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Wave effects on seedling establishment of three pioneer marsh species: survival, morphology and biomechanics

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• **Background and Aims** It is important to have an in-depth mechanistic understanding of tidal marsh establishment and dynamics to ensure the long-term persistence of these valuable ecosystems. As wave forcing may be expected to impact seedling establishment, we studied the effect of water-imposed drag forces on seedling survival, morphology and biomechanical properties of three marsh pioneer species that are dominant along the salinity gradient in many areas around the world: *Spartina anglica* (salt to brackish), *Scirpus maritimus* (brackish) and *Phragmites australis* (brackish to fresh).
• **Methods** Using a newly developed plant-shaking mesocosm (PSM) that mimicked water-imposed wave drag forces, the effect of wave stress on seedling survival was examined, together with impacts on morphology and biomechanical properties.
• **Key Results** After 7 weeks of exposure to wave stress, lowered seedling survival and growth for all species was revealed. Wave treatments increased the root/shoot biomass ratio to enhance anchorage and made seedlings more flexible (i.e. reduced flexural rigidity), which might be regarded as a mixed outcome between a stress avoidance and stress tolerance strategy.
• **Conclusions** The different biomechanical responses between the three dominant marsh pioneer species, overall, make them less resistant to external stress. Therefore, our results indicate that the likelihood of marshes becoming established is reduced if wave energy increases. Despite the different biomechanical response of these three pioneer species to waves, the seedlings of all species were found to have low resistance to external stresses.

Keywords: Tidal marshes, establishment, seedling, wave effects, biomechanical response, *Spartina anglica*, *Scirpus maritimus*, *Phragmites australis*.

INTRODUCTION

Tidal marsh ecosystems are increasingly valued for their wave attenuation service (Bouma *et al.*, 2005, 2010) even under extreme storm conditions (Möller *et al.*, 2014). In coping with the growing risk of coastal flooding under accelerating sea-level rise (Craft *et al.*, 2008; Lin *et al.*, 2012; Kirwan and Megonigal, 2013), the conservation and restoration of tidal marshes are now widely appreciated as long-term sustainable protective solutions (Borsje *et al.*, 2011; van Slobbe *et al.*, 2013; Temmerman *et al.*, 2013). Tidal marshes are also valued for many other valuable ecosystem services such as conservation of biodiversity, regulation of nutrients via nutrient cycling, and regulation of climate via organic carbon sequestration (Gedan *et al.*, 2009; Barbier *et al.*, 2011; Burden *et al.*, 2013). Although there is strong evidence that tidal marshes can survive sea-level rise due to sediment-trapping plant-growth feedbacks (Kirwan and Megonigal, 2013; Kirwan *et al.*, 2016), over the last few

decades tidal marshes have rapidly degraded on a global scale (Silliman *et al.*, 2009; Tonelli *et al.*, 2010; Temmerman *et al.*, 2012). The high value of tidal marshes and the loss of such important ecosystems emphasize the needs to conserve and restore them. The latter requires in-depth understanding of both tidal marsh (re)establishment and lateral expansion under external stressors (Fagherazzi *et al.*, 2013; Bouma *et al.*, 2014; van Belzen *et al.*, 2017; and references therein).

In many coastal areas around the world, seedling establishment is of particular importance for the (re)colonization of large bare tidal flats. This may be especially important for those erosional marsh edges where the height of the erosional marsh-cliff may prevent plants that are growing on top of this cliff from expanding clonally onto the lower tidal flat in front of the cliff. This kind of disconnection between marsh and tidal flats may be especially important in macro-tidal systems. In these areas, seedling establishment is regarded as the first critical

threshold that needs to be surpassed to initiate the transition from a bare mud flat to vegetated state (Wang and Temmerman, 2013; Balke *et al.*, 2014; Bouma *et al.*, 2016). Once seedlings have established, further marsh expansion may be expected by lateral clonal growth (van der Wal *et al.*, 2008; Vandenbruwaene *et al.*, 2011). Recent studies have shown that for some species waves may hamper seedling establishment (Silinski *et al.*, 2015, 2016) via wave-induced sediment dynamics (Callaghan *et al.*, 2010; Hu *et al.*, 2015; Bouma *et al.*, 2016). However, the direct effect of waves on newly establishing seedlings remains poorly understood. We still lack insight as to whether this may vary among marsh pioneer species that occur along the salinity gradient of estuaries, where wave exposure may also be expected to decrease due to narrowing of the system.

Wave exposure is known to be of critical importance for understanding long-term marsh development (Callaghan *et al.*, 2010; Fagherazzi *et al.*, 2012; Bouma *et al.*, 2014). For example, recent studies have demonstrated that wave exposure can induce cliff formation at the marsh edge, causing lateral erosion (Callaghan *et al.*, 2010; Marani *et al.*, 2011; Fagherazzi *et al.*, 2013; Wang *et al.*, 2017). In contrast, surprisingly little is known on how wave stress affect tidal marsh plants during the establishment phase. Previous studies indicate that waves can affect individual plants, either directly by imposition of drag/pull on the plants (Coops *et al.*, 1991, 1994, 1996; Coops and Van der Velde, 1996; La Nafie *et al.*, 2012; Silinski *et al.*, 2018), or indirectly via sediment scouring around the stems (Bouma *et al.*, 2009a; Silinski *et al.*, 2015). As yet, the direct mechanisms that limit or allow marsh establishment are only starting to be quantified by experimental studies (Silinski *et al.*, 2015).

Current knowledge gaps on the direct effects of waves on plants may be partly due to methodological limitations. Studies assessing the interaction between waves and vegetation have conventionally used wave flumes of different scales (Bouma *et al.*, 2005, 2009b, 2010; Möller *et al.*, 2014; Silinski *et al.*, 2015, 2018). Whereas wave flumes typically provide a perfect method to mimic hydrodynamic conditions, the high construction and operational costs of such infrastructure makes these experiments generally too expensive to do long-term growth experiments or have high numbers of replicates. Long-term studies are commonly carried out in wave tanks (Coops *et al.*, 1996; Coops and Van der Velde, 1996; La Nafie *et al.*, 2012; Wang *et al.*, 2017), in which hydrodynamic conditions are typically less ideal than in flumes, but due to lower costs some level of replication is possible. In this study, we provide an even more simple approach to assess wave effects on plants by designing a tidal plant-shaking mesocosm (PSM). The PSM mimics wave-induced drag stress on the seedlings, by moving the plants (seedling) back and forth through the water, rather than by moving the water around the plants.

Species-specific insight into the responses of pioneers to enhanced wave climate is important to inform future restoration projects. As wave exposure may be expected to decrease with salinity, due to the typical narrowing of the estuarine system, species with a higher salt tolerance will perhaps be better adapted to wave exposure. The present study aims to extend current knowledge by studying the direct effect of wave stress on the survival, morphology and biomechanical properties of newly establishing seedlings of marsh pioneers, using novel tidal PSM. We compare this for three dominant marsh pioneer species that occur along the estuaries with decreasing salinity gradient: *Spartina anglica* (salt to brackish), *Scirpus maritimus* (brackish) and *Phragmites australis* (brackish to fresh).

MATERIALS AND METHODS

Plant material

Seedlings of *Sp. anglica*, *Sc. maritimus* and *P. australis* were obtained from seeds that had been collected from the Scheldt estuary (The Netherlands) in November 2016. Seeds were cool stored in a fridge at 4 °C until being germinated in a container with an alternating temperature condition (25 °C during the day and 30 °C at night to speed up germination, practical choice). Seeds with a visible germ were identified as seedlings suitable for transplantation. All seedlings were prepared 1 week before transplantation and stored in an incubator with the same germination conditions as described above. The plants were regarded to be in the seedling stage during the whole period of the experiment, as they remained short compared to fully grown individuals (longest shoots = 17.7 ± 2.4 cm for *Sp. anglica*; 34.9 ± 4.3 cm for *Sc. maritimus*; 44.5 ± 7.2 cm for *P. australis*). For each species, we transplanted 24 seedlings individually to PVC pots (160 mm height × 110 mm inner diameter) by planting them at 1 cm depth of the sediment. The sediment (with an average D50 of 29.93 µm) used was macrobenthos-free (by sieving) and selected in a primary test to have negligible scouring under our experimental conditions.

Experimental design

The experiment was carried out using four tidal mesocosms in a climate room (NIOZ Royal Netherlands Institute for Sea Research, Yerseke, The Netherlands), where light was provided with 12 h d⁻¹ (550 µmol m⁻² s⁻¹ photosynthetically active radiation), and temperature was controlled at 25 °C during the day and 18 °C during night (for more details see references in Cao *et al.*, 2018). In each mesocosm, a semi-diurnal 1.5-h inundation regime was mimicked with a mix of fresh water and Scheldt sea water (which gives a salinity of 12.61 ppt). All pots were equally distributed in the four systems with six seedlings of each species per mesocosm, which gives 12 replicate seedlings per species for each treatment. The water depth of all mesocosms was 21 cm from the sediment top during high tide.

Two of the mesocosms were set as controls (C) and the other two were equipped with plant-shakers to impose continuous drag forces during mimicked high tide. Each PSM consists of a piston-moved cuboid metal frame with a cribriform bottom that can hold many pots with plants. To simulate a wave like stress (W treatment), the plant-shaker was inserted onto the top tank of a tidal mesocosm. The piston was provided with air pressure that was controlled by a timer, to allow the device to move back and forth during tidal inundation. The distance of the motion was 30 cm in both directions, over a period of 2.6 s in the experimental tank, during the semi-diurnal 1.5 h of inundation. In this way, we approximated the drag imposed by regular waves with an average frequency of 0.38 Hz. This is to mimic the measured windy weather wave conditions at tidal marshes

in the Scheldt estuary (this compares with ~0.5 Hz for similar significant wind waves at tidal marsh fronts in the Scheldt estuary field sites, see references in Callaghan *et al.*, 2010; Wang *et al.*, 2017).

Plant morphology and biomechanical properties measuring

The mesocosm experiment lasted for 7 weeks, during which seedling health (survived, toppled or dead) and plant height (soil to leaf top) were surveyed weekly. To compare the overall growth rate of seedlings, the plant height change at each week interval was calculated. To test plant stem flexibility under bending forces, as occurs under wave forcing (Rupprecht *et al.*, 2015), all survived seedlings were carefully cleaned from the sediment at the end of the seventh week.

We measured biomechanical traits by three-point bending tests on the 5-cm-long basal stem fragments from survived seedlings, using a universal testing machine (Instron 5942, Canton, MA, USA). The tests were performed by applying a constant extension rate to the midpoint of each stem sample on two supports of the test machine jaw. The following biomechanical traits that related to bending were calculated:

Young's modulus (*E*; kPa), which quantifies the stiffness of the stem and describes how much force is needed to bend a stem (a higher *E* value indicates a stiffer stem), is calculated as the slope of the stress–strain curve in the elastic deformation region;

the *second moment of area* (*I*; m^4), which quantifies the distribution of material around the axis of bending (increases with diameter), is calculated using the formula for triangular cross-sections: $I = (bh^3)/36$, in which *b* and *h* are the base and height of the cross-section;

flexural stiffness (*EI*, N m^{-2}), which was calculated by multiplying *E* (Young's modulus) and *I* (the second moment of area) to compare the overall flexural rigidity of the seedlings (a higher *EI* value indicates less flexibility of individual plants).

The stem was considered to break or fold when it reaches the maximum bending stress.

The *breaking force* (*F*, N) was then calculated to compare the maximum force that samples can withstand before mechanical failure (a higher *F* value indicates more resistance to tensile stress).

The breaking force is thus a measurement of the external stress that seedlings can resist during the establishment phase (Rupprecht *et al.*, 2015; Silinski *et al.*, 2015, 2018; Vuik *et al.*, 2018). Due to mortality, we used five replicates for each species per treatment for the biomechanical test. For calculation, all the stem samples were deemed as circular in cross-section because hollow stems had not yet developed for all young seedlings in our experiment. Finally, the dry biomass of survived seedlings was measured after oven-drying at 60 °C for 72 h to compare root/shoot biomass.

Statistics

Seedling survival was analysed with the Kaplan–Meier method applying the log-rank for treatment comparisons, with seedling toppling and death set as hazard events. Seedling

growth rates were calculated based on the average change in plant height after 7 weeks. Two-way ANOVAs were carried out to analyse the effects of wave disturbance and species on the growth rate, root/shoot biomass and biomechanical parameters. All results were tested in SPSS 18.0 software (SPSS, Chicago, IL, USA) with a significance level of 5 %. We tested for normality and homoscedasticity of the data, and these assumptions were passed.

RESULTS

Seedling survival and growth

In the control groups (C), all seedlings survived (100 %) throughout the experiment, regardless of species (Fig. 1). However, when subjected to mimicked wave stress (W), the percentage of survived seedlings decreased over time. Kaplan–Meier tests showed that wave treatment significantly reduced overall seedling survival (Fig. 1, Table 1; *P* < 0.05). Although growth of seedlings was non-linear in our experiment (Fig. 2A), for all three pioneer marsh species, the average growth rate calculated from the change in plant height (soil to leaf top) during the 7 weeks was also significantly lower under wave treatments as compared to the control groups (Fig. 2, Table 2; *P* < 0.05). No significant difference in survival or growth rate was observed between the three pioneer marsh species (Figs 1 and 2). These results show that wave exposure is a common important factor that hampers marsh establishment at the seedling phase.

Seedling biomechanical and morphological traits

When comparing the biomechanical strength of the survived seedlings, two-way ANOVAs shows significant main effects of both wave treatment and species (Table 2). In particular, measurements of Young's modulus of elasticity (*E*; kPa) indicate that seedlings exposed to the wave treatment were significantly more flexible (i.e. reduced *E*) (Fig. 3A, *P* = 0.022 for *Sp. anglica*, *P* = 0.014 for *Sc. maritimus*, *P* = 0.003 for *P. australis*). A less stiff stem material together with a reduced second moment of area (*I*, Fig. 3B, *P* = 0.01 for *Sp. anglica*, *P* = 0.011 for *Sc. maritimus*, *P* = 0.347 for *P. australis*) led to significantly decreased overall flexural stiffness (*EI*) values of seedling stems for all three marsh species in response to wave treatments (Fig. 3C, *P* = 0.017 for *Sp. anglica*, *P* = 0.002 for *Sc. maritimus*, *P* = 0.003 for *P. australis*). Consistently, we also observed that the breaking force (*F*) of all three marsh seedlings showed a significant decrease after wave treatments (Fig. 3D, *P* = 0.041 for *Sp. anglica*, *P* = 0.033 for *Sc. maritimus*, *P* = 0.008 for *P. australis*). Thus, the overall results of the three-point bending test showed that marsh seedlings became more flexible and less resistant to tensile stress after continuous wave exposure due to different material properties as well as reduced stem diameter.

However, in spite of these general biomechanical trends, the pattern of parameters shifted between marsh species. For example, *Sp. anglica* seedlings showed the lowest flexural stiffness (*EI*, Fig. 3C) and lowest resistance to tensile stress (*F*, Fig. 3D). Stem diameter (*I*, Fig. 3B) was highest for *Sc. maritimus*
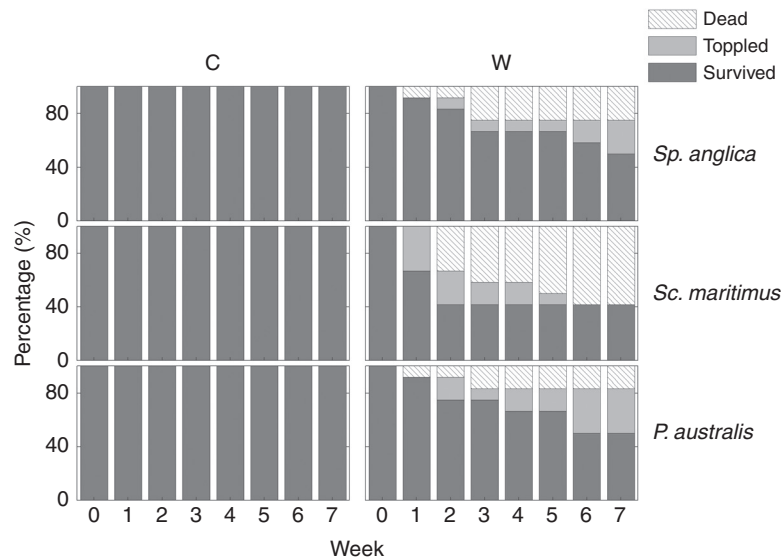
FIG. 1. Percentage of survived, toppled and dead seedlings of three marshes in the mesocosm experiment (C and W indicate the control and wave treatment groups separately).

TABLE 1. *Results of Kaplan–Meier log-rank test on the effects of wave treatments and species on seedling survival during the course of the mesocosm experiment. Both toppling and death of seedlings were set at hazard events during the test*

Variable	χ^2	d.f.	P
Wave treatment	25.98	1	<0.001
Species	0.32	2	0.85

seedlings, giving them the highest mechanical resistance (*F*, Fig. 3D). For *P. australis* seedlings, although stem diameter (*I*, Fig. 3B) was comparable between the control (C) and wave treatment groups (W), the significant decrease in stem flexibility (*E* and *EI*; Fig. 2A, C) nevertheless reduced the external stress that they were able to withstand (Fig. 3D).

Measurements of morphological traits at harvest showed that constant wave exposure also significantly increased the root/shoot biomass ratio of survived seedlings (Fig. 4, Table 2, *P* < 0.05). Interestingly, the root/shoot biomass ratio of *Sp. anglica* seedling was significantly higher than for the other two species after wave stress treatment (Fig. 4, *P* = 0.023 compared to *Sc. maritimus*; *P* = 0.016 compared to *P. australis*).

DISCUSSION

An in-depth understanding of how various physical and biological factors affect thresholds of tidal marsh establishment is essential for enabling restoration and sustainable management of these valuable ecosystems (Moreno-Mateos *et al.*, 2012; Bouma *et al.*, 2014). As yet, mechanistic studies on the direct effect of waves on the probability of establishment and developmental consequences for marsh seedlings are only starting to emerge (see references in Silinski *et al.*, 2015 for the influence of different wave regimes on the survival chances of *Sc. maritimus* from different life stages; see references in Rupprecht *et al.*, 2015 for field observations of biophysical
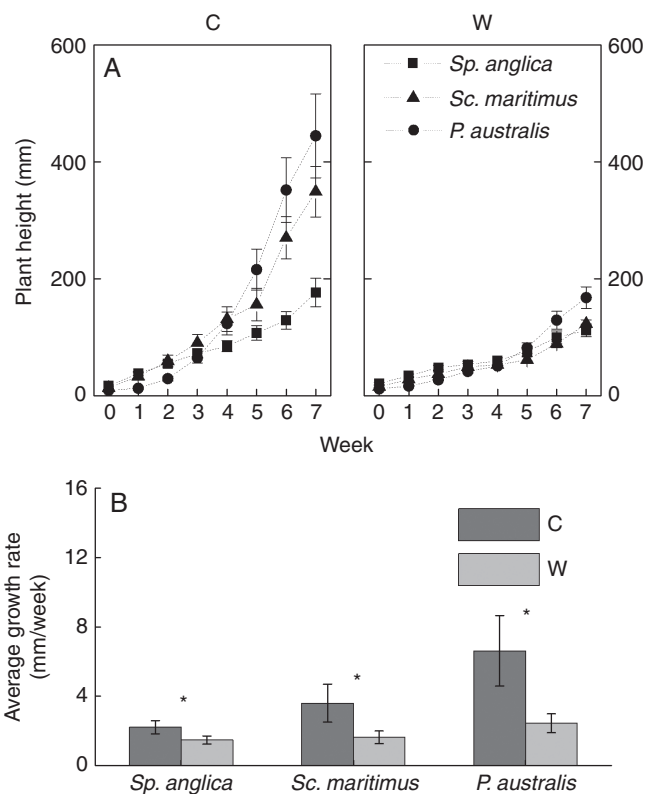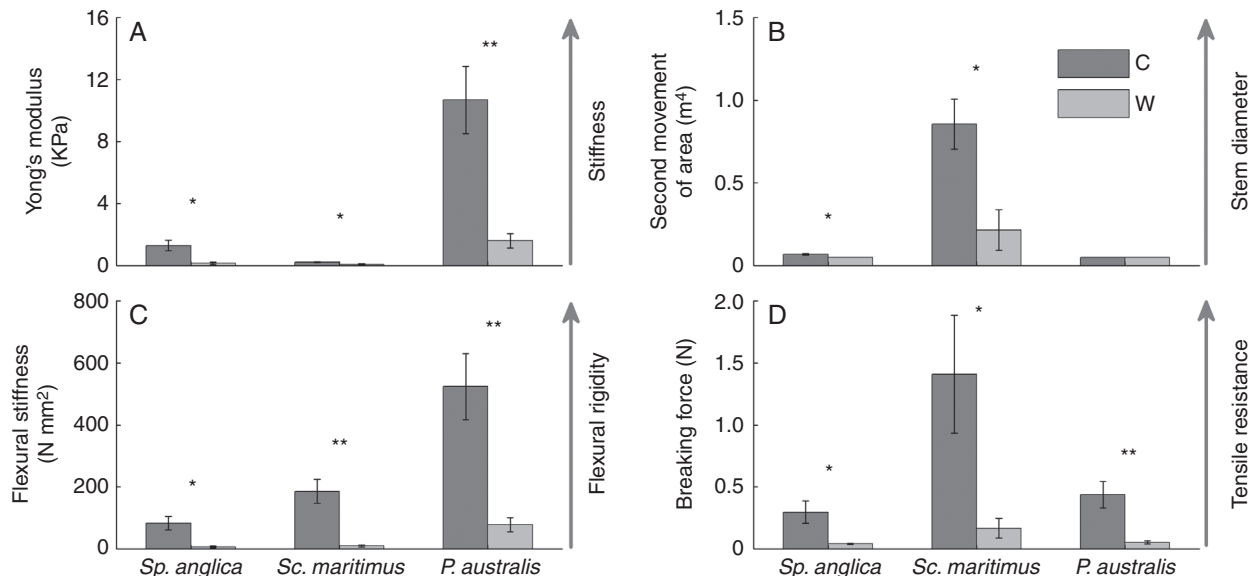

FIG. 2. Plant height change and average growth rate over 7 weeks of three marsh seedlings (data are means ± s.e.; C and W indicate control and wave treatment groups separately, *P* < 0.05).

properties of NW European salt marshes; but also see references in Zhu *et al.*, 2019 for the effects of salinity, inundation and seasonality on the biomechanical properties of marsh vegetation). The present study shows, for the first time, the direct effects of wave treatments on the early seedling establishment of three pioneer marsh species, by using tidal PSMs. We found

TABLE 2. *Two-way ANOVAs table of main effects of wave treatment and species and their interactions on the plant traits of seedlings*

Response variable	Deviance source	d.f.	Mean square	F	P
Growth rate (cm per week)	Wave treatment	1	55.2	8.84	0.005
	Species	2	17.1	2.73	0.077
	Wave treatment × Species	2	6.9	1.10	0.341
Yong's modulus (kPa)	Wave treatment	1	86.2	19.67	<0.001
	Species	2	107.8	24.59	<0.001
	Wave treatment × Species	2	59.3	13.52	<0.001
Second moment of area (m⁴)	Wave treatment	1	0.35	10.56	0.004
	Species	2	0.76	22.98	<0.001
	Wave treatment × Species	2	0.33	9.93	0.001
Flexural stiffness (N mm²)	Wave treatment	1	390 940	32.56	<0.001
	Species	2	176 809	14.73	<0.001
	Wave treatment × Species	2	87 799	7.31	0.003
Breaking force (N)	Wave treatment	1	2.83	12.82	0.002
	Species	2	1.11	5.03	0.015
	Wave treatment × Species	2	0.70	3.17	0.061
Root/shoot biomass	Wave treatment	1	1.85	9.84	0.003
	Species	2	0.33	1.78	0.180
	Wave treatment × Species	2	0.69	3.68	0.033

FIG. 3. Biomechanical traits of survived seedlings showing more flexibility after the continuous wave exposure: (A) Young's modulus; (B) second moment of area; (C) flexural stiffness; and (D) breaking force of three pioneer marsh seedlings (data are means ± s.e.; C and W indicate control and wave treatment groups separately; * $P < 0.5$, **$P < 0.01$).
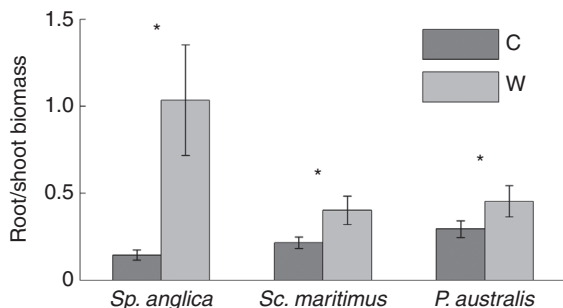
FIG. 4. Root/shoot biomass ratio of survived seedlings at harvest showing increased below-ground biomass investment after the continuous wave exposure (data are means ± s.e.; C and W indicate control groups and wave treatment groups separately; *$P < 0.05$).

that wave treatments directly reduced seedling survival, enhanced the root/shoot biomass ratio to enlarge anchorage, and made seedlings more flexible (reduced flexural rigidity). The different biomechanical responses between the three dominant marsh pioneer species: *Sp. anglica* (salt to brackish), *Sc. maritimus* (brackish) and *P. australis* (brackish to fresh), overall, make them less resistant to external stress.

Effects of wave stress on seedling establishment and growth

Our results clearly show for all three pioneer marsh species that waves are a stress that may create a threshold that directly hampers both seedling establishment and seedling growth. Marsh recovery has been regarded as a critical transition

between two alternative stable states (the bare tidal flat state versus the vegetated state; Wang and Temmerman, 2013; van Belzen *et al.*, 2017). The present results thus underline the requirement for wave-free periods to provide 'windows of opportunity' for a marsh to establish (Balke *et al.*, 2014; Bouma *et al.*, 2014). Whereas previous studies related the need for a wave-free period to prevent uprooting due to sediment dynamics (Bouma *et al.*, 2014, 2016; Cao *et al.*, 2018), we show here that there are also direct effects on seedling growth. As the windows of opportunity concept for seedling establishment requires a disturbance-free period for the seedlings to grow large enough to be able to resist hydrodynamic disturbances (e.g. by having longer roots and stronger shoots; Balke *et al.*, 2013, 2014; Hu *et al.*, 2015; and references therein), reduced seedling growth implies that marsh establishment could be much more vulnerable to waves than previously anticipated. Moreover, as ship-generated waves and extreme weather events may be expected to increase in the near future (Silinski *et al.*, 2015), wave-free windows of opportunity will become more rare. In these cases, site-specific conditions will have to be taken into account (e.g. how much wave stress would be tolerable, or how many windows of opportunity are available) for engineering or management measures that focus on creating mashes by reducing waves on the foreshore.

Response of seedlings in biomechanical traits

Plascticity indiced by wave stress can be beneficial to enhance the performance of aquatic plants in coping with external forces (Puijalon *et al.*, 2005, 2008, 2011). For example, a significant increase in root/shoot biomass ratio indicates an increased investment of seedlings to below-ground biomass to reinforce anchorage strength: a 'tolerance strategy' to resist greater drag force. This is in line with more commonly observed anchorage increase under flow in other aquatic plant species, such as *Luronium natans*, *Mentha aquatica*, *Potamogeton coloratus* and *Sparganium emersum* (Puijalon *et al.*, 2008). Unlike the seedlings in the control group that can tolerate higher breaking force, a decrease in flexural rigidity of seedlings under wave stress enables them to expose less frontal surface areas to incoming waves: an 'avoidance strategy' to experience lower drag forces. This supports a well-recognized adaptation of plants to cope with exposure to strong hydrodynamics (see references in Gaylord *et al.*, 2003 for kelp canopies; Bouma *et al.*, 2005 for salt marshes; Bal *et al.*, 2011; Puijalon *et al.*, 2011 for freshwater aquatic plants and La Nafie *et al.*, 2012 for seagrasses). In an earlier study by Silinski *et al.* (2015), the authors reported that a shift between avoidance and tolerance strategies may occur between life stages within one species. We found that both strategies are, to some extent, adopted particularly during the early seedling phase.

In addition to the above-mentioned similar adjustment in biomechanical traits for all three pioneer marsh seedlings, we also compared interspecific differences in mechanical characteristics, as they may play a role in the shifts of species along estuaries with varying wave exposure (Callaghan *et al.*, 2010; Wang *et al.*, 2017). For example, the significantly higher root/shoot biomass ratio of *Sp. anglica* seedlings under wave

stress can be expected to enable their establishment at higher hydrodynamic conditions (e.g. higher wind exposure or under sea-level rise) than the other two pioneer marsh species. The notably higher stiffness for *P. australis* seedlings in the control groups might be due to nodal stabilization in their stems (Spats *et al.*, 1990). This means that when living in sheltered areas with less oscillating stress, aquatic plants of *P. australis* should be more tolerant to wave attack than the other two species by following a 'tolerance strategy'. The present results extend current knowledge that salinity is an important factor governing spatial species distribution and plant growth (Pennings *et al.*, 2005; Crain *et al.*, 2008), in showing that waves may reduce seedling survival by around 50 %. It should be noted that in our experiment, the species were not always grown at their predominant salinity. That is, *Sp. anglica* was grown at a relatively low salinity whereas *P. australis* was grown at a relatively high salinity compared to their predominant habitat. The latter may have caused a somewhat higher growth rate for *Sp. anglica* and a somewhat lowered growth rate for *P. australis*, with potential effects on exact survival percentages. The general trend that waves hamper seedling survival for pioneer marsh species remains clear. Moreover, recent year-round field observations by Zhu *et al.* (2019) showing that salinity did not result in major changes in plant biomechanical properties suggest that the present findings of wave-effects on (biomechanical) plant traits is not strongly affected by using a single salinity.

Our results that *Sp. anglica* seedlings had the least flexural rigidity and resistance to tensile stress (as compared to change in biomechanical properties for the other two species seedlings) seem controversial given that *Sp. anglica* is well known as having wave attenuation value with stiff shoots (Bouma *et al.*, 2005). However, considering that individual seedlings were used in the present study and the high variability in the flexural rigidity of difference life cycles of marsh plants (Rupprecht *et al.*, 2015), the stem biomass of dense mature plants canopies may compensate for the stiffness in wave dissipation (Bouma *et al.*, 2005; Möller *et al.*, 2014; Rupprecht *et al.*, 2015). Therefore, the present findings are in agreement with earlier studies that highlight the importance of considering plant size and morphology when determining plant biomechanical characteristics for their wave attenuation service at different locations and times (Niklas, 1992; Rupprecht *et al.*, 2015; Silinski *et al.*, 2018; Zhu *et al.*, 2019).

Implications for tidal marsh restoration

In recent decades, physical constraints have been widely recognized to be the overarching control for marsh establishment (Adam, 2002; Friess *et al.*, 2012). The mechanisms responsible for determining critical a hydrodynamic threshold for marsh establishment and long-term evolution have mainly been elucidated by modelling studies (Mariotti and Fagherazzi, 2010). For example, wave stress and the sediment surface erosion they induce are predicted to be the essential processes behind marsh lateral retreat (Callaghan *et al.*, 2010; Tonelli *et al.*, 2010; Marani *et al.*, 2011; Francalanci *et al.*, 2013). Recent experimental studies have extensively looked at the threshold constraints in terms of sediment dynamics (Balke *et al.*, 2014;

Bouma *et al.*, 2016; Cao *et al.*, 2018) or elevation-related inundation (Wang and Temmerman, 2013; van Belzen *et al.*, 2017). In this regard, our results reveal direct wave-stress effects on seedling survival and growth (both restriction and response) and provide a quantitative support for modelling studies to elucidate hydrodynamically driven tidal marsh dynamics. However, wave characteristics (direction, length, height) can vary strongly in space and time (Nielsen, 2009). Due to the impact of increasing ship traffic or deeper water levels following sea-level rise, we may expect more severe wave forcing in an era of global change (Curtiss *et al.*, 2009; Houser, 2010). To better understanding the effect of wave stress on vegetation, it is thus also important to quantify actual wave forcing in the field, and how this changes over time. Such data can then be used to design process-based studies that investigate the effect of waves with varying energy on marsh expansion both by seedling establishment and rhizome expansion (Bouma *et al.*, 2014, 2016; Silinski *et al*., 2015, 2018).

Overall, the present study indicates that the likelihood of marsh establishment is reduced if wave energy increases. Despite the different biomechanical response of the three pioneer species studied, the seedlings of all species have low resistance to external stresses. This type of experimental knowledge is essential to understand the role that coastal vegetation plays in mitigating risk and defending coastlines under sea-level rise, and to build more reliable process-based models to predict long-term marsh ecosystem dynamics, and evaluate measures for site-specific management and restoration schemes.

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