Propositions

1. In salt marsh restoration, seed availability in the growing season must not be assumed but examined. (this thesis)
2. Vertical accretion of the salt marsh does not ensure lateral expansion of the area. (this thesis)
3. Unfortunately, having basic statistical knowledge does not ensure simple scientific experiments and studies.
4. A higher sensitivity to historical baselines would boost re-establishment goals for large megafauna.
5. Mankind should learn from nudibranch regarding the irrelevance of gender and beauty.
6. Oligochaetes lack the cuddle factor.

Propositions belonging to the thesis, titled:

Pioneering the Edges

Unravelling how bioturbation, seeds and sediment dynamics affect initial establishment of pioneer vegetation at the salt marsh edge

Marin van Regteren
Wageningen, 9 October 2020

Pioneering the Edges

Unravelling how bioturbation, seeds and sediment dynamics affect initial establishment of pioneer vegetation at the salt marsh edge

Marin van Regteren

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Pioneering the Edges

Unravelling how bioturbation, seeds and sediment dynamics affect initial establishment of pioneer vegetation at the salt marsh edge

Marin van Regteren

Thesis

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Dedicated to Ak en Joop Riedeman

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CHAPTER 1

General introduction

Marin van Regteren

Salt marshes

Salt marshes form natural transitions from land to the sea, present globally on coastlines (McOwen et al. 2017). They are vital areas in coastal systems that provide ample ecosystem services, among which: harbouring unique flora and fauna, supporting nursery areas, recreational use, sequestering and storing carbon, and attenuating wave energy (Bouma et al. 2005; Reise et al. 2010; Temmerman et al. 2013). Salt marshes are increasingly recognized as a sustainable and cost-effective form of nature-based coastal protection (Temmerman et al. 2013). The width of such ecosystems determines its protective value (Barbier et al. 2008; Kirwan et al. 2016b). Salt marshes are physically able to keep up with sea level rise through vertical accretion when sufficient sediment is available in the water column (Kirwan et al. 2016a). Salt-marsh vegetation generates a positive feedback for sediment deposition because plant biomass will reduce the water flow thus allowing the suspended sediment to settle (Fagherazzi et al. 2012). Increases in elevation, by sediment deposition, pave the way for salt marsh development, i.e. succession (Olff et al. 1997).

Salt marshes are highly dynamic ecosystems in which vegetation is adapted to saline conditions, hydrodynamic stress, sediment deposition and erosion (Allen 2000). Many bird and invertebrate species are unique to vegetated tidal marshes and bare intertidal flats and therefore protected (as endorsed by the EU bird and habitat directives) (van Klink et al. 2016). Communities of marsh plants and animals develop in close interaction with these hydrodynamic and bio-geomorphic processes (Elschot 2015). Salt marshes are negatively impacted by human-driven disturbances, such as land reclamation, alteration of coastal hydrology and nutrient pollution (Gedan, Silliman & Bertness 2009). Marsh areas have declined by 50% worldwide and are still deteriorating (Barbier et al. 2011). In addition, the future of salt marshes is threatened by climate change and expected sea level rise. This is especially critical in areas that are constrained by hard structures, such as dykes or heavily populated regions, where salt marshes can only develop seawards (van Coppenolle & Temmerman 2019).

Salt marshes exist on an elevation gradient, with similar gradients of inundation characteristics and salinity (Fig. 1.1) (Coldewey & Erchinger 1992; Bertness & Hacker 1994; Olff et al. 1997; Allen 2000; Balke et al. 2016; Veldhuis et al. 2019). This leads to the development of several (partly overlapping) plant communities. Salt marshes are generally subdivided in a high marsh, low marsh and pioneer zone (Petersen, Kers & Stock 2014). The most elevated part of temperate marshes, i.e. the high marsh, is only flooded occasionally during spring high tides and storms. This results in the establishment of late-successional plant species such as red fescue, *Festuca rubra*, sea couch, *Elytrigia atherica*, and in more brackish parts reed, *Phragmites australis* (Olff et al. 1997). The low marsh is situated preceding the high marsh towards the mudflat and commonly occurring species in this area are sea aster, *Aster tripolium*, sea lavender, *Limonium vulgare*, and common salt-marsh grass, *Puccinellia maritima* (Davy et al. 2011). These species are adapted to regular tidal flooding. Common salt-marsh grass is a long-living, perennial species that excels in stabilization of sediment (Langlois, Bonis & Bouzille 2003), and is thus important for the resilience of marshes to erosive forces like

currents and waves. Sediment deposition may result in marsh succession when vertical accretion surpasses relative sea level rise or guarantee marsh stability when accretion and relative sea level rise are in balance. Seawards of the low marsh lies the pioneer zone with a species cover above 5% (Petersen, Kers & Stock 2014). The pioneer zone is characterized by pioneer plant species such as common cordgrass, *Spartina anglica* and glasswort, *Salicornia* spp. *Spartina anglica* is a perennial species that, in silty conditions, accumulates sediment (Balke et al. 2012). Species traits such as plant stiffness and density will influence the extent of flow reduction and therefore the accumulation rate of sediment (Bouma et al. 2013). In addition to flow reduction the annual *Salicornia* spp. has other beneficial traits, it can trap seeds and tillers and aid in the establishment of other plant species thereby promoting marsh succession (Smit van der Waaij et al. 1995; Widdows et al. 2006).

Figure 1.1 Salt marsh zonation related to elevation, inundation characteristics and associated plant communities, adapted from Coldewey and Erchinger (1992).

Lateral marsh expansion in the transition zone

The transition zone of a marsh is the seaward edge of the vegetation where the marsh meets the intertidal flat and vegetation cover lies below 5%. Pioneer vegetation establishment in the transition zone ensures the continued development of (new) salt marshes. A shift from bare intertidal flat towards a vegetated marsh can occur when a minimum elevation of 0.5 m below mean high water is reached (Wang & Temmerman 2013).

To initiate a shift from bare flats to a vegetated marsh, the appropriate elevation is a crucial boundary condition but additionally, initial vegetation establishment must

occur to achieve a vegetated state. Cover of pioneer vegetation may display cyclic behaviour of retreat and expansion (Balke et al. 2016). Climate change may influence these patterns (Balke et al. 2013; Kirwan et al. 2016b). Therefore it is vital to understand the development of these systems to better predict adaptability to the changing climate (Friedrichs & Perry 2001; Bouma et al. 2016). Even to sustain a stable marsh area, pioneer vegetation establishment is necessary when marshes are (partly) dependent on annual and biennial pioneer species. Subsequent successful establishment of perennials can lead to more permanent lateral expansion of the marsh, thus increasing its protective function for the populated areas lying behind the marsh.

The transition zone of a marsh is clearly marked when it contains a cliff (Bouma et al. 2016). As not all marshes contain cliffs, these transitions also occur on gradual slopes (Fig. 1.2). This makes the boundary of these two systems (marshes vs tidal flats) less straightforward. The transition zone accommodates high potential for lateral marsh expansion, but only if pioneer vegetation manages to establish (Zedler, Morzaria-Luna & Ward 2003). Important pioneer species in temperate marshes are the annual *Salicornia* spp. and perennial *Spartina* spp, which are common around the globe. They are well able to colonize anoxic sediments (Davy, Bishop & Costa 2001; Koop-Jakobsen, Fischer & Wenzhofer 2017). Both species reproduce by seeds, although *S. anglica* can also sustain itself through vegetative growth (Wolters, Garbutt & Bakker 2005a).

Seed dispersal and retention

Salt-marsh pioneer species generally disperse their seeds in autumn-winter (September to December) in Northern Europe (Hutchings & Russell 1989; Huiskes et al. 1995; Rand 2000; Wolters, Garbutt & Bakker 2005a). The time window of peak dispersal of seeds is species specific. *S. anglica* has peak seed dispersal times in October and December (Huiskes et al. 1995). For example, *Salicornia* spp., *S. anglica* and *A. tripolium* seeds disperse mainly from October to December (Hutchings & Russell 1989; Huiskes et al. 1995; Wolters, Garbutt & Bakker 2005a), *S. anglica* seeds disperse up to march, with the arrival of seeds primarily in January (Zhu et al. 2014). Several common marsh species, such as *Bolboschoenus maritimus*, *Suaeda maritima*, *Atriplex* spp. and *Puccinellia maritima*, disperse their seeds between September and October (Hutchings & Russell 1989; Huiskes et al. 1995; Rand 2000; Wolters et al. 2004; Espinar, Thompson & Garcia 2005). Seed dispersal is mostly local in salt marshes (Rand 2000), thus when vegetation occurs in proximity, the abundance of seeds is not expected to be limiting. Seed dispersal can be driven by wind, waves and/or tidal flow. Wind-driven dispersal has a greater dispersal range compared to the other agents. Water-driven dispersal is largely dependent on the floating time of seeds. When water-driven, seeds are usually transported higher up the marsh and found in drift-line material (Huiskes et al. 1985; Wolters & Bakker 2002; Chang, Veeneklaas & Bakker 2007). Dispersal of seeds is more frequent within the marsh than between marshes, although export of seeds with the ebb current can be considerable (Huiskes et al. 1995).

Once dispersed, seeds need to be retained in an area in which the appropriate boundary conditions for germination and successful establishment are likely to occur. Seed retention is especially vital for annual and biennial species that are dependent on seeds for establishment. If seeds are retained in the transition zone, salt marsh expansion may occur (Zedler, Morzaria-Luna & Ward 2003). Seeds retained throughout the winter can become available for vegetation establishment in spring. Retention of seeds incorporated in the soil may result in the formation of a seed bank provided that they remain viable (Goodson et al. 2001). Seed banks of marshes are generally considered to be transient, i.e. seeds only remain viable for one calendar year (Bakker et al. 1996; Davy, Bishop & Costa 2001). Although the seeds of some species persist in the soil for germination in subsequent years (Looney & Gibson 1995), for example, *Bolboschoenus maritimus* (Thompson, Bakker & Bekker 1997) and *Salicornia* spp. (Davy, Bishop & Costa 2001). Generally, the longer seeds reside in the soil, the lesser viable they become (Espinar, Thompson & Garcia 2005; Erfanzadeh et al. 2010b). Thus salt marshes remain reliant on yearly retention of freshly dispersed seeds for vegetation establishment.

Figure 1.2 Salt marsh to intertidal flat gradient, the low marsh becomes pioneer zone which is followed by the sparsely vegetated transition zone (mainly *Salicornia procumbens*). Thereafter occurs the intertidal flat with elevated hummocks and waterlogged hollows. The two pairs of black poles on the tidal flat are sedimentation erosion bar (SEB) stations.

Pioneer vegetation establishment

The successful germination of seeds requires a window of opportunity, which is described as a critical minimal disturbance-free period to support seedling establishment (Wiehe 1935; Balke et al. 2011; Balke, Herman & Bouma 2014). For halophytes, this is related to the frequency of inundation. Once germinated, young seedlings need a period of low hydrodynamic energy for sufficient roots to grow and thus withstand tidal flows and waves (Hu et al. 2015a). Other abiotic variables have been shown to influence germination and seedling establishment of pioneer plants as well. For example, *S. anglica* is very sensitive to sediment dynamics, i.e. sediment deposition and erosion (Cao et al. 2018; Poppema et al. 2019). *Salicornia* spp. germination has been found to increase when salinity is reduced (Carter & Ungar 2003), while its seedlings are sensitive to waterlogging (Egan & Ungar 2000a). The windows of opportunity framework focus mainly on physical parameters (Balke et al. 2011; Hu et al. 2015a; Poppema et al. 2019), however, we argue that biological aspects, such as microphytobenthos and bioturbation, may also interfere with successful germination and seedling establishment (Fig. 1.3).

Establishment of vegetation can be severely inhibited by infaunal bioturbation. For example, bioturbation by the polychaete worm *Arenicola maritima* can inhibit the establishment of *S. anglica* and the two are nearly mutually exclusive (van Wesenbeeck et al. 2007). The amphipod *Corophium volutator* has been shown to disturb effective

Figure 1.3 Pioneer seedling establishment of *Salicornia procumbens* in a transition zone, superficial cracks are visible.

rooting of *Salicornia* spp., thus constraining its establishment (Gerdol & Hughes 1993). The polychaete worm *Hediste diversicolor* actively transports cordgrass seeds into its burrows to consume them once sprouted (Zhu et al. 2016a). When not consumed, being inside burrows forms an excellent protection against hydrodynamic forces and seeds are then retained. All these examples show that bioturbating benthos interacts and may interfere with successful initial vegetation establishment. The presence of microphytobenthos may indirectly support establishment of vegetation through sediment stabilization. Microphytobenthos (i.e. algal biofilm) excrete extra polymeric substances that significantly increases the cohesiveness of the sediment (De Deckere, Tolhurst & De Brouwer 2001; Widdows et al. 2006).

The Wadden Sea

The Wadden Sea area is an intertidal system stretching along the coasts of the Netherlands, Germany and Denmark in Northern Europe. This area is heavily used commercially and recreationally for shipping, tourists, dredging, food provision and transportation. The coastal habitats, including tidal areas and salt marshes, provide many direct and indirect benefits for society. The Wadden Sea has acquired a UNESCO World Heritage status in 2009 because of its uniquely large interconnected tidal area with high ecological, economic and societal value (Reise et al. 2010; Schuerch et al. 2014). Massive interventions in the Wadden Sea ecosystem during the last century have changed the coastal morphology and thereby hydrodynamic conditions and suspended sediment concentrations (Allen 2000; Lotze 2005; Elias et al. 2012). In the last 1000 years, half of the original Wadden Sea area has been embanked, which has resulted in a decline of surface area to dissipate waves and water filter capacity (Reise 2005). In addition, the intensified use of coastal habitats has led to a dramatic degradation of food-web complexity (Lotze et al. 2006).

The multi-use of the Wadden Sea makes it an intricate area, vulnerable to many different pressures. One of those is the required dredging to keep shipping channels and harbours navigable. Yearly, about 1.3 million m³ of fine sediments are dredged from the port and channels of Harlingen. The dredged sediment is disposed in close vicinity of the harbour, which has a high probability of being transported back into the channels and port (Baptist et al. 2019a). To increase dredging efficiency, the sediment should ideally be stabilized and retained elsewhere. In recent years, an innovative soft engineering pilot, named the Mud Motor project, has tested the effect of multi-year semi-continuous mud nourishment (2016-2017, Baptist et al. 2019a). The dredger disposed its dredged sediment away from the port, further North-East, at the end of the Kimstergat channel. The Mud Motor aims to stimulate lateral marsh expansion while fixating additional supplied sediment, using a building with nature approach (De Vriend et al. 2015). Building with nature strategy aspires to incorporate hydro-morphological and ecological processes in engineering practices. Salt marshes often form a natural protective barrier in front of 'hard' engineering solutions like dykes. In the next decades, many dykes are in need of increased maintenance and adaptive management due to

sea level rise. However, marshes may provide a more sustainable and cost-effective solution (Temmerman et al. 2013). In the Mud Motor project, the disposed dredged sediment is expected to be transported by the flood current to the intertidal flats and salt marshes north of Harlingen. Salt marsh vegetation should stabilize and retain this sediment (increased vertical accretion), reduce sediment recirculation and promote lateral marsh expansion. This will enhance its coastal protective value (Barbier et al. 2008; Temmerman et al. 2013).

Objectives and thesis outline

Thresholds for the initial establishment of marsh vegetation still need to be identified (Bouma et al. 2014; Poppema et al. 2019) and this is specifically interesting in the transition zone of two adjacent systems: the un-vegetated intertidal flat and the vegetated salt marsh. The potential for marsh expansion prevails here if pioneer plants are able to establish successfully. To study the processes that influence vegetation establishment in the transition zone, the salt marsh to the intertidal flat gradient, two marshes at Westhoek and Zwarte Haan are used as our model systems. These two marshes along the Frisian coast have formed naturally in the last 2 to 3 decades and have shown stepwise lateral expansion (Baptist et al. 2019b). Seawards of these marshes lie gently sloping and wide intertidal flats. This makes them excellent areas for studying initial vegetation establishment in the transition zone. The presence of an existing marsh ensures a seed source nearby. Our general objective is to understand the underlying processes that influence initial vegetation establishment in the transition zone. To increase this understanding, several aspects that may influence vegetation establishment were examined. Ecological and physical influences are in continuous interaction in this dynamic area and thus will be studied in unison (Fig. 1.3).

Figure 1.3. Schematic cross-section of the intertidal flat to salt marsh gradient with an overview of topics discussed in the different Chapters of this thesis.

The following general research questions will be addressed in the Chapters of this thesis:

What abiotic and biotic variables determine the transition zone between the salt marsh and the intertidal flat?

In Chapter 2, we characterize the salt marsh to intertidal flat system in which plant species and cover change along a gradient. Specifically focused on the transition zone, where marsh expansion may occur. Do abiotic variables form gradients similar to the gradient in vegetation or is there a more distinct zonation? How does the benthic community change with the transition from land to sea? Additionally, the abundance of seeds in the transition zone is studied in detail.

How does dispersal timing affect seed retention in the transition zone and hence influence initial vegetation establishment?

The salt-marsh area and pioneer zone at Westhoek did not expand during the Mud Motor experiment (as was expected due to additional sediment load). Seeds of pioneer plants are not expected to be a limiting factor, as the transition zone closely borders the pioneer zone. Seeds, however, may not be present in the transition zone during the time window of pioneer vegetation establishment. Seed retention during and after winter of three marsh species was examined in a field experiment described in Chapter 3, in order to gain insight into the availability of seeds in spring. The seed bank and seedling establishment success were assessed in the field and seed viability was measured in the laboratory.

What is the effect of bioturbating oligochaetes on seed distribution and seedling establishment?

In Chapter 4, the effects of oligochaete bioturbation on the burial of *Salicornia* spp. seeds, germination and seedling establishment were studied with a laboratory experiment. Macrobenthic polychaetes are known to interfere with seed availability and seedling establishment but this is yet unknown for the smaller oligochaete worms. We assessed if bioturbation by oligochaete worms hinders germination and seedling establishment. Additionally, the effect of oligochaetes on sediment properties, such as oxygen penetration depth and bulk density, were determined.

*What are the main factors that drive germination occurrence, success and mortality of *Salicornia procumbens* in the transition zone?*

Once seeds have been retained in a suitable location, germination, seedling establishment and mortality of *Salicornia* spp. get affected by environmental variables. These range from weather conditions, such as temperature and precipitation, to sediment dynamics and inundation regime. In Chapter 5, these factors are examined using a field experiment in the transition zone to elucidate on environmental boundary conditions for successful pioneer establishment.

Finally, in Chapter 6, I discuss how the different Chapters have contributed to our understanding of initial pioneer vegetation establishment in transition zones and how this knowledge can be used in coastal management for the conservation and restoration of our natural barriers, the salt marshes.

The salt marsh at Westhoek with pioneer establishment of mainly *Salicornia procumbens* and a considerable layer of fluid mud (November 2016).

CHAPTER 2

Where does the salt marsh start? Field-based evidence for an abrupt transition between gradually sloping tidal flat and salt marsh

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ABSTRACT

Salt marshes are vegetated ecosystems between land and sea, hosting unique plant and animal communities and providing crucial habitats for birds. They form a sustainable nature-based coastal protection, and its protective value increases with the width of the system. So far, there is a lack of studies focussing on vegetation establishment in the transition zone between tidal flat and salt marsh. Especially, studies including an array of biotic and abiotic variables affecting vegetation establishment would aid in salt-marsh restoration and marsh growth stimulation projects. The aim of this study was to determine where the salt marsh starts and how a sparsely vegetated transition zone relates to the densely vegetated salt marsh and the bare intertidal flat. In a naturally developing salt marsh, we sampled the gradient from tidal flat to the low marsh on benthic species composition, seed availability and environmental variables. The abundance of benthos was highest landwards of the transition zone, in the pioneer zone. Distinct meiobenthic groups occurred in the different zones of the tidal flat to low marsh gradient, macrobenthos was largely absent. In the transition zone, the abundance of salt-marsh seeds was similarly low as at the tidal flat. It is suggested that, even though a seed source was in proximity, seed availability was insufficient to achieve lateral marsh expansion. Clustering and nMDS analyses showed that the sparsely vegetated transition zone resembled the bare tidal flat in terms of its environmental conditions. A distinct border was present between the sparsely vegetated transition zone and the vegetated pioneer zone formed by abrupt shifts in soil oxygenation and seed availability.

INTRODUCTION

Salt marshes form connections between land and sea and host unique plant and animal communities. Similar to dunes and mangroves, salt marshes provide a sustainable and cost-effective nature-based coastal defence, in extension to current practices (Temmerman et al. 2013). The risks posed by sea-level rise and climate change call for improved coastal defences (Möller 2006; Bouma et al. 2014). Salt marshes depend on sufficient sediment accretion rates to keep pace with enhanced sea-level rise. Most studies estimating long-term survival of salt marshes focused on vertical marsh accretion (Kirwan & Guntenspergen 2010; Marani, Da Lio & D'Alpaos 2013; Mariotti & Fagherazzi 2013), but lateral (seaward) expansion is as crucial for the preservation of salt marshes (Bouma et al. 2014; Balke et al. 2016; Bouma et al. 2016). Most marshes nowadays are limited inland by hard structures and lateral expansion can only occur on the seaward side of marshes. The most important step of lateral expansion is the establishment of pioneer vegetation in the transition zone, where the un-vegetated tidal flat meets the vegetated salt marsh. This is where salt marsh expansion potential is highest (Zedler, Morzaria-Luna & Ward 2003). Therefore, pioneer vegetation establishment on a bare intertidal flat is essential (van der Wal, Wielemaker-Van den Dool & Herman 2008), however, most thresholds for successful vegetation establishment in the transition zone still need to be quantified (Bouma et al. 2014).

Vegetation establishment depends on appropriate boundary conditions and thus various factors. Various studies that have identified one or a few factors that affect vegetation establishment are: bioturbation (van Wesenbeeck et al. 2007; van Regteren et al. 2017), an inundation-free period (Wiehe 1935), drought (Silinski et al. 2016b), salinity (Baldwin, McKee & Mendelssohn 1996), temperature (Strain et al. 2017), freshwater (Carter & Ungar 2003), seed abundance (Wolters et al. 2008; Erfanzadeh et al. 2010a), soil oxygenation (Lee 2003), grain size (Koop-Jakobsen, Fischer & Wenzhofer 2017), waves and water depth (Zhu et al. 2019), sediment dynamics (Cao et al. 2018; Poppema et al. 2019), elevation (Wang & Temmerman 2013), and algae (Hulzen et al. 2006). In the field, all these variables are likely to change over the salt marsh to the tidal flat interface. However, this still needs to be determined and may affect pioneer vegetation establishment in the transition zone, the prelude for marsh expansion.

The salt marsh to the tidal flat interface is generally divided into multiple zones based on plant species and vegetation cover (Petersen, Kers & Stock 2014). The high marsh is characterized by one or a few late-successional plant species with high cover and density. At the low marsh, a generally more diverse variety of plant species occur in fluctuating cover. Subsequently follows the pioneer zone, which is dominated by pioneer species with cover > 5%. The border between pioneer zone and tidal flat can be either sharp in case a marsh cliff is present (van der Wal, Wielemaker-Van den Dool & Herman 2008) or gradual on a gently sloping marsh. In front of the pioneer zone lies the transition zone on the tidal flat, with only a few pioneer plants per m² present, i.e. a cover < 5%. Seaward of the transition zone, the bare tidal flats are located. Identification of these individual habitats is generally based on vegetation type and cover, however, both environmental conditions and benthic community composition are expected to

differ between these zones as well.

The aim of this study is to understand the development of abiotic conditions, seed availability and benthic species composition in a gradient in a naturally developing salt marsh. The focus lies in the transition zone, which is fundamental for the expansion potential of marshes. Densely vegetated marshes and bare tidal flats are two alternative stable states (Wang & Temmerman 2013), but it is yet unclear what state the sparsely vegetated transition zone belongs to. The research questions are: 1) is there a gradual transition from tidal flat to pioneer zone based on both physical and biological parameters, or is there a distinct threshold separating two alternative stable states?; 2) how does the benthic community change along this gradient? and 3) can we identify which factors impact vegetation establishment in the transition zone. Once we know what distinguishes a transition zone between marsh and bare tidal flat, we gain insight into the expansion potential of salt marshes.

METHODS

Study site

A naturally developing salt marsh in the Wadden Sea of approximately 30 hectares was used as the field study site, located along the Dutch mainland at Westhoek, The Netherlands (53°16.31N, 5°33.14E). The landward boundary is formed by a dyke. Since its formation in 1996, the marsh has been expanding step-wise, with stable periods alternating with periods of expansion, both along the dyke and onto the intertidal flats (Baptist et al. 2019a). Vertical accretion rate was 1.6 ± 0.7 cm yr⁻¹ (mean $\pm$ se) for the period 2015-2019 (Baptist et al. 2019b). The vegetation in the pioneer zone was dominated by pioneer species *Salicornia* spp. and *Spartina anglica*. Most frequently occurring plants on the low marsh were *Aster tripolium*, *Puccinellia maritima*, *Suaeda maritima*, *Atriplex prostrata* and *Halimione portulacoides*. The high marsh was characterized by *Elymus athericus* and *Phragmites australis*.

Field sampling

In May 2016, field sampling was conducted in 14 transects encompassing 5 predefined zones in a gradient from salt marsh to tidal flat (Fig. 2.1). Four zones were defined by vegetation cover and species composition, following the classification of Petersen et al. (2014). These included: low marsh, pioneer zone, transition zone and tidal flat. The tidal flat was divided into waterlogged hollows and higher elevated hummocks based on visual observation of natural bathymetric patterns (Fig. 2.1). On the north-eastern and south-western extremities of the study site, the low-marsh zone had not yet established (Fig. 2.1). In total, 67 plots were sampled divided over 14 transects. Elevation of each plot was measured with a Real-Time-Kinematic-DGPS with a vertical accuracy of 0.016 m. Depth of the oxygenated layer was measured using a soil corer of 26 cm in

length, replicated thrice and averaged. If the oxygenation reached deeper than 26 cm, this value was still noted as the maximum. Vegetation composition and cover were scored in 2 m by 2 m plots using a decimal scale (Londo 1976). Microphytobenthos, consisting of diatoms and cyanobacteria, was measured on the soil surface with a BentoTorch fluorometer (bbe Moldaenke GmbH), replicated thrice and averaged. Soil cores were taken, 5.6 cm in diameter and 10 cm deep, for benthos and seed abundance analyses, hereafter referred to as benthos cores. They were preserved in a 6% formaldehyde solution containing rose Bengal. Sediment cores, 4.6 cm wide and 5 cm deep, were taken to measure bulk density, water content and organic matter content. These were frozen until further analyses. Additionally, three soil samples were taken to estimate surface soil salinity and a surface sediment sample of 40 mL was taken for grain size analysis.

Laboratory analyses

The sediment cores were weighed to obtain wet weight (WW), dried for 24 hours at 105°C and weighed again to obtain dry weight (DW). Subsequently, they were incinerated for 8 hours at 560°C to obtain the ash-free dry weight (AFDW). To estimate organic and carbonate content of the sediment, loss on ignition (LOI, % of AFDW) was measured. The volume was used to calculate the dry bulk density (g cm^{-3}) and mass water content (%) (Flemming & Delafontaine 2000). Grain size (μm) was analyzed by the Netherlands Institute for Sea Research (NIOZ) with a Coulter Beckman LS 13 320. Soil surface salinity was analyzed by putting 15 g of soil into 60 mL of demi-water. This mixture was shaken vigorously and left overnight for the sediment to settle. Once settled, salinity was measured with a Hach HQ40D multi-meter using the Practical Salinity Scale (‰), and the average of the three measurements was taken as the salinity value.

The benthos and seed cores were sieved over a 250 μm sieve, transferred into a transparent tray and placed on a light table. All organisms and detectable seeds were collected and counted. Species were determined up to order level and stored in 70% ethanol. Nematodes and foraminifera were excluded because the sieve was too coarse to retain them quantitatively. Seeds were identified up to species level when possible, otherwise categorized as unidentified.

Data analyses

The protocol designed by Zuur et al. (2010) was used for data exploration and validating the assumptions. Analyses of variance (ANOVA) were used to test whether zones differed from each other based on abiotic and biotic variables, the abundances of benthic groups and seed availability. Zone was used as a predictor variable. When the ANOVA yielded significant results, post hoc Tukey HSD tests were used to explore which zones differed from each other.

Figure 2.1 Map of sampling stations, zonation indicated with coloured dots. Transects are numbered from north-east to south-west. The triangle indicates the location of the Westhoek salt marsh, The Netherlands. The lower excerpts exemplify the zonation.

Cluster analyses were used to validate the zonation defined by plant species, plant cover and topographic heterogeneity. A selection of non-collinear environmental variables was made based on variance inflation factors. The following environmental variables were used: elevation, dry bulk density, soil salinity, mud content, loss on ignition and microphytobenthos. Soil oxygenation was excluded due to collinearity with elevation, similar to mass water content collinear with dry bulk density and d50 collinear with mud content. Clustering on environmental variables and benthos community was performed using Ward's hierarchical clustering on an Euclidean distance matrix. Environmental data were thus standardised and scaled (Clarke and Warwick, 2001). Benthos community data were also analysed using non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity (Bray and Curtis, 1957). The performance of the nMDS was assessed using a Shepard-Diagram.

The relationship between the benthic community structure and abiotic environmental variables was examined using the BioEnv procedure (Clarke and Ainsworth, 1993) to define which combination of environmental variables had the highest correlation with the benthic species community. The association of the benthic community with the selected combination of environmental variables was tested for significance using a Mantel test. This test related the benthic distance matrix with the environmental distance matrix with Spearman's correlation using a bootstrapping procedure with 999 iterations.

All analyses and design of the figures were performed with the statistical program R (R Core Team, 2018) using additional packages *vegan* 2.5-1, *ggplot2* 3.1.0, *MASS* 7.3-51.1, *cowplot* 0.9.2, *tidyverse* 1.3.0, *raster* 3.0-7, *sf* 0.8-0 and *tmap* 2.3-1.

RESULTS

Plant cover and seed abundance

The low marsh contained the most diverse plant community as well as the highest cover. On average, 5.5 different plant species were present and the cover was nearly 90%. It was dominated by *Puccinellia maritima* and *Aster tripolium*, with a combined cover of 66% on average (Table 2.1). *Salicornia* spp. and *Atriplex prostrata* were often present, though in lower abundance. *Spergularia* spp., *Elymus athericus*, *Triglochin maritima*, *Agrostis stolonifera* and *Spartina anglica* were sparsely present in the low marsh. The low marsh was followed by the pioneer zone with 3.7 species on average and cover close to 40% (Table 2.1). The pioneer plants *Salicornia* spp. and *S. anglica* dominated the pioneer zone. *Suaeda maritima*, *P. maritima* and *A. tripolium* were often present in low cover in the pioneer zone. In the transition zone, solely the annual pioneer *Salicornia* spp. was present and its cover was a maximum of 1%. The tidal flat was unvegetated with the exception of one *Salicornia* spp. seedling on a hummock.

Seed abundance differed across the zones (ANOVA, $F_{df=4}=32.4$, $p<0.001$).

Table 2.1 Mean cover (%) of salt-marsh plants (mean $\pm$ sd) given for each zone.

Plant species	Tidal flat Hollows	Tidal flat Hummocks	Transition zone	Pioneer zone	Low marsh
<i>P. maritima</i>	0	0	0	0.6 $\pm$ 0.8	21.5 $\pm$ 36.6
<i>A. tripolium</i>	0	0	0	1.5 $\pm$ 1.8	45.4 $\pm$ 24.3
<i>A. prostrata</i>	0	0	0	0.14 $\pm$ 0.36	5.1 $\pm$ 11.7
<i>S. anglica</i>	0	0	0	8.8 $\pm$ 13.7	2.0 $\pm$ 3.4
<i>Salicornia</i> spp.	0	0.07 $\pm$ 0.27	0.9 $\pm$ 0.36	26.2 $\pm$ 23.5	11.6 $\pm$ 13.8
<i>S. maritima</i>	0	0	0	0.4 $\pm$ 0.5	0.8 $\pm$ 0.8
<i>Spergularia</i> spp.	0	0	0	0.07 $\pm$ 0.27	0.6 $\pm$ 1.2
<i>E. athericus</i>	0	0	0	0	0.5 $\pm$ 0.8
<i>T. maritima</i>	0	0	0	0	0.09 $\pm$ 0.3
<i>A. stolonifera</i>	0	0	0	0	1.9 $\pm$ 6.0
Total cover (%)	0	0.07 $\pm$ 0.27	0.86 $\pm$ 0.36	37.8 $\pm$ 22.7	89.5 $\pm$ 21.9
Sample size	14	14	14	14	11

The post hoc Tukey HSD test indicated that the low marsh and pioneer zone differed significantly from each other and all other zones. Seed abundance in the soil was lowest at the tidal flat and significantly increased towards the low marsh (Table 2), which contained in a similar pattern as the plant cover (Fig. 2.2). Microphytobenthos abundance (cyanobacteria and diatoms) was similar throughout the tidal flat to salt marsh zones (ANOVA, $F_{df=4}=1.5$, $p=0.22$, Fig. 2.2).

The seed bank did not represent all the species that were present in the aboveground vegetation. Seeds from six different plant species were identified, of which five occurred aboveground in our sampling plots. The sixth seed species belonged to *Phragmites australis*, which was present higher up the marsh and disperses by wind.

Figure 2.2 Boxplots of biotic variables that characterise different zones along the tidal flat to salt marsh gradient. Dots, jittered for clarity, indicate the individual measurements. Information on individual species, vegetation cover and seed species can be found in tables 2.1 and 2.2.

Five plant species present in the vegetation were absent from the seed bank. In the low marsh (and in total), the most common seed species found was *A. prostrata* with on average 180 seeds per sample (246 cm^3). However, with only a 5% cover, this was not the dominating species aboveground (Table 2.1). In the pioneer zone, *Salicornia* spp. seeds prevailed with over 40 seeds per 246 cm^3 , corresponding to the dominant vegetation aboveground. The total number of seeds in the transition zone and the tidal flat was, on average, less than 1 seed per 246 cm^3 , mostly *Salicornia* spp. (Fig. 2.2 and Table 2.2).

Salicornia spp. and *A. prostrata* seeds were found throughout all zones, ranging from salt marsh to tidal flat (Table 2.2). The wind-dispersed *A. tripolium* occurred in all zones as well, although in lower abundance. *S. anglica* seed abundance was remarkably low: only six seeds in the low marsh and one in the pioneer zone, even though in the pioneer zone *S. anglica* was the second most common species aboveground. The total number of seeds of all marsh plants combined was four times higher in the low marsh than in the pioneer zone, whereas the vegetation cover was two times higher in the low marsh than in the pioneer zone (Tables 2.1 and 2.2).

Table 2.2 Mean number of seeds sampled in the top 10 cm soil layer, given per 246 cm^3 for each zone and species (mean $\pm$ sd).

Plant species	Tidal flat Hollows	Tidal flat Hummocks	Transition zone	Pioneer zone	Low marsh
<i>P. maritima</i>	0	0	0	0	6.0 ± 19.9
<i>A. tripolium</i>	0.07 ± 0.27	0.21 ± 0.58	0.07 ± 0.27	1.7 ± 3.1	18.6 ± 27.0
<i>A. prostrata</i>	0.07 ± 0.27	0.14 ± 0.36	0.07 ± 0.27	10.2 ± 15.0	180.0 ± 133.2
<i>S. anglica</i>	0	0	0	0.07 ± 0.28	0.6 ± 0.7
<i>Salicornia</i> spp.	0.36 ± 0.84	0.14 ± 0.36	0.79 ± 1.72	42.2 ± 46.5	20.8 ± 28.4
<i>P. australis</i>	0.07 ± 0.27	0	0	0	0
Seeds unid.	0.36 ± 0.74	0	0.21 ± 0.58	0.43 ± 0.94	0.91 ± 1.45
Total seeds (246 cm^3)	0.5 ± 0.94	0.5 ± 1.09	0.93 ± 1.86	54.2 ± 49.7	219.9 ± 129.8
Sample size	14	14	14	14	11

Abiotic environmental variables

Elevation decreased from the low marsh at 1.5 m above NAP towards the tidal flat at around 0.95 m above NAP and the elevation differed significantly between zones (ANOVA, $F_{df=4} = 103.5$, $p < 0.001$). The post hoc Tukey HSD test showed that the low marsh and pioneer zone differed significantly compared to the other zones (Fig. 2.3). Soil oxygenation differed between zones (ANOVA, $F_{df=4} = 88.3$, $p < 0.001$). It declined sharply between the pioneer and transition zone, from $214 \pm 59 \text{ mm}$ to $31 \pm 44 \text{ mm}$. Oxygenation was significantly higher in the vegetated zones than at the transition zone and intertidal flat, which were not significantly different from each other. Soil oxygenation was on average $12 \pm 10 \text{ mm}$ for the tidal flat hollows and $6 \pm 4 \text{ mm}$ for the tidal flat hummocks.

Soil salinity varied significantly between zones (ANOVA, $F_{df=4}=21.7$, $p<0.001$). It was much lower at the low marsh but comparable across the other zones (Tukey HSD, Fig. 2.3 and Supplementary Table S2.1). Overall, the soil was silty with a mud content between 50% and 80% and median grain size (d_{50}) of $37 \pm 17 \mu\text{m}$. A marginally significant difference in mud content was found between the tidal flat hollows and the low marsh (ANOVA, $F_{df=4}=2.5$, $p=0.048$, Tukey HSD, Fig. 2.3). Median grain size was similar across all zones (ANOVA, $F_{df=4}=1.7$, $p=0.17$).

Figure 2.3 Boxplots of abiotic variables that characterise different zones along the tidal flat to salt marsh gradient. Dots, jittered for clarity, indicate the individual measurements.

Mass water content differed between the zones (ANOVA, $F_{df=4}=64.8$, $p<0.001$). On average, the hollows on the tidal flat were distinctly different from all other zones with a higher mass water content of $\pm 70\%$ vs. $\pm 50\%$ (Fig. 2.3, Tukey HSD). Consequently, a reversed pattern occurred for dry bulk density, which varies across zones (ANOVA, $F_{df=4}=48.8$, $p<0.001$). Dry bulk density was lower in the tidal flat hollows compared to all other zones (Fig. 2.3, Tukey HSD). Organic and carbonate content (LOI) varied across zones (ANOVA, $F_{df=4}=8.5$, $p<0.001$) and was significantly lower on the tidal flat hummocks, but similar for all other zones (Fig. 2.3, Tukey HSD).

The cluster analysis for the abiotic environmental variables did not reflect the same five zones as classified in the field based on vegetation cover and topographic heterogeneity, i.e. hummock and hollows (Fig. 2.3). The cluster analysis indicated that the transition zone and tidal flat hummocks shared similar environmental conditions. Together with the cluster for tidal flat hollows, these two clusters were distinctly different from the two clusters of the vegetated zones (Fig. 2.3). The low marsh and pioneer zone were more alike and some low marsh samples were clustered with the pioneer zone.

Benthic community distribution

The soil samples were dominated in abundance by three species groups: oligochaetes, collembolans and harpacticoid copepods (Fig. 2.4). Collembolans and copepods hardly occurred in the low marsh, where insects were most common. Insects differed significantly across zones (ANOVA, $F_{df=4}=11.1$, $p<0.001$) and were significantly more abundant on the low marsh (Tukey HSD). The insects included multiple species of fly pupae, spiders, chironomids, aphids and one thrips. In the pioneer zone, oligochaetes and harpacticoid copepods were the most common benthic fauna. The oligochaetes numbers contained 95% juveniles and 5% adults. Adults were identified up to species level and belonged to *Paranais litoralis* (most common), *Baltidrilus costatus*, *Nais elinguis* and *Enchytraidae sp.* Few adults but many juvenile oligochaetes were present in May, both differed significantly across zones (ANOVA, respectively $F_{df=4}=6.7$, $p=0.0002$ and $F_{df=4}=31.3$, $p<0.001$). Post hoc tests indicated that oligochaetes occurred in significantly higher abundance in the pioneer zone (Fig. 2.4). The pioneer zone contained on average 365 oligochaetes per sample, which corresponds to approximately $148 \cdot 10^3$ oligochaetes per m^2 . Oligochaete juveniles also occurred in all other zones, with the second highest abundance in the low marsh (Fig. 2.4).

Benthic copepods were found in distinct zones (ANOVA, $F_{df=4}=11.0$, $p<0.001$). Harpacticoid copepod abundance was significantly higher in the pioneer zone and tidal flat hollows (Tukey HSD). Remarkably, they occurred most frequently in two non-bordering zones (Fig. 2.4). Springtails (collembola) *Archisotoma sp.* (likely all *Archisotoma subbrucei*) were dominant in the transition zone and the hummocks of the tidal flat (ANOVA, $F_{df=4}=4.3$, $p=0.004$, Tukey HSD). The transition zone contained on average 299 individuals per sample, which corresponds to $120 \cdot 10^3$ collembolans per m^2 . The tidal flat hummocks contained on average 157 individuals per sample, corresponding to $63 \cdot 10^3$

collembolans per m^2 . Springtails and harpacticoid copepods hardly ever co-occurred. The pioneer zone contained on average 215 copepods per sample, which corresponds to 86×10^3 individuals per m^2 . The tidal flat hollows contained on average 317 individuals per sample, equivalent to 127×10^3 copepods per m^2 .

Figure 2.4 Boxplots of total benthos abundance and abundance per benthic group that characterise different zones along the tidal flat to the salt marsh gradient. Dots, jittered for clarity, indicate the individual measurements. Soil samples were taken 10 cm deep and species abundance is given per 246 cm^3 . Note: the vertical axes contain different scales.

Polychaetes were present in the tidal flat hollow samples (Fig. 2.4). Polychaetes contained individuals of *Nereidinae* (often juveniles, most common), *Streblospio benedicti*, *Aphelochaeta marioni*, *Eteone longi*, *Heteromastus filiformis*, and *Pygospio*

elegans and differed in zonal abundance (ANOVA, polychaetes, $F_{df=4}=10.7$, $p<0.001$). Mollusca were scarce and consisted of mostly *Hydrobia* sp. in the pioneer zone and a few juvenile mussels, *Mytilus edulis*, in the tidal flat hollows. Crustaceans were found in all zones in low abundance, consisting of calanoid copepods, amphipods, ostracoda, one *Crangon crangon* and one tidal flat hollows sample with 66 juvenile *Corophium volutator*. Molluscan and crustacean abundances did not differ significantly between zones (ANOVA, respectively, $F_{df=4}=1.5$, $p=0.21$ and $F_{df=4}=0.9$, $p=0.46$). Adult *C. volutator* and juvenile flatfish were observed in the tidal flat hollows but successfully avoided the sampling gear. Macrobenthos (>1 mm) were scarce throughout the study site. The total abundance of benthic animals differed between zones (ANOVA, $F_{df=4}=5.1$, $p=0.0014$) and was highest in the pioneer zone (Fig. 2.4).

Clustering and nMDS analysis

The cluster analysis for the abiotic environmental variables did not reflect the same five zones as classified in the field based on vegetation cover and topographic heterogeneity, i.e. hummock and hollows (Fig. 2.5). The cluster analysis indicated that the transition zone and tidal flat hummocks shared similar environmental conditions. Together with the cluster for tidal flat hollows, these two clusters were distinctly different from the two clusters of the vegetated zones (Fig. 2.5). The low marsh and pioneer zone were more alike and some low marsh samples were clustered with the pioneer zone.

For the benthic community, four clusters were distinguished. The clusters of the benthic community contained more variability regarding the predefined zonation than the clustering of environmental variables (Fig. 2.5). Generally, separate clusters were observed for the low marsh, the pioneer zone, the tidal flat hummocks and the tidal flat hollows. Part of the transition zone samples, mainly the northern transects, formed a cluster together with the tidal flat hollows. The other transition zone samples clustered with the tidal flat hummocks. Similar to the environmental variables, the benthic community of the transition zone predominantly clustered with the tidal flat hummocks (Fig. 2.5).

The clusters were largely confirmed by the nMDS analysis (Fig. 2.6). The first nMDS ordination axis was associated mostly with elevation and soil salinity. Low marsh, pioneer zone and tidal flat hollow samples followed a gradient along this axis. The transition and tidal flat hummocks were more spread out, they show some overlap and were not restricted on the first axis (Fig. 2.6). The second ordination axis was mainly influenced by dry bulk density (and thus, as negatively correlated, water content). The tidal flat hollows were clearly wettest and associated with polychaetes. The transition zone and tidal flat hummocks were drier and overlapping. The most common species occurring in these two zones were the collembolans.

The BioEnv analyses showed that the benthic community was best correlated with elevation, LOI and dry bulk density ($r=0.49$; Mantel test $p = 0.001$, Table 2.3). Elevation

Figure 2.5 Cluster analysis of abiotic environmental variables (left) and the benthic community (right). The dashed red boxes indicate the clustering. The predefined zonation is indicated in colour with transect number and zone abbreviation: low marsh dark green, LM; pioneer zone light green, Pio; transition zone orange, Tra; tidal flat hummocks brown, Hu; tidal flat hollows blue, Ho.

was the single variable that correlated best with the benthic community ($r=0.29$). Elevation was collinear with soil oxygenation thus the benthic community may influence or be influenced by soil oxygenation. In this set-up, it is not possible to discriminate between the elevation and oxygenation. This could (alone or in combination) influence suitability of an area for benthos and vegetation establishment.

Figure 2.6 nMDS ordination plot based on the benthic community of sampling stations, shown with coloured dots for the 5 zones. Dominant benthic groups are displayed in blue and an overlay of environmental variables is added to the graph in black. The stress was 0.146, indicating a good 2d-representation of the data.

DISCUSSION

Based on the cluster analysis of environmental variables, four zones were confirmed: low marsh, pioneer zone, tidal flat hollows and a combined zone consisting of the transition zone and the tidal flat hummocks. The benthic community formed four general clusters, with the benthic community of the transition zone partially overlapping with the tidal flat hummocks and partially with the tidal flat hollows. Strong shifts in abiotic variables, such as soil oxygenation, the benthic community and seed abundance implicate that there is a distinct threshold between two alternative stable states: the vegetated parts (low marsh and pioneer zone) and the sparsely or un-vegetated parts (transition zone and tidal flat). The waterlogged hollows on the tidal flat seemed to form a separate habitat located within the tidal flat.

Seed bank partly reflects vegetation community

The aboveground vegetation was not wholly reflected by the seed bank (Tables 2.1 and 2.2) which is not uncommon for marsh areas (Wolters & Bakker 2002; Erfanzadeh

Environmental variables	Used in cluster and nMDS	Significant in BioEnv
Elevation (m NAP)	Yes	Yes
Soil oxygenation depth (mm)	* collinear elevation	NA
Soil salinity (‰)	Yes	No
Dry bulk density (g cm ⁻³)	Yes	Yes
Mass water content (%)	collinear dry BD	NA
Mud content (% < 63 µm)	Yes	No
d50 (µm)	* collinear mud content	NA
Loss on ignition (%)	Yes	Yes
Microphytobenthos (µg cm ⁻²)	Yes	No

Table 2.3 Variables used in nMDS analyses and significance in BioEnv. Stars (*) indicate variables not included in the nMDS analysis due to collinearity.

et al. 2010b). Seeds were disproportionally more abundant in the low marsh. This could indicate that net seed transport from the pioneer zone is predominantly towards the low marsh (Fig. 2.2). Landward transport of seeds from the pioneer zone has been observed to be the dominant direction in a marsh in the Scheldt estuary (Huiskes et al. 1995). Additionally, seed retention is higher in denser vegetation (Chang et al. 2008), therefore more seeds are expected to be retained in the low marsh than in the pioneer zone or the nearly bare transition zone and intertidal flats.

Establishment of early pioneer colonizers such as *S. anglica* and *Salicornia* spp. can initiate marsh formation and thus expansion (Smit van der Waaij et al. 1995). However, the establishment of *S. anglica* from seeds seems unlikely, as only seven seeds were found in total. This low abundance of *S. anglica* seeds probably originates from the fungus *Claviceps purpurea* which invades the reproductive spikelets (Marks & Truscott 1985) and also happens at other European marshes (Wolters & Bakker 2002; Erfanzadeh et al. 2010b). *S. anglica* is able to expand clonally through rhizomes (Zhu et al. 2014) and this species was present in the pioneer zone despite the lack of seeds. *Salicornia* spp. seeds were present in all zones with the highest abundance in the pioneer zone, where the cover was likewise highest. In contrast, the transition zone contained on average less than one seed per sample. The viability of these seeds was not studied here but forms an additional potential threshold. A low abundance of *Salicornia* spp. seeds can cause marsh retreat in the Wadden Sea (Houwing et al. 1999). In this study, we found that a lack of seeds is likely to form an important limitation for vegetation establishment in the transition zone.

Changing environmental characteristics

Vegetation cover, seed abundance and elevation followed a gradient from salt marsh to tidal flat, which all were higher on the low marsh and decreased towards the intertidal flat. Surface soil salinity was significantly lower in the low marsh but no soil

salinity gradient was observed over the transects. Salinity is a well-known contributing factor of salt-marsh zonation (Elsey-Quirk, Middleton & Proffitt 2009; Erfanzadeh et al. 2010c; Davy et al. 2011). However, some parameters did not differ between zones. For example, the microphytobenthos cover was similar in all zones in May. Therefore, the tidal flat hummocks and hollows pattern is probably not explained by an increased seasonal diatom growth on the hummocks in spring as shown in the Western Scheldt area (Weerman et al. 2012). In our study site, the topographic pattern occurred throughout the entire year (Baptist et al. 2019b). Furthermore, mud content and median grain size (Supplementary Table 2.1) were similar in all zones. Other variables were distinctly different in specific zones. High water content and low dry bulk density in the tidal flat hollows were caused by standing water remaining after tidal inundation in the depressed areas of the tidal flats (Fig. 2.1). There was a sharp decline in soil oxygenation from pioneer to transition zone. This may be attributed to a combination of oxygenation through plant roots and bioturbation of oligochaetes (Lee 2003; van Regteren et al. 2017).

Changes in the benthic community

The environmental variables elevation (collinear with oxygenation), organic matter content and dry bulk density (collinear with water content) were best correlated to the benthic community ($r=0.49$). A gradient in abundance was only observed for the insects, which were most abundant at the low marsh and decreased quickly towards the tidal flat. The most common groups, oligochaetes, copepods, and collembolans were mainly confined to one or two specific zones (Fig. 2.4). The majority of oligochaetes was found in the pioneer zone. Oligochaetes are known for their ability to cope with anoxic soils (Mermillod-Blondin & Lemoine 2010) and their bioturbation capacity (van Regteren et al. 2017). In an earlier study, it was suggested that the increase in oligochaetes is related to grain size (van der Wal & Herman 2012). However, a decline in grain size at the vegetated marsh edge was not observed in our study: the median grain size was similar in all zones (D50 32–49 μm). The increase in density of oligochaetes in the vegetated area versus the bare flat is more often observed (Rader 1984; Whaley & Minello 2002; Bouma, Ortells & Ysebaert 2009; van der Wal & Herman 2012). Possibly, the evasion of predators such as a juvenile flatfish, dunlins and common shelducks may explain the lower density of oligochaetes outside of the pioneer zone (Quammen 1984; Reible et al. 1996; Seys, Vincx & Meire 1999).

Remarkably, the harpacticoid copepods occurred most frequently in the non-bordering pioneer zone and tidal flat hollows. The distinctive abundance may originate from species level, however, we cannot confirm this as in this study organisms were classified up to order level. Variation in harpacticoid copepods is considerable, up to 21 species in Dutch estuaries (van Damme, Heip & Willems 1984). Harpacticoid copepods found in muddy environments are capable of coping with soil anoxia and harpacticoids are generally able to increase soil oxygenation as well as oxygen fluxes (Bonaglia et al. 2014; Grego et al. 2014). Collembolans (*Archisotoma subbrucei*) also occurred most

frequently in two non-bordering zones, the transition zone and the tidal flat hummocks. This overlap is not surprising, as these zones were clustered in terms of environmental variables. The density of the benthic community has been linked to soil organic matter content, as their food supply. The range of organic matter content was between 0.3 and 13.1%, in North Carolina marshes (Sacco, Seneca & Wentworth 1994). In our study area, the range was between 9 and 43% (Fig. 2.3), so that organic matter as a food resource did not seem to be a limiting factor. This is supported by the total number of individuals found, which had a maximum of 69.203 individuals per m² in the North Carolina marshes versus a more than threefold of 243.120 individuals per m² in the Westhoek pioneer zone.

Polychaetes occurred only in the wettest sites, i.e. the tidal flat hollows. Macrofaunal species, apart from a few (juvenile) polychaetes, *C. volutator* and juvenile flatfish (observed but not sampled), were largely absent from this field site (Fig. 2.4). Wadden Sea broad sampling corroborates this observation (Compton et al. 2013a). Potentially, the mud content is too high and grain size too small for larger burrowing animals to prosper.

Many of the benthic organisms found have bioturbative capacity: harpacticoids, collembolans, polychaetes and oligochaetes (Christian 1989; Delefosse & Kristensen 2012; Bonaglia et al. 2014; van Regteren et al. 2017). Bioturbation during the seedling stage may reduce successful vegetation establishment (van Regteren et al. 2017). When the abundance of soil reworking organisms was summed up, the highest number of bioturbating individuals existed in the pioneer zone with on average 241.200 individuals per m². The lowest number of bioturbating individuals was found in the low marsh with on average 41.920 individuals per m². Bioturbation affects the biogeochemistry of the soil, increasing fluxes and stimulating mineralization of organic matter content (Nascimento, Näslund & Elmgren 2012; Bonaglia et al. 2014). Non-pioneer plant species favour oxygenated soils for establishment (Davy et al. 2011; Mossman et al. 2012) and therefore the presence of benthos may aid in creating boundary conditions appropriate for the establishment of successional vegetation. Bioturbation also reduces sediment stability directly through the mechanical reworking of the soil and indirectly by grazing on microphytobenthos. A lack of soil stability reduces the success of vegetation establishment (Houwing 2000). Whichever process is dominant: facilitation through increasing soil oxygen or harming seedling establishment by reduced soil stability will likely differ in time and space. Negative effects will dominate during the germination and seedling stage, whereas positive effects might dominate the rest of the year.

Marsh expansion potential

The transition zone did not fuse the characteristics of the pioneer zone and tidal flats but reflected the tidal flat hummocks in terms of environmental variables (Fig. 2.5 and 2.6). Soil oxygenation showed a sharp decline from pioneer to transition zone (Fig. 2.3). Anoxia may form a limitation for vegetation establishment (Mossman, Davy & Grant

2012), although both pioneers should be able to cope with anoxic environments as they have the capacity to oxygenate the soil through their root systems (Holmer, Gribsholt & Kristensen 2002; Lee 2003; Davy et al. 2011). Whether vegetation establishment follows after the soil is oxygenated, for example, due to bioturbating benthos, or that the plant roots itself oxygenate the soil remains uncertain (Koop-Jakobsen, Fischer & Wenzhofer 2017). Additionally, it remains difficult to tear apart the effect of elevation (and thus oxygenation and inundation) intertwined with soil anoxia (Mossman, Davy & Grant 2012).

The bed level of this area is very dynamic (Baptist et al. 2019a), which may limit vegetation establishment in the transition zone (Cao et al. 2018). For some tussock forming species such as *P. maritima*, *S. anglica* and *Bolboschoenus maritimus* this may not be detrimental as they spread vegetatively as well (Langlois, Bonis & Bouzille 2003; van der Wal et al. 2008; Silinski et al. 2016b). However, for small annuals, deposition of a layer of sediment can smother small seedlings. Thus, in dynamic areas, clonal development may be vital for permanent marsh succession (Silinski et al. 2016b), in addition to initial vegetation establishment from seeds.

Despite seeming to have the appropriate boundary conditions for vegetation establishment in terms of elevation (Wang & Temmerman 2013), a local seed source (Rand 2000) and appropriate inundation characteristics (Balke et al. 2016), the marsh has not expanded laterally in the last few years (Baptist et al. 2019a). Unfavourable soil conditions are often mentioned as an inhibiting factor for vegetation establishment (Wolters et al. 2008; Erfanzadeh et al. 2010a), although viable seed availability is probably a more prominent bottleneck (Erfanzadeh et al. 2010a). Our results showed that seeds were not present in the transition zone (Table 2.2). Even though mud content was high (Fig. 2.3) (Houwing 1999), erosion of the soil surface may have caused a loss of seeds. Apparently, seed availability did constitute a key limitation for vegetation establishment in the transition zone, combined with several environmental variables and changes in the benthic community that create a sharp border from pioneer zone to tidal flat.

CONCLUSION

The transition zone contained the main characteristics of the tidal flat, as shown by the clustering and nMDS analyses. Indicating that, although sparsely vegetated, the transition zone belongs to the stable state of the tidal flat. A distinct border was present between the tidal flat and pioneer zone, in both physical and biological characteristics mainly determined by oxygenation, the benthic community and seed availability. Benthic bioturbation might reduce establishment success but does not inhibit the establishment of pioneer plants entirely, as the highest density of bioturbators was found in the vegetated pioneer zone. Furthermore, bioturbation may enhance soil conditions for vegetation establishment through oxygenation, an increase of material fluxes and organic matter mineralization. Even though a seed source was in proximity, the main constraining factor for vegetation establishment in the transition zone was the

availability of seeds, which inhibited lateral marsh expansion. The shift to the vegetated stable state may be driven by chance events of seed retention in the transition zone and therefore lead to stepwise marsh expansion.

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SUPPLEMENTARY MATERIAL

Table S2.1 Environmental variables, mean $\pm$ sd for each of the 5 zones.

Environmental variables	Tidal flat hollows	Tidal flat hummocks	Transition zone	Pioneer zone	Low marsh
Elevation (m NAP)	0.93 $\pm$ 0.06	0.96 $\pm$ 0.05	1.04 $\pm$ 0.04	1.26 $\pm$ 0.11	1.47 $\pm$ 0.11
Soil oxygenation depth (mm)	12 $\pm$ 10	6 $\pm$ 4	31 $\pm$ 44	214 $\pm$ 59	215 $\pm$ 61
Soil salinity (‰)	30.0 $\pm$ 4.9	25.0 $\pm$ 2.3	29.0 $\pm$ 4.1	29.0 $\pm$ 6.8	13.9 $\pm$ 5.1
Dry bulk density (g cm ⁻³)	0.36 $\pm$ 0.06	0.71 $\pm$ 0.08	0.70 $\pm$ 0.07	0.61 $\pm$ 0.07	0.57 $\pm$ 0.09
Mass water content (%)	69 $\pm$ 4	47 $\pm$ 3	48 $\pm$ 4	52 $\pm$ 4	53 $\pm$ 5
Mud content (% < 63 μ m)	72 $\pm$ 14	70 $\pm$ 6	68 $\pm$ 8	68 $\pm$ 12	59 $\pm$ 11
d50 (μ m)	33 $\pm$ 26	34 $\pm$ 8	35 $\pm$ 12	37 $\pm$ 17	49 $\pm$ 18
Loss on ignition (%)	16.0 $\pm$ 2.0	13.5 $\pm$ 1.8	16.0 $\pm$ 1.1	16.7 $\pm$ 1.3	16.6 $\pm$ 1.9
Microphytobenthos (μ g cm ⁻²)	0.81 $\pm$ 0.88	0.58 $\pm$ 0.30	0.85 $\pm$ 1.26	0.76 $\pm$ 0.51	1.41 $\pm$ 1.15

INTERMEZZO

**Bed level dynamics of intertidal
flats and salt marshes at Westhoek
and Zwarte Haan, The Netherlands**

Marin van Regteren

This intermezzo describes the vertical sediment deposition and erosion patterns of the marsh and the preceding intertidal flats, in both Westhoek and Zwarte Haan. Furthermore, it shows the total surface area at salt marsh Westhoek in September, throughout the Mud Motor study period.

Bed level dynamics

The overarching project, in which the research of this thesis was conducted, is the Mud motor project introduced in Chapter 1. The aim of the Mud Motor project was to enhance lateral salt marsh expansion using strategic disposal of dredged sediment. Dredged sediment was disposed at the end of the Kimstergat channel during flood tide which is supposed to transport the sediment to the intertidal flats and salt marsh of Westhoek (Fig. I.1). The natural hydro-morphological processes are utilised to relocate the sediment, a building with nature approach (De Vriend et al. 2015). To monitor the effectivity of this approach, bed level dynamics were studied for four years (Sep 2015-Sep 2019). A salt marsh, Zwarte Haan, located northwards of Westhoek was used as a control site. Zwarte Haan is situated on the other side of the tidal divide of the Vlie inlet.

Figure I.1 The purple areas (K1 and K2) indicate the original disposal locations and the yellow, orange and red areas depict the experimental disposal location for, respectively, low, mean and high water, used during upcoming tide. The salt marsh lies between Westhoek and Koehool, the control site Zwarte Haan is situated northeast of Westhoek beyond the tidal divide. Note: reference system used is RD New.

At Westhoek, the Mud Motor experimental site, sedimentation and erosion bars (SEB) have been placed on the salt marsh in September 2015 to measure sediment deposition and erosion. SEB stations were placed in September 2016 on the intertidal flats at Westhoek and both intertidal flats and salt marsh at control site Zwarte Haan. SEBs are a frequently used method to measure sediment deposition and erosion in tidal marshes, with an accuracy of 1.5 mm (Nolte *et al.* 2013).

METHODS

SEB stations were clustered for salt marsh zones to acquire generalised zonal patterns in sediment dynamics. Zones were defined using species composition and cover adopted from Petersen *et al.* (2014), as follows: high marsh, low marsh, pioneer zone and intertidal flat. Westhoek contained 19 tidal flat stations, 5 pioneer stations, 9 low marsh stations and 8 high marsh stations. Zwarte Haan contained 8 tidal flat stations, 4 pioneer stations, 3 low marsh stations and 1 high marsh station. These SEB stations were measured 4 to 5 times per year, to cover seasonal variation. The consolidated surface layer of the soil was used for these measurements. Additionally, a soft and water-rich layer of fine silt, i.e. fluid mud layer, was deposited irregularly on the salt marsh and intertidal flat. Therefore, the thickness of the fluid mud layer was measured as well. For each zone, the average sediment deposition or erosion are used, with the average thickness of the fluid mud layer.

Aerial orthophotos of Westhoek were created by photogrammetry from aerial pictures taken by a drone, in four consecutive years in September. These aerial pictures were used to analyse the surface area of the marsh for each year. The differences in marsh surface area between years were used to indicate if the marsh expanded, retreated or remained stable.

RESULTS

The low and high marsh at Westhoek were steadily accreting the past four years (2015-2019, Fig. I.2). The low and high marsh accreted more sediment than the pioneer zone (Fig. I.2). Average sedimentation of all vegetated zones combined was 1.63 ± 1.05 cm year⁻¹ (average $\pm$ se). Elevation of the Westhoek tidal flat remained stable until winter 2018/2019 and then accreted (Fig. I.2). At Zwarte Haan, the control site, the low marsh steadily accreted, although at a lower rate than Westhoek (Fig. I.2). Furthermore, the pattern of sedimentation at the intertidal flat was similar between the two sites, bed elevation being stable until winter 2018/2019 and showing accretion in 2019. The main difference between the two areas was found in the pioneer zone. At Zwarte Haan pioneer zone, bed level erosion occurred whereas accretion occurred in the pioneer zone of Westhoek (Fig. I.2). The fluid mud layer could locally be as thick as 15 cm and varied between 0 and 15 cm. The fluid mud was usually present during autumn and

Figure I.2 Relative bed level elevation from the start of the measurement period for Westhoek (A) and from 2016 for Zwarte Haan (B). Grey areas indicate the consolidated soil, brown areas indicate the variable fluid mud layer. The pink shading displays the periods in which dredge disposal occurred. Note: in February 2018 it was not possible to measure the fluid mud layer due to frozen soil conditions. Adopted from Baptist et al. (2019b).

Table I.1 Total Westhoek salt marsh area in hectares per year and dredged sediment disposal in m³ during a certain time period. Obtained from aerial orthophoto analysis, adopted from Baptist et al. (2019b).

Year	Salt marsh area (Ha) in September	Sediment volume disposed (x 10 ³ m ³)	Period of disposal
2015	28.2	0	No disposal
2016	29.9	300	Sep '16 – Feb '17
2017	26.4	170	Sep '17 – Dec '17
2018	27.9	0	No disposal

winter. This layer was thickest and most frequently present on the intertidal flat and the pioneer zone. Although the deposition of the fluid mud layer is spatially and temporally variable, it appears to occur on the low and high marsh after it was first present at the intertidal flat and the pioneer zone, at Westhoek (Fig. I.2).

An increase in sediment deposition was not observed in years when dredged sediment was disposed, i.e. a nourishment, at the end of the Kimstergat channel (Sep-Feb 2016 and Sep-Dec 2017) and the years before or after. During the Mud Motor experimental period, one year with nourishments (Sep 2016 - Feb 2017) showed sediment deposition at both the salt marsh and the preceding intertidal flats. The second year with nourishments (Sep 2017 - Dec 2017), however, showed sediment erosion during the disposal period (Table I.2).

The marsh has not expanded laterally since the start of the Mud Motor project, although the vegetated marsh area has been accreting vertically (Table I.1 and I.2). The period in which the marsh retreated by 3.5 Ha coincided with the highest vertical accretion of both the marsh and the tidal flat (Table I.2). The subsequent year, the salt marsh expanded, 1.5 Ha while the tidal flat and all marsh zones apart from the high marsh showed vertical erosion. Despite a few exceptions, Westhoek and Zwarte Haan predominantly showed similar patterns in erosion and accretion and the amount of sediment dynamics throughout the measurement period (Fig. I.2 and Table I.2).

Table I.2 Average sedimentation or erosion $\pm$ se (cm) for each zone in Westhoek and Zwarte Haan. Years and zones with erosion or retreat are indicated in red, the dashed boxes indicate years with additional sediment disposal. Adapted from the underlying data used in Baptist et al. (2019b).

Year	Area	High Marsh	Low Marsh	Pioneer zone	Tidal Flat	Marsh Total	Marsh expansion or retreat (Ha)
2015-2016	WH	1.9 $\pm$ 0.8	2.3 $\pm$ 0.6	2.0 $\pm$ 0.4	NA	2.1 $\pm$ 0.4	+1.7
	ZH	NA	NA	NA	NA	NA	NA
2016-2017	WH	1.9 $\pm$ 0.5	3.9 $\pm$ 1.4	6.2 $\pm$ 1.3	5.9 $\pm$ 0.6	3.9 $\pm$ 0.7	- 3.5
	ZH	1.3 (n=1)	5.5 $\pm$ 1.7	3.0 $\pm$ 2.7	0.3 $\pm$ 0.8	3.7 $\pm$ 1.5	NA
2017-2018	WH	0.9 $\pm$ 1.6	-2.2 $\pm$ 1.7	-2.3 $\pm$ 2.1	-3.8 $\pm$ 0.6	-1.1 $\pm$ 1.0	+ 1.5
	ZH	-0.9 (n=1)	-5.4 $\pm$ 1.1	-4.2 $\pm$ 0.2	2.1 $\pm$ 1.3	-4.4 $\pm$ 0.9	NA
2018-2019	WH	2.7 $\pm$ 0.7	0.8 $\pm$ 1.0	-0.4 $\pm$ 0.5	2.6 $\pm$ 0.5	1.6 $\pm$ 0.6	NA
	ZH	0.8 (n=1)	2.3 $\pm$ 1.4	0.4 $\pm$ 2.3	4.4 $\pm$ 0.4	1.2 $\pm$ 1.2	NA

DISCUSSION

Sediment deposition and erosion patterns were quite similar at Westhoek and Zwarte Haan. This similarity between Westhoek and the control site Zwarte Haan can have two probable causes. The first, sediment dynamics at Westhoek were not influenced by the disposal of dredged sediment and thus showed natural patterns, as was expected at Zwarte Haan. The second, Zwarte Haan was not an appropriate control site and it has been influenced by the Mud Motor disposals as well. The tidal divide between Westhoek and Zwarte Haan may not be so strict as was expected beforehand.

Much of the dynamics of the bed level elevation could be explained by the presence of the fluid mud layer. Both areas showed a seasonal dynamic in the deposition and erosion of the fluid mud layer. This is likely to be influenced by wind forcing (speed and direction), which strongly affects sediment transport (Schulz & Gerkema 2018). Fluid mud was usually present in autumn and winter and then eroded or consolidated in spring and summer (Fig. I.2). Spring and summer are warmer and the tidal flats and marshes are less often inundated, which is favourable for consolidation of mud (Colosimo et al. in prep). Consolidation of the fluid mud layer would lead to more permanent vertical accretion. However, it was observed that a single storm was capable to erode a 10 cm thick layer of fluid mud within a day. At least some of the deposited fluid mud during winter has probably consolidated and contributed to bed level accretion since multiple salt marsh zones accreted in the majority of the years. The boundary conditions for tidal flat accretion probably occur less often due to the lower elevation.

At Westhoek, accretion occurred in one year while erosion occurred in the next year, during the two periods (Table I.1) in which dredge disposal took place. Both vertical and lateral accretion were expected to increase due to the dredge disposal nourishments in the Kimstergat channel (Baptist et al. 2019a). Whereas vertical accretion occurred in most zones and years (Table I.2), lateral expansion did not (Table I.1). We suspect that it is unlikely for the dredge disposals in the Kimstergat channel to affect the local sediment dynamics at Westhoek, considering the natural variation in sediment deposition and erosion measured before and after the nourishment periods and the presence of fluid mud in periods without sediment nourishments. As previously demonstrated for cliffed salt marshes (van der Wal & Pye 2004), we can conclude that vertical accretion does not necessarily ensure lateral expansion of the marsh (Table I.2). Furthermore, we deem it unlikely for the Wadden Sea area to be constrained by sediment availability for its salt-marsh development. What does constrain lateral salt-marsh expansion is discussed in the chapters of this thesis.

A 2 m by 2 m permanent quadrat for summerly vegetation assessment and the SEB pole for relative bed level change measurements, at the salt marsh Westhoek. *Spartina anglica* and flowering *Aster tripolium* are clearly visible (September 2017).

The Westhoek salt marsh completely submerged during a storm. Only the tips of the tall reed (*Phragmites australis*) are still emerged (September 2017).

CHAPTER 3

Limited seed retention during winter inhibits vegetation establishment in spring, affecting lateral marsh expansion capacity

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ABSTRACT

Coastal systems worldwide deliver vital ecosystem services, such as biodiversity, carbon sequestration and coastal protection. Effectivity of these ecosystem services increases when vegetation is present. Understanding the mechanisms behind vegetation establishment in bio-geomorphic systems is necessary to understand their ability to recover after erosive events and potential adaptations to climate change. In this study, we examined how seed availability affects vegetation establishment in the salt marsh-intertidal flat transition zone: the area with capacity for lateral marsh expansion. This requires vegetation establishment, therefore seed availability is essential. In a 6-month field experiment, we simulated a before and after winter seed dispersal at two locations, the salt-marsh vegetation edge and the intertidal flat and studied seed retention, the seed bank, and the seed viability of three pioneer marsh species: *Salicornia procumbens*, *Aster tripolium*, and *Spartina anglica*. During winter storm conditions, all supplied seeds eroded away with the sediment surface layer. After winter, supplied seeds from all three species were retained, mostly at the surface while 9% was bioturbated downwards. In the natural seed bank, *A. tripolium* and *S. anglica* were practically absent while *S. procumbens* occurred more frequently. The viability of *S. procumbens* seeds was highest at the surface, between 80% and 90%. The viability quickly decreased with depth, although viable *S. procumbens* seeds occurred up to 15 cm depth. Only when seeds were supplied after winter, many *S. procumbens* and some *S. anglica* individuals did establish successfully in the transition zone. Viable seed availability formed a vegetation establishment threshold, even with a local seed source. Our results suggest that, although boundary conditions such as elevation, inundation and weather conditions were appropriate for vegetation establishment in spring, the soil surface in winter can be so dynamic that it limits lateral marsh expansion. These insights can be used for designing effective nature-based coastal protection.

INTRODUCTION

Coastal areas and estuaries worldwide deliver vital ecosystem services, such as nursery areas for fish, carbon sequestration, food, habitat for unique species, recreation and flood protection (Harley et al. 2006; McLeod et al. 2011). Nature-based flood protection is most effective when vegetation is present and it increases with the width of the vegetated area (Barbier et al. 2008; Bouma et al. 2014). Expansion of natural vegetated barriers can occur in the transition zone in front of the system, through the establishment of pioneer vegetation. Due to erosion, pioneer plant cover exhibits cyclic behaviour of expansion and retreat (Balke et al. 2016). This cyclic pattern can be influenced by climate change in bio-geomorphic systems, such as mangroves (Balke et al. 2013), riparian environments (Karrenberg, Edwards & Kollmann 2002), dunes (Huiskes 1977), and salt marshes (Elsey-Quirk, Middleton & Proffitt 2009; Kirwan et al. 2016a). To predict the adaptability of estuarine bio-geomorphic systems to climate change and ecosystem recovery after erosion, it is necessary to understand the formation and development of these systems (Friedrichs & Perry 2001; Bouma et al. 2016). The last century, estuaries and coastal areas are suffering from depletion and degradation worldwide (Lotze et al. 2006), and 50% of salt marshes have been lost or degraded (Barbier et al. 2008). Essential requirements for the establishment of pioneer plants and expansion include a suitable habitat, appropriate environmental conditions and the availability of propagules, often seeds (Garbutt et al. 2006; Erfanzadeh et al. 2010a). Once seeds are produced, their availability will be determined by dispersal, retention, the seed bank and viability of the seeds (Rand 2000; Wolters, Garbutt & Bakker 2005a; Chang et al. 2008; Balke et al. 2011).

In salt marshes of North-Western Europe, plants generally have ripened seeds in autumn and reported dispersal periods range from autumn to winter (Hutchings & Russell 1989; Huiskes et al. 1995; Rand 2000; Wolters, Garbutt & Bakker 2005a). Seed dispersal in salt marshes is mostly local (Ellison 1987b; Rand 2000). The bare intertidal flats in front of the salt marsh may provide suitable areas for colonization of pioneer marsh plants, i.e. the initial step of lateral marsh expansion. However, seed availability can potentially form a bottleneck for pioneer vegetation establishment (Wolters, Garbutt & Bakker 2005a; Friess et al. 2012).

Once dispersed to a suitable habitat, seed retention is affected by environmental variables which ultimately control successful plant establishment. Factors shown to influence seed retention and the local seed bank are sedimentation (Balke et al. 2013) and erosion rates (Houwing 2000; Zhu et al. 2014), topographic heterogeneity (Chang et al. 2008) and bioturbating infauna (Delefosse & Kristensen 2012). The burial of seeds, through sedimentation or bioturbation, can increase seed retention, as they are protected against erosive forces under a layer of sediment (Zhu et al. 2014). However, these seeds may end up buried too deep in the soil (Erfanzadeh et al. 2010a), disabling their ability to meet their light requirement (Ungar & Riehl 1980) or their ability to reach the soil surface after sprouting in spring (Jurik, Wang & Vandervalk 1994). The availability of seeds closer to the surface is heavily reduced by erosion of the upper soil surface layer (Houwing 2000). The seeds at the surface are transported away by currents and waves (Groenendijk 1986; Huiskes et al. 1995). Both the temporal and spatial differences in

sediment dynamics in the field and their effect on the pre-seedling stage of salt-marsh pioneers remain under-studied at present.

It is possible that the retention of seeds in the soil may result in a persistent seed bank. Although seed banks have been recognized as key for the success of restoration and conservation efforts, research on riverine, estuarine and marine seed banks and related processes has remained sparse (Goodson et al. 2001). Currently, it is uncertain how dependent salt marshes are on a seed bank for lateral expansion (Bossuyt & Honnay 2008; Erfanzadeh et al. 2010a). Furthermore, a seed bank can only advance lateral expansion when seeds remain viable. Studies on viable seed availability in areas where vegetation expansion is prone to occur, such as the salt marsh to the intertidal flat transition zone, are scarce. A seed bank was found to be important for the annual *Salicornia europaea* in years with low seed abundance (Ungar 1987), but this plant species occurs higher up the marsh (Davy, Bishop & Costa 2001). The seed bank and its viability in the marsh transition zone remain to be examined on a deeper level.

The objective of this study is to examine whether seed availability can form a threshold for vegetation establishment in the transition zone even with a source population in proximity. These issues were addressed in a Wadden Sea salt marsh which has been naturally accreting and expanding the past three decades (Baptist et al. 2019b). Three frequently occurring species with discriminant seed size and growing seasons were used to account for variable life histories. In order to gain insight into potential lateral expansion of salt marshes in the transition zone, the specific aims were to assess 1) how seed retention is affected by dispersal time, before winter and after winter; 2) the abundance of seeds in the naturally present seed bank; 3) the viability of seeds retrieved from different depths; and 4) vegetation establishment success in the transition zone.

METHODS

Study area

The salt marsh at Zwarte Haan, The Netherlands (53°18'36N, 5°37'08E), was used as a model system. It is a naturally developing salt marsh in the Wadden Sea located along the Dutch main coast. Current habitat conditions, such as elevation, hydrodynamic regime and presence of a source population, are appropriate for the establishment of pioneer vegetation in the transition zone (Rand 2000; Wang & Temmerman 2013; Balke, Herman & Bouma 2014), giving us the opportunity to study essential processes that affect initial vegetation establishment. This tidal marsh is dominated by plants typical for pioneer, low and high marshes (Petersen et al. 2014). The focus lies on the pioneer species and their ability to colonize the preceding transition zone. Many of the pioneer species are annuals that reproduce by seed, *Salicornia* spp., *Suaeda maritima*, and *Atriplex portulacoides*. *Aster tripolium* is a biennial that also reproduces by seeds. *Spartina anglica* is a pioneer species that can spread vegetatively and through rhizomes as well as reproduce with seeds. In brackish marshes, *Scirpus maritimus* is a pioneer species

that can spread vegetatively, through rhizomes and with seeds (Silinski et al. 2016b). This pioneer zone is mostly dominated by the annual *Salicornia procumbens* (glasswort), patches of the perennial *Spartina anglica* (common cordgrass) and individuals of the biennial *Aster tripolium* (sea aster). As in other marshes (Egan & Ungar 2000b), seed-reproducing species are dominant in this pioneer zone. The astronomical tidal range is approximately 3 m during spring tide and 1.5 m during neap tide, and the elevation at the study site was approximately 1 m above NAP (Dutch Ordnance Datum). The mud content ($<63 \mu\text{m}$) of the sediment was around 68%. The bathymetry contained a gentle slope of 0.0035‰. On the nearby intertidal flat, Westhoek, located approximately 10 km South-West from our field site, sediment dynamics were extensively monitored from December 2017 until February 2018.

Seed-sediment mixture

To simulate seed dispersal, a seed-sediment mixture was created for the manual seed-additions (Table 3.1). This mixture consisted of sediment with a surplus of seeds from *S. procumbens*, *A. tripolium* and *S. anglica*. Seeds were collected in autumn 2017 to ensure that they were ripe, though not yet dispersed. *S. procumbens* and *A. tripolium* seeds were collected in the pioneer zone of Westhoek (53°25'03N, 6°22'49E). Dead standing biomass of *S. procumbens* containing seeds was cut into smaller branches. Additionally, individual seeds were added to the seed mixture, as *S. procumbens* is known to spread with branches holding seeds and individual seeds (Smit van der Waaij et al. 1995). Flower heads containing *A. tripolium* seeds were gathered at Westhoek. *S. anglica* seeds were collected at the Balgzand salt marsh (52°53'56N, 4°50'46E, The Netherlands) in October 2017, dried and loosened from their spike to obtain individual spikelets, mimicking its dispersal strategy. The plants were dried in a climate chamber with continuous ventilation for 14 days. Approximately 40 litres of sediment, 68% mud ($<63 \mu\text{m}$), was collected at the study site. A surplus of seeds was homogenously mixed into the sediment and divided into forty even portions.

Table 3.1 Seed characteristics, based on this study combined with data adapted from Rand (2000) and Wolters et al. (2004).

Species	Shape	Size (mm)	Surface	Dispersal
<i>S. procumbens</i>	Oval	1-2	Hairs	Water
<i>A. tripolium</i>	Elongated	5-7	Hairs, plume	Wind
<i>S. anglica</i>	Elongated	15-20	Smooth	Water

Experimental design

The field experiment lasted from December 2017 through May 2018. Two zones were included, the vegetation edge of the marsh pioneer zone, identified by the presence of dead standing material of *S. procumbens*, and the bare intertidal flat 10 m seawards in front of the vegetation edge, to assess expansion potential. Each

zone consisted of 10 replicate blocks with approximately 25 m between them, each containing three 1 x 1 m experimental plots (Fig. 3.1). These consisted of a control plot and two seed treatment plots. The seed treatments consisted of two temporally distinct manual seed-additions, to simulate different dispersal times. The first seed addition was conducted before winter, December 2017, the second seed-addition was executed after winter, March 2018. A control treatment was included to examine natural seed availability. The treatments will hereafter be referred to as control (no seed-addition), December (December seed-addition) and March (March seed-addition). Twenty seed mixtures were applied in a thin layer (± 1 mm thick) on the surface of 10 plots at the vegetation edge and 10 plots on the intertidal flat in December 2017. The remaining 20 mixtures were stored in a dark and cool environment (3°C) until the seed-addition in March 2018. No seeds were added to the 20 control plots. Final sediment cores were taken in May 2018 to assess seed depth distribution.

Figure 3.1 The experimental design of the field experiment. Two of the ten replicate blocks are displayed. Each block consisted of two locations: the intertidal flat and the vegetation edge. Each zone contained three 1 m x 1 m plots corresponding to control (C), December (D) and March (M) seed dispersal treatments. Three sediment cores were taken from each plot. One CTD-diver measured water levels for the duration of the experiment, a second CTD-diver located on the dyke in permanent dry conditions was used to correct for air-pressure variability.

Seed retention, seed bank and viability of seeds

To assess seed retention and the seed bank, in May 2018, three randomly placed sediment cores with 5.8 cm diameter were taken, up to 15 cm deep in each plot. The three sediment cores together covered a total surface area of 80 cm^2 . This resulted in 180 sediment cores in total, 60 cores for each treatment (control, December and March). Of these 60 cores, 30 cores were collected at the vegetation edge and 30 cores were collected at the intertidal flat to estimate location effects.

The top 5 cm of the soil was sliced into layers of 1 cm, the next 10 cm (5-15 cm deep) of soil was sliced into layers of 2 cm thick. This resulted in the following layers: 0-1, 1-2, 2-3, 3-4, 4-5, 5-7, 7-9, 9-11, 11-13 and 13-15 cm. In the statistical analysis, the centre of each layer is used to represent the depth. Each sediment layer was sieved over

a 500 μm mesh to filter out all seeds from the sediment. Everything larger than 500 μm was transferred to a transparent gridded tray, placed on a light table and analysed for seeds. For the surface layer (0-1 cm), the number of seedlings for each of the three plant species was recorded. In each succeeding layer, the number of seeds for each of the three plant species was recorded. Thereafter, all seeds were transferred to the climate chamber for germination to determine seed viability, following the germination protocol by Ter Heerdt et al. (1996).

Bed level elevation and water level monitoring

The surface elevation was measured at the four corners of each experimental plot using a RTK-DGPS. The averaged elevation of these four measurements was taken as the bed level elevation for each plot. The average elevation of all treatment plots at the vegetation edge was 1.00 ± 0.01 m above NAP and elevation of intertidal flat plots was 0.96 ± 0.01 m above NAP. The elevation measurement was repeated at the beginning of each treatment (i.e. December 2017 and March 2018) and at the end of the entire experiment (May 2018). The elevation of the December treatment plots was additionally measured in December 2017 and March 2018 and the elevation of the March treatment plots was measured in March 2018. One CTD-diver (Conductivity-Temperature-Depth meter, DI27x, Eijkelkamp Soil&Water) was installed to acquire water levels, providing information on the inundation conditions throughout the experiment. Another CTD was placed on top of the dyke to correct for the first CTD for local air-pressure variability.

At Westhoek, hydrodynamic forcing and bed-level changes were measured at four locations of the intertidal flat. The most elevated site was located about 400 m seaward of the vegetation edge at an elevation of 0.15 m NAP. The water level and bed level change at this location is used in this study to show the effect of two storms occurred during the winter experiment. The water level was measured continuously using a pressure sensor (OSS1-010-003C, Ocean Sensor Systems) at 10 Hz frequency, installed at 2 cm from the seabed. This pressure data was corrected for the air-pressure variability using a second CTD. The second CTD was installed at the measurement location, avoiding inundation of the instrument. The bed level data were collected using an ADV (Acoustic Doppler Velocimeter, Nortek) in burst mode, a method frequently used to measure sediment erosion and deposition (Andersen, Fredsoe & Pejrup 2007) and applicable for tidal marsh research (Nolte et al. 2013). It was installed ± 20 cm from the seabed and measured the distance between the probe and the seabed at the beginning and end of each 10 minute measurement interval, with an accuracy of ± 1 mm. Zwarte Haan and Westhoek are similar regarding bed material, i.e. grain size distribution (Boer 2015), the bathymetry, e.g. the slope of the bed in the intertidal zone and the geographical orientation of the coastline. These conditions suggest a similarity in the exposure to the hydrodynamic forcing. However, given the differences in bed elevation (1 m NAP at Zwarte Haan and 0.15 m NAP at Westhoek), the bed level variation at Westhoek was not used to extrapolate the bed level variation at Zwarte Haan. The bed level changes at Westhoek exemplify the effect of storm conditions on sediment erosion and deposition.

Data analyses

Three replicate sediment cores from each plot were pooled to obtain seed abundance data. For the top 5 cm, abundances were counted per 80 cm³, for the lower 10 cm abundances were counted per 160 cm³. For samples between 5 and 15 cm deep, abundances were estimated per 80 cm³, by dividing total abundance with the thickness of the layer (2 cm). Viability was calculated based on the fraction of the recovered seeds that germinated successfully in laboratory conditions. This was repeated for all treatment and depth combinations. Samples without seeds present were excluded from the viability analysis.

The elevation of the experimental plots at Zwarte Haan was used to estimate total bed-level change during and after winter. The high-resolution bed level change at Westhoek was calculated as the difference in distance to the bed from the instrument probe at the beginning and end of each measurement burst (10 min frequency). The measurements of bed level change and water level were then averaged over, respectively, 1 h and 10 minute intervals.

The abundance of *A. tripolium* and *S. anglica* seeds in the control and December treatments as well as below 3 cm in the March treatment was insufficient for statistical analyses. The results of *A. tripolium* and *S. anglica* seed abundance will, however, be presented and qualitatively discussed. Analyses on seed viability and depth distribution of the seed bank were performed on *S. procumbens*.

Firstly, seed abundance was analyzed with a Generalized Additive Model (GAM) (Wood 2006). GAM was selected as the modelling instrument as the effect of depth was not linear. Furthermore, a GAM was applied to smoothen expected autocorrelation between depth layers. The fixed factors were: smoothed depth (interaction with treatment), location and treatment. Block was considered to be a random effect (Wood 2008). The smooth function used penalized regression splines with 5 knots, where the number of knots reflects the degrees of freedom (i.e., the flexibility of the curve) required by the spline (Wood 2008). An offset was used to correct for varying sample thickness at different depths. Because a Poisson distribution resulted in an overdispersed fit, the data was analyzed with a log-linked negative binomial distribution. To correct for the sampled depth layer thickness, both model outcome and observed counts were eventually expressed in numbers per 80 cm³ soil.

Next, viability, expressed as the fraction of successfully germinated seeds/seedlings of the total seeds, was analyzed using a GAM with a binomial distribution with a logit link function. The fixed factors were: smoothed depth (interaction with treatment), location and treatment. The block was again considered to be a random effect. The smooth function used penalized regression splines with 8 knots. Only sampled depth layers with *S. procumbens* seeds present were used for the viability analysis.

Finally, seedling establishment was analyzed with a Generalized Least Squares (GLS) to allow for unequal variances (heteroscedasticity) (Pinheiro and Bates 2000). The

fixed factors were: species, location and weighted treatment. Block was considered to be a random effect.

All confidence intervals were plotted as $1.96 \times \text{standard error}$ (i.e. 95% confidence). Likelihood Ratio tests (LR) were used to assess the significance of individual factors. Model assumptions were assessed graphically (Zuur & Ieno 2016). All statistical analyses were performed with the statistical program R (R Core Team 2015) using additional packages: 'ggplot2' for plotting (Wickham 2009), 'nlme' and 'lme4' for linear mixed effect models (Bates et al. 2015; Pinheiro et al. 2018), and 'mgcv' for GAM models (Wood 2011).

RESULTS

Seed retention

In May, the total abundance of seeds retrieved was similar for the December treatment (272 seeds) and control (233 seeds), while the March treatment was much higher (3755 seeds). For the March treatment, the highest abundances of seeds for all three species were found in the top 2 cm of the surface layer (Fig. 3.2). Most of these seeds belonged to *S. procumbens* (Fig. 3.2). Retention of *S. procumbens* seeds was affected by dispersal time (Fig. 3.3 and Table 3.2). Seeds manually added before winter (December treatment) were not retained. Seeds manually added after winter (March treatment) were retained until the following establishment period (May).

At the surface, retention of *S. procumbens* seeds was similar within the transition zone. However, deeper in the soil (4–9 cm), natural *S. procumbens* abundance differed between the vegetation edge and the intertidal flat (Fig. 3.3). The average abundance of seeds between 4 and 9 cm was higher at the vegetation edge than at the intertidal flat (Fig. 3.3).

The seed bank

The control treatment provided information on the naturally occurring seed bank. For *S. anglica*, no seed bank was present: nearly all *S. anglica* seeds found came from the March seed-addition treatment (0 from the control, 3 from December and 386 from March). In the March treatment, *S. anglica* seeds were most abundant in the surface layers, 0–1 cm (361 seeds) and 1–2 cm layer (21 seeds).

A. tripolium seeds occurred naturally in the transition zone (18 from the control, 21 from December and 766 from March) and seeds were found up to 13 cm deep. For the March treatment, *A. tripolium* seeds, similar to *S. anglica*, were most abundant in the surface layer (676 seeds) and 1–2 cm (59 seeds) layer.

Figure 3.2 Seed abundance (log-scale on the horizontal axis) along depth for three seed dispersal treatments (columns): control, December and March; and three pioneer species (rows): *S. procumbens*, *A. tripolium* and *S. anglica*. Means with 95% confidence intervals (CI) are indicated, pioneer and intertidal flat data were combined for brevity, n=60.

S. procumbens had a higher natural abundance of seeds than *S. anglica* and *A. tripolium* (233 from the control, 279 from December and 3755 from March). *S. procumbens* seeds in the March treatment were, like *A. tripolium* and *S. anglica*, most abundant in the surface layer (3213 seeds) and at 1-2 cm (247 seeds) (Fig. 3.2). In the control as well as in both treatments, *S. procumbens* seeds were found at all depths. The highest natural abundance occurred at depths between 4 and 9 cm. Abundances were slightly but significantly higher at the vegetation edge than on the intertidal flat (Fig. 3.3, Table 3.2). *S. procumbens* seed abundance was significantly higher in the March treatment in the upper 3 layers (0-3 cm) (Fig. 3.3).

Figure 3.3 *S. procumbens* seed abundance of the vegetation edge and the intertidal flat with 95% CI for three seed dispersal treatments along depth, as predicted from the GAM model. Jittered dots indicate individual samples (log-scale on the horizontal axis), n=30.

Seed viability

Natural seed abundance for *S. anglica* was low; in the control treatment, *S. anglica* was absent from all depths. In the December treatment, three seeds were found of which one germinated. For the March treatment, 10% of the 386 *S. anglica* seeds were viable. Viability of *A. tripolium* was naturally low, of the seeds found in the control treatment, one out of 18 germinated. In the December treatment, 21 *A. tripolium* seeds were found, of which none germinated. For the March treatment, 6% of the 766 *A. tripolium* seeds were viable.

The viability of *S. procumbens* seeds decreased over depth and differed between treatments (Depth: $LR_{df=1}=255$, $p<0.0001$; Treatment: $LR_{df=2}=94.15$, $p<0.0001$, Fig. 3.4 and Appendix Fig. 3.1). Seed viability decreased more rapidly with depth in the control and December treatments than in the March treatment (Fig. 3.4). Apart from the surface layer, viability was higher up to 6 cm deep in the March treatment (Fig. 3.4). Viability of seeds was similar at the vegetation edge and the intertidal flat (Table 2). Viability was highest (80% and 90%) in the surface layer (0-1 cm) (Fig. 3.4 and Supplementary Fig. S3.1). Viable *S. procumbens* seeds were found throughout the 15 cm of soil sampled. In the deepest sampled layers, below 9 cm, the uncertainty around the predicted viability of *S. procumbens* seeds becomes larger due to lower seed abundance (Fig. 3.4, supplementary material Fig. S3.1 and Table 3.3).

Figure 3.4 The proportion of viable *S. procumbens* seeds of two seed dispersal treatments and the control along depth with the 95% confidence interval, as predicted from the GAM model.

Table 3.2 Diagnostics of Generalized Additive Models of abundance and viability analyses. Estimates presented with standard error, $s(\text{depth})$ indicates the fitting of a smoothing function and edf are the degrees of freedom used by the smooth.

Abundance <i>S. procumbens</i> seeds			Adj. R^2 0.67
Parametric coefficients	Estimates	z-value	p
Intercept	-1.64 $\pm$ 0.16	-10.32	2.00*10 ⁻¹⁶
December treatment	0.007 $\pm$ 0.19	0.034	0.97
March treatment	1.47 $\pm$ 0.17	8.68	2.00*10 ⁻¹⁶
Location	-0.41 $\pm$ 0.13	-3.08	0.002
Smooth terms	edf	Chi sq.	p
Random effect block	4.5	8.7	0.041
$s(\text{depth})$:Control	3.4	48.8	1.41*10 ⁻⁹
$s(\text{depth})$:December	3.5	49.9	8.07*10 ⁻¹⁰
$s(\text{depth})$:March	3.8	376.0	2.00*10 ⁻¹⁶
Viability <i>S. procumbens</i> seeds			Adj. R^2 0.77
Parametric coefficients	Estimates	z-value	p
Intercept	-2.33 $\pm$ 0.54	-4.29	1.79*10 ⁻⁵
December treatment	0.87 $\pm$ 0.61	1.43	0.15
March treatment	1.95 $\pm$ 0.55	3.51	4.36*10 ⁻⁴
Location	0.04 $\pm$ 0.08	0.48	0.63
Smooth terms	edf	Chi sq.	p
Random effect block	6.9	29.6	1.45*10 ⁻⁵
$s(\text{depth})$:Control	3.4	34.1	5.41*10 ⁻⁶
$s(\text{depth})$:December	3.5	32.4	8.60*10 ⁻⁷
$s(\text{depth})$:March	1.7	211.5	2.00*10 ⁻¹⁶

Germination of seedlings

When sampling in May, seedlings had established in the field (Fig. 3.5). The abundance of seedlings was significantly affected by both treatment and species (GLS, Treatment: $LR_{df=2}=23.9$, $p<0.0001$; Species: $LR_{df=2}=42.0$, $p<0.0001$). There were no *A. tripolium* seedlings in the field. *S. anglica* seedlings were present solely in the March treatment (Fig. 3.5). *S. procumbens* seedlings were present in similar abundances in

Table 3.3 The total number of seeds found in every depth * treatment combination, the number of germinated seeds and the number of samples that contained seeds (max. 60).

cm depth	Total seeds			Germinated seeds			In n samples		
	Total seeds	Germinated seeds	In n samples	Total seeds	Germinated seeds	In n samples	Total seeds	Germinated seeds	In n samples
0-1	11	10	10	18	15	10	3213	2569	60
1-2	6	1	5	3	2	2	247	199	36
2-3	4	0	4	9	1	6	37	25	18
3-4	7	0	6	11	0	4	16	12	9
4-5	40	2	12	26	2	13	54	13	18
5-7	77	3	27	84	6	25	61	29	23
7-9	61	11	23	98	16	28	71	18	26
9-11	17	4	11	24	2	15	31	7	18
11-13	6	0	4	4	2	4	17	1	8
13-15	4	0	3	2	0	2	8	2	3

the control and December treatment, while the number of germinated *S. procumbens* seedlings was significantly higher in March treatment (Fig. 3.5). The relative proportion of seeds added to the experimental plots was 15:5:1 for, respectively, *S. procumbens*, *A. tripolium* and *S. anglica*. Location (vegetation edge vs intertidal flat) had no significant effect on the number of established seedlings (GLS, Location: $LR_{df=1}=0.0008$, $p=0.98$).

Figure 3.5 The abundance of established seedlings for three pioneer salt marsh species, for control, December and March treatments. Boxplots with individual plots depicted as dots, $n=60$. Vegetation edge and intertidal flat data were combined since seedling establishment was similar throughout the transition zone.

Bed level elevation and water level

The RTK-DGPS measurements showed that on average, the elevation of the experimental plots eroded with 10.9 ± 6.1 mm, from December to March, whereas the plots experienced slight sedimentation 4.4 ± 13.4 mm, from March to May. The erosion of ± 10 mm at each plot resulted in the loss of seeds supplied in the December treatment, as the seed-sediment mixture was 1 mm thick.

Information about bed level variation during the experiment could not be derived from bed level elevation measured at the experimental plots at the start and end of the experiment. To elucidate on bed level variation, high-resolution measurements of the Westhoek tidal flat were used. On January 3rd, a storm with wind speeds exceeding 10 m/s from the South-West generated waves with significant wave height up to 1 m. This resulted in a substantial water-level set-up, with a high-water level of 2.5 m NAP (i.e. 1.5 m set-up). The storm lasted for 24 h, i.e. two tidal cycles. The water level during low tide was 1 m NAP (coinciding with the mean high tide level of this zone), meaning

that the experimental plots, not usually submerged extended periods, were continuously inundated for 24 h. Exemplifying the consequences of such a storm, the bed level change and water level data obtained at Westhoek intertidal flat are shown in figure 6. Higher water levels occurred due to wind-induced water level set-up, resulting in bed erosion. This storm was followed by two weeks of calm weather, which coincided with neap tide. These conditions are favourable for sediment accretion (Fig. 3.6). The fresh material deposited during this period was easily eroded during a second storm (January 16th, Fig. 3.6).

Figure 3.6 The water level (blue) and the relative bed level (brown) from the start of the experiment until February 13. Two storm events with high water levels in January (3rd and 16th) caused sediment surface erosion. A calm period in between storms resulted in sediment accretion.

DISCUSSION

Seed retention: temporal and spatial patterns

One of our aims was to assess seed retention with different simulated dispersal times in the transition zone. Salt-marsh pioneer species generally ripen and disperse their seeds in autumn-winter (September to December) in the Northern hemisphere (Hutchings & Russell 1989; Huiskes et al. 1995; Rand 2000; Wolters, Garbutt & Bakker 2005a). However, each species has its own dispersal window. *S. anglica* has peak seed dispersal times in October and December (Huiskes et al. 1995). *S. anglica* seeds still disperse up to march, with the arrival of seeds primarily in January (Zhu et al. 2014). *Salicornia* spp. seeds disperse from October to December, although the months January to spring were not measured (Hutchings & Russell 1989; Huiskes et al. 1995; Wolters, Garbutt & Bakker 2005a). *A. tripolium* disperses from September to December (Hutchings & Russell 1989; Huiskes et al. 1995; Wolters, Garbutt & Bakker 2005a). Other common marsh species, such as *Scirpus maritimus*, *Suaeda maritima*, *Atriplex* spp. and *Puccinellia maritima*, mainly disperse their seeds between September and October (Hutchings & Russell 1989; Huiskes et al. 1995; Rand 2000; Wolters et al. 2004; Espinar, Thompson & Garcia 2005). On higher elevated marshes, storms have been identified to have a significant influence on seed rain, with year to year variation in peaks, varying from March to December (Chang, Veeneklaas & Bakker 2007). Summarizing, seed dispersal mainly occurs between September and December but most studies do not monitor after De-

ember (exceptions: Zhu et al. 2014 who look at *S. anglica* specifically and Chang et al. 2007 who focused on the inner marsh). This is an important knowledge gap in salt marsh research. We showed that dispersal time is crucial in seed retention. Seeds present in December suffered seed loss due to sediment erosion, but seeds, dispersed after winter, were retained (Fig. 3.2). Houwing (2000) suggested that such a seed loss occurs with a high sand/mud ratio, because sandy sediments have a lower erosion threshold and therefore seeds disperse away more easily together with the sediment (Houwing 1999; Houwing et al. 1999). Our study area has a high mud content, 68%, and a loss of seeds due to soil surface erosion was still found during winter, thus grain size alone cannot explain the loss of seeds. Although the three species studied each have different seed size and characteristics, the retention patterns were similar for all (Fig. 3.2 and Table 3.1). This implies that also seeds of other marsh species are likely to suffer from the bed elevation sediment dynamics in transition zones.

At Westhoek intertidal flat, spring tides in combination with high wind speeds caused sediment erosion of the upper 1.5-2 cm of the bed (Fig. 3.6). The calm period that followed the erosion event resulted in sediment deposition, indicating that the cumulative bed level change in the period was much higher. Although we cannot predict the magnitude of the bed level change at Zwarte Haan, we expect that a similar dynamic occurred at this tidal flat, with erosion following from storm conditions and sedimentation occurring in between storms. Multiple storms occurred during the winter months which are likely to cause sediment erosion of the seabed around mean high water, i.e. our experimental location (Janssen-Stelder 2000; Friedrichs 2011). At the experimental site (± 1 m NAP), CTD data showed that water level reached 1.5 m above the sediment surface. Waves of 1 m on 1.5 m water depth cause wave breaking and thus erosion (Friedrichs 2011). The storm of January 3rd lasted for 24 hours, i.e. two tidal cycles, implying an unusual submergence duration of the site which may result in a decrease in critical shear stress for erosion (Colosimo et al. in prep.). We, therefore, expect that the bed level variation was larger than the 1 cm erosion measured with the RTK-DGPS during winter. Currents can lead to seed burial and thus to retention as shown in flume studies (Zhu et al. 2016b), however, in combination with tides and waves it can also lead to seed loss (this study). A dynamic regime can significantly affect seeds transport, i.e. burial and loss of seeds, and therefore seed retention and seed bank formation. Additionally, in dynamic areas, measuring elevation before and after experiments may not suffice as bed-level variation in between could be much greater than the net difference in bed-level elevation. The sediment fluxes in these shallow intertidal areas are significantly influenced by wind speed and direction, even in moderate wind conditions (Colosimo et al. in prep.). Consequently, if plants have peak seed dispersal before winter, the risk of seed loss increases. Subsequently, low availability of seeds in the consecutive spring thus limits vegetation establishment. Zhu et al. (2014) show dispersal of *S. anglica* up until march, which may form an adaptation for seed persistence in dynamic systems. Further studies on seed dispersal and retention in the transition zone are still lacking but would greatly contribute to salt marsh restoration and conservation purposes.

Depth was a significant factor in seed retention and vertical seed distribution. Seed retention was similar at the vegetation edge and intertidal flat plots. For the March

treatment, most supplied seeds, of all species, were present in the surface layer of the sediment (Fig. 3.2). In the 1-2 cm layer (March), *S. anglica* and *A. tripolium* seeds were also present in significantly higher abundances than in the control. *S. procumbens* was present, up to 3 cm deep (March) in significantly higher abundances than the control (Fig. 3.3). We hypothesize that downward transport of these seeds into the soil occurred through benthic bioturbation (Zhu et al. 2016a; van Regteren et al. 2017). Burial of seeds through bioturbation may increase seed retention and create a mechanism for seed bank formation. Superficially buried seeds may aid vegetation establishment in dynamic areas (Ungar 1987; Goodson et al. 2001; Zhu et al. 2014).

The seed bank

Because seed banks are a key component in restoration and conservation efforts (Goodson et al. 2001), we investigated the naturally present seed bank in the transition zone. This was remarkably different for the three species. *S. anglica* was nearly absent (3 seeds in total) from the entire transition zone. A likely culprit is the fungus *Claviceps purpurea*, which invades the spikelets and impairs the development of *S. anglica* seeds (Boestfleisch et al. 2015). Additionally, the longevity of *S. anglica* seeds is known to be generally low (Wolters & Bakker 2002), while floating times are relatively long (days) (Koutstaal, Markusse & De Munck 1987). Natural dispersal of *S. anglica* seeds may, therefore, be away from the transition zone. This is corroborated by Zhu et al. (2014), that state *S. anglica* to be dependent on seeds for long-distance dispersal. *A. tripolium* seeds were sparse in both pioneer and intertidal flat zone. A greater dispersal range is expected because *A. tripolium* is mainly wind-dispersed (Rand 2000). *A. tripolium* can produce many flowers and thus seeds per plant (Nolte, Esselink & Bakker 2013), which may compensate for its low viability.

S. procumbens seeds were present throughout the entire 15 cm of soil. Remarkably, there was an increase in seed abundance between 4-9 cm subsurface. This was consistent in all treatments. These seeds may have been retained and added to the natural seed bank through burial in previous successful years by sedimentation or bioturbation. Additionally, *S. procumbens* seeds occurred in slightly (but significantly) higher abundances at 4-9 cm deep in the vegetation edge than on the intertidal flat. This location effect was not visible in the upper or deeper layers of the soil. The increased seed abundance at the vegetation edge may have originated in years where high reproduction success coincided with local dispersal and less secondary dispersal. So, we can conclude that a seed bank was present for *S. procumbens*, the most important pioneer species in the transition zone. Such a seed bank may be more crucial in dynamic areas, where it influences the local establishment success of the species (Ungar 1987). However, these *S. procumbens* seeds do not germinate when buried below 1 cm (Huiskes et al. 1985). Thus, for *S. procumbens* to benefit from the seed bank for vegetation establishment, they must occur in the upper layer or come to the sediment surface by sediment erosion or crack propagation (Burmeier et al. 2010).

Seed banks in transitions zones are not often sampled. It would aid our understanding of vegetation retreat or expansion if future research would focus on the

study of the available seed bank and its viability in transition zones. Experimental seed-additions in these areas will reveal if vegetation establishment is limited by windows of opportunity (Balke et al. 2011) or the availability of seeds (Erfanzadeh et al. 2010a; Ma, Zhou & Du 2011).

Seed viability

To determine the expansion potential of the salt marsh into the transition zone, we examined the seed viability of both naturally occurring and supplied seeds. For *S. anglica* and *A. tripolium*, viability estimation for naturally occurring seeds was impossible, because abundance was too low. The March treatment did contain sufficient seeds, showing that for *S. anglica*, overall viability was 10%. This corresponds to estimates found in literature stating that many *S. anglica* spikelets are not filled and therefore cannot germinate and that the presence of the fungus *C. purpurea* lowers viability (Marks & Truscott 1985). *A. tripolium* viability was even lower at 6% overall, and only one of the naturally occurring seeds was viable. For perennial species, such as *S. anglica* and *S. maritimus*, a low viability is less of an issue due to rhizomatous and vegetative spread (Zhu et al. 2014; Silinski et al. 2016b). For annual and biennial species, a low viability may be compensated by producing large amounts of seeds, to ascertain successful vegetation establishment.

Viability was highest for *S. procumbens*: between 80% and 90% at the surface layer. This was consistent for naturally occurring and supplied seeds (Fig. 3.5 and table 3.3). In the natural seed bank viability dropped below 1 cm, except for the March treatment, where viability stayed above 50% up to 4 cm deep. A higher seed abundance with a higher viability below 1 cm found in the March treatment (Table 3.3 and Supplementary Fig. S3.1), is an indication that these seeds originated from the experimental seed-addition. They have moved downwards into the soil, most likely through bioturbation (van Regteren et al. 2017). Natural *S. procumbens* seed abundance increased at a depth of 4–9 cm, while viability remained low (between 10% and 40% viability). This indicates that these seeds are older (Espinar, Thompson & Garcia 2005), although most studies do not report on viability in relation to seed age (Wolters & Bakker 2002). Viability of marsh seeds can decrease under anoxic conditions (Mossman et al. 2012) and these conditions are quite common below the soil surface in the transition zone (personal observation). Ultimately, we can conclude that there was a seed bank present for *S. procumbens* in the transition zone and it contained a portion of viable seeds. This dormancy in *Salicornia* seeds may provide a survival tactic for less fruitful years (Davy, Bishop & Costa 2001).

Vegetation establishment and implications for management

Boundary conditions for *S. anglica* and *S. procumbens* establishment are appropriate at this location. In May, seedlings had established, with the highest cover

in the March seed-addition plots (Fig. 3.5). *A. tripolium* did not successfully establish (0%, Fig. 3.5), most likely due to a combination of factors. Viability was low with 6% and because it usually occurs in the lower marsh zone (Petersen et al. 2014) and the soil conditions in the transition zone may have been unfavourable (Erfanzadeh et al. 2010a). Although the number of seeds was not supplied evenly for the three species, the percentage of successful establishment was similar for *S. anglica* and *S. procumbens*. Total establishment success in the field was around 8% for *S. anglica* and 9% for *S. procumbens*. Despite *S. anglica* germination and establishment in the transition zone, natural *S. anglica* establishment from seeds is improbable, as no seed bank is present. Supplying *S. anglica* seeds in transition zones would likely lead to increased abundance in areas that cannot be reached clonally. Once established, *S. anglica* can reproduce vegetatively (through rhizomes) thereby inducing marsh succession, as seen in other marshes and with other perennials, typical for low and high marshes.

In the March seed-addition plots, *S. procumbens* seedlings were abundant. They also showed natural establishment in the control and December treatment (Fig. 3.5). The establishment mainly depended on seeds present in the surface layer. Only a few *S. procumbens* seeds were present in the surface layer of the control plots or December treatment but viability was high (Table 3.3). We suspect that those seeds naturally dispersed to our experimental plots after winter sediment erosion events took place. Possible adaptations for highly dynamic areas could be keeping seeds in dead stands until spring (observed in the field) inducing a prolonged dispersal period. Vegetation at the edge of the pioneer zone would benefit from a slow release of seeds. Additionally, seed viability was highest in more recent seeds, and thus the greatest potential for expansion comes from seeds dispersed in the previous fall. The period from January to spring still forms a knowledge gap in terms of seed dispersal and retention for most marsh species. When aiming to stimulate initial vegetation establishment, further research on seed dispersal times and retention is essential for success.

Annual pioneers produce many seeds and are more dominant than perennials when colonizing new areas or the bare intertidal flat (Bossuyt & Honnay 2008; Erfanzadeh et al. 2010c). For marsh expansion or marsh restoration, annuals will likely precede the establishment of perennials. Their initial establishment can aid subsequent marsh succession. Perennial pioneers, such as *S. anglica* and *S. maritimus*, are able to expand clonally onto the intertidal flat forming a slower but stable base. For longer distance dispersal and establishment, seeds are a vital component even for perennials (Zhu et al. 2014).

Salt marshes are excellent examples of nature-based coastal defence (Temmerman et al. 2013) and their development can be actively promoted (Baptist et al. 2019a). We showed that seed availability can form a threshold for pioneer vegetation establishment, even with a source population in proximity. Successful management strategies aiming to promote salt-marsh expansion should ensure the appropriate boundary conditions regarding seed availability, elevation and sediment dynamics are met. They should take into account that natural seed availability may be reduced by secondary dispersal due to storms, particularly since higher storm frequencies are

expected due to climate change (Woth, Weisse & von Storch 2006). More frequent storms, combined with expected sea-level rise, will alter hydrodynamic regimes (Kirwan et al. 2010) and can thus further constrain lateral expansion and even decrease vegetated areas through cliff erosion (van der Wal & Pye 2004). Seed availability is especially vital in highly dynamic transition zones, not only for salt marshes but also on the edges of mangroves or along vegetated riverbanks. If seed availability is a constraining factor, coastal management could consider, shortly before the establishment period, supplying seeds to strengthen natural barriers.

CONCLUSIONS

Overall, this study contributes to an advanced understanding of seed availability in the salt marsh-intertidal flat transition zone and how this can affect lateral expansion dynamics of salt marshes. Our results showed that: (1) seed retention was successful with seed dispersal after winter, but unsuccessful with seed dispersal before winter due to sediment erosion during winter storms; (2) For *S. anglica* and *A. tripolium*, a natural viable seed bank was hardly present, for *S. procumbens* a seed bank existed up to 15 cm depth; (3) Seeds from *S. anglica* and *A. tripolium* had low viability, respectively 10% and 6%, whereas seeds from *S. procumbens* had a high viability, around 85% in the surface layer, and viability decreased with soil depth; (4) In the field, windows of opportunity for vegetation establishment were met for *S. procumbens* and *S. anglica* but not for *A. tripolium*. Low glasswort seed availability resulted in low seedling establishment for *S. procumbens*, and the absence of cordgrass seeds resulted in a lack of natural seedling establishment of *S. anglica*. However, for *A. tripolium* natural establishment was inhibited due to low viability of seeds and probably inappropriate boundary conditions. In transition areas where high dynamic sedimentary regimes are prone to occur, seed availability should be considered as a potential threshold for initial vegetation establishment.

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SUPPLEMENTARY MATERIAL

Figure S3.1 Percentage of viable *S. procumbens* seeds in three treatments over depth. Dots indicate individual samples (277). Red triangles depict the average viability of the samples and red circles depict the total viability (derived from Table 3).

CHAPTER 4

Biogeomorphic impact of oligochaetes (Annelida) on sediment properties and *Salicornia* sp. seedling establishment

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ABSTRACT

Oligochaetes (Annelida) are active bioturbators that can be present in high densities in the transition zone between intertidal flats and salt marshes, though their occurrence and functional role remains understudied. This study aims to clarify the biogeomorphic role of oligochaete bioturbation in facilitating or hindering vegetation establishment. Two microcosm experiments were performed to assess the effect of oligochaete bioturbation on sediment properties, oxidation depth, algal biomass, seed distribution and germination success of pioneer species *Salicornia* spp. Oligochaetes created burrow networks in the sediment matrix which, together with upward conveyor belt feeding, leads to substrate mixing. Sediment reworking rates compare to those of polychaete macrofauna. Bioturbation and bio-irrigation of burrows can stimulate resource flows into the sediment. Oxidation depth increased almost tenfold in the presence of oligochaetes. Their bioturbation did not seem to affect sediment properties such as dry bulk density, porosity and organic matter content. Sediment reworking, however, significantly reduced algal biomass at the surface with possible cascading effects on sediment stability and erodibility. Oligochaete conveyor belt feeding buried *Salicornia* spp. seeds until below the critical germination depth, thus negatively affecting *Salicornia* spp. germination and seedling establishment. Our study indicates that small, though numerous, oligochaete bioturbators may reduce lateral expansion potential of salt marshes by hindering the establishment of pioneer vegetation in the transition zone. Additionally, in dynamic fine-grained habitats these oligochaetes have the feature to quickly oxygenate the sediment top layer.

INTRODUCTION

Salt marshes and their adjoining intertidal flats are vital areas in coastal systems. They harbor unique plant communities, host migratory and breeding birds, and also function as coastal protection through wave attenuation (Bouma et al. 2005; Reise et al. 2010; Temmerman et al. 2013; Schuerch et al. 2014). Sea-level rise and increased inclemency call for improved coastal defenses (Möller 2006; Bouma et al. 2014). Ecosystem-based coastal defense, such as salt marshes, provide a sustainable and cost effective improvement (Temmerman et al. 2013). Salt marshes are often fixed on their landward side by hard structures, such as dikes, which cause salt-marsh expansion to be possible only on the seaward side (Elias et al. 2012). Lateral expansion is critical for the preservation of salt marshes (Balke et al. 2016; Bouma et al. 2016). Most studies, however, have focused on vertical marsh accretion (Kirwan & Guntenspergen 2010; Mariotti & Fagherazzi 2010; Fagherazzi et al. 2012; Marani, Da Lio & D'Alpaos 2013). Lateral salt-marsh expansion is broadly considered to be a dynamic process with alternating periods of erosion and re-establishment of vegetation (van de Koppel et al. 2005; Schuerch et al. 2014).

Lateral salt-marsh expansion is governed by sediment dynamics and vegetation establishment in the transition zone (Friedrichs & Perry 2001; Meysman, Middelburg & Heip 2006; van Wesenbeeck et al. 2007; Schuerch et al. 2014). Salt-marsh vegetation increases sedimentation rates by impeding water flow, and increases soil stability by binding sediment with their roots, aiding vertical as well as lateral expansion (Gerdol & Hughes 1993; van Wesenbeeck et al. 2008b; van der Wal & Herman 2012). In many salt-marsh transition zones the main pioneer plant species is the strong ecosystem engineer *Spartina anglica*. Once established, its tussocks induce a local positive feedback, initiating bed-level elevation, tussock expansion and, eventually, marsh succession (Bouma et al. 2005; van Wesenbeeck et al. 2008a; Bouma et al. 2013). In many other transition zones, the first colonizer of bare intertidal flats is the annual species *Salicornia* spp., that also can facilitate the accretion of fine sediment (Boorman, Hazelden & Boorman 2001; Wolters, Garbutt & Bakker 2005b). However, sedimentation can also be detrimental for its emergence: *Salicornia* cannot survive to germinate when burrowed deeper than 1 cm below the sediment surface (Gerdol & Hughes 1993; Wirtz 1994). A reduction in *Salicornia* development as a result of high accretion or burrowing rates may inhibit lateral salt-marsh expansion.

Both vegetation establishment and sediment dynamics are affected by interactions between vegetation and benthos (Fagherazzi et al. 2012). For example, *Corophium volutator* can inhibit *Salicornia* development directly through burial of seeds, but also indirectly by disturbing the sediment matrix, which prevents root anchorage (Gerdol & Hughes 1993). This, in turn, reduces sediment stability and deposition. Similarly, the lugworm *Arenicola marina* destabilizes the sediment, which impairs root growth and anchorage of *S. anglica* (van Wesenbeeck et al. 2007). On the other hand, *S. anglica* patches are high in silt content and contain a dense root network, which is unfavorable for the bioturbating lugworm. This results in a clear distinction between areas dominated by *S. anglica* and those by *A. marina*, because both species modify

their habitat to their own benefit, negatively affecting the other (van Wesenbeeck et al. 2007). *Hediste diversicolor* buries cordgrass seeds and consumes them once sprouted (Zhu et al. 2016a). Though, when not consumed, these buried seeds are retained in the soil and thereby protected from hydrodynamics (Zhu et al. 2016a). *Tubifex tubifex*, a limnetic oligochaete, has been shown to exert a positive influence on macrophyte growth through oxygen-stress amelioration (Mermillod-Blondin & Lemoine 2010). As pioneer-vegetation establishment on the intertidal flats can be limited by macrofauna bioturbation (Gerdol & Hughes 1993; van Wesenbeeck et al. 2007), it might similarly be obstructed by bioturbation of small, though numerous, oligochaetes.

Sediment-reworking rates by oligochaetes range from 0.003 (Ravera 1955) to 0.49 (Matisoff, Wang & McCall 1999) cm/day/100.000 individuals/m² and abundances can range up to a million per m² (Bagheri & McLusky 1982; Giere 2006). Although oligochaetes are significant bioturbators, their abundance and ecological role are hardly ever studied (Evans et al. 1979; McCann 1989; Giere 1993; Seys, Vincx & Meire 1999). A likely cause is that these worms belong to meiofauna, that are regularly not included in research (Seys, Vincx & Meire 1999; Chen et al. 2016). Most studies performed on their bioturbation capacities are of the freshwater model species *T. tubifex* (Fisher et al. 1980; Reible et al. 1996; Mermillod-Blondin et al. 2004; Dafoe et al. 2011). Sediment reworking, bioturbation and bio-irrigation, cause cascading effects on resource flows and microbial activity in the sediment (Mermillod-Blondin et al. 2005; Kristensen et al. 2012; Pigneret et al. 2016). Understanding the interaction effects between sediment reworking and aboveground vegetation helps clarify processes that govern lateral marsh expansion (Meysman, Middelburg & Heip 2006).

We studied the role of bioturbating oligochaetes on facilitating or hindering vegetation establishment in the transition zone from salt marsh to intertidal flats. The salt marsh at Westhoek, Friesland (The Netherlands) is a naturally expanding marsh and therefore an excellent location to study expansion processes in the transition zone. A pilot study in the transition zone at Westhoek revealed that a major component of the species abundance and diversity consisted of marine or brackish oligochaetes. Using microcosm lab experiments, this study examines whether oligochaetes play a biogeomorphic role in pioneer seedling establishment. For this purpose, we tested whether oligochaete bioturbation affects algal biomass, chemical and bulk sediment properties, and whether it affects pioneer *Salicornia* seed germination and seedling emergence.

METHODS

The study consisted of two microcosm experiments. The first experiment was conducted to test the effects of oligochaete bioturbation on abiotic sediment characteristics. The second experiment consisted of a full-factorial design to determine the role of oligochaetes in *Salicornia* germination and seedling emergence. Algal biomass measurements were non-invasive and therefore performed in both experiments.

Sediment properties: experimental set-up

Sediment was collected at the intertidal flats at Westhoek, province of Friesland (53°16.31N, 5°33.14E, The Netherlands, 2016). A naturally expanding salt marsh in its early stages is located at Westhoek, predominated by pioneer, low and middle marsh species and inundated on average once per day. The salt marsh is located in the Wadden Sea area and water is at times brackish through freshwater discharge at Kornwerderzand sluices. Sediment was sieved through a 1 mm sieve to remove all large particles and fauna. Sediment, 70% mud ($<63 \mu\text{m}$), was mechanically homogenized and left at 3°C until further use. Anoxic sediment was transferred into 28 transparent microcosm cores of 4.4 cm diameter and 20 cm long. Microcosms with anoxic sediment were left overnight to oxidize the top layer, after which oxidation depth was measured from 4 fixed points. Subsequently, the microcosms were transferred into a tidal basin and subjected to a simulated tidal regime of 2 hours of submergence every 24 hours and a 12 hour light/dark period, corresponding to spring conditions at the source. Oligochaetes were collected at Westhoek by gathering clumps of sediment with worms visible. Extraction of oligochaetes was performed by diluting sediment clumps with brackish seawater (15 PSU), after which suspended sediment was transferred into a transparent tray and placed on a light table. Live oligochaetes were selected from the tray, counted and introduced into the treatment microcosms the same day ($n=14$, 200 oligochaetes per microcosm: resembling a density of 131.493 individuals m^2). To the control microcosms ($n=14$), no worms were added.

It was in this stage not possible to discriminate between species of oligochaetes (Van Haaren 2016). Species composition was, afterwards, determined to be approximately 60% *Heterochaeta costata* and 40% *Enchytraidea* spp. Visual inspection revealed that all worms had burrowed within a few hours. The microcosms were systematically placed, control and treatment microcosms alternating, into a tidal basin in a 15°C climate chamber. Water salinity was $16.4 \pm 0.7\text{‰}$ and water temperature was $14.0 \pm 0.2^\circ\text{C}$ during the experiment. After 36 days the experiment was terminated. Burrows were clearly visible by brown oxidized sediment in the otherwise black anoxic matrix (Mermillod-Blondin et al. 2008; Mermillod-Blondin & Lemoine 2010; De Lucas Pardo et al. 2013). Oxidation depth was measured from the same four fixed points and split up into solid oxidation and deep burrow oxidation (Fig. 4.1a). Solid oxidation refers to the uninterrupted brown oxidized layer, measured from the surface. Deep burrow oxidation was the depth of the interrupted brown oxidized burrows at four fixed points (Fig. 4.1a). Solid and deep burrow oxidation had the same value when burrows were absent. Algal biomass, consisting of diatoms, cyanobacteria and green algae, was measured at the surface with a Benthos fluorometer (bbe Moldaenke GmbH). Subsequently, all microcosms were frozen to halt ammonium formation and breakdown processes and stored at -20°C until further processing.

Measurements

From the 28 experimental units, 10 control and 10 treatment cores were sliced to determine bulk sediment properties. Slice intervals were 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7 and 7-8 cm. The remaining sediment in the microcosm (8-20 cm) was not analyzed because the oligochaetes concentrate mainly in the upper 5 cm (Pers. obs., Appleby & Brinkhurst 1970; McCann 1989; Seys, Vincx & Meire 1999). The outside of the frozen cores was first briefly thawed with hot water, then with a piston we gently extruded the sediment bottom upwards until the specific interval was reached and sawn off. Slice thickness was measured at four points to calculate the volume of each individual slice (V). Each slice was weighed to obtain the wet weight (WW) and samples were dried for 24 hours at 105°C to obtain dry weight (DW) (Compton et al. 2013b). Subsequently they were incinerated for 6 hours at 560°C to obtain the ash free dry weight ($AFDW$) (Compton et al. 2013b). Mass water content and dry bulk density were calculated as follows (Flemming & Delafontaine 2000):

$$w_a (\%) = 100 \times \left(\frac{WW - DW}{WW} \right) \quad (1)$$

$$BD_{dry} = \frac{DW}{V} \quad (2)$$

The density of the solids (ρ_s) in natural sediment mixtures is assumed to be 2.65 g/cm³ (De Groot et al. 2009). Porosity was calculated as follows (De Groot et al. 2009):

$$\varepsilon = \frac{\rho_s - BD_{dry}}{\rho_s} \quad (3)$$

Organic matter content in weight percent was calculated with the loss on ignition (LOI) method as follows (Heiri, Lotter & Lemcke 2001):

$$LOI = 100 \times \left(\frac{DW - AFDW}{DW} \right) \quad (4)$$

Water and organic matter content values were more accurate than those of bulk density and porosity as they were not influenced by possible inaccuracy in volumetric measurements.

Pore-water ammonium

The remaining 4 control and 4 oligochaete treatment microcosms were used for pore-water ammonium (NH_4^+) analysis. Cores were sliced into intervals at 0-1, 1-2, 2-3, 3-4 and 4-5 cm. Sediment from each slice was transferred into a tube and centrifuged for 60 minutes at 3800 rpm. Pore-water was drained, filtered and diluted threefold. Analysis of NH_4^+ was performed with a Spectroquant Merck kit (Ammonium Cell Test 1.14558.0001). Analysis results from one control microcosm were discarded because of a dilution error.

Salicornia germination: experimental set-up

28 microcosm sediment cores were collected at Westhoek. Microcosms were defaunated by freezing them at -20°C for 15 days. The sediment was purposely not homogenized to resemble the field situation. Oligochaetes were collected in the same way as described in the previous experiment. *Salicornia* seeds were collected in October 2015. The experiment was conducted in a full-factorial design with 7 microcosms per treatment and two factors: Oligochaetes and *Salicornia* seeds (Table 4.1). 75 seeds were added to the seeds (S) and seeds and oligochaetes (SO) treatments, 200 oligochaetes were added to the oligochaetes (O) and seeds and oligochaetes (SO) treatments respectively. The control treatment (C) contained neither seeds nor oligochaetes. The microcosms were exposed to the same conditions as the previous experiment. Water salinity was $15.9 \pm 0.8\text{‰}$ and water temperature $13.9^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ during the experiment. After 3 days, oligochaetes were introduced into the O and SO treatment microcosms. On day 4, *Salicornia* seeds were added to the S and SO treatments. Studies indicate that a window of opportunity without flooding is necessary for the establishment of pioneer vegetation on an intertidal flat (Balke et al. 2011). To simulate this, the cores were not submersed for the first 5 days after seed addition and to initiate germination, 3 ml of freshwater was added to each core during the first three days (Wirtz 1994; Hu et al. 2015a). Subsequently, the tidal cycle with 2 hours submergence every 24 hours continued for the remainder of the experiment and 1 ml fresh water was added to each core three days a week to simulate rainfall. The experiment was terminated 33 days after seed addition, germinated seeds and seedlings were counted and algal biomass was measured. To terminate Oligochaete activity and fix their position in the sediment matrix, microcosms were frozen at -80°C and stored at -20°C until further processing. The microcosms were sectioned into slices of 0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–5, 5–6 and 6–12 cm. Each layer was sieved through a $300\text{ }\mu\text{m}$ sieve and placed in a transparent tray on a light table. Oligochaetes and *Salicornia* seeds were counted. *Salicornia* seeds were classified into three different groups: intact seeds, germinated seeds and seedlings. Seeds were classified as germinated when the radicle emerged (Khan, Gul & Weber 2000; Carter & Ungar 2003), and seeds were considered a seedling when the capsule was shed and the cotyledons were visible (Davy, Bishop & Costa 2001).

Table 4.1 Amount of oligochaetes and *Salicornia* seeds retrieved from the microcosms after experiment termination. ‘Outside microcosms’ were seeds and worms that have drifted out of the microcosms with the simulated tide.

Treatment	Oligochaetes		<i>Salicornia</i> seeds	
	added	retrieved	added	retrieved
Seeds and Oligochaetes (SO)	1400	1194	525	345
Oligochaetes (O)	1400	1338	0	0
Control (C)	0	16	0	0
Seeds (S)	0	20	525	426
Outside microcosms	-	85	-	41
Total	2800	2653	1050	812

Data analyses

Data was analyzed taking into account several possible sources of correlation between the data. Spatial correlation may exist between slices at different depths in the same microcosm. To model these sources of variation we used Linear and Additive Mixed Effect Models (Pinheiro & Bates 2000; Wood 2006). Additionally, to allow for heterogeneity between different treatments, control and oligochaetes, in these models a variance factor was included. Oxidation depth and algal biomass data were analyzed using Generalized Least Squares (GLS) to allow for unequal variances (heteroscedasticity) in the treatments (Pinheiro & Bates 2000). Likelihood Ratio (LR) tests were used for model selection and to assess significance of individual factors. All mean values are accompanied by their 95% confidence limits (CL). The protocol by Zuur et al. (2010) was used for data exploration and assessing the assumptions of the analyses (Zuur, Ieno & Elphick 2010).

Distribution of oligochaetes is known to be non-linear over depth (Fisher et al. 1980; Selys, Vincx & Meire 1999; Mermillod-Blondin & Lemoine 2010), therefore their effect on the sediment matrix might also be non-linear. To allow for nonlinearity, models were constructed using Generalized Additive Mixed Models (GAMM) for the analyses of mass water content and ammonium. Microcosm was added as a random effect to allow for correlation between samples from the same microcosm. Mean slice depth was used to construct an autocorrelation structure to model spatial (i.e. sediment depth) correlation among observations (continuous autoregressive model, corCAR1 class, Pinheiro and Bates 2000). Essentially, this means that the correlation between samples decreases exponentially as a function of the distance between them. Model assumptions (e.g. homogeneity of variances and normality of residuals) were assessed graphically. Exploratory analyses gave no indication for non-linearity in the remaining sediment properties (bulk density, porosity and organic matter content) which were therefore analyzed using Linear Mixed Effects models. All statistical analyses were performed with the statistical program R (R Core Team 2015) using additional packages 'lattice' (Sarkar 2008), 'mgcv' (Wood 2006) and 'nlme' (Pinheiro et al. 2016).

RESULTS

Sediment properties: Chemical sediment properties

Oligochaetes significantly increased the solid oxidation depth of the sediment matrix (GLS, Likelihood Ratio_{df=1}=23.9, $p<0.0001$, Fig. 4.1). All microcosms had an oxidized layer of 1 mm before the addition of worms. After five days, a burrow network became apparent, which expanded over time. Oxidation depth gradually increased in microcosms with oligochaetes present. At the end of the experiment, mean oxidation depth was 1.2 mm (CL 1.1,1.3, $n=14$) in the control treatment, while solid oxidation was 10.0 mm (CL 7.7,12.4) and deep burrow oxidation was 25.4 mm in the oligochaete treatment (CL 20.5,30.2; GLS, LR_{df=1}=32.2, $p<0.0001$, Fig. 4.1). Variance in solid oxidation

depth was approximately 22 (CL 13, 39) times greater and the variance in deep burrow oxidation was 48 times greater (CL 28, 84) with bioturbating worms than without (Fig. 4.1).

Figure 4.1 Example of oxidation depth, solid oxidation and deep burrow oxidation are indicated, in a core a) with oligochaetes and b) control treatment without oligochaetes after 36 days. Oxidation depth (log-scale) after 36 days for (c) solid oxidation and (d) deep burrow oxidation ($n=14$ per treatment). Treatments are control, i.e. without oligochaetes, and oligochaetes with on average 200 worms per microcosm. Diamonds indicate the mean, grey bars the 95% confidence limits and black dots depict individual measurements.

There was no difference in ammonium concentrations with and without oligochaetes (Anova, $LR_{df=1}=1.09$, $p=0.30$, Fig. 4.2). Mean pore water ammonium concentrations were 0.52 mmol/l (CL 0.41,0.62) and 0.44 mmol/l (CL 0.35,0.54) for respectively control and oligochaetes. In either treatment ammonium concentration increased nonlinearly with depth (Fig. 4.2). Ammonium concentrations were lower closer to the sediment surface and gradually increased below 2 cm depth. Statistical

models needed a correlation structure because of the dependence among samples. Generally, samples from two adjacent slices have a correlation of 0.86 (CL 0.64,0.95) which decreases exponentially with the distance between slices.

Figure 4.2 Ammonium concentrations over depth in a) control treatment n=3 microcosms and b) oligochaete treatment n=4 microcosms. The dotted lines indicate the 95% confidence limits and black dots depict individual measurements.

Figure 4.3 Mass water content over depth in a) control treatment and b) oligochaete treatment (n=10 per treatment). The dotted lines indicate the 95% confidence limits and black dots depict individual measurements corrected for the random effect.

Sediment properties: Bulk sediment properties

Oligochaetes significantly lowered the mass water content in the upper 2 cm of the sediment matrix (Anova, $LR_{df=1}=42.4$, $p<0.0001$, Fig. 4.3). A correlation structure to model dependence between observation was necessary ($\phi=0.77$, CL 0.55,0.90).

Table 4.2 Results of linear regression analyses on sediment properties with factors depth and treatment (n=10 microcosms per treatment).

Sediment properties	Mean	95% CL	Depth		Oligochaetes	
			$LR_{df=1}$	p-value	$LR_{df=1}$	p-value
Dry bulk density (g cm ⁻³)	0.49	0.48 - 0.50	0.8	0.37	0.99	0.32
Organic matter content (%)	16.4	16.1 - 16.6	2.5	0.11	0.10	0.75
Porosity (%)	81.4	81.0 - 81.8	0.8	0.37	0.99	0.32

Exploratory analyses gave no indication for non-linearity in dry bulk density, porosity and organic matter content but a spatial correlation structure with depth was incorporated in the statistical model. There was no impact of oligochaetes on dry bulk density, porosity or organic matter content, neither was there an effect of sediment depth (Table 4.2).

Sediment properties: Algal biomass

The presence of oligochaetes significantly decreased algal biomass at the surface (Fig. 4.4). At the end of the experiment (36 days), algal biomass was $6.1 \mu\text{g}/\text{cm}^2$ (CL 5.5,6.8) without worms and $2.6 \mu\text{g}/\text{cm}^2$ (CL 2.4,2.7) with worms (GLS, $\text{LR}_{\text{df}=1}=33.9$, $p<.0001$). Algal biomass consisted mainly of diatoms, approximately 95%, while the remaining 5% were green algae and cyanobacteria. The presence of oligochaetes led to a decrease in the variation of algal biomass, i.e. 0.30 (CL 0.18,0.52) times less variation than without worms.

Salicornia germination: Algal biomass

The second experiment, including seeds, also showed a clear and similar effect of the worms (Fig. 4.5), indicating a strong decrease in algal biomass in the presence of oligochaetes (GLS, $\text{LR}_{\text{df}=3}=44.6$, $p<.0001$). The variation of algal biomass was approximately 2 times smaller in the treatments with oligochaetes (variance reduction SO: 0.45 (0.20, 1.0); O: 0.44 (0.20, 0.97) opposed to treatments without oligochaetes (Fig. 4.5). In both experiments the pattern of lower algal biomass in the presence of worms was consistent, though the sediment properties experiment had higher biomass in general. This

Figure 4.4 Algal biomass in $\mu\text{g}/\text{cm}^2$, $n=14$ per treatment. Diamonds indicate the mean, grey bars the 95% confidence limits, and black dots depict individual measurements.

Figure 4.5 Algal biomass in $\mu\text{g}/\text{cm}^2$, $n=7$ per treatment. Diamonds indicate the mean, grey bars the 95% confidence limits, and black dots depict individual measurements.

was probably caused by the different methods for defaunating the sediment, i.e. sieving and homogenising for sediment properties and freezing for *Salicornia* germination.

Salicornia germination: Oligochaete and *Salicornia* seed distribution

The distribution of worms in the two oligochaete treatments (SO and O) was very similar (Fig. 4.6), indicating that *Salicornia* seeds did not influence the distribution of worms. We mainly observed burrows in the upper 4 cm of the microcosms, although oligochaetes sporadically occur as deep as 10 cm. The number of worms differed between the depths; peak densities of oligochaetes occurred at a depth of 1-2 cm, and then gradually decreased down to the lowest level at 6-12 cm depth (Fig. 4.6). Approximately 75% of the worms concentrated between 1 and 4 cm depth, and only 3% was recovered below a depth of 6 cm. At the end of the experiment about 95% of all introduced worms were recollected (Table 4.1). Of the *Salicornia* seeds 77% was retrieved (Table 4.1). 3% of oligochaetes and 3.9% of seeds were found in the tidal basin, as they drifted out of the microcosms with the simulated tide (Table 4.1).

Figure 4.6 Vertical distribution of oligochaetes from the a) seeds and oligochaetes treatment and b) oligochaetes treatment, $n=7$ per treatment. Diamonds indicate the mean, grey bars the 95% confidence interval and black dots depict individual measurements.

After 33 days there were clear differences in the distribution of intact seeds, seedlings and germinated seeds in microcosms with and without oligochaetes (Fig. 4.7). Seeds and seedlings were not found below the upper 0.5 cm without worms and not below 2 cm if worms were present. When oligochaetes were absent we found 98.7% of the seeds in the top layer (S treatment), opposed to 9.0% when worms were present (SO treatment). Without oligochaetes, all germinated seeds were found in the uppermost sediment layer (0-0.5cm), in contrast with oligochaetes, most germinated seeds were

present below a depth of 0.5 cm (Fig. 4.7, S and SO treatment). Absence of worms, meant that only 1.3% of the seeds were retrieved from the 0.5-1 cm sediment layer, opposed to 45.2% with worms. Without worms no seeds were found below 1 cm, while with worms 45.8% were found below 1 cm. The worms transported most of the germinated seeds from the surface into the sediment matrix (Fig. 4.7). With seedlings this effect was less pronounced, though some were found at 1-2 cm depth. Bioturbation led to toppling of established seedlings and washing away or subsequent burial through upward conveyor belt deposition. Visual observation of the microcosms indicated that seeds germinated first and were subsequently buried.

Figure 4.7 Distribution of intact seeds, seedlings and germinated seeds over depth a) with and b) without oligochaetes. *Salicornia* seeds were not retrieved below 2 cm depth therefore these data points are excluded for clarity. Values are expressed as mean percentage of total retrieved seeds (n=7 per treatment). Diamonds indicate the mean, grey bars the 95% confidence limits and black dots depict individual measurements, when only one black dot is displayed all measurements were zero.

DISCUSSION

Algal biomass

Less algal biomass was present at the sediment surface in sediment with oligochaetes (Fig. 4.6). By reducing diatom biomass oligochaete bioturbation has an indirect effect on sediment erodibility. Diatoms excrete extra-polymeric substances (Widdows & Brinsley 2002; Weerman et al. 2012), creating a biofilm that stabilizes the sediment. Disturbance of algal biofilms increases erosion (Montserrat et al. 2008; Weerman et al. 2010; Weerman et al. 2012; De Lucas Pardo et al. 2013). Observations during our experiment revealed that a thin layer of sediment eroded during rising tide, when oligochaetes were present and not when worms were absent. Active grazing of the algae by oligochaetes does not seem likely as they feed on detritus and bacteria and showed upward conveyor belt feeding, i.e. ingestion at depth and deposition of sediment at the surface (Giere 2006; Dafoe et al. 2011). Diatoms are able to migrate vertically and thus are capable of returning to the sediment surface upon burial (van Colen et al. 2014). In the current study, the rate of sediment deposition at the sediment surface appeared to exceed diatom production and migration rate so that a biofilm could not fully establish on the surface. In addition, erosion can be temporally increased by the production of fecal pellets by oligochaetes (De Lucas Pardo et al. 2013). Both palletization and bioturbation lead to a rougher micro-topography, increasing hydrodynamic roughness and thus potentially increasing erosion (Meysman, Middelburg & Heip 2006). The current set-up was not suited to quantify the amount of erosion, but the observations suggest that the presence of oligochaetes in typical field densities may increase sediment erodibility. This in turn affects sedimentation processes and salt-marsh vegetation establishment (Dijkema et al. 1990; Houwing 1999; Widdows & Brinsley 2002).

Sediment properties

To assess potential effects of bioturbation, we examined the depth distribution of worms (Fig. 4.7). Distribution of the marine oligochaetes roughly corresponded to those of *Tubifex tubifex* and a few other freshwater oligochaete species (Appleby & Brinkhurst 1970; Fisher et al. 1980; Seys, Vincx & Meire 1999; Mermillod-Blondin & Lemoine 2010). The distribution pattern we found, simulating spring conditions, resulted in peak density of worms coinciding with the depth of deep burrow oxidation (Fig. 4.1 and Fig. 4.7). Oligochaete bioturbation increased the oxygen diffusion into marine sediment (Fig. 4.1) similar to findings of Davis (1974) in freshwater lake sediments. Because oxidation depth steadily increased, prolongation of the experiment would have likely led to an even deeper solid oxidation depth. Bio-irrigation of the burrow network leads to oxygenated burrows (Kristensen et al. 2012). Additionally, the upward conveyor belt feeding led to even more oxygenated sediment, because anoxic feces was reworked from deeper layers to the surface. There it was locally oxidized by the overlying air when exposed during low tide. Upward conveyor belt feeding indirectly leads to a draw-down

of the organic matter rich top layer. The former top layer becomes suitable for bacteria and oligochaetes are thereby ensuring their own main food resource (Seys, Vincx & Meire 1999). In the field, oxidation of the sediment is also affected by erosion and sedimentation processes. Yet, addition of oligochaetes resulted in a tenfold increase of oxidation depth and therefore seem to be an influential factor in intertidal flat oxygen dynamics.

In fine-grained sediments resource flows are diffusion dominated and both bioturbation and bio-irrigation increase these flows (Reible et al. 1996; Mermillod-Blondin & Rosenberg 2006; Kristensen et al. 2012; Foshtomi et al. 2015). Organic matter mineralization by the microbial community leads to ammonium production. The ammonium measurements showed no significant difference between bioturbated and non-bioturbated microcosms (Fig. 4.3). Ammonium concentration was lowest in the upper 2 cm and then increased with depth (Fig. 4.3) (Mermillod-Blondin et al. 2005; Anschutz et al. 2012). There are two mechanisms for ammonium to disappear from the pore water, either it diffuses upwards from pore water into the water column (when subjected to tide) or it is used and transferred from nitrite to nitrate (Foshtomi et al. 2015). Unfortunately, nitrate could not be taken into account in this study because too little pore water was left after ammonium measurements to perform nitrite/nitrate analyses. In the top layer oligochaetes as well as microbial activity are responsible for the ammonium release (Mermillod-Blondin et al. 2005). It seems that in the current study the effects of the microbial community on ammonium are more influential than the worms. Reports on the impact of oligochaetes on biogeochemical cycles are inconclusive and not uni-directional (Reible et al. 1996; Mermillod-Blondin et al. 2005; Mermillod-Blondin & Rosenberg 2006; Mermillod-Blondin et al. 2008; Anschutz et al. 2012; Pignieret et al. 2016). Especially the cascading effect of organic matter recirculation and nutrient availability on vegetation establishment are of biogeomorphological interest.

Against our expectations, oligochaete bioturbation did not affect bulk sediment properties such as dry bulk density, porosity and organic matter content. Organic matter content is generally high in silty fine-grained sediments and was probably not a limiting factor for biogeochemical processes. Theoretically, burrows should increase sediment porosity (Giere 2006; De Lucas Pardo et al. 2013). The sediment used in our experiments was already very porous, on average 81%. High porosity creates room for consolidation and rapid dewatering of the sediment (Bray & Karig 1985). Bioturbation may form a burrow while adjacently consolidating the sediment, resulting in the same net amount of sediment being present in a slice. In the upper 2 cm, sediment with oligochaetes had lower mass water content (Fig. 4.5), probably because water evaporated from the burrows. Without worms, a diatom biofilm developed that sealed the sediment surface from evaporation. Differences in water content caused by oligochaetes are small, and the ecological relevance of a slightly higher water content is debatable in an intertidal area.

Oligochaete sediment reworking appears substantial, comparable to those of macrofauna, when up-scaled to field values. Transforming bioturbation measurements into a sediment rework-rate is complex because studies contain various methods and

calculations to up-scale rework rates to field situations (Appleby & Brinkhurst 1970; Cadée 1976; Mermillod-Blondin et al. 2004; Solan et al. 2004; Maire et al. 2006; Teal et al. 2008). We estimated, using solid and deep burrow oxidation depth as a proxy, a rework-rate of 0.0213 to 0.0562 cm/day/100.000 individuals/m². This extrapolates to a sediment turnover roughly between 7.8 to 20.5 cm per year (for 100.000 individuals/m²). This falls within the estimations for freshwater oligochaete reworking of Appleby and Brinkhurst (1970) of 1.9 to 25 cm of sediment every year (Cadée 1976). Estimation of oxidation depth is based on the color change from black anoxic to brown oxic sediment, and this results in an underestimation of the actual particle movement (Teal et al. 2008). Our estimation method is therefore precautionary and likely an underestimation of the actual impact. The oligochaete rework-rate is then comparable to that of macrofaunal polychaetes such as *Hediste diversicolor*, *Heteromastus filiformis* and *Arenicola marina* (Cadée 1976; Mermillod-Blondin et al. 2004). These macrofaunal species were hardly present at the study location. Especially *A. marina* does not reside in soft sediments with high mud content (Cadée 1976). Possibly oligochaetes fulfil the role of bioturbator in highly muddy, anoxic environments where macrofauna cannot thrive. Though oligochaetes occur in all sediment types (Giere 1993) and their relative contribution is likely to be underestimated also in other areas. Additionally, the indirect effects of oligochaetes, such as increased erodibility, decreased stability and the draw-down of organic matter, cascade in biogeomorphological sediment processes. We recommend to include meiofauna in biogeomorphic studies because small, though numerous organisms can have profound effects on the sediment matrix (Seys, Vincx & Meire 1999; Meysman, Middelburg & Heip 2006).

Salicornia distribution, germination and emergence

To our knowledge, this study is the first to suggest that oligochaetes can have a negative impact on the establishment of salt marsh pioneer vegetation, specifically *Salicornia*. Oligochaete bioturbation reduced the number of seedlings at the surface and buried intact and germinated seeds, up to 2 cm deep (Fig. 4.7). The critical germination depth for *Salicornia* is estimated to be 1 cm, as deeper burial halts the germination process (Gerdol & Hughes 1993; Wirtz 1994). This indicates that oligochaetes might also hinder the establishment of *Salicornia* seedlings in the field. Seedlings that established with worms present, were later often toppled and subsequently buried. Toppling and burial of seedlings is a major disturbance and can inhibit further development and succession (Jensen & Jefferies 1984; Ellison 1987a; van Wesenbeeck et al. 2007; Volkenborn et al. 2007). Additionally, sediment disturbance, i.e. bioturbation, can cause loss of root anchorage, which increases the seedling's chance of washing away with the tide (Gerdol & Hughes 1993). We observed this in our experiments as well. Oligochaetes are bacterial and deposit feeders, therefore direct seed or seedling predation is not a mechanism for reduced seedling emergence unlike findings of Zhu et al. (2016) for macrofauna. Oligochaete bioturbation is hereby suggested to shape the biogeomorphic environment by reducing pioneer vegetation establishment and survival.

There is high variation in seedling establishment success in the field due to a limited window of opportunity (Balke et al. 2011; Zhu et al. 2014; Hu et al. 2015a). Balke et al. (2011) suggests that this window for salt-marsh vegetation is mainly determined by physical disturbances. Others, however, have shown that biological disturbance of benthic fauna can also play a definite role in seed bank dynamics, both positive and negative (Delefosse & Kristensen 2012, this study; Zhu et al. 2014; Zhu et al. 2016a). Burial of seeds can also function as a mechanism to retain seeds in erosion prone periods (Zhu et al. 2016a). There is a complex interplay of direct and indirect effects that determine vegetation establishment. This can vary per species and developmental stage. Pioneer vegetation may, once sufficiently anchored, benefit from the oligochaete-induced increased oxic conditions (Keiffer, McCarthy & Ungar 1994; Mermillod-Blondin & Lemoine 2010; Schrama et al. 2015). However, this was not assessed in this study and remains to be further investigated. Nonetheless, first the negative effects of bioturbation on seedling establishment have to be withstood. We suspect that bioturbation in the field can limit *Salicornia*'s window of opportunity. Especially as *Salicornia* is a pioneer species and the first to colonize the oxygen poor intertidal flats. Density of worms used in these experiments correspond to 131.493 individuals per meter square, and as field densities of oligochaetes can range up to a million per m² it seems likely that they could influence *Salicornia* establishment in the field. As oligochaetes are not uniformly distributed (Seys, Vincx & Meire 1999) we suspect a negative interaction to probably occur in the field as a patchy effect. Field studies will prove valuable in assessing these results on salt marsh scale and will reveal the possible positive effects of increased oxygen in the sediment.

In conclusion, the current study shows that oligochaete bioturbation and upward conveyor belt feeding is a significant biogeomorphological process. Depth of oxygen availability in the sediment increased substantially by oligochaete bioturbation. Diatom biofilm production was limited by oligochaete bioturbation which probably promoted erodibility of the sediment surface. The worms burrowed seeds and reduced *Salicornia* seedling emergence at the sediment surface. Meiofauna needs to be included in biogeomorphic studies because these small, though numerous organisms can have profound effects on the sediment. Specifically, oligochaetes in fine-grained habitats are an overlooked group that can influence erodibility of the sediment as well as vegetation establishment, and thereby eventually lateral salt marsh expansion.

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CHAPTER 5

Multiple environmental variables affect germination and mortality of an annual salt marsh pioneer: *Salicornia procumbens*

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ABSTRACT

Salt marshes, providing numerous ecosystem services, are degrading worldwide. To effectively aid conservation and restoration efforts, increased insight regarding marsh expansion processes and the initial establishment of pioneer vegetation is essential. In this study, we disentangle environmental drivers at the salt-marsh edge that affect the lifecycle of the annual pioneer *Salicornia procumbens*. In a field experiment, the effect of various environmental variables on the start of germination, germination success and mortality before seed-set were determined. Our results showed that temperature and sedimentation inhibited the initiation of germination. Once germination occurred, higher precipitation rates increased germination success. In contrast, sedimentation rates above 0.5 mm day^{-1} halved germination success through burial of freshly sprouted seedlings. Unexpectedly, natural germination was low, indicating that seed availability may have been limited, despite a seed source nearby. Too frequent inundation, extended periods without inundation (desiccating the soil) and a too dynamic bed-level increased mortality before seed-set. Consequently, sediment dynamics decrease seed production and thus potential reproduction success. A high reproduction success is crucial for annual seed-reproducing species, such as *S. procumbens*, because it determines seed availability for the following year. Consequently, to assess the potential of a given site for salt-marsh restoration, it is important to take local sediment dynamics (and not only hydrodynamics) into account. Furthermore, year-to-year variations in multiple environmental factors can have a strong impact on the colonisation potential of annual pioneer species.

INTRODUCTION

Salt marshes and intertidal flats are essential parts of coastal systems, that provide protection against floods through wave attenuation (Temmerman et al. 2013; Schuerch et al. 2014). They are vital habitats for migratory and breeding birds, and are host to a variety of unique floral and faunal communities (Reise et al. 2010). Currently, many salt marshes are degrading due to human-driven change, such as conversion to land, pollution and alterations to coastal hydrology and morphology (Gedan, Silliman & Bertness 2009). Therefore, increasing our knowledge of how to restore salt marshes or even stimulate expansion is imperative. Most studies focus on the vertical accretion rates of salt marshes and their ability to keep up with sea level rise (Stoddart, Reed & French 1989; Temmerman et al. 2013; Kirwan et al. 2016a). Equally, or maybe even more important, are the processes that drive the lateral expansion of salt marshes (Bouma et al. 2016; Cao et al. 2018). Years with a successful seed-set in the transition zone may promote marsh expansion. However, environmental thresholds for successful initial establishment of pioneer vegetation still need to be identified (Bouma et al. 2014). This is specifically relevant in the transition zone of the two adjacent systems (un-vegetated intertidal flat and vegetated salt marsh), where salt-marsh expansion will occur, especially when marshes are constrained by dykes and unable to migrate landwards.

Crucial for marsh expansion as well as marsh restoration is the successful establishment of pioneer vegetation in the transition zone (Olf et al. 1997; Zedler, Morzaria-Luna & Ward 2003). In case of annual plants, this requires a seed source in proximity, either from adult plants or from a local seed bank (Rand 2000). Salt-marsh seeds and seedlings have to germinate and survive in a dynamic environment. In earlier studies, the effect of one or two particular parameters on germination and survival of intertidal pioneer vegetation were often studied in laboratory experiments: including sediment dynamics (Boorman, Hazelden & Boorman 2001; Cao et al. 2018), inundation regime (Egan & Ungar 2000a; Boorman, Hazelden & Boorman 2001), salinity (Noe & Zedler 2001), temperature (Khan, Gul & Weber 2000; van Regteren et al. 2019), and/or bioturbation (Zhu et al. 2016a; van Regteren et al. 2017). Field experiments are much scarcer, but are important as they can provide valuable information on realistic combinations of these environmental parameters. Additionally, most research is restricted to the germination phase of pioneer plants (Khan, Gul & Weber 2000; Noe & Zedler 2001; Carter & Ungar 2003; Elsey-Quirk, Middleton & Proffitt 2009), whereas, understanding seedling establishment and mortality before seed-set is equally important.

The annual pioneer *Salicornia procumbens* is a key species in NW-European salt marshes, as it is often the first plant species to colonize the transition zone (Hulzen et al. 2006; Davy et al. 2011; Bouma et al. 2013). This species affects faunal assemblages (van der Wal & Herman 2012) and facilitates the subsequent establishment of other perennial salt-marsh plants (Smit van der Waaij et al. 1995; Langlois, Bonis & Bouzillé 2001). Stands of *S. procumbens* trap vegetative tillers of *Spartina anglica* and *Puccinellia maritima*, a process that even continues when stands are deceased. Common salt-marsh grass (*P. maritima*) is a key species for moving towards a diversified community (Langlois, Bonis & Bouzille 2003), and in general vegetation contributes to erosion resistance (Lo et al.

2017).

Establishment of *Salicornia* spp. is influenced by multiple environmental variables. An inundation-free period of at least 2 days is essential to initiate germination (Wiehe 1935). Temperature is a key factor and low temperatures prevent seeds from germinating in winter (Khan, Gul & Weber 2000). Whereas extended high temperatures coinciding with an inundation-free period lead to low soil moisture which inhibits germination as well. Fresh water increases germination success and seedling survival by reducing soil salinity (Noe & Zedler 2001). Additionally, *S. europaea* mortality spikes when the soil is waterlogged (Egan & Ungar 2000a). Successful establishment can also be impeded by too strong sediment dynamics in several ways: 1) during winter storms the surface layer including newly dispersed seeds erodes, reducing seed availability in spring (van Regteren et al. 2019), 2) high sedimentation and erosion rates in the initial life stages lead to increased perennial seedling mortality (Bouma et al. 2016; Cao et al. 2018), and 3) erosion of the soil will wash away seedlings due to insufficient root anchorage (Smit van der Waaij et al. 1995). Thus, interactions between multiple environmental variables will affect the successful completion of the *Salicornia* spp. life cycle.

This field study aims to disentangle the multiple environmental factors that control i) initiation of germination (i.e. presence or absence of germination), ii) germination success (i.e. germinated seeds that survive into seedlings) and iii) mortality (i.e. loss of seedlings) before seed-set of *Salicornia procumbens*. In the transition zone, seeds were supplied in 1 m² plots in at the salt-marsh edge and further seawards on the intertidal flat, where germination and mortality were monitored throughout the growing season. We hypothesize that 1) increased temperature and fresh water (precipitation) will have a positive effect on the initiation of germination; 2) germination success for both natural and supplied seeds of *S. procumbens* will be higher at the vegetation edge than on the intertidal flat; 3) high sediment dynamics will increase seedling mortality.

METHODS

Study area

We used a naturally developing salt marsh in the Wadden Sea, Westhoek, The Netherlands as our model system (Fig. 5.1). In the past two decades the marsh has been expanding step-wise laterally, with stable periods alternating with periods of expansion, while the marsh is also accreting vertically (Baptist et al. 2019a). The median grain size in the transition zone was on average between 40 and 100 µm, which is silty soil. The tidal amplitude is approximately 2 m and mean high water in 2017 was 0.95 m above NAP (Dutch Ordnance Datum). Seawards of the salt marsh a transition zone is present, dominated by *S. procumbens* individuals.

Experimental design

A field experiment in the transition zone was performed during the growing season of *S. procumbens*, from April to September 2017 (Davy, Bishop & Costa 2001). The experiment was repeated simultaneously at four locations distributed along a 1.2 km stretch of marsh parallel to the dike (Fig. 5.1). Each location contained two experimental blocks, with four plots in a randomized block design. Blocks were located in the transition zone, one block at the vegetation edge and one block 10 m seaward on the bare intertidal flat (Fig. 5.1). Each block contained two replicates of two treatments: seed-addition and control, amounting to 16 seed-addition plots and 16 control plots. Surface elevation of each plot was measured with a real-time kinematic DGPS at the start of the experiment. Difference in elevation between the vegetation edge and bare intertidal flat was on average 5.5 ± 0.5 cm.

Figure 5.1 Four experimental locations, with 300–400 m in between, placed perpendicular to the coastline. Experimental design of one location, containing 2 blocks with a randomized block design, is shown in the zoomed area. Two seed plots (yellow squares) and two control plots (blue squares) were placed at two positions: at the vegetation edge and 10 m seawards at the intertidal flat, experimental plots were 1 m². To measure inundation, one Conductivity Temperature Depth device (black triangles, CTD), was placed in the middle of the two blocks. To measure bed-level height, two Surface Elevation Dynamic devices (red circles, SED), were placed in the middle of the block (2 control and 2 seed plots). The study area Westhoek (black diamond), is located in the Dutch Wadden Sea.

Experimental seed-addition

S. procumbens vegetation was collected at the vegetation edge of the same marsh in fall 2016, dried and stored in the dark until use. Dry plants were abraded to remove the seeds from the adults plants. Seeds were divided into 18 portions and mixed with 500 mL of locally obtained, fine-grained sediment. Two portions were evenly spread out in a climate chamber and germination was followed for 5 weeks to check viability of the seeds. Each mixture contained approximately 400 viable seeds. In the field, the remaining 16 seed mixtures were spread on the soil surface in a thin layer mixed with sediment, to avoid immediate wind dispersal. Seeds were added to the experimental plots in the 3rd week of April 2017, the other 16 plots served as controls (Fig. 5.1).

Assessment of germination occurrence, germination success and seedling mortality

Germination and mortality before seed-set (here-after referred to as mortality) of *S. procumbens* were assessed 12 times from April till September 2017, for each period and plot (Table 5.1). Each 1 m² plot was subdivided into 16 subplots of 0.25*0.25 m in size. Subplots allowed discrimination between germination and mortality within plots. The number of individuals in each subplot was recorded at each visit. The subplot data were then used to determine the number of newly germinated individuals as well as the number that had died during each interval, within each plot. For example, at time x in plot y , one seedling was recorded in each of three subplots: B, E and N (amounting to 3 individuals in total). The next visit, time $x + 1$ at this plot, two seedlings in subplot B and one in E, L and P were counted (amounting to 5 individuals in total). In that interval, the number of germinated individuals in plot y was 3, because subplots B, L and P have each acquired one new seedling. Mortality of plot y was 1, because the individual in subplot N had died, the probability of dying was thus 33%.

Sediment dynamics, hydrological and meteorological conditions

To measure bed-level variation a Surface Elevation Dynamics (SED) instrument (Hu et al. 2015b) was placed in the centre of each block (Fig. 5.1). The SEDs measured the bed level every 30 minutes, when emerged and during daylight hours. The SED data was converted into bed-level change information (Willemssen et al. 2018). SED time series were subdivided into the same intervals as those in which the vegetation was assessed. For each plot and each interval, values for bed-level difference, sedimentation and erosion were derived relative to the initial bed level (average of first five bed level measurements in that interval). The average of the five highest consecutive measured bed level values was subtracted from the initial bed level, to obtain a sedimentation value (similar for lowest and final bed-level values, respectively for erosion and bed-level difference). This was repeated for all eleven intervals and plots.

Additionally, one Conductivity Temperature Depth instrument (CTD-diver,

Eijkelpolder Soil & Water) was placed in the middle of the two blocks (Fig. 5.1). The CTDs measured at an interval of 3 minutes to monitor accurate on-site water levels and temperature. CTD time series were subdivided into the same intervals and processed to gather data on temperature, longest inundation-free period (days), inundation frequency (times day⁻¹) and inundation duration (hours day⁻¹) for each block and interval. Meteorological data from weather station Leeuwarden (<http://www.knmi.nl/kennis-en-datacentrum>) was used to correct for air pressure variation in CTD data, and to obtain precipitation data used as a proxy for fresh water input.

Statistical analyses

The protocols by Zuur et al. (2010) and Zuur & Ieno (2016) were used for data exploration and assessing the assumptions of subsequent analyses. Several measured environmental variables were tested to distinguish their effect on the start of germination, germination success and mortality of *S. procumbens*. Possible variables included average temperature, precipitation, inundation-free period, inundation frequency, inundation duration, sedimentation, erosion, bed-level difference and elevation. Inundation duration was not simultaneously used with inundation frequency due to slight collinearity between these covariates, as was the case for sedimentation and bed-level difference. Plot ID was added as a random effect to correct for the correlation between measurements on the same plot (Pinheiro & Bates 2000). Location was added as an explanatory variable to identify potential nesting effects. Position (vegetation edge or intertidal flat) was added as an explanatory variable to check for possible effects of distance to a seed source. However, neither position nor location had a significant effect on the model results, therefore these variables were removed to simplify the models. Although this experiment contained a time component, time was excluded from the statistical models and focus was put on the potential effects of environmental variables (that may or may not be correlated to time). Additionally, the residuals were checked for any patterns in time, which did not occur. Forward selection was performed to determine which variables significantly affected germination and mortality. AIC scores were used to perform model selection (Brooks et al. 2017), and likelihood ratio tests (LR) to determine the significance of individual parameters in each model. Model assumptions (e.g., homogeneity of variances and normality of residuals) were assessed graphically (Zuur, Ieno & Elphick 2010; Zuur & Ieno 2016).

Hardly any *S. procumbens* individuals germinated in the control plots and differences between the treatment and control were assessed graphically. Therefore, solely seed-addition plots were selected for analysis of germination occurrence and success so we could exclude seed limitation as a possible cause for the observed differences. Exploratory analyses gave no indication for non-linearity and germination was therefore analysed using a generalized linear mixed model using template model builder (glmmTMB) (Brooks et al. 2017). The germination analysis was split in two parts, first occurrence of germination was analysed using a binomial model. The final equation for this model reads as:

$$G(\text{bin}) = \text{temp} + \text{sed} + \text{plotID} \quad (1)$$

Where $G(\text{bin})$ stands for germination (absence/presence), temp is temperature ($^{\circ}\text{C}$), sed is the sedimentation rate (mm day^{-1}) and plotID is the random effect.

Secondly, once germination was initiated, we analysed which environmental variables determined germination success. We used an offset ($\text{offset} = \log(\text{days})$) to correct for the different sampling intervals (more time between two visits can lead to an increase in germination success) together with a log-link and a negative binomial family for the error distribution. A negative binomial distribution was chosen because the poisson data was overdispersed. The final equation for this model reads as:

$$G(\text{nr}) = \text{offset} + \text{sed} + \text{precip} + \text{inunfree} + \text{plotID} \quad (2)$$

Where $G(\text{nr})$ stand for germination success (number of individuals), offset is the mean offset, sed is the sedimentation rate (mm day^{-1}), precip is precipitation rate for each period (mm day^{-1}), inunfree is the maximum inundation-free period (days) and plotID is the random effect. A simulation study was performed to assess if simulations from the model complied with the observed data. The negative binomial model is well equipped to cope with the relatively large number of zeros (48%).

Mortality was analysed in all plots with vegetation present, including both seed-addition and control plots. Exploratory analyses gave no indication for non-linearity and mortality was therefore analysed using a generalized linear mixed model (glmmTMB). A weights factor was added to incorporate the total number of individuals for each plot. A zero-inflated binomial model was used to ensure that the model could cope with many zeros. The final zero-inflated binomial model was:

$$M = \text{inunfree} + \text{freq} + \text{bldif} + \text{plotID} \quad (3)$$

Where M stand for probability of mortality, inunfree is the maximum inundation-free period (days), freq is inundation frequency (average times day^{-1}), bldif is the bed-level difference (mm) and plotID is the random effect.

All model predictions were executed with a mean random effect (i.e. 0) and if more than two variables were modelled the mean of the remaining, least influential variable was used. Model predictions were displayed with 95% confidence intervals. All statistical analyses were performed with the statistical program R (R Core Team 2018) using additional packages 'plyr' (Wickham 2011), 'ggplot2' (Wickham 2009), 'lme4' (Bates et al. 2015) and 'glmmTMB' (Magnusson et al. 2017).

RESULTS

Natural seed germination was low with 20 germinated seedlings in the control plots as opposed to 260 individuals in the seed-addition plots (Fig. 5.2). The

seed-addition plots thus contained 12.5 times more individuals than natural control plots. Of the 20 seedlings in the control treatment, 16 germinated at the vegetation edge and 4 on the intertidal flat. Although it could not be tested statistically due to low germination rates, this could indicate that natural seed availability was higher at the vegetation edge. Supplying additional seeds led to an increase of *S. procumbens* individuals in the transition zone, both at the vegetation edge and the intertidal flat (Fig. 5.2). Germination of *S. procumbens* started halfway May and occurred until end of August. Mortality occurred from the moment seedlings were present but was highest in August and September. From the naturally present seedlings, one individual survived until the end of the experiment. Of the 260 seedlings established in the seed-addition plots, 26 individuals reached the seed-set stage. The variability of the environmental factors during the experimental period is displayed in figure 5.3.

Figure 5.2 Germination (positive) and mortality (negative) of *S. procumbens* individuals in each plot (1 m²) and period. Both treatments are shown, control with downward triangles and seed-addition with upward triangles. The colour of the circles indicates the position, green for plots at the vegetation edge and orange for plots on the intertidal flat. Plots are jittered on the x-axis to reduce overlap.

Germination occurrence and germination success

Temperature and sedimentation significantly influenced the absence and presence of germination (Table 5.1). Once germination was initiated, average temperature had increased by 6°C, from 10.5 °C to 16.5 °C (Fig. 5.3). Temperature had a positive effect, and sedimentation had a negative effect (Table 5.1). Sedimentation rates varied between 0 and 4.8 mm day⁻¹ and total sedimentation ranged from 0 to 110 mm. Germination success was halved at sedimentation rates of 0.5 mm day⁻¹ and nearly diminished above 2 mm day⁻¹ (Fig. 5.4).

Sedimentation, precipitation and inundation-free period were significant factors in determining germination success (Table 5.1). Comparing germination success across a sedimentation gradient and for three precipitation levels showed that the highest number of germinated individuals were found at low sedimentation and high precipitation rates (Fig. 5.4). Low (1 mm day⁻¹), mean (3.4 mm day⁻¹) and high (7 mm

Table 5.1 Model variables and results with respect to i) initiation of germination (i.e. absence or presence), ii) germination success (i.e. seeds that establish) and iii) mortality (seedlings that die before seed-set). LR are likelihood ratio tests to estimate the significance of each variable included in the final model. Est stands for the estimates $\pm$ SE. N.S. stands for not significant, meaning that the final model did not contain those variables based on forward model selection using AIC. The random effect was always added; zero-inflation was only required in the mortality analysis.

<i>Environmental variables</i>		Germination occurrence	Germination success	Mortality
Inundation frequency (times day ⁻¹), fig. 4	LR _{df=1}			18.5
	p	N.S.	N.S.	<0.0001
	Est.			2.73 $\pm$ 0.64
Inundation duration (hrs day ⁻¹)	LR _{df=1}	N.S.	N.S.	N.S.
Inundation-free period (days), fig. 4	LR _{df=1}			51.2
	p	N.S.	N.S.	<0.00001
	Est.			0.84 $\pm$ 0.12
Temperature (°C), fig. 2	LR _{df=1}	63.1		
	p	< 0.00001	N.S.	N.S.
	Est.	0.63 $\pm$ 0.15		
Precipitation (mm day ⁻¹), fig. 3	LR _{df=1}		6.1	
	p	N.S.	0.014	N.S.
	Est.		0.018 $\pm$ 0.07	
Sedimentation (mm day ⁻¹), fig. 3	LR _{df=1}	12.4	30.4	
	p	0.0004	<0.00001	N.S.
	Est.	-1.12 $\pm$ 0.38	-1.54 $\pm$ 0.31	
Erosion (mm day ⁻¹)	LR _{df=1}	N.S.	N.S.	N.S.
Bed-level difference (mm)	LR _{df=1}			5.7
	p	N.S.	N.S.	0.017
	Est.			0.024 $\pm$ 0.01
Elevation (m NAP)	LR _{df=1}	N.S.	N.S.	N.S.
Plot ID (random effect)	LR _{df=1}	0.18	5.1	22.88
	p	0.67	0.023	<0.0001
Zero-inflation	LR _{df=1}			81.4
	p	NA	NA	<0.00001
	Est.			-1.11 $\pm$ 0.30
n		176	112	132

Figure 5.3 Environmental variables measured throughout the experiment. Precipitation and temperature, minimum (blue), mean (black) and maximum (red), were measured on a marsh scale (top 2 graphs). Sediment and inundation characteristics are displayed per block (location/position combination), locations are indicated using shapes and position using colour (green at the marsh edge and orange for the intertidal flat).

day⁻¹) precipitation levels per interval were based on field values measured during the experiment. When seeds were added, initiation of germination as well as germination success were similar between the vegetation edge and the intertidal flat and were not significantly affected by initial elevation. Experimental plots were nested together (Fig. 5.1), which did not affect germination occurrence nor success (Table 5.1).

Figure 5.4 Predicted germination success (number of germinated individuals per m²) depending on sedimentation rates at three different precipitation rates: high, mean and low, respectively depicting 7.5, 3.4 and 1 mm precipitation day⁻¹. The shaded grey areas correspond to the 95% confidence interval for the predictions.

Seedling mortality

Mortality of *S. procumbens* increased by a longer inundation-free period (i.e. drought stress), increased inundation frequency (drowning), and with increasing bed-level difference (dislodgement) (Table 5.1). Probability of mortality was modelled along an inundation-free gradient, ranging from 1.7 to 7 days, and three levels of inundation frequency were included (Fig. 5.5). High, mean and low frequencies were based on measured field values, respectively 1.4, 1.1 and 0.6 inundations per day. Greater bed-level variations slightly increased mortality (Table 5.1). The measured bed-level difference within one period ranged up to 45 mm (Fig. 5.3). Mortality was similar between the vegetation edge and the intertidal flat, implicating that mortality was not affected by initial elevation.

DISCUSSION

The aim of this study was to identify the environmental variables that determine the establishment success of the annual *S. procumbens* in the highly dynamic transition zone located between salt marsh and intertidal flat (Fig. 5.2 and 5.3). The experiment confirmed our first hypothesis that temperature is crucial for the occurrence of germination. However, the impact of fresh water was not significant on the initiation of germination, but did increase germination success. In contrast to our second hypothesis, we did not find any significant effect from location (vegetation edge vs. intertidal flat) on germination or mortality. In line with our third hypothesis, we found that sedimentation

had a strong negative effect on germination (initiation and success). Additionally, we found that both frequent inundation and an extended inundation-free period increased seedling mortality significantly (Table 5.1).

Difference between seed-addition and control treatment

A similar germination and mortality patterns between the vegetation edge and intertidal flat may be explained by the lack of variation in elevation. Pioneer vegetation can establish at an elevation of 0.5 m below mean high water (Wang & Temmerman 2013). At this study site, mean high water was 0.95 m above NAP and elevation in the transition zone ranged between 0.88 and 1.04 m above NAP, fulfilling the elevation requirement at all experimental plots. Germination success and mortality were therefore not affected by the initial elevation (Table 5.1). Unexpectedly, natural establishment of *S. procumbens* in the control treatment was very low. This may indicate that seed availability is limiting natural colonization of *S. procumbens* in the transition zone, as was already shown for a similar marsh area nearby (van Regteren et al. 2019). It is often assumed that seeds are not limited when a seed source is close by (Rand 2000; Wolters et al. 2005; Erfanzadeh et al. 2010c; Friess et al. 2012; Zhu et al. 2014). *S. procumbens* seeds do not have extensive floating capacity and disperse at a close range (Huiskes et al. 1985; Rand 2000). A local seed source was present, attested by dead standing material from the previous year (Ellison 1987a; Rand 2000). In the field, however, only 20 individuals germinated naturally, as opposed to 260 when seeds were added (Fig. 5.2).

Figure 5.5 Predicted seedling mortality (probability) before seed-set as a function of an increasing inundation-free period (i.e. potential drought build up) for three different inundation frequencies: high, mean and low, respectively depict 1.4, 1 and 0.6 inundations day⁻¹. The shaded grey areas correspond to the 95% confidence interval for the predictions.

It is probable that seed availability is limited (van Regteren et al. 2019). Causes for seed limitation could be low viability of seeds (Erfanzadeh et al. 2010a), erosion after seed dispersal (van Regteren et al. 2019) or burial through sedimentation or bioturbation (Zhu et al. 2014; van Regteren et al. 2017).

Temperature and precipitation promote germination

Temperature largely determined whether seed germination was initiated. Germination only occurred after average temperature had risen above 10.5°C (Table 5.1 and Fig. 5.2). This is in line with previous studies showing that low temperatures can inhibit germination, by forcing seeds to remain dormant (Khan, Gul & Weber 2000). When temperatures increase early in the season, the growing season is prolonged. The length of the growing season will likely affect the size of *S. procumbens* individuals and its reproductive success. Larger plants carry more seeds and thus have a higher reproductive output (Boorman, Hazelden & Boorman 2001).

Precipitation had no significant effect on the initiation of germination but had a strong positive effect on the germination success of *S. procumbens* (Fig. 5.4 and Table 5.1). This neutral effect on the initiation is probably because precipitation happened frequently. There was only one interval that contained no precipitation, which was in early May when germination was not yet initiated (Fig. 5.2 and 5.3). From *Salicornia europaea*, *Salicornia bigelovii* and *Salicornia virginica* it is known that fresh water input stimulates seedling germination and establishment, which is likely related to decreased soil salinity and soil moisture (Noe & Zedler 2001; Carter & Ungar 2003; Zedler, Morzaria-Luna & Ward 2003).

Inundation regime increases seedling mortality

Inundation frequency has been suggested as the main factor that regulates the seaward extent of vegetation establishment (Balke et al. 2016) and the inundation-free period provides an essential hydrodynamically undisturbed window for germination (Wiehe 1935). In our study, germination (occurrence and success) was not directly influenced by inundation frequency (ranging from 0.5 to 1.5 times per day), and the inundation-free period only had a small effect on germination success (Table 5.1). This contrast is probably because the observed inundation-free period during the experiment ranged from 1.7 to 7 days (Fig. 5.3) which complies with the minimum requirement of two days free of disturbance (Wiehe 1935).

Mortality of *S. procumbens* with increasing inundation frequency as well as with increasing length of inundation-free periods (Fig. 5.5 and Table 5.1). High hydrodynamic stress, due to frequent inundation, enlarges the risk of seedlings getting washed away by strong waves and currents (Houwing 2000). An extended inundation-free period excludes hydrodynamic stress but does contribute to soil dehydration and increased

soil salinity. Soil desiccation may lead to desiccation cracks and thus less strongly rooted vegetation (Noe & Zedler 2001) and severe drought might even change the entire species composition in a salt marsh (Strain et al. 2017). Seedling mortality in our study was highest when an inundation-free period was followed by frequent inundation. The disappearance of *Salicornia* spp. can be regarded as an early warning signal for changing inundation regimes (Balke et al. 2016).

Sedimentation reduced establishment

Sedimentation prevented the initiation of germination and high sedimentation rates also reduced germination success (Fig. 5.4 and Table 5.1). For *Salicornia* spp. the maximum depth from which germinated seeds were able to reach the surface was found to be 10 mm (Huiskes et al. 1985). As total sedimentation ranged from 0 to 110 mm, this becomes precarious when sediment deposition occurs in sudden events. Sedimentation had no direct significant effect on the mortality, but the bed-level variation did significantly increase mortality (Table 5.1). Research on seedling response related to sediment dynamics is scarce and limited to the lab. Larger seedlings are better at outgrowing deposited sediment as shown for *Spartina anglica* (Cao et al. 2018). *S. europaea* can cope with low sedimentation rates as well (Boorman, Hazelden & Boorman 2001), but the rates used in their experiment (0.16 mm day^{-1}) were much lower compared to our field values (average 1.01 mm day^{-1}). Establishment success of marsh plants will be undermined when individuals cannot outgrow sedimentation rates. Other species, for example the perennial *P. maritima*, thrive on sediment deposition and form accretion hummocks which enables a stable and diversified marsh community (Langlois, Bonis & Bouzille 2003). Freshly sprouted *S. procumbens* are only a few mm tall and will be buried completely when sudden sedimentation events occur. In addition to plant size, plant stiffness is also an influential factor (Bouma et al. 2013), with flexible species being more vulnerable. A stable soil generally facilitates vegetation establishment (Bouma et al. 2016), thus a dynamic bed-level is expected to set additional pioneer vegetation establishment boundaries.

Neither *S. procumbens* germination nor mortality were significantly affected by erosion (Table 5.1). This is in contrast with pioneer species *S. anglica*, which was more negatively affected by erosion than by sedimentation (Cao et al. 2018). However, erosion rates were highest at the start of the experiment, when germination did not yet occur (Fig. 5.2 and 5.3), which can explain the lack of effect suffered from erosion. Higher erosion rates will probably show a different response, especially when seedlings have just germinated. Young established seedlings were observed to have deep roots in the transition zone and we suspect the root system to be adaptive regarding the stressful conditions at the marsh edge. A similar response in root investment was shown for *S. anglica* seedlings regarding wave stress (Cao et al. 2019).

The vital area for salt marsh expansion is the transition zone, even though that is where bed-level is most dynamic (Widdows, Pope & Brinsley 2008; Willemsen

et al. 2018). There is an intricate interplay between the sedimentation and erosion at the salt marsh – intertidal flat transition zone. It is essential to distinguish between sedimentary processes, erosion, sedimentation and bed-level dynamics, to increase the understanding of vegetation establishment thresholds. For the initial establishment phase, annuals and perennials are both vulnerable to high sediment dynamics (Cao et al. 2018, this study). Once established, however, perennials are expected to be less fragile than annuals. Surmountable sedimentation, erosion and sediment dynamics are species specific but also life stage specific and depend on temporal and spatial manifestations of sediment dynamics (Langlois, Bonis & Bouzille 2003; Bouma et al. 2013; Lo et al. 2017; Cao et al. 2018).

Implications for salt marsh expansion

Multiple factors affected the establishment success of *S. procumbens* in our field site. However, the impact of each factor depends on a combination of other factors and their intensity. Additionally, effects will differ between marsh sites and between plant species and the life stage that the species is in, resulting in a complex interplay. The density of halophyte seedlings can be quite variable between years (Morzaria-Luna & Zedler 2007). For example, asynchrony in weather conditions (temperature and precipitation) can greatly influence the start of the growing season, affecting plant size and numbers. This can have proliferating effects on the next years reproduction potential (especially for annuals) and hence for salt-marsh expansion and stability. Perennial pioneers such as *S. anglica* may be less affected by years with unfavourable weather conditions due to their ability for rhizomatous and vegetative growth (Zhu et al. 2014), although large expansions of vegetated areas or colonization of new areas are more likely to happen through seed dispersal.

Salt-marsh management that aims to restore or stimulate marsh growth through the establishment of pioneer species in the transition zone need to appoint sites which provide appropriate boundary conditions. In addition to the well-known effects of inundation regime and elevation, our study showed that sediment dynamics, temperature and seed availability should be included when assessing the potential of areas for salt-marsh restoration. Even a narrow stretch of marsh vegetation functions as a coastal protection buffer through wave attenuation (Yang et al. 2012). Supplying seeds led to an increase in successful establishment of *Salicornia procumbens*. Although, the effect of seed supply will differ per area and species used (Rand 2000; Lindig-Cisneros & Zedler 2002; Morzaria-Luna & Zedler 2007), especially marsh restoration sites may benefit from adding seeds to expedite marsh development, provided that they contain the appropriate physical boundary conditions (Lindig-Cisneros & Zedler 2002).

In the Wadden Sea, wind conditions exert a strong influence on sediment transport (Schulz & Gerkema 2018). Dynamics in intertidal areas may inhibit effective pioneer establishment by reducing germination or increasing mortality before seed-set (Cao et al. 2018; Cao et al. 2019). Furthermore, the role of natural variability in external

forcing for the long-term dynamics of biogeomorphic systems deserves emphasis (Balke, Herman & Bouma 2014). Short-term variability in environmental conditions can thereby exert considerable impact on vegetation establishment, especially for annuals, such as *S. procumbens*. An additional challenge in salt-marsh management is that vegetation establishment opportunities are likely to be influenced by climate change, through increased disturbances by wind and waves (Donat et al. 2011), changes in the precipitation regime (Alley 2004) and altered sediment dynamics (Cahoon & Reed 1995).

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CHAPTER 6

General synthesis

Marin van Regteren

This thesis addressed the establishment of initial pioneer vegetation in the transition zone between the vegetated salt marsh and the un-vegetated intertidal flat. The transition zone holds the potential for marsh expansion if pioneer plants are able to establish successfully. Field studies, as well as laboratory studies, were carried out to unravel critical factors that affect initial vegetation establishment of salt marsh pioneer vegetation. Establishment of pioneer vegetation may rely on multiple boundary conditions of both abiotic and biotic factors, such as elevation, seed availability, physical disturbance and benthos. In this synthesis, the research questions and main findings for each Chapter are summarised. Followed by a discussion on the outcomes of the research including recommendations for future ecological research. These insights will advance the success of restoration and conservation projects. Finally, implications for coastal management are given.

THESIS OVERVIEW

What abiotic and biotic variables determine the transition zone between the salt marsh and the intertidal flat?

In Chapter 2, we characterized the low marsh to the intertidal flat gradient in which plant species and cover change from almost fully vegetated to bare intertidal flat. We determined elevation, the benthic community, seed abundance, microphytobenthos density and soil characteristics (surface salinity, grain size, bulk density, water content, organic matter content). We concluded that the sparsely vegetated transition zone is not a separate zone with properties in between those of the pioneer salt marsh and unvegetated tidal flat. Instead, the transition zone shows high similarity to the upper intertidal flat both regarding the benthic community and environmental conditions.

How does dispersal timing affect seed retention in the transition zone and hence influence initial vegetation establishment?

In Chapter 3, seed retention was examined for three salt marsh species, *S. procumbens*, *S. anglica* and *A. tripolium*, using a field simulated seed dispersal before and after winter. Seed dispersal was simulated at the marsh edge and on the bare intertidal flat, to gain insight in the availability of seeds for vegetation establishment in spring. The pioneer establishment success was assessed in the field and the seed bank, i.e. seed depth distribution, as well as viability of the seeds were measured in the laboratory. Our experiment showed that a window of opportunity for establishment occurred for *S. procumbens* and *S. anglica* but natural seed availability was low and formed an important threshold for vegetation establishment in the transition zone.

What is the effect of bioturbating oligochaetes on seed distribution and seedling establishment?

In Chapter 4, we studied the effects of oligochaete bioturbation on algal biomass production, vertical seed distribution, *Salicornia* spp. germination and on facilitating or hindering seedling establishment in a microcosm laboratory experiment. Additionally, the effect of oligochaetes on sediment properties, such as oxygen penetration depth, water content and bulk density, were determined in a second microcosm experiment. Our laboratory-scale experiment indicated that oligochaete bioturbators, even though they are small, may reduce the establishment of pioneer vegetation in the transition zone by toppling of seedlings. Conveyor belt feeding (bioturbation) partially transported seeds into the soil, incorporating them into the seed bank. Active burrowing massively increased the soil oxygenation depth. Furthermore, bioturbation caused a reduction in microphytobenthos biomass which may have subsequent effects on soil stability and erosion.

*What are the main factors that drive germination occurrence, success and mortality of *Salicornia procumbens* in the transition zone?*

Germination, seedling establishment and mortality of *Salicornia* spp. are affected by hydrodynamics and other environmental variables. These range from weather conditions, such as temperature and precipitation, to sediment dynamics and inundation regime. All combined factors affect the windows of opportunity for vegetation establishment. In Chapter 5, multiple environmental variables were examined to elucidate on boundary conditions for successful pioneer establishment. In a field experiment in the transition zone, we assessed germination occurrence, germination success and seedling mortality in plots with and without seed addition, during the growing season. Our results showed that the most critical factors for *S. procumbens* germination were the sedimentation rate, temperature and precipitation. Mortality was primarily driven by the inundation regime and bed-level variation. Natural viable seed availability was low, demonstrated by the sparse establishment of vegetation in the plots where seeds were not supplied.

Vegetation establishment in transition zones

The first and most important step of lateral expansion of a salt marsh is the establishment of pioneer vegetation in the transition zone. The establishment of pioneer vegetation depends on multiple environmental and physical variables (Karrenberg, Edwards & Kollmann 2002; Gurnell et al. 2006; Balke et al. 2011; Ma, Zhou & Du 2011). In Chapter 5, the main factors that drive germination and mortality of *Salicornia procumbens* in a marsh transition zone were studied. Surface elevation is a key boundary condition and a minimum of 0.5 m below MHT is appropriate for vegetation establishment (Wolters et al. 2005; Wolters et al. 2008; Wang & Temmerman 2013).

This condition was amply met in the transition zones of our studies (Ch. 2, 3 and 5). Furthermore, a sufficient inundation-free period (Wiehe 1935; Balke et al. 2011) and a period of low hydrodynamic disturbance must occur for successful establishment (Hu et al. 2015a). Weather conditions, i.e. an increase in precipitation and temperature (Ch. 5), supported vegetation establishment. Physical boundary conditions, such as an inundation-free period and low hydrodynamic energy were also met (Ch. 3 and 5). Our results showed that, in the field, *S. procumbens* seedlings suffered from sedimentation, as small sprouts get entirely buried (Ch. 5).

Concurring with Rand (2000), we found that adding seeds increased vegetation establishment success (Ch. 3 and 5). Nevertheless, this study contradicts Rand's (2000) conclusion that post-dispersal factors (light availability, soil salinity and oxygenation) are more important than seed availability, especially in previously un-colonized zones. Using seed additions in the transition zone, we have shown that *S. procumbens* and *S. anglica* successfully established (Ch. 3 and 5), even though the soil in this zone was anoxic below the upper 3 cm (Ch. 2). Light availability is ample in the transition zone and soil salinity was comparable to the vegetated pioneer zone (Ch. 2). This indicates that, in the transition zone, seedling recruitment is not limited by a lack of windows of opportunity for establishment but by seed availability. Only *A. tripolium*, a biennial species less adapted to anoxic soil (Davy et al. 2011), was constrained in its establishment by environmental factors in the transition zone. This resulted in no establishment of *A. tripolium* seedlings (Ch. 3). Once *S. procumbens* has established, *A. tripolium* may gradually replace *S. procumbens* stands, indicating early succession (van der Wal & Herman 2012). *Suaeda maritima* could be more promising than *A. tripolium* in the transition zone, being an early colonizer with a high seed production (Mossman et al. 2012). Pioneer tussock forming species such as *S. anglica* and *Bolboschoenus maritimus* may be less dependent on seeds as they spread also vegetatively, with plant parts or through rhizomatous growth (van der Wal, Wielemaker-Van den Dool & Herman 2008; Silinski et al. 2016a). Still, they are likely to depend mostly on seeds for long-distance dispersal (Zhu et al. 2014). Many annual and biennial pioneer species depend fully on seeds for successful establishment emphasizing seed limitation as a potential threshold to vegetation establishment.

Bioturbation affected seedling establishment

The role of bioturbating oligochaetes in facilitating or hindering vegetation establishment in the transition zone was examined in Chapter 4. Oligochaete bioturbation toppled freshly sprouted *Salicornia* spp. seedlings, in microcosm lab experiments (Ch. 4). The burrowing of oligochaete worms disturbed effective rooting, thereby inhibiting successful establishment (Fig. 6.1). These effects were not so strong as to cause a mutual exclusion in the field, as shown for cordgrass (*Spartina anglica*) and the polychaete *Arenicola maritima* (van Wesenbeeck et al. 2007). In the salt marsh, the highest density of oligochaetes was found in the vegetated pioneer zone (Ch. 2). The pioneer zone had both successful establishment of multiple salt marsh pioneer species and the highest

number of bioturbating benthos. Thus, meiofaunal bioturbation may locally inhibit the establishment of *Salicornia* spp. seedlings, but on a marsh scale its impact does not seem to be substantial enough to completely inhibit the establishment of pioneer vegetation in the transition zone. Wolters et al. (2005) similarly conclude, for numerous managed realignments sites, that marsh erosion cannot be attributed to invertebrate bioturbation, other factors such as strong winds, increased wave heights and high tides are contributing to marsh retreat.

Bioturbation reduces sediment stability (De Deckere, Tolhurst & De Brouwer 2001), and this may limit initial establishment from seeds (Houwing 2000). Without oligochaetes present, a smooth microphytobenthos biofilm had formed on the surface, stabilizing the soil in lab conditions. With oligochaetes present, microphytobenthos abundance was much lower and the soil surface was rough (Ch. 4). Although not quantified, we observed this to have subsequent effects on the erodibility of the soil surface. Stable soils are favourable for initial vegetation establishment from seeds (Houwing et al. 1999). An increase in root density further enhances soil stability (De Battisti et al. 2019), creating a positive feedback for additional vegetation establishment. The soil surface of our field site, however, was not stable due to high dynamics in sediment deposition and erosion (Intermezzo). Variation in bed-level height of multiple centimetres can even occur within days (Ch. 3). In areas with a dynamic sedimentary regime, therefore, the effect of benthic bioturbation is expected not to exceed the effect of sediment deposition and erosion. However, stable areas with low deposition or erosion rates, such as the back-barrier marshes of the Wadden Sea islands, are likely to be more affected by bioturbation. The amphipod *Corophium volutator* is known to reduce sediment stability and increase erodibility, while its presence in transition zones is common (Gerdol & Hughes 1994). Smaller benthic fauna is studied in marshes (Kneib 1984; Sacco, Seneca & Wentworth 1994; Craft & Sacco 2003) or in the transition zone (Sterzynska & Ehrnsberger 2000; Whaley & Minello 2002; Lange et al. 2018) but neither in relation to subsequent interactions with sediment dynamics.

Contrary to the negative effect of bioturbation on soil stability and vegetation establishment, bioturbation can also have beneficial impacts. The burrow network of oligochaetes resulted in at least a ten-fold increase of the soil oxygenation depth over a 2-month period (Ch. 4). Soil reworking of oligochaetes, harpacticoid copepods and collembolans increases soil oxygenation and this enhances organic matter mineralization and nutrient availability (Christian 1989; Mermillod-Blondin et al. 2004; Mermillod-Blondin & Rosenberg 2006; Bonaglia et al. 2014). The bioturbated burrows cause bio-irrigation which stimulates the microbial community and biogeochemical cycles in the soil (Mermillod-Blondin & Rosenberg 2006). Consequentially, soil reworking may provide essential requirements for plants in the transition zone, where nutrient availability can be low (Daleo et al. 2008; Alberti et al. 2010). The oxygenated soil is favourable for the further development of plant communities (Ursino, Silvestri & Marani 2004), with later successional plant species such as *Suaeda maritima*, *Atriplex portulacoides* and *Aster tripolium*. Additionally, stimulation of nutrient cycling and organic matter mineralization by meiofauna may pave the way for zonal succession in the pioneer zone, dominated by *S. procumbens* and *S. anglica*. Although microcosm experiments show reduced pioneer

vegetation establishment due to bioturbation, we suggest that, overall, the positive effects of small bioturbating benthos may be stronger than the negative influence. Benthos may enhance the establishment of later-successional plants due to their positive influence on the soil environment. Additionally, not all seedlings were toppled (Ch. 4) and once sufficient root anchorage is reached the negative effect dissipates. To properly assess the effects of oligochaete bioturbation on vegetation establishment at a marsh scale, field or mesocosm experiments should be performed that include and exclude bioturbation but also incorporate other natural variation such as sediment and inundation dynamics.

Figure 6.1 Graphical depiction of pioneer vegetation establishment in spring. Sediment dynamics, bioturbation, inundation regime and weather conditions affect the successful establishment of *S. procumbens*.

The seed bank in transition zones

Pioneer seedling establishment may occur from yearly dispersed seeds or from a seed bank. In a seed bank, germination is delayed until conditions become more appropriate. When there is a deficiency of newly dispersed seeds, vegetation establishment becomes dependent on a seed bank. *S. anglica* and *S. procumbens* are the main pioneer species able to colonize the transition zone (Erfanzadeh et al. 2010a). In the transition zone and on the bare tidal flats, the abundance of seeds in the seed bank other than some *Salicornia* spp. was low to non-existent (Ch. 2 and 3). *S. anglica* seeds were not present in the natural seed bank (Ch. 2 and 3). For *S. anglica*, absence from the seed bank but its presence in the aboveground vegetation is observed more often (Wolters & Bakker 2002; Chang, Veeneklaas & Bakker 2007; Erfanzadeh et al. 2010b). A lack of viable seeds may originate from a fungus, *Claviceps purpurea*, that invades the spikelets and impedes viable seed production (Marks & Truscott 1985). Although *S. anglica* can expand with rhizomes, for its long-distance dispersal it is mainly dependent on seeds

(Zhu et al. 2014), emphasizing the importance of seeds for vegetation establishment even in vegetative species.

For *Salicornia* spp. a natural seed bank was observed. Viable seeds occurred up to 15 cm deep. Seed abundance usually decreases with depth (Wolters & Bakker 2002; Erfanzadeh et al. 2010b). We observed a natural peak of *Salicornia* spp. seeds between 4 and 9 cm deep (Ch. 3). Therefore, these seeds may provide a source of viable seeds for *Salicornia* spp. establishment in years that severe erosion takes place. Then these seeds may re-emerge on the soil surface. In wetland and flood meadow systems, desiccation cracks were the cause of a bimodal distribution of seeds (Espinar, Thompson & Garcia 2005; Burmeier et al. 2010). Viability of the seeds revealed that only the seeds that dispersed during the presence of cracks contained viable seeds at depth (Espinar, Thompson & Garcia 2005). On the intertidal flat such desiccation cracks also occur (visible in Fig. 2.2) but usually in the warmer and dryer spring/summer period and not during the dispersal time of *Salicornia* spp. seeds. Generally, deeper seeds are older and show a gradual decline in viability (Espinar, Thompson & Garcia 2005). A similar result was found in our study, the viability of the seeds at depth was much lower than at the surface. Indicating that the peak of *Salicornia* spp. seeds found at depth were likely from previous fruitful years.

The size of the seed bank increases when seeds get buried by sediment deposition or through bioturbation. In both marshes and hydrodynamic riverine systems, seed retention is positively correlated to sediment deposition (Goodson et al. 2003; Zhu et al. 2014). The vertical distribution (i.e. burial depth) of seeds affects the availability of seeds for germination and establishment. Shallow burial of seeds can result in persistence in the soil but the maximum depth of emergence for salt marsh vegetation establishment is species dependent. *Salicornia* spp. seeds cannot emerge when buried below 1 cm deep and have a light requirement for germination (Philipupillai & Ungar 1984; Huiskes et al. 1985). *S. anglica* seeds do not reach the surface when buried below 5 cm deep, but shallow burial (below 2 cm) yields a higher success rate (Groenendijk 1986; Zhu et al. 2014). From the two main pioneer species, *S. anglica* is better equipped than *Salicornia* spp. to handle sediment deposition. Prairie wetland seeds, which are generally smaller (0.05–1 mm vs. Table 3.1), are inhibited to reach the surface for germination already with deposition of 2.5 mm of sediment (Jurik, Wang & Vandervalk 1994). There seems to be a relationship between seed size and maximum burial depth for emergence (Huiskes et al. 1985; Groenendijk 1986; Jurik, Wang & Vandervalk 1994; Zhu et al. 2014). Thus, surmountable burial depth is species-specific and the maximum is dependent on the locally present pioneer species. When sedimentation surpasses this burial depth limit, vegetation establishment will be impeded. Consequentially, ecosystems with high sediment accretion rates may have reduced establishment of vegetation from seeds.

Bioturbating benthos affects vertical seed distribution

Bioturbation by macrofauna can affect vertical seed distribution (Gerdol &

Hughes 1993; Delefosse & Kristensen 2012; Zhu et al. 2016b) but this was not yet known for oligochaetes (Ch. 4). In Chapter 2, we examined all benthos larger than 250 μm on a salt marsh to tidal flat gradient. Benthos smaller than 1 mm were most common. The dominant groups were 1) oligochaetes in the pioneer zone, 2) harpacticoid copepods in the pioneer zone and tidal flat hollows and 3) collembolans (springtails) in the transition zone and tidal flat hummocks. Oligochaetes, harpacticoid copepods and collembolans are known for their sediment reworking capacity (Christian 1989; Mermillod-Blondin et al. 2004; Bonaglia et al. 2014). Even though macrofauna is largely absent from our study area, the smaller benthic community effectively bioturbated the soil (Ch. 2, 3 and 4). In Chapter 4, we found that oligochaetes transported seeds up to 3 cm deep through conveyor belt reworking. The impact of bioturbation was also observed in Chapter 3. Two months of field bioturbation transported approximately 10% of seeds up to 3 cm deep in the transition zone (Ch. 3). It is unlikely that benthos will rework all available seeds, therefore we expect that sufficient seeds remain in the surface layer to germinate and establish (unless other processes cause seed loss). Seed burial was irrespective of seed size, *S. procumbens*, as well as *A. tripolium* and *S. anglica* seeds were all evenly transported downwards. In contrast, in chalk grasslands, bioturbation by earthworms causes an upward transport of seeds (Willems & Huijsmans 1994). Functional feeding and reworking strategies are species-dependent (Quintana, Tang & Kristensen 2007; Delefosse & Kristensen 2012; Zhu et al. 2016a), illustrating that the consequences of soil reworking will depend on the system studied and the agent of bioturbation.

Seed retention in transition zones

In Chapter 3, we studied how dispersal timing affected seed retention and how this may influence initial vegetation establishment in the transition zone. Retention of seeds was a key factor that limited vegetation establishment in the transition zone. Seeds that were manually dispersed in December were not retained in the transition zone (Ch. 3). Seeds manually dispersed after winter (in March) were retained in the transition zone (Ch. 3). Different seed species, with differential sizes and characteristics, were used, but no influence on retention was found. September to December is the period in which salt marsh pioneer species generally ripen and disperse their seeds in Northern Europe (Hutchings & Russell 1989; Huiskes et al. 1995; Rand 2000; Wolters, Garbutt & Bakker 2005a). Very few studies, however, include the months January to March. Zhu et al. (2014) surveyed January to March for *S. anglica* seed arrival, which occurred until March, but the peak arrival was in January. The extent of dispersal time after December of many salt marsh plant species remains unstudied, but is of vital importance for subsequent establishment success, especially in highly dynamic areas. When early dispersal is followed by surface soil erosion, the abundance of seeds available for vegetation establishment in the transition zone becomes limited (Fig. 6.2). Indicating that lateral expansion of the marsh becomes inconceivable. At these marshes, the direction of secondary dispersal of seeds is unknown, although the main direction of transport is usually landwards (Huiskes et al. 1995). Those seeds may end up in drift-line material, which provides inadequate microhabitats for the successful establishment of

pioneer marsh species (Wolters & Bakker 2002; Chang et al. 2005). The dispersal of seeds into unsuitable habitats can have severe consequences for areas that are dependent on seeds for their stable persistence or expansion. Additionally, newly dispersed seeds have a higher chance of resulting in successful vegetation establishment than seeds from previous years (Wolters & Bakker 2002; Espinar, Thompson & Garcia 2005; Erfanzadeh et al. 2010a).

Mobility of the surface layer has been pinpointed as an important cause for seed loss (Houwing et al. 1999). However, this study related seed retention to mud content (mud content above 30% increased seed retention by 66%). Sandy sediments have a lower erosion threshold and therefore seeds disperse away more easily together with the sediment (Houwing 1999; Houwing et al. 1999). Our study area has a high mud content, between 60% and 72% (Ch. 2), and a loss of seeds due to soil surface erosion was still found during winter (Ch. 3). Thus, grain size alone cannot explain the loss of seeds. Regardless of the cause for seed loss, the natural abundance of viable pioneer seeds was low in the transition zone during the vegetation establishment period (spring) (Ch. 2, 3 and 5). This indicated that, although a seed source was in proximity, seeds were not retained in the area where salt marsh expansion could occur. Sediment erosion after seed dispersal will strongly reduce the availability of viable seeds. The potential growth of the marsh was not limited by windows of opportunity for vegetation establishment, since environmental factors such as salinity, temperature, elevation and inundation regime, provided appropriate windows of opportunity (observed in Ch. 3 and 5). When seed availability is low to non-existent then vegetation establishment will not follow.

Figure 6.2 Graphical depiction of environmental processes that affect seed availability. High tides, waves and surface sediment erosion cause secondary dispersal of seeds from multiple marsh species (Ch. 3).

This may seem evident, yet many salt marsh ecologists presume seeds are not limited when a seed source is close by (Rand 2000; Wolters et al. 2005; Erfanzadeh et al. 2010c; Friess et al. 2012; Zhu et al. 2014). We have shown that this presumption does not hold true for all areas (Ch. 2, 3 and 5).

A seed limitation seems to be most profound in marsh-flat transition zones as opposed to the inner areas of salt marshes that usually do not lack seeds. Denser vegetation usually retains more seeds than open vegetation (Chang et al. 2008). The higher marsh is supplied with seeds that are gathered within drift-line material and which is deposited during spring high tides or storm surges (Wolters & Bakker 2002; Chang et al. 2005). Additionally, marsh restoration efforts have shown that if restoration sites are close to existing marshes, vegetation establishment is often not limited by seeds (Wolters, Garbutt & Bakker 2005a; Elsey-Quirk, Middleton & Proffitt 2009). Other areas with dynamic water to land transition, such as river banks, are usually not limited by seed availability as they get supplied from upstream riverbanks or by wind-dispersal (Skoglund 1990; Vogt, Rasran & Jensen 2004; Gurnell et al. 2006). Additionally, some plant species have adapted to establish in highly dynamic areas. For example, *Salicaceae* (willow) seeds germinate immediately on moist surfaces after dispersal, thus preventing secondary dispersal (Karrenberg, Edwards & Kollmann 2002). Our field site, in which salt marsh expansion was the objective, illustrated that seeds can be limiting, even with a seed source in proximity. Local thresholds for successful pioneer vegetation establishment will differ between areas, therefore locations that are proposed for salt marsh restoration or to stimulate lateral expansion must previously be surveyed for its sedimentary regime and seed availability, to increase the chance of a favourable outcome.

Management implications

In Chapter 2, we examined if the transition from the tidal flats to the low marsh is gradual or whether there is a distinct threshold between the bare intertidal flats and the vegetated marsh, based on physical and biological parameters. We found a sharp transition from the vegetated marsh to the bare tidal flat, probably originating from an acute decrease in soil oxygenation and moreover the absence of pioneer seeds. In forests, early successional colonizers are reliant on chance events of seed availability (Leps et al. 2000). This was contradicted by Erfanzadeh et al. (2010c) for salt marshes, however, we must conclude that pioneer seeds are not ubiquitous on all marshes (Ch. 2, 3 and 5). The development of Westhoek has shown stable periods alternating with years of expansion (Baptist et al. 2019b). Indicating that a successful seed-set in the transition zone in one year may shift the system from bare tidal flat to vegetated marsh state (Ch. 2). This would result in step-wise marsh expansion instead of gradual expansion. This would fit the observation of the development at Westhoek (Baptist et al. 2019b). Windows of opportunity for vegetation establishment were present in multiple years, e.g. 2017 and 2019 (Ch. 3 and 5) but the main threshold for marsh expansion was seed limitation (Ch. 2, 3 and 5).

Salt marshes may keep pace with sea-level rise by vertical accretion, provided that sufficient sediment is available or marshes can migrate inland (Kirwan et al. 2016a). Salt marshes in Northern Europe are often constrained by dykes, thus migration inland is impossible. Evidently, to retain salt marsh areas they must accrete vertically considering relative sea level rise. Prospected sea level rise, following the IPCC AR5 scenario, for the Wadden Sea area is 0.07 ± 0.06 m for the period 2018-2030 (Vermeersen et al. 2018). this corresponds to 0.6 ± 0.5 cm year⁻¹. With an accretion rate of 1.63 ± 1.05 cm year⁻¹ (Intermezzo), it is likely that Westhoek will keep pace with sea level rise in the near future. The Mud Motor initiative aimed at stimulating marsh expansion by providing additional sediment (Baptist et al. 2019a). This research showed that even when salt marshes accrete vertically this does not automatically lead to horizontal expansion of the marsh (Intermezzo). High sedimentation rates and/or a dynamic bed-level may interfere with seed availability and establishment success of pioneer species (Cao et al. 2018, Ch. 3 and 5), potentially reducing windows of opportunity for seedling establishment (Balke et al. 2011; Poppema et al. 2019, this thesis). Thus, when nature managers aim to stimulate marsh expansion using dredged sediment disposal, they may additionally need to provide seeds in the transition zone in spring.

Especially with rising sea levels and global climate change, nature-based coastal protection and building with nature schemes will become of increasing importance due to its more sustainable and cost-effective character (Temmerman et al. 2013; De Vriend et al. 2015). The width of nature-based flood defence determines its protective capacity (Barbier et al. 2008). Therefore, understanding the initial pioneer vegetation establishment is essential and will provide insights on how to improve and stimulate establishment and potentially enhance marsh expansion. This will aid sustainable management of these valuable and generally degrading salt marsh ecosystems. Climate change is expected to increase storm frequencies and alter hydrodynamic regimes (Woth, Weisse & von Storch 2006; Kirwan et al. 2010). Wind conditions exert a strong influence on sediment transport, in the Wadden Sea (Schulz & Gerkema 2018; Colosimo et al. in review). Strong winds, high tides and increased wave heights are a determinant factor for marsh erosion (Wolters et al. 2005). For cliffed marshes, an increase in storms may enhance vertical accretion while simultaneously causing lateral marsh erosion (van der Wal & Pye 2004). In these marsh types, the rate of retreat will increase without pioneer vegetation establishment in the transition zone (van de Koppel et al. 2005; van der Wal, Wielemaker-Van den Dool & Herman 2008). More frequent storms and altered hydrodynamics will likely impact sediment dynamics by increasing erosion which may further reduce seedling establishment and seed availability. When studying pioneer vegetation establishment, seed availability in the growing season must not be assumed but has to be examined. Subsequently, to reach effective management in salt marsh restoration, seeds could be supplied in the transition zone to increase the salt-marsh area and/or width.

A wide-angle photograph of a body of water, likely a bay or fjord, during a dramatic sunset or sunrise. The sky is filled with large, dark, billowing clouds that are illuminated from below by the low sun, creating a vibrant orange and pink glow. The water is dark and calm, with numerous small, irregular ice floes scattered across its surface. The horizon line is visible in the distance, where the water meets the sky. The overall mood is serene yet powerful, capturing a moment of natural beauty.

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SUMMARY & SAMENVATTING

Importance of salt marshes

Positive effects of salt marshes on biodiversity, nature-based coastal protection and other ecosystem services are unequivocal. The future of salt marshes is threatened by climate change and sea level rise, which could result in salt marsh degradation and erosion. In addition, the presence of dykes constrains the retreat of salt marshes inland. Salt marshes can keep pace with sea level rise through vertical accretion if sufficient sediment is available and vegetation establishment at the salt marsh edge is an essential requirement to keep marshes vital and resilient. The aim of this thesis was to better understand the ecological and physical processes that determine successful initial establishment of pioneer vegetation beyond the marsh edge.

Initial pioneer vegetation establishment

The focus of this thesis lies at the interface of the salt marsh and the intertidal flats. This transition zone has the highest potential for lateral marsh expansion. Expansion requires successful vegetation establishment, which is influenced by many factors including soil conditions, environmental variables, such as inundation regime and sediment dynamics, bioturbation and seed availability. In this thesis, these factors were studied using a combination of descriptive research, laboratory and field experiments.

Based on vegetation cover we found a gradual change from densely vegetated salt marsh to bare intertidal flat (Figure S1). However, we found a distinct border between the pioneer zone and the sparsely vegetated transition zone, based on distinct benthic communities and environmental characteristics (Ch. 2). A strong increase in soil oxygenation and a much higher abundance of seeds characterised the border between the transition zone and the pioneer (Ch. 2). Although the transition zone has the appropriate elevation for vegetation establishment, it much more resembled the bare intertidal flat than the salt marsh pioneer zone.

If seeds are available in an appropriate area the following spring, a window of opportunity is crucial for seed germination and successful establishment (Ch. 3 and 5). Successful germination of the annual pioneer *Salicornia procumbens* was mainly driven by increased temperature and precipitation with a low sedimentation rate (Ch. 5). Experimental seed-additions in the transition zone demonstrated that boundary conditions for successful pioneer vegetation establishment were met for *S. procumbens* and *Spartina anglica* but not for *Aster tripolium* (Ch. 3 and 5). Sudden sedimentation events were detrimental as they buried small *S. procumbens* seedlings entirely (Ch. 5).

The seed bank and seed retention

Seeds of multiple pioneer and low marsh plant species were present in the soil of the vegetated marsh (Ch. 2). In the transition zone, the availability of natural

viable seeds was low (Ch. 2, 3, and 5). Although seed dispersal of marsh vegetation is mostly local, seeds of the perennial pioneer *S. anglica* were practically absent from the transition zone (Ch. 3 and 5). Seeds of the other main pioneer, the annual *S. procumbens*, were present in the seed bank but the peak abundance of seeds occurred too deep for successful germination.

Dispersal timing affected seed retention. Seeds manually dispersed before winter eroded away but seeds dispersed after winter were retained in the transition zone (Ch. 3). Storm conditions during winter, high tides, wind speed and waves, caused the loss of seeds (Ch. 3). Natural seed availability in spring may also be low because freshly dispersed seeds suffer from secondary dispersal during winter (Fig. S2).

Figure S1. Processes that affect successful initial vegetation establishment and thus lateral salt marsh expansion onto the intertidal flat. Underlined are the most crucial mechanisms in dynamic transition zones. NL: Processen die vestiging van pioniersvegetatie beïnvloeden en zo doorwerken in de mogelijke groei van de kwelder richting het wad. Onderstreept zijn de meest cruciale processen in dynamische overgangszones.

The role of benthos

Numerous, small bioturbators occurred from the pioneer zone to the intertidal flat, mostly oligochaetes, harpacticoid copepods and collembolans (springtails) (Ch. 2). Benthic bioturbators were capable of transporting seeds downwards into the soil, i.e. seed burial, in the field (Ch. 3) and in the lab (Ch. 4). Seeds from different sizes (1-20 mm) and species, *S. procumbens*, *Spartina anglica* and *A. tripolium*, were buried up to 3 cm in 2 two-month period (Ch. 3). Bioturbation of oligochaetes also hindered

pioneer vegetation establishment, toppling and burying small *S. procumbens* seedlings (Ch. 4). Oligochaetes are generally understudied due to their size, although they can have a significant impact on the soil by reworking the sediment. Our studies revealed that oligochaetes can cause a ten-fold increase in soil oxygenation depth (Ch. 4). It is improbable that meiobenthic bioturbation will inhibit vegetation establishment on a marsh scale, because benthic bioturbators, mainly oligochaetes, were most abundant in the pioneer zone, the area with ample establishment of annual pioneer vegetation.

Implications and conclusions

The main conclusion of this thesis is that, even with a seed source in proximity (the pioneer zone), seed availability can still form a threshold for initial pioneer vegetation establishment in the transition zone. In spring, the natural availability of viable seeds was low beyond the marsh edge (Ch. 2, 3, and 5). The dynamic nature of the bed level (Intermezzo) in combination with winter storms induced a loss of seeds (Ch. 3). Bioturbating benthos, small but numerous, may indirectly influence vegetation establishment success, either through seed retention by burial or intermediated by altering soil conditions (Ch. 2 and 4). When aiming to promote resilient and healthy salt marshes, the appropriate boundary conditions regarding seed availability and sediment dynamics must be ensured (Figure S1). To account for imminent climate change, coastal management should implement the possibility that seed availability may be further reduced, caused by soil erosion due to storms. This is particularly important since increased storm frequencies are expected. Together with sea level rise, these effects will alter hydrodynamic regimes and can thus constrain lateral expansion or even cause salt marshes erosion.

Belang van kwelders

Kwelders zorgen voor positieve effecten op lokale biodiversiteit, natuurlijke kustbescherming en vele andere ecosysteemdiensten zoals het vastleggen van CO₂ en water-zuivering. De toekomst van kwelders wereldwijd ligt onder druk door klimaatverandering en zeespiegelstijging die verslechtering van kwaliteit of erosie kunnen veroorzaken. Op veel plekken kunnen de kwelders niet richting het binnenland migreren door de aanwezigheid van dijken. Wanneer er genoeg sediment beschikbaar is in de waterkolom, is het waarschijnlijk dat kwelders mee omhoog groeien met de stijging van de zeespiegel, mits dit niet te snel plaatsvindt. Ook is de vestiging van vegetatie aan de rand van kwelder essentieel op de kwelder vitaal en veerkrachtig te houden. Het doel van dit proefschrift was om de ecologische en fysische processen die vestiging van pioniersvegetatie aan de kwelderrand beïnvloeden beter te begrijpen.

Vestiging van pioniersvegetatie

De focus van dit onderzoek ligt op de overgangszone van kwelder naar wad. Deze transitiezone is de meest waarschijnlijke kandidaat voor kwelderuitbreiding. Kwelderuitbreiding is afhankelijk van succesvolle vestiging van pioniersvegetatie. Dit wordt beïnvloed door verscheidene factoren zoals de bodem hoogte, de bodem condities, het getij, sediment dynamiek, omwoeling door bodemdieren en de beschikbaarheid van zaden. Al deze factoren zijn bestudeerd in een combinatie van beschrijvend en experimenteel onderzoek.

De bedekkingsgraad van kwelder vegetatie neemt af in een gradiënt van kwelder tot wad, met hier en daar plantjes in de overgangszone (Fig. S1). Wanneer we echter naar de omgevingsfactoren en bodemdieren kijken, zien we een strikte scheiding tussen de pionierszone en de transitiezone (Hoofdstuk 2). Het meest opvallend is het verschil in zuurstofpenetratie in de bodem en de beschikbaarheid van zaden. In de transitiezone is de zuurstofrijke laag veel oppervlakkiger en zijn veel minder zaden beschikbaar dan in de pionierszone (Hoofdstuk 2). Alhoewel de hoogte boven het zeeniveau in de transitiezone aan de pionierszone grenst en geschikt is voor de vestiging van planten, lijkt deze zone qua bodemcondities meer op het onbegroeide wad.

Wanneer zaden beschikbaar zijn op de juiste plaats in het voorjaar, zijn ze afhankelijk van 'windows of opportunity'. Dit zijn periodes, met geen tot zeer lage verstoring, waarin zaden kunnen ontkiemen en zich vestigen (Hoofdstuk 3 en 5). Kieming van de eenjarige zeekraal (*Salicornia procumbens*, Fig. 1.2) komt op gang wanneer de temperatuur omhooggaat, er zoet water beschikbaar is en er weinig sediment wordt afgezet (Hoofdstuk 5). Experimenten waarbij zaad is toegevoegd bovenop het natuurlijk aanwezige sediment hebben laten zien dat de vestigingscondities voor zeekraal en Engels slijkgras (*Spartina anglica*) goed zijn in de transitiezone maar dat dit niet geldt voor zee aster (*Aster tripolium*) (Hoofdstuk 3 en 5). Plotselinge depositie van sediment was schadelijk voor jonge zeekraal zaailingen, aangezien zij volledig werden bedolven onder een laag sediment (Hoofdstuk 5).

De zaadbank en het behoud van zaden na zaadval in de herfst

Zaden van verschillende pioniers en lage kwelder soorten waren aanwezig in de bodem van de begroeide kwelder (Hoofdstuk 2). In de transitiezone was de natuurlijke beschikbaarheid van levensvatbare zaden laag (Hoofdstuk 2, 3 en 5). Alhoewel de verspreiding van kwelderzaad voornamelijk lokaal is, waren zaden van het meerjarige Engels slijkgras bijna geheel afwezig in de transitiezone (Hoofdstuk 3 en 5). Zaden van de andere belangrijke pionier soort, zeekraal, kwamen wel voor in de zaadbank, echter zat de piek van deze zaden te diep om te zorgen voor succesvolle vestiging.

Het moment van verspreiden heeft effect op het behoud van de zaden. Zaden die we voor de winter experimenteel verspreid hadden zijn weg geërodeerd. Zaden die na de winter verspreid zijn, waren in het voorjaar nog aanwezig en konden zich vestigen (Hoofdstuk 3). Stormachtige omstandigheden gedurende de winter, zoals hoge getijden, windsnelheden en golven, hebben het verlies van zaden tot gevolg (Hoofdstuk 3). Natuurlijke beschikbaarheid van zaden in het voorjaar is laag om dat de verspreide zaden weer weg eroderen in de winter (Fig. S2).

Figuur S2. Grafische weergave van processen die beschikbaarheid van kwelder zaden beïnvloeden (Hs 3).

Rol van bodemdieren

Talrijke, kleine bodemdieren komen voor van de pionierszone tot aan het wad, merendeels bestaat dit uit oligochaeten (aquatische ringwormen), harpacticoid copepoden (benthische kreeftachtigen) en collembola (springstaarten) (Hoofdstuk 2). Deze benthische organismen woelen de bodem om en oligochaeten graven netwerken van tunnels (bioturbatie). Bioturbatie van de bodem zorgt ervoor dat zaden het sediment in getransporteerd worden, zowel in het veld (Hoofdstuk 3) als in het lab (Hoofdstuk 4). Zaden van verschillende groottes (1-20 mm) en drie verschillende

soorten werden tot 3 cm diep getransporteerd binnen een periode van twee maanden (Hoofdstuk 3). Bioturbatie door oligochaeten hinderde ook de vestiging van pioniervegetatie, jonge zeekraal zaailingen werden begraven en omgewoeld (Hoofdstuk 4). Oligochaeten worden niet veel bestudeerd omdat ze klein en lastig te determineren zijn maar hebben een significante impact op de bodem door het omwoelen van het sediment. Oligochaeten veroorzaakte een tienvoudige penetratie diepte van zuurstof in vergelijking met sediment zonder deze wormen (Hoofdstuk 4). Op kwelder schaal lijkt het onwaarschijnlijk dat deze bodemdieren de vestiging van planten verhinderen. De meeste bodemdieren zijn namelijk gevonden in de pionierszone waar eenjarige pioniersvegetatie zich goed vestigt.

Implicaties en conclusies

De belangrijkste conclusie van dit onderzoek is dat zelfs met een zaadbron nabij (de pionierszone), zaden een limiterende factor kunnen zijn voor vestiging van vegetatie. In het voorjaar is de beschikbaarheid van natuurlijke en levensvatbare zaden aan de kwelderrand laag (Hoofdstuk 2, 3 en 5). Het dynamische karakter van de hoogteligging van de kwelder en het wad (Intermezzo) in combinatie met winterstormen heeft verlies van zaden opgeleverd (Hoofdstuk 3). Sediment omwoelende bodemdieren, die klein maar wel talrijk zijn, beïnvloeden succesvolle vestiging indirect door het begraven van zaden of het veranderen van de chemische samenstelling van de bodem (Hoofdstuk 2 en 4). Wanneer men het voorkomen van gezonde en veerkrachtige kwelders tracht te stimuleren, zijn de juiste randvoorwaarden betreft beschikbaarheid van zaden en sediment dynamiek essentieel (Fig. S1). Beheerders van kustsystemen zullen rekening moeten houden met de mogelijkheid dat beschikbaarheid van zaden verder daalt doordat, in verband met klimaatverandering, waarschijnlijk meer stormen en hoge getijden zullen voorkomen. Dit in combinatie met zeespiegelstijging zal impact hebben op hydrodynamische dynamiek en kan de groei van kwelders belemmeren of zelfs voor kwelder erosie zorgen.

SEB poles on the tidal flat at Westhoek (December 2017).

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- o Multivariate Analysis (2015)
- o Introduction to zero inflated models with R, Highland statistics (2018)

Other PhD and Advanced MSc Courses

- o NCK summer school: Estuarine and coastal processes in relation to coastal zone management (2015)
- o Brain Training, Wageningen Graduate Schools (2015)
- o Scientific writing, VU Amsterdam (2016)

External training at a foreign research institute

- o Antarctic expedition CCAMLR cooperation (2018)
- o Linear mixed effects models and GLMM in R, Highland statistics, Norway (2017)
- o Spatial, temporal and spatio-temporal models using R-INLA, Highland statistics, Belgium (2018)
- o Basic Safety, Falck Safety Services, The Netherlands (2018)
- o Time management, ExplainIT, The Netherlands (2018)

Management and Didactic Skills Training

- o Supervision of eight BSc and MSc students (2016-2018)
- o STW biennial user and stakeholder committee meetings (2015-2019)

Selection of Oral Presentations

- o *Oligochaete impact on pioneer seedling establishment*. NCK, 16th of March 2017, Den Helder, The Netherlands
- o *Ecological aspects of a mud motor*. Coastal Ecology workshop, 25th of October 2017, Westerhever, Germany
- o *Oligochaetes inhibit *Salicornia* germination and seedling emergence*. Coastal Ecology Workshop, 31st of October 2016, Fuhlendorf, Germany
- o *The biogeomorphic impact of *Oligochaetes**. ECSA 57, 5th of September 2018, Perth, Australia

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