



Watching grass grow:

Bottlenecks in seagrass survival

Wouter Suykerbuyk

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Wouter Suykerbuyk
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Watching grass grow: bottlenecks in seagrass survival

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As the seas writes on the sandy shore:
Writes-wipes, writes-wipes
Forever the same,
- Never the same way.

Fodor Ákos



Contents:

Chapter 1	8
<i>Watching grass grow: Bottlenecks in seagrass survival: introduction</i>	
Chapter 2	28
<i>Living in the intertidal: desiccation and shading reduce seagrass growth, but high salinity or population of origin have no additional effect</i>	
Chapter 3	52
<i>Suppressing antagonistic bio-engineering feedbacks doubles restoration success</i>	
Chapter 4	70
<i>Unpredictability in seagrass restoration: analysing the role of positive feedback and environmental stress on <i>Zostera noltii</i> transplants</i>	
Chapter 5	102
<i>Rhizome starch as indicator for temperate seagrass winter survival</i>	
Chapter 6	124
<i>Surviving in changing seascapes: Sediment dynamics as bottleneck for long-term seagrass presence</i>	
Chapter 7	152
<i>Watching grass grow: Bottlenecks in seagrass survival: Synthesis</i>	
Summary / Samenvatting	174
Dankwoord	184
Curriculum vitae	190

Chapter 1

Introduction



Life in estuaries requires the ability to cope with stress

An estuary is the part of a coastal water body where fresh water river currents meet the more salty tides. Estuaries therefore form a unique transition zone, where tides, waves and saline input from the sea interact with the fresh water and sediment effluent from the river, and therefore result in a gradient of brackish conditions (Day Jr. et al. 2012) From fresh to salt, a range of distinct ecosystems can be found with organisms (plants and animals) well adapted to and characteristic for the salinity conditions at that point along the gradient (e.g. Telesh and Khlebovich 2010) and references therein). Along the length axis of the estuary, hydrodynamic forces that derive from sea (tidal currents, waves) and from the river (river currents) mix and produce gradients of hydrodynamic energy. Within the constraints of the geological setting and sediment availability, these forces are shaping the estuarine morphology.

At any point along the estuarine gradient, a vertical gradient can be found, structuring the zonation of organisms according to their ability to cope with either emergence or submergence. In the subtidal zone, organisms will be found that are capable of living permanently submerged, whereas at the other extreme end in the supra intertidal zone, only organisms will be found that are capable of being permanently emerged. In between, in the intertidal zone, organisms have to cope with submergence of up to 12h per tide in the lower tidal zone, to only a few hours per tide in the upper intertidal zone (lower salt marsh) or to as little as a few hours per month or year in the extreme upper intertidal (higher salt marsh). Mobile organisms can in essence move away from the submergence or emergence stress they experience. The majority of the mobile organisms (especially the smaller ones, such as crabs, crustacean, shellfish, snails, diatoms and worms) will not move with the tides, and some not even abandon their burrows to escape this twice-daily stress, as that would require energy. They rather “hide” for the stress, e.g. by digging (further) into the sediment, and more mobile organisms may move to nearby moist areas or tidal pools to wait until the emergence stress disappears when the tide is coming in. In contrast, sessile organisms cannot move away from emergence stress and thus are forced to resist and mitigate it, e.g. by closing their shells as bivalves do or by regulation of osmotic stresses in salt tolerant plants such as seagrasses and salt marsh plants (Reise 1985, Nedwell and Raffaelli 1999).

Apart from adaptation or specific behavior to overcome the above-mentioned abiotic stresses, some species have traits that enable them to reduce the stress levels by modifying their physical environment. This so-called “ecosystem engineering” capacity, as first described by Jones and co-workers (Jones et al. 1994), is divided in allogenic and autogenic engineering. Allogenic engineering is an active process of the engineer by which it alters its

environment. For instance, the sediment reworking activities of a bioturbating lugworm makes its sedimentary environment loose and keeps it sandy. On the contrary, autogenic engineering is the process of a species altering its environment just by its presence, e.g. oyster reefs blocking currents and reducing wave height and thus creating ameliorated, calmer conditions, within and behind the reef. Although a single individual of an ecosystem engineering species contains all traits to bio-engineer its environment, only after a certain density or size is attained a (self-) facilitating bio-engineering effect is established (e.g., Reise 2002, van der Heide et al. 2007, van der Heide et al. 2008, Bouma et al. 2009). Ecosystems containing ecosystem engineers are therefore prone to threshold behavior and generally are stable once the threshold density or size that lead to the facilitating feedback is bridged (Jones et al. 1994, Scheffer et al. 2001, Volkenborn et al. 2009). Such threshold behavior with (self) - facilitation feedbacks may cause alternative stable state dynamics. That is, ecosystems with positive ecosystem-engineering feedbacks can be highly stable and resilient to disturbances. However, once a critical threshold is surpassed the positive feedback is lost and the coastal ecosystems can collapse without any prior warning, causing non-linear dynamics (Silliman et al. 2005, van der Heide et al. 2007, Hughes et al. 2010). Once (self)-facilitating feedbacks are lost, (re)-establishment is difficult. It is no surprise, therefore, that recovery and restoration of these ecosystems is found to be extremely difficult (Bakker et al. 2002, Orth et al. 2006, Halpern et al. 2007, Omori 2011). Seagrass dominated ecosystems are clear examples showing such dynamics, and this thesis aims to understand how to preserve and restore seagrass ecosystems in the face of these difficulties.

Seagrasses

Seagrasses are marine angiosperms found in different species, varieties and appearances worldwide (Short et al. 2007). Seagrass meadows offer numerous ecosystem services (among others, carbon sequestration, food source, nursery and habitat, sediment stabilizer) and are therefore highly valued (Costanza et al. 1997, Barbier et al. 2011). Unfortunately, seagrass meadows suffer significant losses over the last century, worldwide (Waycott et al. 2009), and also in Dutch deltas (Database Ministry of Infrastructure and Environment). The decline is generally related to anthropogenic activities, but it is not always clear what activities and conditions are the direct causal factor. In the Dutch coastal waters, only 2 species are present: *Zostera marina* and *Zostera noltii hornem* (respectively in Dutch “Groot and Klein zeegras”). *Zostera marina* can be found in both subtidal and intertidal waters, whereas *Zostera noltii* is mainly found in intertidal systems. This thesis focuses on intertidal *Z. noltii*, which suffered a major decline in the Oosterschelde, coinciding with the completion of the Delta Works. The area covered decreased from >1000 ha in the late 1970’s

Figure 1: Above: Submerged *Zostera noltii* plants in summer (Location



Dortsman, Oosterschelde Below: Emerged flowering Zostera noltii plants at the end of the season (Location Oostdijk).

to around 75 ha at present. Recent dike reinforcement works posed an additional threat to the remaining *Zostera noltii* meadows, resulting in the mitigation measure of transplanting the endangered seagrass to suitable spots in the Oosterschelde. In this thesis, we aimed to identify environmental preferences and bottlenecks for establishment and maintenance of dwarf eelgrass *Zostera noltii* in the Oosterschelde tidal basin in order to better understand and predict

the dynamics of existing seagrass meadows and to ensure and optimize re-establishment of *Zostera noltii* after transplantation.

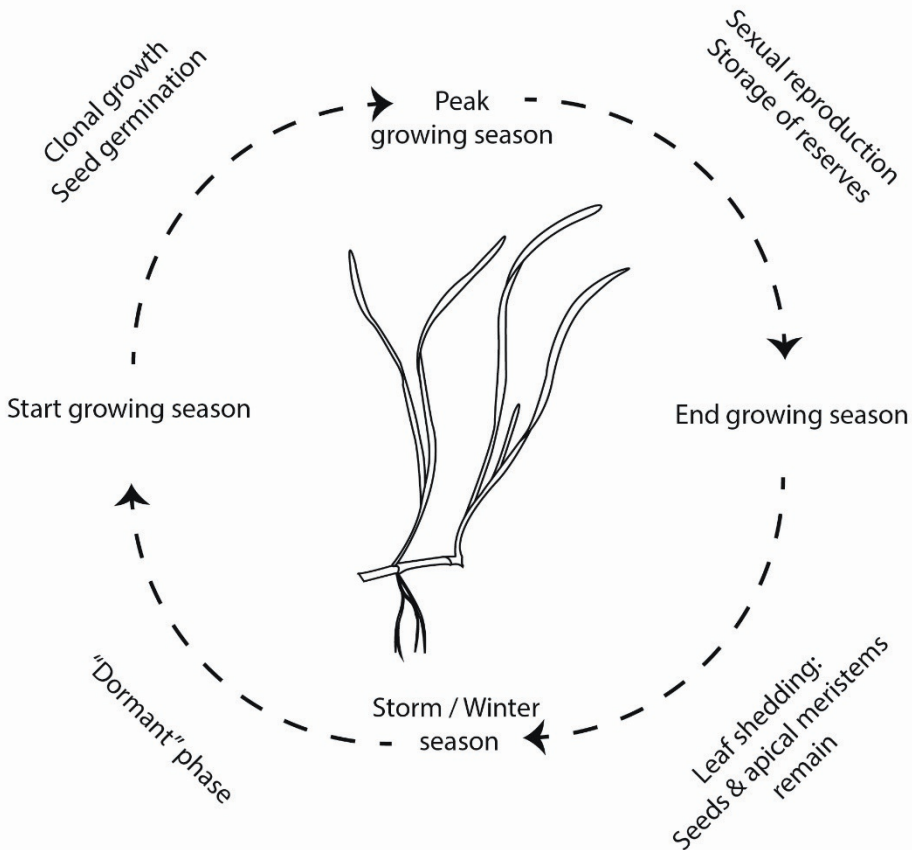


Figure 2: Annual life cycle of temperate *Zostera noltii*. With schematic drawing of *Zostera noltii* morphology (shoots, rhizome and roots).

In the Oosterschelde, *Zostera noltii* is the dominant seagrass with its typical morphology of rhizomes with appending roots and relatively flexible shoots (Figure 1 & 2)(Den Hartog 1970). In Northwest Europe it usually grows in the mid upper intertidal, roughly corresponding with an emergence time of 35 to 70 % of the tidal cycle. During emergence the flexible leaves spread out on the sediment. The populations have a growing season from May to October, when they grow vegetative via expanding and branching their rhizome network at the apical meristems (Brun et al. 2006). They flower and produce seeds that are released and survive in the sediment until the next spring. Additionally, at the end of the season carbohydrates (starch and sucrose) are stored in the rhizomes as a reserve to survive winter when most of the leaves are lost and photosynthesis

stops (Vermaat and Verhagen 1996). In spring, seagrass beds are re-established from overwintering seeds and rhizome fragments (Hootsmans et al. 1987, Vermaat and Verhagen 1996).

The spatial distribution of the seagrass meadows is structured by the stresses it encounters throughout the season and by the ability of the plants to deal with those stresses. In general, seagrasses are predominantly affected worldwide by eutrophication, climate change and coastal reconstructions and their consequences (e.g. Orth et al. 2006). This also holds for *Z. noltii* (e.g., Vermaat et al 1987, Philippart 1995a, Cabaco and Santos 2007, Cabaco et al. 2008, Martinez-Crego et al. 2014, Schumacher et al. 2014, Valle et al. 2014). In the Oosterschelde, likely stressors are light stress, desiccation, salinity stress, and sediment dynamics, as we will explain in the study area description.

Light stress

Light is essential to plants for performing photosynthesis. While being emerged, intertidal seagrass will not be light limited. Contrastingly, low light conditions (and thus reduced photosynthetic rates), could occur when seagrass is submerged by turbid waters that prevent light to reach the plant's leaves. These turbid waters typically result from resuspension of fine material (sediment and organic matter) from the sediment due to wave action or from high levels of phytoplankton in the water column. In addition, low light conditions can be induced by epiphytic algae that settle on the seagrass leaves. (Williams and Ruckelshaus 1993, Hughes et al. 2004). Seagrass plants subject to low light conditions are limited in growth and typically have decreased shoot densities. Aboveground biomass and number of leaves per shoot are reduced to prevent self-shading, while individual leaves become longer and pigment concentration increases to maintain their photosynthetic rates (Goodman et al. 1995, Hemminga 1998, Holmer and Laursen 2002, Cabaco et al. 2009).

Desiccation stress

Periods of air exposure are beneficial to photosynthesis by intertidal seagrass as they provide good light conditions and the opportunity to assimilate CO₂ (Leuschner et al. 1998). However, air exposure might result in desiccation twice a day. *Zostera noltii*'s morphology is well adapted to avoid desiccation stress as the leaves are highly flexible so that they can lay flat on the moist sediment while being emerged (Harmsen 1936). While lying on top of each other and retarding the ebbing water flow, seagrass leaves can mitigate desiccation stress up to a certain level (Fox 1996). However, desiccation stress increases with increasing irradiance and temperature (Perezllorens and Niell 1993, Lee et al. 2007) and with decreasing water content of *Zostera noltii* leaves (Leuschner et al. 1998). Desiccation effects, such as limited growth, reduced canopy height,

leaf width and irreversible leave damage or breakage (Vermaat et al. 1993, Boese et al. 2003), increased with tidal height (Leuschner et al. 1998, Boese et al. 2003, Boese et al. 2005). Air exposure of only 5 hours resulted in a 50% loss of leaf water content (Leuschner and Rees 1993). At higher elevation, desiccation stress can be escaped by growing in tidal pools. Nevertheless, the upper limit of intertidal *Zostera noltii* is usually controlled by the air exposure time and thus the desiccation stress that the plants experience.

Salinity stress

Although seagrass plants are well adapted to their saline habitat, they experience salinity stress while they are exposed to regular (short-term) salinity fluctuations, including hyposaline and hypersaline conditions. Initial acclimation to osmotic changes in marine macrophytes takes place by adjustment of turgor-pressure by up and down regulation of simple ions (mostly Na^+ , K^+ and Cl^-) and leaf water content so that cell-size is maintained (Touchette 2007). When salinity stress continues after these adjustments, the synthesis or breakdown of organic osmotically active compounds may occur. Tolerating fluctuations in salinity requires energy that cannot be invested in plant growth and potentially could reduce the plants' fitness in the long-term. The long-term effects of increased or decreased salinity levels on survival, growth and photosynthesis of *Zostera noltii* has not extensively been studied. In the limited amount of studies available, detrimental effects of a high salinity on survival of *Z. noltii* were found: substantial mortality (25 – 60 %) was found after exposure (for 28 weeks) to seawater of 35 PSU, whereas low salinity conditions (down to 2 PSU) did not show significant (lethal) plant response (Vermaat et al. 2000).

Sediment dynamics

Apart from the above-introduced physiological stresses, seagrasses are also subject to physical stresses related to the sediment and induced by the water and biota in their vicinity. The direct effects of hydrodynamic and burial processes on short-term seagrass performance are well studied (e.g., Fonseca and Kenworthy 1987, Gambi et al. 1990, van Katwijk and Hermus 2000, Cabaco et al. 2008, van der Heide et al. 2010). In contrast, relatively little is known on how altered sediment dynamics may affect long-term seagrass persistence. This question may be particularly relevant for seagrasses in temperate areas, where plants cannot adapt to burial or erosion during winter when they do not grow (Han et al. 2012). The actual sediment stress depends on the nature and thus susceptibility of the sediment to the hydrodynamic forces. As an example, well-consolidated cohesive sediment is much harder to erode or to mix than loose fine sand, resulting in smaller sediment stress for inhabiting plants under comparable bottom shear stress. In Germany and the Netherlands, seagrass *Zostera noltii* can therefore be found on stable or sheltered sediments that provide a stable substrate

or lack (excessive) disturbing forces, respectively (Philippart 1994, Reise and Kohlus 2008, Suykerbuyk et al. 2012, Suykerbuyk et al. 2016). Furthermore, capturing of fine sediment from the water column by the leaves or preventing sediments to re-suspend by dense seagrass roots mats can stabilize the sediment and outcompete infauna and thus reduce sediment stress (Philippart 1994, Christianen et al. 2013). However, these effects can be lost during winter, when the aboveground material is lost and some of the belowground material naturally degrades (Vermaat and Verhagen 1996, Govers et al. 2015).

Study area the Oosterschelde: anthropogenic induced changes and seagrass

Anthropogenic activities of the over 40 % of the Earth's population that lives along the coastline can lead to enhanced stresses. Next to environmental pollution (e.g. heavy metals) and eutrophication (leading to algal blooms and/or causing toxic levels of ammonium or sulfide), coastal constructions for flood protection leads to habitat degradation for seagrass and other marine life (Temmerman et al. 2013). 'Hard' constructions change seascapes, through temporary or permanent changes in hydrology and sedimentology, as is the case in the Oosterschelde estuary (e.g., ten Brinke et al. 1994, Vroon 1994, Louters et al. 1998). After a severe flood in 1953, plans were made to protect the inland from extreme high water levels. The plans included damming the fresh water river inlets and partial closure of the seaside inlet by building a storm-surge barrier. The main effects of these so-called Delta-works were i) the loss of a typical estuarine fresh - salt gradient, ii) a reduced tidal volume and tidal amplitude, and iii) a loss of inward sediment transport and thus a lack of fines in the partially closed estuary (ten Brinke et al. 1994, Louters et al. 1998). The two last mentioned effects lead to a net erosion of the tidal flats in the system (so-called "Zandhonger") (Louters et al. 1998).

Coinciding with the completion of the Delta-works (mid 80's), our study area suffered a period of widespread decline in seagrass area. The adverse conditions that caused the initial decline, three extremely cold winters (1985 - 1987) in succession in combination with artificial lowered high tides, are no longer present at these former seagrass sites (de Jong and van der Pluijm 1994). Nevertheless, seagrass did not recover spontaneously in the Oosterschelde in the following years. The authors attributed the lack of recovery to (i) lack of seagrass seeds and rhizome fragments, considering that propagules are sparsely distributed (the Oosterschelde harbours approximately 75 ha of seagrass beds scattered over a total area of 35,000 ha, of which 11,000 ha are littoral), ii) increased sediment dynamics due to the changed tidal regime (Louters et al. 1998), and in particular to (iii) increased microrelief on the intertidal flats, caused by fecal casts of lugworms that are no longer flattened and redistributed by the (reduced) tidal currents. However, these proposed bottlenecks for recovery were

never experimentally tested. Other factors that coincide with the seagrass decline in the Oosterschelde are reduced input of freshwater in the Oosterschelde, with consequently lower nutrient inputs and higher salinities (Wetsteyn & Kromkamp 1994). Also, pH initially increased during and after closure of the storm surge barrier in 1986, followed by a decreasing pH (Provoost et al. 2010).

Seagrass mitigation project due to dike reinforcement

Nowadays, the Oosterschelde system still has an unbalanced sediment budget with net erosion of the intertidal flats. The decline stabilized at an amount of 75 ha *Zostera noltii* (out of >1000 ha in the 80's) spread over about a dozen of meadows. Seagrass withdrew to stable sediments (consolidated old saltmarsh remnants or shell banks) that usually have reduced bioturbating infauna and are located relatively high in the intertidal, fringing the dikes (figure 3). Reinforcing of the dikes posed a new threat to the remaining seagrass meadows, because a 15 m wide stretch along the dike needed to be excavated. European regulations require that the works should not have a significant effect (> 1 % loss) on seagrass meadows as seagrass is a red list and NATURA 2000-target species for the system. Authorities (Rijkswaterstaat / Projectbureau Zeeweringen and the Province of Zeeland) therefore decided to move the endangered seagrass from the 15m zone to other suitable places within the estuary. Seagrass transplantations generally have low succes rates (globally 37%), which may partly be attributed to the very small scale that is usually applied in transplantation projects (van Katwijk et al. 2016). Considering that (i) seagrasses are typically ecosystem engineers that require a critical mass to establish, combined with (ii) the highly unpredictable environment which requires spreading of risks, the low success rates are not surprising (van Katwijk et al. 2016). The mitigation project gave us the unique possibility to apply a large transplantation scale. Despite a body of literature and local experience on how to successfully transplant *Zostera marina*, less was known regarding the do's and don'ts of translocating *Zostera noltii*. In the Wadden Sea, transplantations lead to slowly expanding *Z. noltii*-patches in Balgzand during 13 years after which they rapidly disappeared, which may be related to freshwater pulses from the canal nearby (van Katwijk et al. 2006, 2009) coinciding with *Ruppia* expansion. In Basque country, two small scale transplantations lead to variable success during 2 years monitored (Valle et al. 2015).



Figure 3: Areal picture of the natural seagrass meadow of Oostdijk (sept 2014, facing Yerseke, photo Bas Oteman)

Outline of the thesis:

This thesis aims to better describe the environmental preferences of dwarf eelgrass *Zostera noltii* and to identify the bottlenecks for its restoration. Moreover, we investigated the ecological processes related to seagrass presence and performance and highlighted their non-linear nature with regard to ecosystem engineering feedback thresholds. We generalised our findings in the broader contexts of ecosystem engineering and ecosystem predictability. With this knowledge, we attempted to optimize *Zostera noltii* transplantation successes at new potential seagrass sites.

Following the correlations between the seagrass decline and the environmental changes in the Oosterschelde as described at the study site description, field manipulations and accompanying laboratory experiments focused on (i) salinity, light and desiccation being determinant regarding local habitat requirements, i.e., for the depth zonation and proximity to freshwater inputs. Regarding system-scale changes, we focused on (ii) sediment dynamics as the main factor that has changed in the Oosterschelde coinciding with the seagrass decline.

First, we checked to what extent seagrass in the Oosterschelde estuary is limited in growth during the growing season. In a series of lab experiments, we investigated the interactive or synergistic effects of desiccation, light and salinity stress on seagrass growth in populations that are adapted to different local conditions (**Chapter 2**). For our transplantation efforts, this allowed us to choose environmental conditions to optimize the transplantation successes. Furthermore, we experimentally checked to what extent restoration of *Zostera noltii* meadows at formerly suitable seagrass habitats could benefit from removal of negative effects by the bioturbating lugworm *Arenicola marina* (**Chapter 3**). In ten large-scale transplantations efforts from 3 donor populations to 6 tidal flats, performed between 2007 and 2012 and monitored until 2013, we tested the effect of our handling and the differences in transplantation success of two transplantation methods (single plants vs. sods), three transplant configurations (small, large or compact), and two elevation levels (lowered vs. normal). This allowed us to determine if we could promote or accelerate the establishment of self-facilitating feedbacks in our restoration efforts by optimising intrinsic and external processes (**Chapter 4**). In addition, we investigated to what extent the next year's growth in natural meadows and our experimental transplants depends on 1) shoot densities in the preceding growth season, 2) the presence of leaves in the winter, and 3) the level of starch reserves (**Chapter 5**). In **Chapter 6**, we performed a correlative study to determine the sediment parameters that related best to long-term seagrass persistence of natural beds. In addition, we studied whether these parameters have critical threshold values outside of which seagrass cannot be found. These chapters quantify the factors that determine long-term seagrass presence and the predictive value of these factors regarding (long-term) seagrass presence of natural meadows and seagrass transplants.

Note that we did not focus on eutrophication or climate change as these factors had a lower likelihood of being vital for succesful restoration in the Oosterschelde. Eutrophication can limit *Z. noltii*, either via direct toxicity (e.g. Brun et al. 2002, 2003) or via shading following algal overgrowth (Philippart 1995b, Schanz et al. 2002) was not considered in this thesis as nutrient loads have decreased rather than increased (Wetsteyn & Kromkamp 2004). Nutrient limitation is not likely as porewater concentrations of nutrients are relatively high (Govers et al. 2014). Climate change such as increased water temperature was also not considered as a priority research topic as the Oosterschelde is located in the middle of its distribution range, although effects can not be excluded (see e.g. Repolho et al. 2017). Acidification was also not likely to have caused the decline in the late 1980s, as there was a temporary pH increase at that time, (pH 7.9 -> 8.2), rather than acidification, later going down to 7.9 again (Provoost et al. 2010). Also, *Z. noltii* is probably not susceptible to lower pH, as was shown in a study comparing pH 7.6 and pH 8.0 (Repolho et al. 2017). First studies to interactive effects between eutrophication and climate change show a more positive effect of the interaction than expected (Martinez-Crego et al. 2014). Table 1: Research questions and hypothesis.

	Question?	Hypothesis:
Ch2	What are the interactive or synergistic effects of desiccation, light and salinity stress and do these effects differ among populations that are adapted to different local conditions?	<p>H1: Emergence has a negative effect on the growth rate of the intertidal temperate <i>Zostera noltii</i>.</p> <p>H2: Growth is further reduced when an additional stressor is present: e.g. shading or high salinity.</p> <p>H3: Seagrass origin may determine the salinity stress response; plants of populations that grow under high salinity will be less affected in their growth response than those of low-salinity populations.</p>
Ch3	To what extent can restoration of <i>Zostera noltii</i> meadows at formerly suitable seagrass habitats benefit from removal of negative effects by the bioturbating lugworm <i>Arenicola marina</i> and how will this depend on abiotic conditions (sheltered vs. wave exposed)?	<p>H1: Minimizing lugworm-induced sediment disturbance will enhance seagrass growth, unless other sources of abiotic stress cause sediment disturbance.</p>
Ch4	Can temperate seagrass meadow restoration benefit from optimising intrinsic and external processes? And if so, to what extent?	<p>H1) Enhanced intrinsic processes benefit seagrass transplant development: (H1a) Transplanted sods will establish and survive better than bare-root single plant transplants, (H1b) Larger or more compact planting configuration and (H1c) higher initial shoot density will have a positive effect on the transplant survival.</p> <p>H2) Optimizing external processes benefit seagrass transplant development: (H2a) Reduction of important bioturbators (biotic external stresses), (H2b) Lowering initial transplant elevation and thus decreasing</p>

		<p>desiccation stress (local abiotic external stress) will promote transplant development and (H2c) sheltered sites that experience less environmental wave-forcing will develop better than more exposed transplant sites (abiotic external disturbances).</p> <p>H3) Intrinsic processes are more important for restoration development and survival in summer than in winter, while in winter restoration success is relatively more related to external</p>
Ch5	<p>To what extent does seagrass growth success depend on 1) shoot densities in the preceding growth season, 2) the presence of leaves in the winter, and 3) the level of starch reserves?</p>	<p>Next year's growth success will be higher:</p> <p>H1) at higher shoot densities in the preceding season</p> <p>H2) when leaves are present during the winter</p> <p>H3) at higher starch levels</p>
Ch6	<p>What sediment related factor determines long-term seagrass persistence?</p> <p>And how does this relate to actual seagrass cover of the investigated meadows? Is there a critical threshold value above which seagrass cannot be found?</p>	<p>H1) Long-term seagrass presence depends on the nature of the sediment, where H1a) hotspot sediments have a more cohesive nature than sediments where seagrass is lost (coldspots), and H1b) hotspot seagrass cover increases with cohesiveness and lack of disturbances (i.e., bioturbation)</p> <p>H2) Actual seagrass cover of hotspots is negatively affected by sediment <i>dynamics</i> in <i>winter</i>, rather than in summer, or by winter <i>static</i> sediment properties or biota.</p> <p>H3) Furthermore, we expect the presence of a critical threshold value of the maximum sediment disturbance depth for actual seagrass presence, above which seagrass presence is affected by sediment disturbances (leading to uprooting).</p>

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Chapter 2



***Living in the intertidal:
desiccation and shading reduce seagrass growth,
but high salinity or population of origin have no additional effect***

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Abstract

The limiting effects of stressors like desiccation, light and salinity on seagrass growth and distribution are well-studied. However, little is known about their interactive effects, and whether such effects might differ among populations that are adapted to different local conditions. In two laboratory experiments we tested a) if growth and development of intertidal, temperate *Zostera noltii* is affected by emergence time (experiment 1 and 2), and b) how this is affected by an additional, second stressor, namely shading (experiment 1) or high salinity (25, 30 and 35 ‰, experiment 2). In addition, we tested c) whether the effects of emergence time and salinity varied between three different European seagrass populations (Saint-Jacut / France, Oosterschelde / The Netherlands, and Sylt / Germany), which are likely adapted to different salinity levels (experiment 2).

In both experiments, emergence of 8 hours per tidal cycle (of 12 hours) had a negative effect on seagrass relative growth rate (RGR), and aboveground biomass. Emergence furthermore reduced either rhizome length (experiment 1) or belowground biomass (experiment 2). Shading (experiment 1) resulted in lower RGR and a two-fold higher aboveground / belowground ratio. We found no interactive effects of emergence and shading stress. Salinity (experiment 2) did not affect seagrass growth or morphology of any of the three populations. The three tested populations differed greatly in morphology but showed no differential response to emergence or salinity level (experiment 2).

Our results indicate that emergence time and shading show an additive negative effect (no synergistic or antagonistic effect), making the plants still vulnerable to such combination, a combination that may occur as a consequence of self-shading during emergence or resulting from algal cover. Emergence time likely determines the upper limit of *Z. noltii* and such shading will likely lower the upper limit. Shading resulted in higher aboveground / belowground ratios as is a general response in seagrass. *Z. noltii* of different populations originating from salinity 30 and 35 ‰ seem tolerant to variations in salinity within the tested range. Our results indicate that the three tested populations show morphotypic rather than ecotypic variation, at least regarding the salinity and emergence, as there were no interactive effects with origin. For restoration, this implies that the salinity regime of the donor and receptor site of *Z. noltii* is of no concern within the salinity range 25-35 ‰.

Introduction

Desiccation due to air exposure imposes a stress to marine life in the intertidal zone. Whereas mobile species can escape to moist places during low tide, sessile intertidal organisms need to cope with hours of air exposure. In the seagrass species *Zostera noltii*, short periods of air exposure are utilized to its advantage to assimilate CO₂, as long as the leaves remain moist (Leuschner *et al.* 1998). However, adverse effects of emergence rapidly increase with duration to air. Emergence periods of only 5 hours air exposure per tide may result in a 50% water loss in the leaves, concomitant with 50% reduction in photosynthetic rates (Leuschner *et al.* 1998). Besides the physiological effects of drought stress, desiccation of the leaves after low tide exposure decreases the mechanical strength and subsequently the probability of leaf sloughing (Vermaat *et al.* 1993). In addition, this may result in shorter leaf lengths as desiccated leaf points are prone to break, resulting in a decreased capacity of photosynthesis (Boese *et al.* 2003), and reduced water retention by the leaves (e.g. Fox 1996), which is density dependent (de Fouw *et al.* 2016).

Desiccation is usually the limiting factor controlling the upper limit of seagrass growth on the intertidal flat (Philippart & Dijkema 1995; Leuschner *et al.* 1998; van Katwijk & Hermus 2000; van der Heide *et al.* 2010). However, risk of desiccation varies over tides, days, seasons and latitude (i.e. Perez-Llorens & Niell 1993), and actual emergence stress will depend on the temperature, wind conditions, sediment water content and seagrass density. For example, although intertidal *Z. noltii* in the sub-tropical Mauretania has to cope with an air temperature of 40 ° Celsius during six hours of emergence per tide, meadows still have high (>75% coverage) shoot density due to the facilitating effects of such high densities on water retention of meadows (de Fouw *et al.* 2016). On the other hand, the productivity during emersion is lower than during submergence in these beds (Clavier *et al.* 2011), which was also found in more temperate beds at the atlantic coast of France (Quisse *et al.* 2011). In both cases, this was largely attributed to self-shading by the leaves laying flat, covering each other in the dense beds. It may seem counterintuitive that light could be limiting in an intertidal bed, but in addition to self-shading, also very turbid water may limit productivity during the high tide hours, and cover by epiphytes and green macroalgae can be severe in intertidal beds, particularly since seagrasses facilitate the presence of these algae by providing substrate and shelter (Michael *et al.* 2008).

In temperate regions, light availability varies over the year and controls the start and end of rhizome branching during the growing season (Vermaat & Verhagen 1996, Govers *et al.* 2015). Photo-inhibition is not likely, as *Z. noltii* is very tolerant to high light levels (Jimenez *et al.* 1987), also in combination with emergence (Clavier *et al.* 2011, Ouisse *et al.* 2011). To compensate for light limitation, shading typically results in an increased aboveground belowground biomass ratio with longer shoots compared to plants grown in ambient light conditions (e.g. Abal *et al.* 1994; Philippart 1995; Vermaat *et al.* 2000; Peralta

et al. 2002). Longer shoots may however make intertidal seagrass plants more vulnerable to desiccation when growing at low density.

In general, several stressors may cause a conditional outcome of emergence stress. For example, in addition to light limitation due to self-shading or algal cover mentioned above, a stress like a high salinity may influence the tolerance to desiccation. Salinity stress could be one of the most ubiquitous stressors that marine life and thus also seagrasses encounter. Marine macrophytes can counteract osmotic stress on the short-term by internal adjustments of turgor-pressure by up- and down-regulation of simple ions and on the long-term by synthesis or breakdown of osmotically active compounds (Touchette 2007). Both physiological mechanisms require energy and could therefore reduce plant fitness. A salinity range as wide as 10 to 35 does not cause increased mortality in seagrass *Zostera marina* (Kamermans *et al.* 1999; van Katwijk *et al.* 1999; Nejrup *et al.* 2008), whereas salinities of 5 and lower increase mortality (Nejrup *et al.* 2008). With increasing temperature, salinity stress effects become even more pronounced (Salo & Pedersen 2014). Within the non-mortal salinity conditions, a salinity range of 22-23 was found to be the optimal *ex situ* for temperate eelgrass *Z. marina*. That is, at this salinity maximum production of shoots and leaves was found whereas growth and vitality were reduced at higher salinities (>26) (Kamermans *et al.* 1999; van Katwijk *et al.* 1999). Plants of *Z. marina* populations grown under high salinity seem to better cope with high salinity than plants originating from an estuarine or other low salinity habitat (van Katwijk *et al.* 1998; van Katwijk *et al.* 1999), and reversely, low-salinity grown plants tolerate lower salinities than high salinity grown plants (Salo *et al.* 2014). Also, in *Posidonia oceanica* distinct differences in response to salinity between plants from different origins are found (Fernandez-Torquemada & Sanchez-Lizaso 2005). In *Z. noltii*, different origins were tested on high salinity tolerance, comparing 15 and 35 (Vermaat *et al.* 2000). Responses to salinity stress are similar to those of *Z. marina*: increased mortality was found at extreme salinities (e.g. Vermaat *et al.* 2000; Charpentier *et al.* 2005). To our knowledge, the more subtle salinity preferences of *Z. marina* plants of different origins described above were never tested for *Z. noltii*. The outcome of such tests can be important for restoration projects with different donors originating from different salinities. Since critical mass is very important for seagrass restoration success (van Katwijk *et al.* 2016), focus should not only be on *surviving* certain salinities but also about highest growth rates.

Although separate effects of desiccation, light and salinity stress are relatively well studied, little is known about the interactive effects of these stressors, in other words, whether they show an additive effect (= no interaction), or may act synergistic or antagonistic (= interaction), and if these effects differ among populations that are adapted or acclimatized to different local conditions (but see Vermaat *et al.* 2000). Thus, we want to test if the growth rate and morphology of the intertidal *Z. noltii* are negatively affected by emergence, and whether its effect is strengthened by the presence of an additional second

stressor: shading or high salinity. In addition, we want to test whether the effects of emergence and salinity vary between *Z. noltii* populations from three different origins in western Europe, which are possibly adapted to different local salinity levels. We hypothesize that 1) Emergence has a negative effect on the growth rate and size of the intertidal temperate *Zostera noltii* (H1). 2) Growth is further reduced when an additional stressor, i.e. shading or high salinity is present (H2), and shading will result in higher aboveground biomass and longer leaves as is commonly found, e.g. de los Santos *et al.* 2010 (H2b). And 3) Seagrass origin determines the salinity stress response; plants of populations that grow under high salinity will be less affected in their growth and morphology response than those of low-salinity populations (H3).

Material and methods

Experiment 1

To test emergence period and shading and their interaction on *Zostera noltii*, we examined seagrass growth in a range of four emergence periods (0, 4, 6 and 8 hours per 12h), under control or shaded light conditions. Plants were collected from the Goese Sas tidal flat, Oosterschelde basin, SW Netherlands (51° 31.40' N; 3° 56.37' E; average salinity of 29.5) (Fig. 1), with permission of the Province of Zeeland (case NB08.068, reference 08033625), transported free floating and stored free-floating in a container with sand-filtered water (salinity of 30, 17° C, 210 $\mu\text{E m}^{-2} \text{s}^{-1}$ 14 h per day) during 3 days before the start of the experiment at 21 July 2011. Each experimental planting unit (EPU) consisted of the apical shoot (#1), shoot #2, the internode between shoot #1 and #2 and 1cm of the appending internode between shoot #2 and the cut off shoot #3 (Fig. 2). 80 EPUs were weighed (wet) and separately planted in small trays (20x9.5x12cm) filled with an sand/ silt mixture (median grain size 169 μm) from a tidal flat near Bath, Westerschelde estuary, The Netherlands. By having only one EPU per tray, consisting of two shoots, minimal self-shading occurs during emergence. Four trays were in each overflow aquaria (50x39x26cm). Four aquaria, each having a different emergence period, were placed in one container; in total 5 replicate containers were used (so 5 replicates, 4 treatments and 4 pseudoreplicates; set-up see Fig. 2) Overflow was created by pumping (300l/h) sand-filtered Oosterschelde water from the container into the bottom of each aquarium, whereupon water returned into the container. The experimental emergence periods of the seagrass (0, 4, 6, or 8h per 12h) were created by automatically switching off the circulation pump, after which the aquaria drained. Emergence periods were chosen for their ecological relevance to seagrass along the natural desiccation gradient (ranging from no desiccation to extreme desiccation): 0h: subtidal zone (*Z. noltii* grows in this zone

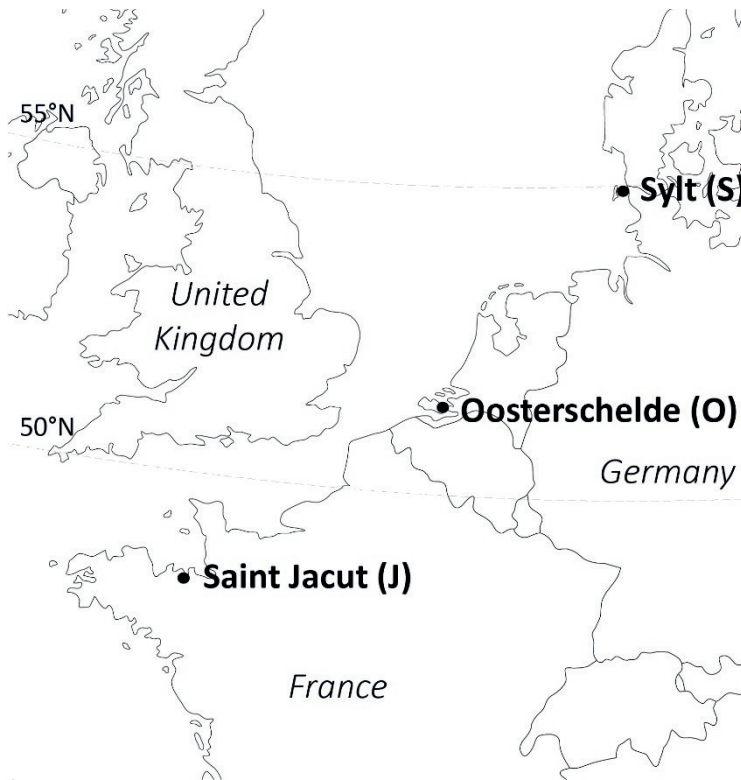


Figure 1: Origin of the *Zostera noltii* plants used in the experiments (experiment 1: only Oosterschelde, experiment 2: all three populations)

in Basque country and Portugal; Valle *et al.* 2011 and Cunha *et al.* 2013, respectively), 4h: lower intertidal seagrass zone in the Oosterschelde; 6h: average *Z. noltii* emergence period in the Oosterschelde, 8h: the upper extreme of *Z. noltii* distribution in the Oosterschelde. Light ($217 \pm 31 \mu\text{E m}^{-2} \text{s}^{-1}$) was produced by beams of LED-lights emitting a photo spectrum with peaks at 450 (blue) and 670 (red) nm, a spectrum optimized to emit the absorption spectrum for plant photosynthesis. To create low light conditions ($51 \pm 10 \mu\text{E m}^{-2} \text{s}^{-1}$), black neutral density filters (which reduce the intensity of all wavelengths of light equally, attenuation coefficient of 0.76 screen^{-1}) were placed over half of each aquarium. The photoperiod was set at 14 hours of light per day, with one emergence period during the light period and one emergence period was during the dark period. The temperature of the climate controlled room was set at 17°C . These abiotic settings resemble natural conditions at the beginning of the experiment. Epiphytes (if any) were gently removed weekly. Every week, the entire volume of water of each of the five water containers was refreshed to maintain constant water quality (i.e. salinity, nutrients, etc.) among the 4 replicates within the containers. At the end of the experiment ($t=92$ days), EPUs were harvested after which their growth response to the treatments was determined by measuring their weight (aboveground and belowground parts

separately) and morphology. Relative growth rate (RGR) was based on EPUs wet weight (WW, in grams) and was calculated as: $RGR = (\ln WW_{\text{end}} - \ln WW_{\text{start}})/(\Delta t)$, Δt is the running time of the experiment in days.

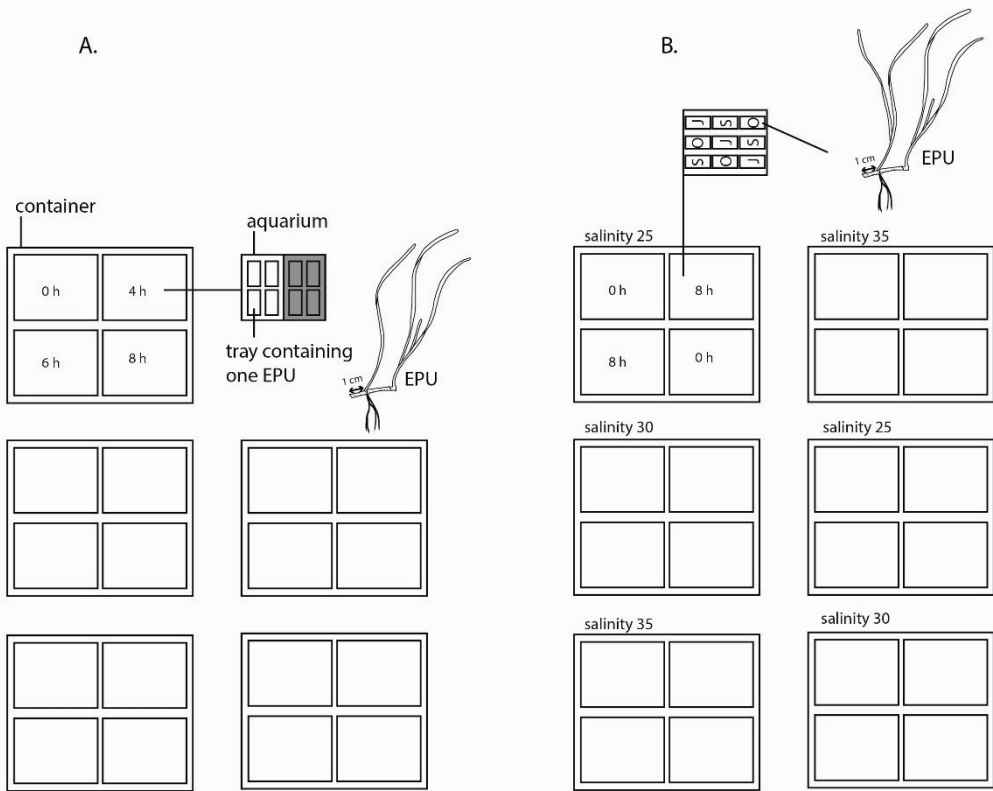


Figure 2: Experimental set-up of experiment 1 and 2. (A) Set-up of experiment 1 testing emergence (0, 4, 6 and 8 h per 12 h) and shading (0 and 75% shading) of $217 \pm 31 \text{ mE m}^{-2}\text{s}^{-1}$, shown as grey shading) in a nested design with five true replicates and four pseudoreplicates. (B) Set-up of experiment two testing three populations of origin (S = Sylt, O = Oosterschelde, J = St Jacut) nested in two replicated emergence regimes (0 and 8 h emergence per 12 h), nested in three salinities (25, 30 and 35). An experimental plant unit (EPU) consists of two *Zostera noltii* shoots (of which one is the apical shoot), plus 1 cm of the adjacent rhizome internode (drawing courtesy of Vanessa González-Ortiz).

Experiment 2

To test the effect of emergence, population of origin and salinity and their interaction on *Z. noltii*, we examined seagrass growth of plants originating from three temperate seagrass populations, at two emergence periods, in three natural range salinities. Plants were collected from three locations in western

Europe: Saint-Jacut, (Brittany, France), 48° 36'14. 79" N, 2° 11'41. 49" W, average salinity of 34.7, Oosterschelde (Southwest Netherlands), 51°53'20. 58" N, 3°93'88.35" E, average salinity of 29.5 and Sylt (Wadden Sea, Germany), 54° 47'50.77" N, 8° 17'43.87" E, average salinity of 29.9 (Fig. 1). At all three locations, water is relatively clear, namely Saint Jacut.: 1.5 – 2 m secchi depth (2 – 4 Formazin Nephelometric Units; Ifremer 2014), Oosterschelde: 1.50 m secchi depth (Data Ministry of Infrastructure and Water Management 2002-2009) and Sylt: 1 - 3.5 m secchi depth (0.5 – 1.9 m⁻¹ light attenuation coefficient at an average tidal range of 1.7m; van Katwijk *et al.* 1998 and frequent personal observation second and last author in later years). Plants from Sylt and Saint Jacut were transported to the laboratory within 24 h, stored in wet tissues, kept at 6°C temp. Plants from Oosterschelde were transported free floating within 3 h. Upon arrival, they were replanted in the laboratory to acclimatize during 2 months in the same sediments as in experiment 1, in sand-filtered Oosterschelde water (salinity of 31-32, 17° C, 210 µE m⁻² s⁻¹ 14 h per day) until the start of the experiment on 30 September 2011, (which is at the end of the growing season in the field, but previous studies had shown vigorous growth of this perennial plant during autumn in the laboratory, Han *et al.* 2012). Except for the applied treatments, the experimental set-up resembled that of the first experiment, i.e. same EPU characteristics, planting trays, overflow aquaria, emergence methods, plant care, artificial lighting, light/ dark cycle and climate controlled temperature were used. In this experiment, 2 containers per salinity treatment were used, in each container 4 aquaria were placed with either 0 or 8 h emergence per 12h, and within each aquarium 3 pseudoreplicates for each population of origin (Fig. 2). EPUs of each population were weighted wet. To test the response of seagrass to different salinities, overflow aquaria were filled with seawater with a salinity of 25, 30 or 35. Seawater with a salinity of 25 was obtained by mixing demineralized water to the ambient (salinity of 30) seawater, whereas a salinity of 35 was obtained by adding artificial seasalt (Instant Ocean® Sea Salt, <http://www.instantocean.com>). pH was assumed not to be influenced by the dilution with demineralized water (as was found in a salinity experiment described in van Katwijk *et al.* 1999; pH data of this experiment are presented in supplementary Table 1). To maintain stable salinity levels, salinity was checked at least twice a week and if needed adjusted by adding demineralized water to compensate for evaporation. The water of each container was refreshed after 5 weeks. Nutrient levels of the water were measured after 28 and 61 days and showed no correlation with salinity treatments (supplementary Table 2). EPUs were evenly divided over all treatments (n=2 true replicates x 3 pseudo replicates x 2 emergence replicates = 12 per treatment). At the end of the experiment (t=75days), EPUs were harvested and measured for weight (aboveground and belowground parts separately) and morphology to determine their growth response. Relative growth rate (RGR) was based on EPUs wet weight (WW, in grams) and was calculated as: $RGR = (\ln WW_{\text{end}} - \ln WW_{\text{start}})/(\Delta t)$, Δt is the running time of the experiment in days.

Statistical analysis

The results of experiment 1 were analysed by linear mixed models with light and emergence time as fixed factors. As aquaria were nested within containers (Fig. 2) and EPU within one aquarium consisted of pseudoreplicates, we included aquarium nested in container (container/aquarium) as a random factor in our models. Rhizome length, shoot length, aboveground biomass, belowground biomass, total biomass, relative growth rate (RGR) and aboveground / belowground biomass ratio were analyzed by general linear mixed models (lme, nlme package, R 3.2.3) and shoot numbers by a generalized linear mixed model with a Poisson distribution (glmer, lme4 package, R 3.2.3).

The results of experiment 2 were also analysed by mixed models with emergence, salinity and origin as fixed factors. Similar to the set-up of experiment 1, aquaria were nested in containers (Fig. 2) and we thus included container/aquarium nested as a random factor in our models. Longest shoot length, rhizome length, aboveground biomass, belowground biomass, total biomass, relative growth rate (RGR,) and aboveground / belowground biomass ratio were analysed general linear mixed models (lme, nlme package, R 3.2.3) and shoot numbers by a generalized linear mixed model with a poisson distribution (glmer, lme4 package, R 3.2.3 © R Core Development Team).

Normal distribution of all data was tested on model residuals by means of a Shapiro test and by looking at the histogram, and data were log- or square root-transformed to meet model assumptions if necessary.

Results

Desiccation stress (emergence) significantly reduced aboveground biomass (Fig. 3A, $P = 0.010$) and relative growth rate (Fig. 3F, $P = 0.045$) (Table 1); aboveground biomass was reduced by 27% and relative growth rate by 32% in the 8h emergence time treatment compared to the completely submerged treatment (0h emergence time). In line with this, emergence also significantly reduced rhizome length (Fig. 3G, $P = 0.049$) in the 6 and 8 h emergence treatment by 26-28%. Next to desiccation stress, Shading (light reduction) also significantly reduced the relative growth rate (Fig 3F, $P = 0.039$) by 18%. In addition, shading significantly reduced belowground biomass by 33% (Fig. 3B, $P < 0.001$) and total biomass by 23% ($P = 0.010$), thereby strongly increasing the aboveground/belowground biomass ratio (Fig. 3D, $P < 0.001$). Plant morphology also changed significantly under light limitation stress; leaves grew 21% longer when shaded (Fig. 3H, $P = 0.011$).

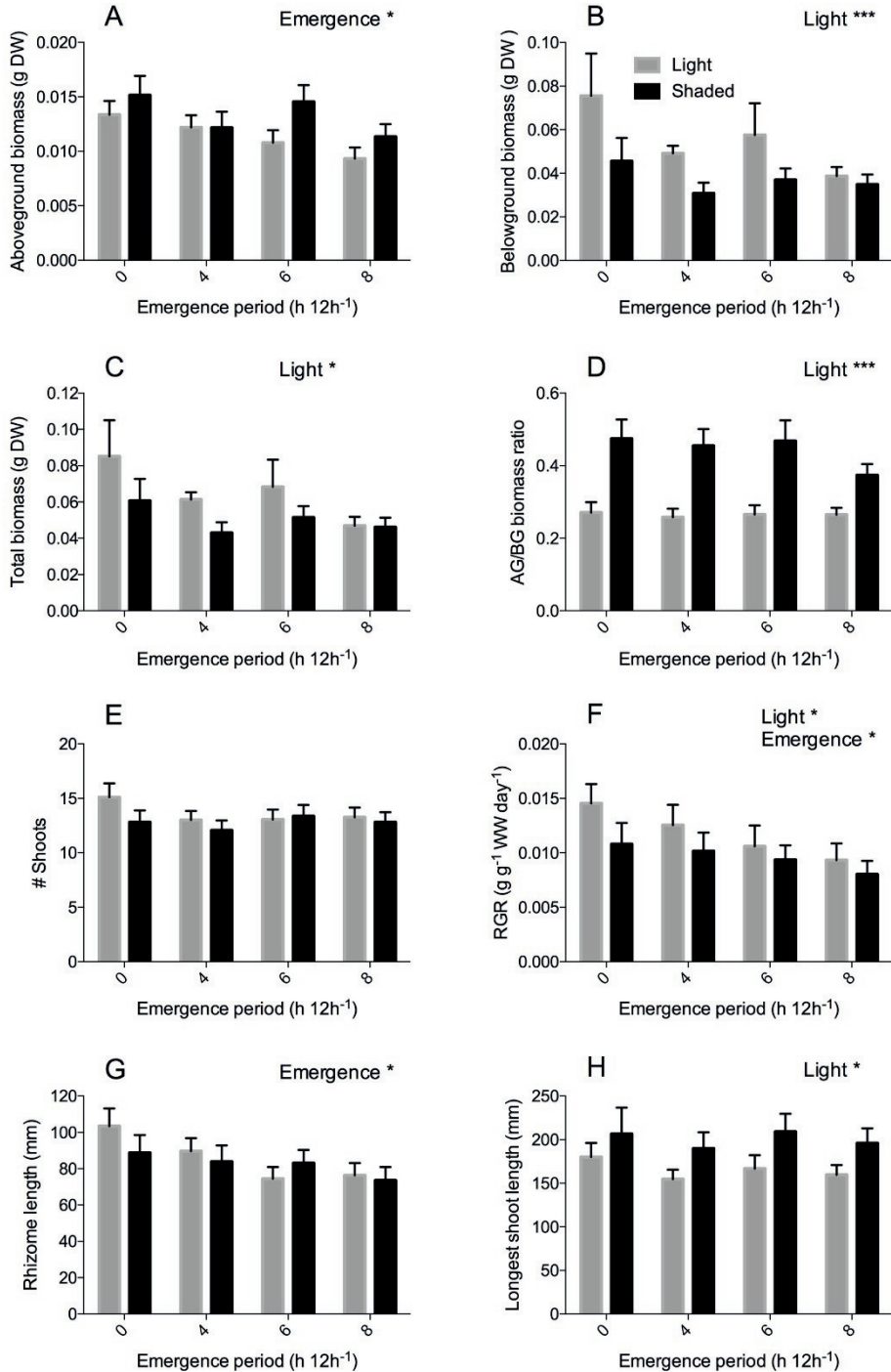


Figure 3: *Zostera noltii* development after 60 days in relation to emergence time (x-axis) under light (grey bars) and shaded (black bars) conditions. A)

Aboveground biomass, B) belowground biomass, C) Total biomass, D) aboveground/belowground (AG/BG) biomass ratio, E) Shoot numbers (# shoots), F) Relative growth rate (RGR) of wet weight (WW), G) Rhizome length and H) Longest shoot length. Statistical results are displayed in the upper right corner of each panel, $0.01 < P < 0.05 = *$, $0.001 < P < 0.01 = **$, $P < 0.001 = ***$. Detailed statistical results are displayed in table 1. Error bars represent standard errors (SEM).

Table 1: Effects of light and emergence on *Zostera noltii*. Statistical results of Experiment 1.

Factor	test	Trans-formation	Treatment	F value	DF	P	
DW AG	lme	log	light	3.531	1	0.060	n.s.
			emergence	11.290	1	0.010	*
			light*emergence	3.965	1	0.265	n.s.
DW BG	lme	log	light	14.728	1	<0.001	***
			emergence	3.022	1	0.388	n.s.
			light*emergence	3.597	1	0.308	n.s.
DW total	lme	log	light	6.569	1	0.010	*
			emergence	3.667	1	0.300	n.s.
			light*emergence	3.498	1	0.321	n.s.
AG/BG ratio	lme	sqrt	light	52.640	1	<0.001	***
			emergence	1.560	1	0.669	n.s.
			light*emergence	2.404	1	0.493	n.s.
# shoots	glmer	-	light	2.185	1	0.139	n.s.
			emergence	3.165	1	0.367	n.s.
			light*emergence	2.545	1	0.467	n.s.
RGR WW	lme	none	light	4.240	1	0.039	*
			emergence	8.042	1	0.045	*
			light*emergence	0.955	1	0.812	n.s.
Rhizome							
Length	lme	none	light	0.480	1	0.489	n.s.
			emergence	7.865	1	0.049	*
			light*emergence	2.169	1	0.538	n.s.
Longest shoot	lme	log	light	6.421	1	0.011	*
			emergence	1.388	1	0.708	n.s.
			light*emergence	0.364	1	0.947	n.s.

Notes: Non-significant test results are marked with “ns”, whereas significant test results are marked with “*”. Abbreviations used: lme = general linear mixed model, glmer = generalized linear mixed model with a Poisson distribution, DF = degrees of freedom, p = p-value, DW = dry weight, WW = wet weight, AG = above ground biomass, BG = below ground biomass, RGR WW = Relative growth rate, # = number, L = length, W = width, ln = natural logarithm, SQRT = square root, log = logarithm.

Table 2: Results of statistical tests testing the main and combined effects of emergence time, salinity and population origin (location) on seagrass growth and morphology.

Factor	Test	Transformation	Treatment	DF	F value	P	
DW AG	lme	sqrt	emergence	1	12.571	0.003	**
			salinity	1	4.109	0.138	n.s.
			origin	2	50.517	<0.001	***
			emergence*salinity	2	1.201	0.328	n.s.
			emergence*origin	2	0.865	0.423	n.s.
			salinity*origin	4	0.358	0.839	n.s.
			emergence*salinity*origin	4	2.042	0.091	n.s.
DW BG	lme	sqrt	emergence	1	20.036	<0.001	***
			salinity	1	0.801	0.7871	n.s.
			origin	2	37.318	<0.001	***
			emergence*salinity	2	1.224	0.322	n.s.
			emergence*origin	2	0.710	0.493	n.s.
			salinity*origin	4	0.664	0.617	n.s.
			emergence*salinity*origin	4	0.591	0.670	n.s.
DW Total	lme	sqrt	emergence	1	15.312	0.001	**
			salinity	1	0.180	0.843	n.s.
			origin	2	39.652	<0.001	***
			emergence*salinity	2	1.150	0.343	n.s.
			emergence*origin	2	0.182	0.834	n.s.
			salinity*origin	4	0.690	0.600	n.s.
			emergence*salinity*origin	4	1.393	0.238	n.s.
AG/BG ratio	lme	sqrt	emergence	1	1.151	0.300	n.s.
			salinity	1	0.460	0.670	n.s.
			origin	2	0.919	0.401	n.s.
			emergence*salinity	2	0.726	0.500	n.s.

			emergence*origin	2	1.577	0.210	n.s.
			salinity*origin	4	0.344	0.848	n.s.
			emergence*salinity*origin	4	1.355	0.252	n.s.
# shoots	glmer	n.a.	emergence	1	0.285 ¹	0.593	n.s.
			salinity	1	1.491	0.222	n.s.
			origin	2	10.793	0.004	**
			emergence*salinity	1	0.013	0.910	n.s.
			emergence*origin	2	0.341	0.843	n.s.
			salinity*origin	2	1.459	0.482	n.s.
			emergence*salinity*origin	2	0.162	0.922	n.s.
			RGR WW	lme	sqrt	emergence	1
salinity	1	1.030				0.457	n.s.
origin	2	1.770				0.174	n.s.
emergence*salinity	2	0.690				0.518	n.s.
emergence*origin	2	0.290				0.746	n.s.
salinity*origin	4	0.730				0.572	n.s.
emergence*salinity*origin	4	2.230				0.068	n.s.
Rhizome length	lme	none				emergence	1
			salinity	1	2.948	0.161	n.s.
			origin	2	8.581	<0.001	***
			emergence*salinity	1	0.481	0.489	n.s.
			emergence*origin	2	0.101	0.903	n.s.
			salinity*origin	2	0.250	0.779	n.s.
			emergence*salinity*origin	2	0.800	0.451	n.s.
			Longest shoot	lme	none	emergence	1
salinity	1	1.721				0.260	n.s.
origin	2	9.918				<0.001	***
emergence*salinity	1	0.543				0.462	n.s.
emergence*origin	2	0.450				0.638	n.s.
salinity*origin	2	0.332				0.718	n.s.
emergence*salinity*origin	2	0.670				0.513	n.s.

Notes: Non-significant test results are marked with “ns”, whereas significant test results are marked with *** for $P < 0.001$, ** $0.001 < P < 0.01$ and * $0.01 < P < 0.05$. Abbreviations used: DF= degrees of freedom, P = p-value, DW = dry weight, WW = wet

weight, AG = above ground biomass, BG = below ground biomass, RGR WW= Relative growth rate, # = number, L = length, W = width, sqrt = square root, log = logarithm, lme = general linear mixed model, glmer = generalized linear mixed model. ¹Indicates chi-square values of the generalized linear mixed model rather than F values.

The second experiment included exposing *Z. noltii* from three different locations in Western Europe (France, the Netherlands, Germany) to desiccation stress (0 vs. 8h emergence) and three different salinities (25, 30, 35 ppt). Similar to the first experiment, we found that desiccation stress (8h emergence) reduced relative growth rate (Fig. 4F, $P < 0.001$) and aboveground biomass (Fig. 4A, $P = 0.003$) by 25% and 19% respectively (Table 2). In addition, belowground biomass (Fig. 4B, $P < 0.001$) and total biomass ($P = 0.001$) were also reduced by desiccation stress by 32% and 29% (Table 2). Location of origin affected morphology and biomass of the plants; rhizomes (Fig. 4G, $P < 0.001$) and leaves (Fig. 4H, $P < 0.001$) were smaller and aboveground (Fig. 4A, $P < 0.001$), belowground (Fig. 4B, $P < 0.001$) and total biomass (Fig 4C, $P < 0.001$) lower in a gradient from France (Saint Jacut) to Germany (Sylt) (Table 2). Leaves and rhizomes from the French *Z. noltii* were 22% longer than the German plants and above- and belowground biomass was approximately 2.4 times larger as compared to Sylt plants at the end of the experiment (pooled results, Fig. 4). As RGR did not differ between the origins of the populations (e.g. RGR of Saint Jacut was 1.06 times the RGR of Sylt), the size differences at the end of the experiment reflect the differences at the beginning of the experiment. Similar plant size differences between these populations are observed in other years or seasons (Soissons *et al.* 2018, Govers *et al.* in press). Plants originating from the Netherlands (Oosterschelde) were in the middle for all measured traits. In addition, total shoot numbers were also significantly higher (Fig. 4E, $P = 0.004$, Table 2) in units with plants from France than in units with Dutch and German plants. Despite these differences in morphology and biomass, seagrass plants from different origins were similarly affected by emergence and salinity as we found no interactive effects for any of our parameters (salinity * origin * emergence). Surprisingly, we found no effect of our salinity treatment on any growth or morphological parameters, indicating a broad salt tolerance of *Z. noltii* (Table 2).

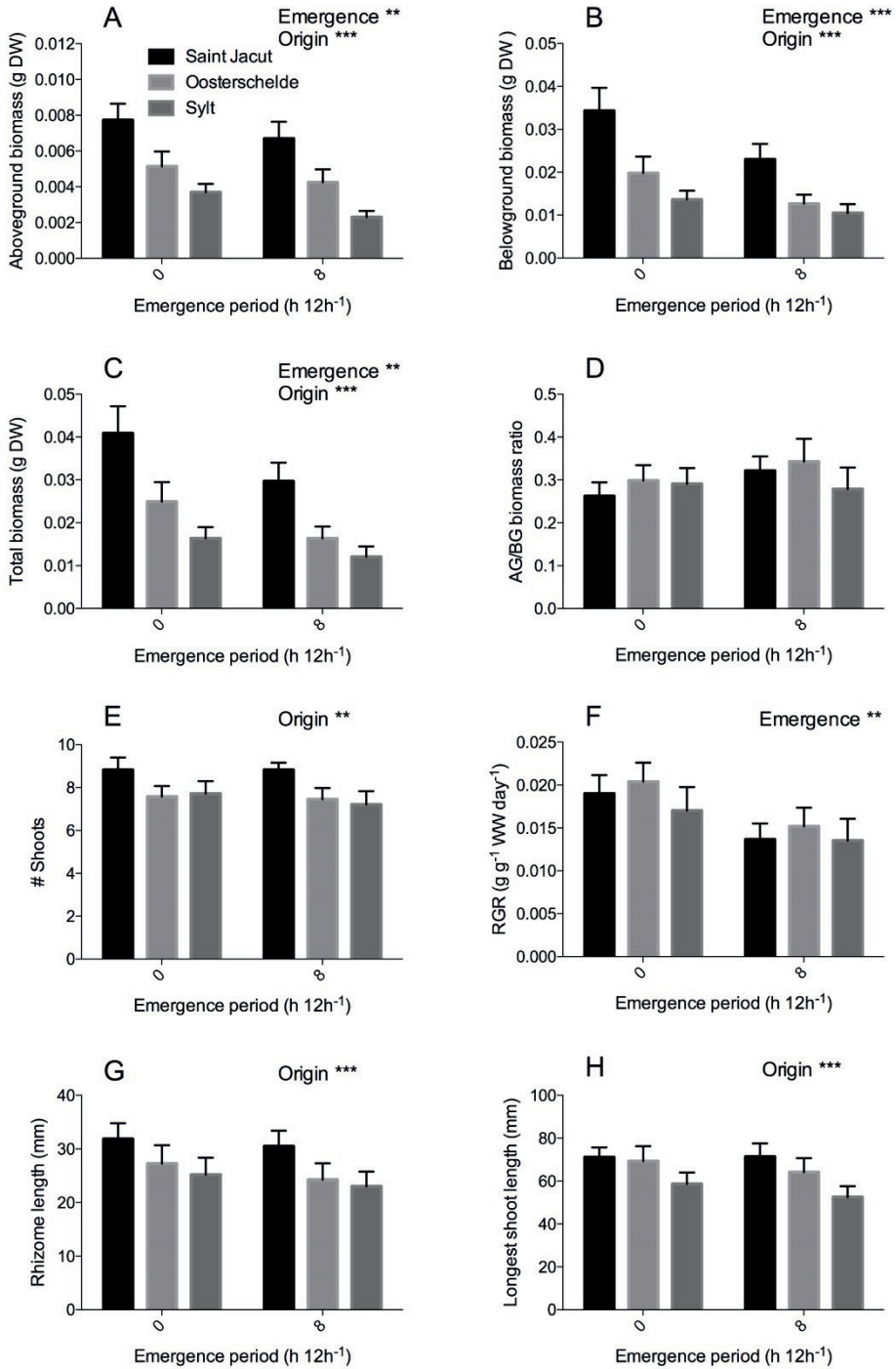


Figure 4: *Zostera noltii* growth response after 75 days of combined salinity and emergence treatment. Salinity treatments are pooled as no significant differences

were found between treatments of all measured parameters (Table 2). Locations of origin are displayed in the following colors: Saint Jacut in black, Oosterschelde in light grey and Sylt in dark grey. A) Aboveground biomass, B) belowground biomass, C) Total biomass, D) aboveground/belowground (AG/BG) biomass ratio, E) Shoot numbers (# shoots), F) Relative growth rate (RGR) of wet weight (WW), G) Rhizome length and H) Longest shoot length. Statistical results are displayed in the upper right corner of each panel, $0.01 < P < 0.05 = *$, $0.001 < P < 0.01 = **$, $P < 0.001 = ***$. Error bars represent standard errors (SEM).

Discussion

Emergence had a negative effect on seagrass growth in both experiments confirming our hypothesis (H1). Increasing duration of air exposure hampered seagrass growth, reflected by a smaller RGR and reduced aboveground biomass of the plants as compared to the control group that was never subjected to emergence. The reduced growth might be the consequence of loss of photosynthetic capacity due to desiccation damage to the leaves (Leuschner *et al.* 1998; Vermaat *et al.* 2000; Fernandez-Torquemada & Sanchez-Lizaso 2005; Shafer *et al.* 2007). In the field, the negative effect of desiccation was demonstrated by the higher biomass found in depressions (which retain water during low tide) as compared to elevations (dry at low tide) within the same bed (van Tussenbroek *et al.* 2016). Concurrently, these authors found lower sexual reproductive efforts in the depressions, in line with the general notion that reproductive efforts increases with increasing stress in seagrasses (Cabaco and Santos 2012). Further growth reduction from desiccation can be expected in situ as sediment trapping by *Z. noltii* often causes the plant to grow on elevations, making them more prone to desiccation (Reise & Kohlus 2008, van der Heide *et al.* 2010, van Tussenbroek *et al.* 2016). Furthermore, desiccation damaged leaves may be easier torn by waves than undamaged leaves, leaving significantly shorter leaves for photosynthesis (Vermaat *et al.* 1993; Boese *et al.* 2003), and reducing water retention by the leaves (e.g. Fox 1996), which is density dependent (de Fouw *et al.* 2016).

Shading resulted in longer leaves, but reduced belowground biomass and RGR. Aboveground/belowground biomass ratio increased (experiment 1), similar to previous studies without emergence treatments (Vermaat *et al.* 1993; Peralta *et al.* 2002; Cabaco *et al.* 2009). Plants apparently invested more in aboveground biomass than in belowground biomass. Confirming our hypothesis (H2), shading added to the negative effect of emergence on RGR. Effects were additive (no interactive effect, so no antagonistic or synergistic effects). The combination is thus more stressful for the plants than singular effects and may explain the strong reduction in net photosynthesis in *Z. noltii* beds during low tide as compared to high tide, due to self-shading by the leaves lying on top of each other during low tide (Clavier *et al.* 2011, Ouisse *et al.* 2011). When shading in the intertidal is caused by algal overgrowth, desiccation of the plants during

low tide may be less severe as the leaves are kept wet by the algal cover. However, in such cases, suffocation, sulfide and ammonium toxicity pose a threat to seagrasses (Goodman *et al.* 1995; Den Hartog 1996; Holmer & Nielsen 2007; Govers *et al.* 2014). In addition, a stronger reduction in net growth may be expected in the field as the resulting longer leaves experience more wave induced drag force than the shorter leaves that develop under ambient light conditions (Bouma *et al.* 2005; La Nafie *et al.* 2012).

The tested salinity range (salinities of 25, 30 and 35, experiment 2) did not affect plant growth over the course of the experiment, which contrasts our hypothesis (H3). Apparently, *Z. noltii* was not stressed by salinities of 30 and 35, probably due to its ability to acclimatize to salinity changes (Touchette 2007), not resulting in reduced growth (production of biomass) as observed for *Z. marina* by Kamermans *et al.* (1999). This is supported by presence of dense *Z. noltii* beds in Banc d'Arguin, Mauritania with salinity levels of over 40 (Vermaat *et al.* 1993), whereas *Z. marina* has an optimum at salinities as low as salinity 25 (Nejrup & Pedersen 2008). Whereas hypothesis H2 (additional stressors aggravate the effects of emergence) was confirmed with regards to shading, it was not confirmed regarding salinity in the range tested. Considering that salinity did not have an effect at all within the tested range, this is not surprising. Perhaps this implies that *Z. noltii* is more of a generalist than a specialist in terms of the environmental extremes it can withstand, making it more of an opportunistic pioneer species instead of a climax community species, as compared to *Z. marina*.

The effects of salinity and desiccation stress (experiment 2) did not differ between plants of different origin. This contrasts our expectation that plants that are used to relatively low salinities would encounter more osmotic stress than those that are already used to relative high salinity conditions, as was shown for *Z. noltii* comparing a Spanish and Dutch population in salinity 15 and 35 (Vermaat *et al.* 2000) and for *Z. marina* in a narrower or lower salinity range (salinity 22-29, van Katwijk *et al.* 1999; salinity 2-25, Salo *et al.* 2014). Thus, *Z. noltii* plants that normally grow at a salinity of 35 grew equally well at salinities of 25 or 30, and plants used to salinities of 29-30 grew equally well at salinities of 25 or 35. Perhaps this flexibility may be explained by plants originating from estuarine and shallow coastal conditions (as we used in our experiments) being more used to frequent variations in osmotic stress and thus better able to make fast physiological adaptations, compared to plants from more osmotically stable, true marine environments. Although plants from the three populations differed in plant size and morphology (from big (Saint Jacut), medium (Oosterschelde) to small (Sylt)), in line with the trend over a broader latitudinal gradient for this species (South Spain to Sylt; Soissons *et al.* 2018), their plant size did not influence their ability to cope with emergence or salinity stress.

Conclusions and ecological implications

In this study, we found that desiccation stress imposed by emergence and shading have a negative effect on *Z. noltii*. When combined, they show additive effects, there are no synergistic or antagonistic effects. This makes plants vulnerable to such combination (although synergistic effects would enhance this vulnerability even more). The three populations tested show distinctive size difference, but did not respond differentially to emergence and salinity. This indicates that the populations show morphotypic rather than ecotypic variation regarding emergence and salinity, within the range tested.

Desiccation is likely to determine the upper distribution of *Z. noltii*, although other factors than physiological factors (such as predation, competition for space and resources) can also be important under field conditions. The question rises why plants don't grow towards the mean low water level in our research areas, where emergence times are shorter? Our study shows that shorter and absent emergence periods are favourable for *Z. noltii*. Although seagrass meadows are often light limited at increasing depth (Philippart 1995; Ralph *et al.* 2007; Cabaco *et al.* 2009; van der Heide *et al.* 2010), light is not likely a limiting factor in the habitats of the three tested populations (see Materials and Methods). Probably, water and/or sediment dynamics may explain the absence of *Z. noltii* in the lower ranges of the intertidal (Suykerbuyk *et al.* 2016a, b). Still, in more southern ranges of its distribution, *Z. noltii* occupies the whole intertidal range and expands even in the higher subtidal (e.g. Basque country, Valle *et al.* 2011, Portugal, Cunha *et al.* 2013) and submerged in seas where tides are near absent (Mediterranean, Green & Short 2003). Further research is required to assess key factors in determining the depth limit of mid-intertidal *Z. noltii* beds. From our study, it is however clear that (i) emersion period is a factor controlling the upper limit, (ii) shading (for example by self-shading during emersion) likely lowers the upper limit, as the effects shading and emergence were additive, and (iii) this upper limit is not affected by salinity or origin of the population. For restoration purposes, there is no need to carefully select donor populations regarding salinity regime within the range tested, as the plants of different origins (salinity 30 and 35) were not influenced by applied salinities (25, 30, 35).

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Chapter 3



Suppressing antagonistic bio-engineering feedbacks doubles restoration success

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Abstract

In a seagrass restoration project, we explored the potential for the enhancing restoration process by excluding antagonistic engineering interactions (i.e., biomechanical warfare) between two ecosystem engineers: bioturbating lugworm *Arenicola marina* and the sediment stabilizing seagrass *Zostera noltii* Hornem. Applying a shell layer underneath half our seagrass transplants successfully reduced adult lugworm density by over 80%, and lugworm induced microtopography (a proxy for lugworm disturbance) at the wave sheltered site. At the wave exposed site adult lugworm densities and microtopography were already lower than at the sheltered site, but were further reduced in the shell treated units. Excluding lugworms and its bio-engineering effects corresponded well with a strongly enhanced seagrass growth at the wave sheltered site, which was absent at the exposed site. Enhanced seagrass growth in the present study was fully assigned to the removal of lugworms negative engineering effects and not to any (indirect) evolving effects such as an altered biogeochemistry or sediment stabilising effects by the shell layer. The context dependency implies that seagrass establishment at the exposed site is not constrained by negative ecosystem engineering interactions only, but also by overriding physical stresses causing poor growth conditions. Present findings underline that, in addition to recent emphasis on considering positive (facilitating) interactions in ecological theory and practice, it is equally important to consider negative engineering interactions between ecosystem engineering species. Removal of such negative interactions between ecosystem engineering species can give a head start to the target species at the initial establishment phase, when positive engineering feedbacks by the target species on itself are still lacking. Though our study was carried out in a marine environment with variable levels of wave disturbance, similar principles may be expected to apply to other ecosystems that are inhabited by ecosystem engineers.

Introduction

About 30% to 50% of the Earth's coastal ecosystems have been severely degraded in the last decades, often due to anthropogenic disturbances such as eutrophication or over-exploitation (Orth *et al.* 2006; Barbier *et al.* 2008). Even though these areas make up just 4% of the Earth's total surface, systems like salt marshes, coral reefs and seagrass meadows are of great economic and ecological importance, because large human populations depend on them for storm buffering, fisheries and enhanced water quality (Orth *et al.* 2006; Barbier *et al.* 2008). Moreover, they serve as key-habitat in the life cycles of many marine animal species. Although these ecosystems were initially considered as highly resilient to human disturbance, we now know that most coastal ecosystems do not respond linearly to change, but may often collapse without warning (Silliman *et al.* 2005; van der Heide *et al.* 2007; Hughes *et al.* 2010). It has also emerged that recovery or restoration of coastal ecosystems has turned out to be extremely difficult (Bakker *et al.* 2002, Orth *et al.* 2006; Halpern *et al.* 2007; Omori 2010).

Various studies have shown that sudden collapse and lack of recovery in coastal ecosystems may follow alternative stable state behaviour, and can be attributed to disturbance of positive feedback mechanisms (e.g. van de Koppel *et al.* 2001; van der Heide *et al.* 2007). Such feedbacks typically arise from the ability of the foundation species (e.g., corals, seagrasses, reef-forming shellfish, marsh plants) to ameliorate environmental stress (i.e. "ecosystem engineering") (e.g. Jones *et al.* 1994; Madsen *et al.* 2001). Because these (self-) facilitation mechanisms typically act above a certain density or size (e.g. van der Heide *et al.* 2007, 2008; Bouma *et al.* 2009), coastal ecosystems are prone to threshold behaviour. The ecosystem seems stable above the threshold size or density, while recovery and restoration are nearly impossible when the threshold is not bridged (Hobbs & Norton 1996; Halpern *et al.* 2007; Suding & Hobbs 2009).

Today, the importance of (self-) facilitation and positive feedbacks are well recognized as important factors to consider for coastal ecosystem conservation and restoration (Crain & Bertness 2006; Halpern *et al.* 2007). The potential role of negative interactions between contrasting ecosystem engineers in restoration efforts, has recently been neglected, despite of the early recognition of its potential importance in marine habitats (see review Peterson 1980, 1991). For example, this has been shown for the negative effect of deposit feeders on filter feeders by making sediment more erosive, causing filter feeding to be hampered by more turbid water (i.e., trophic group amensalism; Rhoads and Young 1970). Several studies have also demonstrated that negative interactions between benthos and vegetation can be very strong (Philippart 1994; Hughes *et al.* 2000; Hughes & Paramor 2004; Siebert & Branch 2006; Berkenbusch *et al.* 2007; van Wesenbeeck *et al.* 2007). For instance, "biomechanical warfare" between ecosystem engineering salt marsh vegetation and infauna can cause a bi-modal species distribution on the salt marsh - mudflat interface, as bioturbation prevented plant colonization in the invertebrate dominated state,

and invertebrates were excluded by sediment compaction in the plant dominated state (van Wesenbeeck *et al.* 2007).

Meadows of the seagrasses *Zostera noltii* Hornem are notoriously hard to restore, and especially initial establishment is difficult as positive feedbacks are still lacking (i.e. Byers *et al.* 2006; van der Heide *et al.* 2007; van Katwijk *et al.* 2009). Historically, *Zostera* meadows are found to co-occur with the bioturbating lugworm *Arenicola marina* (Jacobs *et al.* 1983), though they may encounter negative worm effects (i.e., Philippart 1994; Reise & Kohlus 2008). At present, in some areas of NW Europe the *Zostera noltii* biomass distribution appears to be correlated to the presence of shallow layers of clay, compressed peat or shells that naturally exclude bioturbating lugworms (Reise 2002, Reise & Kohlus 2008, pers. obs. in Scheldt Estuary). Moreover, settlement of *Zostera noltii* has been observed in plots where lugworms were excluded for experimental purposes (Reise 1983, Reise & Kohlus 2008 and references therein). The absence of seagrass recovery on former seagrass grounds that are now inhabited by lugworms, despite of the improved water quality with respect to increased transparency and reduced nutrient concentrations over the last decades, may suggest that the lugworm presence and/or activity may hamper recolonization by seagrass within our study area.

In this study we test to what extent restoration of *Zostera noltii* meadows at formerly suitable seagrass habitats would benefit from removal of negative effects by the bioturbating lugworm *Arenicola marina*, and how this depends on abiotic conditions (sheltered vs. wave exposed). Removal of these negative ecosystem engineering effects on initial seagrass establishment was tested in a large-scale *Zostera noltii* restoration, by comparing sod transplantations with and without lugworm reduction by means of a shallow shell layer (cf. Reise 2002). To test the general relevance of excluding such negative engineering interactions, we tested this method under contrasting abiotic conditions (wave exposed vs. wave sheltered) that may cause other sources of sediment disturbance. We hypothesize that minimizing lugworm induced sediment disturbance will enhance seagrass growth, unless other sources of abiotic stress cause sediment disturbance.

Methods

Study area

Our experiment was performed in the Eastern Scheldt estuary (SW Netherlands), a former arm in the river Scheldt delta (Fig. 1a). After a severe flood in 1953, the estuary was heavily engineered to secure safety against flooding: fresh water input openings in the eastern part of the estuary were cut off by dams and the estuary's mouth in the west was partially closed by a storm surge barrier. Since then, the system changed (e.g. Louters *et al.* 1998) and the extensive seagrass meadows (up to 1000 hectares) decreased during the building of these so-called Delta Works. More surprisingly, this seagrass decline persisted

also long after the completion of the Delta Works, and are still ongoing today, despite the higher transparency of the water column and lower nutrient loading,

Nowadays, only several dozen of hectares of seagrass *Zostera noltii* Hornem are left (data Dutch Ministry of Infrastructure and the Environment), which appear to be concentrated on relatively stable sediments without lugworms being present (e.g., natural shell layers, heavy clay from former dikes or salt marsh remnants (personal observations). Exact mechanistic causes of this massive and still ongoing seagrass decline have not yet been identified. It was observed that the lugworm relief had increased at many intertidal former seagrass locations (personal observations). No previous seagrass transplantation or restoration efforts have been performed in the Eastern Scheldt estuary. Renewal of the defense walls stone cladding requires mitigating measures for the direct endangered seagrass following EU regulations, which led to the present transplantation project.

Experimental set-up

A total of 441 m² of native *Zostera noltii* Hornem sods (196 sods of 1.50 x 1.50m) with a vegetation cover ranging from 10 to 70% (mean sod vegetation cover 33%, standard error of mean 2%) were transplanted to two tidal flats, one sheltered (Krabbenkreek flat) and one relatively more exposed (Slikken van den Dortsman), in the Eastern Scheldt estuary (SW Netherlands) early in the growing season (June 2007) (Fig. 1b,c).

Sods were mechanically harvested in custom-made wooden boxes from a natural meadow at the donor site Slikken van Viane. They were protected against desiccation during transport and replanted within 24 hours to 28 plots divided over the sheltered (16) and exposed (12) site. Plots were distributed evenly 10 meter apart in rows at an average emerging time of 50 to 60% (Fig. 1b,c). To test the effect of plot size, plots consisted of either 5 or 9 sods placed in a checkerboard configuration (Fig. 1d). Planting configurations were evenly applied over the control and exclusion plots at both locations (Fig. 1b,c). In every second transplantation plot, adult lugworms (*Arenicola marina*) were excluded by placing a 10 cm thick shell layer (local cockle shell fragments till Ø 4cm) at a depth of 18 to 8 cm below the sediment surface, both underneath the seagrass sods and the surrounding plot sediment (Fig. 1e,f) (after Reise 2002). In the control plots, sediment was similarly removed and replaced to adjust for sediment handling.

Basic transplant characteristics (i.e. plant coverage and area), as well as lugworm and other biological parameters were monitored at monthly intervals during the growing season from June till October. The area covered by seagrass (m²) is considered as the main indicator for transplantation success (Schanz & Asmus 2003) with area being measured and cover being estimated by eye using a 25 x 25 cm grid and cross-checking between workers.

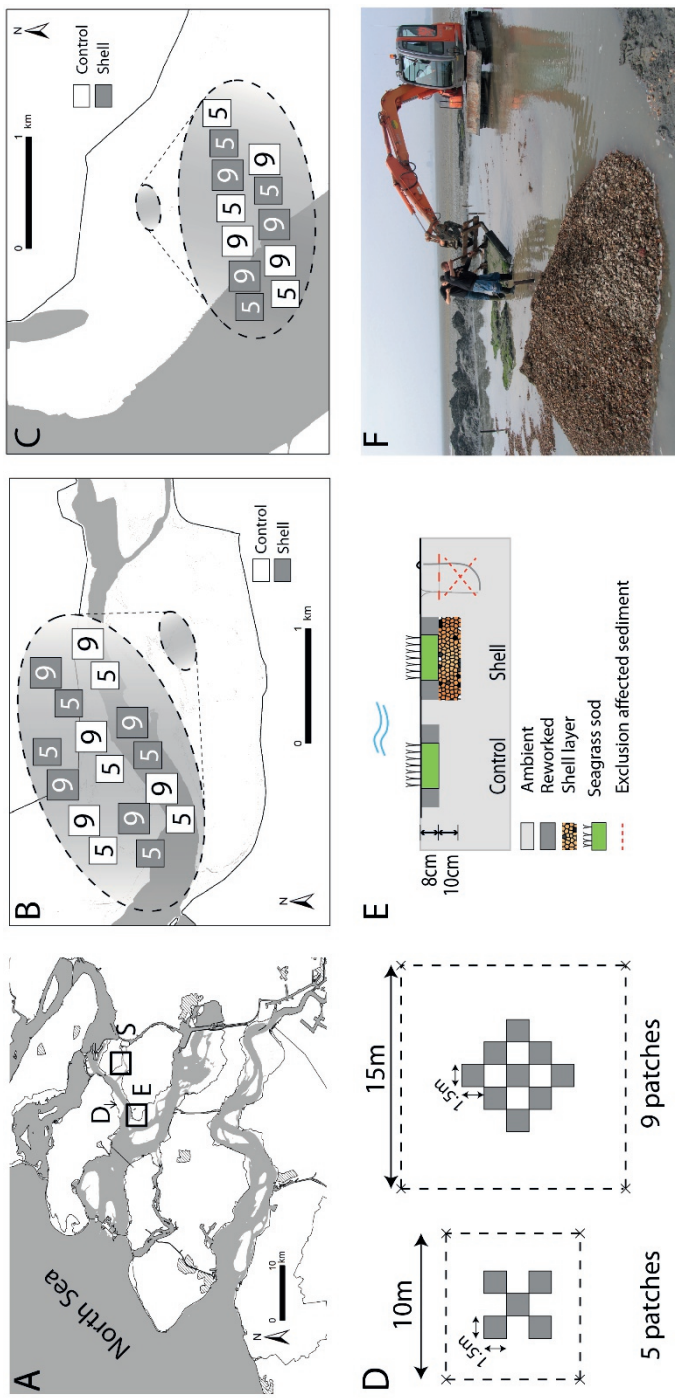


Figure 1: A. Location of the study areas in the south-west delta area of the Netherlands. Letters indicate the donor site (D), wave exposed (E) and sheltered (S) seagrass transplantation sites in the Eastern Scheldt estuary. B. Detailed top view of the wave sheltered Krabbenkreek flats. Colours indicate anti-lugworm treatments, numbers indicate planting configuration. C. Detailed top view of the wave exposed Slikken van den Dorstman flats. Colours indicate lugworm treatments, numbers indicate planting configuration. D. Schematic top view of plots with two different planting densities (5 and 9 patches), their configuration (checker board pattern) and the plot area that was reworked for the anti-lugworm treatment. E. Schematic cross section of the plots indicating how sediments are reworked and organized. Colours indicate different sediment origins or treatments. At the far right a schematic lugworm burrow to indicate the significance of the anti-lugworm treatment on lugworm burrowing. F. Large scale seagrass transplantation in progress at the sheltered site. In front: Shells that are applied to exclude lugworms. In the back: Placement of seagrass sod on the shell treatment.

Lugworm fecal cast counts per area were used as a quantitative proxy for the number of worms present (cf. Farke & Berghuis 1979; Flach & Beukema 1994). Juvenile and adult lugworms were divided by the diameter of the cylindrical shaped cast; rule of thumb: juvenile < diameter cast 1mm < adult. In order to prevent severe underestimation of lugworm numbers, cast counts were not performed on a rainy day or prior than 2 hours after the transplants emerged from the water (sensu Cadee 1976). Lugworm induced microtopography (also referred to as lugworm relief) was measured as the vertical height difference between a lugworms' pit and adjacent mound. This parameter was used as a simple integrative proxy for the potential negative engineering effects of lugworm induced sediment disturbance (e.g., direct burial, unearthing rhizomes and sediment instability) on seagrass survival, as it accounts for size and activity differences between local lugworm communities and can easily be compared across sites with other sources of sediment disturbance like wave exposure.

Sediment characteristics

Additional measurements on pore water nutrients (alkalinity, pH, NO_3^- , NH_4^+ and o-PO_4 , sheltered sites only) and sediment composition (grain size distribution and organic content, both sites) were performed just after maximum seagrass biomass and area were reached in the growing season (early September). Alkalinity (by titration with 0.01 mol l^{-1} HCL to pH 4.2 (Lamers, Tomassen & Roelofs 1998) and pH were determined directly after sampling, whereupon samples were frozen (-20°C) until further analysis. Ammonia and ortho-phosphate concentrations were measured colorimetrically on a Bran+Luebbe auto-analyzer, using hypochlorite (Berthelot reaction) and ammonium-molybdate, respectively. Organic matter content in freeze dried sediments (upper 5cm) was estimated as weight loss by ignition at 550°C after acidifying samples with HCL. Grain size distribution on the same sediment sieved over 1mm was measured by laser diffraction on a Malvern Particle Sizer.

Statistical analysis

All results are summarized by means \pm standard error. In advance to statistical analysis, data were checked for normality and if necessary transformed (Table 1). As planting configuration had no effect on seagrass survival (data not shown), plots are treated as replicates in further statistical analysis. Differences between transplantations sites and anti-lugworm treatments were analysed using analysis of variances (ANOVA, treatment nested in location), followed by post-hoc Tukey's honest-significant-differences (HSD) multiple means comparison tests at the 0.05 confidence limit. Effects of control and shell treatment on pore water and sediment data were tested by independent samples t- test. All tests were performed using Statistica (StatSoft).

Results

Shell layers successfully reduced the mean numbers of adult lugworms at both the sheltered ($P < 0.01$) and the exposed ($P < 0.01$) transplantation sites (Fig. 2a). Although initially, the ambient number of lugworms at the exposed site was almost 2.5 times lower than that at the sheltered location, the proportional reduction of adult worms due to the shell layer was of the same order of magnitude (81.3% vs 86.8% for sheltered and exposed, respectively). The shell treatment significantly promoted ($P < 0.05$) settlement of juvenile lugworms in absence of adult worms at both sites (277.8% vs 290.4 % increase for shell treated compared to control plots, respectively, both at wave sheltered and wave exposed site) (Fig. 2b). Adult lugworm numbers remained stable during at least three more years (data not shown).

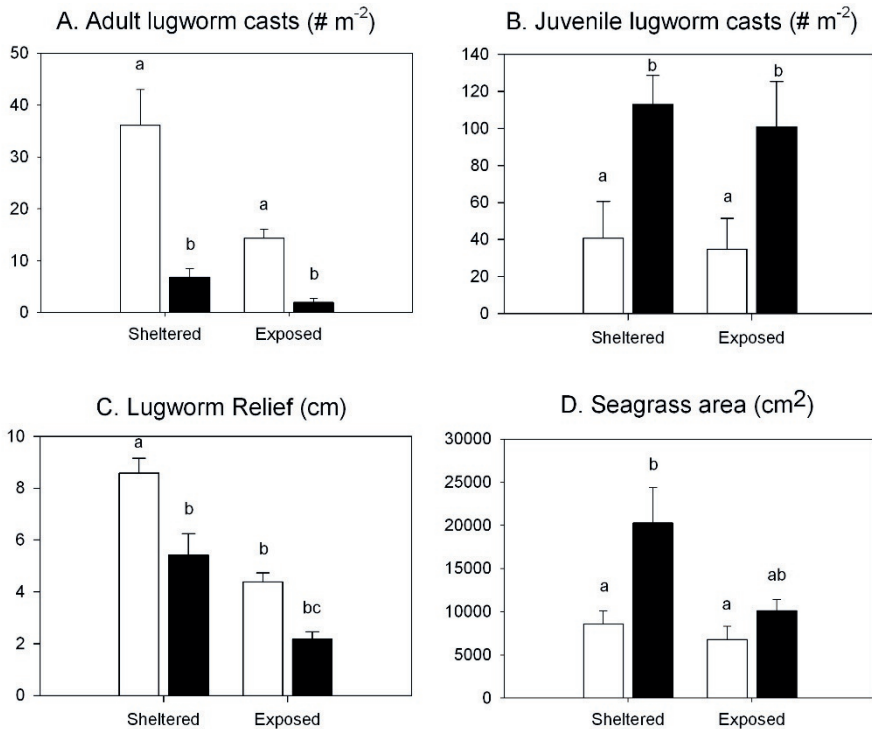


Figure 2: Effects of hydrodynamic forcing (wave sheltered vs wave exposed) and lugworm exclusion method (control in white vs exclusion in black) on A. adult lugworm density and B. juvenile lugworm density (both represented as number of casts per m²), C. lugworm relief (used as proxy for lugworm disturbance) and D. seagrass area per plot (used as proxy for restoration success). Data of 17+18 July 2008, 13 months after transplantation. Means with different letters are significantly different (Tukey's HSD, $p < 0.05$).

Table 1: Results of nested ANOVA on the number of adult and juvenile lugworms, their induced microtopography and the related seagrass area detected at each treated plot on two tidal flats

Dep. Variable	Transform.	Factor	SS	DF	MS	F	P
Adult lugworms	Square root	Location	23.14	1	23.14	8.28	0.006
		Treatment(Location)	122.74	2	61.37	21.96	<0.001
		Error	120.16	43	2.79		
Juv. lugworms	Fourth root	Location	1.75	1	1.75	1.53	0.223
		Treatment(Location)	14.39	2	7.19	6.29	0.004
		Error	49.21	43	1.14		
Lugworm relief	Fourth root	Location	128.31	1	128.31	22.84	<0.001
		Treatment(Location)	71.81	2	35.91	6.39	0.004
		Error	235.91	42	5.62		
Seagrass area	Log	Location	1.23	1	1.23	4.12	0.054
		Treatment(Location)	3.67	2	1.84	6.13	0.007
		Error	7.19	24	0.30		

Lugworm induced microtopography, and thereby the negative engineering effect of lugworms on seagrass, was significantly reduced by the shell treatment at the sheltered location (Fig. 2c; $P < 0.01$). Exclusion plots could

be easily recognized by the reduction of this characteristic pit and mound landscape. At the exposed site, similar anti-lugworm measures and their proportional effects on lugworm numbers resulted in relative higher reduction of lugworm relief between control and treated plots of the exposed site compared to the sheltered site (50.5% vs 37.0% reduction, respectively). Surprisingly, this reduction in relief was not found significantly different like found at the sheltered site.

Table 2: Sediment and pore water composition in control and exclusion plots at sheltered and exposed transplantation sites. Time of sampling: early September 2007, 3 months after transplantation. Significant differences between treated and untreated plots are indicated in bold (*t*-test, $p < 0.05$)

Parameter	Treatment	Sheltered		Exposed	
		Mean	SE	Mean	SE
D50 _{SED} (µm)	Control	159.13	8.54	179.08	1.19
	Shell	168.70	5.74	175.40	0.98
Org C _{SED} (%)	Control	1.27	0.13	0.55	0.09
	Shell	1.04	0.14	0.69	0.12
NH ₄ (µmol L ⁻¹)	Control	91.82	11.18	-	-
	Shell	104.17	13.15	-	-
o-PO ₄ (µmol L ⁻¹)	Control	13.14	2.62	-	-
	Shell	14.24	1.34	-	-
pH	Control	7.48	0.02	-	-
	Shell	7.49	0.03	-	-
Alkalinity (meq L ⁻¹)	Control	2.75	0.12	-	-
	Shell	3.03	0.10	-	-

Although reduction of lugworm microtopography was comparable at both sites, surprisingly no such comparable effects on seagrass growth were found. That is, at the sheltered site, seagrass growth was significantly promoted in *Arenicola*-excluded by shells plots as compared to the control plots (Fig. 2d, $P < 0.05$) (data 17+18 July 2008). However, no such difference was found at the exposed transplantation site, where seagrass growth was low both in the presence or absence of worm-exclusion. Seagrass growth responses at the sheltered site could not be ascribed to shell layer effects on sediment and pore water characteristics, as these were unaffected (Table 2) except for a slightly lower median grain size (D50_{SED}) in the exposed lugworm-exclusion plots ($t(14)=2.38$, $P < 0.05$). Planting configuration (5 or 9 sods) had no effect on seagrass survival at both sites at any point in time (data not shown).

Discussion

The use of facilitative interactions is now a common recommendation in restoration and conservation efforts, and ecosystem engineers causing such interactions are increasingly considered as conservation and restoration target species (Boogert *et al.* 2006; Byers *et al.* 2006; Crain & Bertness 2006; Halpern *et al.* 2007). Surprisingly, these papers however neglect the potential restoration benefits of the removal of negative interactions originating from ecosystem engineers, despite that such interactions can play an important role in marine habitats (see references intro). In this study, we show that suppressing such negative ecosystem engineering effects could be a useful approach in restoration and conservation efforts by giving at least a better start during the initial establishment phase. Using the seagrass (biostabiliser) – lugworm (biodestabiliser) interaction as a model system, we showed that seagrass growth was significantly enhanced by excluding lugworms in a lugworm-dominated area. Removing negative engineering interactions may especially be important for re-establishing those species, from which initial establishment is hampered by thresholds but later on benefit from self-facilitating positive feedbacks. The latter is clearly the case in seagrass (van der Heide *et al.* 2007; van Katwijk *et al.* 2010; van der Heide *et al.* 2011).

Enhanced seagrass growth in the present study was fully assigned to the lugworm exclusion and not to any (indirect) evolving effects such as an altered biogeochemistry (Table 2) or sediment stabilising effects by the shell layer. From previous studies on *Zostera* it is known that sediment stabilisation improves success at relatively exposed locations (van Katwijk & Hermus 2000; Reise & Kohlus 2008), but that this is not always the case at sheltered locations (van Katwijk & Hermus 2000). In our study the reverse holds: the success was only promoted at sheltered sites; therefore this explanation does not hold for our situation. At the relatively exposed site no positive effect of lugworm exclusion on seagrass growth was found, although the proportional reduction of lugworm disturbance was comparable to that observed at the sheltered site. We expect this to be due to poorer growth conditions at the exposed site, due to direct physical disturbances by waves and currents (dislodgement or mechanical stress), or to indirect effects of increased hydrodynamics, like increased sediment dynamics (resulting in burial or erosion) (e.g. Cabaço & Santos 2007; Han *et al.* 2012) or decreased epiphyte grazer densities (increasing algal overgrowth and thus light reduction for the seagrass plants) (Schanz and Asmus 2003). This indicates that excluding negative ecosystem engineering interactions to start up a population, is only useful in areas where growth is not limited by other overriding (physical) stresses.

Feasibility and long-term consequences of method

Our large-scale shell treatment showed to be a good measure to promote the initial seagrass establishment success by excluding adult lugworms and their negative effects, which is an important step in restoration. Extra costs for the

application of a shell layer were ~20 % of the total transplantation costs. For restoration purposes, lugworm exclosure by shells has major advantages over using nets, as has been used in scientific studies on lugworm exclosure (Reise 1983; Philippart 1994; Volkenborn & Reise 2006). Firstly, shells are natural material that can generally be easily obtained locally. Secondly, in contrast to nets as used by Volkenborn & Reise (2006), shell layers only exclude large lugworms but allows smaller ones to establish. This is advantageous because the full exclusion has several side effects, like accumulation of fine sediment and blocking interstitial pores causing enhanced nutrient and sulphide levels (Volkenborn *et al.* 2007) which may have negative effects on seagrass performance (i.e. Touchette & Burkholder 2000; Calleja *et al.* 2007).

The present approach of removing negative engineering interactions was specifically aimed at improving the initial establishment for eco-systems that are difficult to restore due to establishment thresholds, but later on can maintain themselves by self-facilitating positive feedbacks. Maximal establishment is the first requirement to enhance long-term success. However, although the treatment most likely will persist to diminish negative engineering interactions over the long-term (the shell layer will not easily get lost), this does not necessarily warrant long-term success. During winter, when lugworms are less active and seagrass is dormant, grazing or physical disturbances from storm driven waves, freezing and ice rafting may form a second bottle neck to meadow persistence and thus restoration success. Unfortunately, these processes appeared to be important at our restoration sites, as seagrass transplantation suffered large losses during subsequent winters. Still, during summers, the shell-treatment had a consistent positive effect on seagrass survival and growth similar to shown in Fig. 2. Present observations suggest that the proposed method of removing negative engineering interactions will be most beneficial in those areas, where biological activity causing such interactions is the main driver restricting seagrass growth year round.

Conclusions

Dynamic ecosystems that do not respond linearly to changes but are characterized by threshold behaviour (like coastal ecosystems) are notoriously difficult to restore (Rappport *et al.* 1998; Hobbs *et al.* 2006; van der Heide *et al.* 2007; Suding & Hobbs 2009); and are also sensitive to environmental perturbations (Scheffer *et al.* 2001; Pascual & Guichard 2005). Thorough understanding of system feedbacks, criticalities, shifts and thresholds is needed to predict and thereby preventing transitions towards a barren state (Pascual & Guichard 2005; Scheffer *et al.* 2009; Firn *et al.* 2010). We provide compelling experimental evidence demonstrating that exclusion of negative ecosystem engineering interactions may be useful to initiate the re-establishment of those species, from which initial establishment is hampered by thresholds but later on benefit from self-facilitating positive feedbacks. Present findings underline that in addition to the recent growing awareness of the need to consider positive

(facilitating) interactions in ecological theory (ie Bruno *et al.* 2003; Brooker & Callaway 2009) and ecological conservation and restoration projects (Crain & Bertness 2006; Byers *et al.* 2006; Halpern *et al.* 2007; van Katwijk *et al.* 2009), it is equally important to also consider negative engineering interactions between ecosystem engineering species in restoration projects. This result re-emphasizes and (by specifically focusing on negative engineering interactions) extends on a wealth of older theory (see i.e. Rhoads *et al.* 1970; Peterson 1980, 1991; Reise 1985; Wilson 1991 and references therein). Similar to positive feedbacks, such negative engineering interactions may also cause threshold dynamics. Though our study was carried out in a marine environment, similar principles may be expected to apply to other ecosystems dominated by ecosystem engineers (Rietkerk *et al.* 2004; Scheffer *et al.* 2009).

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Chapter 4



Сробица 1

Unpredictability in seagrass restoration: analysing the role of positive feedback and environmental stress on *Zostera noltii* transplants

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Summary

1. Restoration of key species in dynamic coastal ecosystems benefits from reduction of environmental stress. This can be realized by promoting positive feedback (intrinsic processes) or by reducing extrinsic negative forcing.
2. In a seagrass (*Zostera noltii*) restoration project in the southwestern Netherlands, we investigated transplantation success in relation to intrinsic processes (i.e. comparing sods versus single shoots, transplant size, transplant configuration, and transplant density) and extrinsic forcing (i.e. bioturbation by *Arenicola marina*, desiccation and exposure to water dynamics). In total, 2600 m² of seagrass sods were mechanically transplanted to six intertidal flats over the course of five years.
3. In total, 43% of sod transplants (2.25 m²) survived at the long term, whereas single shoot transplants failed within the first three months. The use of larger, or more compact (sod) transplant configurations had no long-term effect on survival, and initial densities did not affect transplantation success either. Reducing desiccation stress increased the transplantation success during the first growing season. Shielding transplants from bioturbating lugworms had a positive effect on long-term survival.
4. Seagrass abundance in summer was related to spring abundance, whereas winter survival was not related to prior seagrass abundance. At four of the six intertidal flats transplants gradually decreased in size over time. At the other two, extensive colonization occurred around the transplant areas in some years and is still partly present in 2015. A correlation to the studied environmental parameters was not found.
5. *Synthesis and applications.* Intrinsic processes favour transplantation development during the growing season, allowing positive feedback. Extrinsic processes favour the development at a longer time scale (i.e. reduction of bioturbation, thus breaking the positive feedback of the bare state). Most surprisingly, the starting colonization of two out of six tidal flats could not be related to environmental factors (hydrodynamics, light, emergence time, sediment characteristics, macro-algae and grazing). Environmental managers can improve transplantation success by restoring the positive feedback, reducing stress, but also via risk-spreading by performing transplants over wider areas. They thereby accept the complexity of processes and unpredictable temporal and spatial variation in which transplantation sites turn out to be successful.

Introduction

Coastal ecosystem deterioration caused by persistent anthropogenic pressure is an unfortunate but dominant phenomenon worldwide (Lotze *et al.* 2006). Loss of a single ecosystem engineering keystone species within a coastal ecosystem often results in the loss of multiple species and associated valuable services (Orth *et al.* 2006; Waycott *et al.* 2009). In practice, lost ecosystems are difficult to restore (Suding 2011). This is particularly true in stressful environments, where the target species often require positive feedback to ameliorate environmental stresses (Jones, Lawton & Shachak 1994; Madsen *et al.* 2001; van de Koppel *et al.* 2001; van der Heide *et al.* 2007). These self-facilitating feedback mechanisms only arise above a certain critical density or size threshold (van der Heide *et al.* 2007; Bouma, Ortells & Ysebaert 2009), which should be surpassed to obtain successful and long-term restoration of the target species (Halpern *et al.* 2007; Suding & Hobbs 2009). In addition to crossing thresholds for self-facilitating feedback, successful restoration of target species in stressful environments may depend on breaking antagonistic feedback mechanisms that may hamper establishment of the target species. Enhancing positive feedback (Crain & Bertness 2006; Hastings *et al.* 2007; van Katwijk *et al.* 2009) and suppressing negative feedback from neighbouring ecosystem engineering species (Suykerbuyk *et al.* 2012) can promote fast and sustainable establishment of the target species, though site selection and timing remain critical for any restoration project (Halpern *et al.* 2007).

Seagrasses are among the most well-studied marine ecosystem engineers (e.g. see Bos *et al.* 2007) that generate intraspecific positive feedback mechanisms and follow threshold behaviour (e.g. van der Heide *et al.* 2007; Carr *et al.* 2012). Next to crossing thresholds for self-facilitating feedback and (temporarily) suppressing negative feedback, a special challenge may be involved in the restoration of seagrass meadows in temperate zones where the plants follow seasonal cycles. Most positive feedback loops are annually lost due to the reduction of above-ground biomass during winter. Moreover, this reduction occurs when physical disturbances are highest in terms of, for example, storms causing frequent and intense water dynamics and sediment mixing, or ice scouring causing sediment disturbances (Vermaat & Verhagen 1996). This raises several questions concerning the bottlenecks for restoration of temperate seagrass meadows: (1) can we restore *intrinsic processes* related to positive feedback by optimizing (1a) transplant size, (1b) transplant configurations, and (1c) transplant density?, and (2) can we optimize *extrinsic processes* such as (2a) excluding negative biotic interactions, (2b) minimizing abiotic stresses during the growing season, and (2c) site selection to reduce winter disturbances.

In this study we report the results of a multi-year, large-scale, *Zostera noltii* transplantation project in the Oosterschelde sea inlet (south-west Netherlands) as part of a mitigation programme for dike reinforcement. To assess the importance of *intrinsic processes*, such as self-facilitation, we compared (1a) the developments of single plant versus sod transplantations, (1b) small, large

and compact patch configurations, and (1c) the transplant development in relation to the initial shoot density. The role of (2a) biotic *extrinsic stresses* was investigated by manipulating sediment stability by excluding important bioturbators. The role of (2b) *extrinsic abiotic stresses* was studied by varying the water cover (i.e. varying desiccation stress) via different initial transplant elevations. The role of (2c) *extrinsic abiotic disturbances* during the winter season was studied by comparing sites with different wave exposures.

We hypothesize that:

(H1) Enhanced intrinsic processes benefit seagrass transplant development: (H1a) transplanted seagrass sods establish and survive more often than bare-root single plant transplants, (H1b) larger, or more compact patch configuration and (H1c) higher initial shoot density have a positive effect on the transplant survival.

(H2) Optimizing extrinsic processes benefits seagrass transplant development: (H2a) reduction of important bioturbators (biotic extrinsic stresses) and (H2b) lowering initial transplant elevation and thus decreasing desiccation stress (local abiotic extrinsic stress) promote transplant development, and (H2c) sheltered sites that experience less environmental wave-forcing have higher success rates and develop better than more exposed transplant sites (abiotic extrinsic disturbances).

(H3) Intrinsic processes are more important for restoration development and survival in summer than in winter, while in winter restoration success is relatively more dependent on extrinsic processes.

Materials and methods

Multi-year, large-scale, mechanical transplants

During the period of 2007–2012, 10 large-scale seagrass transplantations were performed in the Oosterschelde sea inlet (Fig. 1A & B) in early June (the growing season runs from early May through mid-September). Mitigation locations were selected based on their suitability for seagrass growth to ensure long-term transplantation success, i.e. long-term survival of the transplanted sods and colonization of the tidal flat by scattered seagrass patches resulting from the transplants. We considered all locations with former seagrass occurrence and selected those that had suitable emergence times and hydrodynamics (ranging from wave-sheltered to relatively exposed, see Table S1 in Supporting Information), and were not prone to intensive tourist or bait digging activities, or other threats such as construction or dredging activities. In total, 2326 seagrass sods (2617 m²) of 1.5 x 0.75 x 0.1 m were mechanically harvested in custom-made boxes at the donor site and protected against desiccation during transport (Fig. 1C). They were replanted from those boxes in pairs to form a patch of 1.5 x 1.5 m, within 24 h after harvesting (Fig. 1D). To allow for ingrowth between the patches during the summer season, the seagrass patch configuration was designed in a checkerboard pattern of alternating patches of seagrass and equally

sized patches of bare sediment (Fig. 1E, for an overview per site, see Table S2 and Fig. S1). To test the effect of configuration size, we planted a smaller (five patches) and a larger (nine patches) configuration. During the course of our transplantation efforts, gaps between the patches were not always vegetated from the transplanted seagrass in the first two years. Therefore, we added the more compact, doughnut-shaped 8-patch configuration in the third year of transplantations and for comparison also again the smaller configuration (five-patch) (Fig. 1E). The minimum spacing between the seagrass configurations of neighbouring plots always exceeded 5 m (Fig. S1) to isolate treatments, to spread risks, and to allow covering a larger area in case of rapid expansion. The number

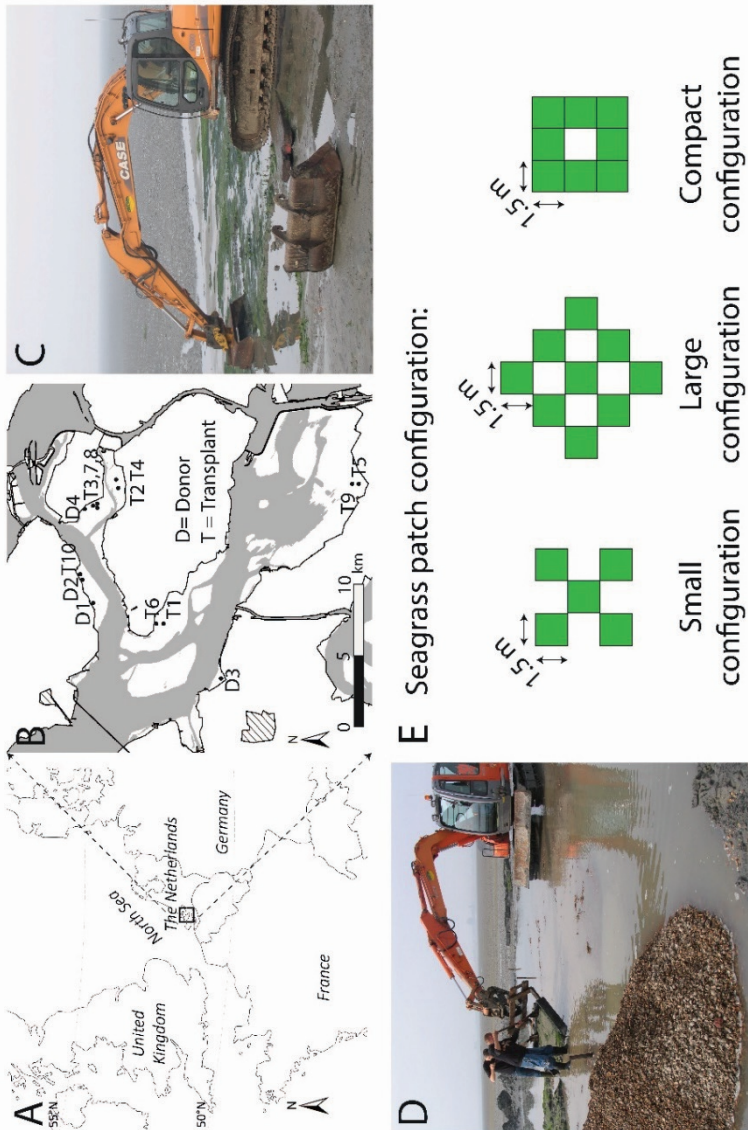


Figure 1: Experimental set-up. *A.* Location of the Oosterschelde sea inlet (study area) in the south-western Netherlands, north western Europe. Mean tidal range is 3,5m. *B.* Overview of donor (D) and transplant (T) sites within the study area. *C.* Mechanical seagrass harvest in custom-made boxes. *D.* Mechanical planting of the harvested sods. *E.* Seagrass patch configuration and dimensions.

of plots of each transplantation was determined based on the availability of seagrass at the donor site of that particular year, making sure that there were minimally three replicates of each combination of treatments. Treatments were evenly applied over the transplanted plots. To investigate the timing of planting, one transplant (T7) was performed before the start of the growing season (March). Mean transplanting costs were approximately 85 euro (indexed to 2015, ~ 90 U.S. dollar, exchange rate 20 March 2015) per square meter transplanted seagrass. We experimentally tested the handling effects of our transplantation method in 2012 by a reciprocal transplantation experiment, which was evaluated at the peak of the growing season (early September).

To assess the importance of intrinsic processes we tested the potential positive effects of initial shoot density and patch configuration on the development of the transplants (for an overview, see Table S2 and Fig. S1). Firstly, to test the potential positive feedback caused by shoot density and belowground integrity, we compared the effect of bare-root versus sod transplants (H1a). We transplanted 225 plant fragments (rhizome fragments several centimetres in size with appending shoots) in 8 plots at T3 in 2008 and compared their survival with seagrass sods that were transplanted simultaneously at the same location. Secondly, to test whether plot size and patch density could cause a positive feedback, we assessed the role of smaller, larger and compact patch configuration on seagrass development (H1b). We compared the smaller and larger patch configuration of the transplants of 2007 and 2008 (T1–T6), and the small and the compact patch configuration of transplant T9 (2011). Note: T7, which was transplanted before the start of the growing season, was almost entirely lost at the start of the growing season and was therefore not taken into account in the comparison of the small versus compact patch configuration. Other transplants contained only one type of patch configuration. Thirdly, to test the potential positive feedback of initial shoot density on transplant development (H1c) we recorded and compared the numbers of shoots per transplanted plot at the beginning and peak of the growing season (June and September respectively). Due to the patchy seagrass distribution of the donor sites, initial shoot density varied among transplanted sods. To be able to compare the development of transplants of different patch configuration, the number of shoots per plot (our main parameter for transplant success) was normalized to the initial transplanted area and will be referred to as the Normalized Shoot Number (NSN).

To test the importance of minimizing negative extrinsic processes, we first experimentally improved sediment stability, by excluding sediment destabilizing, bioturbating (adult) lugworms *Arenicola marina* from our plots (H2a). During transplants T1–T6 (in 2007 and 2008) a 10 cm thick shell layer was installed beneath the seagrass sods and the surrounding sediment, at every second transplantation plot (i.e. 46 of 92 plots) (Reise 2002; Suykerbuyk *et al.* 2012). To control for sediment handling, sediment was removed and replaced in the same way in the bioturbation control plots. In transplants T7, T8 and T9 (in

2010 and 2011), this bioturbation suppressing shell layer was used in every plot (Table S2 and Fig. S1). The shell layer was not installed during transplant T10 (2012) where the local sediment already naturally excluded bioturbators and lugworm densities were close to zero (personal observation). Secondly, we experimentally tested the importance of minimizing extrinsic abiotic stress, i.e. desiccation stress by manipulating the initial elevation of transplanted sods. Half of the plots of transplant T10 (8 out of 16) were laid out at a slightly lower depth (around 3 cm) to prevent water drainage and thus create a small layer of water above the seagrass while exposed at low tide (H2b).

Transplant monitoring and site comparison

Transplanted plots were monitored from the moment they were transplanted to the end of the 2013 growing season. Plant characteristics (i.e. number of shoots, plant cover and area covered) were monitored at least two times a year; containing the start and the peak of the seagrass growing season (early June and end of August to early September, respectively). The total number of shoots per plot is used to evaluate the transplant development and success. For analysis of transplant development we differentiate between initial (first growing season) and long-term development (> 1 year.). The area adjacent to the transplanted plots was inspected for seagrass presence at each monitoring visit in summer. For site comparison (H2c and H3), we assessed a suite of environmental variables. Hydrodynamic exposure, light availability and emergence time were assessed from models; sediment composition, salinity, pore-water nutrients, sulphide concentration, macro-algal cover, grazing and ice scour were monitored (materials and methods, see Appendix S1). Lugworm faecal cast counts per area were used as a quantitative proxy for the number of worms present (cf. Suykerbuyk *et al.* 2012). Juvenile and adult lugworms were divided by the diameter of the cylindrical shaped cast; rule of thumb: juvenile < diameter cast 1 mm < adult.

Statistical analysis:

Data points representing means are displayed as means \pm standard error of the mean (SEM), unless differently stated. Data were checked for normality and if necessary transformed prior to statistical analysis. Effects of the different configurations, anti-lugworm treatments and wave-exposure categories (classification see Table S1) on short-term transplant development were analysed using ANOVA, whereas all other effects on short-term development were analysed using either a *t*-test (normal distributed data) or a Mann-Whitney U Rank Sum test (if the variance of the data was not normally distributed). Effects on long-term development of the transplants were first analysed using Repeated Measures ANOVA (RPM), after which time steps were separately analysed using *t*-tests (normal distributed data) or Mann-Whitney U Rank Sum tests (non-normal data). We used an alpha level of 0.05 for all statistical tests. All tests were

performed using Sigmaplot v12.0 (Systat Software Inc.), repeated measure ANOVAs were performed using IBM SPSS Statistics 21.

Results

Short term transplantation results

Seagrass sods were successfully transplanted within the intertidal zone using a large-scale, mechanical transplantation method; initial sod survival was 100% and the number of shoots increased during the first growing season (Fig. 2). The reciprocal test showed that transplantation method did not negatively affect shoot densities, as shoot densities of the transplant site (T10), transplant control, and untouched natural donor meadow were similar at the shoot density peak of the first growing season (one-way ANOVA, $F(2, 45) = 2.803$, $P = 0.071$). Across all years and locations, shoot numbers of transplanted sods increased over the course of the first growing season by 256% (SEM 39%; Fig. 2C). In our assessment of the importance of intrinsic processes on transplant development during the first growing season, we found that single plant fragments (rhizome + appending shoot) disappeared right after planting, whereas the simultaneously transplanted sods of T3 had a good survival and shoot number increased by about 50% (Table S3). This was in line with hypothesis H1a. Secondly, in contrast to our expectation (H1b), larger patch configurations did not promote seagrass development when comparing survival of the small 5-patch versus the large 9-patch configuration (in 2007 T1 & T2; and 2008 T3–T6; Fig. 2A). However, the growth of the compact patch configuration as compared to the small 5-patch configuration was enhanced during the first growing season (t -test, $t(31) = -2.374$, $P = 0.024$; Fig. 2B). Thirdly, transplant successes were, in contrast to our hypothesis (H1c), only log-linearly related to initial planting densities, thus showing no density dependent positive feedback (Fig. 2C). Overall (regardless of patch configuration or sediment treatment), the seagrass covered area increased during the first growing season by 44.6% (mean), ranging from –100% to 480% (Fig. S2). During the first growing season, the development of the number of shoots per plot was negatively correlated (around 25% lower) with exposure to wind-driven waves (three-way ANOVA, $F(1, 84) = 5.494$, $P = 0.02$, data: T1–T6, factors: configuration, shell treatment, exposure category). We enhanced seagrass shoot development with more than a factor of two in the first growing season by lowering the initial elevation of seagrass sods (mean 3 cm; Fig. 2D; T10, Mann–Whitney $U = 11.0$, $n_1 = n_2 = 8$, $P = 0.028$). Desiccation stress was alleviated by the tidal pool (with a depth of a few cm) that was created in the lowered plots, while control plots drained naturally. In the lowered plots 44.3% (SEM 14.2%) of the seagrass area was covered by around 1.75 cm of water, while in the control plots only 1.4% (SEM 0.7%) of the seagrass area was continuously submerged. We reduced lugworms (one of the main bioturbators) to less than 25 adult worms m^{-2} in the shell treated plots. However, seagrass development was not yet promoted by the shell treatment during the first growing season (Fig. 2A, two-way ANOVA, $F(1, 88) = 0.013$, $P = 0.910$).

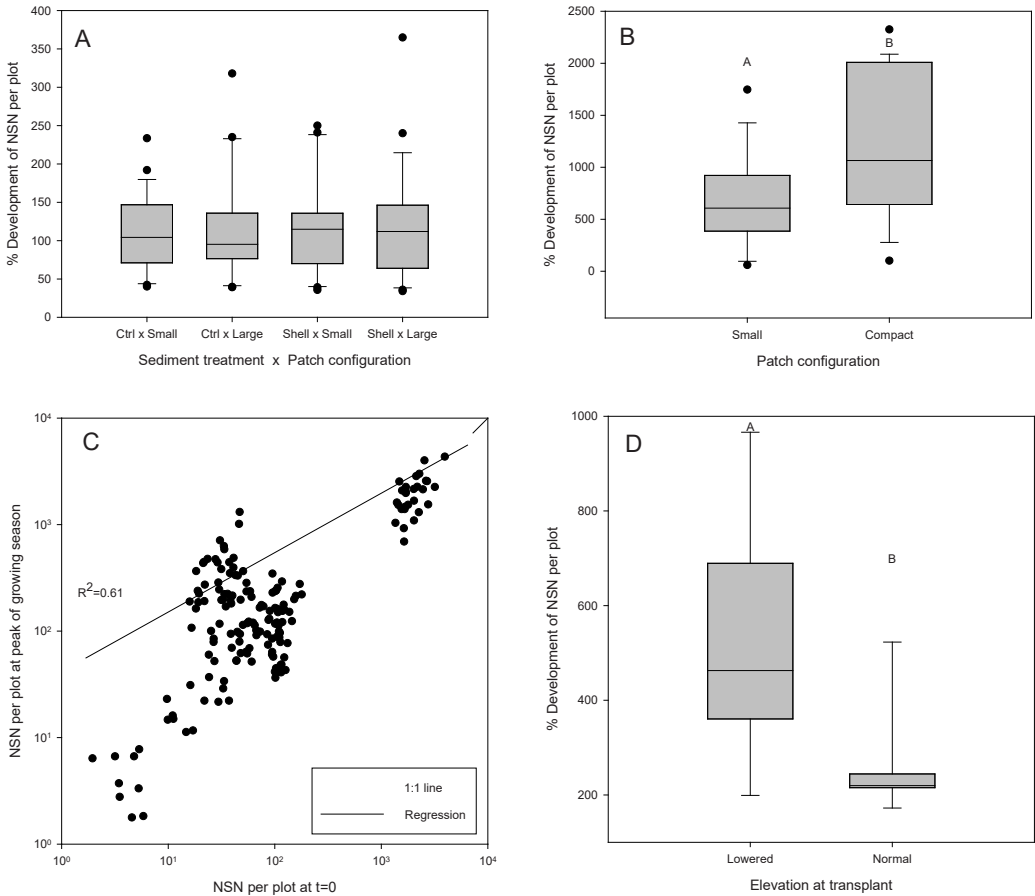


Figure 2: Short-term (1st growing season) development of transplanted seagrass sods presented as the relative decrease/increase of the normalized shoot numbers (NSN) per plot. NSN = total numbers of shoots per plot divided by the transplanted area at $t=0$. Box-whisker plots: box shows the 25th/75th percentile, the line inside the box the median value, the whiskers the 5th/95th percentile and the solid dots the outliers. Capital letters indicate statistical differences ($P < 0.05$). (A) The development of NSN per plot of the transplants T1–T6 at 4 nested sediment treatments (ctrl/shell) and patch configurations (small, large or compact) ($n=23$). (B) The development of NSN per plot of the transplant T9 (small vs. compact patch configuration, respectively $n=15$ & 18 , t -test, $P = 0.024$). (C) Relation of NSN per plot at beginning (horizontal axis) and at the peak of the first growing season (vertical axis) for all ($n=173$) plots. The dashed line indicates the 1-on-1 line, the solid line the overall trend (Note the logarithmic scale) (D) The development of NSN per plot of the transplant T10, at two initial transplantation elevations (normal & lowered, $n=8$, for each) (t -test, $P < 0.028$).

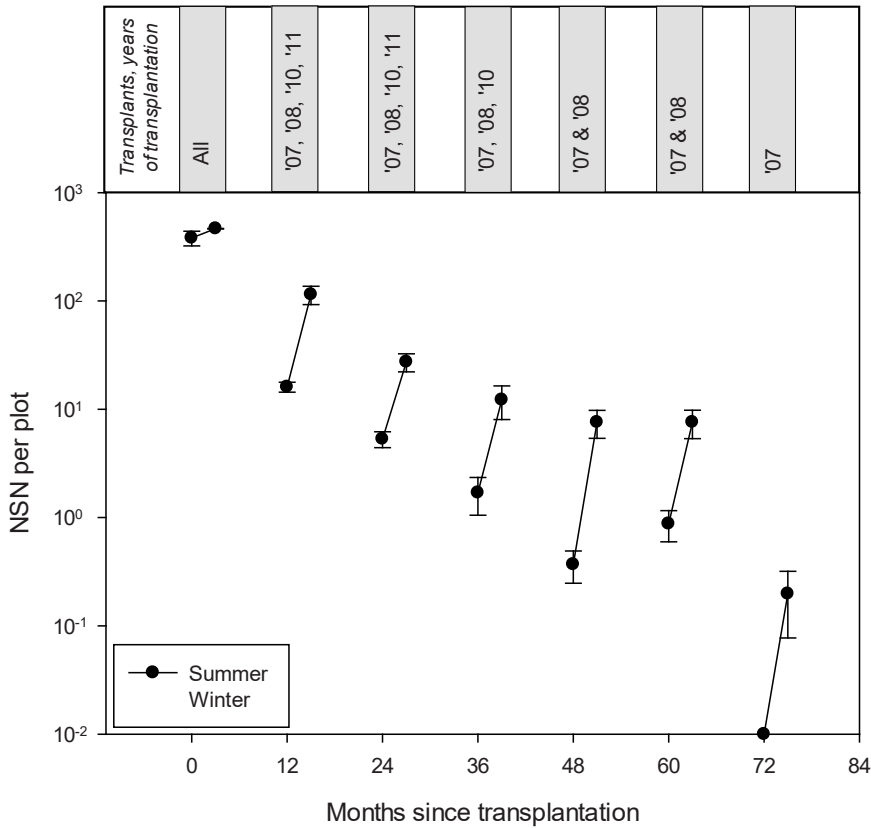


Figure 3: Overall long-term development of seagrass transplants. Mean normalized shoot numbers (NSN) per plot \pm standard errors are presented by black dots, the growing seasons are indicated by the solid lines. Note that the transplant efforts were not performed in the same year. As a result, the number of plots comprising one data point decreases from $n=173$ (10 transplantations during 2007–2012) during the first growing season, to $n=30$ (two transplantations in 2007) after six years.

Long-term transplantation results

After the expansion during the first growing season, seagrass transplants lost most of their aboveground tissues and survived winter on their rhizome reserves (Govers *et al.* 2015). In June of the second growing season, shoot numbers were found to be reduced to 0% to 62.1% (mean 7.1%) of the shoot numbers that were recorded in August/September of the previous year (Fig. 3). During growing seasons, transplants consistently increased in shoot numbers, but suffered larger shoot losses in the subsequent winter (Fig. 3). As a consequence, at four out of six tidal flats, transplants steadily declined over the years (though in 2013 43% of all plots still contained seagrass; Fig. 3). In contrast, at two tidal flats (containing T5 + T9 and T6), the number of seagrass

shoots strongly increased after an initial decline (Fig. S3A). This occurred inside the transplanted area, and also outside the transplanted area as scattered patches colonized the formerly unvegetated flats surrounding the transplanted area. At T6 this occurred in 2010, 2011 and 2013 (Figs S3A & B), coinciding with an expansion of a natural bed nearby. As the expansions consisted of scattered patches (Fig. S3B), it was not possible to know whether they originated from the adjacent natural bed or from the transplantation. At T5 and T9, in 2013, a total area of about 20 ha became extensively colonized by scattered patches of seagrass, in total amounting to more than 3000 m² and over 2000 seagrass patches (Figs S3A & C). As neighbouring meadows were absent at T5 and T9, the new colonization likely originated from the transplantation.

In the long term, a larger or more compact patch configuration did not result in more shoots per plot (Repeated measures ANOVAs Control vs. Shell, $F = 2.06$, $P = 0.139$; Small vs. Large configuration, $F = 0.68$, $P = 0.493$; Small vs. Compact configuration, $F = 0.40$, $P = 0.679$, Figs 4A & B, H1b). In contrast, minimizing biotic, extrinsic stresses (by reducing the number of bioturbating adult lugworms via a shell treatment) was effective in the long-term, as the number of shoots was higher in the treated plots (Fig. 4C, H2a, second growing season t -test, $t(90) = -2.015$, $P = 0.047$, third growing season Mann–Whitney $U = 707.5$, $n_1 = n_2 = 46$, $P = 0.005$). Lugworm numbers increased over time at the shell treated plots (from mean 4.8 to 36.0 individuals m⁻² from 2008 until 2013), but were lower than the control numbers (14.4 to 45.4 individuals m⁻² from 2008 until 2013). Furthermore, the depth of the shell treatment remained shallow enough (mean 14.1 cm depth in 2013) to effectively exclude adult lugworms. In contrast to the first growing season, artificial local lowering of the bed level (preventing water drainage during low tide and thus minimizing drought stress), did not result in an increased number of shoots in the second summer (Fig. 4D, H2b).

Shoot numbers at the peak of the first growing season were closely correlated to the shoot numbers at the start of that season ($R^2 = 0.805$, Fig. S4A, summer development). In contrast, shoot numbers at the start of the next growing season were hardly correlated at all with the shoot numbers at the peak of the preceding growing season ($R^2 = 0.145$) (Fig. S4B, winter survival).

Starting colonization of tidal flats and site comparison

Two out of six tidal flats started to become colonized by small patches in some years, particularly in 2011, 2013 and 2014 (Fig. S3). In 2015, many were still present (personal observations). These long-term transplantation successes could not be attributed to any characteristic measured or observed, nor to any event recorded (Table S4). Exposure and sediment composition were very contrasting between the two relatively successfully transplanted flats (Table S4), ice scouring was observed only once, at one tidal flat (one of the two relatively successfully transplanted flat), macro algal cover remained low over all years and tidal flats (< 10% cover), adult lugworm densities varied among tidal flats

from means of 1 to 70 individuals per m², geese pits were observed at all tidal flats in October and November, and salinity, pore water nutrients and sulphide concentrations did not differ between sites (Table S4).

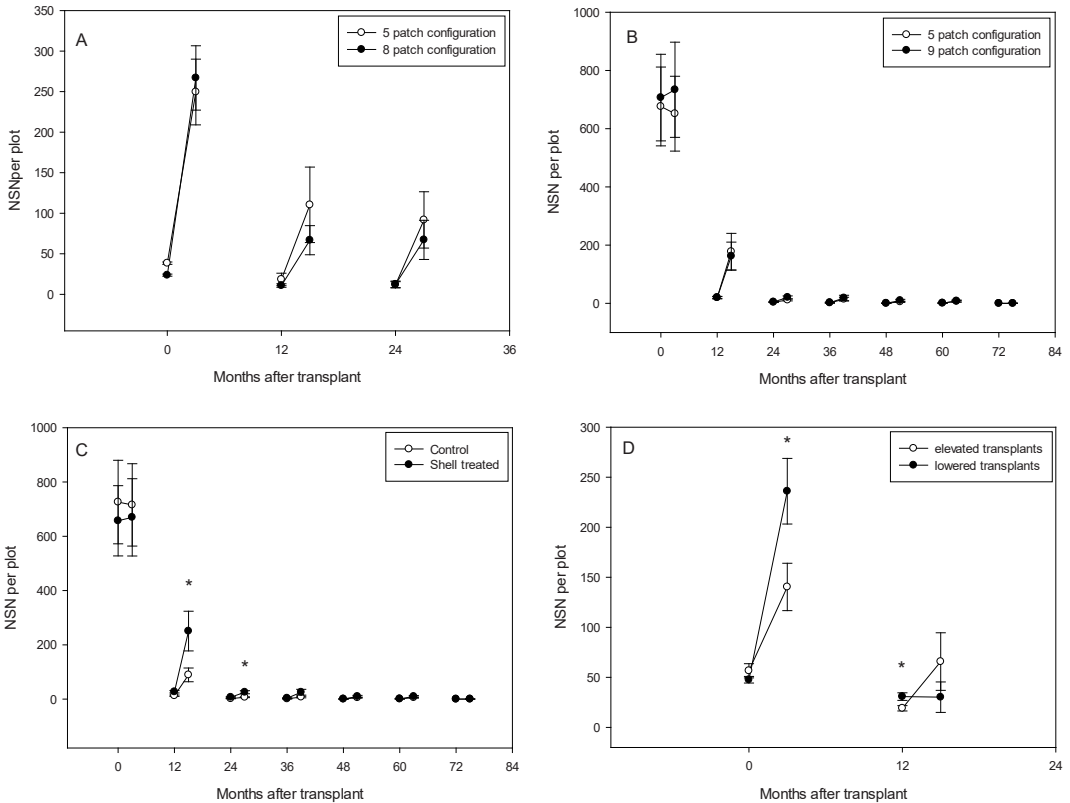


Figure 4: Effects of patch configuration and sediment treatments on long-term transplantation successes. A&B. Mean NSN per plot + Standard error of mean (SEM) in time for T9 (Panel A, $n=15$ for the 5-patch and $n=18$ for 8-patch configuration) and for T1–T6 transplants (panel B, $n=46$ per configuration) (C) Effect shell treatment improving sediment stability by excluding adult lugworms. Mean NSN per plot + SEM for T1–T6 transplants ($n=46$ per treatment) * indicate significant difference at that particular time step ($t=15$, t -test $P = 0.047$, $t=27$, Rank sum test $P = 0.005$). (D) Effect of mitigating desiccation stress by bed level manipulations to prevent water drainage during low tide (in the initially lowered transplants). Mean NSN per plot + SEM in time for T10, ($n=8$ per treatment). * indicate significant difference at that particular time step (t -test, $t=3$, $P = 0.033$, $t=12$, $P = 0.024$).

Discussion

The large-scale *Zostera noltii* transplantations that were carried out in the intertidal flats of the Oosterschelde over the period 2007–2012 showed variable success. Most of the transplanted sods survived 43% (reference date: September 2013), but shoot numbers declined over time. However, in the long run, at two out of six tidal flats, the transplantations and surrounding areas were extensively colonized by new patches of seagrass. In this study we show: (i) intrinsic processes favour the transplantation development during the growing season (supporting the importance of positive feedbacks), (ii) extrinsic processes favour the development at a longer time scale (i.e. reduction of bioturbation, supporting the importance of breaking the positive feedback maintaining the bare state (cf. Suykerbuyk *et al.* 2012), whereas (iii) the long-term transplantation successes (starting colonization of two out of six tidal flats) could not be related to any exposure to environmental factor (i.e. hydrodynamics, light availability, sediment composition, emergence time, macro-algal cover, grazing, salinity, pore-water nutrients and sulphide toxicity). Our study involves large-scale transplantations with several years and sites of planting, long and intensive monitoring of plants and environment and a number of manipulations. Despite this, no correlations were found between starting colonizations of two out of four tidal flats and their site characteristics or manipulations. We apparently cannot predict or deduce habitat suitability completely from the ample available environmental monitoring data. Thus, the typically high environmental variability that governs seagrass habitats requires spreading of risks in time and space in the transplanting set-up and scheme, as we did.

Intrinsic processes in the transplanted sods

Positive feedback arising from intrinsic plant properties that have been identified in seagrass systems are for example based on enhanced water clarity (Carr *et al.* 2012) due to plant-induced reduced resuspension or alleviated NH(x) toxicity at high shoot density (van der Heide *et al.* 2008). Moreover, some of these intrinsic feedback loops follow threshold behaviour, which can lead to self-accelerating processes once the threshold is reached (e.g. van der Heide *et al.* 2007; Carr *et al.* 2012). In our mesotrophic intertidal system with high water clarity, feedback related to turbidity or toxicity is unlikely to occur (Wetsteyn & Kromkamp 1994). However, water and sediment dynamics are relatively strong at intertidal sites like our study area (Louters, van den Berg & Mulder 1998). Positive feedback in the seagrass system can therefore be expected from a root/rhizome system that is sufficiently large and entangled to hold sediments and prevent erosion and dislodgement (e.g. Madsen *et al.* 2001; Bos *et al.* 2007; Christianen *et al.* 2013). The immediate disappearance after planting of single, bare-root shoots and contrasting survival of seagrass sods observed in our study, support the idea that this intrinsic process is important, as does the larger expansion of the compact configuration as compared to the small configuration. The seagrass in the sods always survived and expanded during the first growing

season. In addition to sediment stabilization by sod-vegetation, there may have also been an initial effect of the sod sediments being more cohesive at some but not all of the locations. However, these differences in sediment composition rapidly disappear due to local sediment dynamics in winter (Giesen *et al.* 2012).

Further up-scaling of transplant size within the limits of what is practically and economically feasible does not seem worth pursuing as a larger discontinuous patch configuration (nine vs. five) did not improve transplantation success, although the difference between the two configurations might not have been large enough to establish an effect. The compact arrangement of seagrass patches improved short-term transplantation success, but not long-term success. Apparently, the gaps in our configurations do not have adverse effects on the seagrass patches, confirming flume and field studies (Folkard 2005; Folkard 2011; Christianen *et al.* 2014). In addition to the lacking influence of patch configuration size or compaction, the development of our transplanted patches was only log-linearly correlated to the initial shoot density. From this we conclude that all transplanted patches (except the single shoots) were sufficiently large and dense for self-facilitating processes to occur. Long-term success is likely to be determined by processes other than self-facilitation processes, such as extrinsic forcing. Alternatively, an even larger transplant scale could promote self-facilitation at a landscape scale as postulated by van de Leemput and co-workers (2015), although this may not be (economically) feasible.

Extrinsic forcing in the transplanted sods

In our system, minimizing extrinsic stress, i.e. improving the sediment stability by suppressing bioturbating adult lugworms, was proven to promote initial seagrass transplantation success in two transplantations (Suykerbuyk *et al.* 2012). The present study shows that this effect is consistent over years and locations. Secondly, seagrass development was stimulated by minimizing desiccation stress, i.e. by the prevention of water drainage. The negative effect of desiccation stress was already shown in several *Zostera* systems (Leuschner, Landwehr & Mehlig 1998; Boese, Robbins & Thursby 2005; van der Heide *et al.* 2010). This effect only lasted during the first growing season, as the sediment relief (that was manipulated to locally reduce desiccation) levelled during the following winter.

Summer expansion versus winter survival of transplanted sods

Seagrass abundance at the peak of the growing season (September) generally correlated well to the seagrass abundance in June of the same year ($R^2=0.805$). This strong correlation in summer implies that intrinsic processes may be more important than extrinsic forces during summer. In contrast, seagrass abundance at the peak of the growing season showed a low overall correlation with seagrass abundance in June of the next year ($R^2=0.145$). This suggests that during the near-absence of aboveground biomass in winter, intrinsic processes are less important than extrinsic forcing. The extrinsic forcing is likely resulting

from increased water and sediment dynamics that are generally higher in winter, and may cause erosion and subsequent loss of seagrass rhizomes. Particularly fast-growing, shallow-rhizomed (~1 cm) species like *Z. noltii* adapt to bed level changes by growing their rhizomes to the optimal sediment depth (Han *et al.* 2012); during winter growth is nearly absent (Vermaat & Verhagen 1996), thus this adaptation is slowed down.

What drives long-term transplantation successes?

Although we could firmly establish that intrinsic processes favour the transplantation development during the growing season, and extrinsic processes consistently favour the development at a longer time scale, the long-term transplantation results could not be related to any factor in spite of the intense monitoring of a broad range of environmental factors. Two out of six tidal flats became colonized by scattered seagrass patches, and the transplant areas themselves developed reasonably well, whereas the four others diminish every year and show no colonization. The differences in transplantation success could not be ascribed to site- to- site differences in factors associated with seagrass losses, like light limitation, eutrophication, high hydrodynamics, grazing, bioturbation, desiccation, storms, ice-scour (e.g. Calumpong & Fonseca 2001; Short *et al.* 2002; Orth *et al.* 2006). Pore-water nutrient levels and sediment composition at the transplant sites were in the range of naturally occurring seagrass beds (Giesen *et al.* 2012). In our study area light was ample, porewater nutrients and sulphide did not differ between sites and were below toxic levels (Govers *et al.* 2014), exposure to hydrodynamics and storms, sediment composition and bioturbation were contrasting at the two successful sites (and the non-successful sites had similar and intermediate values), macro algal cover, and grazing events were all similar between sites and ice scour only happened once, notably at a successful site (Table S4).

What else could have caused the expansion at two, and decline at four out of six tidal flats? When discussing this, we have to keep in mind that (i) the success varied between years (this study), and (ii) also the natural populations at the Oosterschelde basin are characterized by large non-synchronous variability in expansion and decline. It is therefore unlikely that one simple, overlooked factor is responsible. More likely, successes and failure result from the complex interplay of tidal dynamics, annually varying weather conditions, and/or biological processes related to colonization, such as seed production, timing of release, germination induction and germination, as shown for several biogeomorphic ecosystems by Balke and coworkers (2014). Alternatively, or in addition, success may be influenced by large distance, landscape-scale interactions (Gillis *et al.* 2014; van de Leemput, van Nes & Scheffer 2015); in our case, for example, the outflow of seawater from the neighbouring saltmarshes during low tide, in combination with a limited drainage of the local tidal flat may have prevented desiccation at the two successful sites in some years better than at the four unsuccessful sites. In former times, the lush seagrass beds

at the unsuccessful sites may have flourished due to the accumulation of fines and the subsequent development of several layers of seagrass leaves that may concertedly have kept the beds moist (personal observation DJ de Jong in the Oosterschelde). A starting bed does not yet have this positive feedback; moreover, suspended fines have been reduced in the water layer of the Oosterschelde since the construction of the storm-surge barrier. In short, complex interacting, unpredictable environmental factors likely influence long-term transplantation results, even in big and repeated transplantations like ours. Such factors may also help to explain the variable success of other seagrass transplantations around the world (cf. Orth *et al.* 2010; van Katwijk *et al.* in press).

Synthesis and applications

Although sod transplantation shows significantly better initial survival than transplanting individual plants, further up-scaling by increasing the patch configuration size or compaction did not improve the transplantation success, and there was only a simple, linear relationship between transplantation success and initial density of the sods. This implies that sediment stabilization by the rhizomes (self-facilitation) favours the initial establishment of individual plants, whereas, at patch scale (here 2.25 m²), extrinsic processes such as enhanced sediment dynamics caused by bioturbating lugworms and desiccation hamper the long-term transplantation survival. However, long-term transplantation successes (starting colonization of the tidal flat) could not be related to any studied environmental factor. The processes determining site-to-site and year-to-year success may be unknown due to lack of knowledge, but may likely at least partly result from variability that can rise from the unpredictable outcome of complex and interacting processes, such as hydrodynamics and recruitment biology (e.g. Balke, Herman & Bouma 2014). Thus, in dynamic, temperate systems like our study area, overall extrinsic forcing (i.e. sediment dynamics but also complexly interacting, unpredictable processes) is more important for long-term seagrass transplantation success than intrinsic processes, although a positive feedback from root/rhizome anchoring is required for initial survival. The same may hold for other dynamic ecosystems with ecosystem engineering based self-facilitation feedbacks, like saltmarshes (Crain & Bertness 2006), shellfish beds (van de Koppel *et al.* 2001), and various freshwater and terrestrial ecosystems (Madsen *et al.* 2001; Scheffer *et al.* 2001; Rietkerk *et al.* 2004). Our results clearly show that seagrass restoration in the Oosterschelde is feasible but the long-term result is highly variable. Overall, our study emphasizes that managers restoring seagrass beds: (i) need to account for the unknown and unpredictable part of the variability by spreading risks in space and time, and (ii) should realize that suitable restoration sites in temperate zones not only have optimal (growing) conditions in summer *but* also enable survival in the more dynamic winter. Environmental management plans must account for a long evaluation time (incorporate patience), and the unpredictability of the precise

locations and years of successes and failures (so spread risks and partially submit to nature).

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Supporting information:

Appendix S1: Materials and methods of the abiotic monitoring of the transplantations

Figure S1: Transplants experimental design: visual

Figure S2: Effect of treatments and configuration on short term seagrass survival

Figure S3: Detailed and visual long-term transplantation results of two successful transplants

Figure S4: Shoot density correlations between specific moments of the growing season

Table S1: Sediment characteristics and hydrodynamic exposure of the transplants

Table S2: Transplants experimental design: numbers and timing

Table S3: Transplant development of T1–T6

Table S4: General characteristics of tidal flats with seagrass expansion and decline

Appendix S1:

Materials and methods of the abiotic monitoring of the transplantations

All transplants were visited multiple times a year to monitor their development. Next to monitoring the development of the transplanted seagrass, we monitored related environmental parameters. During the winter, a basic monitoring routine was performed at least twice and included the recording of the macro-algal cover per plot, recording of the presence of grazing or ice-scour damage to the transplants, and recording of any other remarkable sedimentary changes. During the growing season, sediment characteristics, pore water nutrients, and pore water sulphide concentrations were recorded at least once near the shoot density peak in the growing season. In most cases these parameters were also measured early in the growing season (June). Ammonia and ortho-phosphate concentrations were measured colorimetrically on a Bran+Luebbe auto-analyser, using hypochlorite (Berthelot reaction) and ammonium-molybdate, respectively. Nitrate was determined by sulphanilamide, after reduction of nitrate to nitrite in a cadmium column. Within 5 hours after sampling, total sulphide concentration in the pore water was measured in a mixture of 50% sample and 50% Sulphide Anti-Oxidation Buffer (SAOB), using an ion-specific silver-sulphide electrode (Govers et al. 2014). Grain size distribution of the sediment sieved over 1mm was measured by laser diffraction on a Malvern Particle Sizer. The hydrodynamic exposedness of the transplant sites was calculated or derived from models. Exposedness to stormy winds is presented as the fraction of stormy wind directions the transplant is exposed to. Maximum bottom shear stress (Pascal) is calculated using a model using the bathymetry, wind fetches and actual weather data of a normal year (2005, model courtesy Bregje van Wesenbeeck). Emergence time was calculated by the tidal curve and the tidal elevation of the transplant site. The tidal range in our study area varies between 2.4 and 3.5 m, maximum current velocities range from around 0.3 m s⁻¹ in the shallow areas to 1 to 1.5 m s⁻¹ in the tidal channels, and waves are generated within the system by wind (Louters, van den Berg & Mulder 1998).

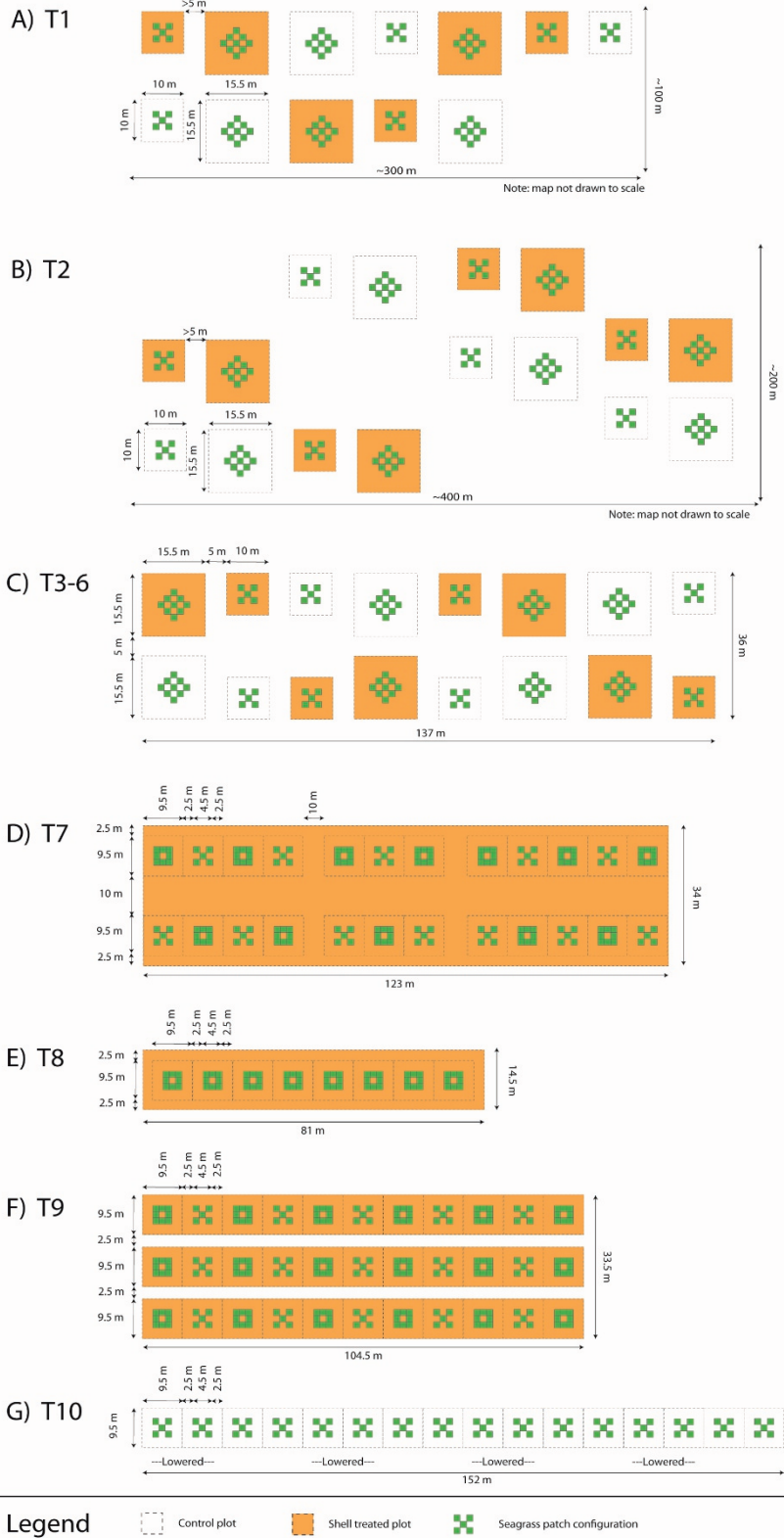


Figure S1: Schematic top view of the transplants T1-T10, their plot and patch arrangements and the sediment treatments applied.

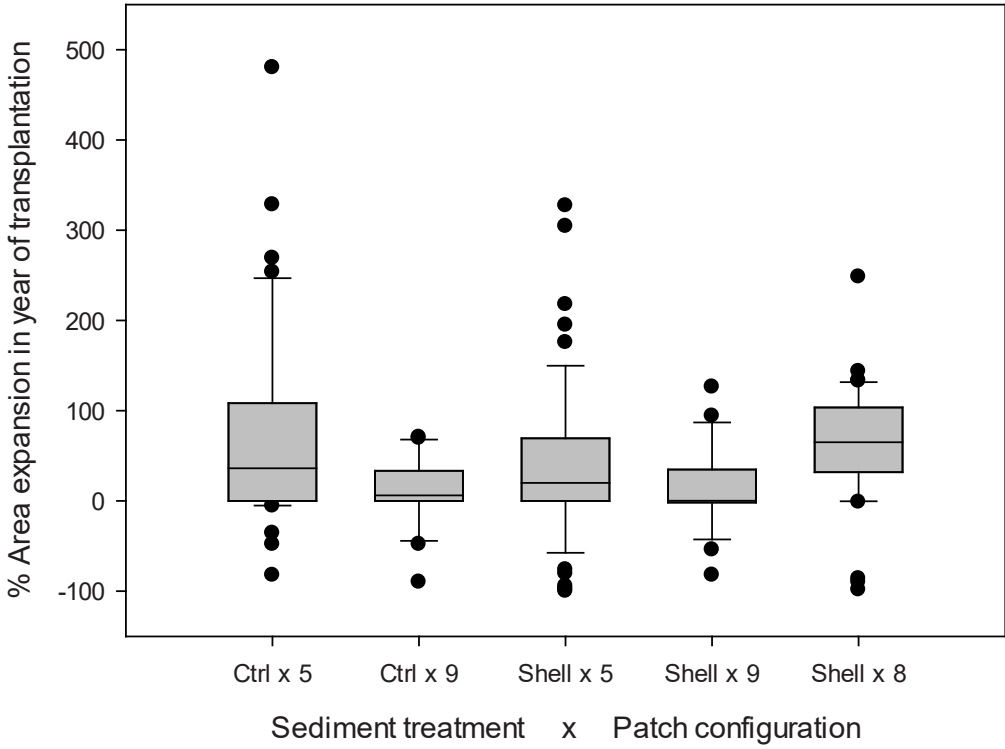


Figure S2: Expansion in seagrass area during the first growing season after transplantation, expressed as the percentage of change compared to the initial seagrass area transplanted.

Figure S3 A:

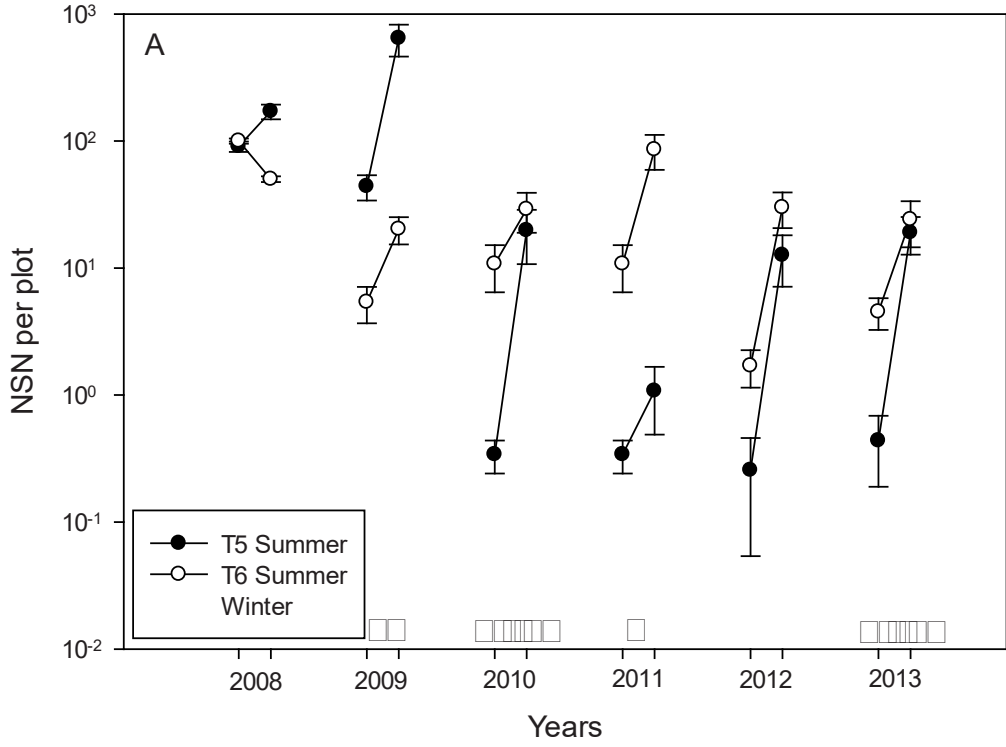


Figure S3 B:

B

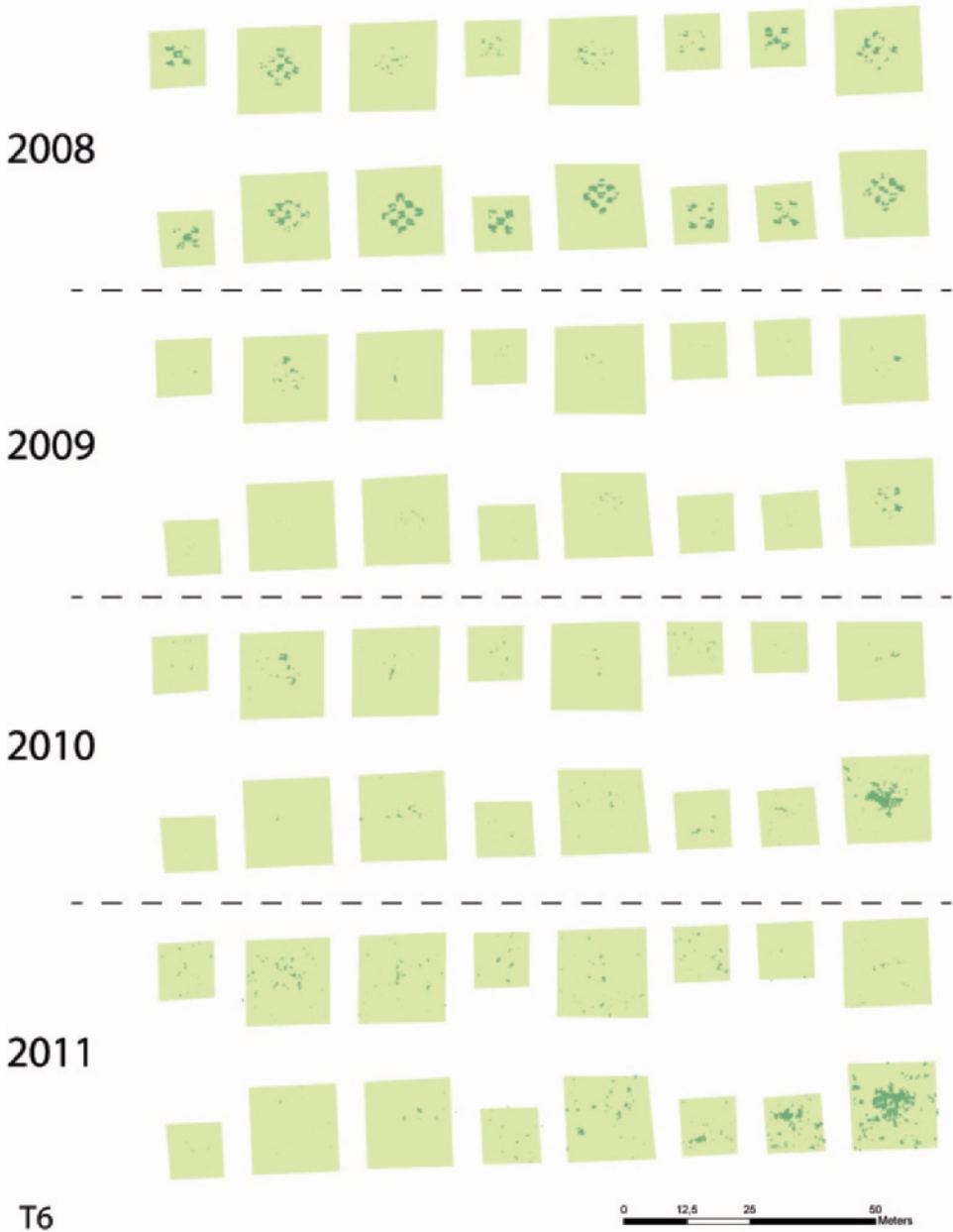


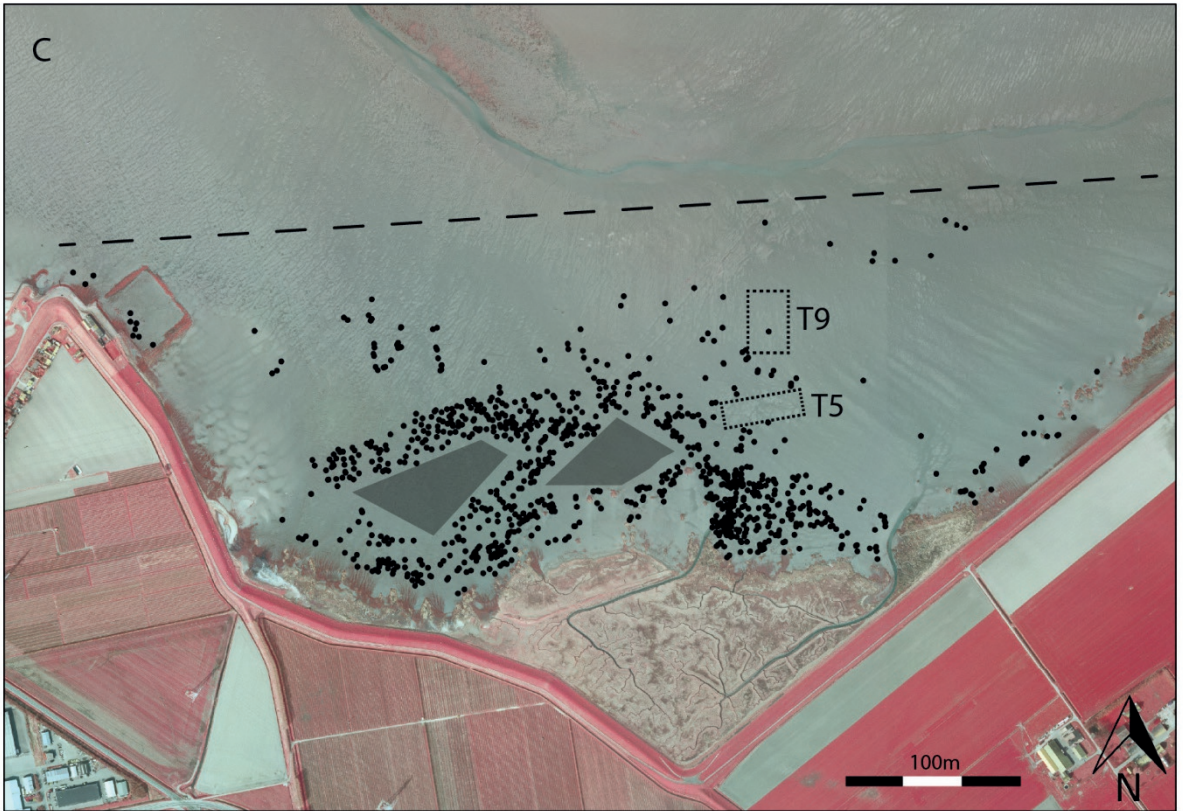
Figure S3 C:

Figure S3: Development and long-term transplantation successes of two (partly) successful transplantations. Panel A. Mean normalized shoot numbers (NSN) per plot per growing season of transplant T5 and T6. * and ** indicate the presence of newly colonized seagrass patches (order of 1000s) around the transplanted plots in the given growing season, respectively for T6 and T5. Panel B. Seagrass development within the transplant plots of T6. Light green squares indicate transplant plots, dark green shading indicates seagrass presence derived from dGPS mapping. Four years are depicted. The area outside the transplant plots was not mapped C. Newly colonized seagrass patches (black dots) at the tidal flat adjacent to T5 & T9 (dashed rectangles). Gray polygons indicate areas that were not mapped. No mapping occurred north of the long dashed line.

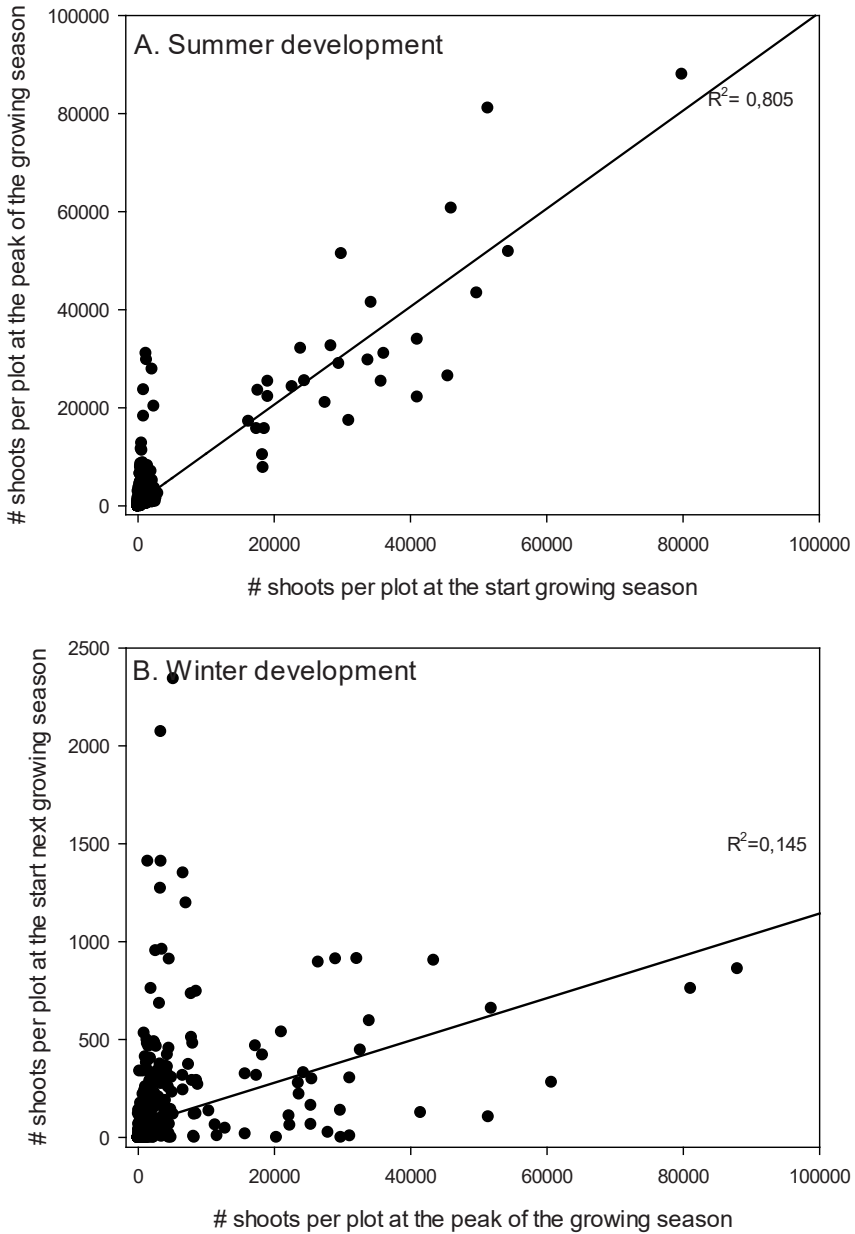


Figure S4: Correlation of number of shoots per plot between. A) Beginning and peak of the growing season ($n=659$) and B) the peak of the growing season and the start of the following growing season ($n=478$). Data: all transplants, all plots. Regression of the data is given by the solid lines as well as the R^2 .

Table S1: Sediment characteristics and hydrodynamic exposure of the transplants. D50 = Median grain size in μm , % Silt = fraction of the sediment smaller than 63 μm . Stormy winds are classified as winds from Beaufort 7 and higher deriving from south to North West directions (clockwise), usually occurring in the winter season. Exposedness to stormy winds is presented as the fraction of stormy wind directions the transplant is exposed to. Maximum bottom shear stress (Pascal) is calculated using a model using the bathymetry, wind fetches and actual weather data of a normal year (2005, model courtesy Bregje van Wesenbeeck)

Transplant ID	Mean air exposure time (% of total time)	D50 (μm)	% Silt (<63 μm)	Exposedness to stormy wind directions (%)	Max. bottom shear stress (Pa)	Exposedness (classification)
T1	65	171	0.3	100	1.54	Exposed
T2	47	162	4.8	60	0.37	Intermediate
T3	61	132	5.7	80	1.53	Exposed
T4	57	175	3.6	60	0.33	Intermediate
T5	59	112	10.3	60	0.85	Intermediate
T6	67	153	0.01	100	1.46	Exposed
T7	62	121	10.4	80	1.69	Exposed
T8	64	128	9.6	80	1.54	Exposed
T9	51	120	10.8	60	0.58	Intermediate
T10	69	120	14.3	20	1.42*	Sheltered

*Overestimation of the model, while the tidal flat in reality is protected from southerly and westerly winds by a mole

Table S2: Overview of all transplant characteristics; time of transplant, donor site used, the number of plots, their seagrass patch configuration and sediment treatment. “Shell” refers to the anti-lugworm shell layer that was applied beneath the transplants, “Ctrl” to the absence of this layer

Transplant ID	Year	Month	Donor ID	Patch configuration & Sediment treatment						Totals	
				Small		Large		Compact		Sum plots	Area (m ²)
				Ctrl	Shell	Ctrl	Shell	Ctrl	Shell		
T1	2007	June	D1	3	3	3	3			12	189
T2	2007	June	D1	4	4	4	4			16	252
T3*	2008	June	D2	4	4	4	4			16	252
T4	2008	June	D1 + D2	4	4	4	4			16	252
T5	2008	June	D1	4	4	4	4			16	252
T6	2008	June	D2	4	4	4	4			16	252
T7	2010	March	D2		12					24	351
T8	2010	June	D4							8	144
T9	2011	June	D3		15					33	492.8
T10**	2012	June	D4***	16						16	180
										173	2616.8

* Additional test: individual plants vs. sods
 ** Differing in elevation: 8 normal vs. 8 initially lowered
 *** Simultaneous reciprocal transplant at D4: 8 sods, kept in boxes overnight, protected against desiccation

Table S3: Development of NSN per plot of the transplants T1 - T6 over the first growing season, expressed in percentages (numbers > 100% represent an increase in NSN, numbers < 100% a decrease)

Exposedness	Treatment Patch configuration	Control		Shell	
		5	9	5	9
Intermediate	T2	106.9	108.8	90.6	114.6
	T4	108.3	86.8	118.1	71.6
	T5	145.9	200.8	199.0	219.6
	Subtotal	120.4	132.1	135.9	135.3
Exposed	T1	87.2	94.0	94.2	86.8
	T6	47.0	47.5	50.6	70.5
	T3	167.1	155.8	151.0	145.2
	Subtotal	101.7	99.5	99.0	102.1

Table S4: General characteristics of tidal flats with seagrass expansion and decline. Means based on monitoring between 2007 and

Tidal flats categorized on seagrass dynamics	Transplants located on the tidal flat	Hydrodynamic exposure	Median grain size (D50)	Adult lugworm density	Ice scouring	Porewater				
						ammonium	nitrate	phosphate	sulphide	
			μm	$\# \text{m}^{-2}$	presence (+) absence (-)	$\mu\text{mol/l}$	$\mu\text{mol/l}$	$\mu\text{mol/l}$	$\mu\text{mol/l}$	$\mu\text{mol/l}$
Seagrass expansion	T6	Exposed	153	1	-	116	3	21		53
	T5	Intermediate	112	70	+	71	1	16		22
	T9	Intermediate	120	15	+	98	1	18		2
Seagrass decline	T1	Exposed	171	2	-	30	9	4		2
	T2	Intermediate	162	56	-	70	2	18		0
	T4	Intermediate	175	54	-	55	4	11		2
	T3	Exposed	132	55	-	157	ND	29		60
	T7	Exposed	121	48	-	57	10	13		1
	T8	Exposed	128	16	-	90	11	18		1
	T10	Sheltered	120	3	-	69	3	15		56

Notes:

- Macro-algal cover: always < 10%
- Pore water salinity: mean 29.5 PSU, not different between and within seagrass locations
- Geese feeding pits: frequently in October - November at all sites

A full-page background image showing a sunset over a body of water. The sun is low on the horizon, creating a bright orange and yellow glow that reflects on the water. The sky transitions from a deep orange near the horizon to a pale purple at the top. In the distance, a city skyline is visible against the horizon line.

Chapter 5

Rhizome starch as indicator for temperate seagrass winter survival

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Ecological Indicators 2015

Abstract

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

Introduction

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

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

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

For seagrasses, the most important non-soluble carbohydrate for long-term storage is starch (Pirc, 1989). Starch is mainly stored in the rhizomes, and produced in periods with a positive carbon balance (Olive et al., 2007; Zimmerman and Alberte, 1996). Wintering seagrasses are thus dependent on the production of starch reserves in the preceding growing season. The amount of starch needed for winter survival depends on both internal and external factors; respiration can be affected by temperature and light, but also by biomass and photosynthetic activity (Alcoverro et al., 1999; Fourqurean and Zieman, 1991; Marsh et al., 1986; Vermaat and Verhagen, 1996). In addition, environmental stressors such as ammonium toxicity and sediment anoxia can increase carbon

demand (Brun et al., 2008). Growth is another important process that contributes to carbon demand (Alcoverro et al., 1999). Although growth is marginal during the winter months ($0.015 \text{ cm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ versus loss rates of $-0.110 \text{ cm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ in *Zostera noltii*) (Vermaat and Verhagen, 1996), carbon demand for growth in early spring can deplete carbohydrate reserves.

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

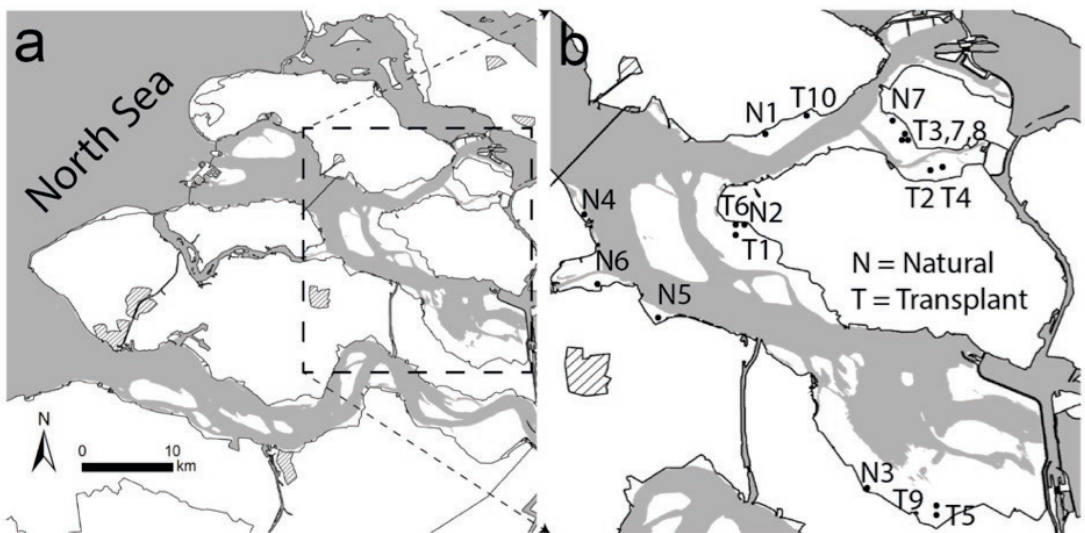


Figure 1: Maps of (a) the Dutch Delta, and (b) the locations of the transplant sites (T1-10) and natural seagrass beds (N1-7) in the Oosterschelde.

Materials & Methods

Location

We studied the winter survival of the natural *Zostera noltii* beds and transplanted *Zostera noltii* beds in the Oosterschelde Delta, The Netherlands (Fig. 1a, $51^{\circ}39' \text{ N}$, $4^{\circ}01' \text{ E}$). The Oosterschelde has a surface area of 351 km^2 , a

tidal amplitude of 2.5-3 m (Troost et al., 2009) and freshwater input is highly limited, resulting in a salinity of 30 PSU (Nienhuis and Smaal, 1994). Intertidal *Zostera noltii* meadows cover around 75 ha of the mudflats in the Oosterschelde and are located on relatively stable sediments such as clay banks, saltmarsh remnants and natural shell layers (Suykerbuyk et al., 2012). Intertidal *Zostera noltii* in the Oosterschelde, which is sometimes referred to as *Zostera noltei*, are generally not nutrient limited (Govers et al., 2014a) and light availability (when submersed) is around 3-4.5 m secchi disc visibility in summer (Wetsteyn and Kromkamp, 1994).

Monitoring transplants and natural seagrass beds

As a part of a large-scale seagrass transplantation project, aiming to mitigate seagrass damage caused by dike reinforcements, seagrass sods were mechanically transplanted to 10x10 m or 15x15 m plots on several mudflats in the Oosterschelde (for details, see Suykerbuyk et al. (2012)) (Fig. 1). Plots were monitored in spring and summer from spring 2009 – autumn 2011; we counted shoot densities and determined seagrass surface area plot⁻¹ with a Real Time Kinematic differential GPS (RTK-dGPS, Trimble, USA). Samples for starch analysis were taken each spring (end of April / start of May) and each autumn (end of October, start of November). Simultaneously, samples for starch analysis were taken from a number of natural seagrass beds in the Oosterschelde. In addition several abiotic characteristics (sediment grain size, sediment organic C, leaf %C, leaf %N, porewater NH₄, NO₃, PO₄, and H₂S) were determined every summer (Table 1). For detailed methodological details on these measurements, please see Govers et al. (2014b).

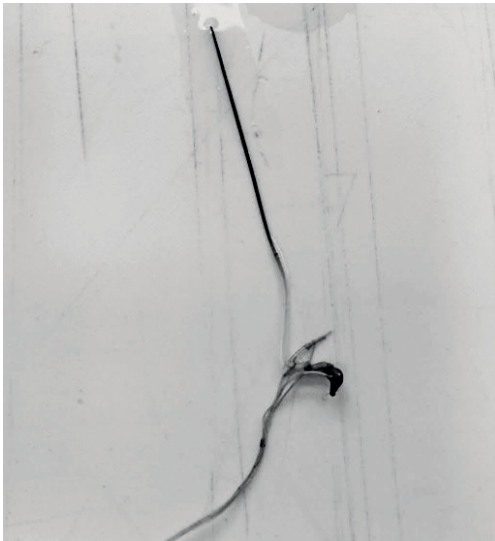


Figure 2: Photograph of wintering unit of *Zostera noltii* with one single shoot and an active meristem.

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

unsuccessful plots and vice versa. A single transplant site could thus be classified as successful in one year and unsuccessful in the next.

Leaf removal experiment set-up

Winter survival of *Zostera noltii* occurs by a short rhizome with a single shoot, which encloses an active meristem (Vermaat & Verhagen 1996) (Fig. 2). To test the effect of leaf presence and autumn starch levels on seagrass winter survival, we selected three locations based on their differential starch concentration of rhizomes in November 2012. The average starch concentrations were 57.3 mg g⁻¹ DW in Dortsman Noord (DMN – high starch, Fig. 1b N2), 38.8 mg g⁻¹ DW in Oostdijk (OD – medium starch, Fig. 1b N3), and 18.8 mg g⁻¹ DW in KATS (Kats – low starch, Fig. 1b N4). Next to starch concentration, the length and biomass of the rhizome fragments (in November) also varied between sites at the start of the experiment: rhizome fragments were longer and heavier at OD (medium) and Kats (low) (14 ± 0.44 mm, 3.7 ± 0.07 mg DW and 14 ± 0.40 mm, 4.7 ± 0.08 mg DW respectively) than at DMN (high) (11 ± 0.23 mm, 3.2 ± 0.01 mg DW). A more detailed description of abiotic site characteristics is given in Table 2.

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

Processing leaf removal experiment samples

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

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

Site	Grain size	Sediment C	Leaf %N	Leaf %C	NH ₄	NO ₃	PO ₄	H ₂ S	Hydrodynamic Exposure							
Natural populations	115 ^a	4.6	0.44 ^a	0.06	3.33 ^a	0.06	36.1 ^a	0.4	55 ^{ab} / 1	2.0 ^a	0.8	13.16 ^b	1.8	1.49 ^a	1.0	Exposed to sheltered
Successful transplants	133 ^a	7.9	0.24 ^b	0.02	3.28 ^a	0.08	36.6 ^a	1.2	88 ^{ab} / 17	47.1 ^b	22.2	17.97 ^a	2.6	22.97 ^b	1.1	Exposed to sheltered
Unsuccessful transplants	122 ^a	4.7	0.26 ^b	0.03	3.32 ^a	0.04	36.3 ^a	0.9	89 ^{ab} / 9	26.7 ^b	3.6	17.46 ^b	1.0	26.65 ^b	1.2	Exposed to sheltered

Table 2: Characterization of the leaf experiment sites. Abbreviations: DMN = Dortsman Noord, OD = Oostdijk, Kats = Kats. Sed. = sediment. Units are: starch (mg g⁻¹ DW), sediment median grain size (D50, μ m), sediment organic C (%), leaf total nitrogen (% DW), and porewater NH₄, NO₃, PO₄ and H₂S were measured in the porewater.

Location	Code map	Starch ¹ characterization	Starch	Grain size	Sediment C	Leaf %N ¹	NH ₄ ²	NO ₃ ²	PO ₄ ²	H ₂ S ³	Hydrodynamic exposure
DMN	N2	57.3	high	103	0.49	3.1	119.97	0.85	19.67	11.56	exposed
OD	N3	38.8	medium	78	0.44	2.8	22.75	0.00	10.55	23.48	sheltered
Kats	N4	18.8	low	117	NA	3.0	26.75	0.70	7.65	3.35E-05	intermediate

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

photochemical efficiency of PSII ($(F_m - F_0)/F_m$). F_m is the maximum phytofluorescence after dark-adaptation and F_0 is the minimum fluorescence of the chlorophyll. Photosynthetic yield was measured on the middle part of each leaf (in the middle between the leaf tip and leaf base).

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

Starch analysis of transplants, natural beds & experiment

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

Statistical analysis

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

Results

Transplants and natural beds

We found no correlation between the shoot densities in summer and the shoot densities in the following spring in the transplant plots ($R^2 = 0.02$, Fig S1). These results indicate that other processes than the preceding year's growth success play a role in predicting growth success in June. Subsequently, we compared rhizome starch concentration of transplanted seagrass patches with seagrass from the natural seagrass beds in the Oosterschelde (Fig. 3b). Starch concentration varied seasonally, with the highest starch concentration in autumn and lowest

starch concentration in spring. Starch concentration of all the unsuccessful transplant sites was always significantly lower than rhizome starch concentration from the successful transplants and the natural beds (Linear mixed model, $F_{2, 160} = 23.63$, $P < 0.001$), which not differed mutually (Fig. 3b). This difference was greater at the end of the growing season (autumn) than at the start, which suggests that seagrasses of both natural beds and successful transplants had more reserves to spend during the winter months than the unsuccessful transplants. In contrast, we did not find any significant differences in measured abiotic characteristics between successful and unsuccessful transplant sites (Table 1)

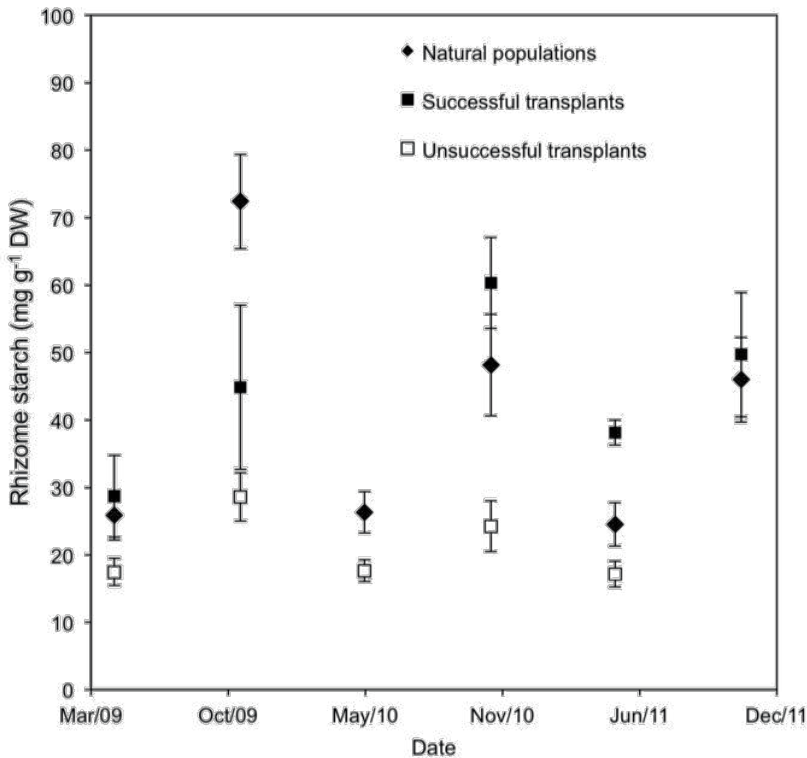


Figure 3: Rhizome starch concentration in spring and autumn in the period 2009-2011 of natural populations, successful transplants and unsuccessful transplants in the Oosterschelde. Displayed values are means and error bars represent standard errors (SE).

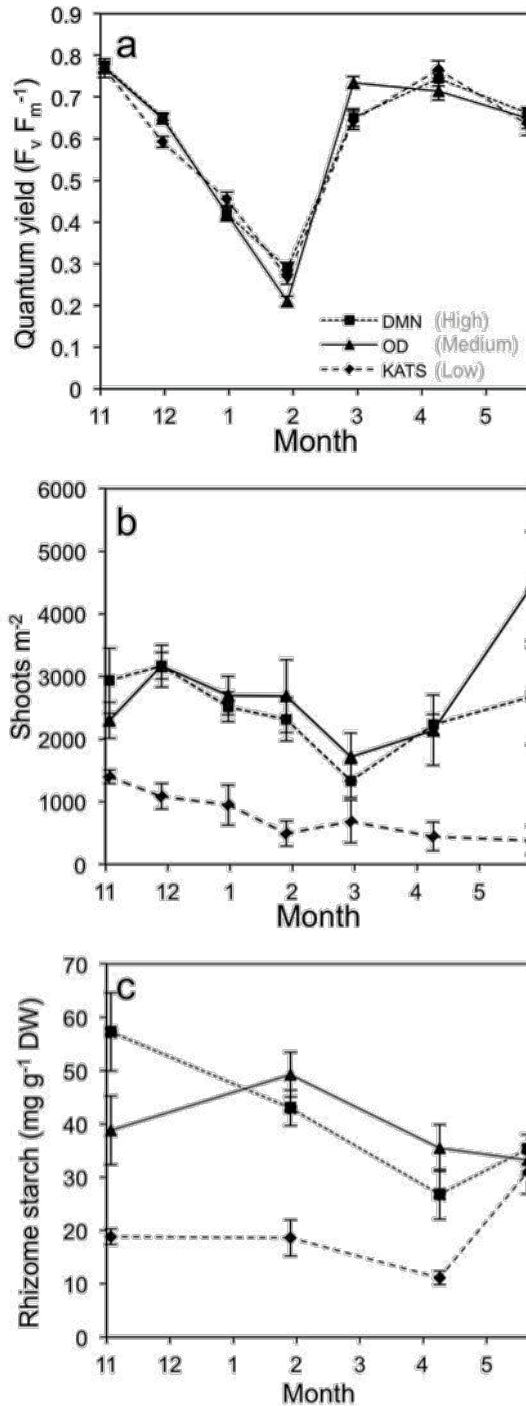


Figure 4: a) PAM yield, b) shoots m^{-2} , and c) rhizome carbohydrate concentration of *Zostera noltii* on three different locations. Displayed values are means and error bars represent standard errors (SE).

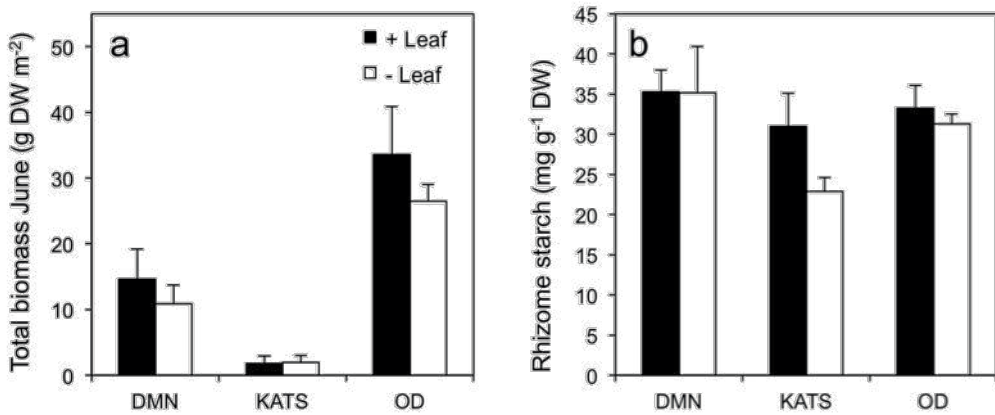


Figure 5: Effects of the leaf removal treatment (+leaf and -leaf) on a) seagrass biomass, and b) rhizome carbohydrate concentrations in June, at the end of the experiment on three locations: DMN (high), Kats (low), and OD (medium). Displayed values are means and error bars represent standard errors (SE)

Leaf removal experiment

Seagrass photosynthetic performance (maximum quantum yield) decreased dramatically in the winter (RPM ANOVA, $F_{3,6} = 282.25$, $P < 0.001$), with the lowest quantum yield in February 2013. In March (2013), photosynthetic performance strongly increased, back to growing season levels (Fig. 4a). Photosynthetic performance did not vary between locations ($F_2 = 0.54$, $P = 0.562$, $n=20$). In contrast, shoot density did vary between locations ($F_2 = 21.23$, $P = 0.001$, $n=5$), and the lowest shoot densities were observed in KATS (low), with 2-3 times lower shoot densities than DMN (high) and OD (medium) (Fig. 4b). Shoot density did not differ significantly between the latter two locations (Fig. 4b). Shoot density also decreased during winter ($F_{1,78} = 7.496$, $P = 0.019$), but at least some aboveground biomass was present at all locations during the entire winter (<2000 shoots m⁻² compared to >10,000 shoots m⁻² in summer). Shoot density strongly increased after the winter at OD (medium) and DMN (high), but further decreased at KATS (low). The latter may reflect a lack of starch to enable regrowth. Rhizome starch concentration (Fig. 4c) dropped during winter on all locations ($F_2 = 11.054$, $P = 0.001$, $n=5$), and although DMN (high) and OD (medium) started off with different rhizome starch concentration in November 2012, there was no difference between the two locations during winter.

Rhizome starch however remained significantly lower at Kats (low) ($F_2 = 43.77$, $P < 0.001$) than at the other two locations during the entire winter. Starch concentration increased again from May (2013) onwards. Although

photosynthetic performance (Fig. 4a) and shoot densities started to increase again around March (2013), in DMN (high) and OD (medium) (Fig. 4b), rhizome starch concentration dropped from November (2012) onwards to the end of April (2013), and only started to increase again from May (2013) onwards.

Leaf removal treatments resulted in lower shoot densities at DMN (high) (Linear mixed model, $F_{1,53} = 7.547$, $P = 0.008$) and OD ($F_{1,53} = 23.58$, $P < 0.001$), where shoot densities dropped by 700-1500 shoots m^{-2} in the -leaf (leaf removal) treatments. The leaf removal treatment however, did not result in a significant decrease in shoot density at Kats (low) ($F_{1,53} = 0.607$, $P = 0.439$), where standing biomass was very low to begin with (Fig. 4b). Although we successfully reduced shoot densities, we did not observe any effect of this treatment on seagrass biomass at the end of our experiment in June 2013, on any of the locations (Linear mixed model, $F_{1,26} = 0.861$, $P = 0.362$) (Fig. 5a). Seagrass biomass differed significantly between all three locations (ANOVA, $F_{1,27} = 31.68$, $P < 0.001$), with biomass OD (medium) > DMN (high) > Kats (low) and Kats having a total biomass m^{-2} that was 10x lower than at OD (medium) (Fig. 5a). Rhizome starch concentration did however not differ anymore between locations in June 2013 (ANOVA, $F_{2,26} = 3.207$, $P = 0.057$), despite the differences in autumn. Leaf removal treatment did not affect rhizome starch concentration in June 2013 (Linear mixed model, $F_{1,25} = 1.81$, $P = 0.191$) (Fig. 5b).

Rhizome starch concentration in November 2012 was positively related to shoot density in June 2013 ($R^2 = 0.51$, $P = 0.006$) (Fig. 6), showing the importance of a good start-off before winter for the success in next growing season. The relationship between November rhizome starch and shoot densities in the next June could be described according to the following equation (Fig. 6):

$$y = 67.69x - 723.43 \quad eq. 1$$

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

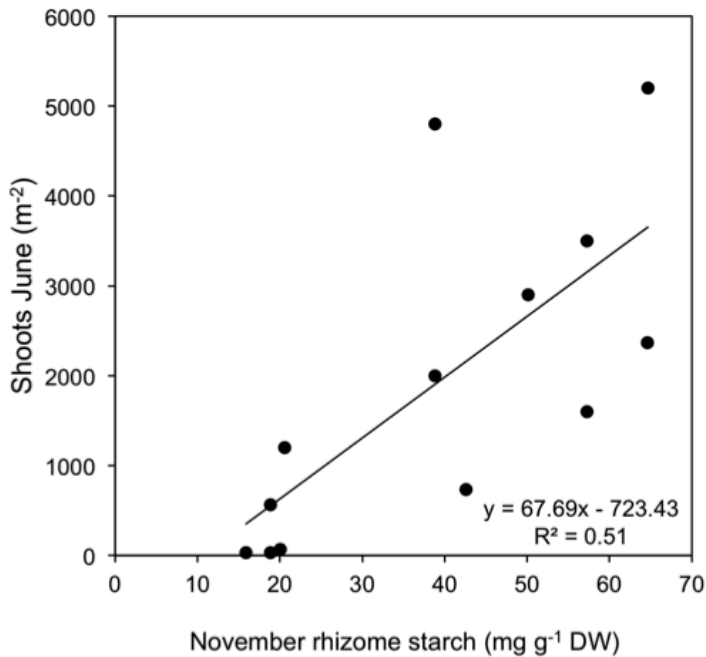


Figure 6: The relationship between rhizome carbohydrate concentration in autumn and next year's shoot density in June.

Discussion

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

Starch reserves and carbon balance

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

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

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

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

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

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

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

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

Conclusions & perspectives

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

Indicators, such as autumn starch reserves may not only be used to predict transplant success, but also to determine the timing of transplanting, as to make sure that transplanted plants have enough reserves for recovery and regrowth (Zimmerman et al., 1995). From these seagrass wintering mechanisms, lessons may be learnt with respect to other processes that may drain carbohydrate

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

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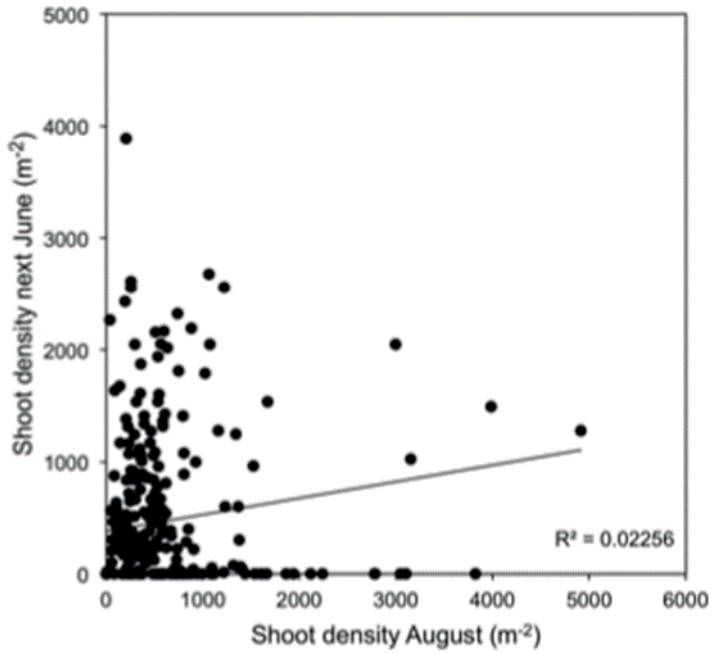


Figure S1: Correlation between shoot densities in summer and shoot densities in the following spring of the transplants in the first 36 months after transplanting

Chapter 6



***Surviving in changing seascapes:
Sediment dynamics as bottleneck for long-term seagrass presence***

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Abstract

Changes in the seascape often result in altered hydrodynamics that lead to coinciding changes in sediment dynamics. Little is known on how altered sediment dynamics affect long-term seagrass persistence.

We studied the thresholds of sediment dynamics in relation to seagrass presence by comparing sediment characteristics and seagrass presence data of seven separate seagrass meadows. All meadows had a long-term (>20 years) presence. Within these meadows we distinguish so-called “hotspots” (areas within a meadow where seagrass was found during all mapping campaigns) and “coldspots” (with infrequent seagrass presence). We monitored static sediment characteristics (median grain size, bulk density, silt content) and sediment dynamics (i.e., bed level change and maximum sediment disturbance depth), bioturbation (i.e., lugworm densities and induced fecal pit and mound relief), and seagrass cover. We statistically analysed which sediment characteristic best explains seagrass cover.

Densely vegetated hotspots were shown to have lower sediment dynamics than sparsely vegetated hotspots and coldspots, while static sediment characteristics were similar (grain size, bulk density). The vegetation cover was either low (2 – 15%) or high (> 30%) and sediment dynamics showed a threshold for vegetation cover. From this correlative finding, we postulate a self-sustaining feedback of relatively dense seagrass via sediment stabilisation and accordingly a runaway feedback once the seagrass cover becomes too sparse. The sensitivity for sediment dynamics shown in our study implies that future existence of seagrass meadows may be at risk as ongoing climate change might directly (increased environmental extremes) or indirectly (changing seascapes) negatively affect seagrass beds.

Introduction

There is an overwhelming body of literature describing the massive and still ongoing losses of seagrass over the last decades, predominantly due to anthropogenic impacts (e.g., Orth *et al.* 2006a; Waycott *et al.* 2009). The dominant anthropogenic impacts are related to the effects of light limitation, via either enhanced turbidity or eutrophication. A much less studied aspect is the more indirect effect of changes to the coastline due to infrastructural works. Such changes in the seascape may alter natural seagrass structuring processes, and thereby indirectly threaten continued seagrass existence (i.e., Lotze *et al.* 2006). Changes in the seascape often result in altered hydrodynamics that in turn lead to changes in sediment dynamics. The direct effects of hydrodynamics and burial processes on short-term seagrass performance are well-studied (e.g., Fonseca & Kenworthy 1987; Gambi *et al.* 1990; van Katwijk & Hermus 2000; Cabaco *et al.* 2008). In contrast, relatively little is known on how altered sediment dynamics may affect long-term seagrass persistence. This question may be particularly relevant for seagrasses in temperate areas, where plants cannot adapt to burial or erosion during winter when they do not grow (Han *et al.* 2012).

Restoration efforts of seagrass species, particularly *Zostera* species, have aimed at mitigating the adverse effects of sediment related processes, either by reducing hydrodynamic forces (van Breedveld 1975; Lewis *et al.* 2006), increasing seagrass anchoring (e.g. Davis & Short 1997; Fonseca & Bell 1998; Bastyan & Cambridge 2008; Zhou *et al.* 2014), increasing planting density (e.g. Bos & van Katwijk 2007) or reducing the adverse effect of bioturbating infauna (Fonseca *et al.* 1995; Hammerstrom *et al.* 1998; Sheridan *et al.* 1998; Hughes *et al.* 2000; Bos & van Katwijk 2007; Suykerbuyk *et al.* 2012) and enhancing sediment stabilisation (van Katwijk & Hermus 2000; Campbell & Paling 2003; van Keulen *et al.* 2003; Irving *et al.* 2010).

However, there has been little attention on how sediment dynamics may affect long-term seagrass persistence. In Germany and the Netherlands temperate intertidal seagrass *Zostera noltii* is mainly found on intrinsically stable or sheltered sediments (Philippart 1994; Reise & Kohlus 2008; Suykerbuyk *et al.* 2012; Suykerbuyk *et al.* subm.-a). Sediments are intrinsically stable when they consist of clay-rich and compact remnants of former salt marshes or dikes, or when they consist of a shell layer. In such sediments lugworm densities are low (Philippart 1994). Sediment dynamics may then be low even if exposure to hydrodynamics is relatively high, and seagrass presence may relate to sediment dynamics even more than to hydrodynamics (van Katwijk & Hermus 2000; Reise & Kohlus 2008). Alternatively, dense seagrass rhizomes and roots may stabilise the sediments (Christianen *et al.* 2013) and outcompete the common bioturbator *Arenicola marina* (Philippart 1994; Berkenbusch *et al.* 2007; Reise & Kohlus 2008; Eklöf *et al.* 2011). *Arenicola marina* is widespread destabiliser of intertidal sandy sediments at northwest European coasts (Cadée 1976; Reise 1985; Coosen

et al. 1994); its bioturbation hampers *Zostera* restorations in these areas (Valdemarsen et al. 2011, Suykerbuyk et al. 2012; subm.).

In the Eastern Scheldt tidal basin (SW Netherlands), long-term monitoring of a large-scale seagrass transplantation project clearly indicated winter survival as the main bottleneck for long-term persistence (Suykerbuyk et al. 2012; Govers et al. 2015; Suykerbuyk et al. Subm.-a). Winter losses could not be attributed to adverse negative environmental conditions (e.g. nutrients, light availability) as seagrass area expanded during all summers (Suykerbuyk et al. Subm.-a). Han et al. (2012) showed that erosion or sedimentation events can be quickly counteracted by *Zostera noltii* during summer, by growing back to a rooting depth of around 0.6 ± 0.3 cm. During the stormy winter season, erosion events are however typically more pronounced and more frequent than during summer, and likely to reach deeper than the typical rooting depth. Erosion events above a critical threshold depth may result in uprooting and loss of overwintering seagrass rhizomes, and hence the loss of both transplanted and natural meadows.

We hypothesize that high sediment dynamics reduce winter survival and consequently long-term seagrass persistence, although evidence for the existence of thresholds of sediment dynamics in relation to seagrass presence and insights into how they work are still lacking. Thresholds may be dynamic but site specific, depending on the interaction between local environmental forcing from currents and waves and the susceptibility of the sediment to that force, which depends on the nature of the sediment (i.e., packing, cohesiveness, shell content) and the presence of sediment loosening activities (e.g. bioturbating infauna). We aim to fill this knowledge gap on thresholds of sediment dynamics in relation to seagrass presence by comparing sediment characteristics and seagrass presence data of seven separate seagrass meadows. All meadows had a long-term (>20 years) presence. Within these meadows we distinguish so-called “hotspots” (areas within a meadow where seagrass was found during all mapping campaigns) and “coldspots” (areas with infrequent seagrass presence during 1989 – 1998, and absence during 1999-2009). Static sediment characteristics (median grain size, bulk density, silt content) were measured in the summer of 2012. Between April 2012 and May 2013, we monitored sediment dynamics (i.e., bed level change and maximum sediment disturbance depth), bioturbation (i.e., lugworm densities and induced fecal pit and mound relief), and seagrass cover. The resulting dataset was statistically analysed to identify which sediment characteristic(s) would best explain seagrass cover. We specifically aimed to test the hypotheses that:

- H1) Long-term seagrass presence depends on the nature of the sediment, where H1a) hotspot sediments have finer sediments than sediments with infrequent seagrass presence (coldspots), and H1b) hotspot seagrass cover increases with decreasing grain size and lack of disturbances (i.e. bioturbation).
- H2) Actual seagrass cover of hotspots is negatively affected by winter sediment processes, rather than summer sediment processes.

Furthermore, we expect the presence of a critical threshold value of the maximum sediment disturbance depth for actual seagrass presence, above which seagrass presence is affected by sediment disturbances (leading to uprooting). As maximum sediment disturbance depth is the result of several sediment characteristics and processes, the relationship between these characteristics and the maximum sediment disturbance depth is investigated as well.

Methods and Materials

Sample point selection

Seven intertidal *Zostera noltii* seagrass meadows with more than 20 years of seagrass presence were selected across the Eastern Scheldt tidal basin (Fig. 1a + b). These seagrass meadows were mapped by Rijkswaterstaat (part of the Dutch Ministry of Infrastructure and the Environment) every second year. The meadows were still present in the year prior to the measurements (2011). We identified so-called seagrass “hotspots”; areas within a meadow where seagrass was found during all mapping campaigns and that thus seem to have the best conditions for seagrass growth (Fig. 1c-i, in black). Recently, 4 out of the 7 selected meadows suffered a significant decline in seagrass cover and area, with only sparse seagrass cover remaining in seagrass hotspots. We therefore distinguished hotspots with either dense or sparse seagrass cover. We also identified seagrass “coldspots”: areas with infrequent seagrass presence during 1989 – 1998 (presence only 30-40% of the years monitored), and absence during 1999-2009. Coldspots lie within the range of colonisation by vegetative growth or seedfall, but are probably not (or only very marginally) suited for seagrass growth (Fig. 1c-i, in white). Sampling points were selected within the hotspots (3 replicates per hotspot, white bullets) and coldspots (4 to 6 replicates per coldspot, black bullets). The meadows’ air exposure time ranged from 50 to 70 % of the tidal cycle; tidal amplitude was 3 m. Exposure to hydrodynamics for each meadow were assessed by a 2D wave and current velocity model by Rijkswaterstaat, Ministry of Infrastructure and Environment. The Eastern Scheldt is a relatively sheltered system with a reduced tidal movement, little wind fetch and small waves compared to coastal systems (Louters et al. 1997). Temperature does not differ much between meadows in the Eastern Scheldt (Govers unpublished results). Suspended sediment concentrations are very low throughout the Eastern Scheldt (data Ministry of Infrastructure and Environment).

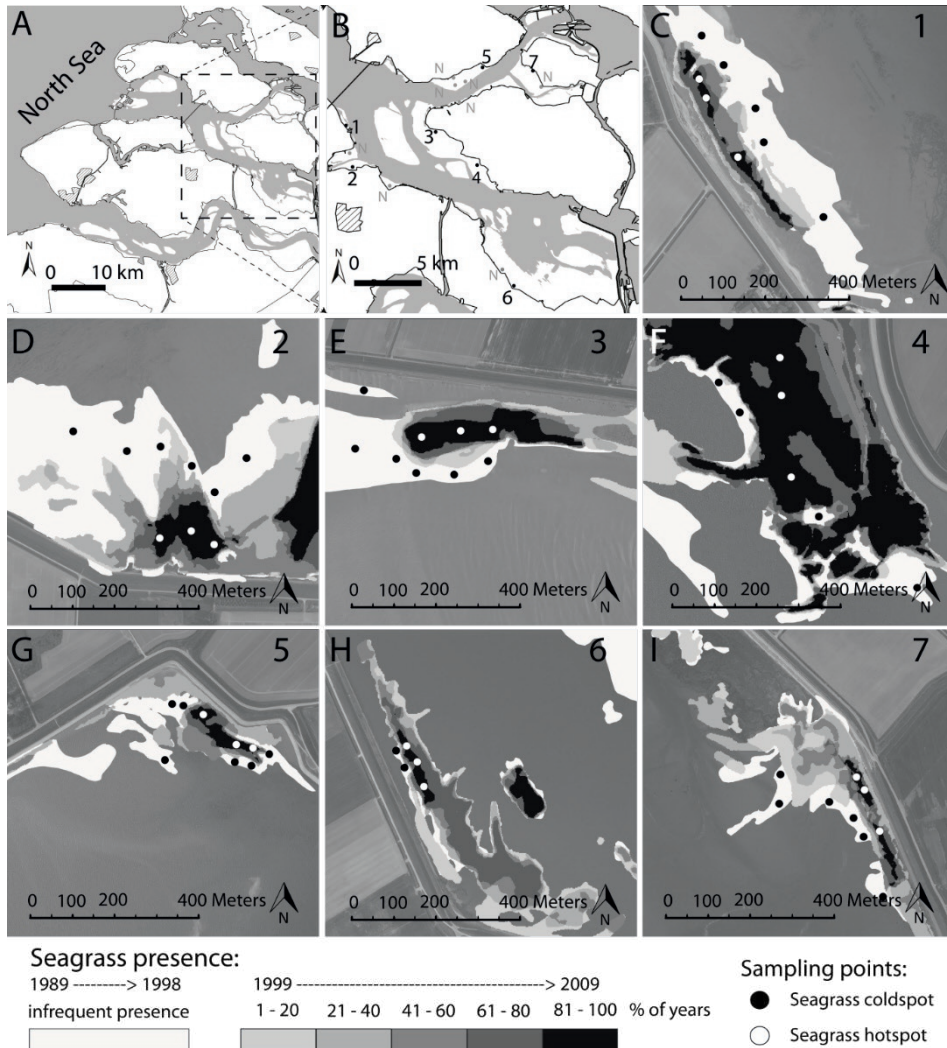


Figure 1: A & B Location of the monitored seagrass meadows across the Eastern Scheldt tidal basin, SW Netherlands. (Numbers indicate the selected meadows, “N” all other). Panels C-I; seagrass presence maps for each selected meadow (top right numbers correspond with numbers in panel B). Grayscale colors represent the percentage of years that area had >5 % coverage at mapping surveys during 1999-2009 (data Rijkswaterstaat). Black areas are considered as seagrass hotspots (areas within a meadow where seagrass was found during all mapping campaigns). White areas are considered as seagrass coldspots (areas with infrequent seagrass presence during 1989 – 1998, and absence during 1999-2009). Sampling points of coldspot and hotspot areas are indicated by white and black bullets, respectively

Monitoring

To assess how seagrass presence correlated with several sediment properties, we measured static sediment properties and seagrass presence in the summer of 2012, and we measured dynamic sediment properties from April 2012 up until May 2013, with an interval of approximately 7-9 weeks. At the beginning of the summer of 2012, static sediment characteristics were measured by taking 20 ml sediment cores from the top 5 cm that were further processed (wet weighted, freeze-dried, dry weighted, sieved over 1 mm) prior to grain size distribution analysis by laser diffraction on a particle sizer (Malvern, Worchester, UK) and dry bulk density calculation

Eq. 1

$$\text{Sediment bulk density}_{dry} = \frac{\text{Dry weight (gr)}}{\text{Total volume (cm}^3\text{)}}$$

At the same time, the elevation (Amsterdam Ordnance Datum) of each sampling point was measured using a real time kinetic differential GPS (RTK-dGPS, Leica Viva GS12 GNSS receiver and CS15 controller), with a vertical accuracy of less than 2 mm). To separate the effects of seasons and aboveground seagrass biomass, data were pooled per season: summer (growing season, April – November 2012 and March – May 2013) and winter (non-growing season, November 2012 – March 2013). To assess sediment dynamics, first, bed level change was measured as the vertical height difference between initial bed level (marked on 2 separate bamboo sticks per sampling point) and the bed level at the time of measurement. For analysis, bed level changes were pooled per sampling point. Secondly, maximum sediment disturbance depth (an integrative measure for sediment dynamics) was quantified using fluorescent dye sediment tracer rods after (Runte 1989). Universal rods were used for all locations, consisting of a 17:1 volume ratio mix of moist sediment from the Tholseinde tidal flat (Median grain size: $D_{50} \sim 100 \mu\text{m}$) and dry inert, fluorescent tracer (luminophores, $D_{50} = 41 \mu\text{m}$; Environmental Tracing Systems, UK; <http://www.environmentaltracing.com>). The prepared, frozen tracer rods (10 cm long x 1.5 cm diameter) were vertically positioned in the sediment in a pilot hole and leveled with the surrounding bed level. At the next sampling visit the (remaining) rod was retrieved by taking a large core (30 cm depth x 4.0 cm diameter) using a template, and cores were kept upright until being frozen at the institute. Frozen cores were cut lengthwise to measure (under black light) the depth of the bottom of the tracer rod, its remaining length, and the depth of the top of the remaining rod (Suppl. Fig. 1). The maximum sediment disturbance depth was then calculated from the bed level change and the depth of the top of the remaining tracer rod (Eq. 2 and 3).

In case of sedimentation:

Eq. 2

$$\text{Sediment disturbance depth}_{max} = \text{Tracer rod depth}$$

In case of erosion:

Eq. 3

$$\begin{aligned} \text{Sediment disturbance depth}_{max} \\ = [\text{Bed level change}] + \text{Tracer rod depth} \end{aligned}$$

At each monitoring visit, seagrass presence and cover were recorded as well as adult lugworm densities and their induced pit and mound relief. For analysis in relation to seagrass cover, we used the maximum seagrass cover that was recorded during summer.

Statistical analyses:

A comparison of sediments from the coldspot- and sparsely vegetated hotspot categories was used to reveal differences between seagrass-suitable and non-suitable sediments without any potential ecosystem engineering effects of seagrass (H1a). The comparison of sparsely and densely vegetated hotspots was used to test what characteristics are most correlated to seagrass cover. Both comparisons were tested separately using One-Way ANOVAs in Sigmaplot v12.0 (Systat Software Inc.). The data were analysed with ANOVA on ranks if normalised data could not be obtained after data transformation. Results are graphically shown as Tukey boxplots, unless differently stated. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. All outliers are represented by dots. Prior to statistical analysis, data were checked for normality and if necessary transformed. To find the relative importance of the effect of each parameter on seagrass cover and on maximum sediment disturbance depth in winter (H1b & H2), first Pearson's product-moment correlation coefficients were calculated and statistically tested using R 2.15., and second a backward stepwise multiple regression analysis was performed in R. For the latter, the final adjusted models were the most reduced models, which were not significantly worse ($P < 0.05$) than the full model that included all factors. Model selection was based on the Akaike Information Criterion (AICs). Models were checked for independence of errors and multicollinearity.

Results

The seven selected seagrass meadows showed two distinct levels of vegetation cover in the year of our study. Three hotspots had high seagrass cover in summer ($>30\%$), whereas four hotspots had sparse cover ($2 - 15\%$). In coldspots seagrass cover was between 0 and 3%, except for a recently expanding meadow showing a cover of 30% (Supplementary data).

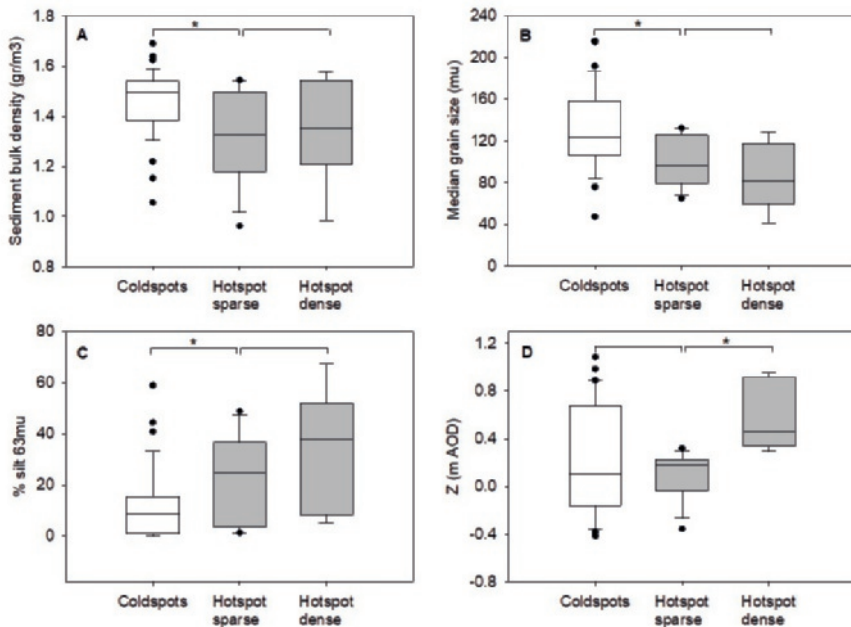


Figure 2: Static sediment characteristics and absolute elevation of sampling points in natural meadows, split into 3 seagrass categories, namely coldspots (historical, but no recent seagrass cover), hotspot_low (recent, low seagrass cover) and hotspot_high (recent, high seagrass cover). Significant difference between the seagrass categories (one-Way ANOVA, significance level: $p < 0.05$) are indicated with asterisk.

Comparing coldspots and sparse vegetated hotspots

To find differences between coldspots and hotspots *irrespective of seagrass cover* (thus without relevant potential ecosystem engineering effects of seagrass), we compared the coldspots with the four sparsely vegetated hotspots for all measured sediment and meadow characteristics. Sparsely vegetated hotspots had smaller median grain size, lower sediment bulk density and a higher silt content than seagrass coldspots (Fig. 2A, B & C, Tab 2). Elevation did not consistently differ between seagrass hotspots and coldspots (Fig. 2D, Tab 2). Measures related to sediment dynamics (maximum sediment disturbance depth, lugworm density and lugworm relief) did not differ between coldspots and sparsely vegetated hotspots, in winter nor summer (Fig. 3, Tab 2).

Comparing sparsely and densely vegetated hotspots

To gain insight into whether characteristics of hotspots that distinctly differ in actual seagrass cover are structurally different, we compared sparsely vegetated hotspots (2 – 15 % cover) with densely vegetated hotspots (> 30 % cover). Static sediment characteristics, i.e., median grain size, sediment bulk

density and silt content did not differ between the two types of hotspot (Fig. 2A, B & C, Tab 2). Densely vegetated hotspots were found at an elevation that was

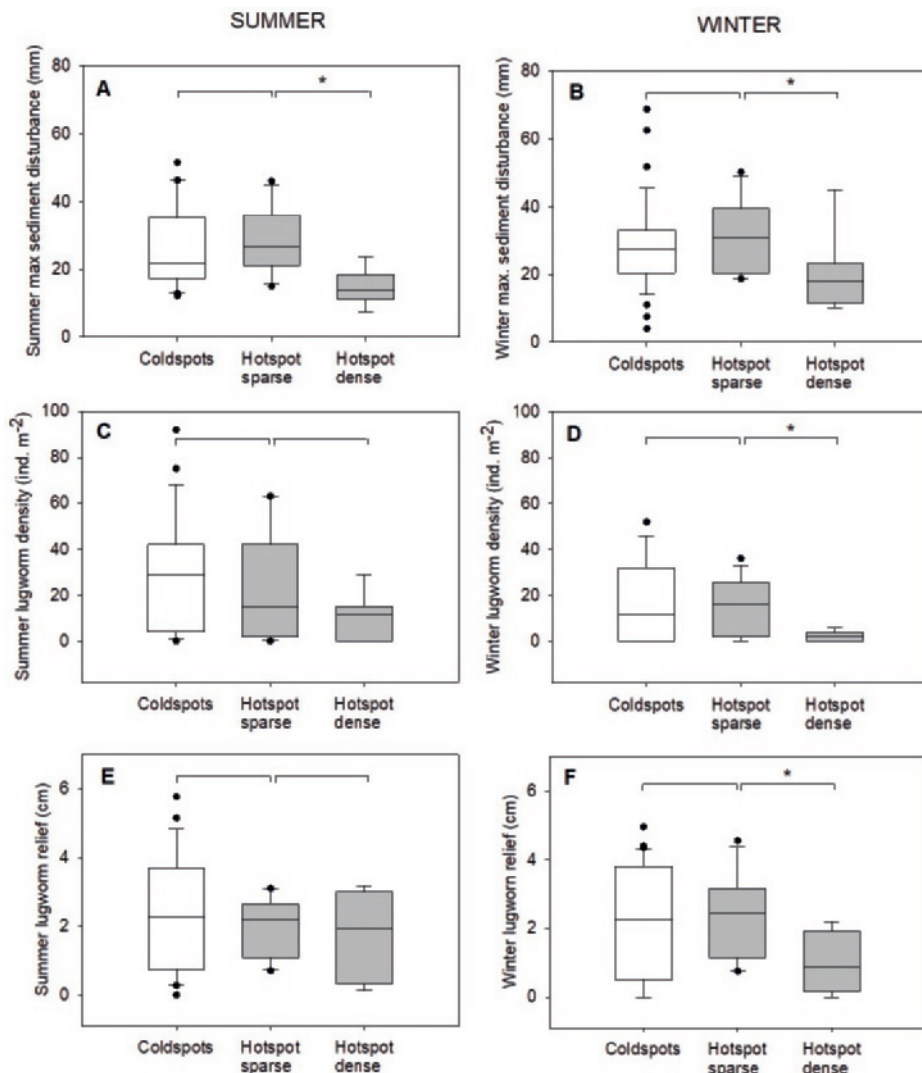


Figure 3: Maximum sediment disturbance, lugworm densities and lugworm relief, split into summer (left panels) and winter (right panels). Significant difference between the seagrass categories (one-Way ANOVA, significance level: $p < 0.05$) are indicated with asterisk.

around 60 cm higher than that of sparsely vegetated hotspots. As general hydrodynamic exposure of the meadows was similar (Tab 1, unpaired T-test $p > 0.1$), the higher elevated beds experienced hydrodynamics during shorter

periods, thus contributing to lower sediment dynamics (i.e. van Katwijk & Hermus 2000). The maximum disturbance depth was lower at densely vegetated hotspots as compared to sparsely vegetated hotspots, both in summer (14 versus 29 mm) and in winter (19 versus 32 mm) (Fig. 3A & B, Tab 3). Densely vegetated hotspots had lower lugworm densities and relief in winter compared to sparsely vegetated hotspots, whereas in summer, lugworm densities and relief were comparable (Fig. 3C-F, Tab 4). Bed level change did not differ significantly between sparse and dense hotspots at any moment in time (Tab 3).

Table 1: *Current velocity (cm s⁻¹) and significant wave height (cm) of the seven hotspots. Data derived from SCAROOST 2D model from Rijkswaterstaat, Dutch Ministry of Infrastructure and Environment. The model uses the systems bathymetry (2010 data) and is validated by field measurements. Current velocity is the maximum current velocity at spring tide. Significant wave height is the wave height under stormy conditions. The tidal range in our study area varies between 2.4 and 3.5 m, maximum current velocities range from up to 0.3 m s⁻¹ in the shallow areas to 1 to 1.5 m s⁻¹ in the tidal channels, and waves are generated within the system by wind (Louters, van den Berg & Mulder 1998)*

<i>Hotspot category</i>	<i>Hotspot no.</i>	<i>Current velocity (cm s⁻¹)</i>	<i>Significant wave height (cm)</i>
Dense	3	18.3	16.0
Dense	6	22.8	19.4
Dense	7	17.5	15.0
Sparse	1	25.1	44.6
Sparse	2	16.7	26.6
Sparse	4	14.6	21.3
Sparse	5	11.1	11.0

Explaining seagrass cover by sediment characteristics

To assess what parameter explains seagrass cover best, we used multiple backward stepwise regressions, using all static, dynamic and faunal sediment variables. This showed that maximum seagrass cover is best (62 %) explained by a model containing maximum sediment disturbances in winter, lugworm relief in winter and median grain size (Tab 5). Note that lugworm winter density was strongly correlated with lugworm winter relief ($r=0.78$) and was therefore deleted from the regression analysis to meet collinearity rules. Seagrass cover also correlates with elevation ($r=0.39$, $p < 0.05$, Tab 4), but this does not significantly enhance the explanation by the model.

Plotting the significantly correlated maximum disturbance depth and the maximum seagrass cover against each other revealed a clear critical disturbance threshold both in summer and winter (Fig. 4). Disturbance depths of more than 25 mm almost always coincided with sparsely vegetated hotspots, whereas hotspots with disturbance depths below that “critical” threshold can be dense as well as sparse.

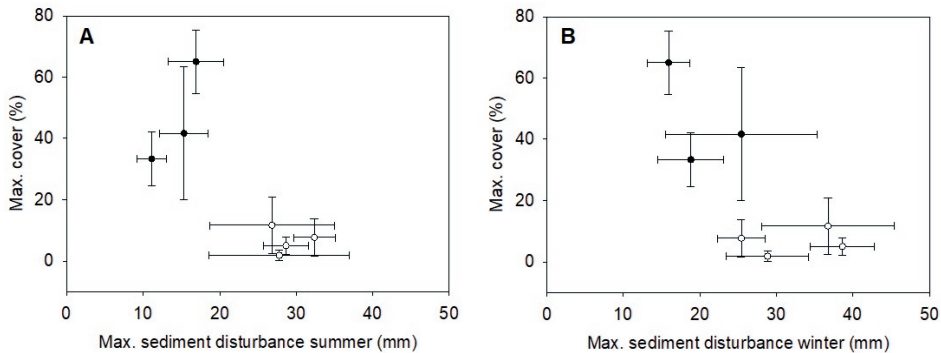


Figure 4: Relation between the maximum sediment disturbance (mm, x-axis, summer; panel A, winter; panel B) and the maximum seagrass cover in summer (% , y-axis). Filled symbols are hotspot averages per location \pm standard error. Open symbols represent sparsely vegetated hotspots, filled bullets densely vegetated hotspots. Densely vegetated hotspots are on average found at small maximum winter sediment disturbances (less the 25 mm).

Explaining maximum sediment disturbance depth

As maximum sediment disturbance is a parameter integrating several environmental characteristics (i.e., abiotic sediment characteristics, erosion/sedimentation processes and bioturbation), we performed a multiple backward stepwise regression on this parameter as well. We found that the maximum sediment disturbance depth was best explained by dry bulk density of the ambient sediment and lugworm density in winter (Tab 6). Note that during the regression analysis, median grain size (D50) was excluded in the process to further simplify the model without losing strength, followed by excluding “elevation” after a violation of independence with bulk density.

Table 2: 1-way ANOVA significance test outcomes of the comparison of seagrass coldspots with sparsely vegetated hotspots for sediment and lugworm characteristics. Sediment and lugworm characteristics and were split into summer (April – November 2012 and March – May 2013) and winter (November 2012 – March 2013) when data were available.

Variables	Coldspot vs Hotspot sparse	
	P	Sign.
Absolute elevation (m AOD ^s)	0.764 ^r	
Median grain size (mu)	0.017	*
Dry bulk density (gr cm ⁻³)	0.022 ^r	*
% silt <63 mu	0.029 ^r	*
Bed level change _{summer} (mm)	0.920 ^r	
Bed level change _{winter} (mm)	0.450 ^r	
Max. sediment disturbance _{summer} (mm)	0.096 ^r	
Max. sediment disturbance _{winter} (mm)	0.280 ^r	
Lugworm density _{summer} (# m ⁻²)	0.519 ^r	
Lugworm density _{winter} (# m ⁻²)	0.937 ^r	
Lugworm relief _{summer} (cm)	0.781 ^r	
Lugworm relief _{winter} (cm)	0.747 ^r	

^s = Amsterdam Ordnance Datum

* = significance level $p < 0.05$

^r = 1 way Anova on ranks

Table 3: 1-way ANOVA significance test outcomes of the comparison of seagrass hotspots of low (*Hotspot_sparse*) and high plant density (*Hotspot_dense*) for sediment and lugworm characteristics. Variables were split into summer (April – November 2012 and March – May 2013) and winter (November 2012 – March 2013) when data were available

Variables	Hotspot sparse vs Hotspot dense	
	P	Sign.
Absolute elevation (m AOD ^s)	0.001	**
Median grain size (mu)	0.306	
Dry bulk density (gr cm ⁻³)	0.751	
% silt <63 mu	0.227 ^r	
Bed level change _{summer} (mm)	0.175	
Bed level change _{winter} (mm)	0.152	
Max. sediment disturbance _{summer} (mm)	0.813	
Max. sediment disturbance _{winter} (mm)	0.021	*
Lugworm density _{summer} (# m ⁻²)	0.110	
Lugworm density _{winter} (# m ⁻²)	0.017 ^r	*
Lugworm relief _{summer} (cm)	0.496	
Lugworm relief _{winter} (cm)	0.015	*

^s = Amsterdam Ordnance Datum

* = significance level $p < 0.05$

** = significance level $p < 0.01$

^r = 1 way Anova on ranks

Variables						Summer				Winter			
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Cover	Elevation	D50	Bulk	Silt	dZ	Disturb.	Lugworm	Relief	dZ	Disturb.	Lugworm	Relief
1	X												
2	Max. seagrass cover	0.39*	x										
3	Absolute elevation	-0.31	0.03	x									
4	Median grain size	-0.18	0.31	0.71***	x								
5	Dry bulk density	0.29	-0.03	-0.99***	-0.71***	x							
6	% silt <63 mu	0.19	0.01	-0.03	-0.15	-0.03	x						
7	Bed level change	-0.44*	-0.61**	0.24	-0.06	0.06	0.06	x					
8	Max. sediment disturbance	-0.26	-0.25	-0.26	-0.42*	0.26	-0.13	0.12	x				
9	Lugworm density	0.13	-0.34	-0.55**	-0.65**	0.52*	0.16	0.12	0.63**	x			
10	Lugworm relief	0.07	0.47*	0.51*	0.44*	-0.58**	0.13	-0.35	-0.37	-0.52*	x		
11	Bed level change	-0.53*	-0.31	0.04	0.17	-0.05	-0.01	0.26	0.20	0.23	-0.27	x	
12	Max. sediment disturbance	-0.48	-0.26	-0.1	-0.23	0.12	-0.19*	0.17	0.74***	0.56**	-0.36	0.56**	x
13	Lugworm density	-0.37	-0.34	-0.41	-0.42	0.46*	-0.20*	0.34	0.67***	0.63**	0.68***	0.78***	0.33
13	Lugworm relief												x

* = significance level p<0.05

** = significance level p<0.01

*** = significance level p<0.001

Table 4 (previous page): Pearson product-moment correlation coefficient (r) for seagrass related sediment and lugworm characteristics. Input data are averages from all sampling points within seagrass hotspots ($n=22$). Significant correlations are in bold and marked with * ($p<0.05$), ** ($p<0.01$) or *** ($p<0.001$). All other correlations are found to be not significant ($p>0.05$).

Table 5: Summary of multiple backward stepwise regression statistics for the predictor variables to maximum seagrass cover. ΔR^2 =fit of the reduced model, B = un-standardised beta coefficient, $SE B$ = standard error, P = significance value, $Sign.$ = significance level. (Full model: all variables listed in

Reduced model	ΔR^2	B	SE B	P	Sign.
Adjusted model	0.62	14.31	2.27	<0.001	***
Max sediment disturbance _{winter}		-0.094	0.04	0.046	*
Median grain size		-0.067	0.01	0.008	**
Lugworm relief _{winter}		-1.34	4.43	0.007	**

* = significance level $p<0.05$

** = significance level $p<0.01$

*** = significance level $p<0.001$

Table 6: Summary of multiple backward stepwise regression statistics for the predictor variables to maximum sediment disturbance_{winter}. ΔR^2 =fit of the reduced model, B = unstandardised beta coefficient, $SE B$ = standard error, P = significance value, $Sign.$ = significance level, $Sqrt$ = square root. (Model: All variables listed in the column headings of table 3 except those of summer)

Reduced model	$\square R^2$	B	SE B	P	Sign.
Adjusted model	0.42	-16.87	18.9 4	0.385	
Sqrt Lugworm density _{winter}		3.73	1.09	0.003	**
Dry bulk density		25.88	13.2	0.067	

** = significance level $p<0.01$

Discussion

Monitoring seagrass meadows yielded clear evidence for sediment related feedbacks affecting seagrass survival and provided insights into how these feedbacks work. Twenty years of monitoring by the Dutch Ministry of Infrastructure and the Environment allowed us to distinguish coldspots (where seagrass was almost never present) and hotspots (where seagrass was always present during this monitoring period). In the year of this study, hotspots were either sparsely (<15 % cover) or densely (>30 % cover) vegetated. Sparsely vegetated hotspots had a higher maximum sediment disturbance depth in winter and a higher lugworm winter relief as compared to densely vegetated hotspots. These two factors explain 62% of the variation in vegetation cover (multiple backward stepwise regression). Obviously, other factors correlate with these factors as well. For instance, maximum sediment disturbance depth was best explained by dry bulk density of the ambient sediment and lugworm density in winter. Tidal elevation, although not having a prominent role in explaining variation by stepwise regression, in general contributes to sediment stability by affecting (i) the time interval of exposure to hydrodynamics, (ii) sediment compaction and (iii) potentially (indirectly) the lugworm relief and density.

Building on our results and on literature, we pose a conceptual model containing a self-sustaining feedback loop for densely vegetated meadows and a runaway feedback loop for sparsely vegetated seagrass meadows (Fig. 5).

Self-sustaining feedback loop

Seagrass ecosystem engineering capacities are widely acknowledged and reported; they are related to their canopy, i.e., flow and wave reduction, sediment trapping, reduced re-suspension (Bos & van Katwijk 2007), but also related to their belowground biomass, i.e., improving sediment stability (Christianen *et al.* 2013) and outcompeting bioturbators (Philippart 1994; Berkenbusch *et al.* 2007; Reise & Kohlus 2008; Eklof *et al.* 2011). A comparison between sparse and dense hotspots showed that sparsely vegetated hotspots (<15 % cover) have higher sediment dynamics (deeper disturbance depth, more lugworms, and more lugworm relief) than dense hotspots (> 30 % cover), at comparable static sediment properties (i.e., median grain size and bulk density, Fig. 3). This implies that densely vegetated hotspots may limit sediment dynamics, which in turn facilitates seagrass (Cabaco *et al.* 2008; Han *et al.* 2012; Suykerbuyk *et al.* 2012), thus establishing a self-sustaining feedback loop (Fig. 5).

Runaway feedback loop

With decreasing seagrass densities, the seagrass sediment may be more disturbed (increased lugworm densities and less protection from wave impact) and more susceptible to disturbances (sediments getting more mobile) (i.e., Cabaco *et al.* 2008). In our study, sparsely vegetated hotspots, while having static

sediment characteristics similar to densely vegetated hotspots and a history of high seagrass cover, showed greater sediment dynamics than densely vegetated hotspots ; sparsely vegetated hotspot dynamics were in fact comparable to those of coldspots. This may inhibit seagrass recovery, thus establishing a runaway feedback loop (Fig. 5). Eventually, this runaway loop might result in total loss of seagrass and its habitat. An accelerated decline may have occurred in the sparsely vegetated hotspots in recent years. Such declines could have been initiated by any large-scale stochastic anthropogenic or climatological disturbance (e.g. prolonged storms from unfavorable directions combined with increased water levels, or frequent lugworm digging, or deep ice-scour), thus breaking the self-sustaining feedback related to sediment dynamics. The presence of either low or high cover seagrass beds, as well as the threshold for sediment dynamics supports the conceptual runaway feedback in Figure 5.

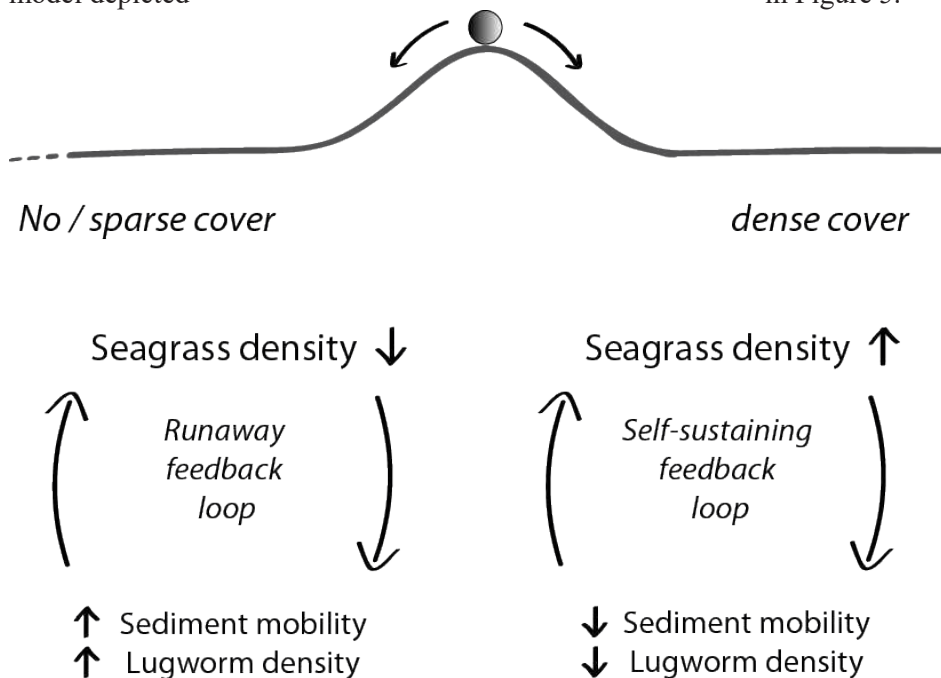


Figure 5: Above: A conceptual diagram showing the bi-modal seagrass density distribution that was found in the Eastern Scheldt tidal basin. Below: the posed positive feedback loops from which the density distribution derives.

Whether the seagrass loss is initiated by structural external forcing, by a more stochastic disturbance initiating a runaway loop or a mix of both, long-term presence of low-cover seagrass meadows indicates that the sites have a minimum suitability for seagrass survival. Following our conceptual model, these beds

may recover as soon as a window of opportunity allows the beds to reach 33% cover or more. Such a window of opportunity should consist of a prolonged period of low sediment dynamics. Alternatively, when the seagrass loss is the result of permanent local changes, such as higher or more frequent sediment disturbances (Nienhuis *et al.* 1994; ten Brinke *et al.* 1994), only a permanent solution, i.e. a man-made structure that reduces the excess of disturbances, could help to recover the seagrass beds.

Seasonality as bottleneck for seagrass survival in temperate climates

In general, winter rather than summer processes were found to be most important for summer seagrass cover in the stepwise multiple backward regression. During winter, seagrass is at the mercy of generally more extreme and varying environmental conditions (i.e., storms and ice events). Moreover, during summer, seagrass is able to quickly react to dynamic environmental processes, like burial and erosion (Cabaco *et al.* 2008; Han *et al.* 2012). In winter, growth is minimal and aboveground seagrass biomass is largely lost. Only rhizomes with winter reserves and some apical shoots remain (Vermaat & Verhagen 1996); these shoots do not contribute to the winter reserves (Govers *et al.* 2015). The relationship between seagrass cover and winter processes may imply a dominant engineering role of the belowground biomass. Our study shows that highest seagrass winter survival and thus highest potential summer seagrass cover is likely to be found at spots that have stable sediments. Such spots are either sheltered from winter storms and therefore experience less wave induced sediment mixing, or are located at higher elevations where exposure to hydrodynamics is interrupted by period of low tide for a longer period, or lack bioturbating animals, or have shell-rich, packed and cohesive sediments that are less prone to disturbances., or a combination of those, which often occurs (correlation matrix see Tab 4). The importance of stable sediments was already suggested by Reise and Kohlus (Reise & Kohlus 2008) for the highly dynamic temperate Northern Wadden Sea. Here we show that this relationship also applies in relatively sheltered delta systems with a reduced tidal movement, little wind fetch and small waves compared to coastal systems.

Maximum sediment disturbance depth

The maximum sediment disturbance depth is strongly correlated to seagrass cover and is very distinct between the sparsely and densely vegetated hotspots, showing a threshold at around 25 mm. The observed maximum disturbance depth itself is best explained by bulk density of the ambient hotspot sediment and lugworm density in winter (Tab 6), and thus integrates both the nature of sediment and its disturbances. The maximum disturbance depth could therefore be a useful indicator for the restoration and long-term survival potential of species that require low sediment dynamics like the shallow-rooted *Z. noltii*, but also for other benthic species, such as mangroves and bivalves (i.e., Bouma *et al.* 2001; Balke *et al.* 2013a). In general, the maximum sediment disturbance

depth could well preclude the persistence of organisms below a certain size and may influence the trade-off between investments in belowground (anchoring) parts versus aboveground parts. For settlement, anchoring has to occur within a certain window of opportunity during which sediment levels remain constant (cf. Balke *et al.* 2011; Balke *et al.* 2014). More specifically, as sediments will be continuously reworked, the anchoring ‘rate’ should exceed the sediment-reworking rate. At this moment, the maximum sediment disturbance depth is rarely quantified. Further research on the maximum sediment disturbance depth may help to explain species compositions of communities, and recruitment traits, size distributions and morphology of organisms in estuarine and coastal environments.

An optimal seagrass habitat in the Eastern Scheldt: sediment dynamics versus desiccation stress

In our study area, the Eastern Scheldt, high potential *Zostera noltii* habitats were shown to have low sediment dynamics and low lugworm numbers. Low sediment dynamics are correlated to a low sediment bulk density (in addition to occurring on locations that are sheltered from winter storms and have small fetches for wave buildup). In our system and most likely in other intertidal systems, such sediments are generally found in the upper intertidal zone. Here, bulk density is lower as a result of settlement or retention of fine sediments due to i) longer emersion periods, ii) shorter durations of exposure to hydrodynamics and ii) extensive emergence periods (>6 h per 12 h tidal period) that largely exclude adult lugworm presence and their bioturbation. For this reason long-term seagrass persistence is expected in these upper intertidal zone, which is actually what we found (Fig. 3D). However, every day growing conditions for seagrass in this zone are sub-optimal due to desiccation stress (i.e., Boese *et al.* 2005; Shafer *et al.* 2007; van der Heide *et al.* 2010; Suykerbuyk *et al.* Subm.-b).

Future perspective of hotspots

With ongoing climate change, it is expected that environmental extremes like large winter storms will increase in strength and/or occur more frequently (Harley *et al.* 2006; Björk *et al.* 2008). As a direct result, sediment dynamics may be expected to increase in size and frequency (i.e., alternating erosion and deposition events). For seagrass that is naturally sheltered from winter abiotic disturbances or that inhabits sediments that are non-susceptible to sediment dynamics (i.e., shell banks or extremely cohesive sediments with low bulk density), this might not have any adverse effects. However, for seagrass living under sub-optimal conditions, this may lead to declines, as rhizomal (winter) survival will become limited. Recruitment from seeds may then become more important for meadow resilience and persistence (Zipperle *et al.* 2009). When beds have disappeared, seed availability may become an important bottleneck (Orth *et al.* 2006b). Provided seeds are available, Seedling development will simultaneously decrease with increasing sediment dynamics (i.e., Infantes *et al.*

2011; Balke *et al.* 2013b; Balke *et al.* 2014). In addition, antagonistic ecosystem engineers like lugworms may benefit from enhanced sediment dynamics (van Wesenbeeck *et al.* 2007; Suykerbuyk *et al.* 2012).

Besides direct effects, climate change will also indirectly affect seagrass persistence. Sea level rise and increased weather extremes will call for ongoing coastal constructions that protect us and our economic activities against flooding (Temmerman *et al.* 2013). Following such ‘hard’ constructions, seascapes will change, often resulting in temporarily or permanently increased sediment dynamics. As our study shows, this may threaten seagrass persistence. Installing ecosystem-based flood defenses by ecosystem creation and restoration of ecosystem engineering species such as shellfish beds and saltmarshes, and eventually seagrasses may prove to be more sustainable and cost-effective (Borsje *et al.* 2011; Temmerman *et al.* 2013). Our study provides valuable insights in the boundary conditions that are needed to ensure long-term persistence of high density seagrass meadows, along with their ecosystem and coastal protection services.

Summarising, from this correlative study, we conclude that long term temperate, intertidal *Zostera noltii* presence is restricted by winter sediment dynamics. Densely vegetated hotspots were shown to have lower sediment dynamics than sparsely vegetated hotspots and coldspots, whereas static sediment characteristics (grain size, bulk density) were similar across the different spots. The vegetation cover was either low (2 – 15%) or high (> 30%) and sediment dynamics showed a threshold for vegetation cover. From this correlative finding, we postulate a self-sustaining feedback of relatively dense seagrass via sediment stabilisation, and accordingly a runaway feedback once the seagrass cover becomes too sparse. Recovery after losses require a window of opportunity that can be stochastic, or can be created by temporal or permanent habitat measures (i.e. creating sediment stabilisation). The sensitivity for sediment dynamics shown in our study implies that future existence of seagrass meadows may be at risk as ongoing climate change might directly (increased environmental extremes) or indirectly (changing seascapes) negatively affect seagrass beds. However, increased insights in the bottlenecks for long-term seagrass survival also open opportunities for setting the boundaries to create successful seagrass restoration projects. So, perhaps in the end, Nietzsche’s saying “That which does not kill us, makes us stronger”, counts for both seagrasses and the scientists studying them.

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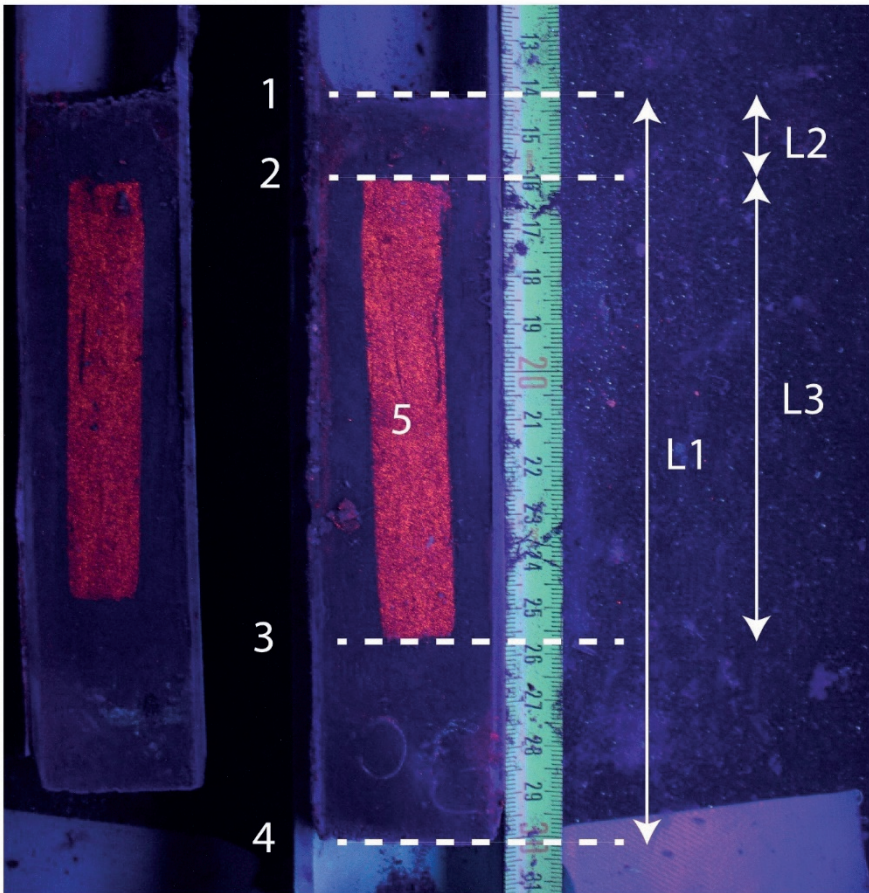
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Supplementary figure 1: Cross section of the retrieved sediment cores containing the dyed tracer rod (indicated by number 5). Numbered lines indicate: 1) bed level, 2) top of retrieved tracer rod, 3) bottom of retrieved tracer rod and 4) bottom of the core. $L1$ = total length of the sediment core, $L2$ = tracer rod depth and $L3$ = the length of the (remaining) tracer rod.

Supplementary table 1: Summarising table showing averages (\pm standard error) per location for each investigated variable. Numbers are rounded off to the nearest integer of decimal. Averages are separated in hotspots (either sparsely or densely vegetated) and coldspots. Variables were split into summer (April – November 2012 and March – May 2013) and winter (November 2012 – March 2013) when data were available.

Location ID		Hotspot								Coldspot							
		Dense			Sparse												
		6	3	7	1	4	5	2	6	3	7	1	4	5	2		
Max. cover (%)	Avg	65	42	33	12	8	5	2	30	0	3	0	0	0	0		
	SE	16	9	2	22	6	3	10	3	0	0	0	0	0	4		
Dry bulk density (g cm ⁻³)	Avg	1.2	1.3	1.6	1.4	1.5	1.2	1.2	1.4	1.5	1.6	1.3	1.5	1.4	1.4		
	SE	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0		
Median grain size (μ)	Avg	80	62	121	111	127	84	80	106	182	124	134	129	97	131		
	SE	16	12	11	11	3	3	16	16	27	13	9	2	7	1		
% Silt <63 μ	Avg	36	52	7	18	2	33	38	9	1	6	23	1	19	16		
	SE	12	9	8	9	1	3	13	6	9	6	1	0	7	0		
Absolute elevation (m AOD)	Avg	0.4	0.4	0.9	0.2	-0.1	0.0	0.2	0.4	0.5	0.9	-0.1	-0.3	0.2	-0.1		
	SE	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.2	0.1	0.1	0.0		
Summer	Bed level change (mm)	Avg	-1	-6	-5	-6	-5	-2	-6	-7	2	-5	-6	-4	-2	-3	
		SE	2	1	2	1	1	2	2	2	3	1	4	1	1	2	
	Max. sed. Disturbance (mm)	Avg	17	15	11	27	32	29	28	27	38	18	20	26	27	28	
		SE	6	8	9	3	3	3	4	5	3	5	5	2	8	3	
Lugworm density (# m ⁻²)	Avg	8	1	19	28	41	24	1	32	9	25	14	53	34	45		
	SE	11	17	0	1	13	11	4	10	8	13	6	5	10	4		
Lugworm relief (cm)	Avg	3	0	2	3	2	2	1	4	1	3	1	3	3	4		
	SE	1	0	0	0	0	0	1	1	0	0	0	0	1	1		
Winter	Bed level change (mm)	Avg	-1	-13	11	-8	-2	-4	-13	5	-12	-8	-11	-4	-2	-2	
		SE	5	6	2	3	2	1	1	4	5	1	8	1	4	1	
	Max. sed. Disturbance (mm)	Avg	16	25	19	37	25	39	29	18	43	23	24	26	32	25	
		SE	7	9	5	10	3	4	3	5	6	2	8	2	6	0	
Lugworm density (# m ⁻²)	Avg	2	0	4	15	23	25	0	7	1	17	2	37	30	23		
	SE	7	4	0	0	3	6	1	7	1	5	1	4	7	2		
Lugworm relief (cm)	Avg	1	0	2	2	4	3	1	1	1	2	0	4	4	3		
	SE	1	0	0	0	0	0	0	1	0	1	0	0	0	0		

Chapter 7

Synthesis



7.1 Introduction:

Coastal ecosystems are under pressure by anthropogenic activities of the over 40 % of the Earth's population that lives along the world's coastline (e.g., Lotze et al. 2006, Halpern et al. 2007). Moreover, as these dynamic ecosystems are known for non-linear responses to changes and threshold behaviour, they can be lost without any warning (Silliman et al. 2005, van der Heide et al. 2007, Hughes et al. 2010). Restoration of these ecosystems, often consisting of eco-engineering species, is notoriously hard (Bakker et al. 2002, Orth et al. 2006, Halpern et al. 2007, Omori 2011) as thresholds need to be bridged to ensure reestablishment of the self-sustaining positive feedback loops of the eco-engineering species. Despite the difficulties faced, numerous efforts have been performed globally to conserve and restore key ecosystem species, like seagrasses, because of their high economic and ecological value (e.g. van Katwijk et al. 2016).

In this thesis, I – while watching seagrass grow - focused on the effects of combined physiological stressors (salinity, desiccation and light), bioturbation and sediment dynamics on seagrass ecosystem dynamics. More specifically, I aimed to identify environmental preferences and bottlenecks of dwarf eelgrass *Zostera noltii* in the Oosterschelde tidal basin in order to better understand and predict the dynamics of existing seagrass meadows and to ensure and optimize reestablishment of *Zostera noltii* at new potential seagrass sites by transplantation. I aimed to generalise these findings in the broader context of ecosystem engineering and predictability of system dynamics. This chapter integrates the main findings of my work and discusses (future) challenges for seagrass research, conservation and restoration.

7.2 Seagrass dynamics: Growth versus loss

Seagrass presence is a function of environmental conditions that enhance or restrict seagrass growth and survival. More specifically, the spatial seagrass distribution observed in the field is the complex outcome of consecutive annual cycles of seed germination, seedling development, expansion by clonal growth during the growing season, sexual reproduction and reserve storage at the end of the growing season, and winter survival of remaining apical meristems and seeds. Each of these life phases is shaped by environmental stresses. We propose that, in the Oosterschelde estuary, biomass patterns can be explained by (i) synergistic stresses that cause growth limitation (Chapter 2 & 6) and (ii) stresses and disturbances that significantly reduce winter reserves and survival (Chapter 3, 4, 5 & 6).

7.2.1 Q1: Is seagrass growth limited during the growing season?

Seagrass meadows are shaped by the stresses they encounter. Some stresses increase or decrease along the tidal gradient and thereby control seagrass distribution. For instance, desiccation stress controls the upper elevation limit (Vermaat et al. 1993, Philippart and Dijkema 1995, Leuschner et al. 1998, van Katwijk and Hermus 2000, van der Heide et al. 2010) whereas low light conditions usually control the lower limit of seagrass distribution (e.g., Ralph et al. 2007, van der Heide et al. 2009); water or sediment dynamics may also determine the lower depth limit (e.g., van Katwijk and Hermus 2000, Reise and Kohlus 2008). Stresses like high salinity or sediment dynamics might affect the seagrass distribution through separate or synergistic effects on growth. We found that growth of *Zostera noltii* apical shoots (under controlled conditions) was mainly constrained by desiccation stress (emergence) and not by any separate nor interactive or synergistic effects with low light or salinity stress (Chapter 2). Our results show that seagrass can grow in the zone ranging from the subtidal up to elevations equivalent to 6 hours of emergence; desiccation constrains growth when plants grow higher and dryer. Consistent with these laboratory findings, transplantations in the field showed that locally lowering the planting depth with a few centimetres (and thus creating a tidal pool preventing desiccation stress) resulted in a significantly better development of the plants compared to plants that were planted at the same level as the surrounding sediment (Chapter 4). This implies that under field conditions seagrass growth can be promoted by a local topography of tidal pools.

The omnipresent physical sediment disturbances induced by waves, currents and biota were found to be of minimal importance during the growing season. Suppressing the antagonistic bioengineering lugworms from ambient densities of 40 to 60 individuals m⁻² to numbers below 15 individuals m⁻², led to significantly better winter survival, but did not result in enhanced development of seagrass transplants during the growing season (Chapter 3 & 4). In addition, wave-sheltering did not promote development of anchored rhizomes compared to controls, during an additional experiment in the growing season of 2012 (unpublished results). The sediment disturbance depths in sediments adjacent to seven natural meadows in the Oosterschelde during the growing season of 2013 and 2014 were in the order of a few centimetres (Chapter 6, fig 6.3). According to the sediment disturbance threshold that separates *Z. noltii* meadows in high and low density beds (Chapter 6, figure 6.5), this might indicate that seagrass growth in these natural beds is not restricted by sediment dynamics.

Eutrophication or nutrient limitation as one of the main growth factors for seagrass worldwide, were not part of the present study. In the

Oosterschelde, the transparency is high due to very low plankton and suspended matter concentrations (Wetsteyn and Kromkamp 1994). Nutrient limitation is unlikely as sediments contain sufficient nutrients (Govers et al. 2014). Epiphytes, a common indicator of eutrophication, are rare. Finally, macroalgae accumulate at sheltered locations, but rarely in seagrass beds or transplantations (personal observations).

Growth limitation by environmental stressors might be counteracted when ecosystem engineering results in self-facilitation through positive feedback. Once a critical mass (density or area) has been reached, these processes may reinforce growth and expansion. Several positive feedback processes have been identified in seagrass systems. In particular, enhanced water clarity due to plant-induced reduced resuspension has been proposed (Carr et al. 2012). Alleviated NH(x) toxicity at high shoot density is another proposed mechanism (van der Heide et al. 2008). These feedback loops follow threshold behaviour, leading to self-accelerating processes once the threshold is reached (e.g., van der Heide et al. 2007, Carr et al. 2012). In our mesotrophic intertidal system with high water clarity, feedback related to turbidity or toxicity is unlikely to occur (Wetsteyn and Kromkamp 1994). However, water and sediment dynamics are relatively strong at intertidal sites in our study area (Louters et al. 1998). Positive feedback in the seagrass system can therefore be expected from a root/rhizome system that is sufficiently large and entangled to hold sediments and prevent erosion and dislodgement (e.g., Madsen et al. 2001, Bos et al. 2007, Christianen et al. 2013). The immediate disappearance after planting of single, bare-root shoots and contrasting survival of seagrass sods observed in our study (Chapter 4), supports the idea that this intrinsic process is important, as does the larger expansion from the compact configuration as compared to the small configuration (Chapter 4).

7.2.2 Q2: *Is clonal winter survival limited?*

In temperate systems, a characteristic period of low temperatures and low light levels during the winter make growth conditions for the plant suboptimal. Temperate seagrass *Zostera noltii* exhibits two mechanisms to overcome this harsh period till the next spring: 1) investing in, and surviving on the apical meristems (Vermaat and Verhagen 1996), Chapter 5) or 2) surviving on seeds that are produced during the summer and stored in the sediment. The first option is characterised by storage of carbohydrate reserves at the end of the growing season and shedding of unnecessary energy demanding plant material (non-apical shoots and rhizomes). Watching our seagrass transplants grow, we found winter to be the bottleneck in their life

cycle (Chapter 3, 4, 5, 6). The number of shoots at the start of the growing season was not correlated to the number of shoots at the end of the preceding season (Chapter 4 & 5), indicating that the losses encountered during winter correlate to external factors rather than the amount of biomass available in autumn (Chapter 4, fig 3 and 6b). As our transplants gradually declined in seagrass biomass over the winters, these losses were not related to single stochastic events (Chapter 4).

The low winter survival rates may be explained by high physical losses due to uprooting of the wintering small rhizome fragments. Reducing the numbers of the bioturbating lugworms by applying a shell layer underneath our transplants did not result in promoted development of the seagrass during the growing season, but after winter, the shoot numbers in plots protected from bioturbation were always higher than those of control plots (Chapter 3 & 4), implying a vital role of sediment stability for seagrass winter survival. This was confirmed by our study in natural meadows (Chapter 6), revealing long-term seagrass persistence on consolidated cohesive sediments (low impacts of lugworms, waves and currents), having a maximum sediment disturbance depth less than 3 cm deep. These results are in analogy with studies in the Dutch and German Wadden Sea, that point out that low dynamic, cohesive sediments are the key to seagrass presence (Philippart 1994, Reise and Kohlus 2008).

We found starch content of the rhizomes at the end of the growing season to be indicative of next year's growth success of transplants (i.e. the number of shoots in June) (Chapter 5). Possibly, in unsuccessful transplants starch reserves may have been insufficient to keep up with the respiration costs of the below- and aboveground tissues. Unfavourable environmental conditions that increase respiration and reduce growth (i.e. adverse sediment dynamics) may prevent sufficient starch reserve storage at the end of the growing season (Chapter 5, Burke et al. 1996, Herzka and Dunton 1997, Alcoverro et al. 2001, Ruiz and Romero 2003). We also found leaves that remain in winter still performed photosynthesis, though at a lower level than in summer. However, the presence or absence of leaves during winter did not correlate with subsequent biomass in June (Chapter 5). The possibility of starch storage restriction should also be taken into account when selecting suitable sites for transplantation, and the starch levels in autumn can be a suitable indicator to select them.

Seagrass eco-engineering (further referred to as intrinsic processes) is considered to be minimal during winter as a consequence of near absence of aboveground biomass, disintegration of the dense root mat and strongly reduced growth (Vermaat and Verhagen 1996, Chapter 5). Contrastingly, extrinsic stresses and disturbances are generally high in winter by increased water and

sediment dynamics. Our study suggests that during winter, intrinsic processes (read: seagrass eco-engineering) are less important than extrinsic forcing (Chapter 4 & 6). This may cause erosion and subsequent loss of seagrass rhizomes as e.g. the fast growing, shallow-rhizomed *Z. noltii* cannot grow its rhizomes to its optimal sediment depth to keep up with bed level changes (Han et al. 2012).

7.3 Q3: How did transplantations and the natural meadows develop?

The large-scale *Zostera noltii* transplantations that were carried out in the intertidal flats of the Oosterschelde over the period 2007-2012 showed variable success. Most of the transplanted sods survived, but shoot numbers declined over time. However, in the long run, at two out of six tidal flats, the transplantations and surrounding areas were extensively colonised by new patches of seagrass. In Chapter 4 we show (i) intrinsic processes favour the transplantation development during the growing season (supporting the importance of positive feedbacks), (ii) extrinsic processes favour the development at a longer time scale (i.e., reduction of bioturbation, supporting the importance of breaking the positive feedback maintaining the bare state (cf. Chapter 3), whereas (iii) final long-term transplantation success (starting colonisation of two out of six tidal flats) could not be related to any exposure to measured environmental factors (i.e., hydrodynamics, light availability, sediment composition, emergence time, macro-algal cover, grazing, salinity, pore-water nutrients and sulphide toxicity).

Natural meadows in the Oosterschelde tidal basin are monitored by the Ministry of Infrastructure and the Environment. Area and cover (categories 0-5% or >5% cover) were estimated on a two-yearly basis in the past, and since 2010 on a three-yearly basis. Shoot dynamics and local environmental conditions are not monitored. The Oosterschelde estuary was scattered with over 1000 ha of *Zostera noltii* prior to the completion of the Delta Works in the mid 80's. Coinciding with the completion of the storm-surge barrier, seagrass decreased over the course of 10 years to approximately 75 ha. This decline was most likely induced by two coinciding adverse incidents: three extremely cold winters in succession in combination with a period of five months of declining high tides followed by seven months with extremely low high tides during the last two winters. These exceptional tides artificially regulated and were not repeated since (Louters et al. 1998). The declining seagrass meadows lost the associated thick layer of fine sediments and the remnants now grow on the sandy sediment that emerged after loss of the muddy layer (personal observations D.J. de Jong). In addition, seagrass meadows retreated upwards in tidal elevation to mainly relatively stable

sediments (old dike or salt marsh remnants or shell banks) (Chapter 6). Recovery has not occurred, although seagrass meadows in some years expand and adjacent tidal flats can be scattered with seedling patches. Unfortunately, those expansions are in most cases not structural and persistent and can be in different years for different meadows. Between 2007 and 2014 we frequently visited natural beds and witnessed the disappearance of one (Krabbenkreek Noord- Zuid) and decline of several beds (Dortsman Zuid, Zandkreek Zuid, Goese Sas, Viane West). One bed, near Oostdijk, appeared and expanded during our study period (see also Giesen et al. 2015).

7.4. Q4: Sustainability of the transplants and populations: lessons learnt

Zostera noltii in temperate systems (having a clear growing season and “dormant” life stage) can only be sustainable when the winter losses are compensated by clonal growth over the growing season. In the Oosterschelde, this thesis shows that winter losses determine long- term survival of transplants and natural populations (Chapter 3, 4, 5, 6). Conservation and restoration of seagrass in the Oosterschelde should focus on reduction of winter losses (i.e. application of a shell layer, Chapter 3 & 4), but, to counterbalance losses, also by further promoting summer growth and expansion.

This thesis has shown that growth and expansion can be enhanced by improving growing conditions (e.g., prevent desiccation, Chapter 2 & 4) but also by restoring the self-facilitating feedback (Chapter 4 & 6). Although we could firmly establish that intrinsic processes favour the transplantation development during the growing season, and extrinsic processes (e.g. reduction of sediment dynamics / bioturbation) consistently favour the development at a longer time scale, the final long-term transplantation success could not be related to any factor in spite of the intense monitoring of a broad range of environmental factors (listed in Table 1). Two out of six tidal flats became colonised by scattered seagrass patches, and the transplant areas themselves developed reasonably well, whereas the four others diminished every year and showed no colonisation. The differences in transplantation success could not be ascribed to site- to- site differences in factors associated with seagrass losses, like light limitation, eutrophication, high hydrodynamics, grazing, bioturbation, desiccation, storms, ice-scour (e.g., Calumpong and Fonseca 2001, Short et al. 2002, Orth et al. 2006). In our study area light was ample, porewater nutrients and sulfide did not differ between sites and were below toxic levels (Table 1, Chapter 5, see figure 1 for transplantation sites), exposure to hydrodynamics and storms, sediment composition and bioturbation were contrasting at the two successful sites (and the non-successful sites had similar and intermediate values), macro algal cover, and

grazing events were all similar between sites and ice scour only happened once, notably at a successful site (Table 1, Giesen et al. 2015).

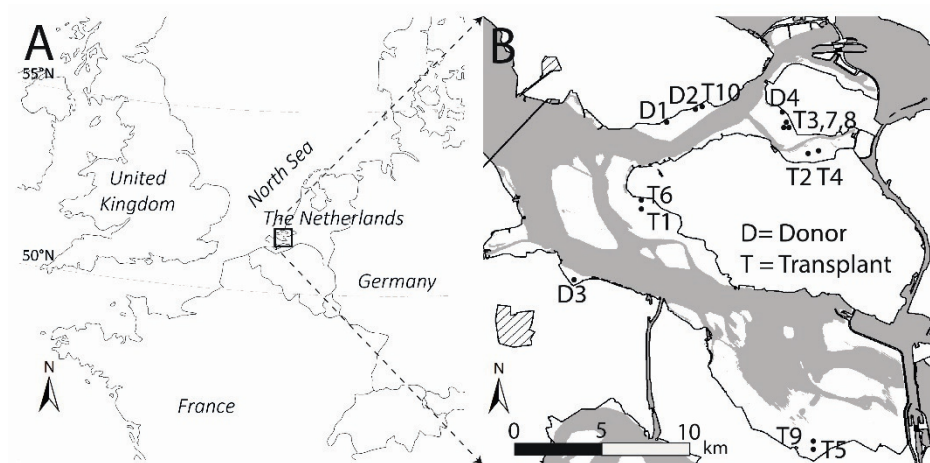


Figure 1: (a) Location of the Oosterschelde sea inlet (study area) in the south-western Netherlands, north-western Europe. (b) Overview of donor (D) and transplant (T) sites within the study area.

What else could have caused the final success at two, and decline at four out of six tidal flats? When discussing this, we have to keep in mind that (i) the success varied between years (Chapter 4), and (ii) also the natural populations at the Oosterschelde basin are characterised by large non-synchronous variability in expansion and decline. It is therefore unlikely that one simple, overlooked factor is responsible. More likely, successes and failure result from the complex interplay of tidal dynamics, annually varying weather conditions, and/or biological processes related to colonisation, such as seed production, timing of release, germination induction and germination, as shown for several biogeomorphic ecosystems by Balke and coworkers (Balke et al. 2014). Alternatively, success may be influenced by large distance, landscape-scale interactions (van de Leemput et al. 2015). In our case for example, the outflow of seawater from the neighbouring saltmarshes during low tide, in combination with a limited drainage of the local tidal flat may have prevented desiccation at the two successful sites in some years better than at the four unsuccessful sites. In former times, the lush seagrass beds at the unsuccessful sites may have flourished due to the accumulation of sediment fines and the subsequent development of several layers of seagrass leaves that may concertedly have kept the beds moist (personal observation D.J. de Jong in the Oosterschelde; the leaf-moistening effect of dense beds was convincingly demonstrated in Irish beds by Fox (Fox 1996). A starting bed does not yet have this positive feedback, moreover, suspended fines have

Table 1: General characteristics of tidal flats in the Oosterschelde with seagrass expansion and decline. Means based on monitoring between 2007 and 2013

Tidal flats categorized on seagrass dynamics	Transplants located on the tidal flat <i>(locations see figure 1)</i>	Hydrodynamic exposure	Median grain size (D50) μm	Adult lugworm density $\# \text{ m}^{-2}$	Ice scouring <i>presence (+) absence (-)</i>	Pore water				Pore water sulphide $\mu\text{mol/l}$
						ammonium $\mu\text{mol/l}$	nitrate $\mu\text{mol/l}$	phosphate $\mu\text{mol/l}$	phosphate $\mu\text{mol/l}$	
Seagrass expansion	T6	Exposed	153	1	-	116	3	21	53	
	T5	Intermediate	112	70	+	71	1	16	22	
	T9	Intermediate	120	15	+	98	1	18	2	
Seagrass decline	T1	Exposed	171	2	-	30	9	4	2	
	T2	Intermediate	162	56	-	70	2	18	0	
	T4	Intermediate	175	54	-	55	4	11	2	
	T3	Exposed	132	55	-	157	ND	29	60	
	T7	Exposed	121	48	-	57	10	13	1	
	T8	Exposed	128	16	-	90	11	18	1	
	T10	Sheltered	120	3	-	69	3	15	56	

Notes:

- Macro-algal cover: always < 10%
- Pore water salinity: mean 29.5 PSU, not different between and within seagrass locations
- Geese feeding pits: frequently in October – November at all sites

been reduced in the water layer of the Oosterschelde since the construction of the storm-surge barrier (ten Brinke et al. 1994). At least one of the two successful sites seems to be ‘fuelled’ by local input of suspended fines. The new bed at Oostdijk and the Roelshoek- transplants may receive suspended sediments from the slow erosion of the newly appeared bank of compact mud at Oostdijk (Dick J. de Jong, personal observations). The intertidal *Zostera noltii* in the Oosterschelde system would thus likely benefit from the return of the fine sediments in the system, by which they can use their ecosystem engineering capacity to the max, including natural exclusion of bioturbating worms and natural protection against eroding processes by protecting the stable sediment from disturbing forces (like was the case in the past in the Oosterschelde and is still the case in the Wadden Sea, personal observations).

In short, complex, interacting, unpredictable environmental factors likely influence final transplantation success, even in big and repeated transplantations like ours. Such factors may also help to explain the variable success of other seagrass transplantations around the world (cf. Orth et al. 2010, van Katwijk et al. 2016).

7.5 Future perspectives?

Sediment dynamics versus desiccation stress

In the Oosterschelde, high potential *Zostera noltii* habitats were shown to have low sediment dynamics and low lugworm numbers. Low sediment dynamics are correlated to a low sediment bulk density (in addition to occurring on locations that are sheltered from winter storms and have small fetches for wave build-up; chapter 6). In our system and most likely in other intertidal systems, such sediments are generally found in the upper intertidal zone. Here, bulk density is lower as a result of settlement or retention of fine sediments due to i) longer emersion periods, ii) shorter durations of exposure to hydrodynamics and ii) extensive emergence periods (>6 h per 12 h tidal period) that largely exclude adult lugworm presence and their bioturbation. For this reason long-term seagrass persistence is expected in these upper intertidal zone, which is actually what we found (Chapter 6). However, every day growing conditions for seagrass in this zone are sub-optimal due to desiccation stress (e.g., Boese et al. 2005, Shafer et al. 2007, van der Heide et al. 2010, Chapter 2).

Lack of downward expansion?

Natural meadows up until now never expanded downwards in the intertidal towards more optimal conditions with respect to desiccation. Although clonal expansion from the stable meadow and recruitment from seeds have been

recorded, they never lasted very long. Possibly, in both cases, sediment dynamics were too high to ensure long-term survival due to the lack of stable sediments, the high number of bioturbating lugworms or possibly the bigger impact of wave energy in that zone (Hu et al. 2015). In addition, shoot numbers of these colonisations possibly did not reach the threshold density yet to establish a self-facilitating feedback (i.e. resulting in stabilising the sediment, outcompeting lugworms, or reducing desiccation stress and thus preventing uprooting or loss of essential winter reserves) that potentially promotes long-term survival.

Improving the sediment stability by human action (like we did in our transplants) could be a step forward in facilitating downward expansion, but will in itself not lead to the desired effect, regarding our non-sustainable transplants. However, such techniques are very useful and feasible to expand and re-inforce the edge of existing seagrass meadows, like suggested by the first trial results along the natural meadow of Dortsman Noord (Giesen et al. 2015). Using landscape scale interactions, such as cascades of ecosystem engineering species (cf. Borsje et al. 2011), could be effective in our system. Saltmarshes, oyster reefs and mussel bed may i) prevent fast water run-off and thus reduce desiccation stress and/or shellfish reefs may additionally ii) reduce the waves that likely induce the sediment stress that is at the base of our winter bottleneck.

Additional potential future threats

With ongoing climate change, environmental extremes like large winter storms may increase in strength and/or occur more frequently (Harley et al. 2006, Björk et al. 2008). As a direct result, sediment dynamics may be expected to increase in size and frequency (i.e., alternating erosion and deposition events). For seagrass that is naturally sheltered from winter abiotic disturbances or that inhabits sediments that are non-susceptible to sediment dynamics (i.e., shell banks or extremely cohesive sediments with low bulk density), this might not have any adverse effects. However, for seagrass living under sub-optimal conditions, this may lead to declines, as rhizomal (winter) survival will become limited. Recruitment from seeds may then become more important for meadow resilience and persistence (Zipperle et al. 2009b). However, seedling development may simultaneously decrease with increasing sediment dynamics (e.g., Infantes et al. 2011, Balke et al. 2013, Balke et al. 2014). In addition, antagonistic ecosystem engineers like lugworms may benefit from enhanced sediment dynamics (van Wesenbeeck et al. 2007, Chapter 3).

Besides direct effects, climate change will also indirectly affect seagrass persistence. Sea level rise and increased weather extremes will call for ongoing coastal constructions for flood protection (Temmerman et al. 2013). Following such ‘hard’ constructions, seascapes will change, often resulting in temporarily

or permanently increased sediment dynamics. As our study shows, this may threaten seagrass persistence. Installing ecosystem-based flood defences by ecosystem creation and restoration of ecosystem engineering species such as shellfish beds and saltmarshes may prove to be more sustainable and cost-effective (Borsje et al. 2011, Temmerman et al. 2013). It may also be key to the creation of favourable seagrass habitat. Our study provides insights in the boundary conditions that are needed to ensure long-term persistence of high density seagrass meadows, along with their ecosystem and coastal protection services. Moreover, it shows that seagrass restoration in the Oosterschelde is feasible but success is highly variable.

7.6 Recommendations

1) Seagrass habitat awareness

Our study shows that managers restoring or conserving seagrass beds should realise that suitable seagrass (restoration) sites in temperate zones not only have optimal (growing) conditions in summer but should also enable survival in the more dynamic winter (chapter 3, 4, 5, 6). For restoration, such areas should be selected.

2) Seagrass habitat protection

For conservation, seagrass suitable areas should not be disturbed by human activities, even in the absence of seagrass, as it is a potential habitat. The appearance of Oostdijk bed has shown that such areas can become (re-) colonized. Our studies have shown that successful colonization cannot always be predicted (Chapter 4), which is also evidenced by the dynamics of the seagrass beds (Chapter 6, Philippart & Dijkema 1995, Dolch et al. 2013).

3) Optimising seagrass habitat

Next to considering positive (facilitating) interactions in ecological theory and practice, we showed it is equally important to consider negative engineering interactions between ecosystem engineering species (Chapter 3, 4, 6, Philippart 1995, van Weesenbeeck et al. 2007). Removal of such negative interactions between ecosystem engineering species can give a head start to the target species at the initial establishment phase, when positive engineering feedbacks by the target species on itself are still lacking. For restoration, applying a shell layer can help to convert lost seagrass habitat towards its former “seagrass” state (Chapter 3, 4). For conservation, shell armouring near the edges of these beds may be beneficial (observations 2014) to expand or reinforce these existing beds.

4) Spreading risks in space and time

Our study emphasises that managers restoring or conserving seagrass beds need to account for the unknown and unpredictable part of the variability by spreading risks in space and time (Chapter 4). As our study involves large-scale transplantations with several years and sites of planting, long and intensive monitoring and a number of manipulations, we cannot come much closer to ‘proving’ that we apparently cannot predict or deduce habitat suitability completely from the ample available environmental monitoring data (Chapter 4). Thus, the typically high environmental variability that governs seagrass habitats requires spreading of risks in time and space in the transplanting set-up and scheme.

5) Patience and awareness of complexity

In addition to recommendation 4, environmental management plans must account for a long evaluation time (incorporate patience), and the unpredictability of the precise locations and years of successes and failures (so spread risks and partially submit to nature, Chapter 4). Successes and failure can result from the complex interplay of tidal dynamics, annually varying weather conditions, and/or biological processes related to colonisation, such as seed production, timing of release, germination induction and germination.

6) Awareness of and incorporating landscape-scale interaction

Success may be influenced by large distance, landscape-scale interactions (Gillis et al. 2014, van de Leemput et al. 2015). Seagrass restoration might benefit from other ecosystem engineers that can reduce wave impact on the seagrass habitat (i.e. mussels beds or oyster reefs, Bos & van Katwijk 2007) or reduce desiccation stress (a salt marsh that slowly releases it water over the tidal flat or natural reefs that prevent natural run-off, pers. observation). The potential of such interactions need to be further investigated. Coastal managers could facilitate the potential of such interactions by conserving the natural seascapes with cascades of ecosystems along the tidal gradient, instead of creating seascapes with lots of “hard constructions” that restrict such natural cascade gradient and like in our case (dams and a storm surge barrier) have major impact in the balance of sediment fines.

7) Upscaling of transplant size

The fast establishment of a self-facilitating feedback loop could promote restoration works and their long-term survival (van Katwijk et al. 2016). Upscaling the transplant size should therefore be considered to enforce intrinsic processes, like we showed by transplanting sods instead of single plants (Chapter 4).

8) Further upscaling of transplant size

Further upscaling of transplant size within the limits of what is practically and economically feasible does not seem worth pursuing, although an even larger scale may be recommendable as this could promote self-facilitation at a landscape scale as postulated by van de Leemput and co-workers (van de Leemput et al. 2015).

9) Starch: a useful indicator for seagrass winter survival

Winter survival of temperate seagrasses is largely determined by plant carbohydrate reserves, and autumn starch concentrations can be used as indicators for the success of seagrass transplants in the next growing season (Chapter 5). This indicator may be very useful, since abiotic site characteristics seem to give a very limited indication of transplant success and winter survival (Chapter 4).

10) Seagrass cover: an indicator for seagrass health?

Seagrass meadows with a low cover seem to be unsustainable on the long term (Chapter 6). Seagrass cover could therefore be used by managers as a rough, visible first indicator for a meadow's health status. Measures such as shell armouring (Chapter 3, 4), or extra protection from local human activities could then be subsequently applied to support the meadow and prevent it from sliding down the runaway feedback loop (Chapter 6).

11) Role of seeds in seagrass persistence

Regarding the bottleneck in clonal winter survival, recruitment from seeds may become more important for meadow resilience and persistence (Zipperle et al. 2009b). Recruitment from seeds can boost the biomass within the meadow and contribute to re-establish the self-facilitation feedback. The establishment of seedlings near our transplantations confirms the importance of expansion by seeds. However, seeds were found to account for not more than 30 percent of the recruitment (Zipperle et al. 2009a) and have their own winter bottle necks (like deep burial as found for *Z. marina* by Greve et al. 2005, Delafosse & Kristensen 2012).

The importance and potential of seeds in *Zostera noltii* resilience and restoration efforts could be further explored.

12) Long term effects of foraging geese

Seagrass decline in the Oosterschelde tidal basin coincided with the increase of Brent geese (*Branta bernicla*). These geese typically arrive in our system each year from the mid of September onwards. Upon arrival, they feed

on the leaves and / or rhizomes, the latter resulting in shallow tidal pools. These pools can be beneficial to seagrass as they can act as a sink for seeds (Zipperle et al. 2010). On the contrary, they may be a starting point for erosion processes. However, our personal observations did not show a promoting nor restricting effect of these geese feeding pits in the Oosterschelde. The exact role of (enhanced) geese foraging on long-term seagrass persistence needs further investigation. However, taking effective measures to support seagrass persistence is likely only feasible on the small (meter) scale (i.e. for transplants, personal observations) and not at the large (meadow) scale.

Lessons learned

Lessons learned:	Question?	Findings:
Ch2	What are the interactive or synergistic effects of desiccation, light and salinity stress and do these effects differ among populations that are adapted to different local conditions?	1: Emergence has a negative effect on the growth rate of the intertidal temperate <i>Zostera noltii</i> . 2: Growth is NOT further reduced in the presence of an additional stressor like shading or high salinity. 3: Seagrass origin does not determine the salinity stress response;
Ch3	To what extent can restoration of <i>Zostera noltii</i> meadows at formerly suitable seagrass habitats benefit from removal of negative effects by the bioturbating lugworm <i>Arenicola marina</i> and how will this depend on abiotic conditions (sheltered vs. wave exposed)?	1: A stabilizing shell layer enhances survival during winter, but cannot prevent large losses over the winter.

Ch4	<p>Can temperate seagrass meadow restoration benefit from optimising intrinsic and external processes? And to what extent?</p>	<p>Sod transplants (2.25 m²) survived more often than single shoot transplants (no survival). Using larger or more compact transplant configurations had no long-term effect on survival, and initial densities were not related to transplantation success either. Reducing desiccation stress promoted the transplantation success during the first growing season and shielding transplants from bioturbating lugworms had a positive effect on long-term survival. Final success was not predictable from a large suite of environmental variables monitored during 8 years</p>
Ch5	<p>To what extent does growth success depend on 1) shoot densities in the preceding growth season, 2) the presence of leaves in winter, and 3) the level of starch reserves?</p>	<p>Growth success was not correlated to 1) shoot densities in the preceding season and 2) presence of photosynthetic active leaves in winter Starch levels at the start of winter are an indicator for winter seagrass survival</p>
Ch6	<p>What sediment related factors determine long-term seagrass persistence? And how does this relate to actual seagrass cover of the investigated meadows? Are there critical threshold values for seagrass presence?</p>	<p>Densely vegetated hotspots were shown to have lower sediment dynamics than sparsely vegetated hotspots and coldspots, while static sediment characteristics were similar (grain size, bulk density). The vegetation cover was either low (2 – 15%) or high (> 30%) and sediment dynamics showed a threshold for vegetation cover.</p>

7.7 Conclusions

In this thesis, I aimed to identify environmental preferences and bottlenecks of dwarf eelgrass *Zostera noltii* in the Oosterschelde tidal basin in order to better understand and predict the dynamics of existing seagrass meadows and to ensure and optimize reestablishment of *Zostera noltii* at new potential seagrass sites by transplantation. We found that growth was not limited during the growing season by sediment dynamics (Chapter 3, 4 & 6) nor by single, interactive or synergistic effects of low light, high salinity or desiccation stress, though desiccation occurs at the higher tidal levels (Chapter 2). Intrinsic processes (i.e. seagrass ecosystem engineering) leading to self-facilitation were found to be important during summer, whereas overall extrinsic forcing (i.e., sediment dynamics but also complexly interacting, unpredictable processes) is more important for long-term seagrass transplantation success than intrinsic processes (Chapter 4 & 6). We showed that seagrass growth and expansion can be enhanced by improving growing conditions (in particular, by preventing desiccation and stabilising the sediment, Chapter 2 & 4) but also by restoring the self-facilitating feedback (Chapter 4 & 6). However, transplants success varied between years and also the natural populations in the Oosterschelde basin are characterised by large non- synchronous variability in expansion and decline. This made us realise that sustainable transplants, colonisations from seedlings or meadows are most likely the result of a complex interplay of conditions instead of just one single parameter, and final success remains partly unpredictable. Future perspectives of seagrass in the Oosterschelde basin are thought to be good, although input of fines in the system might return the full use of seagrass' ecosystem engineering capacity. Natural return of seagrass to its former sediments can possibly be facilitated by i) preserving (not disturbing) former and still potential seagrass sediments ii) preserving or facilitating natural cascades of ecosystem engineers, and iii) preserving natural processes that are key to ecosystem engineering when seascapes change, i.e. keep fines in the water layer.

Overall, our study emphasises that managers conserving and restoring seagrass beds (1) need to account for the unknown and unpredictable part of the variability by spreading risks in space and time, (2) should realise that suitable restoration sites in temperate zones not only have optimal (growing) conditions in summer *but* also enable survival in the more dynamic winter, by suitable site selection (for which rhizome starch contents in autumn can serve as an indicator) and shell armouring, (3) could further counterbalance winter losses by enhancing summer growth by incorporation of facilitating landscape scale interactions of other ecosystem engineers, (4) should be aware that low vegetation cover (below 30%), might indicate that seagrasses are at danger, and (5) could realise a

headstart for transplantation in restoration by using sods in compact arrangement and create desiccation refugia.

Environmental management plans must account for a long evaluation time (incorporate patience), and the unpredictability of the precise locations and years of successes and failures (so spread risks and partially submit to nature).

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Chapter 8

Summary & Samenvatting



GROTE
GRIEZELENDE
GROEISELSI
ZEEGRASI!

Summary:

Coastal ecosystems are put under pressure by anthropogenic activities of the over 40 % of the Earth's population that lives along the world's coastline. Moreover, as these dynamic ecosystems are known for not responding linearly to changes, but for following threshold behavior, they can be lost without any warning. Restoration of these ecosystems that often consist of eco-engineering species has proven to be notoriously hard as thresholds need to be bridged to ensure reestablishment of the self-sustaining positive feedback loops of the ecoengineering species. Despite the difficulties that have to be overcome, globally numerous efforts are performed to conserve and restore key ecosystem species and their high economic and ecological value, like is the case for seagrasses.

In this thesis, I – while watching seagrass grow - focused on the effects of combined physiological stressors (salinity, desiccation and light), bioturbation and sediment dynamics on seagrass dynamics. Moreover, I aimed to identify environmental preferences and bottlenecks of dwarf eelgrass *Zostera noltii* in the Eastern Scheldt tidal basin in order to better understand and predict the dynamics of existing seagrass meadows and to ensure and optimize reestablishment of *Zostera noltii* at new potential seagrass sites by transplantation efforts.

Environmental stresses limiting seagrass growth

In two laboratory experiments (**chapter 2**), I tested 1) if growth and development of intertidal, temperate *Zostera noltii* is affected by emergence time, and 2) how this is affected by an additional, second stressor (shading or high salinity). In addition, I tested 3) whether the effects of emergence time and salinity varied between three different European seagrass populations (Brittany France, Oosterschelde The Netherlands and Wadden Sea Germany), which are likely adapted to different salinity levels.

The results indicate that emergence time and shading show an additive negative effect (no synergistic or antagonistic effect), making the plants still vulnerable to such combination, a combination that may occur as a consequence of self-shading during emergence or resulting from algal cover. Emergence time likely determines the upper limit of *Z. noltii* and such shading will likely lower the upper limit. Shading resulted in higher aboveground / belowground ratios as is a general response in seagrass. *Z. noltii* of different populations originating from salinity 30 and 35 seem tolerant to variations in salinity within the tested range. These results indicate that the three tested populations show morphotypic rather than ecotypic variation, at least regarding the salinity and emergence, as there were no interactive effects with origin. For restoration, this implies that the salinity regime of the donor and receptor site of *Z. noltii* is of no concern within the salinity range 25-35.

Optimizing seagrass restoration: promoting positive feedback, reducing extrinsic forcing and risk-spreading

Restoration of key species in dynamic coastal ecosystems benefits from reduction of environmental stress. This can be realised by promoting positive feedback (intrinsic processes) or by reducing extrinsic negative forcing.

In a seagrass (*Zostera noltii*) restoration project in the south western Netherlands, a total of 2600 m² of seagrass sods were mechanically transplanted to six intertidal flats over the course of five years. In **chapter 3 and 4**, I investigated transplantation success in relation to intrinsic processes (i.e., comparing transplant size, transplant configuration, and transplant density) and extrinsic forcing (i.e., bioturbation by *Arenicola marina*, desiccation and exposure to water dynamics).

Sod transplants (2.25 m²) survived more often than single shoot transplants (no survival). Using larger or more compact transplant configurations had no long-term effect on survival, and initial densities were not related to transplantation success either. Reducing desiccation stress promoted the transplantation success during the first growing season and shielding transplants from bioturbating lugworms had a positive effect on long-term survival.

Seagrass presence in summer was related to spring presence, whereas winter survival was not related to prior seagrass presence. At four of the six intertidal flats transplants gradually decreased in size over time. At the other two, extensive spontaneous colonisation occurred around the transplant areas in some years and is still partly present. This success could not be linked to any environmental parameter investigated.

I concluded that intrinsic processes favour the transplantation development during the growing season, supporting the importance of positive feedback. Extrinsic processes favour the development at a longer time scale (i.e., reduction of bioturbation, supporting the importance of breaking the positive feedback of the bare state). Most surprisingly, final long-term transplantation success at two out of six tidal flats could not be related to any obvious environmental factor, such as exposure to hydrodynamics, light availability, sediment composition, emergence time, macro-algal cover, grazing, salinity, pore-water nutrients and sulphide toxicity. Environmental managers can improve restoration success by restoring the positive feedback, reducing stress, but also via risk-spreading by performing transplants over wider areas, thereby accepting the complexity of processes and unpredictable temporal and spatial variation in which transplantation sites turn out to be successful.

Identifying bottlenecks in seagrass survival

In **chapter 5**, I attempted to elucidate some important plant-related bottlenecks for wintering survival in temperate areas of the intertidal model species *Zostera noltii*, using a three-year transplant-monitoring dataset and a field experiment. I found that next year's transplant success could not be predicted based on the preceding year's growth success, emphasizing the winter as a crucial period for survival of seagrass transplants. In addition, transplant success was neither determined by abiotic site characteristics. Low autumn rhizome starch concentrations in unsuccessful transplants, compared to successful transplants and natural beds, hinted at the importance of starch for winter survival. Hence, I tested the importance of starch, accumulated in autumn, versus the importance of the presence of sparse aboveground photosynthetic winter biomass for winter survival of seagrass transplants in a field experiment. I clipped the overwintering-leaves of three natural beds that naturally varied in their autumn rhizome starch concentrations. Decreased leaf densities in winter did not affect seagrass biomass in June, nor did this treatment affect rhizome starch concentration in June. Autumn rhizome starch reserves did however provide a good indication of next year's growth success, confirming the importance of starch reserves for winter survival. I thus conclude that autumn rhizome starch can be a good predictor of next year's growth success, whereas the preceding growing season shoot density and the presence of leaves during the winter were bad indicators of next year's growth success.

In **chapter 6**, I studied the thresholds of sediment dynamics in relation to seagrass presence by comparing sediment characteristics and seagrass presence data of seven separate seagrass meadows. All meadows had a long-term (>20 years) presence. Within these meadows I distinguish so-called "hotspots" (areas within a meadow where seagrass was found during all mapping campaigns) and "coldspots" (with infrequent seagrass presence). I monitored static sediment characteristics (median grain size, bulk density, silt content) and sediment dynamics (i.e., bed level change and maximum sediment disturbance depth), bioturbation (i.e., lugworm densities and induced fecal pit and mound relief), and seagrass cover. I statistically analysed which sediment characteristic best explains seagrass cover.

Densely vegetated hotspots were shown to have lower sediment dynamics than sparsely vegetated hotspots and coldspots, while static sediment characteristics were similar (grain size, bulk density). The vegetation cover was either low (2 – 15%) or high (> 30%) and sediment dynamics showed a threshold for vegetation cover. From this correlative finding, I postulate a self-sustaining feedback of relatively dense seagrass via sediment stabilisation and accordingly a runaway feedback once the seagrass cover becomes too sparse. The sensitivity for

sediment dynamics shown in this study implies that future existence of seagrass meadows may be at risk as ongoing climate change might directly (increased environmental extremes) or indirectly (changing seascapes) negatively affect seagrass beds.

Conclusion:

This thesis shows that seagrass sites in temperate zones not only have optimal (growing) conditions in summer but should also enable survival in the more dynamic winter.

Winter survival of temperate seagrasses is largely determined by plant carbohydrate reserves, and autumn starch concentrations can be used as indicators for the success of seagrass meadows or transplants in the next growing season. This indicator may be very useful, since abiotic site characteristics seem to give a very limited indication of transplant success and winter survival.

Intrinsic processes in seagrass meadows were found to be important for sustainable seagrass growth. Upscaling the transplant size could be considered to enforce such positive feedbacks. In addition, seagrass meadows with a low cover seem to be unsustainable on the long term; they slide down the runaway feedback loop.

Next to positive (facilitating) interactions, I showed it is equally important to consider negative engineering interactions between ecosystem engineering species. As this study involves large-scale transplantations with several years and sites of planting, long and intensive monitoring and a number of manipulations, I cannot come much closer to ‘proving’ that I apparently cannot predict or deduce habitat suitability completely from the ample available environmental monitoring data. Thus, the typically high environmental variability that governs seagrass habitats requires spreading of risks in time and space in the transplanting set-up and scheme.

For managers conserving and restoring seagrass beds I recommend that they (1) account for the unknown and unpredictable part of the variability by spreading risks in space and time, (2) realise that suitable restoration sites in temperate zones not only have optimal (growing) conditions in summer *but* also enable survival in the more dynamic winter, by suitable site selection (for which rhizome starch contents in autumn can serve as an indicator) and shell armouring, (3) further counterbalance winter losses by enhancing summer growth by incorporation of facilitating landscape scale interactions of other ecosystem engineers, (4) be aware that low vegetation cover (below 30%), might indicate that seagrasses are at danger, and (5) realise a headstart for transplantation in restoration by using sods in compact arrangement and create desiccation refugia.

Samenvatting:

Meer dan 40 procent van de mensen op aarde leeft langs de kust. Hun activiteiten zorgen ervoor dat kustecosystemen onder druk staan. In deze dynamische systemen is het vaak lastig om te overleven voor flora en fauna. Vaak zie je er dan ook biobouwers; organismen die hun omgeving aanpassen doordat ze daar zijn of door wat ze doen. Als ze eenmaal een drempel over zijn en genoeg positieve terugkoppeling op elkaar hebben, kunnen ze blijvend (over)leven op plekken waar het normaal uiterst moeilijk of zelfs onmogelijk is. De keerzijde is dat deze ecosystemen ook plots kunnen verdwijnen (in plaats van gelijkmatig) als het systeem verandert en de positieve terugkoppeling verdwijnt. Restauratie van systemen met dit soort onrechtlijnigheid is dan ook niet eenvoudig omdat de positieve terugkoppelingen van biobouwers die zorgen voor duurzaam overleven moeten worden teruggebracht. Ondanks de uitdagingen die dit met zich meebrengt, worden wereldwijd veel acties ondernomen om belangrijke sleutel ecosystemen (zoals zeegrasvelden) en hun economische en ecologische waarde te behouden en te restaureren.

In dit proefschrift heb ik onderzocht wat de effecten zijn van gecombineerde fysiologische stressen (zoals zoutgehalte, droogvalduur en licht), bioturbatie (omwoeling van het sediment door fauna) en sediment dynamiek (beweging van het sediment onder invloed van bijvoorbeeld golven en stroming) op zeegrasgroei. Bovendien heb ik geprobeerd te bepalen welke condities zeegrasgroei mogelijk maken en waar de bottlenecks voor groei en overleving zitten voor klein zeegras *Zostera noltii* in de Oosterschelde. Met deze kennis hoop ik de dynamiek van bestaande zeegrasvelden beter te kunnen begrijpen en voorspellen en daarnaast restauratie successen op nieuwe geschikte plekken te vergroten.

Wat remt zeegrasgroei?

In hoofdstuk 2 beschrijf ik 2 laboratoriumexperimenten. Hierin testte ik 1) of groei en ontwikkeling van droogvallend zeegras beïnvloed wordt door de tijd die het per dag droogvalt en 2) of dit anders is met een extra, tweede stress (beschaduwning of hoog zoutgehalte). Daarnaast heb ik onderzocht of de effecten van droogvalduur en zoutgehalte op zeegrasgroei verschillen tussen 3 Europese zeegraspopulaties (Bretagne Frankrijk, Oosterschelde Nederland en Waddenzee Duitsland) die elk aangepast zijn aan verschillende zoutgehaltes.

Uit de experimenten blijkt dat droogvalduur en beschaduwning beide een negatief effect hebben op zeegrasgroei. Als deze stressen tegelijkertijd voorkomen zijn hun effecten additief en niet synergistisch of antagonistisch. In het veld is het zeer waarschijnlijk dat een dergelijke combinatie voorkomt bij bijvoorbeeld zelfbeschaduwning van de zeegrasbladeren of door algen die het zeegras bedekken. Droogvalduur bepaalt voor een groot deel de bovengrens van het voorkomen van *Z. noltii* in het intergetijdengebied. Een toevoeging van

beschaduwning zal deze bovengrens hoogstwaarschijnlijk verlagen, naast dat het resulteert in de gebruikelijke verhoging van de bovengronds vs. ondergronds biomassa ratio.

De verschillende Europese populaties zeegras bleken tolerant voor zoutgehaltes binnen onze testrange. Dit duidt er erop dat de 3 geteste populaties eerder morfotypische dan ecotypische variatie vertonen ten op zichte van zoutgehalte en overstromingsduur. Uit statistische analyse bleek namelijk steeds geen effecten voor de oorsprong van de planten. Bij zeegrasrestauratie zouden geen groeiproblemen verwacht kunnen worden zolang zoutgehaltes op de donor- en aanplantlocatie tussen 25 en 35 PSU zitten.

Optimalisering van zeegrasrestauratie: ondersteunen van positieve terugkoppeling, verminderen van extrinsieke krachten en risicospreiding.

Het verbeteren van de leefomstandigheden draagt bij aan de restauratie van sleutelsoorten in dynamische kustsystemen. Dit kan bereikt worden door positieve terugkoppeling binnen de soort (intrinsieke processen) te ondersteunen of door het verminderen van extrinsieke, negatieve krachten.

In een grootschalig zeegras (*Z. noltii*) restauratieproject in de Oosterschelde (zuidwest Nederland) zijn gedurende 5 jaar niet minder dan 2600m² zeegrasplaggen verplaatst. In **hoofdstuk 3 en 4** onderzocht ik het transplantatiesucces in relatie het verbeteren van de intrinsieke processen (o.a. vergelijking van aanplantgroottes, aanplant lay outs en aanplantdichtheden) en het verminderen van extrinsieke krachten (o.a. omwoeling door wadpieren, uitdroging en waterdynamiek).

Gebleken is dat zeegras getransplanteerd in plaggen (2.25 m²) beter overleeft dan los getransplanteerde zeegrasscheuten (0% overleving). Het gebruik van grotere en meer compacte aanplant lay outs had op de lange termijn geen effect op zeegrasoverleving. Ook een grotere aanplantdichtheid bij de start was geen sleutel tot transplantatiesucces. Daarentegen, het verminderen van droogtestress verbeterde het transplantatieresultaat in het eerste jaar. Het verminderen van omwoeling door wadpieren had zelfs een langdurig positief effect op zeegrasoverleving.

Verder bleek dat zeegras aanwezigheid in de zomer gerelateerd was aan aanwezigheid in de lente. Echter, winteroverleving was niet gerelateerd aan eerdere aanwezigheid. Dit werd in het veld zichtbaar doordat 4 van de 6 aanplanten in de loop van de tijd geleidelijk in omvang afnamen. De overige 2 lieten op enkele momenten juist enorme spontane kolonisatie zien rondom de aanplanten, die deels nog steeds overleven. Dit succes heb ik niet kunnen verklaren door een van de vele (a)biotische parameters die ik gemeten heb.

Hieruit concludeerde ik dat gedurende het groeiseizoen het ondersteunen van intrinsieke processen de ontwikkeling van de transplantatie ten goede komt, wat

het belang van positieve terugkoppeling benadrukt. Op de langere tijdschaal is het verminderen van extrinsieke processen (bijvoorbeeld omwoeling door wadpieren) belangrijk voor het aanplantsucces. Verrassend genoeg heb ik het positieve transplantatie succes op 2 van de 6 aanplantlocaties niet kunnen toeschrijven aan een duidelijke (a-)biotische factor, zoals stroming, golven, lichtsterkte, sedimentsamenstelling, droogvalduur, bedekking van macro-algen, begrazing, zoutgehalte, bodemnutriënten en sulfide toxiciteit. In de praktijk kan het restauratiesucces vergroot worden door het herstellen van positieve terugkoppelingen en het verminderen van (a-)biotische stressen. Maar zeker ook door het spreiden van risico's in tijd en ruimte. Immers, de processen zijn complex en variatie in succes is onvoorspelbaar gebleken in tijd en ruimte.

Bottlenecks in zeegrasoverleving

In **hoofdstuk 5** probeerde ik enkele aan de klein zeegrasplant gerelateerde bottlenecks voor winteroverleving in gematigde klimaten te ontrafelen. Hiertoe monitorde ik een zeegrasaanplant gedurende 3 jaar en deed ik een veldexperiment. Analyse van de monitoringsdata leerde me dat het succes van transplantatie niet te voorspellen was op basis van het groeisucces in het voorgaande jaar. Dit benadrukte dat de winter een cruciale periode in zeegrasoverleving is. Daarnaast kon het succes van transplantatie niet toegeschreven worden aan abiotische condities van de transplantatielocatie. Zeegrasoverleving in de winter leek afhankelijk van zetmeelconcentraties in de rhizome; onsuccesvolle transplantaties hadden lagere zetmeelconcentraties in het najaar dan succesvolle transplantaties en natuurlijke velden. In het veldexperiment dat ik hierna ontwierp, testte ik in welke mate zeegraswinteroverleving afhing van de grootte van de zetmeelvoorraad in de rhizomen aan het einde van het najaar en het al dan niet hebben van fotosynthetisch actieve bladeren in de winter. Hiertoe heb ik overwinterende bladeren afgeknipt in 3 natuurlijke velden die van nature verschilden in rhizoom zetmeelconcentraties. Het afknippen van overwinterend bladmateriaal resulteerde niet in minder zeegrasbiomassa of minder zetmeelgehalte in de rhizomen aan het begin van de zomer (juni). De grootte van de zetmeelvoorraad in het najaar bleek wel een goede indicator te zijn voor het groeisucces in het volgende jaar. Dit bevestigde ons vermoeden van het belang van een zetmeelvoorraad in de rhizome voor de winteroverleving van klein zeegras. Ik concludeerde dat zetmeelconcentratie in het rhizoom in het najaar een goede indicator kan zijn voor het groeisucces in het opvolgende jaar. Dit geldt niet voor bladdichtheden in het voorgaande jaar en de aanwezigheid van blad gedurende de winter.

In **hoofdstuk 6** bestudeerde ik de rol van sediment dynamiek in relatie tot zeegrasvoorkomen door sedimentkarakteristieken en zeegrasvoorkomen van 7

zeegrasvelden te vergelijken. Alle bestudeerde zeegrasvelden bestaan al geruime tijd (meer dan 20 jaar), maar hebben in die jaren niet steeds evenveel zeegrasbedekking gehad, zo blijkt uit karteringen. Plekken binnen de zeegrasvelden waar tijdens alle karteringen zeegras is gevonden noemde ik “hotspots” en plekken waar minder frequent zeegras aangetroffen is “coldspots”. Op deze hotspots en coldspots bepaalde ik statische sediment karakteristieken (mediane korrelgrootte, bulkdensiteit en slibgehalte), de sediment dynamiek (d.w.z. hoogteveranderingen en de maximale verstoringsdiepte), de mate van bioturbatie (d.w.z. wadpieraantallen en het reliëf dat zij maken) en zeegrasbedekking. Daarna testte ik welke van deze bepaalde karakteristieken het beste de zeegrasbedekking verklaarde.

Ik vond dat dichtbegroeide hotspots een lagere sedimentdynamiek kende dan hun begroeide hotspots of coldspots, terwijl de statische sediment karakteristieken (korrelgrootte en bulkdensiteit) vergelijkbaar waren. Zeegrasbedekking was steeds of laag (2-15%) of hoog (boven de 30%) en een gerelateerde hoog-laag tweedeling was ook te zien in de sediment dynamiek (hoge dynamiek bij lage bedekking en lage dynamiek bij hoge bedekking). Uit deze correlatie, opperde ik i) een zichzelf in stand houdende positieve terugkoppeling van dichtbegroeide zeegrasvelden via de sediment dynamiek en ii) het afglijden langs een zichzelf versterkende negatieve spiraal zodra zeegrasbedekking te laag is geworden. De gevonden gevoeligheid voor sedimentdynamiek impliceert dat het toekomstperspectief van zeegrasvelden niet al te rooskleurig kan zijn. Immers voortdurende klimaatveranderingen kunnen zeegrasvelden bedreigen: direct door grotere weersextremen en indirect door landschappelijke veranderingen.

Conclusie:

Dit proefschrift toont aan dat in gematigde klimaten zeegrashabitat niet alleen gekenmerkt moet worden door optimale (groei-) condities gedurende de zomer, maar ook geschikt moet zijn voor zeegrasoverleving in de altijd dynamische winter. Winteroverleving in gematigde gebieden wordt voornamelijk bepaald door zetmeelvoorraden in de rhizomen, waarbij de zetmeelgehalten in het najaar als een goede indicator kunnen worden gebruikt voor het groeisucces in het navolgende jaar. Zo'n indicator lijkt extra bruikbaar omdat abiotisch karakteristieken van zeegraslocaties weinig voorspelbare waarde blijken te hebben ten aanzien van transplantatie succes en winteroverleving.

Positieve terugkoppeling binnen zeegrasvelden blijkt van groot belang voor duurzame zeegrasgroei. Bij transplantaties kan deze terugkoppeling worden versterkt door aanplantgroottes op te schalen. Daarbij lijken zeegrasvelden met een lage plantdichtheid op de lange termijn gedoemd te verdwijnen omdat ze langzaam wegglijden langs de zichzelf versterkende negatieve spiraal. Daarnaast moet altijd rekening gehouden worden met negatieve terugkoppeling door

buurbiobouwers. Dankzij de gespreide, jarenlange grootschalige transplantaties, de lange en intensieve monitoring hiervan en de vele experimenten is een stuk duidelijker welke condities zeegrasgroei mogelijk maken en waar de bottlenecks voor groei en overleving zitten voor klein zeegras *Zostera noltii* in de Oosterschelde. Helaas, kan ik vanuit alle beschikbare data de geschiktheid en daarmee het groeisucces van zeegras op een locatie op voorhand niet voorspellen. Door de hoge variatie in condities binnen zeegrashabitats lijkt spreiden van risico's in tijd en ruimte bij transplantaties het devies.

Voor het behoud en restauratie van zeegrasvelden beveel ik het volgende aan: 1) Spreid risico's in ruimte en tijd i.v.m. onbekende en onvoorspelbare variatie in zeegrasvelden en omstandigheden, 2) Kies geschikte aanplantlocaties: zetmeelgehalte in de rhizomen in het najaar is hier een goede indicator voor, maar realiseer dat geschikte aanplantlocaties niet alleen gedurende de zomer geschikte condities hebben, maar ook gedurende de winter. Breng eventueel een schelpenlaag in om omwoeling te verminderen en zo sediment stabiliteit te vergroten, 3) versterk de groei in de zomer door gebruikt te maken van positieve terugkoppeking van buurbiobouwers op landschapschaal, 4) ben je bewust dat een lage zeegrasbedekking (beneden 30%) vaak betekent dat het veld in een neerwaartse spiraal zit en 5) geeft transplantaties een voorsprong door te restaureren in zoden die compact bij elkaar geplaatst worden en voorkom dat ze uitdrogen.



DANK

WOORD

Goede muziek ✓, goed glas bier ✓, vlizotrap achter me dichtgetrokken ✓
De ideale cocktail om de laatste woorden te schrijven van dit proefschrift.

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En dan “Team Spatial Ecology”: Peter, Tjeerd, Johan, Daphne, Tom, Annette, Jos, Bas, Lennart, Jeroen, Aniek, Ellen, Francesc, Christian, Paul, Nienke, Thorsten, Arnon, Yuyu, Monique, Jim, Qi Hui, Laura, Sil, Hélène, Lucy, Francesco, Zhegang, Loreta, Mr. Zhu, Bas, Tisja, Isabelle, Marije, Olivier, Oliver, Alinda, Rebecca, Simeon, studenten, gasten en iedereen die ik eventueel vergeten ben. Het was een feest om tot dit team te behoren. Ik koester de vele mooie momenten met deze mooie club mensen. Nooit te beroerd om een handje te helpen in het veld. Memorabel zijn de postersessies voor de NAEM en NIOZ days, het opzetten van de open dagen, de babyboom en de yes-my-paper-is-published-cakes. Een team dat elkaar op en buiten de werkvloer vond en aanvulde. Simeon, ik ga je nooit bijhouden met de fiets, ook niet als die racefiets

er ooit nog gaat komen. Maar die coastal-ecology-tocht moet nog een keer van mijn bucket list af. Sil, Snarky Puppy was awesome om in jouw bewoordingen te blijven. Dank voor het delen van de vele funky muziek. Ideale schrijfmuziek op de vrijdagmiddag. Jos, je was mijn langste roomie. Dank voor de vele tussen de bedrijven door gesprekken over zeegras, het veld, marktplaats, pepers en nog zoveel meer. En nee, het is geen toeval dat je naam stiekem vereeuwigd is in chapter 2. Gewoon omdat het kon, net als andere verborgen funnies in dit proefschrift ;-). Helaas Jim, voor jou heb ik dat niet kunnen regelen, maar je bent dan ook niet zolang mijn roomie geweest. Dat neemt niet weg dat het er niet minder om klikte, al was je wel een rare snuiter met je modelletjes en theorie'tjes. Maar gelukkig hadden we o.a. wel een bierklik en een goed gevoel voor droge humor.

Ook grote dank aan “Team NIOZ” met de geweldige werksfeer en drive voor de beste en mooiste wetenschap. Collega’s van MM, ES, MON, ANALAB, TD, Administratie in de breedste zin en Laura en Jan natuurlijk. Het was tof om met jullie te werken. Dank voor alle support, analyses, verzorgde lunches, lunchtalks, kennisdeling, koffie- en pauzemomenten, PV-momenten, borrels etc. etc. U was buutegewoon. Excuses van mijn kant dat ik nooit de collega-van-de-maand-bokaal heb uitgereikt, terwijl ik al die jaren heb gestrooid met bonuspunten voor deze verkiezing. Jan, al verzorg je nog 100 lunches en borrels, na het doorprikken van mijn auto met de heftruck heb je zoveel minpunten dat je niet in de top 10 voorkomt. Eveneens buiten de boot: Het analytisch lab dat structureel bonuspunten verzilverde voor appeltaart, maar gelukkig wel analyses vooruit schoof. Bert en Louis voor jullie een mooie gedeelde 2^e plek voor alle klakkeloze, maar waardevolle technische support voor experimenten en privé-restauratieprojecten. Veronique voor jou de hoogste ranking. Misschien nooit zo uitgesproken, maar ik koester onze koffiemomenten en social talks. Soms moet je gewoon even je ei kwijt of een paar minuten niet aan de wetenschap denken. Dank.

Special thanks to “Team NIOZ-outside-NIOZ” for all the fun moments after working hours. Legendary are the bbqs and parties at the Keete, the Friday afternoon special beer drinks and the carnival workshops in Bergen op Zoom. Love you guys!

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En de eer van de laatste woorden die ik voor dit proefschrift schrijf zijn voor mijn trouwste steun en toeverlaten. Hoewel wij Suykerbuyken niet erg talkative zijn met betrekking tot gevoelens, hierbij toch een poging. Pa en ma, dank voor

de eeuwige steun, het tot vervelens toe vragen naar de promotiedatum, het zijn van trotse opa en oma en voor alle andere grote en kleine dingen in het leven. In goede en uitdagendere tijden. Broertje en zus, dank voor de broeder- en zusterschap. Het leven zou een stuk saaier zijn zonder jullie om me heen, En dan mijn 2 kanjers (of zijn jullie nu boeven, soepkippen, oelwappers of blij eien). Roel en Stef, ik kan me geen leven meer voorstellen zonder jullie. Het was elke dag een feest om weer thuis te komen en jullie neuzen tegen de ruit van de voordeur gedrukt te zien. Zet de lego maar klaar, papa hoeft nu niet meer op zolder te werken! Roel, voor jou een belangrijke taak: niemand mag de aula in zonder ons wachtwoord!

En als laatste dan mijn grootste steun en toeverlaat. Diana, dank voor alle jaren samen, onze geweldige kids, het niet zeuren over een korreltje zand meer of minder in de auto, het zorgen voor kids, je kookkunsten, de gezelligheid, de schoppen onder mijn kont etc. etc. etc. Zonder jouw onvoorwaardelijke steun was dit alles nooit mogelijk geweest. Love you!

Kortom, iedereen bedankt jullie bijdrage, hoe klein dan ook. Het was om nooit te vergeten. Of zoals bezongen in de betere Nederpop:

Het zien duurt een seconde, de gedachte blijft voor altijd!





Curriculum vitae



ZEEGRASSTRAAT

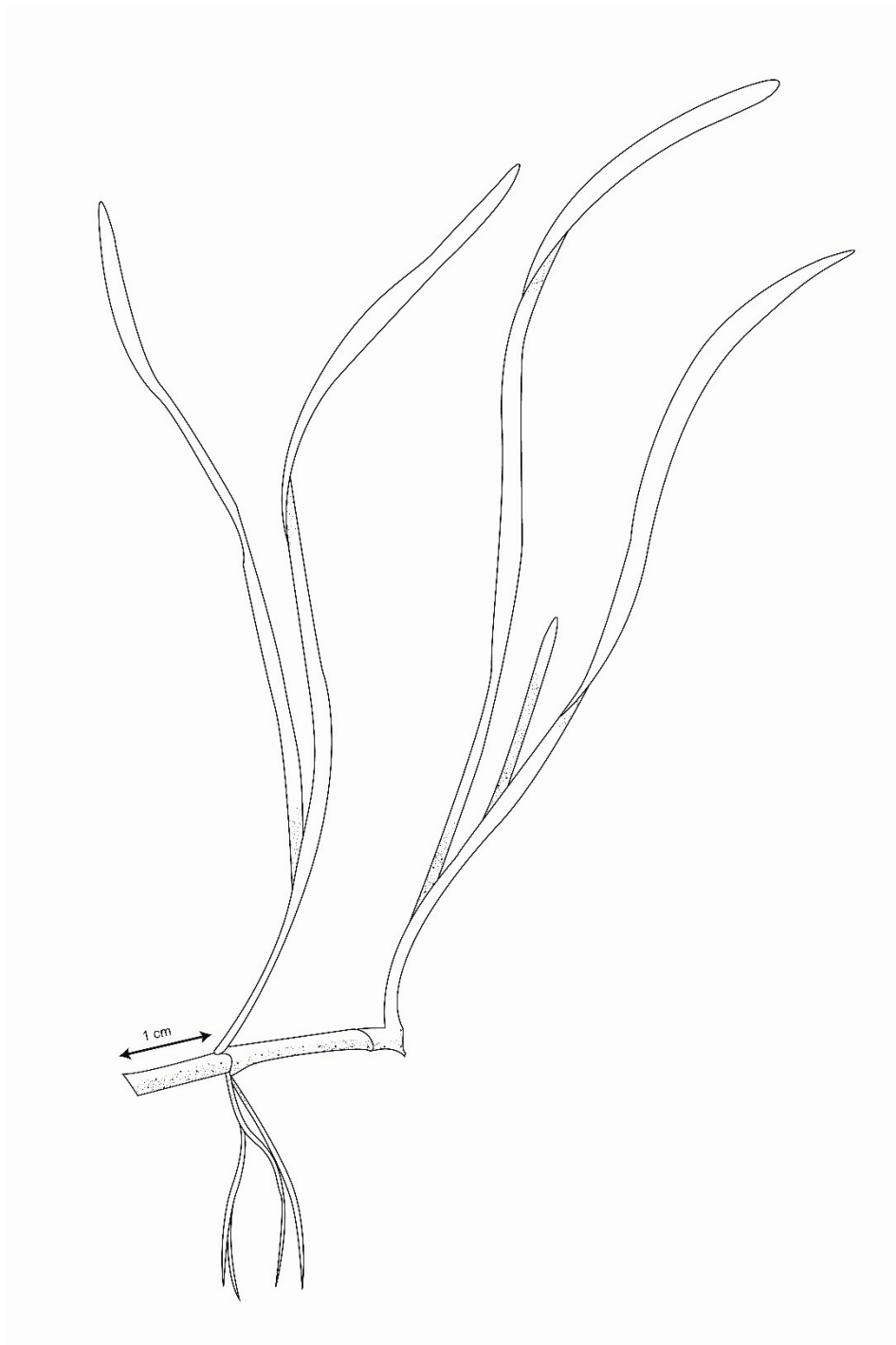


About the author:

Wouter Suykerbuyk (official named Cornelis Johannes Petrus Adrianus) was born in Bergen op Zoom, the Netherlands, on January 12, 1982. He grew up on top of “de Brabantse Wal”, surrounded by its beautiful diverse landscapes; fields, forests, polders, heathland, fens, lakes and the Oosterschelde tidal basin. His passion for the outdoors and interest in the “mysteries” of flora and fauna most likely originated from the visits to his grand parents farm. During his childhood and adolescence his “how life and nature work”-interest was fed by being outdoors, being a boy scout and even more by his classes in natural sciences at highschool. Biology classes were always ranked number one. After graduating, he choose to continue feeding his broad interests by starting the bachelor Biology at the Radboud University Nijmegen. After a scientific side trip into animal physiology during his first bachelor internship, he developed a growing interest in marine ecology. During his masters, he investigated i. the wave-dampening effect of mussel beds on *Zostera marina* seagrass meadows, ii, the effect of *Zostera marina* on the sediments it inhabits within a restoration project in the Dutch Wadden Sea, iii. the effect of the bioturbating lugworm *Arenicola marina* on the composition of the sediments and iv. the wave-dampening effects of oyster reefs.

After finishing his masters, he joined a new seagrass (*Zostera noltii*) project from the Radboud University Nijmegen and NIOZ, Yerseke. The practical goal of the project was to successfully transplant seagrass patches within the Oosterschelde tidal basin, as they were directly endangered by dike reinforcements. Via multiple test transplantations, field and lab experiments and monitoring of natural seagrass fields he investigated the physical needs and boundaries of sustainable healthy seagrass. With this knowledge the team aimed to better understand (non-)successes of seagrass transplantation in order to develop succesfull transplantation techniques and to improve selection of transplantation locations. At the end of his PhD, he helped to investigate the sediment dynamics within a new *Zostera marina* restoration project in the Dutch Wadden Sea using the techniques and expertise he developed during his PhD.

Outside the office and field, Wouter enjoyed his free time leading a group of scouts, playing percussion and giving drum classes and workshops. After his PhD, he took the opportunity to turn his teaching hobby into a profession by starting a traject to become a primary school teacher. From 2016, he returned to the Oosterschelde intertidal flats. He now aims to grow the best looking and tasting off-bottom oysters in the Oosterschelde, incorporating his farmer genes, his marine expertise and passion for the Oosterschelde system and its (a)biotic beauty. As always, combining seriousness with crazyness and humour.



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