate, and further vacuum-dried. The content of OC and TN was determined with an elemental analyzer (FlashEA 1112, Thermo Electron, Bremen, Germany). We repeated the measurement with another bulk sample (prepared in the same way) for sprouts, dehusked seeds and seed husks, respectively. The muddy sediment was not included in the repeated measurement, as its nutritional quality in terms of OC and TN has already been shown hugely different from the rest from the data of the abovementioned measurements (Table. 6.2). The new sample was analyzed in the same method but in a different machine (vario MICRO cube, Elementar, Hanau, Germany).

6.2.4 Statistics

Prior to analysis, we conducted Shapiro–Wilk tests to check for data normality. One-way ANOVA was used to detect the effects of *H. diversicolor* handling and burial on seed viability. We conducted ANCOVAs to compare the weight change of *H. diversicolor* between diet treatments, using initial weight as the covariate. To satisfy the assumption of normality, the data were log (x+0.5)-transformed. We performed a Pearson's chi-square test to examine if worm mortality differed between the diet treatments. All statistical analyses were conducted in R (http://www.R-project.org), applying a significance level of $\alpha = 0.05$.

6.3 Results

6.3.1 Food preference experiment

Our experiment revealed that *H. diversicolor* had a marked preference for sprouted seeds. All sprouts were found to be missing after 3 weeks, whereas all the sterile seeds were recovered intact, indicating lack of germination or predation (Fig. 6.2). During the experiment, we observed *H. diversicolor* attempting to collect both the sterile and sprouted seeds around their burrows, and pulling some of them inside. The video recordings revealed *H. diversicolor* cutting the sprout off a germinated seed and eventually pulling it into its burrow.

Table.6.1 Initial and final weight (fresh, mg) of H.diversicolor used for the different diet treatments: 1) muddy sediment + intact seeds (M+S), 2) muddy sediment + dehusked seeds (M+DS), 3) muddy sediment + sprouting seeds (M+SP), and 4) muddy sediment only (M).

Weight class	M+S		M+DS		M+SP		M	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
90 ~150 mg	104	127	99	Dead	99	Dead	90	70
	114	143	116	207	113	107	96	111
	117	Escaped	122	135	116	139	108	119
	128	92	126	141	120	122	116	136
	129	112	130	271	122	133	117	155
	137	136	133	Dead	124	Escaped	130	Dead
	140	121	140	144	138	133	137	133
	141	125	143	Dead	143	195	141	140
	144	161	147	161	144	212	145	Dead
	150	Dead	149	210	148	261	148	Dead
150~240 mg	151	132	153	Dead	151	Dead	152	172
	155	148	159	131	158	170	153	Dead
	162	190	162	Dead	160	Dead	163	132
	165	Dead	165	Dead	161	Dead	166	Dead
	168	164	167	156	163	263	170	Escaped
	177	193	175	187	175	Dead	175	Dead
	178	Escaped	184	193	181	223	183	151
	189	161	191	Escaped	190	220	187	Dead
	205	155	192	221	192	Dead	195	195
	214	Dead	210	Dead	235	Dead	228	282
Mean	149		150		145		144	
Stardard deviation	27		25		25		42	

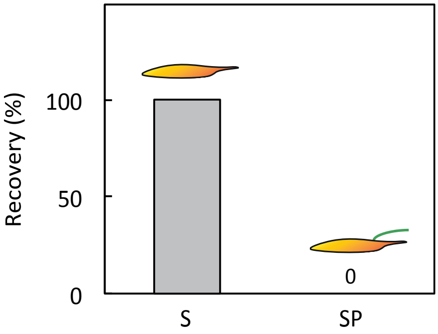

Fig.6.2 Recovery of sterilized seeds (S) and sprouting seeds (SP) by *H. diversicolor* after 3 weeks. All sprouts were excised, whereas all the sterile seeds were recovered intact.

6.3.2 Seed redistribution and viability after handling

Seed viability was not affected by the handling and burial of the seeds by *H. diversicolor* (Fig. 6.3). For the 'worm + surface seeds' (WS) treatment, all the seeds were redistributed by *H. diversicolor* and ca. 25% of the seeds were recovered from their burrows, mostly within the top 1 cm (Fig. 6.3 b). For the treatment of 'worm + buried seeds', only 1% of the seeds were found deeper (1-2cm) than the original depth (0-1cm), suggesting very limited vertical redistribution by *H. diversicolor*. Neither the handling nor the shallow seed burial affected seed viability (Fig. 6.3 c), as the subsequent germination test showed that there was no significant difference (One-way ANOVA, $P = 0.89$) between the treatment and control groups.

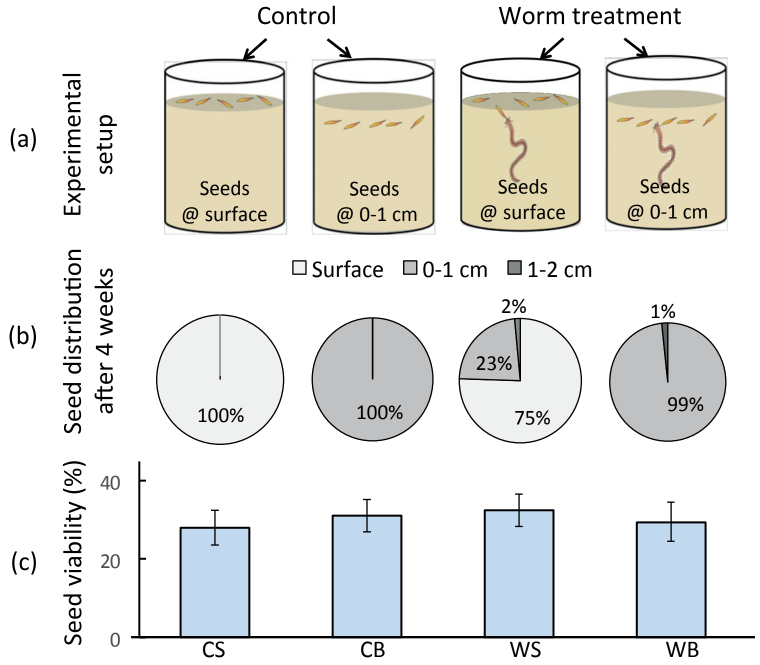

Fig. 6.3 (a) Experimental setup. (b) Vertical distribution of the seeds retrieved from the 2 treatments 'worm + surface seeds' (WS) and 'worm + buried seeds' (WB), and 2 controls with surface seeds (CS) or buried seeds (CB). (c) No significance of total germination (Mean ± SE) of these retrieved seeds was found between treatments/controls (ANOVA, P = 0.89).

6.3.3 Consequences of nutrition on ragworm growth

The diet types were important in determining the growth of *H. diversicolor* (Fig. 6.4). The differences in growth rate could be explained by the variation in nutritional values (Table 1). Sprouts and dehusked seeds are both protein-rich, while the basal food source (i.e. particulate organic matter in the mud) and seed husks have much lower nitrogen content. The diet with sprouted seeds led to a significantly higher weight increase (ANCOVA, $P<0.05$) than that with muddy sediment or sterile intact seeds. The dehusked seed itself also proved to be nutritious, contributing to a significantly higher weight increase than the intact seeds (ANCOVA, $P<0.05$), suggesting that the seed husk serves as a physical barrier against direct seed consumption.

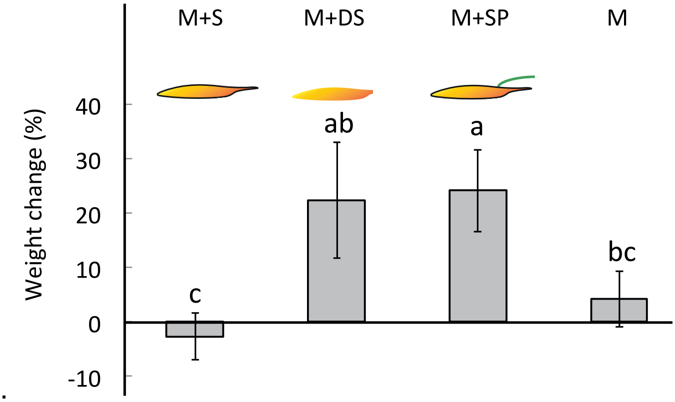

Fig.6.4 Weight change (Mean ± SE) of worms fed with four different diets: 1) muddy sediment + intact seeds (M+S), 2) muddy sediment + dehusked seeds (M+DS), 3) muddy sediment + sprouting seeds (M+SP), and 4) muddy sediment only (M). Means with different letters vary significantly (P < 0.05).

There were no detectable differences in growth between individuals eating sprouted and dehusked seeds, although the mean dry weight of a sprout (1.2 mg) is only 21.8% that of a dehusked seed (5.5 mg). Sprouts have a comparable C:N ratio with the macroalgae species *Enteromorpha intestinalis* (Table 6.2), which has previously been shown to yield rapid growth of H. diversicolor (Olivier et al. 1996). The dehusked seeds have a higher C:N ratio than sprouts (Table 6.2), suggesting that sprouting improves both food accessibility and quality for H. diversicolor. No difference in worm mortality between treatments could be detected (chi-square test, P = 0.79), suggesting that the few dead worms in the experiment did not result from the diet. When all the living worms had been recovered, all the sterile seeds were eventually retrieved intact without the occurrence of germination or predation, while only 8.3 % of the dehusked seeds were recovered. All sprouts had been excised from the sprouted seeds and were missing

Table 6.2. Nutritional quality of dehusked cordgrass seeds , cordgrass sprouts, seed husks and the muddy sediment used in our experiments. The nutritional quanlity data from the literature for one macroalgae species *Enteromorpha intestinalis*, and two marine vascular plants *Spartina anglica* (i.e. cordgras) and *Halimione portulacoides*, were also included for comparsions.

Food item	Organic C (% / dry weight)	Total N (% / dry weight)	C:N ratio
Dehusked seeds	30.5[a]	2.8[a]	10.9
	30.8[b]	2.8[b]	11.0
Sprouts	28.8[a]	3.6[a]	7.9
	28.2[b]	3.7[b]	7.6
Seed husks	45.5[a]	0.7[a]	61.0
	44.4[b]	0.8[b]	55.5
Mud	1.0[a]	0.2[a]	5.3
Enteromorpha intestinalis	45.4[c]	5.17[c]	8.8
Spartina anglica	48.0[c]	1.4[c]	34.4
Halimione portulacoides	1.0[c]	0.2[c]	21.2

[a] Analyzed with FlashEA 1112; [b] Analyzed with vario MICRO cube; [c] Source: Olivier et al. 1996

6.4 Discussion

6.4.1 Sprouting as a gardening strategy: evidence from H. diversicolor

Animal seed dispersers like rodents and earthworms often directly consume seeds as the reward (Brown and Ojeda 1987, Hulme and Benkman 2002); however, we illustrate that the common ragworm *H. diversicolor* can benefit from seed burial by sprouting the buried seeds to produce high-quality food. Sprouting enables *H. diversicolor*, which feeds primarily on poor-quality food (Scaps 2002), to exploit the rich nutrients in cordgrass seeds protected by their husks. We reveal that initial failure in directly consuming intact seeds can result in improved food accessibility, higher nutritional values and associated higher weight increase. After burial, no further action is needed by these worms to tend their gardens besides not moving too far away during the period between seed burial and germination.

The dispersal of cordgrass seeds starts in autumn (October), and extends to the winter and early spring (March) of the following year, with very few seeds germinating during this dispersal period (Huiskes et al. 1995). Cordgrass seedlings mainly emerge in spring (April) and germination may extend to summer as some seeds can remain viable in the seed bank till July, but no seeds were found to survive by the end of the growing season (Groenendijk 1986). Therefore, the waiting time for seed-caching *H. diversicolor* to harvest their sprouts may vary from a few weeks to a few months. Given that this worm lives in a semi-permanent burrow and has a life span of 1-3 years (Scaps 2002), seed caching by this species can reasonably be assumed to result in sprout consumption, provided that the worms are not predated and the seeds remain viable during the waiting period.

As not every seed eventually germinates (Fig. 6.3), seed burial by *H. diversicolor* might not always result in sprout consumption. Given its relatively short seed longevity (< 1 year, Wolters and Bakker 2002), however, it is likely that the buried cordgrass seeds that fail to germinate will decompose in the burrow, and the detritus may eventually be consumed by *H. diversicolor*. The decomposed seeds could still be nutritious because of the high energy storage and richer nitrogen than other plant debris (Table 1), but they would still be inferior to the freshly sprouted food as the latter has a lower C:N ratio (this study), is more easily digested and is richer in micronutrients, e.g. vitamins (Chavan and Kadam 1989). Although ragworms have been previously suggested to stimulate microbial growth in their burrows (Hylleberg 1975, Scaps 2002), our experiments showed that ragworm handling did not actually facilitate seed decay, as neither decomposition of intact seeds nor effects on seed viability were observed. Moreover, microbial activity can be expected to be low during the period between seed dispersal and germination, i.e. winter and early spring, because of the low temperatures.

Seed consumption and seedling herbivory by *H. diversicolor* could, in some cases, form a major bottleneck for vegetation (re-) establishment from seed in low-lying tidal areas (Emmerson 2000, Hughes et al. 2000, Paramor and Hughes 2004). Paradoxically, the inability to directly consume seeds may, however, improve the chances for cordgrass establishment if some ragworms die or are predated before seed germination. Consequently, burial does not necessarily end up with all sprouts being consumed and may incidentally result in directed dispersal, which can increase seed persistence by decreasing seed removal caused by the tide (Chapter 2 & 5).

6.4.2 General relevance

Sprouting may also be found for other ragworm species (e.g. *Alitta virens* and *Neanthes Succinea*), given their comparable feeding behaviors and niches (Blackburn and Orth 2013). Similarly, this phenomenon also likely occurs for seeds of other macrophytes, e.g. seagrass and glasswort, that can be cached by ragworms (Chapter 4 & Paramor and Hughes 2004, Delefosse and Kristensen 2012), provided that their seed coats can prevent immediate seed consumption. Moreover, sprouting may be more widespread as a feeding strategy, given that analogous seed-caching behavior has been seen in earthworms (Regnier et al. 2008, Forey et al. 2011). These terrestrial worms primarily feed on low-quality detritus (Curry and Schmidt 2007) and are able to obtain nutrients from high-quality food sources like seeds and germinated seeds (Shumway and Koide 1994, Eisenhauer et al. 2009, Eisenhauer et al. 2010). Thus, sprout-based gardening involving active seed burial may also have developed in seed-earthworm systems, when seeds are not directly edible due to large sizes or hard seed coats. This could be especially relevant in cases when seed germination is enhanced by earthworm gut passage, as found for some plant species (Eisenhauer et al. 2009).

Sprouting as a solution to utilize the nutrients from well-secured seeds may also apply to other seed-caching animals. Possible candidates are rodents that cache nuts and seeds with hard shells or with tannins that reduce the immediate food value (Hulme and Benkman 2002, Vander Wall 2010), as such animals also consume young seedlings (e.g. Ostfeld and Canham 1993, Ostfeld et al. 1997).

For instance, a study on meadow voles revealed that consumption of fresh green foods like sprouted wheat allowed for rapid growth and development in young meadow voles (Meek et al. 1995). In addition, a previous study on the leaf-cutting ant *Atta sexdens* also suggested that seed-caching ants might also use sprouted seeds as a food source. These ants were found to cut the epicotyl from the sprouting seeds of non-myrmecochorous plants (Silva et al. 2007).

6.4.3 Concluding remarks

Besides humans, very few animal species have been reported to ‘grow' their own food. While most of these farming species have been characterized by cultivation of high-yield fungal or algal crops as their staple food, sprouting by omnivorous seed-caching ragworms, as presented here, serves as a novel type of gardening strategy that yields superior supplementary food. Unlike human agriculture, gardening or farming by invertebrates seems more likely to be an adaptive feeding strategy to obtain nourishment, without such animals being conscious of or intentional in their behavior (e.g. Farrell et al. 2001, Silliman and Newell 2003, Mueller et al. 2005). Seed caching by *H. diversicolor*, however, still yields high-quality sprouted food, analogous to the sprouting practices found in humans (e.g. soy sprouts). We propose both a proximate cause (protection by seed husk) and ultimate cause (nutritional demand) for this feeding behavior. Given the ubiquity of seed-protecting mechanisms (Hulme and Benkman 2002, Vander Wall 2010), sprouting may be a common strategy used by many seed-collecting animals to exploit nutrients from otherwise well-protected seeds.

Chapter 7

7. General discussion

7.1 Towards a holistic understanding of the bottlenecks to the pioneer seedling establishment in coastal wetlands

Seedling recruitment of coastal foundation species is essential for the development of coastal vegetated systems and their long-term dynamics (van de Koppel et al. 2005, Van der Wal et al. 2008, Friess et al. 2012), yet there is still a lack of knowledge on the processes and mechanisms that enable/disable seedling establishment in stressful coastal environments, where seedling recruitment is often problematic due to physical disturbances (Bouma et al. 2009, Friess et al. 2012).

Previous studies mostly focused on the mechanisms that cause seedling dislodgment or mortality (e.g. van Wesenbeeck et al. 2007, Bouma et al. 2009, Schwarz et al. 2011), underscoring the importance of windows of opportunity (WoO), i.e. disturbance free periods (Balke et al. 2011, Balke et al. 2014, Hu et al. 2015) to allow seedlings to surpass the biomass threshold for survival. This study took a step back to investigate seed fate pathways to understand to what extent and in which way seed dispersal and post-dispersal fate can influence reproductive success in dynamic coastal habitats.

Using cordgrass (*Spartina*) as a model plant, I have studied seed fate pathways from primary seed delivery to seed-seedling transition process. Generally, the current study demonstrated that, in addition to low seedling survival caused by environmental harshness such as salinity (e.g. Dethier & Hacker 2005), hydrodynamic stress (e.g. Hammond et al. 2002, Schwarz et al. 2011) and sediment instability (e.g. van Wesenbeeck et al. 2007, Marion & Orth 2012), pioneer seedling establishment in coastal wetlands can also be bottlenecked by seed related processes including effective seed delivery, seed bank formation & persistence, and seed-seedling transition.

7.1.1 Effective seed delivery

Cordgrass seedling establishment occurs mainly in the pioneer zone and fronting tidal flats with the yearly formation of a transient seed bank (Wolters and Bakker 2002, Xiao et al. 2009). The formation of such a seed bank requires in the first place effective seed delivery (quantity x quality). Despite the potential for long-distance dispersal of seeds by tidal currents (Koutstaal et al. 1987), salt marsh plants were found to usually disperse their seeds locally (Rand 2000, Wolters et al. 2005). Seed recruitment of cordgrass on tidal flats can be primarily limited by insufficient seed delivery if a seed source is absent or far away, since seed arrival was found to decrease exponentially with increasing distance from the seed-source area, resulting in the seeds being deposited mainly on the mudflats close to the marsh edge (Chapter 2).

Even if there is a local seed source, reproductive success could still be constrained by low-quality seed delivery. Cordgrass has long been noted for their zonal variation in seed production and seed viability (Marks and Truscott 1985, Mullins and Marks 1987, Xiao et al. 2009). In the current study, both the quantity and viability of the produced cordgrass seeds were found to decrease with lowered elevations. Given a high yearly variation of cordgrass seed production in terms of both quantity and quality (Chapter 3), seed dispersal may be ineffective merely due to the scarcity of viable seeds.

This study further showed that effective seed delivery of cordgrass was episodic, resulting from tidal pulsing and its interactions with seed production patterns along the elevation gradient (Chapter 3). Consequently, effective seed delivery strongly relies on high magnitude events like spring tides and storm surges that contribute disproportionally to the delivery of good seeds towards the fronting mudflats (Chapter 3). Whereas effective seed delivery during spring tides & storm surges might further benefit seed settlement by providing longer waterlogging periods for seeds to become sufficiently heavy, it also risks low seed persistence when coinciding with strong disturbance events, e.g. wind-driven waves that could cause high removal of the deposited seeds (Chapter 5), raising the uncertainty over the formation of a sediment seed bank

7.1.2 Seed bank formation and persistence

Once seeds have settled, their retention is vital to the initiation of the sediment seed bank. This study illustrated that seed bank formation on tidal flats can be bottlenecked by the high seed removal on the surface, as caused by hydrodynamics and found to relate positively with wave-induced bed shear stress (Chapter 2 & 5). These findings stress the importance of seed burial in facilitating seed retention and seed bank formation.

Seed burial can non-linearly enhance seed retention in tidal flats (Chapter 2 & 5). Vertical seed displacement in tidal habitats can result from physical processes, i.e. hydrodynamics-driven seed and sediment redistribution (chapter 4), biological activities including active seed displacement by seed-collecting animals or sediment reworking by ecosystem engineers (Chapter 4, Delefosse and Kristensen 2012, Blackburn and Orth 2013) and/or their interactions (Chapter 4). Especially, the current study demonstrated that the coupling between passive seed burial engineers (e.g. lugworms and bivalves) and physical forcing could act in synergy, resulting in greater seed burial quantity and deeper burial depths (Chapter 4).

However, seed burial is no guarantee of seed persistence as the buried seeds are still vulnerable to sediment disturbance. Seed erodibility in dynamic tidal flats depends on the burial depth (Chapter 2 & 5, Groenendijk 1986, Marion and Orth 2012) and the magnitude of sediment disturbance (Chapter 5). Deeper buried seeds have much higher seed persistence, while greater disturbance depth can turn over the buried seeds that would otherwise remained 'safe' under lower sediment dynamics (Chapter 2 & 5, Groenendijk 1986, Marion and Orth 2012). Sediment dynamics on tidal flats is often induced by hydrodynamics tightly coupled with benthic activities (Herman et al. 1999, Widdows and Brinsley 2002). Therefore, seedling recruitment on tidal flats could in some cases be inhibited by severe seed erosion caused by sediment disturbances, depending on the biological (e.g. bioturbators) and physical conditions (e.g. hydrodynamics and sediment properties).

7.1.3 Seed-seedling transition

Although deep burial is beneficial for seed persistence in tidal flats, it can lead to the failure of seed-seedling transition. Seedling emergence of cordgrass was found to decline with increasing seed burial depth (Bouma et al. 2016), as seeds germinated from deep depth are not able to reach the sediment surface (Greve et al. 2005). Hence successful seed-seedling transition predominantly requires seeds to be positioned in moderate depths that benefit both seed persistence and seedling emergence.

Nevertheless, even when the seeds are buried to the optimal depth, seed-seedling transition can still fail in the presence of herbivores. One such animal is the common ragworm *Heidiste diversicolor,* with overlapping niches with marsh pioneer plants (Olivier et al. 1996, Paramor and Hughes 2007). This species was reported to inhibit seedling establishment of pioneer marsh plants (Emmerson 2000, Paramor and Hughes 2004, 2007) via top-down control. Interestingly, *H. diversicolor* (Delefosse and Kristensen 2012) was also found to actively bury macrophyte seeds e.g. seagrass (Delefosse and Kristensen 2012), glasswort (Paramor and Hughes 2004) and cordgrass (Chapter 4, Emmerson 2000), which can shield them from hydrodynamic induced seed loss (Chapter 2 & 5).

While ragworms were considered as seed predators (Emmerson 2000, Paramor and Hughes 2004), the direct consumption of cordgrass seeds was observed neither in our field (Chapter 2) nor our lab experiments (Chapter 6). Importantly, the active seed transport by *H. diversicolor* does no harm to the viability of cordgrass seeds (Chapter 6). I further illuminate that sprouts that germinated from the buried seeds rather than the seeds *per se,* serve as the reward for the burial of cordgrass seeds (Chapter 6). Such an opportunistic gardening strategy utilized by *H. diversicolor*, analogous to human's sprouting practice, enables this omnivore worm species to obtain supplementary superior food, contributing to enhanced growth (Chapter 6). Although a sprout-based gardening may occasionally turn into directed dispersal when worm mortality occurs, sprout consumption by *H. diversicolor* would generally impose a strong bottleneck to cordgrass seedling recruitment (Chapter 6).

7.1.4 Conclusions:

Taken together, my findings on cordgrass indicate that, successful seedling establishment in dynamic coastal habitats not only requires windows of opportunity to warrant seedling survival, but also necessitates that abiotic (i.e. the tide) and biotic dispersal agents (e.g. benthic animals) effectively deliver the viable seeds to the correct place (suitable elevation and moderate sediment depth), where the physical (e.g. waves) and biological conditions (e.g. herbivores) are proper to enable the seeds to persist till the right moment to fulfill seed-seedling transition.

This thesis highlights the importance of incorporating the detailed knowledge of seed fate in space and time for a more holistic understanding of the bottlenecks to the pioneer seedling establishment in coastal wetlands. My study suggests that, the potential formation of multiple bottlenecks (Fig.7.1) during the entire recruitment process, including both seed and seedling stages, may better explain the rarity and difficulty of pioneer seedling establishment in most coastal habitats.

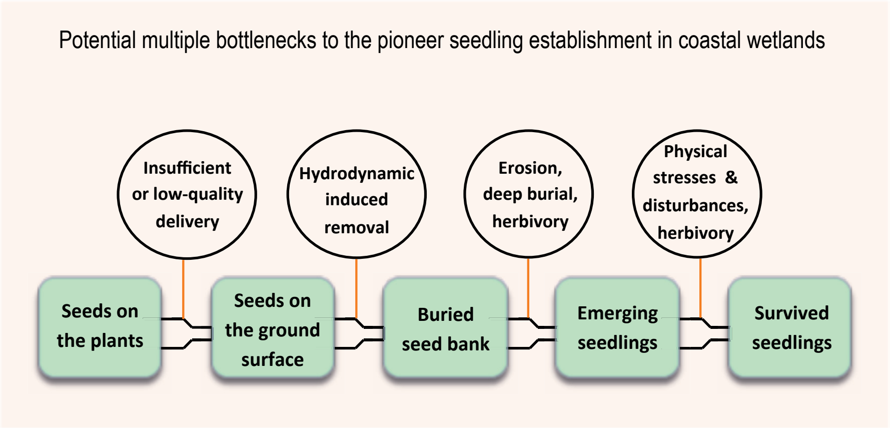

Fig.7.1 Potential multiple bottlenecks (the bottle-like shapes) to the pioneer seedling establishment in coastal wetlands. Rectangles represent physical states, and circles represent the factors that potentially constrain the seed/seedling entry into the next state, and thus form a bottleneck between two states. These factors were identified in literature (e.g. Paramor and Hughes 2004, Friess et al. 2012, Hu et al. 2015) and this study (Chapter 2, 3, 5 & 6).

7.2 Implications for wetland conservation/restoration

Understanding to what extent and in which way pioneer seedling establishment is restricted by seed fate dynamics may hold the key for effective salt marsh restoration/creation measures, given that transplantation efforts can be enhanced by natural recruitment or seed-based restoration techniques.

This study suggests that seed-based marsh restoration would benefit from selecting or regulating the hydrological conditions towards the delivery of sufficient good seeds to the potential recruitment sites (Chapter 3). When seed addition is needed, seeds should be planted to moderate depths to improve seed persistence while not inhibiting seedling emergence (Chapter 2 & 5, Bouma et al., Submitted). Where waves are strong, seed persistence can be enhanced by measures to create more dissipative foreshores e.g. construction of low stone dams that damp wave energy (Van Loon-Steensma and Slim 2012) and disposing dredging materials to promote marsh accretion (Temmerman et al. 2013). Such measures may further facilitate subsequent seedling survival (Hu et al., 2015).

Regardless of physical conditions, seed-based restoration is very likely to fail in tidal flats where seed/seedling hebivores (e.g. ragworms) are abundant (Chapter 6, Emmerson 2000, Paramor and Hughes 2004, 2007). Vegetation restoration or recreation in such habitats may only be possible with the implementation of defaunation or by planting older seedlings or adult plants biomass, since herbivory by ragworms is size dependent (van Belzen et al., In prep.).

Identifying the bottlenecks may also shed light on the control of invasive species, as processes that enable or disable seedling establishment are the same. For instance, top-down control by ragworm *Heidiste diversicolor* and its close relatives might be applicable in the control of invasive cordgrass species (e.g. *Spartina alternifora* and *Spartina anglica*) in the infected areas (Gray et al. 1991, Xiao et al. 2009, Strong and Ayres 2013), provided that the introduction/addition of ragworms will not cause other ecological problems.

7.3 Implications for ecosystem-based management in a shifting climate

While ecosystem-based coastal protection using coastal wetlands has been increasingly valued as a promising measure for mitigating the flooding risk posed by the shifting climate (Gedan et al. 2011, Temmerman et al. 2013, Moller et al. 2014), my study suggests that climate change induced physical changes may increase the uncertainty in the long-term ecosystem persistence, thereby hampering the application of coastal wetlands within coastal defense schemes.

Coastal wetlands like salt marshes are dynamic systems with long-term cyclic alternations between a retreating phase of cliff erosion and an establishment phase by seedling recruitment (van de Koppel et al. 2005). Given a rising sea level and increasing storminess as predicted for many parts of the world (Donat et al. 2011, Young et al. 2011) and the sensitivity of reproductive production to environmental variations, this thesis suggests that the marsh propagation phase through seedling establishment may be considerably influenced by shifting physical conditions.

Amplified physical disturbance under climate change may worsen seed bank formation and seed bank persistence (Chapter 5). Such effect combined with reduced windows of opportunity for seedling survival due to amplified physical disturbances may lead to a substantial reduction of regeneration capacity, thereby ‘locking’ the system in the retreating phase. In this case, a persistent vegetated coastal ecosystem would probably require human-assisted maintenance. For instance, measures can be applied to enhance establishment chances by temporarily reducing physical stresses and disturbances or a ‘jump start’ by planting big seedlings/tussocks that can surpass establishment thresholds (van Wesenbeeck et al. 2008), harnessing positive interactions to improve survival chance (Silliman et al. 2015). Future work should aim at developing knowledge-based bioengineering measures to conserve and regenerate valuable coastal vegetated systems to realize sustainable ecosystem-based coastal protection in the face of a changing climate.

Summary

Coastal wetlands, e.g. saltmarshes and mangroves are among the most valuable ecosystems on the globe. Recently, there has been increasing recognition on their flooding mitigation value, inducing a paradigm shifts towards an ecosystem-based coastal defense. Given a global degradation of vegetated coastal ecosystems and the fact that their rejuvenation strongly depends on sexual reproduction, there is pressing need to understand the mechanisms governing coastal seedling establishment. This is especially relevant in the context of global change, since the recruitment phase is sensitive to environmental changes, which may threaten the sustainability of ecosystem-based coastal protection measures.

Pioneer seedling recruitment is essential for the development of coastal vegetated systems and their long-term dynamics, yet is often problematic due to thresholds and bottlenecks imposed by habitat harshness. Earlier studies centered on the causes for seedling mortality, underscoring the importance of windows of opportunity (WoO) i.e. disturbance free periods for recruitment success. I focused on seed fate pathways to examine whether and how seed dispersal and post-dispersal fates may limit seedling recruitment in coastal environment. Using a common pioneer plant cordgrass as a model, I investigated seed fate dynamics including primary seed delivery, seed bank formation and persistence, as well as seed-seedling transition.

Due to the nature of the transient seed bank, the yearly formation and persistence of a soil seed bank is a prerequisite for cordgrass seedling establishment on tidal flats. Seed bank formation is determined primarily by the presence of a local seed source population and seed delivery by the tide (chapter 2). Effective seed delivery towards the fronting tidal flats is episodic, as controlled by the heterogeneity of maternal seed production and tidal pulsing, with spring tides and storm surges contributing disproportionally to the transport of viable seeds (Chapter 3). Once arrived, seeds need to be buried due to the high seed removal from the tidal flat surface caused by hydrodynamic stress (Chapter 2 & 5).

Seed burial in tidal flats can be achieved by physical processes (e.g. sediment redistribution), biological processes including active seed transport (e.g. ragworms) or sediment reworking by ecosystem engineers. More importantly, the coupling between ecosystem engineers (e.g. lugworms) and physical forcing could act in synergy, resulting in greater seed burial quantity and deeper burial depths (Chapter 4). Yet the buried seeds are still susceptible to erosion, depending on the burial depths relative to the magnitude of sediment disturbance induced by hydrodynamics and tuned by benthic communities (Chapter 5).

Deeper buried seeds have much higher seed persistence (Chapter 2 & 5), but deep burial may hamper seedling emergence, requiring seeds to be positioned in moderate depths to allow successful seed-seedling transition. However, seed-seedling transition can still fail in the presence of herbivores (e.g. ragworms), even though the seeds are buried to the optimal depth (Chapter 6). The top-down control by seed collecting ragworms can act via a peculiar gardening behaviour. Sprouts that germinated from the buried seeds rather than the seeds *per se,* serve as the reward for seed burial service (Chapter 6). Such an opportunistic gardening enables this omnivorous worm species to obtain supplementary superior food, contributing to enhanced growth (Chapter 6).

Overall, this study indicates that, in addition to WoO, successful seedling establishment in coastal habitats necessitates that abiotic (i.e. the tide) and biotic agents (e.g. animals) effectively deliver viable seeds to the correct place (suitable elevation and moderate burial depth), where the physical (e.g. waves) and biological conditions (e.g. herbivores) are proper for the seeds to persist till the right time to realize seed-seedling transition. Such information contributes to a more holistic understanding of the bottlenecks to the pioneer seedling establishment in coastal wetlands. This may serve as a critical step towards the conservation/restoration and sustainable management of valuable but threatened coastal wetlands in the face of global climate change.

Samenvatting

Kwelders en mangroves behoren tot de meest waardevolle ecosystemen op aarde. Zij spelen een belangrijke rol in het vastleggen van koolstof, en kunnen significant bijdragen aan kustverdediging. Deze ecosysteemdiensten maken alternatieve wijzen van kustverdediging, waarbij natuurlijke ecosystemen worden ingezet, steeds aantrekkelijker. Wereldwijd heeft er echter in de afgelopen decennia een sterke achteruitgang van het areaal van begroeide kustecosystemen plaatsgevonden, waardoor de vraag wordt opgeworpen hoe herstel het beste kan worden bevorderd. De verjonging en hervestiging van kwelders en mangroves is afhankelijk van seksuele voortplanting; er is dus dringend behoefte aan een beter begrip van de mechanismen die de vestiging van zaailingen bepalen. De context van klimaatverandering en zeespiegelstijging verleent extra belang aan deze studies. Enerzijds staat de kustbescherming onder druk, anderzijds is bekend dat vestiging van vegetaties afhankelijk is van abiotische condities die met de klimaatverandering zullen wijzigen.

De vestiging van pioniervegetatie is essentieel voor de ontwikkeling van begroeide kustgebieden en hun dynamiek op lange termijn. Vestiging is vaak een knelpunt in de ontwikkeling, omdat de stress in het leefgebied sterker inwerkt op zaailingen dan op volwassen planten. Eerdere studies naar vestiging van vegetatie benadrukken het belang van “windows of opportunity” (Woo), verstoringsvrije periodes waarin vestiging succesvol kan verlopen. Ik concentreerde me op de verspreiding en overleving van zaden om te onderzoeken of en hoe processen van zaadverspreiding en de daarop volgende kieming de vestiging van vegetatie in kustgebieden kunnen beperken. Ik gebruikte de veel voorkomende pioniersoort Slijkgras als model, en onderzocht de processen van zaadzetting, vorming van de zaadbank in het onbegroeide slik, en de kieming en vestiging van zaailingen.

De zaadbank in het slik heeft een korte overlevingstijd, en is daardoor afhankelijk van de jaarlijkse instroom van zaden vanuit de begroeide kwelder. De vorming van een zaadbank hangt daardoor vooral af van de aanwezigheid van een lokale bron van zaden, en van transportprocessen door het getij (hoofdstuk 2). De effectieve bijdrage van zaden aan de zaadbank op het slik is episodisch, afhankelijk van de temporele heterogeniteit van de maternale zaadproductie en de periodiciteit van het getij. Springtij en stormvloeden dragen buiten verhouding veel bij aan het transport van levensvatbare zaden (hoofdstuk 3). Eenmaal aangekomen moeten zaden snel begraven worden omdat ze anders van het slik worden weggespoeld door de waterbeweging (hoofdstukken 2 en 5). De begraving van zaden op getijdenplaten kan het gevolg zijn van fysische processen (mobiliteit van het sediment), of van biologische processen. Actief begraven van zaden is een biobouwer-effect, dat bijvoorbeeld wordt uitgevoerd door zagers (*Hediste diversicolor*). Biobouwers en fysische factoren kunnen in synergie optreden, waardoor grotere hoeveelheden zaad begraven kunnen worden op grotere diepte (hoofdstuk 4). Toch blijven de begraven zaden erosiegevoelig, afhankelijk van de diepte van begraving relatief ten opzichte van de normale diepte van sedimentverstoring door stroming en golven (hoofdstuk 5).

De overleving van zaden neemt toe met de diepte waarop ze zijn begraven (hoofdstukken 2 en 5), maar de kieming en vestiging van zaailingen neemt af met deze diepte. Het hoogste vestigingssucces treedt op bij begraving op intermediaire diepte. Toch kan vestiging ook dan falen, omdat herbivorie door zagers (hoofdstuk 6) tot verlies van zaailingen kan leiden. Deze top-down controle door herbivoren is een nieuw ontdekte vorm van 'tuinieren' door bodemdieren. Het voedsel van de zagers zijn niet de zaden zelf, maar de spruiten die er bij ontkieming uit ontstaan. Het begraven van de zaden is gedrag dat het ontkiemen bevordert, en dat wordt 'beloond' door het opeten van de spruiten. Voor de omnivore worm vormen de spruiten een waardevolle aanvulling op het dieet, waardoor de groei meetbaar toeneemt (hoofdstuk 6).

Dit onderzoek vult het concept van 'windows of opportunity' voor vestiging aan. Succesvolle vestiging is afhankelijk van de juiste abiotische condities (bv. getij, golven), maar ook van biotische factoren. Er moet sprake zijn van effectieve toelevering van levensvatbare zaden op de juiste plaats (geschikte hoogte in het getijvenster, geschikte diepte van begraving), waar de zaden kunnen overleven tot het moment van kieming, en waar herbivorie de kieming en vestiging niet volledig verhindert. Inzicht in deze factoren draagt bij tot het identificeren van de belangrijkste knelpunten voor de vestiging van vegetatie. Daarmee kan deze kennis de basis vormen voor effectieve maatregelen voor behoud, duurzaam beheer en herstel van waardevolle maar bedreigde kustvegetaties in tijden van klimaatverandering.

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Acknowledgement

Although this is ‘my’ thesis, the credits also belong to many others who helped me and supported me during my PhD.

My sincere gratitude first goes to my promoters Prof. Peter Herman and Prof. Tjeerd Bouma. Your guidance directed me to the field of estuarine and coastal science. Despite your busy schedule, you always find time providing me with very helpful feedbacks in my research and writing. Your supervision and inspiration greatly facilitates my growth as a young researcher, enhancing my establishment chance in the highly competitive scientific habitat. I am also very grateful to my copromoter Dr. Tom Ysebaert and my master supervisor Prof. Liquan Zhang from East China Normal University. You built an international bridge that enables me to pursue my PhD in the Netherlands. Thanks to your considerable input and continuous support to my research, this PhD turns out to be a smooth trip.

The work in my thesis also results from collaborations with other fellow PhDs. Many thanks to Francesco Cozzoli for your help in the annular flume experiment, data analysis and writing of Chapter 4. Special gratitude also goes to Jim van Belzen. You are always very supportive and your invaluable inputs substantially contributed to the improvement of Chapter 5 & 6. I also want to extend my appreciation to master students Nanyang Chu, Maria Salvador and Tao Hong for your assistances in the experimental work and data collection. Special thanks to Annick van der Laan for correcting my Dutch summary.

The completion of my PhD research would not be possible without the assistances of the supporting staff in NIOZ-Yerseke. I would like to thank Bas Koutstaal, Jos van Soelen, Kris Giesen, Lennart van IJzerloo and Jeroen van Dalen, Bert Sinke, Lowie Haazen, Annette Wielemaker, Aniek van de Berg, Peter van Breugel and his team for providing logistic and technical support to my research. My appreciation also goes to Anneke van der Endt, Joke van Houte, Christine van der Jagt, Elly van Hulsteijn, Hans Ruissen, Jan Thijs and Jan Megens for their administrative support.

I am grateful to Tadao Kunihiro who not only contributed to my thesis as a co-author of chapter 6, but also took a lot of care of me in life as a close friend. Many thanks to my previous officemates Christian Schwarz and Quanxing Liu, who helped me a lot during the early stage of my PhD, providing all kinds of advices and technical helps. Special thanks also to my current officemates Simeon moons, Rebecca James & Veronica Lo for the pleasant working atmosphere (warm and green), nice conversations we have had during breaks and for correcting my English or Dutch. My gratitude also goes to many other people in the (previous) 'Spatial ecology' department: Johan van de Koppel, Daphne van del Wal, Laura Soissons, Sil Nieuwhof, Loreta Cornacchia, Bas Oteman, Tisja Daggers, Oliver Jewell, Roeland van de Vijsel, Brenda Walles, Olivier Beauchard, Wouter Suykerbuyk, Helene de Paoli, Lucy Gills… for the lovely scientific atmosphere (open and cooperative). Particularity, I learnt a lot from your comments/suggestions to my research and presentations as well as everybody's presentation and subsequent discussion during the department meetings.

As one of the longest (61 months) inhabitants in the guest house, I have been lucky to meet a great amount of international friends: Sven, Jule, Loran, Kris, Michele, Francesc, Silvia, Laurine, Diana, Yayu, Dorina, Heiko, Juliette, Siti, Christine, Clara, Alina, Angelina, Tanja, Daan, Isi, Sandra, Tatiana, Maria, Rick, and many more…Thank you for being helpful in many ways during my PhD and I will never forget the many enjoyable parties, dinners, beer/wine, movies and a lot of fun we had together. My special thanks also go to my Chinese friends I met in Yerseke at different stages: Qi Hui, Haoxin, Zhigang, Cheng Hui, Tao, Nanyang, Zhan Hu, Heng, Haobing, Jiaguo, Maomao and her husband Norbert, for the family-like atmosphere, your help with my research and your care in my life. Your company greatly alleviated my homesickness, and your superb cooking skills keep me away from Chinese restaurants...

I want to further express my deep gratitude to my family, my parents, my sisters and brothers in law for your constant support on all aspects of my life.
感谢父母对我常年在外学习和生活的理解，儿子一定早日学成归国，与家人团聚。感谢姐姐和姐夫们对父母以及大家庭的关怀和照顾，你们是我最坚强的后盾。Thank you Qin, you are not only a great wife that loves me, you also directly contribute to my research as a collaborator. It's my biggest fortune to have met you.

Finally my appreciation goes to the Chinese Scholarship Council for providing the grant to cover my life expenses during my PhD.

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Curriculum Vitae

Zhenchang Zhu was born in July.19th, 1986 in a small village in Jiangxi Province, China. Since 2004, he moved to Shanghai and studied biotechnology in school of life science, East China Normal University (ECNU) for his bachelor degree. In 2008, he switched to wetland ecology for his master research in the state key laboratory of estuarine and coastal research (SKLEC), ECNU. After graduation in 2011, he received a 4-year grant from the Chinese Scholarship council to pursue his PhD in Royal Netherlands Institute for Sea Research, located in Yerseke (NIOZ-Yerseke), with a research focus on the regeneration problem of coastal foundation species. Since September 2015, he has continued to work as a postdoc at NIOZ-Yerseke, on an interdisciplinary 'Building With Nature' project that aims at harnessing the vegetated coastal ecosystems as a solution of nature-based flood defense.

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