# Seed arrival and persistence at the tidal mudflat: identifying key processes for pioneer seedling establishment in salt marshes

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ABSTRACT: Salt marshes are highly valuable ecosystems that provide numerous important ecosystem services. Given the global marsh decline, there is a pressing need to understand the natural bottlenecks and thresholds to their establishment and long-term ecological maintenance. Seed presence in the right place and time is a prerequisite for pioneer establishment. We performed field surveys and manipulative seed-bank experiments on 2 mudflats with different levels of exposure in the Westerschelde, The Netherlands, to identify patterns of seed arrival and persistence and their role in determining seed bank abundance. Seed arrival decreased with distance from the seed-source area, i.e. the salt marsh, resulting in seeds being deposited on the mudflat close to the marsh edge. Once arrived, most of the surface seeds were washed away during subsequent tides, with retention of <20%. However, seed burial enhanced seed retention nonlinearly with increasing burial depth. Predation was not a major cause of seed loss. These results point to the importance of hydrodynamic and morphodynamic processes to both lateral seed dispersal and vertical seed burial on tidal flats and, therefore, suggest that saltmarsh establishment and expansion are closely linked to the physical dynamics of the entire estuarine system.

KEY WORDS: Salt marsh · Mudflat · Seed arrival · Seed persistence · Seed bank · *Spartina*

## INTRODUCTION

Salt marshes are highly valued for their numerous ecosystem services such as carbon storage, biodiversity conservation, nursery function for fish, water purification, flood and shoreline protection, and recreation (Zedler & Kercher 2005). Unfortunately, these ecosystems have been suffering severe and long-term habitat losses due to increasing anthropogenic impact worldwide (reviewed by Gedan et al. 2009). This loss is expected to continue due to sea level rise and 'coastal squeeze'. The former may cause drowning and dieback of salt marshes (reviewed by Gedan et al. 2009) if sediment accretion fails to keep pace with sea level rise because of sediment starvation (Cahoon et al. 2006). Coastal squeeze ensues when marshes are squeezed out of the ecosystem in the presence of seawalls preventing marshes from migrating upward into the tidal range as the sea level rises (Winn et al. 2003). Moreover, recent modeling studies indicate that sea level rise may also enhance the risk of marsh edge erosion (Mariotti & Fagherazzi 2010, Marani et al. 2011). Another worldwide problem facing salt marshes is posed by invasive species (e.g. *Spartina* spp.), whose strong spreading capacity endangers local biodiversity and ecosystem functioning (reviewed by Gedan et al. 2009). These threats to salt marshes emphasize

the need for fundamental insights into how to manage and conserve existing marshes as well as in restoring and/or facilitating the creation of new salt marshes. Within this context, a major issue that needs to be resolved is identifying and unraveling key processes governing seedling establishment (Dethier & Hacker 2005, Bouma et al. 2009, Friess et al. 2012).

Salt marsh development starts with pioneer species colonizing a bare intertidal flat. Species of the genus *Spartina*, either native or invasive, are important salt marsh pioneers worldwide (Gedan et al. 2009). Colonization of mudflats by *Spartina* spp. occurs initially by dispersal and settlement of propagules (e.g. seeds, seedlings and/or rhizome fragments) that are transported by tidal currents. Once established, these propagules can develop into discrete circular domed tussocks separated by open mudflat through clonal growth (Van Hulzen et al. 2007, Balke et al. 2012), which may eventually coalesce into continuous swards (Daehler & Strong 1994, Nehring & Hesse 2008, Zhu et al. 2012). Among the different possible propagule types that can be dispersed, seeds often play a critical role in population expansion through seed dispersal and subsequent seedling recruitment. For example, the long-distance spread of nascent populations of *Spartina alterniflora* along the US Pacific coast was primarily driven by seeds and to a lesser extent by the dispersal of vegetative fragments (Sayce et al. 1997, Ayres et al. 2004). Likewise, recruitment by seed transport and seedling establishment on the mudflat was crucial for the rapid range expansion of the invasive *S. alterniflora* at the fast-accreting salt marshes in the Yangtze Estuary (Zhu et al. 2012). Similar scenarios also occurred in NW Europe, where *Spartina anglica* spread rapidly into the intertidal zones since the formation of a polyploid hybrid around 1900 (Gray et al. 1991, Nehring & Hesse 2008). Within NW Europe, *S. anglica* has been so widespread for such a long time that in some countries it has become a target species for nature conservation and restoration (e.g. the Natura 2000 programme) (Nehring & Hesse 2008).

In spite of considerable seed yield in salt marshes, success of seedling establishment of pioneer species on tidal flats is generally low due to ecological bottlenecks (e.g. fecundity, dispersal ability) and/or thresholds imposed by physical stressors (e.g. inudation, salinity, shear stress) (Bouma et al. 2009, Friess et al. 2012). Seedling establishment can be limited by seed processes (e.g. seed production, dispersal and survival) and/or seedling processes (e.g. seedling emergence and survival). Previous studies mostly concerned thresholds involved in seedling emergence

and survival, which were found to relate to salinity (Dethier & Hacker 2005), inundation regime (Gray et al. 1991), hydrodynamic conditions (Houwing 2000, Hammond et al. 2002, Schwarz et al. 2011), sediment stability (Ayres et al. 2004, Bouma et al. 2013) and bioturbation (van Wesenbeeck et al. 2007, Marion & Orth 2012). Regardless of all abiotic and biotic external conditions, pioneer seedling establishment is only possible if seeds are present at the right location; i.e. at both the appropriate elevation in the intertidal zone (Crooks et al. 2002, van Loon et al. 2007, Dixon et al. 2008) and the appropriate sediment depth (Harrison 1993, Greve et al. 2005) to enable germination—in addition to being there at the right time (Balke et al. 2011). Yet, there is still a lack of knowledge on the dynamics of seed dispersal and seed banks of pioneer species or the follow-on consequences for pioneer seedling establishment in salt marshes. Most work on seed dispersal (e.g. Rand 2000, Chang et al. 2007, Chang et al. 2008) or seed banks (e.g. Bakker et al. 1996, Wolters & Bakker 2002) in salt marshes was done on species from the higher marsh.

Like in terrestrial ecosystems, seed dispersal in salt marshes involves 2 phases (Chambers & Macmahon 1994). First, seeds detach from their parent plant and arrive at the ground. After this initial deposition, they can be secondarily dispersed or remain at the site and gradually be incorporated into the local soil seed bank through seed burial (Wolters & Bakker 2002). The yearly formation of a soil seed bank on the mudflat is essential for the establishment of most salt marsh pioneer plants (e.g. *Spartina* spp.), since they only have transient seed banks (Thompson & Grime 1979) in which seeds persist for <1 yr (Wolters & Bakker 2002, Xiao et al. 2009). Thus, seedling recruitment of such species relies mainly on the arrival of fresh seeds and their short-term persistence in the soil seed bank. Understanding to what extent, and in which way, pioneer seedling establishment is limited by seed dynamics, including seed arrival and seed bank persistence, may hold the key for effective and sustainable management decisions (e.g. extension of established marshes through seedling recruitment, seed-based invasive species control) and salt marsh restoration/creation measures (e.g. seed-based techniques to facilitate seedling establishment).

In this study, we aimed to develop a process-based understanding on the role of seed arrival and persistence in determining seed bank abundance on tidal flats. Through a combination of field surveys and manipulative experiments, we studied patterns of seed arrival and persistence of *S. anglica* at the mud-

flat in front of a marsh. Here, seed arrival is measured as the deposition of seeds at the sediment surface. Seed persistence is defined as the retention of seeds in the sediments; the survival in terms of viability is beyond the scope of the current study. Specifically, we address the following questions: (1) how is seed arrival influenced by the distance from the seed source, (2) how does seed persistence depend on the burial depths of seeds in the sediment and local hydrodynamic conditions (e.g. wave exposure), and (3) is there significant loss from the seed bank due to predation?

## MATERIALS AND METHODS

### Study site

To test how local hydrodynamic conditions can affect seed persistence on the tidal flats, we selected 2 salt marshes with contrasting wave exposure due to their position relative to the prevailing southwesterly winds (Callaghan et al. 2010). One is the relatively sheltered Paulinapolder (southern bank) and the other the relatively wind-exposed Zuidgors (northern bank) in the Westerschelde, The Netherlands (51° N, 4° E) (Fig. 1). The Westerschelde is tide-dominated and experiences a semi-diurnal tide; the spring tidal range varies from 4.4 to 5.5 m (Baeyens et al. 1998). The pioneer vegetation consists mainly of *Spartina anglica*, which was introduced to the Westerschelde in 1925 (Nehring & Hesse 2008). At both field sites, *S. anglica* forms monocultures in the seaward part of the salt marshes. Our field survey and manipulative experiments were conducted in the pioneer zone of the marsh, where vegetation extension of pioneer species through seedling establishment takes place.

This zone is characterized by scattered *S. anglica* tussocks on a predominantly bare mudflat with elevations suitable for the seedling establishment of this species. *S. anglica* flowers from July to October and seeds ripen within 12 wk. Seed release of *S. anglica* starts in autumn, extending to the winter and early spring of the following year (Huiskes et al. 1995). *S. anglica* germinate new seedlings in spring and may extend germination to summer as some seeds can remain viable in the seed bank till July, whereas no seeds survive at the end of the growing season (Groenendijk 1986). Based on this information, we ran our experiments from January to June to cover the period of seed dispersal and deposition.

### Survey of seed arrival and ambient soil seed bank

To assess seed arrival at the mudflat at both field sites, 15 AstroTurf® mats (artificial grass made from polyethylene; Wolters et al. 2004), were deployed at 3 parallel transects located 5, 25 and 45 m in front of the marsh edge (Fig. 2a). These AstroTurf® mats (0.5 × 0.5 m each, Fig. 2b) were deployed and recovered monthly from January to April 2012, with 5 replicates in each transect at an interval of ca. 10 m. These mats were fixed to the ground surface with 5 PVC tubes perforated by steel bars to secure them and prevent them from floating up. To evaluate *S. anglica* seed abundance (expressed as no. m$^{-2}$) in the ambient soil seed bank at the same locations, 5 sediment samples (length × width × depth: 0.25 × 0.5 × 0.05) were excavated from the mudflat surrounding the AstroTurf mats. Sampling was restricted to a depth of 50 mm because experiments have shown that seeds germinating at a depth <50 mm in the sediment have little chance to reach
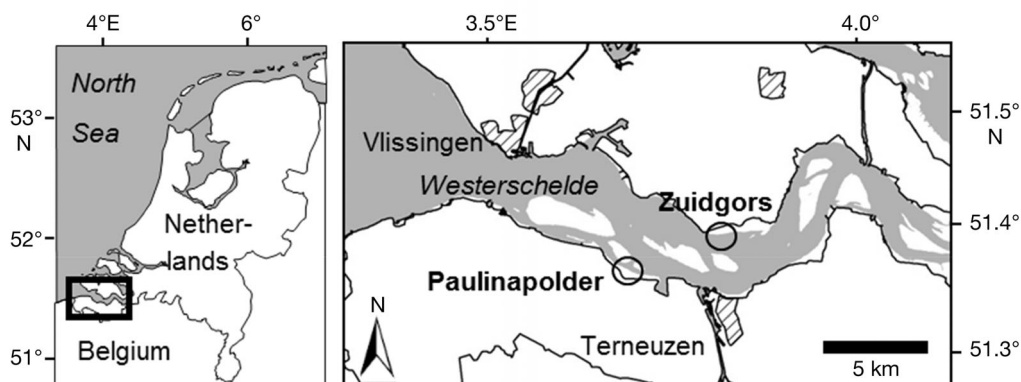


Fig. 1. Location of Paulinapolder (sheltered) and Zuidgors (wind-exposed) salt marshes in the Westerschelde (The Netherlands)
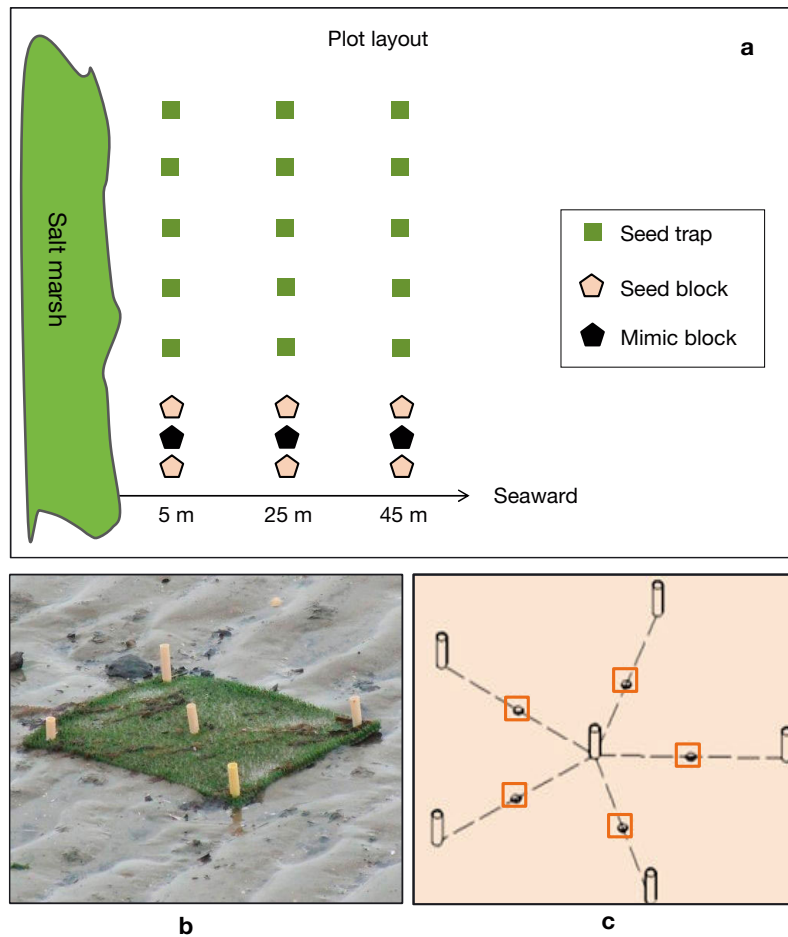
Fig. 2. (a) Experimental setup of transects and experimental plots in front of the salt marsh, (b) AstroTurf® mat, (c) pentagon-shaped seed bank cores deployment, (d) seed bank cores with coloured (Rose Bengal) seeds, and (e) seed bank cores with mimics

the sediment surface (T. J. Bouma et al. unpubl.). Each sampling point was between 0.5 to 2 m from each mat (Fig. 2a), and was marked with PVC tubes after sampling, so as to avoid sampling the same spot twice. The survey of the seed bank was conducted monthly from January to June. Recovered mats and seed bank samples were rinsed and sieved in the lab through a 1 mm sieve and *S. anglica* seeds were identified and counted.

## Manipulative experiment on seed bank persistence

### Manufacture of seed bank cores

To determine the pattern of seed persistence on the mudflat, a manipulated seed bank was established at the same locations as the ambient seed bank survey, by installing layered seed bank cores. These cores were made layer by layer with colored *S. anglica* seeds (Fig. 2d) or seed mimics (Fig. 2e) placed at different depths, simulating seeds that were just

deposited on the surface or were buried to a certain depth. The application of non-digestible mimics in this experiment aimed to detect whether seed predation might occur by a common polychaete in European estuaries, *Hediste* (previously known as *Nereis*) *diversicolor*, as suggested by earlier studies (e.g. Emmerson 2000, Paramor & Hughes 2004). At our study sites, the density of this species at the tidal mudflats was found to range from several hundred to ca. 2000 ind. $m^{-2}$ in Paulinapolder (Van Colen et al. 2008) and 339.9 ± 78.7 ind. $m^{-2}$ in Zuidgors (J. van Belzen unpubl. data). Mimics used were plastic chips with different colors and of similar shape but slightly bigger size than *S. anglica* seeds.

Layered cores were created in 200 ml syringes (36 mm diameter), using homogenized ambient sediment. Five seeds or mimics were placed at the sediment surface (0 mm treatment) and at 5, 15 and 30 mm beneath the sediment surface (Fig. 2d). To prevent seeds/mimics placed on the surface from dropping off during transport, they were lightly pressed into the sediment. Seeds from different

depth layers were distinguished by staining: black or blue (normal ink) or red (Rose Bengal). When a fourth layer was needed for the 5 mm depth, uncolored seeds were used. Each layer was frozen at −20° before adding a new layer of sediment to minimize mixing. When all layers were ready, they were taken out of the syringes while still frozen, and kept in the freezer to facilitate placing them in the field.

## Deployment and recovery

Seed bank cores were transported frozen on dry ice to the field. At each location, 3 pentagon-shaped blocks (Fig. 2c), 3 m apart, were set up, with the middle block for mimic cores and the other 2 for seed cores (Fig. 2a). Within each block, 5 seed bank cores were installed. They were placed through a hole (40 mm diameter) drilled in the middle of a wooden template, that in addition had both endpoints marked with holes that fitted PVC tubes inserted into the soil to allow for accurate relocation of the cores. The cores were deployed by first extracting a sediment core of the same depth from inside a slightly larger concentric tube, preventing the collapse of sediment into the evacuated space. Then the cores were placed with their surface at the same level as the surrounding sediment surface. After 4 wk, these cores were relocated using the same template and recovered through PVC pipes (150 mm length, 50 mm diameter) which were larger than the original cores, to ensure the whole core was re-sampled despite a slight imprecision in locating the original core. After relocating the position of the core, the pipe was slowly inserted into the sediment to a depth of 100 mm, followed by pulling it out with one hand while covering the bottom with the fingers of the other hand.

From January to June 2012, this experiment was repeated monthly in the field except in April. In January and February, seeds or mimics were placed at 3 sediment depths (0, 15 and 30 mm), while an additional depth at 5 mm was established in March and May. To detect whether any seeds had moved from the original position to the adjacent area, additional sediment samples were scooped from the surrounding 0.25 × 0.25 m area to a depth of 50 mm in March. Only seeds that were originally deposited at the sediment surface (i.e. 0 mm treatment) were found in these samples (Table 1), suggesting that the recovery of surface seeds could be slightly underestimated (ca. 2%) due to their movement within the close vicinity. Seeds displaced out of the 0.25 × 0.25 m area were regarded as 'lost'.

Table 1. Recovery of *Spartina anglica* seeds and mimics in additional sediment samples. Sampling took place in March 2012 of sediment adjacent to experimental seed/mimic cores on the mudflat at 5, 25, and 45 m seaward distance from Paulinapolder and Zuidgors salt marshes

| Site | Distance (m) | Seeds | Mimics |
|---|---|---|---|
| Paulinapolder | 5 | 1 | 1 |
| | 25 | 0 | 0 |
| | 45 | 0 | 0 |
| Zuidgors | 5 | 0 | 1 |
| | 25 | 3 | 1 |
| | 45 | 1 | 0 |
| Sum | | 5 | 3 |
| Total deployed | | 300 | 150 |
| Recovery (%) | | 1.67 | 2.00 |

No seeds or mimics were visible on the surface of each plot when recovered. The recovered samples (depth = 100 mm) were transported to the lab, sliced every 5 mm and sieved through a 1 mm sieve to retrieve deployed seeds and mimics, with their layer of origin identified by seed color (see previous section). Many seeds (but no mimics) had turned black *in situ* because of the anaerobic condition within the sediment. To identify the original color of those seeds, they were bathed in fresh water for at least 24 h until their original colors re-appeared. The number of recovered seeds or mimics of each color was counted and recorded. Seed persistence in the seed bank was then calculated (recovered/total deployed).

## Data analysis

### Seed arrival and seed bank abundance

To examine the effects of distance to the marsh edge ('Distance'), and time ('Month') on seed arrival and seed bank abundance, analysis of deviance was implemented by using generalized linear models (GLMs). This was due to the non-normal errors in the count data of these 2 response variables. Each response variable was fitted by a GLM using family 'quasipoisson' to account for overdispersion (Crawley 2007). To simplify each model, we first ran these models for each site separately with 'Distance' as the continuous variable and 'Month' as the categorical variable. In these models, both seed arrival and seed bank abundance showed a pattern of exponential decay with distance and the slopes did not differ with months. Thus 'Month' was not included in the further analysis to detect the differences (1) between seed

arrival and seed bank abundance and (2) between sites. This was done by pooling data of both seed arrival and seed bank abundance from the 2 sites together, followed by comparing their mean values and slopes within one GLM with 'Distance' as the continuous variable.

### Seed persistence

Pairwise Wilcoxon rank sum test was adopted to test for a difference in recovery between seeds and mimics. A linear correlation was used to examine the comparability of the material we used for mimicking *S. anglica* seeds with the actual seeds. GLMs were employed to test the effects of 'Month', 'Distance' and 'Depth' (i.e. vertical position in the sediment) on seed persistence in the manipulated seed bank. These analyses were conducted for each site separately, by specifying a GLM with 'family' = 'binomial' for the proportion data. When necessary, we refitted the model using 'quasibinomial' to account for overdispersion (Crawley 2007). Pairwise Wilcoxon rank sum tests were also applied to detect the differences between sites in seed persistence in each month.

### Statistics

All statistical analyses were run in R (www.r-project.org). Multiple comparisons between slopes or intercepts within each model were achieved through the 'glht' function in the package 'multcomp'. Each GLM started with the maximum model by fitting, for each level of the categorical factors, a line with separate slopes and intercepts; the minimum adequate model was ultimately generated by progressively removing non-significant terms (Crawley 2007). All tests were performed applying a significance level of $\alpha = 0.05$.

### **RESULTS**

### **Seed arrival at the mudflat**

Deviance analysis of the minimum 'quasipossion' model showed that 'Distance' had a significant effect on

seed arrival at the mudflat (Table 2). At both the sheltered and the exposed site, the number of deposited seeds declined exponentially with increasing distance to the marsh edge (Fig. 3a). Since 'Distance' did not have an interactive effect with 'Month', the rate of decrease (slope) in seeds arrived was consistent over time within each site. The wind-exposed site Zuidgors had a higher decay rate (steeper slope) than the relatively sheltered Paulinapolder (p = 0.020). However, there was no significant difference in seed arrival between these 2 sites (p = 0.249).

Seed arrival was also significantly affected by 'Month' (Table 2) at both sites. At the relatively sheltered site, fewer seeds arrived at the mudflat in March than in both January (p < 0.001) and February (p < 0.001), between which there was no significant difference (p = 0.328). At the wind-exposed site, a similar decrease was found, but with seed deposition in January being significantly higher than in February (p < 0.001) and March (p < 0.001).

### **Seed bank abundance on the mudflat**

At both sites, seed bank abundance in the top 50 mm of the ambient sediment fluctuated over time but the effect of 'Month' was significant only at the wind-exposed site (Table 2). Generally, seed bank abundance paralleled the descending trend of seed arrival with distance from the marsh edge (Fig. 3). Differences in the rates of decrease (slopes) between seed arrival and seed bank abundance were not sig-

Table 2. Analysis of deviance table of the minimum adequate models for *Spartina anglica* seed deposition, seed bank abundance, and seed persistence in the manipulated seed bank. Salt marsh sites—sheltered: Paulinapolder; exposed: wind-exposed Zuidgors. Dev: deviance. Significance level: ***p ≤ 0.001, **p < 0.01

| Response variable Site | Source of deviance | df | Dev | Residual df | Residual Dev | p (>Chi) |
|---|---|---|---|---|---|---|
| **Seed deposition** | | | | | | |
| Sheltered | Month | 2 | 205.7 | 42 | 333.7 | <0.001*** |
| | Distance | 1 | 98.0 | 41 | 235.7 | <0.001*** |
| Exposed | Month | 2 | 429.0 | 42 | 824.9 | <0.001*** |
| | Distance | 1 | 559.3 | 41 | 265.6 | <0.001*** |
| **Seed bank abundance** | | | | | | |
| Sheltered | Distance | 1 | 107.5 | 88 | 679.2 | <0.001*** |
| Exposed | Month | 5 | 110.5 | 84 | 813.8 | 0.009** |
| | Distance | 1 | 274.7 | 83 | 539.2 | <0.001*** |
| **Seed persistence** | | | | | | |
| Sheltered | Depth | 1 | 1220.3 | 418 | 1126.0 | <0.001*** |
| | Depth×Month | 3 | 104.8 | 415 | 1021.2 | 0.002** |
| Exposed | Depth | 1 | 1117.6 | 418 | 976.4 | <0.001*** |
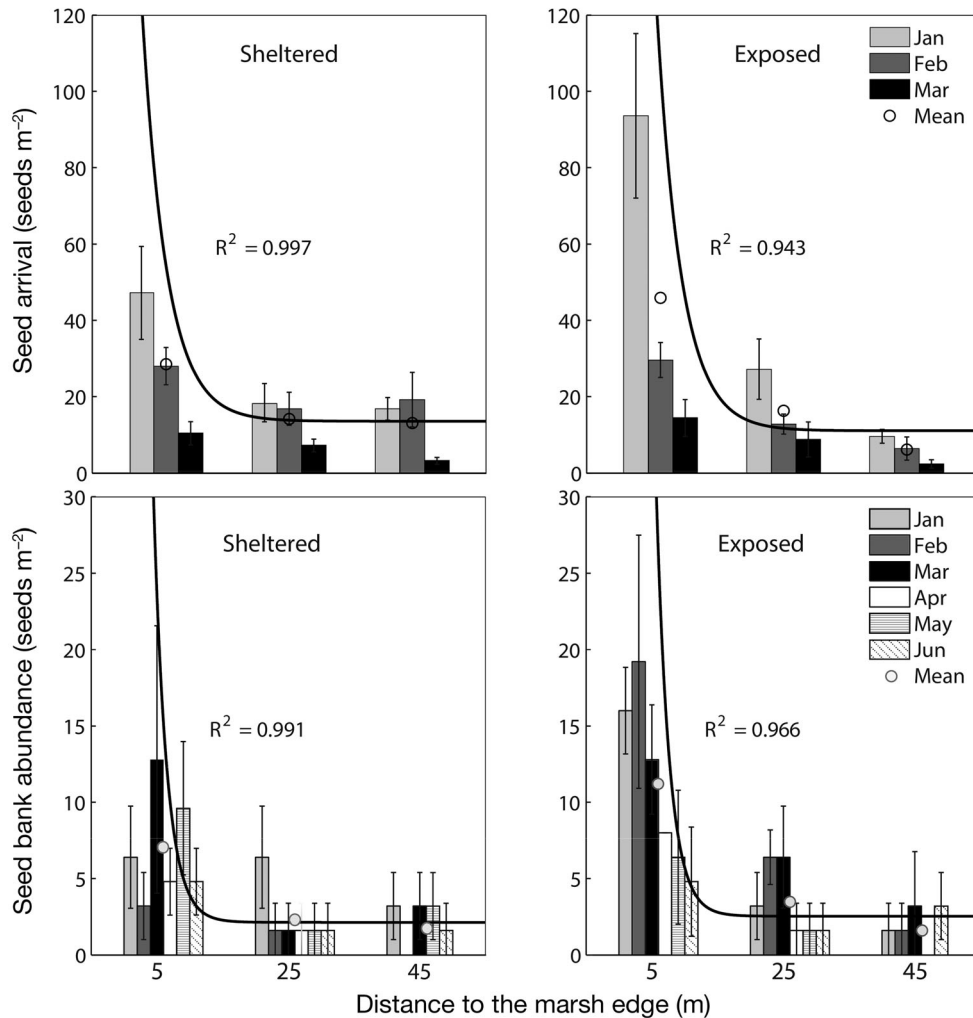
Fig. 3. *Spartina anglica.* Mean (±SE) seed arrival (top) from January to March and seed bank abundance (bottom) surveyed monthly from January to June at Paulinapolder (sheltered, left) and Zuidgors (exposed, right). Both showed a trend of exponential decay as illustrated by the equation $y = a \times \exp(x) + b$, which were fitted by the month-averaged seed deposition or seed bank abundance data

nificant either at the relative sheltered site (p = 0.250) or wind-exposed site (p = 0.946). The number of seeds present in the seed bank at both sites was much smaller than the number deposited on the Astro-Turf® mats during 1 mo at the same sites (Fig. 3). There was no significant difference in seed bank abundance between the 2 sites (p = 0.067).

## Persistence of manipulated seed bank

There were no significant differences between the recovery of seeds and mimics for either the relatively sheltered (pairwise Wilcoxon rank sum test, p = 0.11) or the relatively wind-exposed site (p = 0.24). Recovery of seeds and mimics were highly correlated, with

a correlation coefficient close to 1 (Fig. 4). These results imply that seed loss at both sites was mainly due to physical disturbances (e.g. sediment erosion driven by hydrodynamics) rather than seed predation.

Seed persistence did not significantly change along the gradient of distance from the marsh edge at either the relatively sheltered or the relatively exposed site (Table 2). In contrast, persistence of seeds was significantly affected by their vertical positions in the sediment at both sites (Fig. 5). Overall, the retention of surface (i.e. 0 mm) seeds in the manipulated seed bank was low at both the sheltered (mean ± SE, 11.5 ± 2.2%, n = 120) and exposed site (20.7 ± 3.0%, n = 120). Once buried, seed persistence was greatly improved even if buried as shallow as 5 mm (sheltered site: 45.7 ± 5.6%, exposed site: 89.3 ±
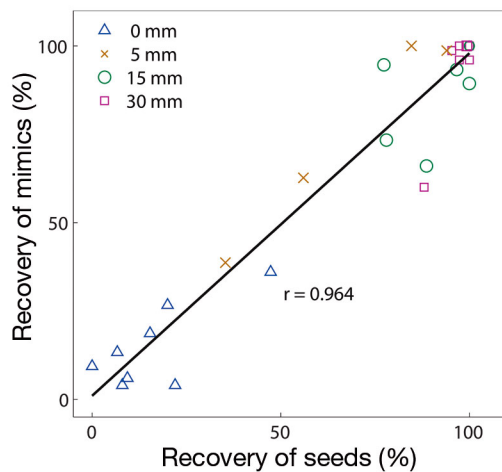
Fig. 4. Correlation between the recovery of *Spartina anglica* seeds vs. mimics. Each data point represents the mean pooled value of the 2 sites Paulinapolder (sheltered) and Zuidgors (exposed) at a given depth in each month

right location at the right time, stressing the relevance of seed arrival and persistence processes in seedling establishment in intertidal ecosystems. The current study indicates that the existence of local source populations and shallow seed burial are essential for generating an annual soil seed bank at tidal mudflats.

Despite the potential for long-distance dispersal of seeds by tidal currents (Koutstaal et al. 1987), salt marsh plants were found to mainly disperse their seeds locally (Rand 2000, Wolters et al. 2005). Medium or longdistance dispersal might occur, but only under extreme weather conditions (Chang et al. 2007). Previous studies have also shown that, at the local scale, seed deposition is inversely related to distance from the source population (Wolters et al. 2005). Our findings at 2 salt marshes with different wind exposure were consistent with that finding. At both of our study sites, *Spartina anglica* seeds were found to

3.0%; n = 60). Seed persistence increased with depth of burial, approximately following a power function (Fig. 6). On average, over 90% of seeds remained in the seed bank at both sites, when placed at a depth of 15 or 30 mm.

There was no difference in seed persistence in the manipulated seed bank between the relatively sheltered and the exposed site in January (p = 0.560) or February (p = 0.580). Nonetheless, seed persistence was surprisingly higher in March (p < 0.001) and May (p < 0.001) at the exposed site than at the relatively sheltered site, even though the onshore winds at the exposed sites would have had a greater impact during these latter months (Fig. 7).

## DISCUSSION

Successful seedling establishment in tidal flats requires specific windows of opportunity, i.e. disturbance-free periods (Balke et al. 2011), due to thresholds imposed by the harsh environments they inhabit. However, opportunity only favors the 'prepared' seeds that arrive or are present in the
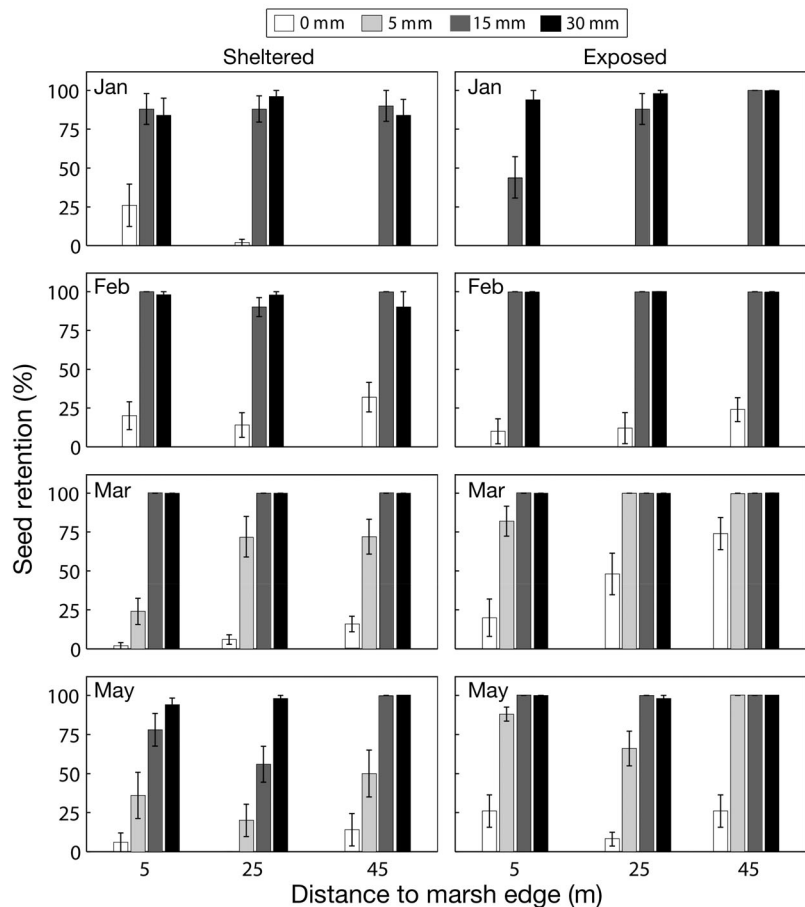


Fig. 5. Persistence of the manipulated *Spartina anglica* seed bank (mean ± SE) at Paulinapolder (sheltered, left) and Zuidgors (exposed, right) in relation to the distance to the salt marsh edge. Seeds were originally deployed at the depth of 0, 15, 30 mm in January and February, and a new layer at 5 mm was added in March and May
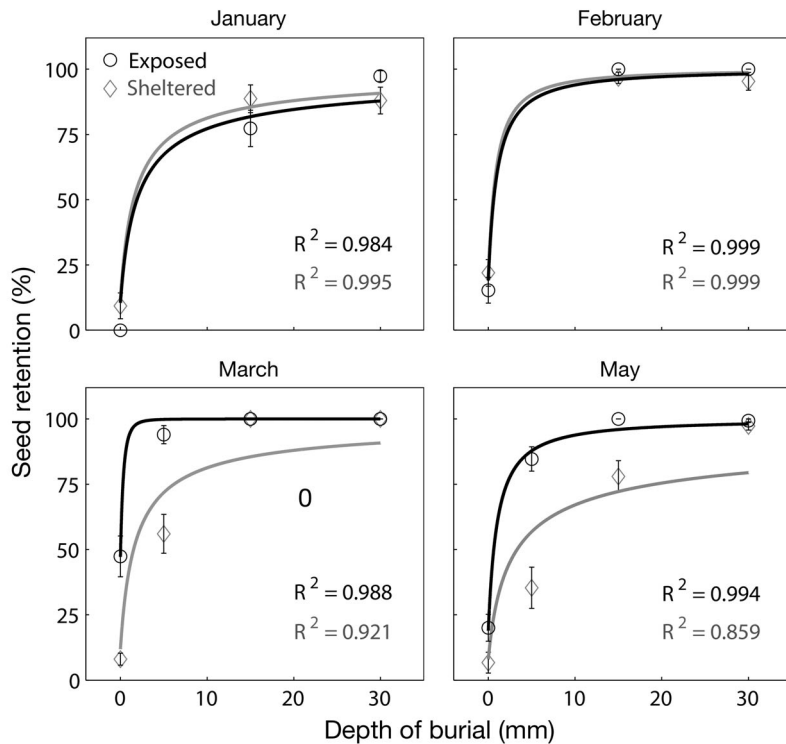
Fig. 6. Persistence of the manipulated seed bank (mean ± SE) at Pauli-napolder (sheltered) and Zuidgors (exposed) in relation to the depth of burial in the sediment. Equation for the fitted curves: $y = 1 - (x + 1.2)^a$ when $a < 0$

deposit close to the marsh edge, and deposition decreased exponentially with increasing distance to the marsh edge, while the amount of deposited seeds was month-specific and the rate of decrease in deposition was site-specific. This might result from the heterogeneity in seed source patterns, seed release dynamics, hydrodynamic forces, and interactions of all of these variables; however, investigating these additional factors was outside the scope of this paper.

We identified the role of seed arrival and persistence for determining seed bank abundance at the tidal flat in front of the salt marsh. Our results clearly indicated that the pattern of seed bank distribution at the adjacent tidal flats was set by initial seed arrival, while seed abundance in the seed bank was mainly constrained by subsequent persistence of arrived seeds, especially those on the surface, that had a high chance (>80% on average in our experiments) to be entrained and progressively transported to farther locations during subsequent tidal cycles. Thus, seedling establishment potential on the mudflat can be limited by insufficient seed arrival when the seed source is far away, whereas high seed loss can serve as a main bottleneck regardless of the distance to the seed source.

Biological events like seed predation by benthic animals were not responsible for seed loss from the manipulated seed bank in our experiments because there was no difference in recovery but a high correlation between seeds and mimics. Given the size (ca. 15 mm in length) and the material of the seed mimics (non-digestible hard plastic) used in our experiments, the probability that seed mimics also suffered losses due to consumption by benthic animals was negligible. This implies that the loss of seeds was mainly due to physical disturbances (e.g. sediment erosion driven by hydrodynamics) rather than biological factors. This result contrasted with the lab experiment (Emmerson 2000) that suggested loss of *S. anglica* seeds was due to the 'grazing' by *Hediste diversicolor*, one of the most frequently found benthic macro-invertebrates in European estuaries. This species was also reported to eat the seeds and seedlings of another salt marsh plant, *Salicornia europaea* (Paramor & Hughes 2004). It is possible that the herbivory by such species was a conditional outcome, e.g. *Spartina* seeds could only be consumed after germination or merely due to the absence of other food sources in their experiments. However, this remains to be verified. Nonetheless, other effects of benthic animals like seed burial, either being directly transported or indirectly displaced through sediment mixing (Delefosse & Kristensen 2012), could play important roles in seed bank dynamics in salt marshes.

The present study specifically highlights the importance of seed burial for seeds to persist in the right location at the right time. Our experiments demonstrated that seed burial generally enhanced seed retention nonlinearly with increasing burial depth. Once seeds moved down and escaped from the water-sediment interface, their persistence was greatly improved even if buried by only a thin layer of sediment. This result lent support to studies on other plants that inhabit tidal flats. Seagrass, for example, benefits from seed burial which provides the seeds with a 'safe site' (Orth et al. 2006). However, deep burial can lead to the failure of the seed-seedling transition. A lab experiment (T. J. Bouma et al. unpubl.) on seedling emergence of *S. anglica* showed that seedling emergence linearly declined
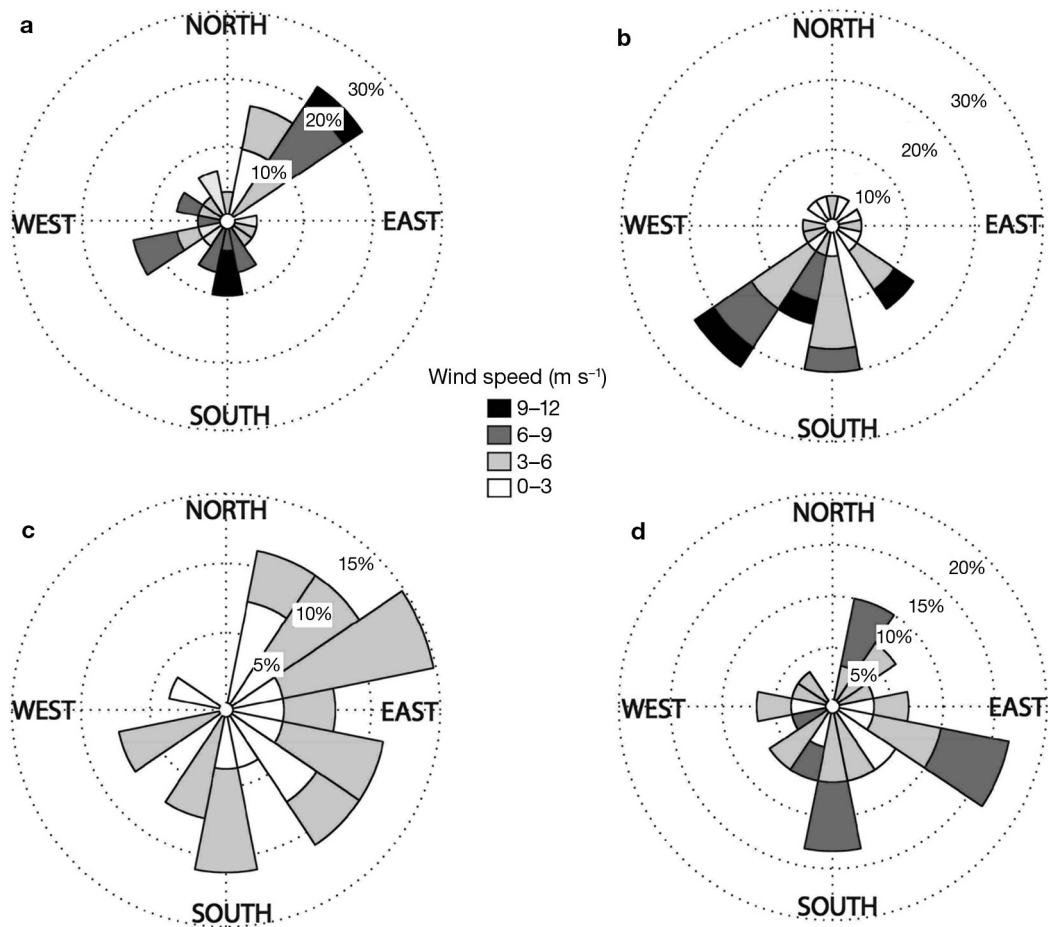
Fig. 7. Wind roses for the experimental periods in (a) January, (b) February, (c) March, and (d) May 2012. Concentric circles with percentages represent the wind frequencies. Data was acquired from the weather station of Vlissingen, The Netherlands (see Fig. 1). The data showed that in Febuary, March and May, onshore winds occurred more frequently at Zuidgors (southerly wind) than at Paulinapolder (northerly wind) due to their geographic locations (see Fig. 1)

with increasing burial depth (regression line equation: $y = -1.12x + 95.8$, $R^2 = 0.96$). In their experiment, >80% of seeds buried in shallow depths (within 0–20 mm) were able to emerge seedlings, while >80% of seeds buried deeper than 50 mm failed. A study on seagrass species *Zostera marina* found that seeds germinated from deeper than 50 mm cannot reach the sediment surface because of insufficient energy stored in hypocotyls (Greve et al. 2005). However, it remains unclear how the survival of seed viability in the buried seed bank is influenced by abiotic and biotic factors in tidal habitats.

It seems that seed persistence in the sediments cannot be simply inferred from wind exposure. The more exposed site did not show higher seed loss than the relatively sheltered site in our experiments. For instance, seeds buried at the depth of 5 mm had a seed loss of >50% in May at the relatively sheltered

site, but only 10% at the wind exposed site (Fig. 6). A previous study suggested that the percentage of seeds washed away from the buried seed bank was depending on the mobility of the top layer of the sediment (van Eerdt 1985). The effects from wave exposure might be confounded by other factors such as sediment dynamics that can be influenced by abiotic factors (e.g. currents, waves, sediment properties) and biotic factors (e.g. bioturbation) being variable in space and time. However, since we only had 2 sites for comparison and did not measure actual hydrodynamic conditions during our experiments at both sites, caution may be warranted when inferring any relationship between hydrodynamics and seed persistence based on our results. Future studies should seek to unravel the effects of hydrodynamics and their potential coupling with sediment dynamics on seed persistence.

In our experiments, short-term (1 mo) persistence of seeds in the buried seed bank was generally high. Nonetheless, the actual season-long (several months) seed persistence might be lower due to the dynamic nature of the tidal mudflats. Seeds that persisted for 1 mo might be eroded when exposed to disturbance for a longer time. For example, an early study on the establishment of *S. anglica* on a tidal mudflat in the Oosterschelde, The Netherlands, showed that >90% of the planted seeds at a depth of 5 mm were lost after ca. 2 mo (Groenendijk 1986). In another *in situ* experiment in the Oosterschelde, most seeds of *S. anglica* buried at 15 mm depth were eroded from mudflats after 6 mo (van Eerdt 1985). In contrast, seeds buried at the same depth (i.e. 15 mm) for a shorter period (i.e. 1 mo) in our experiments at both sites were mostly undisturbed, with a retention of >90% on average (Fig. 6).

Moreover, the bare tidal flats are characterized by short, high-disturbance stochastic events (e.g. storm surges) that alternate with longer periods of relatively low disturbance, i.e. 'normal' hydrological conditions (Deloffre et al. 2006). Extreme hydrodynamic events may have disproportionately greater effects than normal tidal regimes on seed bank dynamics, because they can strongly influence sediment dynamics in tidal flats (e.g. Roman et al. 1997, Bartholdy & Aagaard 2001). One storm event may wipe out the majority of the buried seed bank by mobilizing a few centimeters of sediment. The same event might also do the opposite by transporting more seeds (e.g. Chang et al. 2007) to the tidal flats and burying them into the seed bank through sediment accretion, which was found to have a positive effect on seed deposition (Goodson et al. 2003). Sediment dynamics driven by high energy events can have considerable impact on seed bank dynamics at the tidal flats, especially when matching temporally with seed deposition. Thus one would expect large year-to-year variation in seedling establishment potential, since the timing of occurrence and severity of storms varies considerably between years.

Our findings are highly relevant to the management aspect of restoration and/or creation of salt marshes, given that transplantation efforts can be enhanced by natural recruitment or seed-based restoration techniques. For instance, seed mimics could be employed as an economical tool to help managers find the most suitable locations for successful restoration efforts (Delefosse & Kristensen 2012, Marion & Orth 2012). Persistence of planted seeds can be improved if managers manipulate seeding depths in the sediment. Our findings may also have implica-

tions for the control of invasive species such as *S. alterniflora* (Sayce et al. 1997, Xiao et al. 2009) and *S. anglica* (Nehring & Hesse 2008). Consideration of seed arrival and seed persistence processes might help slow down the spreading of exotic species by applying measures that prevent seed arrival at suitable locations or hinder seed entry into the buried seed bank. However, it still remains a challenge to develop effective measures that are practical in the field.

## CONCLUSIONS

The present study fills an important gap in knowledge on pioneer salt marsh establishment by providing insights into the patterns of seed arrival and seed persistence, which serve as key determinants for seed bank availability and thus colonization potential at the fronting tidal flats. Our findings contribute to developing a more holistic understanding of bottlenecks and thresholds to salt marsh pioneer establishment, which is an important step towards conservation and sustainable management of this valuable and threatened ecosystem. Our findings also point to the importance of hydrodynamic and morphodynamic processes on tidal flats to the establishment potential of salt marshes and suggest that salt marsh establishment and extension are closely linked to the physical dynamics of the entire estuarine system.

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