RESEARCH ARTICLE

# Spatial design improves efficiency and scalability of seed-based seagrass restoration

Max L. E. Gräfnings[1]  |  Jannes H. T. Heusinkveld[2]  |  Nadia Hijner[1]  |
Dieuwke J. J. Hoeijmakers[2]  |  Quirin Smeele[3]  |  Maarten Zwarts[2]  |  Tjisse van der Heide[1,4]  |
Laura L. Govers[1,4]

[1]Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, PO Box 11103m 9700 CC, Groningen, The Netherlands

[2]The Fieldwork Company, Stockholmstraat 2B, 9723BC, Groningen, The Netherlands

[3]Natuurmonumenten, Hoofdweg 251B, 9765 CH, Paterswolde, The Netherlands

[4]Department of Coastal Systems, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB, Den Burg, The Netherlands

**Correspondence**
Max L. E. Gräfnings
Email: maxgrafnings@gmail.com

**Funding information**
Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Number: 016.veni.181.087; Waddenfonds

**Handling Editor:** Emanuela Weidlich

## Abstract

1. Coastal ecosystem restoration is often ineffective and expensive in practice. As a consequence, upscaling restoration efforts to functionally relevant spatial scales remains one of the largest hurdles for coastal restoration practice. On small scales, restoration success of vegetated ecosystems (i.e. salt marshes and seagrasses) can be amplified by spatial designs that harness positive interactions. However, it remains unknown if positive interactions can be harnessed with seed-based approaches, that are considered to be more cost-effective and scalable than traditional shoot-based restoration methods.

2. Here, we investigated with a full-factorial seeding experiment if (1) restoration scale (4, 40 and 400 m$^2$) and (2) seeding density (10 and 50 injections/m$^2$) affected multi-year recruitment efficiency (measured as *restored plants/seed injection*) of annual eelgrass *Zostera marina* in the Dutch Wadden Sea.

3. We found that the largest restoration scale (400 m$^2$) increased second-generation recruitment efficiency by suppressing a sedimentation-related negative feedback. With increased restoration scale, the inner parts of the restoration plots captured less sediment, which decreased the desiccation stress of the restored eelgrass during low tide. Due to this stress alleviation, plants grew larger and produced more seed-bearing spathes, which the following year resulted in two and three times higher recruitment efficiency at the largest restoration scale compared to the smaller scales. Moreover, lower seeding density more than doubled second-generation recruitment efficiency compared to the higher density, supporting recent work showing that the effectiveness of 'clumped' spatial designs is context dependent.

4. *Synthesis and applications*. The efficiency of restoration efforts is seldom taken into account, but can offer restoration projects a valuable metric with which workload, donor material and cost-requirements can be reduced. We demonstrate

---

Tjisse van der Heide and Laura L. Govers contributed equally and share last authorship.

that simple modifications to seed-based coastal restoration designs (e.g. scale and density) can have a substantial impact on recruitment efficiency and multi-year restoration yields. Thus, optimised restoration designs can strongly contribute to the upscaling potential of coastal ecosystem restoration. However, optimal restoration designs are expected to be strongly context dependent and we therefore argue that investigating optimal designs should be adopted as common practice, providing a crucial steppingstone between 'proof-of-concepts' and true large-scale restoration attempts ($km^2$).

**KEYWORDS**

ecological restoration, ecosystem engineer, intraspecific feedback, recruitment efficiency, restoration design, scalability, seagrass, seed based

## 1 | INTRODUCTION

Coastal ecosystems (e.g. seagrass meadows, coral reefs, mangroves, saltmarshes) form the foundation of our coastal seas, supporting highly productive and diverse communities (De Fontaubert et al., 1996; Duarte & Chiscano, 1999; Roberts et al., 2002). In addition to their ecological importance, coastal systems provide humans with a multitude of valuable ecosystem services ranging from coastal protection and fisheries production to carbon sequestration (Barbier et al., 2011). At the same time, these systems rank among the most threatened ecosystems on the globe and have experienced alarming losses during the last century (Halpern et al., 2008). The dramatic degradation of coastal ecosystems has made their conservation a pressing topic for scientists and managers alike. Mitigating anthropogenic threats is a crucial step to aid the recovery of degraded natural systems, but studies have shown that passive conservation initiatives on their own may be insufficient to halt or reverse trajectories of change (Jones et al., 2018; Perrow & Davy, 2002). Active restoration (i.e. the process of actively assisting the recovery of an ecosystem; SER, 2004) is considered a promising supplement to passive conservation initiatives. Thus far, however, restoration success rates of coastal ecosystems have generally been low (Bayraktarov et al., 2016). In addition to low survival rates, coastal restoration trials often struggle with scalability, both in the sense of practicalities (e.g. unscalable methods and insufficient donor material) and insurmountable costs (Bayraktarov et al., 2016; Saunders et al., 2020). As such, upscaling restoration efforts to functionally/ecologically relevant scales remains one of the largest hurdles for coastal restoration efforts (Abelson et al., 2020). Hence there is an urgent need for more efficient, scalable and economically viable restoration methods and strategies.

Coastal restoration ecology is a young discipline compared to its terrestrial counterpart, and successful restoration practices from land usually do not translate directly to the marine realm (Gedan & Silliman, 2009; Silliman et al., 2015). Terrestrial restoration designs often aim to minimise competition between restored individuals, while this strategy is often ineffective in the coastal zone where stress reduction (e.g. wave attenuation) has been shown to be more

important (Silliman et al., 2015). Coastal ecosystem engineers often depend on positive interactions (i.e. self-facilitation and mutualism) to establish and persist in the dynamic coastal zone (Maxwell et al., 2017; van der Heide et al., 2007). Growing evidence suggests that incorporating such interactions into restoration designs may improve restoration outcomes of coastal ecosystems (Renzi et al., 2019; Silliman et al., 2015; Temmink et al., 2020; Zhang et al., 2018). A major benefit of this strategy is that positive interactions can be triggered with small adjustments to planting designs, making the strategy more efficient and very cost-effective (Saunders et al., 2020 and references within). For example, Silliman et al. (2015) showed that clumping saltmarsh transplants close together instead of spreading them sparsely increased restoration yields, without increasing costs or donor material usage. Although promising (Temmink et al., 2022), restoration designs that incorporate facilitation mechanisms have mostly been tested on small scales in the marine realm and the question remains whether positive interactions can also effectively be harnessed in large-scale restoration efforts (Renzi et al., 2019).

For the restoration of coastal plants (e.g. seagrasses, mangroves and saltmarshes), restoration methods that use propagules (e.g. seeds or fruits) are typically considered more scalable than methods that rely on established plants (e.g. saplings or transplants), because they are less expensive and logistically more feasible to use (Kettenring & Tarsa, 2020 and references within). Additionally, these methods can be upscaled more sustainably, as large amounts of donor material can be collected without harming donor sites. However, to be able to restore plants on very large scales (100 to 1000-$km^2$) massive amounts of donor material are needed (for seeds in the order of tens to hundreds of metric tons, Merritt & Dixon, 2011). The massive volumes needed are partly due to the high seed losses (>90%) that often plague restoration efforts (Merritt & Dixon, 2011), illustrating the need for increased efficiency of restoration efforts. To date, most seed-based restoration efforts have used relatively simple methods, such as hand broadcasting or other methods that rely on the natural dispersal of the seeds (Orth et al., 2012; Vanderklift et al., 2020). In calm conditions, methods that rely on passive dispersal can be very successful, allowing the restoration of large areas (e.g. Orth et al., 2020). However, these methods are less likely to be successful in harsher environments
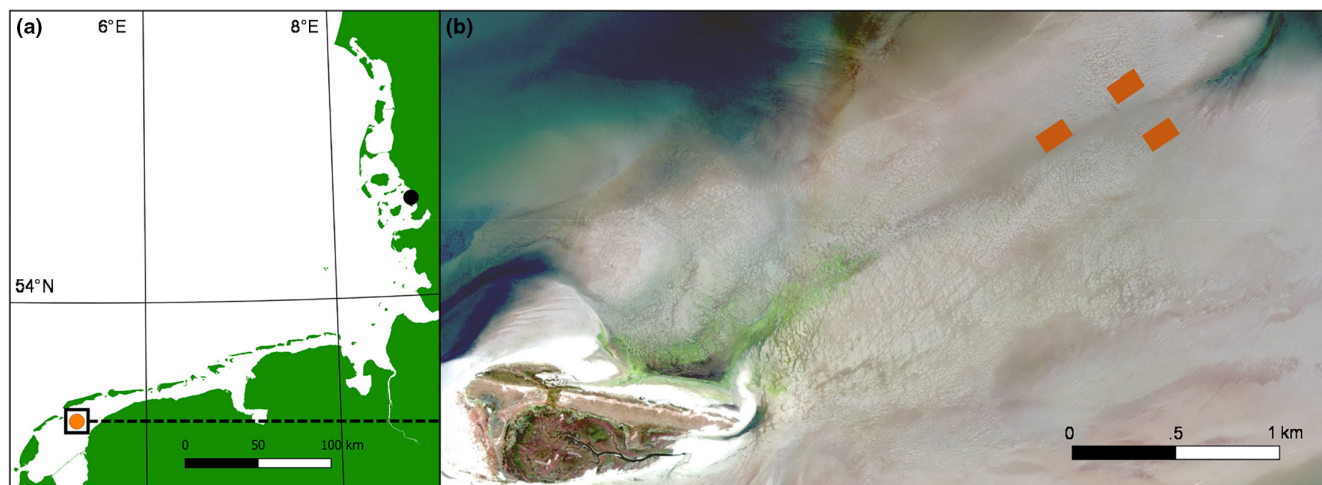
**FIGURE 1** (a) Location of the experimental site (orange) and donor site (black) in the Wadden Sea, (b) satellite photo (July 2020, Netherlands Space Office) of the Griend mudflat with the three experimental blocks highlighted.

where seeds have a higher chance to wash away from the target area or where the plants' long-term survival is dependent on self-facilitation (Govers et al., 2022; van Katwijk et al., 2016). Even so, seed-based restoration trials have rarely (if ever) specifically targeted the incorporation of positive interactions (i.e. facilitation and mutualism) into their designs. Hence, the feasibility of combining seed-based methods with harnessing strategies is uncertain. If positive interactions can be incorporated into seed-based restoration designs it might prove a promising way forward, combining efficiency with scalability.

Here, we investigated if positive interactions can be triggered with specific seeding designs, allowing (more) efficient upscaling of eelgrass *Zostera marina* restoration. Specifically, we studied if (1) restoration scale (4, 40 and 400 m²) and (2) seeding densities (10 and 50 injections/m²) affected recruitment success (adult eelgrass plants/m²) and efficiency (restored plants/seed injection) of annual intertidal eelgrass in the Dutch Wadden Sea. Naturally, the long-term restoration success of annual plants does not merely depend on initial recruitment and survival, but also on successful sexual reproduction and consequent establishment of following generations. Therefore, we followed the restoration yields of both the first (actively seeded) and second generations (established from seeds produced by first-generation plants). We expected self-facilitation mechanisms to be of importance in the dynamic intertidal, as these mechanisms have generally been shown to increase in importance with harsher environmental conditions (Bos & van Katwijk, 2007; Bouma et al., 2009; Maxwell et al., 2017). Larger restoration scales have previously been shown to benefit seagrasses (Paulo et al., 2019; van Katwijk et al., 2016), although the underlying mechanisms of the increased successes are not always clear. Potentially, restored seagrass patches need to reach certain scales to be able to sufficiently modify their environment like natural meadows, improving their own growing conditions and easing the transition from a unvegetated stable state to vegetated (van der Heide et al., 2007). In addition, higher seagrass densities have been shown to increase seed fertilisation and consequently sexual reproduction (Valdez et al., 2020; van Tussenbroek et al., 2016) and we therefore hypothesised that

higher initial densities would increase multi-year restoration yields of our annual plants. Additionally, higher eelgrass densities were expected to increase the survivability of the restored plants (Bos & van Katwijk, 2007).

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

The Wadden Sea is the largest intertidal system in the world, that stretches out from the northern Netherlands to southwestern Denmark. Here, eelgrass beds experienced massive die-offs during the 20th century, due to a wasting disease and anthropogenic impacts (e.g. eutrophication and coastal development; Giesen et al., 1990). This seagrass restoration experiment was carried out over 16 months in 2020–2021 (covering two growing seasons and a winter) in the Dutch Wadden Sea. The experiment was performed on an intertidal sandflat northeast of the island Griend (N 53.2692, E 5.2949; Figure 1), sheltered from wave action and with ~6 h emergence time per 12 h tidal cycle. Permission for field access was granted by the province of Friesland and Natuurmonumenten. The restoration site was selected because previous restoration trials in 2018 and 2019 were very successful at the site (Gräfnings et al., 2022). Initially, the site was selected because spontaneous establishment of dwarf eelgrass *Zostera noltii* and *Ruppia maritima* was observed at the site between 2014 and 2017. Appropriate ethics, permits and other approvals were obtained for the research included in this manuscript.

### 2.2 | Seed collection and storage

Seeds of annual intertidal eelgrass *Z. marina* were collected in September 2019 from a healthy intertidal seagrass meadow in the German Wadden Sea (Hamburger Hallig; N 54.5986, E 8.8111; Figure 1a). Permission to harvest seagrass seeds was granted by

*Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz Schleswig-Holstein*. Seed harvest, treatment and storage were performed in similar fashion as described in Govers et al. (2022), including treating the seeds with copper sulphate (0.2 ppm) to counteract *Phytophthora* spp. infection (Govers et al., 2017).

## 2.3 | Experimental setup and monitoring

With a full factorial field experiment, we tested how restoration scale (4, 40 and 400 m²) and seeding densities (10 and 50 injections/m²) affected first-generation (eelgrass recruitment success, recruitment efficiency, plant dry weight and reproductive success) and second-generation (eelgrass recruitment success and recruitment efficiency) restoration outcomes. The experiment (plot dimensions: 20×20 m, 6.3×6.3 m and 2×2 m) was constructed in a randomised block design (3 blocks, 18 plots in total), in sandy areas that were unvegetated prior to this experiment. Plots were spaced ~20 m apart and blocks 200–300 m apart (Figure 1b). The elevation of the contour of each plot was measured with a rtk-dGPS (Trimble; eight measurement points for the 400 m² plots and four points for the two smaller scales). All experimental plots were located at a similar elevation (+30±1.2 cm NAP, Dutch ordinance level, close to the Dutch mean sea level). Seagrass seeding was performed in March 2020 with the Dispenser Injection Seeding-method (DIS; Govers et al., 2022; Gräfnings et al., 2022). Seeds were injected to a depth of 3 cm and each injection contained ~3 seeds. In order to space injections evenly and in desired densities metal grids (size: 1×1 m, grid size: 0.1×0.1 m) were used during seeding. In total 240,000 seeds were sown in a 2664 m² area.

In July 2020, we estimated recruitment success by counting the number of adult eelgrass plants in four random squares (1 m²) in each experimental plot. All the restored eelgrass plants were annual and clearly separated from each other, which made the identification of individual plants possible. Recruitment of annual plants includes three basic processes: seed germination, seedling survivorship and seedling growth and as the counted plants all had survived these perilous stages, we could assume successful recruitment. The eelgrass plants had visibly trapped sediment and therefore rtk-dGPS measurements were taken in each plot (approximately 80, 25 and 10 measurements were taken evenly spaced in the 400, 40 and 4 m² plots respectively). Additionally, a drone image was taken of each plot during peak low tide (Drone: Dji M210 with X5S camera, 20.5 MP). In August 2020, we harvested five random eelgrass plants from the centre of each plot. In the laboratory, the number of spathes was counted for each plant. Afterwards, plants were dried at 60°C for 48 h. Once dry, the plant material was weighed (g DW⁻ᵖˡᵃⁿᵗ). We were not able to accurately estimate seed numbers per plant since spathes still contained immature seeds in August and when the experiment was revisited 2 weeks later, in early September, most of the seeds had already been released before they could be counted. Seed maturation was most likely very rapid due to a late-summer heat wave (*personal observation*). Hence, spathe numbers were used as an indication of reproductive output (in 2018 we estimated 7.4 developing seeds/spathe at the same location, unpublished

data). To investigate how eelgrass plants affected sedimentation, average elevation change for the full plots was calculated by comparing the average of all dGPS measurements from March (contour measurements) with all measurements from July. Additionally, we calculated elevation change in the centre of each plot, by comparing the average of all dGPS measurements from March and the average of the five most central measurement points/plot in July. To investigate if seagrasses affected low tide mudflat emergence, we first manually highlighted on the drone images areas that were emerged over the water level for each plot, where after we calculated in ImageJ v1.53 how much (%) of the centre (inner 1/9th) and full plot had been emerged.

In August 2021, the experimental plots were revisited and we counted second-generation eelgrass recruitment success in a similar fashion as the year before (# adult eelgrass plants in four random 1 m² squares/plot). This was done to estimate the reproductive success of each treatment, as all plants established in 2021 had their origin from seeds produced by previous year's plants (the annual plants had a 100% mortality rate after the first growing season).

## 2.4 | Recruitment efficiency

In this study, we calculated recruitment efficiency by dividing recruitment success (restored adult eelgrass plants/m²) of the first and second generation with the initial seeding density (injections/m²). This way we acquired an estimate for how many adult eelgrass plants each individual injection resulted in. Thus, recruitment efficiency (restored plants/seed injection) provides a measure for how efficient our treatments (restoration scale + seeding density) were in terms of workload (injections) and donor material usage (~3 seeds/injection).

## 2.5 | Statistical analysis

The experimental data were analysed using linear mixed effect models (R-package: LME4) in R version 4.0.3 (R Core Team, 2020). We analysed how 'Restoration scale', 'Seeding density' and their interaction affected first-generation (eelgrass recruitment success, recruitment efficiency, biomass and spathe production) and second-generation (recruitment success and recruitment efficiency) restoration outcomes. 'Block' was included as a random factor in the models, except when analysing second-generation recruitment success to avoid an overfitted model. Datapoints for each plot were nested together in the random factor to account for pseudo-replication. For each model, a backward stepwise regression based on Akaike's information criterion was used to find the minimal adequate model (Table S1). Residuals of the linear mixed effect models were checked for normality and, if necessary, transformed to fit model assumptions (Table S1). An ANOVA from the CAR package (Fox & Weisberg, 2011) was applied to conduct the Wald chi-squared test on model outputs. When necessary, we performed Tukey post-hoc tests (EMMEANS package) to determine which of the three restoration scales differed significantly from each other. The Tukey post-hoc tests did not always detect differences between restoration scales although

the overarching model did and we therefore also ran the more liberal Fisher's least significant difference (LSD; i.e. no correction of the significance level) to highlight weaker differences.

Further analyses were performed to explore if sedimentation and mudflat emergence over low tide inundation could explain differences in restoration yields and eelgrass fitness between treatments. We analysed how 'Restoration scale', 'Seeding density' and their interaction affected elevation change (proxy for sedimentation) and mudflat emergence % in the centre and full area of each plot from March to July 2020. 'Block' was included as a random factor in all models. The models were optimised in similar fashion as explained above. Additionally, linear regressions were performed to investigate if differences in eelgrass biomass and spathe production could be explained with (1) elevation change and (2) mudflat emergence % in the centre of the restoration plots. All figures show untransformed data.

## 3 | RESULTS

### 3.1 | Recruitment success and efficiency

The higher seeding density (50 injects/m$^2$) increased recruitment success (adult eelgrass plants/m$^2$) for the first generation with 433% compared to the lower density (10 injects/m$^2$, chi$^2$=82.51, $df$=1, $p$<0.001; Figure 2a). This was slightly lower than expected as the difference

between the two seeding densities was fivefold. Neither restoration scale nor the interaction between the two examined variables affected recruitment. Recruitment efficiency (measured as restored plants/seed injection) was significantly affected by restoration scale (chi$^2$=82.51, $df$=1, $p$=0.015; Figure 2b). The initial pairwise comparison (Tukey) was not able to detect significant differences between the restoration scales, but an LSD-based comparison revealed a significant ($p$<0.05) increase in efficiency at the largest restoration scale (400 m$^2$; 0.57 plants/injection ±0.03 SEM) compared to the smallest scale (4 m$^2$; 0.43 plants/injection ±0.04 SEM). Recruitment efficiency of the first generation was not significantly affected by seeding density or the interaction between the two examined variables.

Second-generation recruitment success was also positively affected by higher seeding density (chi$^2$=5.49, $df$=1, $p$=0.019; Figure 2c), but eelgrass densities were only 182% higher than in the plots seeded with 10 injections/m$^2$. Additionally, recruitment was strongly affected by restoration scale (chi$^2$=21.71, $df$=2, $p$<0.001; Figure 2c), with the largest scale (400 m$^2$) harbouring 355% higher eelgrass densities than the 4 m$^2$ plots ($p$<0.01). The interaction between restoration scale and seeding density did not significantly affect second-generation recruitment success. Second-generation recruitment efficiency was strongly affected by both the initial seeding density (chi$^2$=25.89, $df$=1, $p$<0.001) and restoration scale (chi$^2$=38.25, $df$=2, $p$<0.001; Figure 2c), but not their interaction. Lower initial seeding density (10 injections/m$^2$) more than doubled
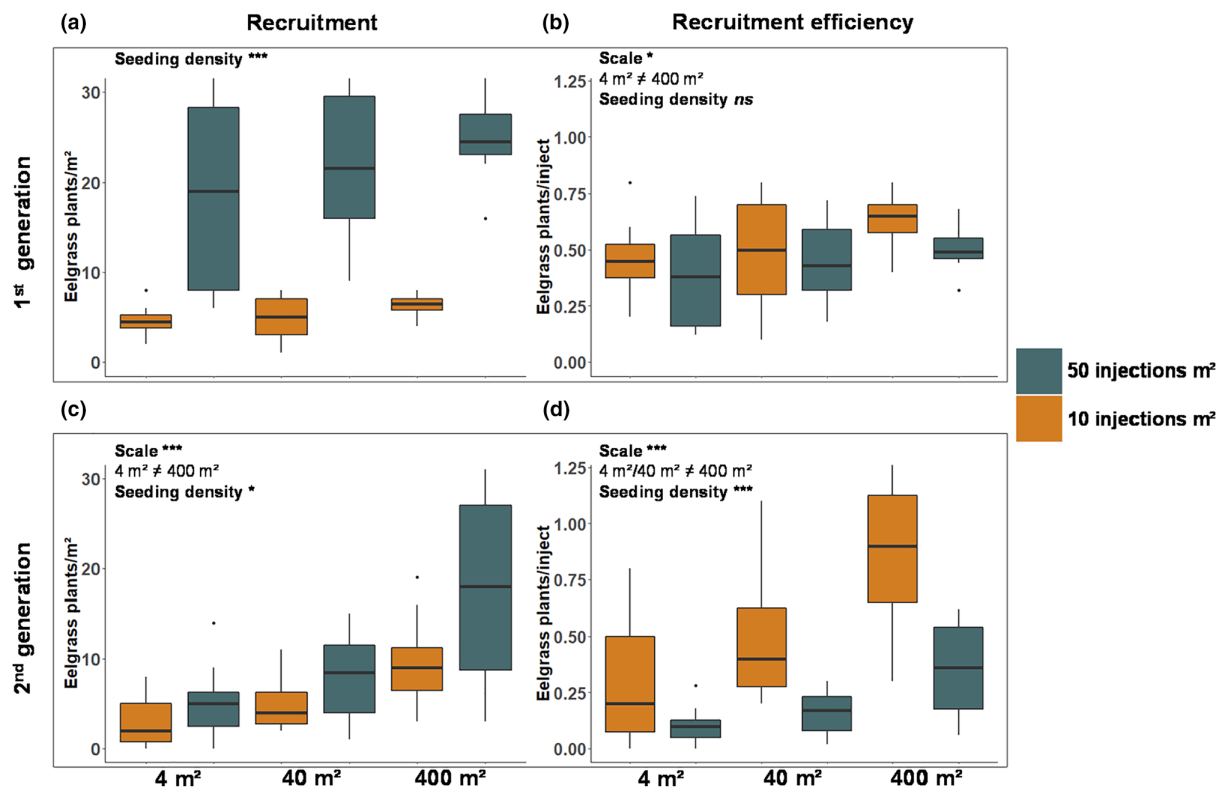


**FIGURE 2** First-generation (July 2020) (a) eelgrass recruitment success and (b) efficiency (restored plants/seed injection) and second-generation (August 2021) (c) eelgrass recruitment success and (d) efficiency (restored plants/seed injection). Boxplots show median (line in box), upper and lower quartile (box), 1.5×interquartile range (vertical line), and outliers (circle). Variables included in the optimised statistical models are presented in the top left corner of each graph. The stars indicate significance: ***$p$<0.001, **$p$<0.01, *$p$<0.05, ns=$p$>0.05.

second-generation recruitment efficiency compared to the higher density (0.57 plants/injection ±0.07 SEM vs. 0.21 plants/injection ±0.03 SEM; Figure 2d). Restoring larger scales (400 m²) significantly increased ($p < 0.01$) the recruitment efficiency compared to the two smaller scales (Figure 2d): in the 400 m² plots, each injection from the year before resulted in 0.65 second-generation plants (±0.09 SEM), while in the 40 and 4 m² plots only 0.33 (±0.06 SEM) and 0.19 (±0.05 SEM) second-generation plants/injection established respectively.

## 3.2 | First-generation eelgrass fitness

In August 2020 (first generation), differences were observed in plant fitness (biomass and reproductive output) between the three restoration scales (Figure 3a,b). At the largest scale, plants grew significantly larger (100%) than in the 4 m² plots (chi² = 8.58, $df = 2$, $p = 0.014$). Moreover, plants in the 400 m² plots produced significantly more spathes than those in the two smaller plot sizes (59% more than 4 m² and 75% more than 40 m²; chi² = 10.085, $df = 2$, $p = 0.006$). Neither seeding density nor the interaction between tested variables significantly affected the plant fitness parameters.

## 3.3 | Eelgrass habitat modification

Restoration scale significantly influenced elevation change in the centre of the plots (chi² = 9.66, $df = 2$, $p = 0.008$) as a result of

sediment entrapment (Figure 4a). In the 4 m² restoration plots, elevation increased on average with 3.2 cm (±0.6 SE) from March to July 2020, which was significantly ($p = 0.028$) more than in the largest plots (0.5 cm ±0.5 SE). Seeding density or the interaction between the two examined variables did not affect this elevation change. Mudflat emergence in the centre of the restoration plots was significantly affected by restoration scale (Figure 4b; chi² = 6.53, $df = 2$, $p = 0.038$). Mudflat emergence was significantly higher (LSD comparison $p = 0.033$; Tukey $p = 0.08$) in the centre of the smallest restoration plots (4 m²; 65.1 ± 20.6%) compared to the 400 m² plots (9.0 ± 4.2%). Mudflat emergence was not affected by either seeding density or the interaction between the two examined variables. Neither elevation change nor mudflat emergence in the full plots differed significantly between restoration scales or seeding densities (Figure S1). Further analysis revealed that increased elevation change between March and July correlated negatively with both eelgrass biomass (Figure 4c; $R = -0.63$, $p = 0.005$) and spathe production (Figure 4e; $R = -0.54$, $p = 0.019$). Similarly, higher mudflat emergence % in the centre of the restoration plots correlated negatively with both eelgrass biomass (Figure 4d; $R = -0.6$, $p = 0.0022$) and spathe production (Figure 4f; $R = -0.63$, $p = 0.005$).

## 4 | DISCUSSION

We here show that simple adaptations to restoration designs can enhance seed-based restoration in coastal ecosystems. Specifically,
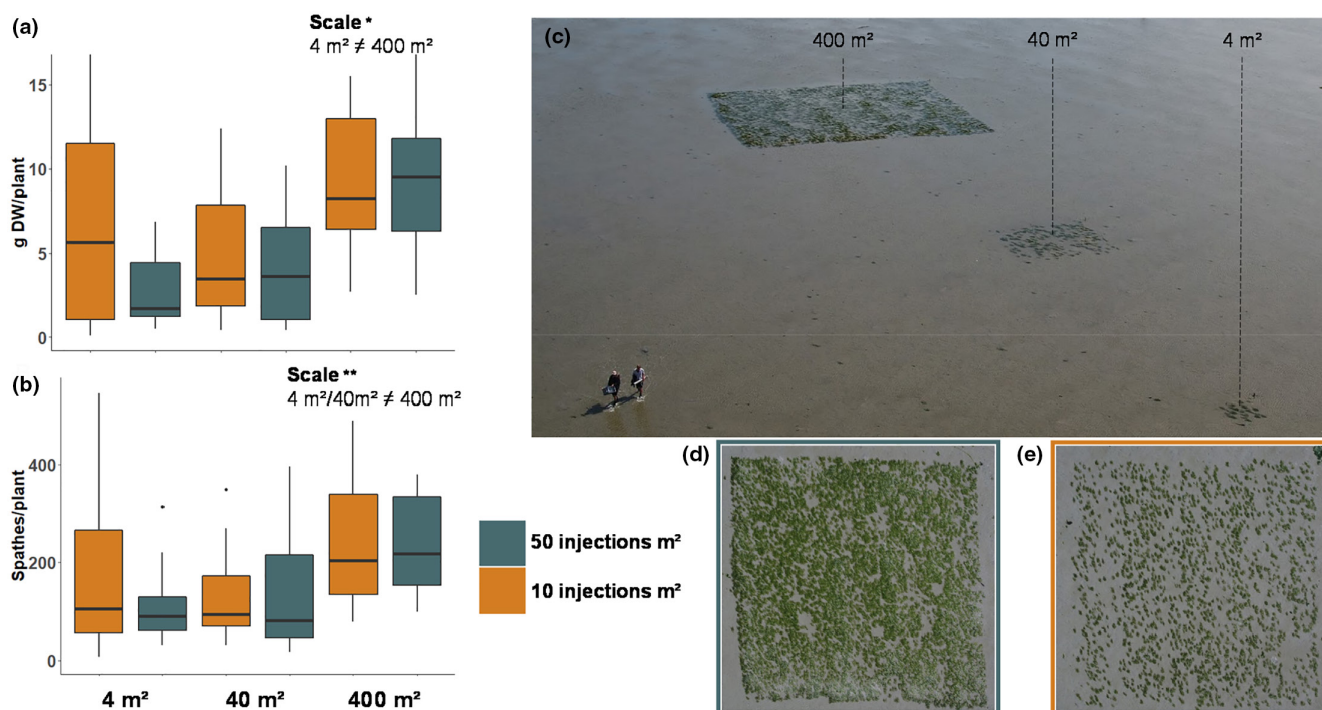


FIGURE 3 First-generation (August 2020) (a) eelgrass dry weight (g) and (b) spathe production. Pictures from July 2020 showcasing the three different restoration scales (C.; 400, 40 and 4 m²) and 400 m² plots seeded with 50 (d) and 10 injections/m² (e). Boxplots show median (line in box), upper and lower quartile (box), 1.5×interquartile range (vertical line), and outliers (circle). Variables included in the optimised statistical models are presented in the top right corner of the graphs. The stars indicate significance: **$p < 0.01$, *$p < 0.05$.
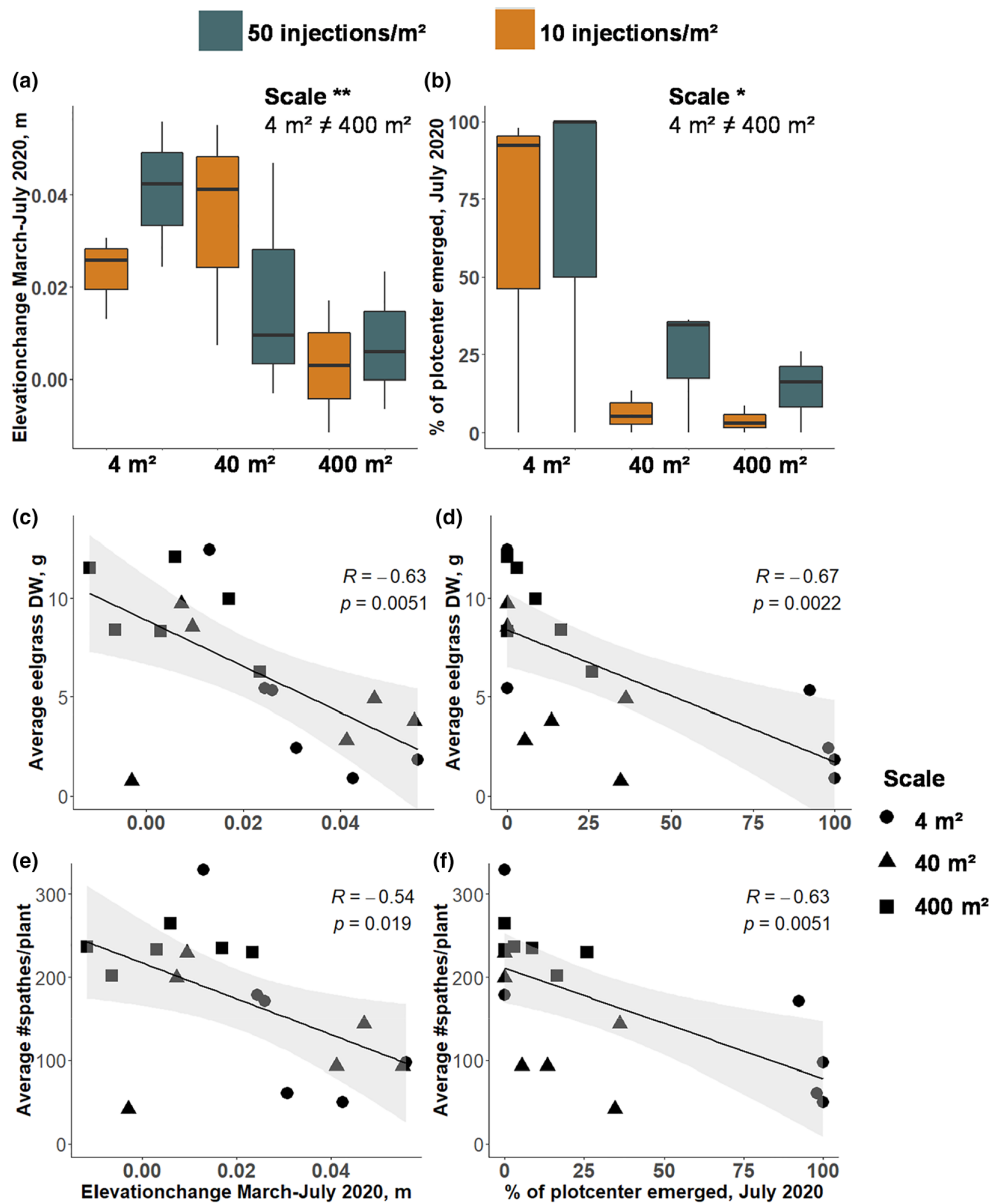
**FIGURE 4** (a) Elevation change in the centre of restoration plots between March and July 2020 and (b) % mudflat emergence during low tide in the centre of plots, July 2020. Relationships between elevation change (March–July 2020) in the centre of restoration plots and (c) first-generation eelgrass dry weight; (e) first-generation eelgrass spathe production. Relationships between mudflat emergence (%) during low tide in the centre of plots (July 2020) and (d) first-generation eelgrass dry weight; (f) first-generation eelgrass spathe production. Boxplots show median (line in box), upper and lower quartile (box), 1.5×interquartile range (vertical line) and outliers (circle). Variables included in the optimised statistical models are presented in the top right corner of the boxplots. The stars indicate significance: **$p < 0.01$, *$p < 0.05$. Grey shaded area depicts the 95% confidence limits.

we found that multi-year recruitment efficiency of intertidal annual eelgrass *Zostera marina* was enhanced by implementing (1) larger restoration scales and (2) lower initial seeding densities. Metrics like recruitment efficiency (here measured as *restored eelgrass plants/ seed injection*) are seldom taken into account in restoration practice, although improving efficiency can greatly enhance the upscaling potential of restoration efforts. Here, the difference in recruitment efficiency of the second generation was 10-fold between the best ($400\,m^2 \times 10$ injections/$m^2$) and worst performing treatment ($4\,m^2 \times 50$ injections/$m^2$) ($0.94 \pm 0.14$ vs. $0.10 \pm 0.02$ restored plants/

seed injection), which in practice means that 10 times less work (injections) and seeds were needed to restore each individual plant. Thus, our results indicate that seed-based restoration projects can be upscaled efficiently, if intraspecific interactions are accounted for in seeding designs.

Restoring seagrasses at larger scales has also previously been shown to improve restoration success (Paulo et al., 2019; van Katwijk et al., 2016). However, the exact mechanisms behind this have remained largely unclear. At larger restoration scales, seagrasses have been hypothesised to more likely function as natural

meadows, thus easing the transition from bare unvegetated areas to vegetated (van der Heide et al., 2007; van Katwijk et al., 2016). Our study provides novel evidence for this hypothesis, as the increased success observed at the largest scale is attributed in part to the restored plants' ability to modify their habitat, specifically sediment entrapment. The ability of seagrasses to trap sediment is often regarded as a positive feedback, as the plants have been shown to facilitate themselves, for example, by improving their own light environment and by entrapping nutrients in nutrient poor systems (Maxwell et al., 2017). However, our results suggest that in the intertidal zone increased sedimentation, can also have a self-inhibitory effect on the desiccation-sensitive eelgrass (Boese et al., 2005). With increased sedimentation, eelgrass plants in our experiment grew smaller and produced less spathes, most likely due increased desiccation stress. A similar negative feedback was observed during the 2018 European heat wave, when eelgrass plants that elevated themselves over low tide inundation level experienced high mortality rates (LL Govers, unpubl data). Here, this proposed negative feedback was suppressed with larger restoration scale, resulting in increased recruitment success and efficiency. In the centre of largest restoration plots, seagrasses caught less sediment compared to the smallest plot size (4 m$^2$), which led to lower mudflat emergence and provided the eelgrass a wetter and less stressful environment. With increased scale, the edge/area ratio decreases which we expect was key for the increased success. Edges are generally considered more vulnerable to disturbance (e.g. hydrodynamics and grazing: Lange et al., 2022; Statton et al., 2015) than the inner parts of a meadow and have also been shown to capture more sediment (Hendriks et al., 2008). Our results suggest that a minimum restoration scale is needed for the edges to function as a barrier protecting (from sedimentation in this case) the rest of the meadow. Due to the suppressed negative feedback eelgrass plants restored at the largest scale grew larger (DW, g) and produced more spathes. Higher plant fitness translated to higher recruitment success and efficiency of the second generation, highlighting that long-term restoration yields can be greatly enhanced with simple context-based seeding design choices.

Restoring high densities of coastal ecosystem engineers has been identified as an easy-to-implement design choice that can enhance restoration success (Silliman et al., 2015; Temmink et al., 2020). Dense or 'clumped' spatial designs have been shown to facilitate coastal restoration success through several different facilitation mechanisms, for example, wave attenuation, enhancing oxygen availability and reduction of ammonium toxicity (Bos & van Katwijk, 2007; Silliman et al., 2015; van der Heide et al., 2008). The restoration success of seagrasses is also hypothesised to increase with higher restored densities, as seagrasses have been found to facilitate their own fertilisation, survival and growth through positive density-dependent feedbacks (Maxwell et al., 2017; Valdez et al., 2020). However, we found that dense spatial designs are not always beneficial for seagrass restoration, similarly to what has recently been shown for saltmarshes (Huang et al., 2022). Huang et al. (2022) found that the saltmarsh plant *Scirpus mariqueter* did not

benefit from clumped spatial designs and highlighted the need to consider biotic attributes (species-specific) context when planning restoration designs. Previously, dispersed spatial designs have also been predicted to outperform clumping for saltmarsh species in the upper intertidal (where hydrodynamic stress is less of an issue; Renzi et al., 2019), but due to different reasons than hypothesised here. Renzi et al. (2019) suggested that dispersed designs will perform better at high elevation due to lower intraspecific competition, while in our study competition was likely not an important driver. According to the stress-gradient hypothesis, positive interactions are more prevalent and important in stressful environments (Bertness & Callaway, 1994). However, for eelgrasses the high intertidal is not a less stressful environment as the plants are just affected by a different kind of stressor (temperature/desiccation). Although we are not able to pinpoint the exact mechanisms underlying the large differences in second-generation recruitment efficiency between initial seeding densities, we suggest that restoring higher eelgrass densities was unbeneficial both due to species- and site-dependent interactions. Eelgrass can survive in the intertidal, but to thrive, plants cannot be exposed for too long (Boese et al., 2005). In contrast, species better adapted to the intertidal (e.g. saltmarsh species or dwarf eelgrass *Zostera noltii*) may be less negatively affected by higher densities (that promote sedimentation) due to the plant's higher drought resistance. At restoration sites situated lower in the intertidal (where desiccation stress is reduced and hydrodynamic stress is expected to increase), higher eelgrass densities are still expected to benefit restoration yields (like shown in Bos & van Katwijk, 2007). Finally, although we were not able to study seed fertilisation, our results indicate that sexual reproduction was successful across all experimental treatments and that the lowest plant densities (~5 plants/m$^2$) were high enough to secure seed fertilisation in our study area. However, we note that high plant densities might still be beneficial to target (in order to secure sufficient seed fertilisation) in other areas or when restoring other species.

It is important to note that the seeding designs only marginally affected first-generation recruitment efficiency, which implies that high initial eelgrass recruitment was enabled by other factors. Often seagrass restoration is very challenging in practice (Bayraktarov et al., 2016; van Katwijk et al., 2016), but failures have taught us what is needed to enable successful restoration efforts. First, the stressors that caused local seagrass decline and that might impede natural recovery should be addressed. This links to site selection, which is perhaps the most important single factor enabling restoration success (Fraschetti et al., 2021). If a site is unsuitable for seagrasses and their restoration the chances for restoration success are very low, while if a site is very suitable the mere addition of donor material to a system can spark restoration success (Orth et al., 2020). Additionally, choosing a suitable restoration method can greatly boost restoration yields, especially in areas where recruitment bottlenecks are present (Govers et al., 2022; Gräfnings et al., 2022). In this study, we attribute the high initial recruitment efficiency to three factors: suitable site selection, high-quality donor material and a functioning restoration method.

Realising the ambitious restoration goals of the UN's 'Decade on Ecosystem Restoration' requires improved efficiency and scalability of coastal restoration initiatives. In this study we show that both aspects can be improved by combining informed spatial designs with seed-based restoration methods. In many restoration trials where positive interactions have been successfully incorporated, facilitation mechanisms have been used to increase the target-species initial establishment, survival or resilience to disturbance. For seed-based methods in general, we suspect that simple changes in spatial designs cannot properly be used to combat early-life bottlenecks due to how vulnerable seeds/seedlings are and how negligible their ability to modify their surroundings is. However, for restoration methods that can counter early-life bottlenecks and that can be used to seed specific spatial designs (e.g. DIS method or burlap bags: Govers et al., 2022; Harwell & Orth, 1999), our results suggest that spatial designs can be used to target interactions that support or inhibit later stages of the life cycle (growth, reproduction, etc). Based on our results, we conclude that to maximise long-term recruitment efficiency of annual eelgrass in the Dutch Wadden Sea future upscaling attempts should aim to restore relatively sparse (5–10 plants/m$^2$) and large areas (≥400 m$^2$). However, optimal seeding designs are context dependent and additional experiments are needed to find the most effective spatial designs for other areas and coastal species. Therefore, we suggest that experiments investigating optimal spatial designs should be implemented more often in restoration projects as a stepping stone between 'proof-of-concept' experiments and large-scale restoration (km$^2$). 'Proof-of-concept' experiments are often performed on small scales (m$^2$) and in light of our results might not provide enough information to enable efficient upscaling. We acknowledge that large-scale restoration experiments are difficult, time-consuming and expensive to replicate, however, we show that the added value (increased efficiency and consequently lowered costs) gained from experiments can definitely be worth it even for nonscientist.

## AUTHOR CONTRIBUTIONS

Max L. E. Gräfnings, Jannes H. T. Heusinkveld, Quirin Smeele, Tjisse van der Heide and Laura L. Govers designed the study. Max L. E. Gräfnings, Jannes H. T. Heusinkveld, Nadia Hijner, Dieuwke J. J. Hoeijmakers, Maarten Zwarts, Tjisse van der Heide and Laura L. Govers conducted the field experiment. Max L. E. Gräfnings analysed the data. Max L. E. Gräfnings wrote the first draft of the manuscript, all authors contributed to revisions.

## ACKNOWLEDGEMENTS

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data available via DataverseNL. https://doi.org/10.34894/FKSY2M (Gräfnings et al., 2023).

## ORCID

*Max L. E. Gräfnings* https://orcid.org/0000-0002-8463-876X
*Laura L. Govers* https://orcid.org/0000-0003-4532-9419

## REFERENCES

Abelson, A., Reed, D. C., Edgar, G. J., Smith, C. S., Kendrick, G. A., Orth, R. J., Airoldi, L., Silliman, B., Beck, M. W., Krause, G., Shashar, N., Stambler, N., & Nelson, P. (2020). Challenges for restoration of coastal marine ecosystems in the anthropocene. *Frontiers in Marine Science*, *7*, 544105. https://doi.org/10.3389/fmars.2020.544105

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, *81*, 169–193. https://doi.org/10.1890/10-1510.1

Bayraktarov, E., Saunders, M. I., Abdullah, S., Mills, M., Beher, J., Possingham, H. P., Mumby, P. J., & Lovelock, C. E. (2016). The cost and feasibility of marine coastal restoration. *Ecological Applications*, *26*, 1055–1074. https://doi.org/10.1890/15-1077

Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology & Evolution*, *9*(5), 191–193. https://doi.org/10.1016/0169-5347(94)90088-4

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. https://doi.org/10.1515/BOT.2005.037

Bos, A. R., & van Katwijk, M. M. (2007). Planting density, hydrodynamic exposure and mussel beds affect survival of transplanted intertidal eelgrass. *Marine Ecology Progress Series*, *336*, 121–129. https://doi.org/10.3354/meps336121

Bouma, T. J., Friedrichs, M., Van Wesenbeeck, B., Temmerman, S., Graf, G., & Herman, P. (2009). Density-dependent linkage of scale-dependent feedbacks: A flume study on the intertidal macrophyte *Spartina anglica*. *Oikos*, *118*, 260–268. https://doi.org/10.1111/j.1600-0706.2008.16892.x

De Fontaubert, A. C., Downes, D. R., & Agardy, T. S. (1996). *Biodiversity in the seas: Implementing the convention on biological diversity in marine and coastal habitats*. IUCN.

Duarte, C. M., & Chiscano, C. L. (1999). Seagrass biomass and production: A reassessment. *Aquatic Botany*, *65*, 159–174. https://doi.org/10.1016/S0304-3770(99)00038-8

Fox, J., & Weisberg, S. (2011). *An {R} companion to applied regression* (second ed.). Sage.

Fraschetti, S., McOwen, C., Papa, L., Papadopoulou, N., Bilan, M., Boström, C., Capdevila, P., Carreiro-Silva, M., Carugati, L., Cebrian, E., Coll, M., Dailianis, T., Danovaro, R., De Leo, F., Fiorentino, D., Gagnon, K., Gambi, C., Garrabou, J., Gerovasileiou, V., … Guarnieri, G. (2021). Where is more important than how in coastal and marine ecosystems restoration. *Frontiers in Marine Science*, *8*, 626843. https://doi.org/10.3389/fmars.2021.626843

Gedan, K. B., & Silliman, B. R. (2009). Using facilitation theory to enhance mangrove restoration. *Ambio*, *38*(2), 10–19. https://doi.org/10.1579/0044-7447-38.2.109

Giesen, W. B. J. T., van Katwijk, M. M., & den Hartog, C. (1990). Temperature, salinity, insolation and wasting disease of eelgrass (*Zostera marina* L.)

in the Dutch Wadden Sea in the 1930s. *Netherlands Journal of Sea Research*, *25*, 395–404. https://doi.org/10.1016/0077-7579(90)90047-K

Govers, L. L., Heusinkveld, J. H. T., Gräfnings, M. L. E., Smeele, Q., & van der Heide, T. (2022). Adaptive intertidal seed-based seagrass restoration in the Dutch Wadden Sea. *PLoS ONE*, *17*(2), e0262845. https://doi.org/10.1371/journal.pone.0262845

Govers, L. L., Van der Zee, E. M., Meffert, J. P., Van Rijswick, P. C. J., Man in 't Veld, W. A., Heusinkveld, J. H. T., & van der Heide, T. (2017). Copper treatment during storage reduces Phytophthora and Halophytophthora infection of *Zostera marina* seeds used for restoration. *Scientific Reports*, *7*, 43172. https://doi.org/10.1038/srep43172

Gräfnings, M. L. E., Heusinkveld, J. H. T., Hijner, N., Hoeijmakers, D. J. J., Smeele, Q., Zwarts, M., van der Heide, T., & Govers, L. L. (2023). Replication Data for: Spatial design improves efficiency and scalability of seed-based seagrass restoration. DataverseNL, V1 https://doi.org/10.34894/FKSY2M

Gräfnings, M. L. E., Heusinkveld, J. H. T., Hoeijmakers, D. J. J., Smeele, Q., Wiersema, H., Zwarts, M., van der Heide, T., & Govers, L. L. (2022). Optimizing seed injection as a seagrass restoration method. *Restoration Ecology*, *31*, e13851. https://doi.org/10.1111/rec.13851

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., & Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, *319*, 948–952. https://doi.org/10.1126/science.1149345

Harwell, M. C., & Orth, R. J. (1999). Eelgrass (*Zostera marina* L.) seed protection for field experiments and implications for large-scale restoration. *Aquatic Botany*, *64*, 51–61. https://doi.org/10.1016/S0304-3770(99)00008-X

Hendriks, I. E., Sintes, T., Bouma, T. J., & Duarte, C. M. (2008). Experimental assessment and modeling evaluation of the effects of seagrass *Posidonia oceanica* on flow and particle trapping. *Marine Ecology Progress Series*, *356*, 163–173. https://doi.org/10.3354/meps07316

Huang, H., Xu, C., & Liu, Q.-X. (2022). 'Social distancing' between plants may amplify coastal restoration at early stage. *Journal of Applied Ecology*, *59*, 188–198. https://doi.org/10.1111/1365-2664.14044

Jones, H. P., Jones, P. C., Barbier, E. B., Blackburn, R. C., Rey Benayas, J. M., Holl, K. D., McCrackin, M., Meli, P., Montoya, D., & Mateos, D. M. (2018). Restoration and repair of Earth's damaged ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20172577. https://doi.org/10.1098/rspb.2017.2577

Kettenring, K. M., & Tarsa, E. E. (2020). Need to seed? Ecological, genetic, and evolutionary keys to seed-based wetland restoration. *Frontiers in Environmental Science*, *8*, 109. https://doi.org/10.3389/fenvs.2020.00109

Lange, T., Oncken, N. S., Svane, N., Steinfurth, R. C., Kristensen, E., & Flindt, M. R. (2022). Large-scale eelgrass transplantation: A measure for carbon and nutrient sequestration in estuaries. *Marine Ecology Progress Series*, *685*, 97–109. https://doi.org/10.3354/meps13975

Maxwell, P. S., Eklöf, J. S., Katwijk, M. M., O'Brien, K. R., Torre-Castro, M., Boström, C., Bouma, T. J., Krause-Jensen, D., Unsworth, R. K. F., van Tussenbroek, B. I., & van der Heide, T. (2017). The fundamental role of ecological feedback mechanisms for the adaptive management of seagrass ecosystems—A review. *Biological Reviews*, *92*, 1521–1538. https://doi.org/10.1111/brv.12294

Merritt, D. J., & Dixon, K. W. (2011). Restoration seed banks—A matter of scale. *Science*, *332*(6028), 424–425. https://doi.org/10.1126/science.1203083

Orth, R. J., Lefcheck, J. S., McGlathery, K. S., Aoki, L., Luckenbach, M. W., Moore, K. A., Oreska, M. P. J., Snyder, R., Wilcox, D. J., & Lusk, B.

(2020). Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. *Science Advances*, *6*, eabc6434. https://doi.org/10.1126/sciadv.abc6434

Orth, R. J., Moore, K. A., Marion, S. R., Wilcox, D. J., & Parrish, D. B. (2012). Seed addition facilitates eelgrass recovery in a coastal bay system. *Marine Ecology Progress Series*, *448*, 177–195. https://doi.org/10.3354/meps09522

Paulo, D., Cunha, A. H., Boavida, J., Serrao, E. A., Goncalves, E. J., & Fonseca, M. (2019). Open coast seagrass restoration. Can we do it? Large scale seagrass transplants. *Frontiers in Marine Science*, *6*, 52. https://doi.org/10.3389/fmars.2019.00052

Perrow, M. R., & Davy, A. J. (2002). *Handbook of ecological restoration: Restoration in practice* (Vol. *2*). Cambridge University Press.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Renzi, J., He, Q., & Silliman, B. R. (2019). Harnessing positive species interactions to enhance coastal wetland restoration. *Frontiers in Ecology and Evolution*, *7*, 131. https://doi.org/10.3389/fevo.2019.00131

Roberts, C. M., McClean, C. J., Veron, J. E. N., Hawkins, J. P., Allen, G. R., McAllister, D. E., Mittermeier, C. G., Schueler, F. W., Spalding, M., Wells, F., Vynne, C., & Werner, T. B. (2002). Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, *295*(5558), 1280–1284. https://doi.org/10.1126/science.1067728

Saunders, M. I., Doropoulos, C., Bayraktarov, E., Babcock, R. C., Gorman, D., Eger, A. M., Vozzo, M. L., Gillies, C. L., Vanderklift, M. A., Steven, A. D. L., & Bustamante, R. H. (2020). Bright spots in coastal marine ecosystem restoration. *Current Biology*, *30*, 1500–1510. https://doi.org/10.1016/j.cub.2020.10.056

SER. (2004). *Society for Ecological Restoration International Science & Policy Working Group. The SER international primer on ecological restoration*. Society for Ecological Restoration International.

Silliman, B. R., Schrack, E., He, Q., Cope, R., Santoni, A., van der Heide, T., Jacobi, R., Jacobi, M., & van de Koppel, J. (2015). Facilitation shifts paradigms and can amplify coastal restoration efforts. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 14295–14300. https://doi.org/10.1073/pnas.1515297112

Statton, J., Gustin-Craig, S., Dixon, K. W., & Kendrick, G. A. (2015). Edge effects along a seagrass margin result in an increased grazing risk on *Posidonia australis* transplants. *PLoS ONE*, *10*(10), e0137778. https://doi.org/10.1371/journal.pone.0137778

Temmink, R. J. M., Christianen, M. J. A., Fivash, G. S., Angelini, C., Boström, C., Didderen, K., Engel, S. M., Esteban, N., Gaeckle, J. L., Gagnon, K., Govers, L. L., Infantes, E., van Katwijk, M. M., Kipson, S., Lamers, L. P. M., Lengkeek, W., Silliman, B. R., van Tussenbroek, B. I., Unsworth, R. K. F., … van der Heide, T. (2020). Mimicry of emergent traits amplifies coastal restoration success. *Nature Communications*, *11*, 3668. https://doi.org/10.1038/s41467-020-17438-4

Temmink, R. J. M., Fivash, G. S., Govers, L. L., Nauta, J., Marin-Diaz, B., Cruijsen, P. M. J. M., Didderen, K., Penning, E., Olff, H., Heusinkveld, J. H. T., Lamers, L. P. M., Lengkeek, W., Christianen, M. J. A., Reijers, V. C., Bouma, T. J., & van der Heide, T. (2022). Initiating and upscaling mussel reef establishment with life cycle informed restoration: Successes and future challenges. *Ecological Engineering.*, *175*, 106496. https://doi.org/10.1016/j.ecoleng.2021.106496

Valdez, S. R., Zhang, Y. S., van der Heide, T., Vanderklift, M. A., Tarquinio, F., Orth, R. J., & Silliman, B. R. (2020). Positive ecological interactions and the success of seagrass restoration. *Frontiers in Marine Science*, *7*, 91. https://doi.org/10.3389/fmars.2020.00091

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. https://doi.org/10.1007/s00442-008-1155-2

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. https://doi.org/10.1007/s10021-007-9099-7

van Katwijk, M. M., Thorhaug, A., Marbà, N., Orth, R. J., Duarte, C. M., Kendrick, G. A., Althuizen, I. H. J., Balestri, E., Bernard, G., Cambridge, M. L., Cunha, A., Durance, C., Giesen, W., Han, Q., Hosokawa, S., Kiswara, W., Komatsu, T., Lardicci, C., Lee, K.-S., … Verduin, J. J. (2016). Global analysis of seagrass restoration: The importance of large–scale planting. *Journal of Applied Ecology*, *53*, 567–578. https://doi.org/10.1111/1365-2664.12562

van Tussenbroek, B. I., Soissons, L. M., Bouma, T. J., Asmus, R., Auby, I., Brun, F. G., Cardoso, P. G., Desroy, N., Fournier, J., Ganthy, F., Garmendia, J. M., Godet, L., Grilo, T. F., Kadel, P., Ondiviela, B., Peralta, G., Recio, M., Valle, M., Van der Heide, T., & van Katwijk, M. M. (2016). Pollen limitation may be a common Allee effect in marine hydrophilous plants: Implications for decline and recovery in seagrasses. *Oecologia*, *182*, 595–609. https://doi.org/10.1007/s00442-016-3665-7

Vanderklift, M. A., Doropoulos, C., Gorman, D., Leal, I., Minne, A. J. P., Statton, J., Steven, A. D. L., & Wernberg, T. (2020). Using propagules to restore coastal marine ecosystems. *Frontiers in Marine Science*, *7*, 724. https://doi.org/10.3389/fmars.2020.00724

Zhang, Y. S., Cioffi, W. R., Cope, R., Daleo, P., Heywood, E., Hoyt, C., Smith, C. S., & Silliman, B. R. (2018). A global synthesis reveals gaps in coastal habitat restoration research. *Sustainability*, *10*, 1040. https://doi.org/10.3390/su10041040

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** Details for the optimised linear mixed effects models used in this article.

**Figure S1.** (A) Elevation change March–July and (B) % mudflat emergence during low tide in the full plots. Boxplots show median (line in box), upper and lower quartile (box) and 1.5×interquartile range (vertical line). Neither restoration scale nor seeding density significantly affected elevation change or % mudflat emergence in the full plots.