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

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Running title: Unpredictability in seagrass transplantations

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 m^2 of seagrass sods were mechanically transplanted to six intertidal flats over the course of five years.
3) 43% of sod transplants (2.25 m^2) 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.

Key-words

abiotic forcing, feedback mechanisms, large-scale, long-term transplantation evaluation, mitigation, multi-year, restoration, self-facilitation, spreading of risks, *Zostera noltii* Hornem

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 m^2) 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 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 ± 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, $F(1, 88) = 0.013$, $P = 0.910$).

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 m^2 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 m^2, 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).

Discussion

The large-scale Z*ostera 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 m^2), 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).

Supporting information

Additional supporting information may be found in the online version of this article.

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

Data accessibility

The data are available at Data Archiving and Networked Services (DANS) doi: http://dx.doi.org/10.17026/dans-234-6epm (Suykerbuyk 2015).

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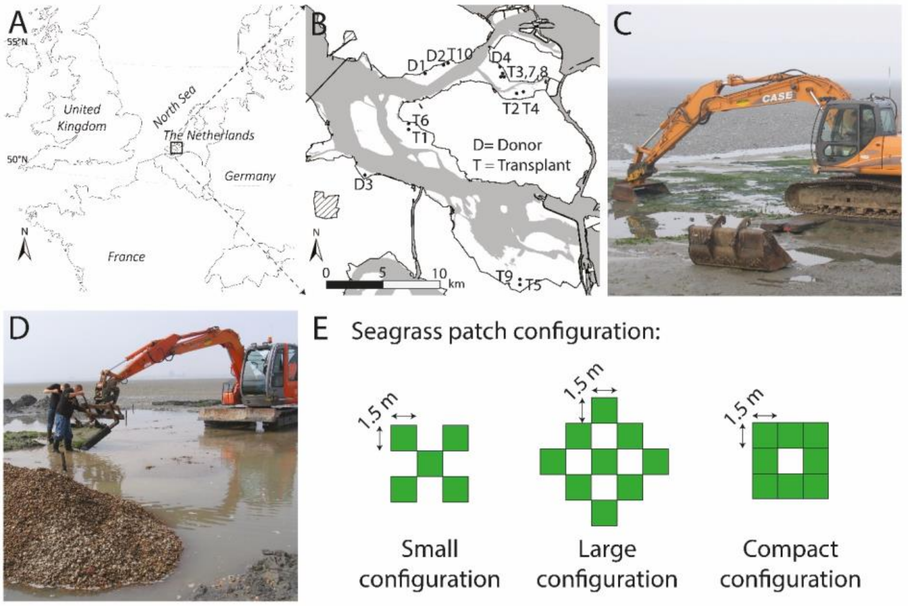


Figure 1 Experimental set-up. A. Location of the Oosterschelde sea inlet (study area) in the south-western Netherlands, north western Europe. Mean tidal range is 3,5m. B. Overview of donor (D) and transplant (T) sites within the study area C. Mechanical seagrass harvest in custom-made boxes. D. Mechanical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

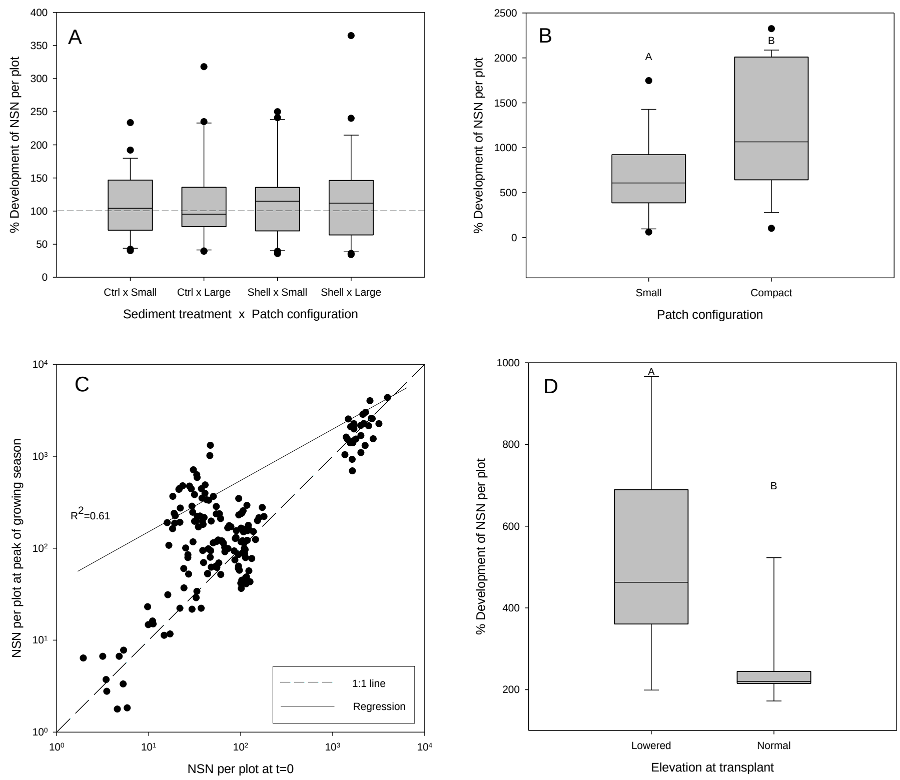


Figure 2 Short-term (1st growing season) development of transplanted seagrass sods presented as the relative decrease/increase of the normalized shoot numbers (NSN) per plot. NSN = total numbers of shoots per plot divided by the transplanted area at t=0.Box-whisker plots: box shows the 25th/75th percentile, 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).

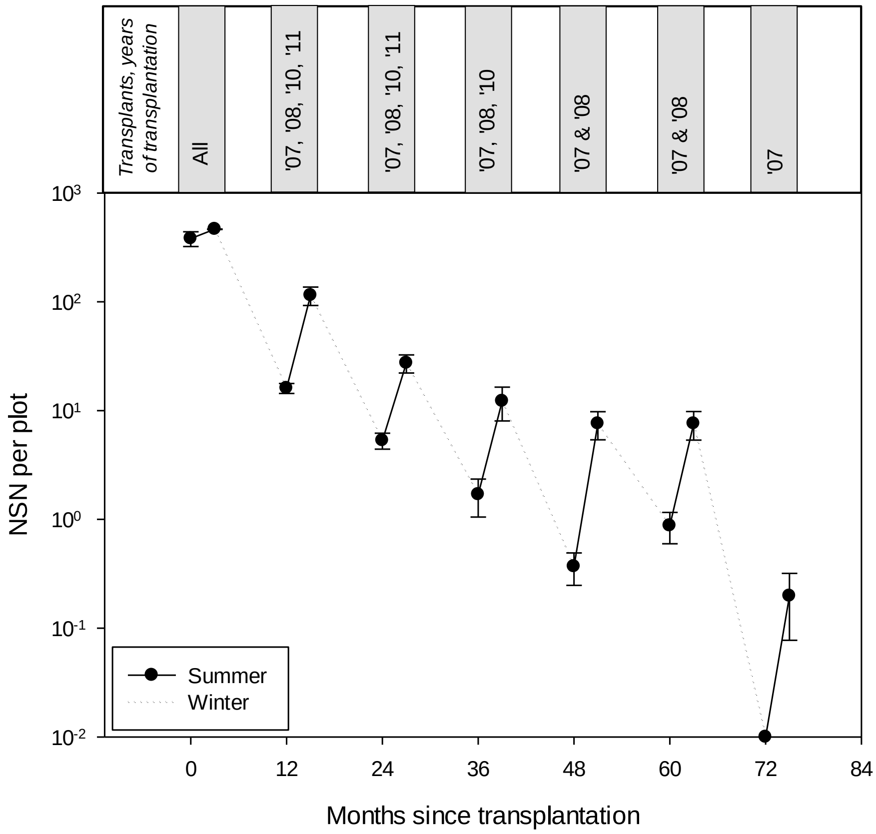


Figure 3 Overall long-term development of seagrass transplants. Mean normalized shoot numbers (NSN) per plot +- standard errors are presented by black dots, the growing seasons are indicated by the solid lines. Note that the transplant efforts were not performed in the same year. As a result, the number 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.

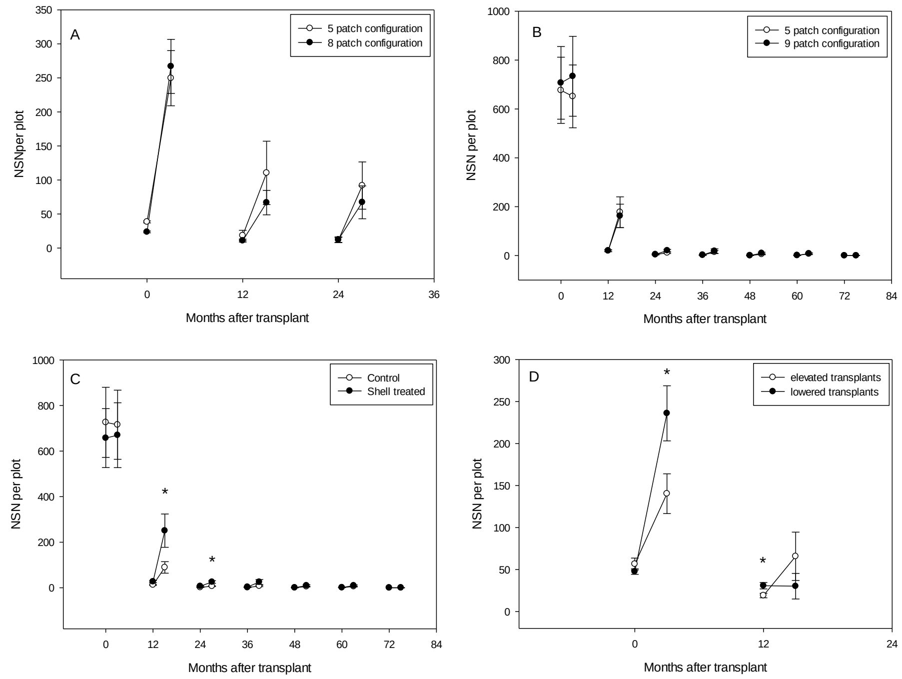


Figure 4. Effects of patch configuration and sediment treatments on long-term transplantation successes. A&B. Mean NSN per plot + Standard error of mean (SEM) in time for T9 (Panel A, n=15 for the 5-patch and n= 18 for 8-patch configuration) and for T1–T6 transplants (panel B, n=46 per configuration) (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).

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).

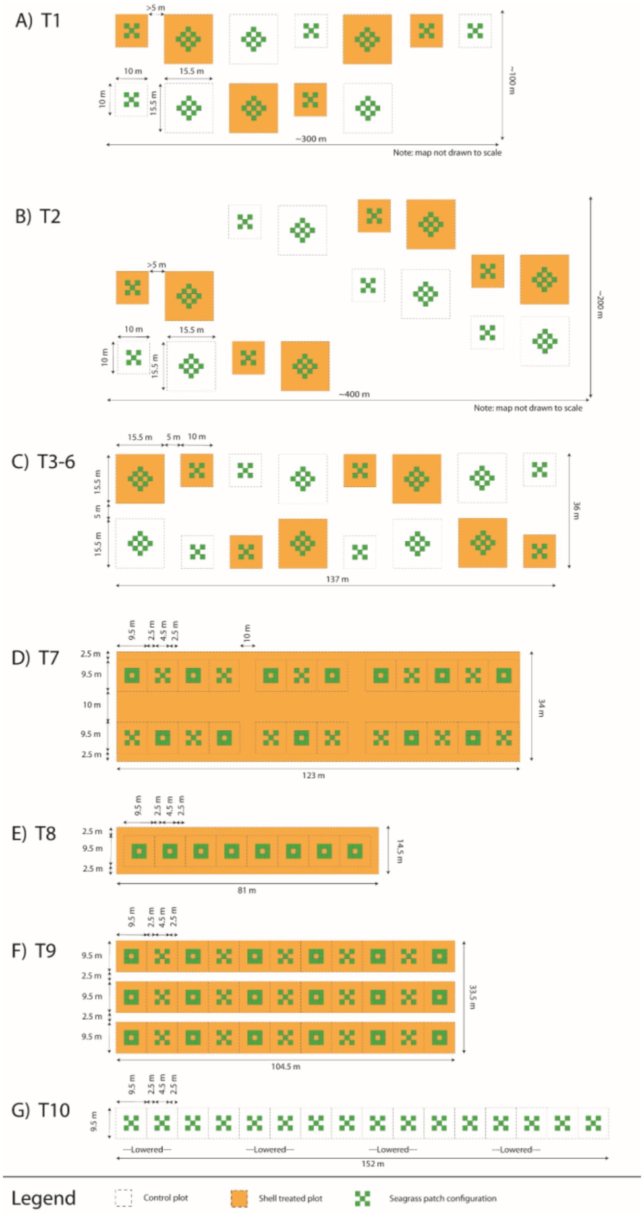


Figure S1 Schematic top view of the transplants T1-T10, their plot and patch arrangements and the sediment treatments applied.

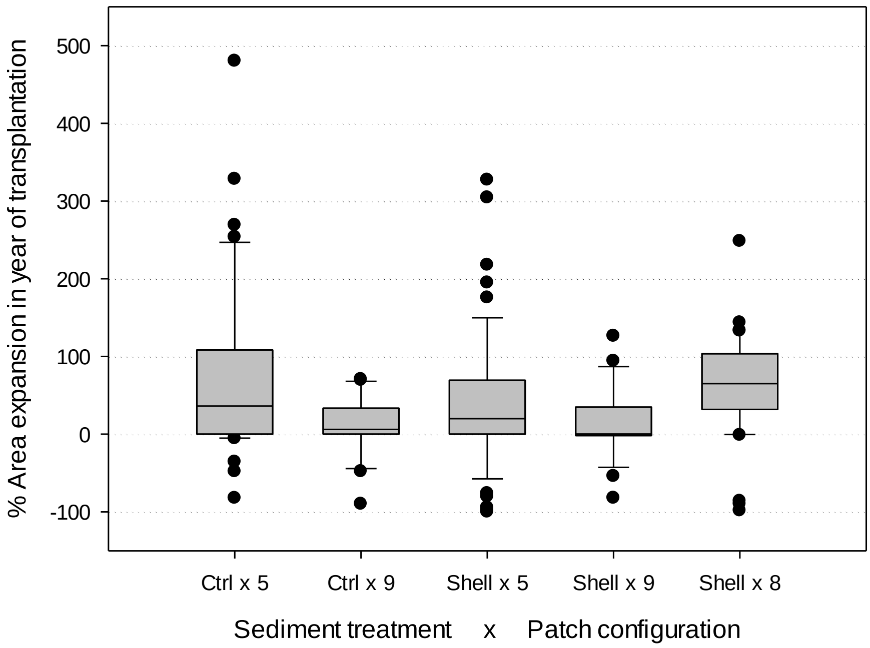
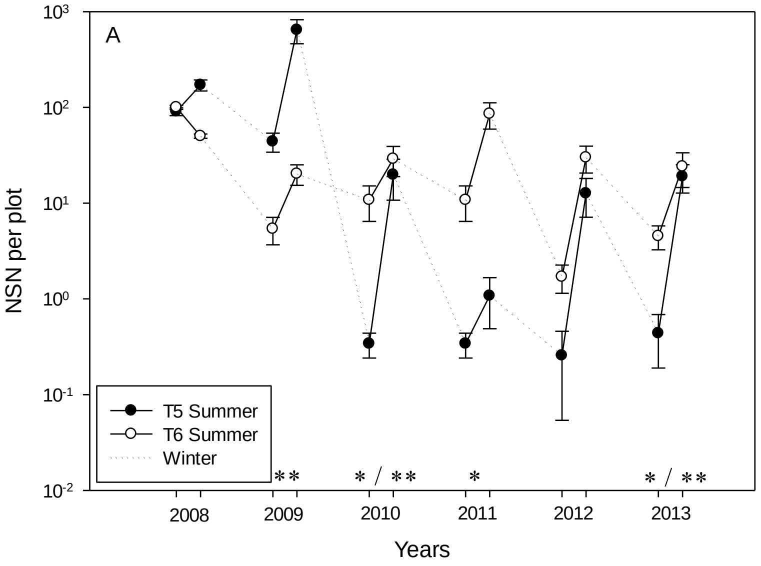


Figure S2 Expansion in seagrass area during the first growing season after transplantation, expressed as the percentage of change compared to the initial seagrass area transplanted.


A
NSN per plot
10^3
10^2
10^1
10^0
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T5 Summer
T6 Summer
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* / **
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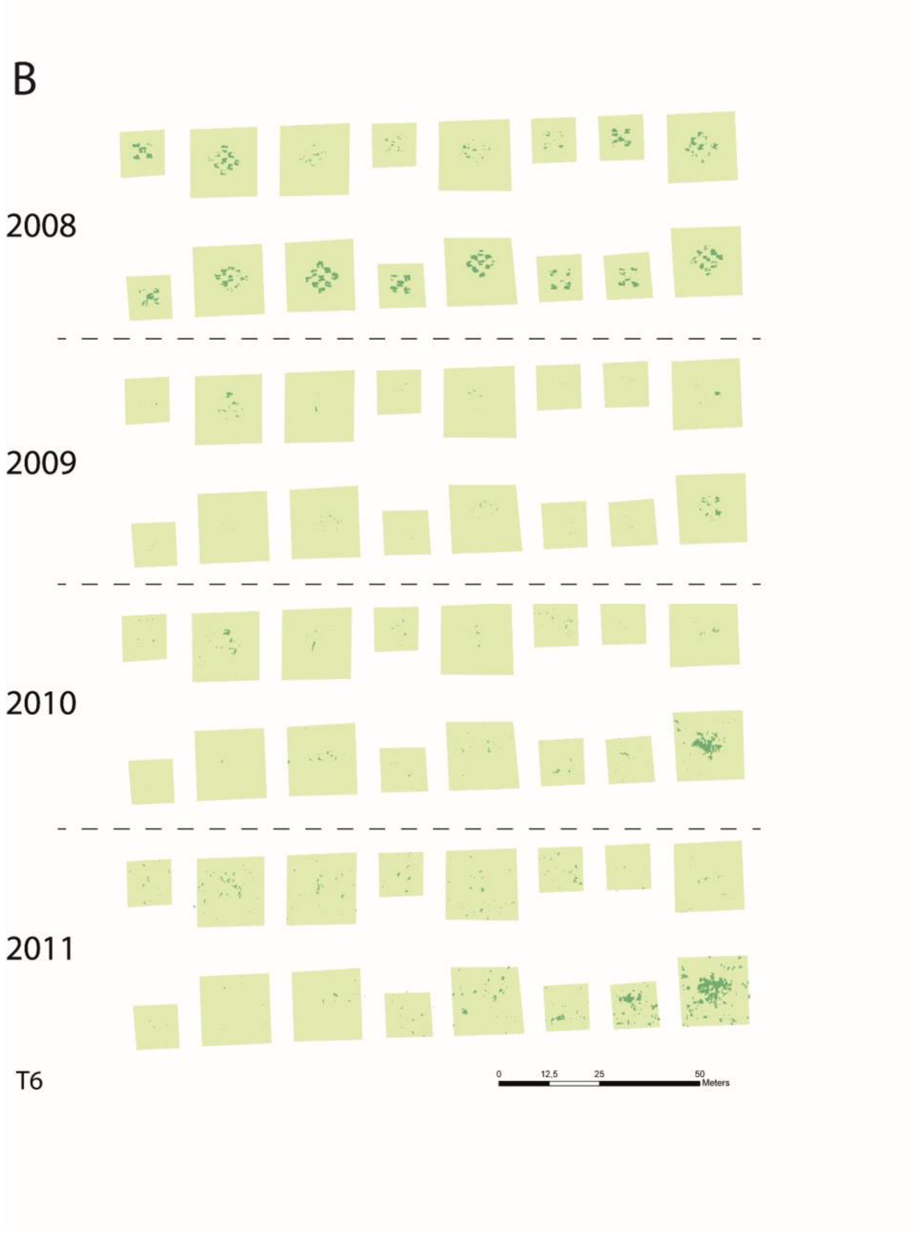
2009
2010
2011
2012
2013
Years

646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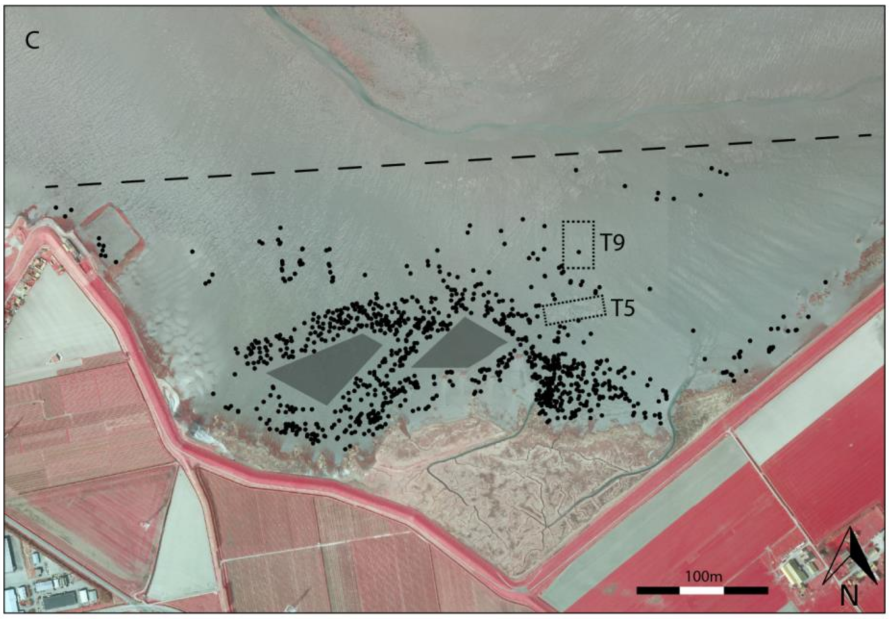

Figure S3 Development and long-term transplantation successes of two (partly) successful transplantations. Panel A. Mean normalized shoot numbers (NSN) per plot per growing season of transplant T5 and T6. * and ** indicate the presence of newly colonized seagrass patches (order of 1000s) around the transplanted 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.

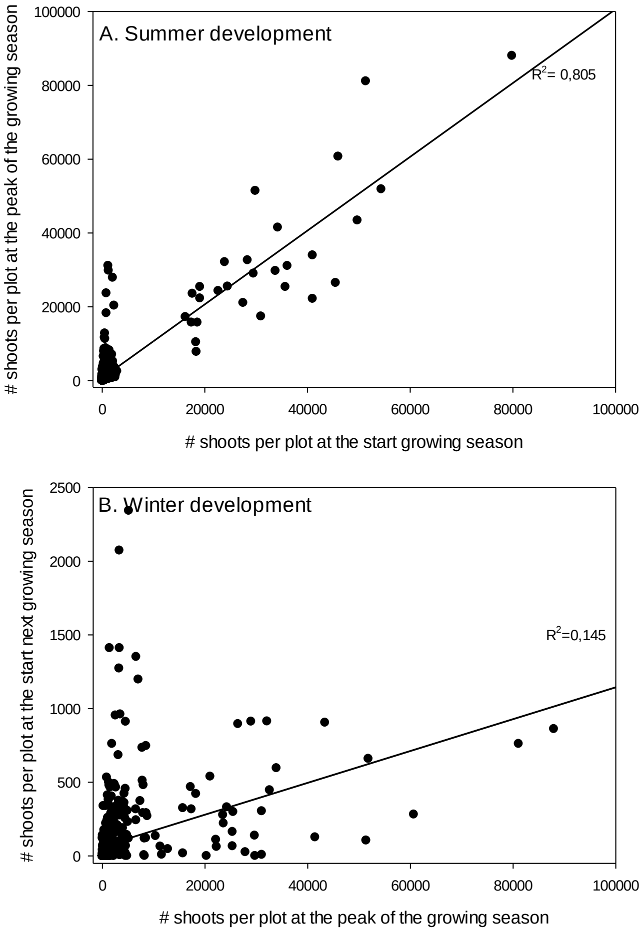

Figure S4 Correlation of number of shoots per plot between. A) Beginning and peak of the growing season (n=659) and B) the peak of the growing season and the start of the following growing season (n=478). Data: all transplants, all plots. Regression of the data is given by the solid lines as well as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

Table S1 Sediment characteristics and hydrodynamic exposure of the transplants. D50 = Median grain size in mu, % Silt = fraction of the sediment smaller than 63 mu). 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 (mu)	% Silt (<63 mu)	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
				Ctrl	Shell	Ctrl	Shell	Ctrl	Shell	Sum plots	Area (m^2)
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				12	24	351
T8	2010	June	D4						8	8	144
T9	2011	June	D3		15				18	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)

	Treatment	Control		Shell	
	Patch configuration	5	9	5	9
Exposedness					
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 2013

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**	**Porewater nitrate**	**Porewater phosphate**	**Porewater sulphide**
			µm	*# m^{-2}*	*presence (+) absence (-)*	*µmol /l*	*µmol /l*	*µmol /l*	*µmol /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