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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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Summary

1. Restoration of key species in dynamic coastal ecosystems benefits from reduction in 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 south-western Netherlands, we investigated transplantation success in relation to intrinsic processes (i.e. comparing sods vs. 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 5 years.

3. In total, 43% of sod transplants (2·25 m^2) survived at the long term, whereas single shoot transplants failed within the first 3 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 was still partly present in 2015. A correlation with 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 in 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

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Introduction

Coastal ecosystem deterioration caused by persistent anthropogenic pressure is an unfortunate but dominant phenomenon world-wide (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; van de Koppel *et al.* 2001; Madsen *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 in aboveground 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 vs. 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, and (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 in 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, that is 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, Supporting

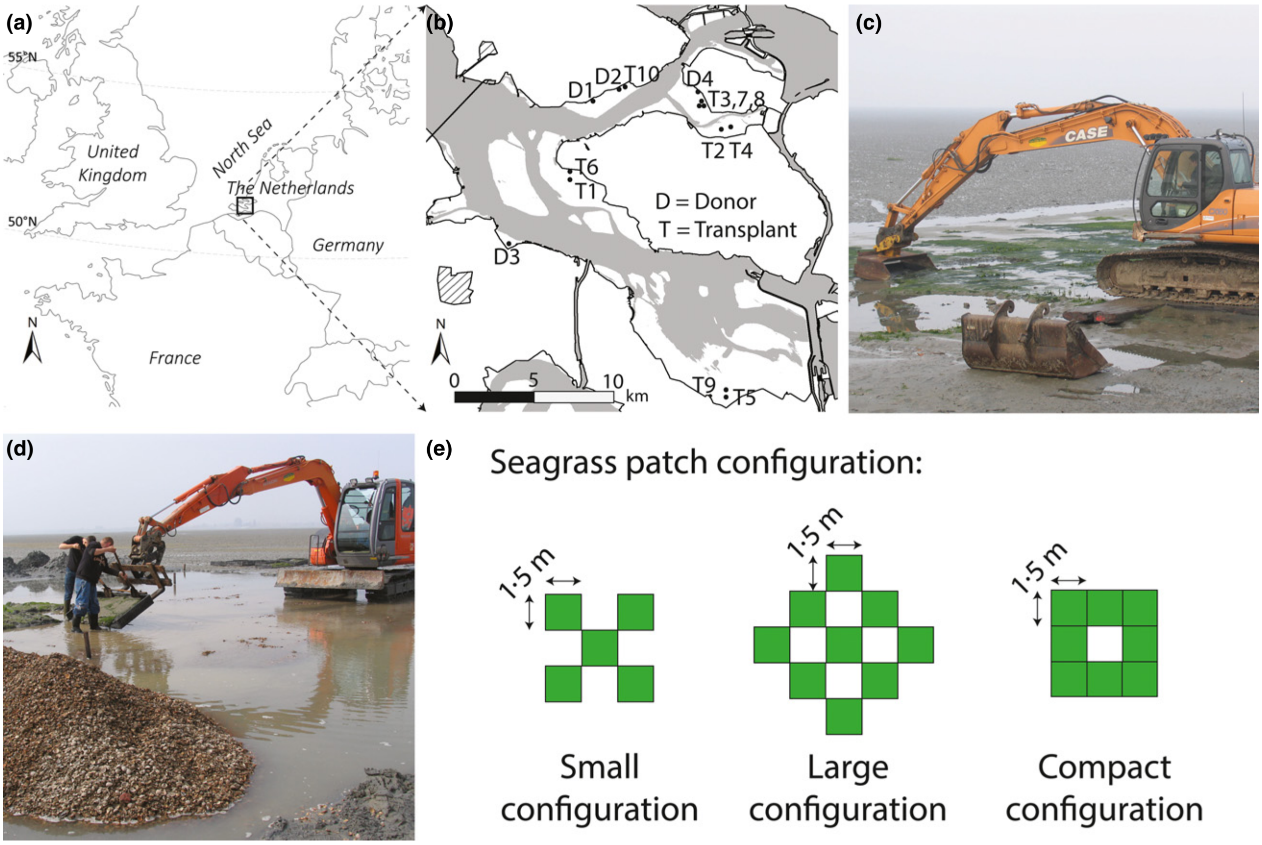

Fig. 1. Experimental set-up. (a) Location of the Oosterschelde sea inlet (study area) in the south-western Netherlands, north-western Europe. Mean tidal range is 3·5 m. (b) Overview of donor (D) and transplant (T) sites within the study area (c). Mechanical seagrass harvest in custom-made boxes. (d)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

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 × 0·75 × 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 × 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 2 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 metre 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). First, to test the potential positive feedback caused by shoot density and below-ground integrity, we compared the effect of bare-root vs. sod transplants (H1a). We transplanted 225 plant fragments (rhizome fragments several centimetres in size with appending shoots) in eight 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). It should be noted that 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 vs. 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, that is desiccation stress by manipulating the initial elevation of transplanted sods. Half of the plots of transplant T10 (eight 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 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 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., San Jose, CA, USA), and repeated-measures ANOVAS were performed using IBM SPSS Statistics 21 (Armonk, NY, USA).

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, $F(2, 45) = 2{\cdot}803$, $P = 0{\cdot}071$). 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 vs. 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, $t(31) = -2{,}374$, $P = 0{\cdot}024$; 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, $F(1, 84) = 5{\cdot}494$, $P = 0{\cdot}02$, data: T1–T6, factors:

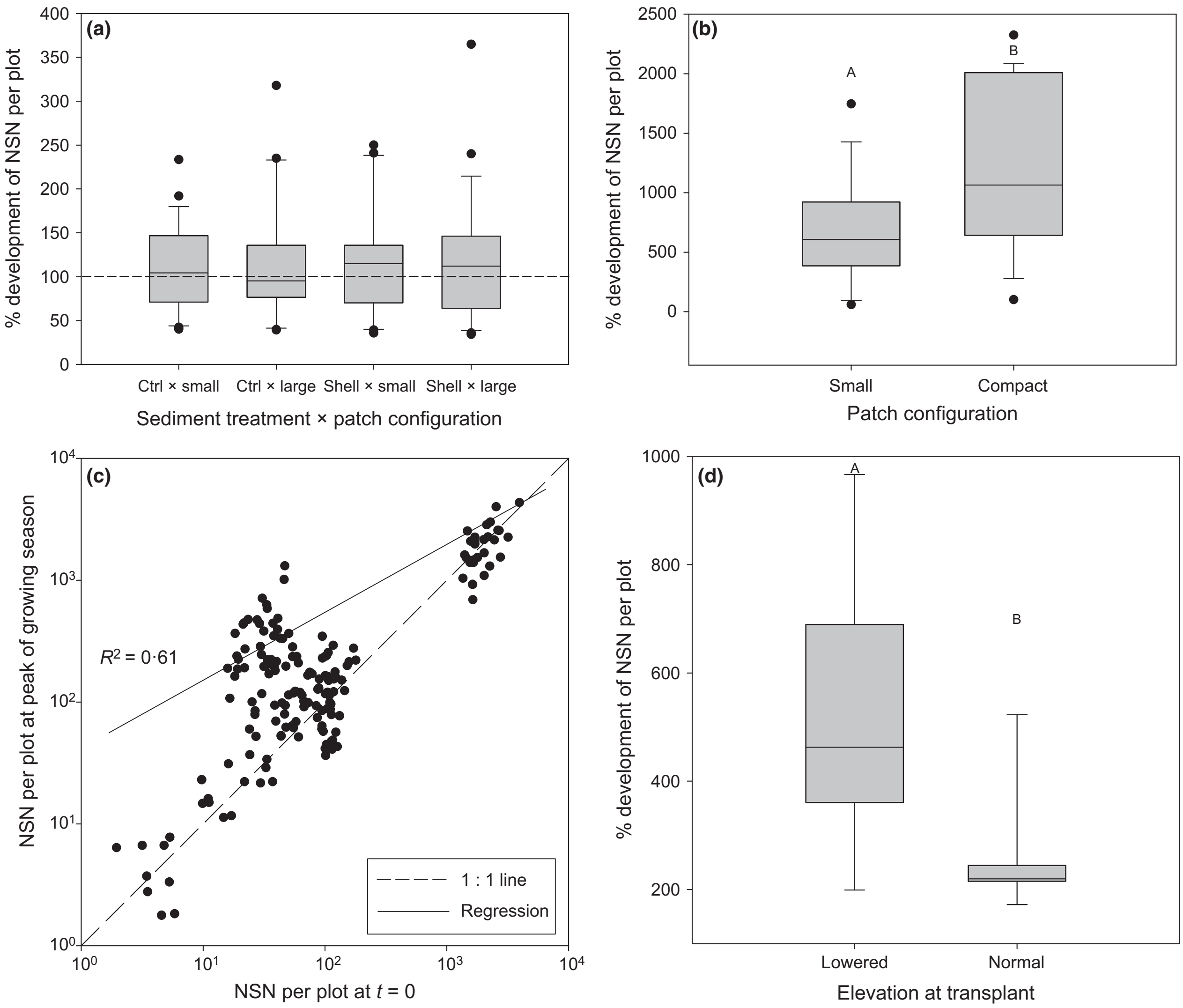

Fig. 2. Short-term (1st growing season) development of transplanted seagrass sods presented as the relative decrease/increase in the normalized shoot numbers (NSNs) per plot. NSN = total numbers of shoots per plot divided by the transplanted area at $t = 0$. Box–whisker plots: box shows the 25th/75th 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 ($P < 0{\cdot}05$). (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, $P = 0{\cdot}024$). (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 and lowered, $n = 8$, for each) (t-test, $P < 0{\cdot}028$).

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-test* = 11·0, $n_1 = n_2 = 8$, $P = 0{\cdot}028$). 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, $F(1, 88) = 0{\cdot}013$, $P = 0{\cdot}910$).

LONG-TERM TRANSPLANTATION RESULTS

After the expansion during the first growing season, seagrass transplants lost most of their above-ground 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

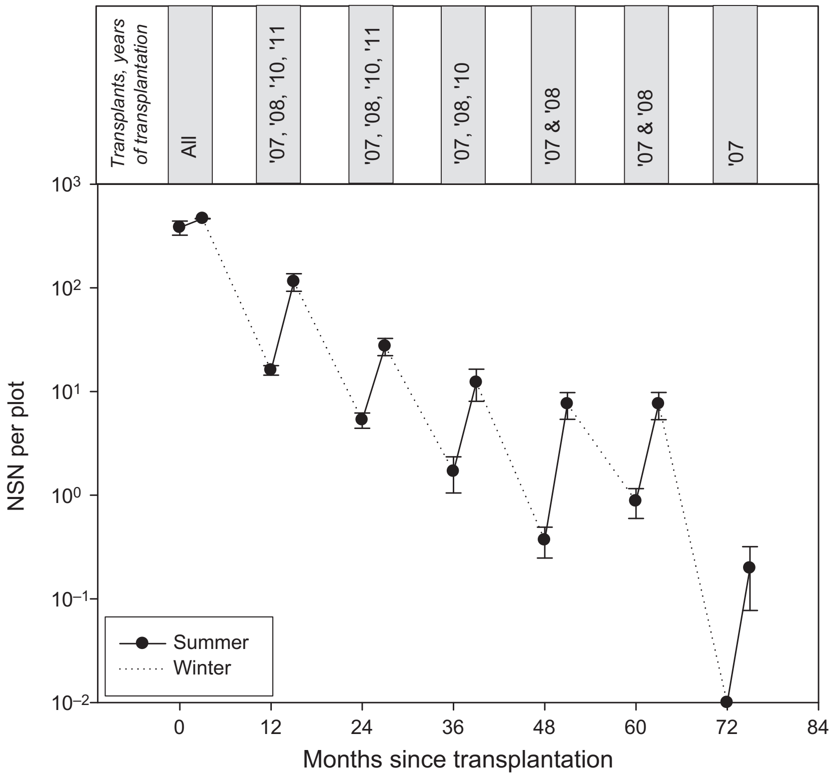

Fig. 3. Overall long-term development of seagrass transplants. Mean normalized shoot numbers (NSNs) per plot ± standard errors are presented by black dots, and the growing seasons are indicated by the solid lines. Note that the transplant efforts were not performed in the same year. As a result, the 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 6 years.

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 (Fig. 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 (Fig. 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, $F = 2{\cdot}06$, $P = 0{\cdot}139$; Small vs. Large configuration, $F = 0{\cdot}68$, $P = 0{\cdot}493$; Small vs. Compact configuration, $F = 0{\cdot}40$, $P = 0{\cdot}679$, Fig. 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, $t(90) = -2{\cdot}015$, $P = 0{\cdot}047$, third growing season Mann–Whitney U-test = 707·5, $n_1 = n_2 = 46$, $P = 0{\cdot}005$). 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 with the shoot numbers at the start of that season ($R^2 = 0{\cdot}805$, 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 ($R^2 = 0{\cdot}145$) (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 (W.B.J.T. Giesen, D.J. de Jong, K. Giesen, P.T. Giesen & M.M. van Katwijk, personal observations). These long-term transplantation successes could not be attributed to any characteristic measured or observed, nor to any event

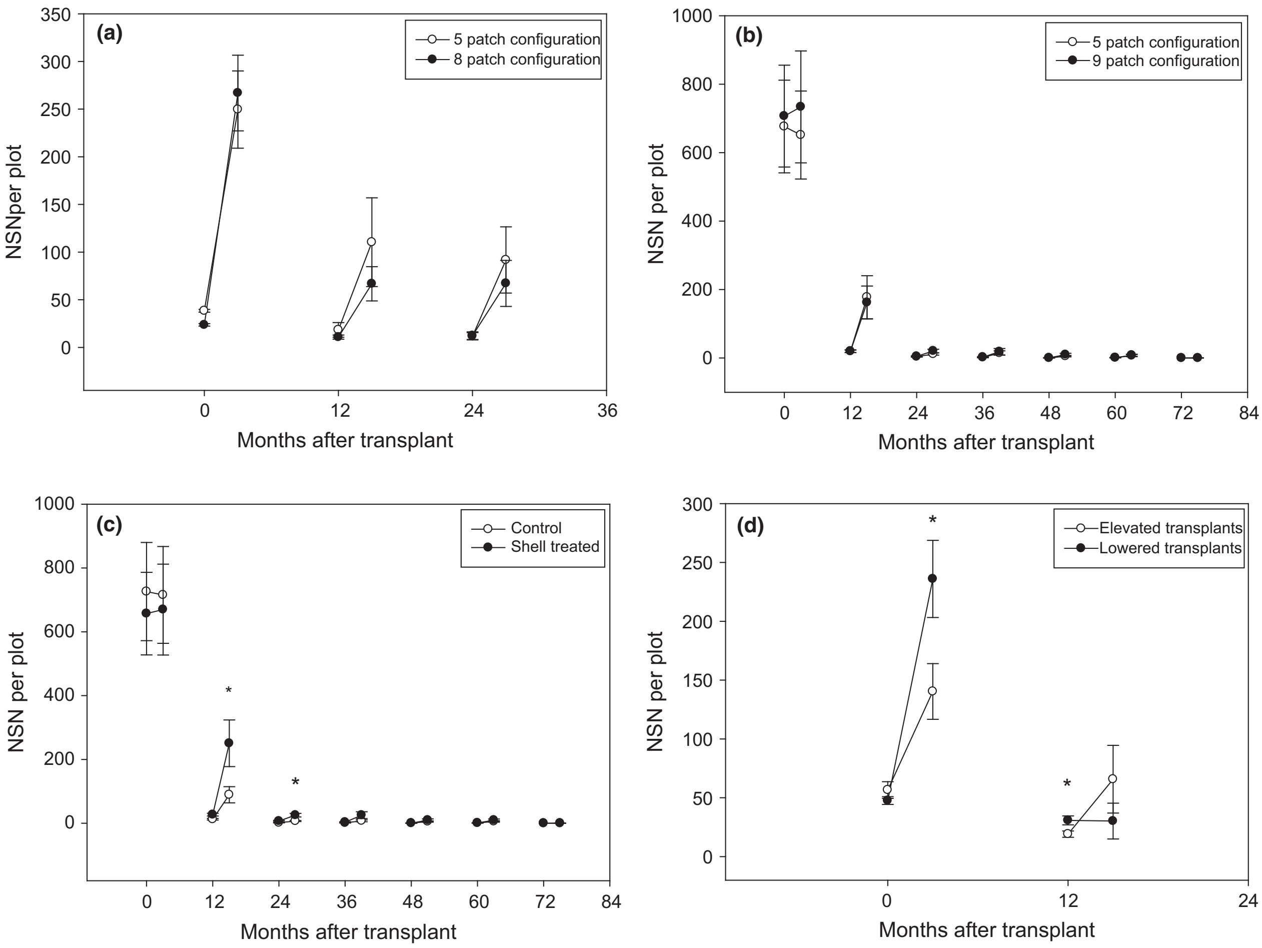

Fig. 4. Effects of patch configuration and sediment treatments on long-term transplantation successes. (a,b) Mean NSN per plot + standard error of mean (SEM) in time for T9 (Panel a, n = 15 for the 5-patch and n = 18 for 8-patch configuration) and for T1–T6 transplants (panel b, n = 46 per configuration). (c) Effect shell treatment improving sediment stability by excluding adult lugworms. Mean NSN per plot + SEM for T1–T6 transplants (n = 46 per treatment) * indicates 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). * indicates significant difference at that particular time step (t-test, t = 3, P = 0·033, t = 12, P = 0·024).

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 *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 in 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 is, 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 upscaling 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, 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 with 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, van Nes & Scheffer (2015), although this may not be (economically) feasible.

EXTRINSIC FORCING IN THE TRANSPLANTED SODS

In our system, minimizing extrinsic stress, that is 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, that is 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 VS. WINTER SURVIVAL OF TRANSPLANTED SODS

Seagrass abundance at the peak of the growing season (September) generally correlated well with the seagrass abundance in June of the same year ($R^2 = 0{\cdot}805$). 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 ($R^2 = 0{\cdot}145$). 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, such as 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, pore water 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), and 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, Herman & Bouma (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*. 2016).

SYNTHESIS AND APPLICATIONS

Although sod transplantation shows significantly better initial survival than transplanting individual plants, further upscaling 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 $2{\cdot}25\ m^2$), 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).

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Data accessibility

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

References

Balke, T., Herman, P.M.J. & Bouma, T.J. (2014) Critical transitions in disturbance-driven ecosystems: identifying Windows of Opportunity for recovery. *Journal of Ecology*, **102**, 700–708.

Boese, B.L., Robbins, B.D. & Thursby, G. (2005) Desiccation is a limiting factor for eelgrass (*Zostera marina* L.) distribution in the intertidal zone of a northeastern Pacific (USA) estuary. *Botanica Marina*, **48**, 274–283.

Bos, A.R., Bouma, T.J., de Kort, G.L.J. & van Katwijk, M.M. (2007) Ecosystem engineering by annual intertidal seagrass beds: sediment accretion and modification. *Estuarine Coastal and Shelf Science*, **74**, 344–348.

Bouma, T.J., Ortells, V. & Ysebaert, T. (2009) Comparing biodiversity effects among ecosystem engineers of contrasting strength: macrofauna diversity in *Zostera noltii* and *Spartina anglica* vegetations. *Helgoland Marine Research*, **63**, 3–18.

Calumpong, H.P. & Fonseca, M.S. (2001) Seagrass transplantation and other seagrass restoration methods. *Global Seagrass Research Methods* (eds F.T. Short, R.G. Coles & C.A. Short), pp. 425–443. Elsevier Science, Amsterdam.

Carr, J.A., D'Odorico, P., McGlathery, K.J. & Wiberg, P.L. (2012) Stability and resilience of seagrass meadows to seasonal and interannual dynamics and environmental stress. *Journal of Geophysical Research: Biogeosciences*, **117**, G01007, doi:10.1029/2011JG001744.

Christianen, M.J.A., van Belzen, J., Herman, P.M.J., van Katwijk, M.M., Lamers, L.P.M., van Leent, P.J.M. & Bouma, T.J. (2013) Low-canopy seagrass beds still provide important coastal protection services. *PLoS One*, **8**, e62413.

Christianen, M.J.A., Herman, P.M.J., Bouma, T.J., Lamers, L.P.M., van Katwijk, M.M., van der Heide, T. *et al.* (2014) Habitat collapse due to overgrazing threatens turtle conservation in marine protected areas. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20132890.

Crain, C.M. & Bertness, M.D. (2006) Ecosystem engineering across environmental gradients: implications for conservation and management. *BioScience*, **56**, 211–218.

Folkard, A.M. (2005) Hydrodynamics of model *Posidonia oceanica* patches in shallow water. *Limnology and Oceanography*, **50**, 1592–1600.

Folkard, A.M. (2011) Flow regimes in gaps within stands of flexible vegetation: laboratory flume simulations. *Environmental Fluid Mechanics*, **11**, 289–306.

Giesen, W.B.J.T., Giesen, K., Giesen, P.T., Govers, L.L., Suykerbuyk, W. & van Katwijk, M.M. (2012) Eindrapport Zeegrasmitigaties Oosterschelde. Proeven met verplaatsen van klein zeegras Zostera noltii in de Oosterschelde: mitigatiemaatregel bij toekomstige dijkwerkzaamheden ZLD-6606A.

Gillis, L.G., Bouma, T.J., Jones, C.G., van Katwijk, M.M., Nagelkerken, I., Jeuken, C.J.L., Herman, P.M.J. & Ziegler, A.D. (2014) Potential for landscape-scale positive interactions among tropical marine ecosystems. *Marine Ecology Progress Series*, **503**, 289–303.

Govers, L.L., de Brouwer, J.H.F., Suykerbuyk, W., Bouma, T.J., Lamers, L.P.M., Smolders, A.J.P. & van Katwijk, M.M. (2014) Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. *Aquatic Toxicology*, **155**, 253–260.

Govers, L.L., Suykerbuyk, W., Hoppenreijs, J., Giesen, K., Bouma, T.J. & Katwijk, M.M.V. (2015) Rhizome starch as indicator for temperate seagrass winter survival. *Ecological Indicators*, **49**, 53–60.

Halpern, B.S., Silliman, B.R., Olden, J.D., Bruno, J.P. & Bertness, M.D. (2007) Incorporating positive interactions in aquatic restoration and conservation. *Frontiers in Ecology and the Environment*, **5**, 153–160.

Han, Q.Y., Bouma, T.J., Brun, F.G., Suykerbuyk, W. & van Katwijk, M.M. (2012) Resilience of *Zostera noltii* to burial or erosion disturbances. *Marine Ecology Progress Series*, **449**, 133–143.

Hastings, A., Byers, J.E., Crooks, J.A., Cuddington, K., Jones, C.G., Lambrinos, J.G., Talley, T.S. & Wilson, W.G. (2007) Ecosystem engineering in space and time. *Ecology Letters*, **10**, 153–164.

van der Heide, T., van Nes, E.H., Geerling, G.W., Smolders, A.J.P., Bouma, T.J. & van Katwijk, M.M. (2007) Positive feedbacks in seagrass ecosystems: implications for success in conservation and restoration. *Ecosystems*, **10**, 1311–1322.

van der Heide, T., Smolders, A., Rijkens, B., van Nes, E.H., van Katwijk, M.M. & Roelofs, J. (2008) Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. *Oecologia*, **158**, 411–419.

van der Heide, T., Bouma, T.J., van Nes, E.H., van de Koppel, J., Scheffer, M., Roelofs, J.G.M., van Katwijk, M.M. & Smolders, A.J.P. (2010) Spatial self-organized patterning in seagrasses along a depth gradient of an intertidal ecosystem. *Ecology*, **91**, 362–369.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994) Organisms as ecosystem engineers. *Oikos*, **69**, 373–386.

van Katwijk, M.M., Bos, A.R., de Jonge, V.N., Hanssen, L., Hermus, D.C.R. & de Jong, D.J. (2009) Guidelines for seagrass restoration: importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Marine Pollution Bulletin*, **58**, 179–188.

van Katwijk, M.M., Thorhaug, A., Marbà, N., Orth, R.J., Duarte, C.M., Kendrick, G.A. *et al.* (2016) Global analysis of seagrass restoration: the importance of large-scale planting. *Journal of Applied Ecology*, **53**, 567–578

van de Koppel, J., Herman, P.M.J., Thoolen, P. & Heip, C.H.R. (2001) Do alternate stable states occur in natural ecosystems? Evidence from a tidal flat. *Ecology*, **82**, 3449–3461.

van de Leemput, I.A., van Nes, E.H. & Scheffer, M. (2015) Resilience of alternative states in spatially extended ecosystems. *PLoS One*, **10**, e0116859.

Leuschner, C., Landwehr, S. & Mehlig, U. (1998) Limitation of carbon assimilation of intertidal *Zostera noltii* and Z-marina by desiccation at low tide. *Aquatic Botany*, **62**, 171–176.

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C. *et al.* (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, **312**, 1806–1809.

Louters, T., van den Berg, J.H. & Mulder, J.P.M. (1998) Geomorphological changes of the Oosterschelde tidal system during and after the implementation of the delta project. *Journal of Coastal Research*, **14**, 1134–1151.

Madsen, J.D., Chambers, P.A., James, W.F., Koch, E.W. & Westlake, D.F. (2001) The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia*, **444**, 71–84.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L. *et al.* (2006) A global crisis for seagrass ecosystems. *BioScience*, **56**, 987–996.

Orth, R.J., Marion, S.R., Moore, K.A. & Wilcox, D.J. (2010) Eelgrass (*Zostera marina* L.) in the Chesapeake Bay region of Mid-Atlantic coast of the USA: challenges in conservation and restoration. *Estuaries and Coasts*, **33**, 139–150.

Reise, K. (2002) Sediment mediated species interactions in coastal waters. *Journal of Sea Research*, **48**, 127–141.

Rietkerk, M., Dekker, S.C., de Ruiter, P.C. & van de Koppel, J. (2004) Self-organized patchiness and catastrophic shifts in ecosystems. *Science*, **305**, 1926–1929.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C. & Walker, B. (2001) Catastrophic shifts in ecosystems. *Nature*, **413**, 591–596.

Short, F.T., Davis, R.C., Kopp, B.S., Short, C.A. & Burdick, D.M. (2002) Site-selection model for optimal transplantation of eelgrass *Zostera marina* in the northeastern US. *Marine Ecology Progress Series*, **227**, 253–267.

Suding, K.N. (2011) Toward an era of restoration in ecology: successes, failures, and opportunities ahead. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 465–487.

Suding, K.N. & Hobbs, R.J. (2009) Threshold models in restoration and conservation: a developing framework. *Trends in Ecology & Evolution*, **24**, 271–279.

Suykerbuyk, W. & van Katwijk, M.M. (2015) Data from: Unpredictability in seagrass restoration: analysing the role of positive feedback and environmental stress on Zostera noltii transplants. DANS, http://dx.doi.org/10.17026/dans-234-6epm.

Suykerbuyk, W., Bouma, T.J., van der Heide, T., Faust, C., Govers, L.L., Giesen, W.B.J.T., de Jong, D.J. & van Katwijk, M.M. (2012) Suppressing antagonistic bioengineering feedbacks doubles restoration success. *Ecological Applications*, **22**, 1224–1231.

Vermaat, J.E. & Verhagen, F.C.A. (1996) Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem: coupling demographic and physiological patterns. *Aquatic Botany*, **52**, 259–281.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S. *et al.* (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 12377–12381.

Wetsteyn, L. & Kromkamp, J.C. (1994) Turbidity, nutrients and phytoplankton primary production in the oosterschelde (the netherlands) before, during and after a large-scale coastal engineering project (1980–1990). *Hydrobiologia*, **283**, 61–78.

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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.

Fig. S1. Transplants experimental design: visual.

Fig. S2. Effect of treatments and configuration on short-term seagrass survival.

Fig. S3. Detailed and visual long-term transplantation results of two successful transplants.

Fig. 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.