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Coping with waves: plasticity in tidal marsh plants as self-adapting coastal ecosystem engineers

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Running head: Marsh plants as self-adapting ecosystem engineers

Key words: wave exposure; phenotypic plasticity; ecosystem-based coastal protection; *Scirpus maritimus*; biogenic silica

28 **Abstract**

29 Tidal marsh vegetation is increasingly valued for its role in ecosystem-based coastal protection
30 due to its wave dissipating capacity. As the efficiency of wave dissipation is known to depend on
31 specific vegetation properties, we quantified in this study how these morphological, biochemical
32 and biomechanical properties of tidal marsh vegetation are, in turn, affected by wave exposure.
33 This was achieved by field measurements at two locations, with contrasting wave exposure, in
34 the brackish part of the Scheldt Estuary (SW Netherlands), where *Scirpus maritimus* is the
35 dominant pioneer. Our results show that shoots from more wave-exposed conditions developed
36 significantly shorter and thicker stems than the ones growing in more sheltered conditions.
37 Furthermore, we show that the more exposed shoots are more flexible whereas the shoots
38 growing in more sheltered conditions are stiffer. This may indicate plasticity in response to wave
39 exposure following a stress-avoidance strategy. Increasing stiffness was shown to be related to
40 enhanced biogenic silica and lignin contents of the shoot tissue. These properties might affect the
41 wave-attenuating capacity of the marsh as stiff plants are known to mitigate waves more
42 effectively than flexible ones. However, we also found higher shoot densities on the exposed site,
43 which may partly explain why higher relative wave attenuation rates were found on the exposed
44 site, despite the presence of more flexible individual shoots. This study highlights that the
45 efficiency of wave attenuation by tidal marsh vegetation ultimately depends on mutual
46 interactions between waves and plasticity in morphological, biochemical and biomechanical plant
47 properties.

Introduction

In the face of global sea level rise, there is a need for sustainable alternatives to traditional coastal protection by engineered constructions. Ecosystem-based solutions, including the conservation and restoration of tidal marshes, are increasingly suggested as long-term self-sustainable protective systems (Borsje et al. 2011; Temmerman et al. 2013): contrary to conventional engineering solutions (dikes, dams), marshes can adapt to increasing sea levels by accumulating sediment and thus grow with the rising sea. Additionally, natural coastal protection by marsh vegetation has been proven to be very effective as the majority of wave energy entering a marsh dissipates over the first few meters after the edge of the marsh (Bouma et al. 2005; Ysebaert et al. 2011; Möller et al. 2014). Even under extreme water levels and wave heights typical for storm surges, when coastal defence is most important, up to 60 % of wave-height attenuation may be attributed to marsh vegetation (Möller et al. 2014).

In the past, research has focussed on current-induced hydrodynamic stresses and their effect on clonal macrophytes and marsh vegetation (Bouma et al., 2009; Puijalon and Bornette, 2006; Puijalon et al., 2005), although recent findings emphasise the role of wave exposure (Tonelli et al. 2010). The capacity of plants to attenuate waves, however, depends on plant morphological traits: for instance, flume experiments and field measurements showed that stiffer shoots as well as a high standing biomass increase wave attenuation (Bouma et al. 2010, 2013; Ysebaert et al. 2011). The acting waves, in turn, have been shown to exert stress on plant growth. Direct wave effects on plants are exerted by drag and pulling forces (Denny 2006; Henry and Myrhaug 2013) and scouring of sediment around plant stems (Bouma et al. 2009; Friess et al. 2012). Indirect effects are the wave-induced sediment texture and composition (Coops et al. 1991, 1994), resulting from hydrological sorting.

In order to cope with hydrodynamic forces, clonal macrophytes are known to produce plastic responses: wave exposure leads, for example, to shorter plants, a greater allocation of below-ground biomass, higher densities of above-ground stems and less daughter plants (Coops and Van der Velde 1996; Blanchette 1997; Doyle 2001). On a biomechanical level, plants typically follow either an avoidance or a tolerance strategy (Coops et al. 1994; Puijalon et al. 2011): enhanced flexibility, which enables the plant to bend when exposed to water flow or waves and thus to reduce experienced drag, can be seen as a typical stress-avoidance strategy (Puijalon et al. 2011; Schoelynck et al. 2013; Silinski et al. 2015). High mechanical resistance to breaking, on the other hand, determines the capacity of the plants to tolerate hydrodynamic stress, usually correlated to higher tissue rigidity (Coops and Van der Velde 1996; Puijalon et al. 2011). On a molecular level, rigidity of the shoot is enhanced when lignin and cellulose fibrils associate and form a secondary cell wall (Turner et al., 2001; Wang et al., 2012). Furthermore, biogenic silica (BSi) can also enhance the rigidity of the shoots (Schoelynck et al. 2012). Given that a stress-avoidance or stress-tolerance strategy can result in the development of different plant morphological properties, the capacity of the plants to attenuate hydrodynamics will be affected as well. Hence, an important remaining question is whether there are mutual feedbacks between plant morphological development and wave intensity and attenuation at tidal marsh shorelines.

The aim of this study is to investigate the response of morphological and tissue properties within a tidal marsh plant species to differences in wave exposure. This was done in a field monitoring campaign in the brackish part of the Scheldt Estuary (SW Netherlands and N Belgium), during the growing season of 2014, at two locations with contrasting wave exposure. The species under investigation was *Scirpus maritimus* L. Palla, which is a clonal, dominant pioneer marsh plant in the brackish parts of estuaries in the temperate zone. The novelty of our study is that we

quantified morphological, biochemical and biomechanical properties of *S. maritimus* between two contrasting sites, and also along a wave exposure gradient within each site, during the entire growing season of 2014. We hypothesize that (i) *Scirpus maritimus* adapts its morphological, biomechanical and biochemical properties depending on wave exposure (varying between the sites and with exposure within each site), and that (ii) these adaptations are the result of phenotypic plasticity and occur progressively over the growing season.

100

For Review Only

Materials and methods

Study sites

Two sites of contrasting wave exposure were chosen in the Scheldt Estuary (Fig. 1): Groot Buitenschoor (Belgium) and Schor van Rilland (The Netherlands), henceforth referred to as the sheltered and exposed site, respectively. The contrasting hydrodynamic conditions of these sites result from the fact that the Belgian site is sheltered by a breakwater from ship and wind waves, while the Dutch site is highly exposed through a long wind fetch to dominant South-westerly winds and the proximity of the shipping channel. Both sites are located in the brackish part of the Scheldt Estuary with an average annual salinity of 9.2 ± 0.17 SE ppt and an average annual suspended sediment concentration of 0.062 ± 0.004 SE g L⁻¹ (Maris et al. 2013). Due to their proximity, tidal characteristics are similar at both sites. Mean tidal amplitude in this part of the estuary is approximately 5 m.

Study species

Scirpus maritimus L. Palla (syn. *Bolboschoenus maritimus*) is the naturally occurring dominant pioneer plant in the study area. It typically forms the dominant vegetation at the lower fringes of marshes in the brackish zones of European estuaries (e.g. Scheldt, Elbe, Weser) (Heukels and van der Meijden 2005). It forms monospecific clonal stands, which produce aboveground shoots with a triangular stem cross-section that can reach heights of up to 2 m (personal observation in the field). This species grows clonally by producing several underground rhizomes per shoot that connect existing and newly produced shoots with each other (Charpentier and Stuefer 1999; Charpentier et al. 2000). During the growing season, tubers are formed which ensure the survival of the clonal, belowground plant in winter, and the regrowth of new shoots in spring, typically by the end of March in the Scheldt Estuary. From September onwards, the aboveground biomass

dries and dies off. In this way, short dead stem stumps of only several tens of centimetres' height are all that remains by the end of winter, when the appearance of new shoots growing from the tubers formed in the previous year begins.

Field campaigns

Monitoring plots

In order to compare site effects of wave exposure, monitoring plots were set up within the *S. maritimus* pioneer zone along a transect at both the sheltered and exposed site (Fig. 1 and 2). Along both transects, the monitoring was performed at three distances (subsequently referred to as plots) into the marsh: at the marsh edge (0 m), at 4 m and at 12 m into the marsh. An 8 m plot was added for the final campaign in September (see below). Each plot was approximately 1 m wide (perpendicular to the marsh edge) and stretched over approximately 10 m parallel to the marsh edge, following lines of equal elevation. Within these plots samples were taken randomly. By stretching the sampling over this area, we avoided negative effects of repeated sampling (e.g. of shoot sampling) on the conditions along the transect. This was done in order to compare within-site differences from the most wave-exposed plot (at the marsh edge) towards wave-sheltered plots (further into the marsh). This within-site gradient in wave exposure results from wave attenuation by the vegetation and potentially also by bottom friction - i.e. by the interaction of the waves with the sediment bed. At both transects the general topography on the tidal flat and within the marsh was a smooth slope without any strong small-scale topography (ridges or troughs) that could affect the hydrodynamic impact on the marsh. The slope over the first 12 m into the marsh was around 3 % and the elevation of the four plots along the transects were approximately -0.66, -0.54, -0.42 and -0.32 m, relative to mean high water (MHW), from the marsh edge towards the 12 m plot. Similar slopes at both transects were required so that there

would not be any differences in topography-induced wave attenuation between the two sites, and equal elevations imply a similar tidal inundation regime. Furthermore, vegetation canopy was continuous and without any bare patches along the transects at both sites. Hence, the only expected difference in hydrodynamics between the two sites resulted from higher incoming waves at the exposed site compared to the sheltered site.

Continuous measurements

Wave monitoring

For practical reasons (availability of three wave sensors), waves were monitored simultaneously at the marsh edge, 4 m and 12 m into the marsh (Fig. 2), one site at a time. From April on the three wave sensors were shifted between the exposed and sheltered site every two weeks, resulting in four blocks of two weeks of wave data per site (one block per month, from April to July). As tidal high water levels vary strongly in the course of a month (astronomical neap and spring tides, wind conditions) each block covered a spring-neap cycle to ensure that inundation depth distribution at both locations was comparable during these alternating monitoring phases. Thereby, we could ensure that differences in incoming wave heights at the marsh edge were not merely a result of differences in inundation depth. We then quantified plant-induced wave attenuation along the transects over the growing season. Additionally, we measured waves simultaneously at the marsh edges of both sites during a period of six weeks starting from early November 2014 in order to quantify the difference in wave exposure between sites over the same period in time. Waves were measured by submerged stand-alone PDCR 1830 pressure transducers (GE Sensing) at a frequency of 16 Hz. Wave data were converted from pressure to water surface elevation and analysed using a LabView script developed by the Department of Civil Engineering of UGent (Versluys and Troch 2010). The conversion was performed using

linear wave theory, in particular by applying the pressure response factor (Dalrymple and Dean 1991). The tidal signal was removed from the data by de-trending and de-meaning the wave measurements. Using the core-functions of R (R Core Team 2014), version 3.1.1, we then further processed the data and calculated significant wave heights (H_s , meaning the arithmetic mean of the highest third of recorded waves) and $H_{1/100}$ (meaning the arithmetic mean over the highest 1 % of recorded waves) for each measured time period and plot. The highest waves as well as relative wave attenuation over the first 12 m into the marsh (i.e. the reduction of wave heights at the 12 m plots compared to the incoming waves at the marsh edge) were also extracted and computed by means of R. Wave attenuation was calculated following the formula $R = (H_{me} - H_{12m}) / H_{me} * 100$ [%], where H_{me} are the wave heights at the marsh edge (= incoming wave height) and H_{12m} are the wave heights at 12 m into the marsh. We calculated wave attenuation for both, H_s and $H_{1/100}$. Multiplication with 100 was applied in order to convert relative wave heights to percentage values. Positive values indicate wave reduction into the marsh. These values were calculated for all wave data gathered at both marsh edges, irrespective of inundation depth (as both sites have the same absolute elevation, are relatively close to each other, and experienced very similar water depths, see Table 1). In this way, wave exposure at the marsh edges - as encountered in the course of full tidal cycles - was quantified.

Ground water monitoring

Piezometers of 2 m depth of which the lowest meter was perforated were installed at both sites at 0 m, 4 m and 12 m into the marsh (Fig. 2). Pressure sensors (Cera-divers, Schlumberger), installed in the bottom of these piezometers, monitored the ground water fluctuations from April to September with one measurement every 2 minutes. Surface water at the marsh edge was also monitored by a Cera-diver, set to the same frequency. Furthermore, a Baro-diver (Schlumberger)

recorded air pressure on the nearby dike which was used for correcting atmospheric pressure fluctuations in the water surface measurements. We analysed the tidal data gathered with the surface water diver with the R package “Tides” (Cox 2014) in order to obtain average inundation times, inundation frequencies and local mean high waters for the monitored period.

Monthly measurements

A monthly monitoring campaign was initiated in April 2014 and was performed until September 2014. Every month, 30 shoots were randomly sampled per plot in order to quantify their morphological properties (Fig. 2). They were clipped off close to the sediment bed. After measuring basal stem diameter, length of the shoots, and determining their fresh biomass, they were dried at 70 °C for 72 h in batches of 10 plants, allowing three replicate measurements per plot and per month for subsequent biochemical analyses. The dry biomass of shoots was quantified. Shoot density was determined per plot by counting the number of living shoots (i.e. shoots produced in 2014) within two fixed squares of 0.4 m x 0.4 m. We calculated average aboveground biomass (shoot density x dry biomass per shoot) per m² per plot.

The dried plant material, grouped per 10 plants (n=3 per plot and month), was ground with a grinding mesh of 1 mm (Retsch ZM2000). Silica, lignin and cellulose contents were determined on the samples of April, June, August and September, applying the DeMaster alkaline (NaCO₃) extraction method (DeMaster 1981) for the first and the Van Soest method (Van Soest 1963) for the two latter. These strengthening compounds influence biomechanical tissue properties such as flexibility and tensile strength. Furthermore, we quantified total NP concentrations with the Walinga method (Walinga et al. 1989), as it allowed us to form N:P ratios, required in order to determine the nutrient status of the shoots. This was done to check whether the different plant

morphologies between sites and along the within-site exposure gradient might simply result from nutrient availability.

September measurements

We performed additional sampling during the last monthly campaign in early September 2014 (Fig. 2) when biomass of *S. maritimus* is typically at its peak. For this campaign, we extended the three sampling plots at 0 m, 4 m and 12 m to four plots, by adding another plot at 8 m from the marsh edge for most measurements (Fig. 2).

Sediment samples

As we expected high disturbance of the plots by taking sediment cores, they were taken in September after all other measurements and sampling had been performed, so that none of the other measurements along the transects were influenced by the sediment core sampling. Three replicate sediment cores of 45 cm depth and 8 cm diameter were taken per plot and divided into three depth portions of 15 cm each, resulting in three core layers representing the top 15 cm, the depth of 15–30 cm and of 30–45 cm, respectively. The replicate depth portions per plot were pooled and well mixed. Grain size analyses were performed on three subsamples per mixed sample with a Mastersizer 2000 (Malvern) after a combined H_2O_2 and HCl treatment to remove organic compounds. Organic matter content was determined with Loss on Ignition (LOI), i.e. by ashing the samples at 550 °C during 6 h. KCl-extractions on fresh sediment allowed the determination of pH-KCl (potential pH), NO_3^- -N and NH_4^+ -N. Ammonium Acetate EDTA extraction allowed to measure PO_4^{3-} -P (Cottenie et al. 1982; Houba et al. 1989). Total P and N were obtained by digestion of the fresh sediment with H_2SO_4 -salicylic H_2O_2 -acid and selenium (Walinga et al. 1989). Quantification of these nutrients was done by colorimetry with a Continuous Flow Analyser (SAN++, Skalar, Breda, The Netherlands). Actual pH (after

extraction with H₂O) was also measured. Three replicate surface sediment samples per plot, taken with a Kopecky ring (4.6 cm in diameter and 5.2 cm high), were used to determine dry bulk density and water contents (after drying at 105 °C during 72 h) of the sediment surface of each plot.

Pore water samples

During the September campaign, pore water samples were taken at both sites from around 5 h after high water onwards: it was extracted from the marsh edge, 4 m, 8 m and 12 m plots by inserting five 9 cm Macrorhizon pore water samplers (pore size 0.15 µm, Rhizosphere Research Products, the Netherlands) per plot vertically into the sediment. These five samples per plot were pooled into one sample per plot in order to have a sufficient volume of each sample for quantification of dissolved silica (DSi) contents with the Continuous Flow Analyser (SAN++, Skalar, Breda, The Netherlands).

Belowground biomass samples

In order to quantify belowground biomass per plot, we took three replicate cores of 10 cm in diameter and of 45 cm depth at each plot. As for the sediment samples, this sampling was done after all other sampling due to the high disturbance of the plots. Similarly, the 45 cm of the core samples were split into three sections of 15 cm each. These core portions were thoroughly rinsed and sieved until only plant material (roots, tubers, belowground parts of stems) remained. It was dried during 72 h at 70 °C. Subsequently, dry belowground biomass per 15 cm core section was quantified. Based on the three replicate cores per plot, belowground biomass per m² marsh surface could be estimated.

Biomechanical traits

In order to quantify biomechanical parameters, 20 shoots per plot were sampled in addition to the regular monthly plant sampling campaign in September. Biomechanical traits were measured through tensile and bending tests on these shoots, using a universal testing machine (Instron 5942, Canton, MA, USA). Both tests (tensile and bending) were carried out on 20 cm long basal stem fragments: for the bending tests, the lowest 10 cm of these basal fragments were used while the tensile tests were performed on the upper 10 cm. For each sample, we measured the height and width of the triangular stem cross-sections at three different points along the sample.

- Bending tests

We performed three-point bending tests, consisting of a force applied at a constant rate of 10 mm min^{-1} to the midpoint of a sample placed on two supports. The support span was adjusted such that it was 8 to 10 times longer than the sample thickness. The following biomechanical traits related to bending were calculated: *Young's modulus* (E in Pa) quantifies the material stiffness, regardless of stem size or shape, and is calculated as the slope of the stress-strain curve in the elastic deformation region. *Second moment of area* (I in m^4) quantifies the distribution of material around the axis of bending, accounting for the effect of the cross-sectional geometry of a structure on its bending stress. I was calculated using the formula for triangular cross-sections (Niklas 1992): $I = (bh^3)/36$, where b and h are the base and height of the cross section (m). The *flexural stiffness* (EI in N m^2) quantifies the stiffness of the fragment and was calculated by multiplying E and I .

- Tensile tests

The stem fragments were clamped into the jaws of the testing machine and a constant extension rate of 5 mm min^{-1} was applied to the upper jaw until the fragments broke. The following biomechanical traits were calculated: *breaking force* (in N), defined as the maximum force that

the sample can bear without suffering mechanical failure, and *tensile strength* (in N m^{-2}) which is calculated as the breaking force per cross-sectional area.

The greenhouse control

We performed a control experiment, so as to mitigate the event of the observed plant morphological differences in the field being dependant upon the position of the tubers within the clonal network. In March 2014, we extracted 15 tubers, from both sites, from the 12 m plot as well as from the marsh edge. We transplanted these 60 tubers into pots of 25 cm height and 12 cm diameter that were filled with natural Scheldt sediment ($d_{50}=320 \mu\text{m}$) mixed with the slow releasing fertilizer Osmocote (Substral). They were randomly put into plastic containers in a greenhouse which served as troughs within which we maintained a water level of around 15 cm below the top of the pots. In this way we simulated the naturally water logged sediment conditions from the field. Furthermore, a daily rain event with 3.0 L m^{-2} assured the required water supply and equal growing conditions for the plants. Newly appearing clonal shoots were cut off weekly in order to maintain only one original shoot per pot. They grew under equal conditions until mid-June, around 8 weeks after transplantation, when we harvested the shoots and performed the same morphological measurements as on the monthly sampled field plants (diameter, stem height, dry biomass). The gap between the final harvest in the greenhouse (June) and in the field (September) results from the fact that the plants had grown faster in the greenhouse and were starting to show signs of senescence (in June) as typically found in the field once the annual biomass peak is passed (in September). We also quantified dry shoot biomass after drying at 70°C during 72h.

Statistical analysis

All statistical analyses were performed with the core functions of R (R Core Team, 2014), version 3.1.1. Two-way-ANOVAs were used in order to compare variables between both sites and the different distances from the marsh edge. Site and distance from the marsh edge were the fixed factors. T-tests were performed on the September data to compare the site effect for one plot (i.e. distance from the marsh edge) at a time. The Pearson's correlation coefficient was used to determine correlations between all measured biotic and abiotic variables. Before performing these tests, some of the data needed to be transformed in order to approach normal distribution: square-root-transformation was applied to basal stem diameter, dry biomass, flexural stiffness, Young's modulus, breaking force and lignin content; and log-transformation was performed on belowground biomass, second moment of area and N:P-ratios of plants.

Results

Physical and chemical parameters

Wave and surface water measurements

The analyses of the tidal surface water fluctuations for the full monitoring period show that the three plots along the transects at both sites experienced similar inundation depths, times and frequencies (Table 1). Furthermore, analysis of tidal levels, recorded during the respective bi-weekly wave monitoring periods, showed that a similar range of inundation depth was covered at the marsh edge plots of both transects during wave measurements. This ensures that wave heights recorded at both sites do not result from differences in water depth between sites. The incoming significant wave height (H_s), recorded during the full growing season at the marsh edge, was twice as high at the exposed site (5.7 cm) than at the sheltered site (2.8 cm) (Fig. 3a and Table 1). Maximum wave heights during that period were 45 cm and 25 cm, respectively. At both sites, the significant wave height and $H_{1/100}$ were reduced as the waves travelled through the vegetation (Fig. 3a, Table 1), with a higher attenuation rate (i.e. relative reduction of wave height at the 12 m plots compared to the incoming wave heights at the marsh edge) at the exposed site compared to the sheltered site (Fig. 3b). The wave attenuation rate for H_s varied over the growing season at both sites (Fig. 3b), corresponding to seasonal variations in aboveground biomass as shown below. This seasonal variation is less apparent for wave attenuation of $H_{1/100}$. The synchronous wave measurements at the marsh edge in November confirmed the difference in wave exposure (H_s of 5.3 cm and 3.2 cm and $H_{1/100}$ of 19.4 cm and 12.1 cm at exposed and sheltered site, respectively).

Ground water fluctuations

Regarding ground water fluctuations, a lowering of the ground water level with low waters in the order of up to 15 cm below sediment surface level, and following the monthly spring-neap tidal cycle, could be seen in our data. A time-lag of drawdown in the order of ten to twenty minutes between the exposed and sheltered site could also be seen, with earlier onset of drawdown at the exposed site, probably due to the sandier sediment corresponding to better drainage. However, the differences between sites were only in the order of centimetres, which was the same order as the range of error of our measurements, making any statistical analyses or definite conclusions difficult.

Sediment samples

Median grain size was coarser at the exposed than at the sheltered site (Table 2), and decreasing over distance into the marsh at both sites (Table 2). Organic matter contents, in contrast, was higher at the sheltered site, and increased with distance into the marsh at both sites. Dry bulk density of the top 5 cm of the sediment surface at the exposed site was almost twice the density of the sheltered site, while water content of the same samples was less than half compared to the sheltered site (Table 2).

In terms of chemical properties of the sediment (Table 3), PO_4^- -P and total P and N contents differed significantly between the sheltered and exposed site (t-test, $t = 7.281$, $df = 11.081$, $p < 0.001$ for PO_4^- -P; $t = 7.1252$, $df = 11.555$, $p < 0.001$ for total P and $t = 5.4337$, $df = 14.032$, $p < 0.001$ for total N), whereas the site-averages of NH_4^+ -N and N:P ratio did not differ significantly. NO_3^- -N content lay below detection limit. Actual and potential pH were lower at the sheltered than at the exposed site and were close to the reported optimum pH for *S. maritimus* of 8.3 (Hroudová et al. 2013)

Pore water samples

Pore water DSi concentrations at the sheltered site were two to three times higher than at the exposed site, with 18.22, 25.35, 20.47 and 20.50 mg L⁻¹ for marsh edge, 4 m, 8 m and 12 m into the marsh, respectively, at the sheltered site and with 6.26, 4.84, 7.57 and 8.59 mg L⁻¹ along that gradient at the exposed site.

Plants

Plant morphology - September

The new shoots of 2014 started to emerge at the end of March 2014. At the end of the growing season, as recorded during the September campaign (Fig. 4), shoots were significantly taller at the sheltered site compared to the exposed site (t-test, $t = 5.0947$, $df = 57.269$ at the marsh edge, $t = 9.7467$, $df = 57.583$ at 4 m into the marsh, $t = 9.8401$, $df = 56.794$ at 8 m into the marsh and $t = 13.3063$, $df = 54.803$ at 12 m into the marsh, with $p < 0.001$ for all). Furthermore, there was a size gradient within each site, with increasing size from the marsh edge into the marsh, which also differed significantly between sites (ANOVA, $F = 8.26$, $df = 1$, $p < 0.01$ for the interaction effect, i.e. a steeper increase of shoot size at the sheltered site compared to the exposed site). At both sites, stem diameter decreased with distance into the marsh (ANOVA, $F = 56.17$, $df = 1$, $p < 0.001$). This decrease was significantly stronger at the exposed site than at the sheltered site (ANOVA, $F = 14.94$, $df = 1$, $p < 0.001$ for the interaction term): at the exposed site the basal stem diameters close to the marsh edge were thicker than at the sheltered site, but similar between sites from 8 m onwards (t-test, $t = -4.7547$, $df = 57.567$, $p < 0.001$ at the marsh edge, $t = -5.1558$, $df = 52.244$, $p < 0.001$ at 4 m into the marsh plots, and $p > 0.05$ (not significant) for the last two plots). Except for the marsh edge plots, shoot density was higher at the exposed site than at the sheltered site (t-test, $t = -3.9476$, $df = 15.032$, $p < 0.01$). Furthermore, at both sites density of shoots was

significantly lower at the marsh edge than within the marsh (t-test, $t = 3.7687$, $df = 9.974$ and $p < 0.01$ at the sheltered site; $t = 6.828$, $df = 7.167$, $p < 0.001$ at the exposed site). In terms of biomass per m^2 , significant differences between sites only occurred at 4 m and 12 m into the marsh (t-test, $t = 3.5142$, $df = 55.618$, $p < 0.001$ and $t = 3.0487$, $df = 51.75$, $p < 0.01$, respectively), with higher standing biomass at the sheltered site.

Plant morphology – seasonal development

The monthly measurements during the entire growing season revealed a progressive development of most measured plant properties (Fig. 4). Shoot length increased over the growing season and differed significantly between sites (ANOVA, $F = 50.280$, $df = 1$, $p < 0.001$) and along the distance gradient (ANOVA, $F = 190.523$, $df = 1$, $p < 0.001$) over the entire growing season. The basal stem diameters only differed significantly between sites in September (ANOVA, $F = 31.12$, $df = 1$, $p < 0.001$), but from June onwards there was a significant distance effect (ANOVA, $F = 40.119$, $df = 1$, $p < 0.001$) as well as a site $\times$ distance interaction effect (ANOVA, $F = 7.045$, $df = 1$, $p < 0.01$), emphasizing the stronger reduction in stem diameter over the first 8 m into the marsh at the exposed site compared to the sheltered site. Shoot density only differed significantly between sites in September (ANOVA, $F = 8.289$, $df = 1$, $p < 0.01$), with a higher density at the exposed site, while a distance effect, with lower densities at the front compared to the back of the marsh, could be seen from June onwards (ANOVA, $F = 6.156$, $df = 1$, $p < 0.05$). After omission of the marsh edge plots, however, within-marsh stem densities at the exposed site proved to be significantly higher in April, May, June (ANOVA, $F = 35.805$, $df = 1$, $p < 0.05$) and September (ANOVA, $F = 13.543$, $df = 1$, $p < 0.01$) and with a trend (ANOVA, $F = 5.771$, $df = 1$, $p < 0.1$) for higher shoot density at the exposed site in July. Dry biomass per m^2 was, except for June, significantly higher at the sheltered site than at the exposed site (ANOVA, $F = 23.303$, $df = 1$,

p<0.001), and distance into the marsh had a significant effect (ANOVA, $F = 551.931$, $df = 1$,
p<0.001), as the biomass increased from the marsh edge into the marsh. However, no site $\times$
distance interaction effect could be shown for either shoot density or dry biomass.

Belowground biomass

At the end of the growing season (September 2014), total belowground plant biomass increased
at both sites over distance into the marsh (ANOVA, $F = 32.263$, $df = 1$, $p<0.001$), while
differences between sites were not significant (Fig. 5). Biomass allocation over depth, however,
differed between sites at 4 and 8 m into the marsh (ANOVA, $F = 9.489$ (4 m), $F = 7.645$ (8 m) ,
 $df = 2$, $p<0.05$ for both), as there was more biomass in the deeper layers of the cores at the
exposed site. Belowground-aboveground biomass ratio per m^2 differed only slightly between sites
except for the marsh edge plots.

Biochemical analyses

Plant N:P ratios did not change over the season and were on average 7.91 ± 0.30 at the sheltered
and 8.69 ± 0.34 at the exposed site (data not shown). When looking at the strengthening
compounds (Fig. 6), cellulose contents remained stable over time and throughout the transects,
and was on average only slightly higher (in the order of 20 mg g^{-1}) at the sheltered site. Lignin
contents, however, more than doubled from April to September. In terms of BSi, big differences
could be seen in time, between sites and along the transects, as BSi contents were significantly
higher at the sheltered than at the exposed site (t-test, $t = 2.5885$, $df = 51.981$, $p<0.05$). From
April to September, it increased at the sheltered site by a factor 5, and almost doubled at the
exposed site. In September, BSi increased at both sites from the marsh edge to the 12 m plot, by
almost tripling at the sheltered site, and more than doubling at the exposed site. For September,
the overall site averages differed significantly, too (t-test, $t = 3.2899$, $df = 12.799$, $p<0.01$).

Biomechanical traits

Biomechanical analyses of the September plant samples showed that the shoots from the sheltered site, except for those growing at the marsh edge, were generally stiffer and stronger than those grown at the exposed site (Fig. 7). There was also a general increase of stiffness from the marsh edge towards the back of the marsh, at both sites. This trend was stronger at the sheltered site compared to the exposed site ($F = 3.24$, $df = 1$, $p < 0.1$ for the site $\times$ distance interaction term of flexural stiffness). While Young's modulus was significantly higher at the sheltered site at the 0 m and 4 m plots (t-test, $t = 2.9998$, $df = 37.486$, $p < 0.01$ and $t = 4.4107$, $df = 30.714$, $p < 0.001$, respectively) and significantly lower at the 12 m plots (t-test, $t = -3.0178$, $df = 26.295$, $p < 0.01$), it did not differ significantly between sites overall. However, it increased at both sites with distance into the marsh (ANOVA, $F = 66.072$, $df = 1$, $p < 0.001$). Breaking force and tensile strength showed a significant site (ANOVA, $F = 45.502$, $df = 1$, $p < 0.001$ for breaking force and $F = 7.204$, $df = 1$, $p < 0.01$ for tensile strength) and distance effect (ANOVA, $F = 44.490$, $df = 1$, $p < 0.001$ for breaking force and $F = 68.312$, $df = 1$, $p < 0.001$ for tensile strength), as well as a significant interaction term (ANOVA, $F = 7.596$, $df = 1$, $p < 0.01$ for breaking force and $F = 4.810$, $df = 1$, $p < 0.05$ for tensile strength), as they both increased more strongly with distance at the sheltered site than at the exposed site. There was a significant effect of site and distance from the marsh edge for flexural stiffness (ANOVA, $F = 52.21$, $df = 1$, $p < 0.001$ for site and $F = 78.27$, $df = 1$, $p < 0.001$ for distance). Differences between sites and distances were not significant for the second moment of area.

Greenhouse control

Differences between sites and position along the distance gradient were much smaller for the greenhouse control than in the field (Fig. 8), overall: e.g. the difference in shoot length between

454 sites was only significant for the 12 m plots (ANOVA, $F = 5.055$, $df = 1$, $p < 0.05$). Within sites,
455 distance led - only at the sheltered site - to significant differences (ANOVA, $F = 19.58$, $df = 1$,
456 $p < 0.001$). Basal stem diameters did not differ significantly between sites nor within sites. Dry
457 weight of shoots differed significantly between plots at the sheltered site (ANOVA, $F = 39.36$, df
458 $= 1$, $p < 0.001$), but not at the exposed site. When comparing between sites, the differences were
459 not significant.

460 Correlations

461 Correlations between wave impact, on the one hand, and sediment and plant properties, on the
462 other hand (Table 4 & 5), need to be viewed with caution, as wave heights measured along each
463 transect are not independent from each other: i.e. the wave heights measured at the 4 and 12 m
464 plots are a function of the wave heights recorded at the respective marsh edge. When testing for
465 correlation, despite this constraint, wave exposure and sediment properties emerge as highly
466 correlated (Pearson's correlation coefficient, with $|r| > 0.86$ for all tested variables). While
467 median grain size and dry bulk density were positively correlated to wave exposure, DSi, organic
468 matter and water content were negatively correlated. Furthermore, plant BSi correlated well with
469 sediment pore water DSi ($r = 0.78$, not shown in the table).

When looking at the correlation between waves and plant properties (Table 5), the strongest correlations were found for BSi, shoot length, breaking force, flexural stiffness and stem diameter (Pearson's correlation coefficients $|r| > 0.75$ with a negative coefficient for all except for stem diameter). Furthermore, biomass, cellulose, lignin and BSi contents increased with shoot length, and decreased with stem diameter. While BSi had the highest correlation with flexural stiffness and tensile strength, cellulose was best correlated with Young's modulus. Cellulose and lignin contents also showed a high positive correlation.

For Review Only

Discussion

In this study we investigated whether plant properties of *Scirpus maritimus* vary between locations with different wave exposure and whether these properties develop progressively over the growing season. The novelty of this approach is that we looked into the relationships between waves and different types of plant properties, i.e. at the level of morphological, biomechanical and biochemical plant properties, and that we related the different levels to each other. Shoots from more wave-exposed conditions developed a stress-avoidance strategy by being shorter, thicker and more flexible than the shoots from more wave-sheltered conditions. Inner marsh stem density was higher at the exposed site than at the sheltered site. Some of these morphological differences developed during the growing season as a potential adaptation to local wave exposure. Plants from more wave sheltered locations were stiffer and stronger. These biomechanical properties were found to be related to biochemical properties of the plants: breaking force and tensile strength of stems correlated best with BSi contents which, in turn, reflected the local DSi contents in the sediment. Shoots grown under equal conditions (greenhouse control) showed only minor differences, indicating that the morphology of *S. maritimus* in the field is a response to the environmental factors that they are exposed to.

Waves have been shown to have direct and indirect effects on tidal marsh plants (e.g. Coops et al. 1991). Direct effects of waves on plants result from the direct physical impact of waves on the plants, leading, for example, to shorter shoots, thicker stems or higher shoot density of tidal marsh plants and aquatic macrophytes in general (e.g. Blanchette, 1997 for *Fucus gardneri*; Coops and Van der Velde, 1996 for different halophyte species including *Scirpus maritimus*; Doyle, 2001 for *Vallisneria americana*; Sun et al., 2002 for *Scirpus mariqueter*; Szmeja and

Galka, 2008 for a range of macrophyte species; see Bornette and Puijalon, 2010 for more references and a general review). The results presented in this paper are, overall, in line with these previous findings, as the shoots from more wave exposed locations were shorter, thicker and denser, and hence showed all the typical signs of morphological adaptations to more intense waves conditions (Fig. 4 and 9). Indirect effects of waves affect sediment properties via hydrodynamic grain size sorting and its effects on related sediment properties. This includes decreasing organic matter, water and nutrient contents and increasing bulk density (with increasing grain size), which could then potentially influence plant growth as well. However, in our case, despite significantly higher total N and P of the sediment at the sheltered site, N:P ratios did not differ between sites, and plant N:P ratios were in the same range, which implies that observed differences in plant morphology do not result from differences in nutrient availability. This is furthermore supported by Coops et al. (1991) who found in a controlled cultivation experiment that sediment type (and thus the corresponding sediment properties, including nutrients) did not affect the growth form of *S. maritimus*. The only influence of sediment properties on plant properties that we could establish was the direct correlation of pore water DSi contents and plant BSi contents: DSi contents were higher at the sheltered site compared to the exposed site, and plant BSi contents reflected the local sediment DSi contents. This “reflectance” of DSi availability in the BSi of plants had already been reported by Struyf et al. (2005) and Schaller et al. (2012). BSi has also been established in recent findings as a low-energy substitute for cellulose in conditions of local hydrodynamic stress (Schoelynck et al. 2012), which, however, cannot be supported by our findings, as cellulose and BSi contents were both negatively correlated to wave exposure (Table 5).

The higher DSi contents at the sheltered site could be an indirect consequence of wave exposure: lower wave exposure at the sheltered site is likely to enhance accumulation of BSi, e.g. by deposition of diatom and plant material. The decomposing diatom and plant material will mainly be retained within the marsh due to low wave action and a longer retention of sediment pore water in the finer sediment at the sheltered site. Dissolution of this retained BSi to DSi will stay *in situ* for longer periods and be available for new shoots (e.g. Struyf et al. 2007) (Fig. 9).

The interesting part of this finding is that plant BSi correlated well with flexural stiffness and tensile strength and also (to a lesser extent) with Young's modulus and breaking force of the shoots (Table 5), which are typically correlated to the presence of strengthening compounds such as cellulose, lignin and BSi (Mullarney and Henderson 2010; Rupprecht et al. 2015; Schoelynck and Struyf 2016). While lignin and cellulose concentrations were similar between plants from different sites (Fig. 6), BSi contents of shoots showed a strong site (and wave) effect, with BSi contents increasing with decreasing wave exposure, implying that the differences in biomechanical properties found between sites and plots may probably result mainly from differences in BSi contents: the more BSi was available (i.e. the more sheltered the location), the stiffer and stronger the shoots.

Biomechanical properties of intertidal plants have already been assessed in a number of publications (e.g. Carus et al., 2016 for *S. maritimus* sampled from the field; Coops and Van der Velde, 1996 for *Phragmites australis* and *Scirpus lacustris* sampled from the field; Heuner et al., 2015 for *S. maritimus* and *S. tabernaemontani* grown under controlled and sheltered conditions; Möller et al., 2014 for *Puccinellia maritima* and *Elymus athericus* sampled from the field; Rupprecht et al., 2015 for *Spartina anglica*, *Puccinellia maritima* and *Elymus athericus* sampled

from the field; Silinski et al., 2015 for *S. maritimus* grown under controlled and sheltered conditions). The results presented in this paper are, overall, in the same range as the ones reported by the above mentioned authors. Furthermore, biomechanical plant properties also relate (together with morphological properties) to survival strategies of plants: flexible shoots experience less drag forces due to their enhanced capacity to bend under flow or wave stress (Puijalon et al. 2011; Pujol and Nepf 2012; Schoelynck et al. 2013), which, in turn, reduces the risk for individual shoots from breaking or from being uprooted. These properties are typical of stress-avoidance strategies by plants as smaller, thinner and more flexible shoots will experience less drag forces than tall, thick and stiff ones, because these plants expose less surface to the incoming waves (e.g. Puijalon et al., 2008; Silinski et al., 2015) (Fig. 9).

While marsh vegetation is increasingly valued for its wave attenuation function and contribution to coastal protection (e.g. Borsje et al. 2011; Temmerman et al. 2013), it has been shown that plant properties such as shoot density and stiffness affect wave dissipation rates (e.g. Bouma et al., 2005; Ysebaert et al., 2011). For instance, higher standing biomass and higher plant stiffness lead to stronger wave attenuation through higher wave energy dissipation. At our field sites, relative wave attenuation rates ranged from 20 to 40 % of wave height reduction over the first 12 m of the marsh, which is in the same range as described for marsh fringes consisting of different marsh species (e.g. Bouma et al. 2005 for *Spartina angelica*; Ysebaert et al. 2011 for *Scirpus mariqueter* and *Spartina alterniflora*; Möller et al. 2014 for a mixed canopy of *Elymus athericus*, *Puccinellia maritima*, and *Atriplex prostrata*). We found that wave attenuation rates were higher at the exposed site. Given the higher flexibility of stems at the exposed site, this seems counter intuitive at first. However, we found higher shoot densities at the exposed site for most of the growing season (except for the marsh edge plots), which may explain the higher

relative wave attenuation rates at the exposed site, despite the higher stem flexibility. This interpretation is supported by findings of Bouma et al. 2010 and Paul et al. 2012: while these authors report that wave attenuation is stronger within stiff vegetation compared to flexible vegetation for a given standing biomass, they also found that flexible vegetation can reach the same effectiveness of wave height attenuation when occurring at higher standing biomass. A potential additional explanation could be that the higher incoming waves - under similar water depth conditions at the exposed site - lead to higher loss of wave energy by a stronger interaction of the waves with the sediment bed (e.g. Callaghan et al., 2010; de Roo and Troch, 2013; Mariotti and Fagherazzi, 2010). However, given the particularly pronounced annual biomass-cycle of *S. maritimus* it needs to be considered that during winter and early spring, when occurrence of storm waves is generally more likely, aboveground biomass of this species will be extremely low. Thus, its contribution to wave attenuation might be limited during the winter season when its wave attenuation service would be most needed.

Conclusions

Scirpus maritimus, when exposed to waves, grows concordantly to a stress-avoidance strategy on individual shoot level. Adaptation to the conditions occurs on a morphological, biochemical and biomechanical level and was shown to develop over the growing season. Comparison with a greenhouse control suggests that these differing plant properties are a result of phenotypic plasticity. Relative wave attenuation was more efficient at the exposed site, probably due to higher stem density, emphasising the wave attenuating potential of marshes. This wave attenuating potential will, however, vary with the annual biomass-cycle of *S. maritimus* dominated pioneer zones.

References

- Blanchette, C. A. 1997. Size and survival of intertidal plants in response to wave action: A case study with *Fucus gardneri*. *Ecology* **78**: 1563–1578. doi:10.1890/0012-9658(1997)078[1563:SASOIP]2.0.CO;2
- Bornette, G., and S. Puijalon. 2010. Response of aquatic plants to abiotic factors: a review. *Aquat. Sci.* **73**: 1–14. doi:10.1007/s00027-010-0162-7
- Borsje, B. W., B. K. van Wesenbeeck, F. Dekker, P. Paalvast, T. J. Bouma, M. M. van Katwijk, and M. B. de Vries. 2011. How ecological engineering can serve in coastal protection. *Ecol. Eng.* **37**: 113–122. doi:10.1016/j.ecoleng.2010.11.027
- Bouma, T. J., M. Friedrichs, P. Klaassen, and others. 2009. Effects of shoot stiffness, shoot size and current velocity on scouring sediment from around seedlings and propagules. *Mar. Ecol. Ser.* **388**: 293–297. doi:10.3354/meps08130
- Bouma, T. J., S. Temmerman, L. A. van Duren, and others. 2013. Organism traits determine the strength of scale-dependent bio-geomorphic feedbacks: A flume study on three intertidal plant species. *Geomorphology* **180–181**: 57–65. doi:10.1016/j.geomorph.2012.09.005
- Bouma, T. J., M. B. De Vries, and P. M. J. Herman. 2010. Comparing ecosystem engineering efficiency of two plant species with contrasting growth strategies. *Ecology* **91**: 2696–704. doi:10.1890/09-0690.1
- Bouma, T. J., M. B. De Vries, E. Low, G. Peralta, I. Tanczos, J. van de Koppel, and P. M. J. Herman. 2005. Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. *Ecology* **86**: 2187–2199. doi:10.1890/04-1588
- Callaghan, D. P., T. J. Bouma, P. Klaassen, D. van der Wal, M. J. F. Stive, and P. M. J. Herman.

2010. Hydrodynamic forcing on salt-marsh development: Distinguishing the relative importance of waves and tidal flows. *Estuar. Coast. Shelf Sci.* **89**: 73–88. doi:10.1016/j.ecss.2010.05.013

Carus, J., M. Paul, and B. Schröder. 2016. Vegetation as self-adaptive coastal protection: Reduction of current velocity and morphologic plasticity of a brackish marsh pioneer. *Ecol. Evol.* **6**: 1579–1589. doi:10.1002/ece3.1904

Charpentier, A., P. Grillas, and J. D. Thompson. 2000. The effects of population size limitation on fecundity in mosaic populations of the clonal macrophyte *Scirpus maritimus* (Cyperaceae). *Am. J. Bot.* **87**: 502–507.

Charpentier, A., and J. F. Stuefer. 1999. Functional specialization of ramets in *Scirpus maritimus*. Splitting the tasks of sexual reproduction, vegetative growth, and resource storage. *Plant Ecol.* 129–136. doi:10.1023/A:1009825905117

Coops, H., R. Boeters, and H. Smit. 1991. Direct and indirect effects of wave attack on helophytes. *Aquat. Bot.* **41**: 333–352.

Coops, H., N. Geilen, and G. van der Velde. 1994. Distribution and growth of the helophyte species *Phragmites australis* and *Scirpus lacustris* in water depth gradients in relation to wave exposure. *Aquat. Bot.* **48**: 273–284.

Coops, H., and G. Van der Velde. 1996. Effects of waves on helophyte stands: mechanical characteristics of stems of *Phragmites australis* and *Scirpus lacustris*. *Aquat. Bot.* **53**: 175–185. doi:10.1016/0304-3770(96)01026-1

Cottenie, A., M. Verloo, L. Kiekens, G. Velghe, and R. Camerlynck. 1982. Chemical analysis of plant and soil laboratory of analytical and agrochemistry, State University Ghent [ed.].

Cox, T. J. S. 2014. Tides: Quasi-Periodic Time Series Characteristics. R package version 1.1.

- 642 Dalrymple, R. A., and R. G. Dean. 1991. Water wave mechanics for engineers and scientists,
643 Prentice-Hall.
- 644 DeMaster, D. J. 1981. The supply and accumulation of silica in the marine environment. *Geochim.*
645 *Cosmochim. Acta* **45**: 1715–1732. doi:10.1016/0016-7037(81)90006-5
- 646 Denny, M. W. 2006. Ocean waves, nearshore ecology, and natural selection. *Aquat. Ecol.* **40**: 439–
647 461. doi:10.1007/s10452-004-5409-8
- 648 Doyle, R. D. 2001. Effects of waves on the early growth of *Vallisneria americana*. *Freshw. Biol.*
649 389–397. doi:10.1046/j.1365-2427.2001.00668.x
- 650 Friess, D. A., K. W. Krauss, E. M. Horstman, T. Balke, T. J. Bouma, D. Galli, and E. L. Webb.
651 2012. Are all intertidal wetlands naturally created equal? Bottlenecks, thresholds and
652 knowledge gaps to mangrove and saltmarsh ecosystems. *Biol. Rev. Camb. Philos. Soc.* **87**:
653 346–66. doi:10.1111/j.1469-185X.2011.00198.x
- 654 Henry, P.-Y., and D. Myrhaug. 2013. Wave-induced drag force on vegetation under shoaling
655 random waves. *Coast. Eng.* **78**: 13–20. doi:10.1016/j.coastaleng.2013.03.004
- 656 Heukels, H., and R. van der Meijden. 2005. Heukels' Flora van Nederland, 23rd ed. Wolters-
657 Noordhoff.
- 658 Heuner, M., A. Silinski, J. Schoelynck, and others. 2015. Ecosystem engineering by plants on wave-
659 exposed intertidal flats is governed by relationships between effect and response traits. *PLoS*
660 *One* **10**: 1–18. doi:10.1371/journal.pone.0138086
- 661 Houba, V. J. G., J. J. van der Lee, I. Novazamsky, and I. Walinga. 1989. Soil and plant analysis, a
662 series of syllabi, *In* Soil Analysis Procedures. Agricultural University, Wageningen.
- 663 Hroudová, Z., P. Zákavský, and M. Flegrová. 2013. The tolerance to salinity and nutrient supply in

- 664 four European *Bolboschoenus* species (*B. maritimus*, *B. laticarpus*, *B. planiculmis* and *B.*
665 *yagara*) affects their vulnerability or expansiveness. *Aquat. Bot.* **112**: 66–75.
666 doi:10.1016/j.aquabot.2013.07.012
- 667 Mariotti, G., and S. Fagherazzi. 2010. A numerical model for the coupled long-term evolution of salt
668 marshes and tidal flats. *J. Geophys. Res.* **115**: 1–15. doi:10.1029/2009JF001326
- 669 Maris, T., L. Oosterlee, and P. Meire, eds. 2013. Onderzoek naar de gevolgen van het Sigmaplan,
670 baggeractiviteiten en havenuitbreiding in de Zeeschelde op het milieu. Geïntegreerd
671 eindverslag van het onderzoek verricht in 2012.,.
- 672 Möller, I., M. Kudella, F. Rupprecht, and others. 2014. Wave attenuation over coastal salt marshes
673 under storm surge conditions. *Nat. Geosci.* **7**: 727–731. doi:10.1038/ngeo2251
- 674 Mullarney, J. C., and S. M. Henderson. 2010. Wave-forced motion of submerged single-stem
675 vegetation. *J. Geophys. Res. Ocean.* **115**: 1–14. doi:10.1029/2010JC006448
- 676 Niklas, K. J. 1992. Plant biomechanics: an engineering approach to plant form and function,
677 University of Chicago Press.
- 678 Paul, M., T. J. Bouma, and C. L. Amos. 2012. Wave attenuation by submerged vegetation:
679 Combining the effect of organism traits and tidal current. *Mar. Ecol. Prog. Ser.* **444**: 31–41.
680 doi:10.3354/meps09489
- 681 Puijalon, S., and G. Bornette. 2006. Phenotypic plasticity and mechanical stress: Biomass
682 partitioning and clonal growth of an aquatic plant species. *Am. J. Bot.* **93**: 1090–1099.
683 doi:10.3732/ajb.93.8.1090
- 684 Puijalon, S., G. Bornette, and P. Sagnes. 2005. Adaptations to increasing hydraulic stress:
685 morphology, hydrodynamics and fitness of two higher aquatic plant species. *J. Exp. Bot.* **56**:
686 777–86. doi:10.1093/jxb/eri063

- 687 Puijalon, S., T. J. Bouma, C. J. Douady, J. van Groenendael, N. P. R. Anten, E. Martel, and G.
688 Bornette. 2011. Plant resistance to mechanical stress: evidence of an avoidance-tolerance trade-
689 off. *New Phytol.* **191**: 1141–1149. doi:10.1111/j.1469-8137.2011.03763.x
- 690 Puijalon, S., T. J. Bouma, J. Van Groenendael, and G. Bornette. 2008. Clonal plasticity of aquatic
691 plant species submitted to mechanical stress: Escape versus resistance strategy. *Ann. Bot.* **102**:
692 989–996. doi:10.1093/aob/mcn190
- 693 R Core Team. 2014. R: A language and environment for statistical computing, R Foundation for
694 Statistical Computing [ed.].
- 695 de Roo, S., and P. Troch. 2013. Field Monitoring of Ship Wave Action on Environmentally Friendly
696 Bank Protection in a Confined Waterway. *J. Waterw. Port, Coastal, Ocean Eng.* 527–534.
697 doi:10.1061/(ASCE)WW.1943-5460.0000202.
- 698 Rupprecht, F., I. Möller, B. Evans, T. Spencer, and K. Jensen. 2015. Biophysical properties of salt
699 marsh canopies — Quantifying plant stem flexibility and above ground biomass. *Coast. Eng.*
700 **100**: 48–57. doi:10.1016/j.coastaleng.2015.03.009
- 701 Schaller, J., C. Brackhage, M. O. Gessner, E. Bäuer, and E. G. Dudel. 2012. Silicon supply
702 modifies C:N:P stoichiometry and growth of *Phragmites australis*. *Plant Biol.* **14**: 392–396.
703 doi:10.1111/j.1438-8677.2011.00537.x
- 704 Schoelynck, J., K. Bal, S. Puijalon, P. Meire, and E. Struyf. 2012. Hydrodynamically mediated
705 macrophyte silica dynamics. *Plant Biol.* **14**: 997–1005. doi:10.1111/j.1438-8677.2012.00583.x
- 706 Schoelynck, J., D. Meire, K. Bal, K. Buis, P. Troch, T. J. Bouma, P. Meire, and S. Temmerman.
707 2013. Submerged macrophytes avoiding a negative feedback in reaction to hydrodynamic
708 stress. *Limnol. - Ecol. Manag. Int. Waters* **43**: 371–380. doi:10.1016/j.limno.2013.05.003
- 709 Schoelynck, J., and E. Struyf. 2016. Silicon in aquatic vegetation. *Funct. Ecol.* 1323–1330.

doi:10.1111/1365-2435.12614

Silinski, A., M. Heuner, J. Schoelynck, and others. 2015. Effects of Wind Waves versus Ship Waves on Tidal Marsh Plants: A Flume Study on different Life Stages of *Scirpus maritimus*. PLoS One. doi:10.1371/journal.pone.0118687

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Agric. Chem. **46**: 829–835.

Struyf, E., S. Van Damme, B. Gribsholt, K. Bal, O. Beauchard, J. J. Middelburg, and P. Meire. 2007. *Phragmites australis* and silica cycling in tidal wetlands. Aquat. Bot. **87**: 134–140.

Struyf, E., S. Van Damme, B. Gribsholt, J. Middelburg, and P. Meire. 2005. Biogenic silica in tidal freshwater marsh sediments and vegetation (Schelde estuary, Belgium). Mar. Ecol. Prog. Ser. **303**: 51–60. doi:10.3354/meps303051

Sun, S. C., Y. L. Cai, and S. Q. An. 2002. Differences in morphology and biomass allocation of *Scirpus mariqueter* between creekside and inland communities in the Changjiang estuary, China. Wetlands **22**: 786–793.

Szmeja, J., and a Galka. 2008. Phenotypic responses to water flow and wave exposure in aquatic plants. Acta Soc. Bot. Pol. **77**: 59–65.

Temmerman, S., P. Meire, T. J. Bouma, P. M. J. Herman, T. Ysebaert, and H. J. De Vriend. 2013. Ecosystem-based coastal defence in the face of global change. Nature **504**: 79–83. doi:10.1038/nature12859

Tonelli, M., S. Fagherazzi, and M. Petti. 2010. Modeling wave impact on salt marsh boundaries. J. Geophys. Res. **115**: 1–17. doi:10.1029/2009JC006026

Turner, S. R., N. Taylor, and L. Jones. 2001. Mutations of the secondary cell wall. Plant Mol. Biol.

732 **47**: 209–219. doi:10.1023/A:1010695818416

733 Versluys, T., and P. Troch. 2010. Description of the conversion algorithm from pressure
734 measurements to surface elevations. Technical Report., Dept. of Civil Engineering, Ghent
735 University.

736 Walinga, I., W. Van Vark, V. J. G. Houba, and J. J. Van der Lee. 1989. Soil and plant analysis, a
737 series of syllabi, part 7: Plant analysis procedures, Wageningen Agricultural University,
738 Wageningen, Netherlands.

739 Wang, X., H. Ren, B. Zhang, B. Fei, and I. Burgert. 2012. Cell wall structure and formation of
740 maturing fibres of moso bamboo (*Phyllostachys pubescens*) increase buckling resistance. J. R.
741 Soc. Interface 988–996. doi:10.1098/rsif.2011.0462

742 Ysebaert, T., S. L. Yang, L. Zhang, Q. He, T. J. Bouma, and P. M. J. Herman. 2011. Wave
743 attenuation by two contrasting ecosystem engineering salt marsh macrophytes in the intertidal
744 pioneer zone. Wetlands **31**: 1043–1054. doi:10.1007/s13157-011-0240-1

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Figure legends

Figure 1: (a) Position of the Scheldt Estuary in the Dutch-Belgian border region (indicated by the black square). (b) Overview of the Scheldt Estuary, with its tidal flats and marshes, downstream of Antwerp to its mouth near Vlissingen. (c) Overview of the location of both monitoring transects (black stars) at both tidal marshes with indications of the breakwater and the shipping channel.

Figure 2: Monitoring field set-up indicating continuous, monthly (April to July for waves; April to September for surface and ground water measurements) and September sampling, along a distance gradient of 12 m from the marsh edge into the marsh.

Figure 3: (a) Significant wave height (H_s), and the average over the highest 1 % of wave heights ($H_{1/100}$) (cm), along the three transect plots at both sites, and (b) wave attenuation rates (%) based on the ratios of wave heights (H_s and $H_{1/100}$), observed at the 12 m plot and incoming wave heights (H_s and $H_{1/100}$, respectively) at the marsh edge per month over the growing season. The error bars in (a) are not visible as they are smaller than the point symbols.

Figure 4: Average $\pm$ SE of morphological plant properties as quantified on 30 stems per plot and month (total length (cm), basal stem diameter (mm), shoot density ($\# \text{ m}^{-2}$) and dry biomass per m^2 (g m^{-2})). September campaign: significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.05 > * > 0.01 > ** > 0.001 > ***$).

Figure 5: (a) Mean dry belowground biomass per m^2 (g m^{-2}) per depth section, averaged over $n=3$; (b) aboveground-belowground biomass ratio of the dry biomass per m^2 of marsh.

Figure 6: Strengthening compounds of shoots in $\text{mg g}^{-1} \pm \text{SE}$ ($n=3$ per plot and month), monthly for the sheltered and exposed site, and for the September sampling for both sites. September

campaign: significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.1 > \# > 0.05 > * > 0.01 > ** > 0.001 > ***$; significance was assumed at $p < 0.05$).

Figure 7: Biomechanical traits of shoots sampled in the September campaign ($n=20$). **Top:** tensile properties; **bottom:** bending properties. Significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.1 > \# > 0.05 > * > 0.01 > ** > 0.001 > ***$; significance was assumed at $p < 0.05$).

Figure 8: Morphological properties for the greenhouse control plants ($n=15$ per plot and site). Significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.1 > \# > 0.05 > * > 0.01 > ** > 0.001 > ***$; significance was assumed at $p < 0.05$).

Figure 9: Schematic view of two marshes with contrasting wave exposure. Wave dynamics (1) induce direct mechanical stress affecting the morphology of the shoots (2A and 3A); indirect effects on the plants are the wave-induced effects on the sediment properties by determining the sediment texture and composition (2B, i.e. dissolved silica contents (DSi), organic matter contents (OM) and water contents (H_2O)). DSi content in the sediment is reflected in the biogenic silica contents of the shoots (BSi) (3B), which in turn is an important source for the DSi pool in the sediment after decomposition of the plant material. Both morphology and tissue composition determine the biomechanical strength of the shoots (4). More exposed shoots are more flexible, and smaller, which suggests a stress-avoidance strategy, whereas the increased belowground biomass at deeper depth might indicate a stress-tolerance strategy.

Tables

Table 1: Overview of significant wave height (H_s , cm), the mean over the highest 1 % of wave heights ($H_{1/100}$, cm), mean inundation depth (id_{mean} , cm), mean inundation time per tidal cycle (it_{mean} , min) and inundation frequency (if, %) for the three plots of the sheltered (shelt.) and exposed (exp.) site, respectively, during the growing season of 2014.

plot	H_s (cm)		$H_{1/100}$ (cm)		id_{mean} (cm)		it_{mean} (min)		if (%)	
	shelt.	exp.	shelt.	exp.	shelt.	exp.	shelt.	exp.	shelt.	exp.
0 m	2.8	5.7	9.7	20.3	81	77	138	127	100	100
4 m	2.7	5.2	9.2	18.0	71	67	125	115	99	99
12 m	2.1	3.5	6.9	12.6	51	47	99	87	94	94

Table 2: Physical sediment properties of Kopecky ring samples (top 5 cm of sediment), averaged over the entire transect, and core samples, averaged over depth at the marsh edge and 12 m plots: median grain size (d_{50} , $\mu\text{m} \pm \text{SE}$), organic matter contents (LOI, $\% \pm \text{SE}$), dry bulk density ($\text{g cm}^{-3} \pm \text{SE}$) and water contents (H_2O -contents, $\% \pm \text{SE}$); $n=12$ for Kopecky ring samples per transect, $n=3$ for core samples per plot.

sample	unit	type/plot	site sheltered	exposed
d_{50}	μm	Kopecky		
		all	24 ± 7	135 ± 3
		core		
		Marsh edge	87 ± 21	121 ± 12
		12 m	31 ± 11	116 ± 15
LOI	$\%$	Kopecky		
		all	8.18 ± 0.87	1.15 ± 0.02
		core		
		Marsh edge	3.71 ± 1.00	1.70 ± 0.59
		12 m	7.87 ± 1.17	2.11 ± 0.81
dry bulk density	g cm^{-3}	Kopecky		
		all	0.76 ± 0.01	1.48 ± 0.01
H_2O -contents	$\%$	Kopecky		
		all	49.15 ± 0.01	23.92 ± 0.01

Table 3: Chemical sediment properties averaged over site (n=12 per site). Concentrations are given in $\text{mg g}^{-1} \pm \text{SE}$. p-value indicates significant differences between sites as obtained from a t-test. Significance was assumed at $p < 0.05$. ns: not significant.

sample	unit	site		p (t-test)
		sheltered	exposed	
$\text{PO}_4^{3-}\text{-P}$	mg g^{-1}	0.530 ± 0.07	0.030 ± 0.01	<0.001
$\text{NH}_4^+\text{-N}$	mg g^{-1}	0.002 ± 0.001	0.001 ± 0.0002	ns
total P	mg g^{-1}	1.020 ± 0.10	0.290 ± 0.02	<0.001
total N	mg g^{-1}	1.280 ± 0.16	0.340 ± 0.06	<0.001
N:P ratio	-	1.22 ± 0.08	1.13 ± 0.12	ns
pH, actual	-	8.20 ± 0.25	8.76 ± 0.16	<0.01
pH, potential	-	7.65 ± 0.20	8.30 ± 0.20	<0.01

Table 4: Correlation matrix for wave activity (waves: mean over the highest 1 % of wave heights) and sediment variables, i.e. dissolved silica (DSi), median grain size (d_{50}), dry bulk density (BD), organic matter contents (OM) and water content (H_2O). Pearson's correlation coefficients are reported. The correlation of dry bulk density with water content is not reported (/) as water content results from the difference of fresh and dry bulk density.

	waves	DSi	d_{50}	BD	OM
DSi	-0.87				
d_{50}	0.86	-0.86			
BD	0.87	-0.96	0.88		
OM	-0.98	0.89	-0.97	-0.90	
H_2O	-0.86	0.96	-0.87	/	0.89

Table 5: Correlation matrix for wave activity (waves: mean over the highest 1 % of wave heights) and plant properties, i.e. plant length (length), basal stem diameter (diam), dry biomass per m² (BM), flexural stiffness (FS), Young’s modulus (YM), tensile strength (TS), breaking force (BF), cellulose (cell), lignin (lig) and biogenic silica (BSi). Pearson’s correlation coefficients are reported. The correlations of flexural stiffness with Young’s modulus and tensile strength with breaking force are not reported (/) as Young’s modulus and breaking force were used to calculate flexural stiffness and tensile strength, respectively.

	waves	length	diam	BM	FS	YM	TS	BF	cell	lig
length	-0.79									
diam	0.76	-0.57								
BM	-0.60	0.85	-0.45							
FS	-0.76	0.64	-0.35	0.51						
YM	-0.57	0.47	-0.35	0.48	/					
TS	-0.46	0.49	-0.31	0.43	0.56	0.37				
BF	-0.77	0.47	-0.33	0.32	0.58	0.26	/			
cell	-0.61	0.86	-0.86	0.89	0.69	0.83	0.65	0.50		
lig	-0.41	0.81	-0.91	0.86	0.55	0.78	0.57	0.36	0.93	
BSi	-0.81	0.88	-0.70	0.74	0.90	0.57	0.74	0.58	0.64	0.59

Coping with waves: plasticity in tidal marsh plants as self-adapting coastal ecosystem engineers

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Running head: Marsh plants as self-adapting ecosystem engineers

Key words: wave exposure; phenotypic plasticity; ecosystem-based coastal protection; *Scirpus maritimus*; biogenic silica

28 **Abstract**

29 Tidal marsh vegetation is increasingly valued for its role in ecosystem-based coastal protection
30 due to its wave dissipating capacity. As the efficiency of wave dissipation is known to depend on
31 specific vegetation properties, we quantified in this study how these morphological, biochemical
32 and biomechanical properties of tidal marsh vegetation are, in turn, affected by wave exposure.
33 This was achieved by field measurements at two locations, with contrasting wave exposure, in
34 the brackish part of the Scheldt Estuary (SW Netherlands), where *Scirpus maritimus* is the
35 dominant pioneer. Our results show that shoots from more wave-exposed conditions developed
36 significantly shorter and thicker stems than the ones growing in more sheltered conditions.
37 Furthermore, we show that the more exposed shoots are more flexible whereas the shoots
38 growing in more sheltered conditions are stiffer. This may indicate plasticity in response to wave
39 exposure following a stress-avoidance strategy. Increasing stiffness was shown to be related to
40 enhanced biogenic silica and lignin contents of the shoot tissue. These properties might affect the
41 wave-attenuating capacity of the marsh as stiff plants are known to mitigate waves more
42 effectively than flexible ones. However, we also found higher shoot densities on the exposed site,
43 which may ~~contribute to partly~~ explain why higher relative wave attenuation rates were found on
44 the exposed site, despite the presence of more flexible individual shoots. This study highlights
45 that the efficiency of wave attenuation by tidal marsh vegetation ultimately depends on mutual
46 interactions between waves and plasticity in morphological, biochemical and biomechanical-~~and~~
47 ~~biochemical~~ plant properties.

Introduction

~~In the Facing face of~~ global sea-level rise, there is a need for sustainable alternatives to traditional coastal protection by engineered constructions. Ecosystem-based solutions, including the conservation and restoration of tidal marshes, are increasingly suggested as long-term self-sustainable protective systems (Borsje et al. 2011; Temmerman et al. 2013): contrary to conventional engineering solutions (dikes, dams), marshes can adapt to increasing sea levels by accumulating sediment and thus grow with the rising sea. Additionally, natural coastal protection by marsh vegetation has been proven to be very effective as the majority of wave energy entering a marsh ~~is dissipated~~ dissipates over the first few meters after the ~~marsh edge~~ edge of the marsh (Bouma et al. 2005; Ysebaert et al. 2011; Möller et al. 2014). Even under extreme water levels and wave heights typical for storm surges, when coastal defence is most important, up to 60 % of ~~wave-wave~~ height attenuation may be attributed to marsh vegetation (Möller et al. 2014).

In the past, research has focussed on current-induced hydrodynamic stresses and their effect on clonal macrophytes and marsh vegetation (Bouma et al., 2009; Puijalon and Bornette, 2006; Puijalon et al., 2005), although recent findings emphasise the role of wave exposure (Tonelli et al. 2010). The capacity of plants to attenuate waves, however, depends on plant morphological traits: for instance, flume experiments and field measurements showed that stiffer shoots as well as a high standing biomass increase wave attenuation (Bouma et al. 2010, 2013; Ysebaert et al. 2011). The acting waves, in turn, have been shown to exert stress on plant growth. Direct wave effects on plants are exerted by drag and pulling forces (Denny 2006; Henry and Myrhaug 2013) and scouring of sediment around plant stems (Bouma et al. 2009; Friess et al. 2012). Indirect effects are the wave-induced sediment texture and composition (Coops et al. 1991, 1994), resulting from hydrological sorting.

In order to cope with hydrodynamic forces, clonal macrophytes are known to produce plastic responses: wave exposure leads, for example, to shorter plants, a greater allocation of below-ground biomass, higher densities of above-ground stems and less daughter plants (Coops and Van der Velde 1996; Blanchette 1997; Doyle 2001). On a biomechanical level, plants typically follow either an avoidance or a tolerance strategy (Coops et al. 1994; Puijalon et al. 2011): enhanced flexibility, which enables the plant to bend when exposed to water flow or waves and thus to reduce experienced drag, can be seen as a typical stress-avoidance strategy (Puijalon et al. 2011; Schoelynck et al. 2013; Silinski et al. 2015). High mechanical resistance to breaking, ~~to the~~ contrary on the other hand, determines the capacity of the plants to tolerate hydrodynamic stress, usually correlated to higher tissue rigidity (Coops and Van der Velde 1996; Puijalon et al. 2011). On a molecular level, rigidity of the shoot is enhanced when lignin and cellulose fibrils associate and form a secondary cell wall (Turner et al., 2001; Wang et al., 2012). Furthermore, biogenic silica (BSi) can also enhance the rigidity of the shoots (Schoelynck et al. 2012). ~~Because a~~ Given that a stress-avoidance or stress-tolerance strategy can result in the development of different plant morphological properties, the capacity of the plants to attenuate hydrodynamics will be affected; ~~too as well~~. Hence, an important remaining question is whether there are mutual feedbacks between plant morphological development and wave intensity and attenuation at tidal marsh shorelines.

The aim of this study is to investigate the response of morphological and tissue properties within a tidal marsh plant species to differences in wave exposure. This was done in a field monitoring campaign in the brackish part of the Scheldt Estuary (SW Netherlands and N Belgium), during the growing season of 2014, at two locations with contrasting wave exposure. The ~~investigated species~~ species under investigation was *Scirpus maritimus* L. Palla, which is a clonal, dominant

pioneer marsh plant in the brackish parts of estuaries in the temperate zone. The novelty of our study is that we quantified morphological, biochemical and biomechanical properties of *S. maritimus* between two contrasting sites, and also along a wave exposure gradient within each site, during the entire growing season of 2014. We hypothesize that (i) *Scirpus maritimus* adapts its morphological, ~~biomechemical~~ biomechanical and biochemical properties depending on wave exposure (varying between the sites and with exposure within each site), and that (ii) these adaptations are the result of phenotypic plasticity and occur progressively over the growing season.

For Review Only

Materials and methods

Study sites

Two sites of contrasting wave exposure were chosen in the Scheldt Estuary (Fig. 1): Groot Buitenschoor (Belgium) and Schor van Rilland (The Netherlands), ~~in the following~~henceforth referred to as the sheltered and exposed site, respectively. The contrasting hydrodynamic conditions of these sites result from the fact that the Belgian site is sheltered by a breakwater from ship and wind waves, while the Dutch site is highly exposed through a long wind fetch to dominant South-westerly winds and the proximity of the shipping channel. Both sites are located in the brackish part of the Scheldt Estuary with an average annual salinity of 9.2 ± 0.17 SE ppt and an average annual suspended sediment concentration of 0.062 ± 0.004 SE g L⁻¹ (Maris et al. 2013). Due to their proximity, tidal characteristics are similar at both sites. Mean tidal amplitude in this part of the estuary is approximately 5 m.

Study species

Scirpus maritimus L. Palla (syn. *Bolboschoenus maritimus*) is the naturally occurring dominant pioneer plant in the study area. It typically forms the dominant vegetation at the lower fringes of marshes in the brackish zones of European estuaries (e.g. Scheldt, Elbe, Weser) (Heukels and van der Meijden 2005). It forms monospecific clonal stands, which produce aboveground shoots with a triangular stem cross-section that can reach heights of up to 2 m (personal observation in the field). This species grows clonally by producing several underground rhizomes per shoot that connect existing and newly produced shoots with each other (Charpentier and Stuefer 1999; Charpentier et al. 2000). During the growing season, tubers are formed which ensure the survival of the clonal, belowground plant in winter, and the regrowth of new shoots in spring, typically by the end of March in the Scheldt Estuary. From September onwards, the aboveground biomass

dries and dies off. In this way, short dead stem stumps of only several tens of ~~centimetres~~centimetres' height are all that remains by the end of winter, when the appearance of new shoots growing from the tubers formed in the previous year begins.

Field campaigns

Monitoring plots

In order to compare site effects of wave exposure, monitoring plots were set up within the *S. maritimus* pioneer zone along a transect at both the sheltered and exposed site (Fig. 1 and 2). Along both transects, the monitoring was performed at three distances (~~called plots in the following~~subsequently referred to as plots) into the marsh: at the marsh edge (0 m), at 4 m and at 12 m into the marsh. An 8 m plot was added for the final campaign in September (see below). Each plot was approximately 1 m wide (perpendicular to the marsh edge) and stretched over approximately 10 m parallel to the marsh edge, following lines of equal elevation. Within these plots samples were taken randomly. By stretching the sampling over this area, we avoided negative effects of repeated sampling (e.g. of shoot sampling) on the conditions along the transect. This was done in order to compare within-site differences from the most wave-exposed plot (at the marsh edge) towards wave-sheltered plots (further into the marsh). This within-site gradient in wave exposure results from wave attenuation by the vegetation and potentially also by bottom friction, ~~—~~ i.e. by the interaction of the waves with the sediment bed. At both transects the general topography on the tidal flat and within the marsh was a smooth slope without any strong small-scale topography (ridges or troughs) that could affect the hydrodynamic impact on the marsh. The slope over the first 12 m into the marsh was around 3 % and the elevation of the four plots along the transects were approximately -0.66, -0.54, -0.42 and -0.32 m, ~~±~~ relative to mean high water (MHW), from the marsh edge towards the 12 m plot. Similar slopes at both transects

were required so that there would not be any differences in topography-induced wave attenuation between the two sites, and ~~same-equal~~ elevations imply a similar tidal inundation regime.

Furthermore, vegetation canopy was continuous and without any bare patches along the transects at both sites. Hence, the only expected difference in hydrodynamics between the two sites resulted from higher incoming waves at the exposed site compared to the sheltered site.

Continuous measurements

Wave monitoring

For practical reasons (availability of three wave sensors), waves were monitored simultaneously at the marsh edge, 4 m and 12 m into the marsh (Fig. 2), ~~at~~ one site at a time. From April on, the three wave sensors were shifted between the exposed and sheltered site every two weeks, resulting in four blocks of two weeks of wave data per site (one block per month, from April to July). As tidal high water levels vary strongly in the course of a month (astronomical neap and spring tides, wind conditions) each block covered a spring-neap cycle to ensure that we verified during the processing of the data that inundation depth covered distribution at both locations was comparable during these alternating monitoring phases. ~~In this way~~ Thereby, we could ensure that differences in incoming wave heights at the marsh edge were not merely a result of differences in inundation depth. We then quantified plant-induced wave attenuation along the transects over the growing season. Additionally, we measured waves simultaneously at the marsh edges of both sites during a period of six weeks starting from early November 2014 in order to quantify the difference in wave exposure between sites over the same period in time. Waves were measured by submerged stand-alone PDCR 1830 pressure transducers (GE Sensing) at a frequency of 16 Hz. Wave data were converted from pressure to water surface elevation and analysed using a LabView script developed by the Department of Civil Engineering of UGent (Versluys and Troch

2010). The conversion was performed using linear wave theory, in particular by applying the pressure response factor (Dalrymple and Dean 1991). The tidal signal was removed from the data by de-trending and de-meaning the wave measurements. Using the core-functions of R (R Core Team 2014), version 3.1.1, we then further processed the data and calculated significant wave heights (H_s , meaning the arithmetic mean of the highest third of recorded waves) and $H_{1/100}$ (meaning the arithmetic mean over the highest 1% of recorded waves) for each measured time period and plot. The highest waves as well as relative wave attenuation over the first 12 m into the marsh (i.e. the reduction of wave heights at the 12 m plots compared to the incoming waves at the marsh edge) were also extracted and computed by the means of R. Wave attenuation was calculated following the formula $R = (H_{me} - H_{12m}) / H_{me} * 100 [\%]$, where H_{me} are the wave heights at the marsh edge (= incoming wave height) and H_{12m} are the wave heights at 12 m into the marsh. We calculated wave attenuation for both, H_s and $H_{1/100}$. Multiplication with 100 was applied in order to convert relative wave heights to percentage values. Positive values indicate wave reduction into the marsh. These values were calculated for all wave data gathered at both marsh edges, irrespective of inundation depth (as both sites have the same absolute elevation, are relatively close to each other, and experienced very similar water depths, see Table 1). In this way, wave exposure at the marsh edges – as encountered in the course of full tidal cycles – was quantified.

Ground water monitoring

Piezometers of 2 m depth of which the lowest meter was perforated were installed at both sites at 0 m, 4 m and 12 m into the marsh (Fig. 2). Pressure sensors (Cera-divers, Schlumberger), installed in the bottom of these piezometers, monitored the ground water fluctuations from April to September with one measurement every 2 minutes. Surface water at the marsh edge was also

monitored by a Cera-diver, set to the same frequency. Furthermore, a Baro-diver (Schlumberger) recorded air pressure on the nearby dike which was used for correcting atmospheric pressure fluctuations in the water surface measurements. We analysed the tidal data gathered with the surface water diver with the R package “Tides” (Cox 2014) in order to obtain average inundation times, inundation frequencies and local mean high waters for the monitored period.

Monthly measurements

A monthly monitoring campaign was initiated in April 2014 and was performed until September 2014. Every month, 30 shoots were randomly sampled per plot in order to quantify their morphological properties (Fig. 2). They were clipped off close to the sediment bed. After measuring basal stem diameter, length of the shoots, and determining their fresh biomass, they were dried at 70 °C for 72 h in batches of 10 plants, allowing three replicate measurements per plot and per month for subsequent biochemical analyses. The dry biomass of shoots was quantified. Shoot density was determined per plot by counting the number of living shoots (i.e. shoots produced in 2014) within two fixed squares of 0.4 m x 0.4 m. We calculated average aboveground biomass (shoot density x dry biomass per shoot) per m² per plot.

The dried plant material, grouped per 10 plants (n=3 per plot and month), was ground with a grinding mesh of 1 mm (Retsch ZM2000). Silica, lignin and cellulose contents were determined on the samples of April, June, August and September, applying the DeMaster alkaline (NaCO₃) extraction method (DeMaster 1981) for the first and the Van Soest method (Van Soest 1963) for the two latter. These strengthening compounds influence biomechanical tissue properties such as flexibility and tensile strength. Furthermore, we quantified total NP concentrations with the Walinga method (Walinga et al. 1989), as it allowed us to form N:P ratios, required in order to determine the nutrient status of the shoots. This was done to check whether the different plant

morphologies between sites and along the within-site exposure gradient might simply result from nutrient availability.

September measurements

We performed additional sampling during the last monthly campaign in early September 2014 (Fig. 2) when biomass of *S. maritimus* is typically at its peak. For this campaign, we extended the three sampling plots at 0 m, 4 m and 12 m to four plots, by adding another plot at 8 m from the marsh edge for most measurements (Fig. 2).

Sediment samples

As we expected high disturbance of the plots by taking sediment cores, they were taken in September after all other measurements and sampling had been performed, so that none of the other measurements along the transects were influenced by the sediment core sampling. Three replicate sediment cores of 45 cm depth and 8 cm diameter were taken per plot and divided into three depth portions of 15 cm each, resulting in three core layers representing the top 15 cm, the depth of 15–30 cm and of 30–45 cm, respectively. The replicate depth portions per plot were pooled and well mixed. Grain size analyses were performed on three subsamples per mixed sample with a Mastersizer 2000 (Malvern) after a combined H_2O_2 and HCl treatment to remove organic compounds. Organic matter content was determined with Loss on Ignition (LOI), i.e. by ashing the samples at 550 °C during 6 h. KCl-extractions on fresh sediment allowed the determination of pH-KCl (potential pH), NO_3^- -N and NH_4^+ -N. Ammonium Acetate EDTA extraction allowed to measure PO_4^{3-} -P (Cottenie et al. 1982; Houba et al. 1989). Total P and N were obtained by digestion of the fresh sediment with H_2SO_4 -salicylic H_2O_2 -acid and selenium (Walinga et al. 1989). Quantification of these nutrients was done by colorimetry with a Continuous Flow Analyser (SAN++, Skalar, Breda, The Netherlands). Actual pH (after

extraction with H₂O) was also measured. Three replicate surface sediment samples per plot, taken with a Kopecky ring (4.6 cm in diameter and 5.2 cm high), were used to determine dry bulk density and water contents (after drying at 105 °C during 72 h) of the sediment surface of each plot.

Pore water samples

During the September campaign, pore water samples were taken at both sites from around 5 h after high water onwards: it was extracted from the marsh edge, 4 m, 8 m and 12 m plots by inserting five 9 cm Macrorhizon pore water samplers (pore size 0.15 µm, Rhizosphere Research Products, the Netherlands) per plot vertically into the sediment. These five samples per plot were pooled into one sample per plot in order to have a sufficient volume of each sample for quantification of dissolved silica (DSi) contents with the Continuous Flow Analyser (SAN++, Skalar, Breda, The Netherlands).

Belowground biomass samples

In order to quantify belowground biomass per plot, we took three replicate cores of 10 cm in diameter and of 45 cm depth at each plot. As for the sediment samples, this sampling was done after all other sampling due to the high disturbance of the plots. ~~Equally~~ Similarly, these 45 cm of the core ~~samples depth~~ were split into three sections of 15 cm each. These core portions were thoroughly rinsed and sieved until only plant material (roots, tubers, belowground parts of stems) remained. It was dried during 72 h at 70 °C. ~~and~~ Subsequently, dry belowground biomass per 15 cm core ~~portion~~ section was quantified. Based on the three replicate cores per plot, belowground biomass per m² marsh surface could be estimated.

Biomechanical traits

In order to quantify biomechanical parameters, 20 shoots per plot were sampled ~~additionally in~~ addition to the regular monthly plant sampling campaign ~~of in~~ September. Biomechanical traits were measured through tensile and bending tests on these shoots, using a universal testing machine (Instron 5942, Canton, MA, USA). Both tests (tensile and bending) were carried out on 20 cm long basal stem fragments: for the bending tests, the lowest 10 cm of these basal fragments were used while the tensile tests were performed on the upper 10 cm. For each sample, we measured the height and width of the triangular stem cross-sections at three different points along the sample.

- Bending tests

We performed three-point bending tests, consisting of a force applied at a constant rate of 10 mm min^{-1} to the midpoint of a sample placed on two supports. The support span was adjusted such that it was 8 to 10 times longer than the sample thickness. The following biomechanical traits related to bending were calculated: *Young's modulus* (E in Pa) quantifies the material stiffness, regardless of stem size or shape, and is calculated as the slope of the stress-strain curve in the elastic deformation region. *Second moment of area* (I in m^4) quantifies the distribution of material around the axis of bending, accounting for the effect of the ~~cross-sectional~~ cross-sectional geometry of a structure on its bending stress. I was calculated using the formula for triangular cross-sections (Niklas 1992): $I = (bh^3)/36$, where b and h are the base and height of the cross section (m). The *flexural stiffness* (EI in N m^2) quantifies the stiffness of the fragment and was calculated by multiplying E and I .

- Tensile tests

The stem fragments were clamped into the jaws of the testing machine and a constant extension rate of 5 mm min^{-1} was applied to the upper jaw until the fragments broke. The following

biomechanical traits were calculated: *breaking force* (in N), defined as the maximum force that the sample can bear without suffering mechanical failure, and *tensile strength* (in N m^{-2}) which is calculated as the breaking force per cross-sectional area.

The greenhouse control

We performed a control experiment, ~~in order so as to exclude mitigate the event of that~~ the observed plant morphological differences in the field ~~being dependant -upon~~ the position of the tubers within the clonal network. In March 2014, we extracted ~~from both sites~~ 15 tubers, ~~from both sites~~, from the 12 m plot as well as from the marsh edge. We transplanted these 60 tubers into pots of 25 cm height and 12 cm diameter that were filled with natural Scheldt sediment ($d_{50}=320 \mu\text{m}$) mixed with the slow releasing fertilizer Osmocote (Substral). They were randomly put into plastic containers in a greenhouse which served as troughs within which we maintained a water level of around 15 cm below the top of the pots. In this way we simulated the naturally water logged sediment conditions from the field. Furthermore, a daily rain event with 3.0 L m^{-2} assured the required water supply and equal growing conditions for the plants. Newly appearing clonal shoots were cut off weekly in order to maintain only one original shoot per pot. They grew under equal conditions until mid-June, around 8 weeks after transplantation, when we harvested the shoots and performed the same morphological measurements as on the monthly sampled field plants (diameter, stem height, dry biomass). The gap between the final harvest in the greenhouse (June) and in the field (September) results from the fact that the plants had grown faster in the greenhouse and were starting to show signs of senescence (in June) as typically found in the field once the annual biomass peak is passed (in September). We also quantified dry shoot biomass after drying at 70°C during 72h.

Statistical analysis

All statistical analyses were performed with the core functions of R (R Core Team, 2014), version 3.1.1. Two-way-ANOVAs were used in order to compare variables between both sites and the different distances from the marsh edge. Site and distance from the marsh edge were the fixed factors. A t-test was performed on the September data to compare the site effect for one plot (i.e. distance from the marsh edge) at a time. The Pearson's correlation coefficient was used to determine correlations between all measured biotic and abiotic variables. Before performing these tests, some of the data needed to be transformed in order to approach normal distribution: square-root-transformation was applied to basal stem diameter, dry biomass, flexural stiffness, Young's modulus, breaking force and lignin content; and log-transformation was performed on belowground biomass, second moment of area and N:P-ratios of plants.

Results

Physical and chemical parameters

Wave and surface water measurements

The analyses of the tidal surface water fluctuations for the full monitoring period show that the three plots along the transects at both sites experienced similar inundation depths, times and frequencies (Table 1). Furthermore, analysis of tidal levels recorded during the respective bi-weekly wave monitoring periods showed that a similar range of inundation depth was covered at the marsh edge plots of both transects during wave measurements. This ensures that wave heights recorded at both sites do not result from differences in water depth between the two sites. The incoming significant wave height (H_s) recorded during the full growing season at the marsh edge was twice as high at the exposed site (5.7 cm) than at the sheltered site (2.8 cm) (Fig. 3a and Table 1). Maximum wave heights during that period were 45 cm and 25 cm, respectively. At both sites, the significant wave height and $H_{1/100}$ were reduced as the waves travelled through the vegetation (Fig. 3a, Table 1), with a higher attenuation rate (i.e. relative reduction of wave height at the 12 m plots compared to the incoming wave heights at the marsh edge) at the exposed site compared to the sheltered site (Fig. 3b). The wave attenuation rate for H_s varied over the growing season at both sites (Fig. 3b), corresponding to seasonal variations in aboveground biomass as shown below. This seasonal variation is less apparent for wave attenuation for $H_{1/100}$. The synchronous wave measurements at the marsh edge in November confirmed the difference in wave exposure (H_s of 5.3 cm and 3.2 cm and $H_{1/100}$ of 19.4 cm and 12.1 cm at exposed and sheltered site, respectively).

Ground water fluctuations

Regarding ground water fluctuations, a lowering of the ground water level with low waters in the order of up to 15 cm below sediment surface level, and following the monthly spring-neap tidal cycle, could be seen in our data. A time-lag of drawdown in the order of ten to twenty minutes between the exposed and sheltered site could also be seen, with earlier onset of drawdown at the exposed site, probably due to the ~~more-sandysandier~~ sediment corresponding to better drainage. However, the differences between sites were only in the order of centimetres, which was the same order as the range of error of our measurements, making any statistical analyses or definite conclusions difficult.

Sediment samples

Median grain size was coarser at the exposed than at the sheltered site (~~Tab.~~Table 2), and decreasing ~~with-over~~ distance into the marsh at both sites (~~Tab.~~Table 2). Organic matter contents, in contrast, was higher at the sheltered site, and increased with distance into the marsh at both sites. Dry bulk density of the top 5 cm of the sediment surface at the exposed site was almost twice the density of the sheltered site, while water contents of the same samples was less than half compared to the sheltered site (~~Tab.~~Table 2).

In terms of chemical properties of the sediment (~~Tab.~~Table 3), PO_4^- -P and total P and N contents differed significantly between the sheltered and exposed site (t-test, $t = 7.281$, $df = 11.081$, $p < 0.001$ for PO_4^- -P; $t = 7.1252$, $df = 11.555$, $p < 0.001$ for total P and $t = 5.4337$, $df = 14.032$, $p < 0.001$ for total N), whereas the site-averages of NH_4^+ -N and N:P ratio did not differ significantly. NO_3^- -N contents lay below detection limit. Actual and potential pH were lower at the sheltered than at the exposed site and were close to the reported optimum pH for *S. maritimus* of 8.3 (Hroudová et al. 2013)

Pore water samples

Pore water DSi concentrations at the sheltered site were two to three times higher than at the exposed site, with 18.22, 25.35, 20.47 and 20.50 mg L⁻¹ for marsh edge, 4 m, 8 m and 12 m into the marsh, respectively, at the sheltered site and with 6.26, 4.84, 7.57 and 8.59 mg L⁻¹ along that gradient at the exposed site.

Plants

Plant morphology - September

The new shoots of 2014 started to emerge at the end of March 2014. At the end of the growing season, as recorded during the September campaign (Fig. 4), shoots were significantly taller at the sheltered site compared to the exposed site (t-test, $t = 5.0947$, $df = 57.269$ at the marsh edge, $t = 9.7467$, $df = 57.583$ at 4 m into the marsh, $t = 9.8401$, $df = 56.794$ at 8 m into the marsh and $t = 13.3063$, $df = 54.803$ at 12 m into the marsh, with $p < 0.001$ at for all-plots). Furthermore, there was a size gradient within each site, with increasing size from the marsh edge into the marsh, which also differed ~~also~~ significantly between sites (ANOVA, $F = 8.26$, $df = 1$, $p < 0.01$ for the interaction effect, i.e. a steeper increase of shoot size at the sheltered site compared to the exposed site). At both sites, stem diameter decreased with distance into the marsh (ANOVA, $F = 56.17$, $df = 1$, $p < 0.001$). This decrease was significantly stronger at the exposed site than at the sheltered site (ANOVA, $F = 14.94$, $df = 1$, $p < 0.001$ for the interaction term): at the exposed site the basal stem diameters close to the marsh edge were thicker than at the sheltered site, but similar between sites from 8 m onwards (t-test, $t = -4.7547$, $df = 57.567$, $p < 0.001$ at the marsh edge, $t = -5.1558$, $df = 52.244$, $p < 0.001$ at 4 m into the marsh ~~$p < 0.001$ for the first two plots~~, and $p > 0.05$ (not significant) for the last two plots). Except for the marsh edge plots, shoot density was higher at the exposed site than at the sheltered site (t-test, $t = -3.9476$, $df = 15.032$, $p < 0.01$).

Furthermore, at both sites density of shoots was significantly lower at the marsh edge than within the marsh (t-test, $t = 3.7687$, $df = 9.974$ and $p < 0.01$ at the sheltered site; $t = 6.828$, $df = 7.167$, $p < 0.001$ at the exposed site). In terms of biomass per m^2 , significant differences between sites only occurred at 4 m and 12 m into the marsh (t-test, $t = 3.5142$, $df = 55.618$, $p < 0.0015$ and $t = 3.0487$, $df = 51.75$, $p < 0.01$, respectively), with higher standing biomass at the sheltered site.

Plant morphology – seasonal development

The monthly measurements during the entire growing season revealed a progressive development of most measured plant properties (Fig. 4). Shoot length increased over the growing season and differed significantly between sites (ANOVA, $F = 50.280$, $df = 1$, $p < 0.001$) and along the distance gradient (ANOVA, $F = 190.523$, $df = 1$, $p < 0.0015$) over the entire growing season. The basal stem diameters only differed significantly between sites in September (ANOVA, $F = 31.12$, $df = 1$, $p < 0.001$), but from June onwards there was a significant distance effect (ANOVA, $F = 40.119$, $df = 1$, $p < 0.001$) as well as a site $\times$ distance interaction effect (ANOVA, $F = 7.045$, $df = 1$, $p < 0.01$), emphasizing the stronger reduction in stem diameter over the first 8 m into the marsh at the exposed site compared to the sheltered site. Shoot density only differed significantly between sites in September (ANOVA, $F = 8.289$, $df = 1$, $p < 0.051$), with a higher density at the exposed site, while a distance effect, with lower densities at the front compared to the back of the marsh, could be seen from June onwards (ANOVA, $F = 6.156$, $df = 1$, $p < 0.05$). After omission of the marsh edge plots, however, within-marsh stem densities at the exposed site proved to be significantly higher in April, May, June (ANOVA, $F = 35.805$, $df = 1$, $p < 0.05$) and September (ANOVA, $F = 13.543$, $df = 1$, $p < 0.01$) and with a trend (ANOVA, $F = 5.771$, $df = 1$, $p < 0.1$) for higher shoot density at the exposed site in July. Dry biomass per m^2 was, except for June, significantly higher at the sheltered site than at the exposed site (ANOVA, $F = 23.303$, $df = 1$,

p<0.001), and distance into the marsh had a significant effect ([ANOVA, \$F = 551.931\$, \$df = 1\$, \$p < 0.001\$](#)), as the biomass increased from the marsh edge into the marsh. However, no site $\times$ distance interaction effect could be shown for either shoot density or dry biomass.

Belowground biomass

At the end of the growing season (September 2014), total belowground plant biomass increased at both sites ~~with over~~ distance into the marsh ([ANOVA, \$F = 32.263\$, \$df = 1\$, \$p < 0.001\$](#)), while differences between sites were not significant (Fig. 5). Biomass allocation over depth, however, differed between sites at 4 and 8 m into the marsh ([ANOVA, \$F = 9.489\$ \(4 m\), \$F = 7.645\$ \(8 m\), \$df = 2\$, \$p < 0.05\$ for both](#)), as there was more biomass in the deeper layers of the cores at the exposed site. Belowground-aboveground biomass ratio per m² differed only slightly between sites except for the marsh edge plots.

Biochemical analyses

Plant N:P ratios did not change over the season and were on average 7.91 ± 0.30 at the sheltered and 8.69 ± 0.34 at the exposed site (data not shown). When looking at the strengthening compounds (Fig. 6), cellulose contents remained stable over time and throughout the transects, and was on average only slightly higher (in the order of 20 mg g⁻¹) at the sheltered site. Lignin contents, however, more than doubled from April to September. In terms of BSi, big differences could be seen in time, between sites and along the transects, as BSi contents were significantly higher at the sheltered than at the exposed site ([t-test, \$t = 2.5885\$, \$df = 51.981\$, \$p < 0.05\$](#)). From April to September, it increased at the sheltered site by a factor 5, and almost doubled at the exposed site. In September, BSi increased at both sites from the marsh edge to the 12 m plot, by almost tripling at the sheltered site, and more than doubling at the exposed site. For September, the overall site averages differed significantly, too ([t-test, \$t = 3.2899\$, \$df = 12.799\$, \$p < 0.01\$](#)).

Biomechanical traits

Biomechanical analyses ~~on of~~ the September plant samples showed that the shoots from the sheltered site, except for those growing at the marsh edge, were generally stiffer and stronger than those grown at the exposed site (Fig. 7). There was also a general trend of increasing increase of stiffness and strength from the marsh edge towards the back of the marsh, at both sites (ANOVA, $F = 78.27$, $df = 1$, $p < 0.001$ for flexural stiffness and $F = 68.312$, $df = 1$, $p < 0.001$ for tensile strength). This trend was stronger at the sheltered site compared to the exposed site ($F = 3.24$, $df = 1$, $p < 0.1$ for the site x distance interaction term of flexural stiffness and $F = 4.810$, $df = 1$, $p < 0.05$ for tensile strength). While Young's modulus was significantly higher at the sheltered site at the 0 m and 4 m plots (t-test, $t = 2.9998$, $df = 37.486$, $p < 0.01$ and $t = 4.4107$, $df = 30.714$, $p < 0.001$, respectively) and significantly lower at the 12 m plots (t-test, $t = -3.0178$, $df = 26.295$, $p < 0.01$), it did not differ significantly between sites overall. However, it increased at both sites with distance into the marsh (ANOVA, $F = 66.072$, $df = 1$, $p < 0.001$). Breaking force and tensile strength showed a significant site (ANOVA, $F = 45.502$, $df = 1$, $p < 0.001$ for breaking force and $F = 7.204$, $df = 1$, $p < 0.01$ for tensile strength) and distance effect (ANOVA, $F = 44.490$, $df = 1$, $p < 0.001$ for breaking force and $F = 68.312$, $df = 1$, $p < 0.001$ for tensile strength), as well as a significant interaction term (ANOVA, $F = 7.596$, $df = 1$, $p < 0.01$ for breaking force and $F = 4.810$, $df = 1$, $p < 0.05$ for tensile strength), as they both increased more strongly with distance at the sheltered site than at the exposed site. There was a significant effect of site and distance from the marsh edge for flexural stiffness (ANOVA, $F = 52.21$, $df = 1$, $p < 0.001$ for site and $F = 78.27$, $df = 1$, $p < 0.001$ for distance). Differences between sites and distances were not significant for the second moment of area.

Greenhouse control

Differences between sites and position along the distance gradient were much smaller for the greenhouse control than in the field (Fig. 8), overall: e.g. the difference in shoot length between sites was only significant, ~~but smaller~~ for the 12 m plots (ANOVA, $F = 5.055$, $df = 1$, $p < 0.045$). Within sites, distance led only at the sheltered site to significant differences (ANOVA, $F = 19.58$, $df = 1$, $p < 0.001$). Basal stem diameters did not differ significantly between sites nor within sites. Dry weight of shoots differed significantly between plots at the sheltered site (ANOVA, $F = 39.36$, $df = 1$, $p < 0.001$), but not at the exposed site. When comparing between sites, the differences were not significant.

Correlations

Correlations between wave impact, on the one hand, and sediment and plant properties, on the other hand (Tab. Table 4 & 5), need to be viewed with caution, as wave heights measured along each transect are not independent from each other: i.e. the wave heights measured at the 4 and 12 m plots are a function of the wave heights recorded at the respective marsh edge. When testing for correlation, despite this constraint, wave exposure and sediment properties emerge as highly correlated (Pearson's correlation coefficient, with $|r| > 0.86$ for all tested variables). While median grain size and dry bulk density were positively correlated to wave exposure, DSi, organic matter and water content were negatively correlated. Furthermore, plant BSi correlated well with sediment pore water DSi ($r=0.78$, not shown in the table).

When looking at the correlation between waves and plant properties (Tab. Table 5), the strongest correlations were found for BSi, shoot length, breaking force, flexural stiffness and stem diameter (Pearson's correlation coefficients $|r| > 0.75$ with a negative coefficient for all except for stem diameter). Furthermore, biomass, cellulose, lignin and BSi contents increased with shoot length, and decreased with stem diameter. While BSi had the highest correlation with flexural stiffness

481 and tensile strength, cellulose was best correlated with Young's modulus. Cellulose and lignin
482 contents also showed a high positive correlation.

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Discussion

In this study we investigated whether plant properties of *Scirpus maritimus* vary between locations with different wave exposure and whether these properties develop progressively over the growing season. The novelty of this approach is that we looked into the relationships between waves and different types of plant properties ~~for types of plant properties~~, i.e. at the level of morphological, biomechanical and biochemical plant properties, and that we related the different levels to each other. Shoots from more wave-exposed conditions developed a stress-avoidance strategy by being shorter, thicker and more flexible than the shoots from more wave-sheltered conditions. Inner marsh stem density was higher at the exposed site than at the sheltered site. Some of these morphological differences developed during the growing season as a potential adaptation to local wave exposure. Plants from more wave sheltered locations were stiffer and stronger. These biomechanical properties were found to be related to biochemical properties of the plants: breaking force and tensile strength of stems correlated best with BSi contents which, in turn, reflected the local DSi contents in the sediment. Shoots grown under equal conditions (greenhouse control) showed only minor differences, indicating that the morphology of *S. maritimus* in the field is a response to the environmental factors that they are exposed to.

Waves have been shown to have direct and indirect effects on tidal marsh plants (e.g. Coops et al. 1991). Direct effects of waves on plants result from the direct physical impact of waves on the plants, leading, for example, to shorter shoots, thicker stems or higher shoot density of tidal marsh plants and aquatic macrophytes in general (e.g. Blanchette, 1997 for *Fucus gardneri*; Coops and Van der Velde, 1996 for different halophyte species ~~among which~~ including *Scirpus maritimus*; Doyle, 2001 for *Vallisneria americana*; Sun et al., 2002 for *Scirpus mariqueter*;

Szmeja and Galka, 2008 for a range of macrophyte species; see Bornette and Puijalon, 2010 for more references and a general review). ~~The~~^{Our} results presented in this paper are, overall, in line with these previous findings, as the shoots from more wave exposed locations were shorter, thicker and ~~more dense~~^{denser}, and hence showed all ~~the~~ typical signs of morphological adaptations to more intense waves conditions (Fig. 4 and 9). Indirect effects of waves affect sediment properties via hydrodynamic grain size sorting, and its effects on related sediment properties, ~~including~~. ~~This includes~~ decreasing organic matter, water and nutrient contents and increasing bulk density (with increasing grain size), which could then potentially influence plant growth, ~~too~~ ^{as well}. However, in our case, despite significantly higher total N and P of the sediment at the sheltered site, N:P ratios did not differ between sites, and plant N:P ratios were in the same range, which implies that observed differences in plant morphology do not result from differences in nutrient availability. This is furthermore supported by Coops et al. (1991) who found in a controlled cultivation experiment that ~~soil-sediment~~ type (and thus the corresponding ~~soil-sediment~~ properties, including nutrients) did not affect the growth form of *S. maritimus*. The only influence of ~~soil-sediment~~ properties on plant properties that we could establish was the direct correlation of pore water DSi contents and plant BSi contents: DSi contents were higher at the sheltered site compared to the exposed site, and plant BSi contents reflected the local sediment DSi contents. This “reflectance” of DSi availability in the BSi of plants had already been reported by Struyf et al. (2005) and Schaller et al. (2012). BSi has also been established in recent findings as a low-energy substitute for cellulose in conditions of local hydrodynamic stress (Schoelynck et al. 2012), which, however, cannot be supported by our findings, as cellulose and BSi contents were both negatively correlated to wave exposure (Table 5).

The higher DSi contents at the sheltered site could be an indirect consequence of wave exposure: lower wave exposure at the sheltered site is likely to enhance accumulation of BSi, e.g. by deposition of diatom and plant material. The decomposing diatom and plant~~and diatom~~ material will mainly be retained within the marsh due to low wave action and a longer retention of sediment pore water in the finer sediment at the sheltered site. Dissolution of this retained BSi to DSi will stay ~~longer in situ~~ for longer periods and be available for new shoots (e.g. Struyf et al. 2007) (Fig. 9).

The interesting part of this finding is that plant BSi correlated well with flexural stiffness and tensile strength and also (to a lesser extent) with Young's modulus and breaking force of the shoots (Table 5), which are typically correlated to the presence of strengthening compounds such as cellulose, lignin and BSi (Mullarney and Henderson 2010; Rupprecht et al. 2015; Schoelynck and Struyf 2016). While lignin and cellulose concentrations were similar between plants from different sites (Fig. 6), BSi contents of shoots showed a strong site (and wave) effect, with ~~increasing~~ BSi contents increasing ~~for~~ with decreasing wave exposure, implying that the differences in biomechanical properties found between sites and plots may probably result mainly from differences in BSi contents: the more BSi was available (i.e. the more sheltered the location), the stiffer and stronger the shoots.

Biomechanical properties of intertidal plants have already been assessed in a number of publications (e.g. Carus et al., 2016 for *S. maritimus* sampled from the field; Coops and Van der Velde, 1996 for *Phragmites australis* and *Scirpus lacustris* sampled from the field; Heuner et al., 2015 for *S. maritimus* and *S. tabernaemontani* grown under controlled and sheltered conditions; Möller et al., 2014 for *Puccinellia maritima* and *Elymus athericus* sampled from the field;

Rupprecht et al., 2015 for *Spartina anglica*, *Puccinellia maritima* and *Elymus athericus* sampled from the field; Silinski et al., 2015 for *S. maritimus* grown under controlled and sheltered conditions). The results presented in this paper are, overall, in the same range as the ones reported by ~~these~~ the above mentioned authors. Furthermore, biomechanical plant properties also relate (together with morphological properties) to survival strategies of plants: flexible shoots experience less drag forces due to their enhanced capacity to bend under flow or wave stress (Puijalon et al. 2011; ~~Bal et al. 2011~~; Pujol and Nepf 2012; Schoelynck et al. 2013), which, in turn, ~~then~~ reduces the risk for individual shoots from breaking or from being uprooted. These properties are typical of stress-avoidance strategies by plants as smaller, thinner and more flexible shoots will experience less drag forces than tall, thick and stiff ones, because these plants expose less surface to the incoming waves (e.g. Puijalon et al., 2008; Silinski et al., 2015) (Fig. 9).

While marsh vegetation is increasingly valued for its wave attenuation function and contribution to coastal protection (e.g. Borsje et al. 2011; Temmerman et al. 2013), it has been shown that plant properties such as shoot density and stiffness affect wave dissipation rates (e.g. Bouma et al., 2005; Ysebaert et al., 2011). For instance, higher standing biomass and higher plant stiffness lead to stronger wave attenuation through higher wave energy dissipation. At our field sites, relative wave attenuation rates ranged from 20 to 40 % of wave height reduction over the first 12 m of the marsh, which is in the same range as described for marsh fringes consisting of different marsh species (e.g. Bouma et al. 2005 for *Spartina angelica* ~~and *Zostera noltii*~~; ~~Koch et al. 2009 for marshes, mangroves, seagrasses, and coral reefs~~; Ysebaert et al. 2011 for *Scirpus mariqueter* and *Spartina alterniflora*; Möller et al. 2014 for a mixed canopy of *Elymus athericus*, *Puccinellia maritima*, and *Atriplex prostrata*). We found that wave attenuation rates were higher

at the exposed site. Given the higher flexibility of stems at the exposed site, this seems counter intuitive at first. However, we found higher shoot densities at the exposed site for most of the growing season (except for the marsh edge plots), which may explain the higher relative wave attenuation rates at the exposed site, despite the higher stem flexibility. This interpretation is supported by findings of Bouma et al. 2010 and Paul et al. 2012: while these authors report that wave attenuation is stronger within stiff vegetation compared to flexible vegetation for a given standing biomass, they also found that flexible vegetation can reach the same effectiveness of wave height attenuation when occurring at higher standing biomass. A potential additional explanation could be that the higher incoming waves under similar water depth conditions at the exposed site lead to higher loss of wave energy by a stronger interaction of the waves with the sediment bed (e.g. Callaghan et al., 2010; de Roo and Troch, 2013; Mariotti and Fagherazzi, 2010). However, given the particularly pronounced annual biomass-cycle of *S. maritimus* it needs to be considered that during winter and early spring, when occurrence of storm waves ~~are~~is generally more likely, aboveground biomass of this species will be extremely low. Thus, its contribution to wave attenuation might be limited during the winter season when its wave attenuation service would be most needed.

5. Conclusions

Scirpus maritimus, when exposed to waves, grows concordantly to a stress-avoidance strategy on individual shoot level. Adaptation to the conditions occurs on a morphological, biochemical and biomechanical level and was shown to develop over the growing season. Comparison with a greenhouse control suggests that these differing plant properties are a result of phenotypic plasticity. Relative wave attenuation was more efficient at the exposed site, probably due to higher stem density, emphasising the wave attenuating potential of marshes. This wave attenuating potential will, however, vary with the annual biomass-cycle of *S. maritimus* dominated pioneer zones.

6. References

- Blanchette, C. A. 1997. Size and survival of intertidal plants in response to wave action: A case study with *Fucus gardneri*. *Ecology* **78**: 1563–1578. doi:10.1890/0012-9658(1997)078[1563:SASOIP]2.0.CO;2
- Bornette, G., and S. Puijalon. 2010. Response of aquatic plants to abiotic factors: a review. *Aquat. Sci.* **73**: 1–14. doi:10.1007/s00027-010-0162-7
- Borsje, B. W., B. K. van Wesenbeeck, F. Dekker, P. Paalvast, T. J. Bouma, M. M. van Katwijk, and M. B. de Vries. 2011. How ecological engineering can serve in coastal protection. *Ecol. Eng.* **37**: 113–122. doi:10.1016/j.ecoleng.2010.11.027
- Bouma, T. J., M. Friedrichs, P. Klaassen, and others. 2009. Effects of shoot stiffness, shoot size and current velocity on scouring sediment from around seedlings and propagules. *Mar. Ecol. Ser.* **388**: 293–297. doi:10.3354/meps08130
- Bouma, T. J., S. Temmerman, L. A. van Duren, and others. 2013. Organism traits determine the strength of scale-dependent bio-geomorphic feedbacks: A flume study on three intertidal plant species. *Geomorphology* **180–181**: 57–65. doi:10.1016/j.geomorph.2012.09.005
- Bouma, T. J., M. B. De Vries, and P. M. J. Herman. 2010. Comparing ecosystem engineering efficiency of two plant species with contrasting growth strategies. *Ecology* **91**: 2696–704. doi:10.1890/09-0690.1
- Bouma, T. J., M. B. De Vries, E. Low, G. Peralta, I. Tanczos, J. van de Koppel, and P. M. J. Herman. 2005. Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. *Ecology* **86**: 2187–2199. doi:10.1890/04-1588
- Callaghan, D. P., T. J. Bouma, P. Klaassen, D. van der Wal, M. J. F. Stive, and P. M. J. Herman.

2010. Hydrodynamic forcing on salt-marsh development: Distinguishing the relative importance of waves and tidal flows. *Estuar. Coast. Shelf Sci.* **89**: 73–88. doi:10.1016/j.ecss.2010.05.013
- Carus, J., M. Paul, and B. Schröder. 2016. Vegetation as self-adaptive coastal protection: Reduction of current velocity and morphologic plasticity of a brackish marsh pioneer. *Ecol. Evol.* **6**: 1579–1589. doi:10.1002/ece3.1904
- Charpentier, A., P. Grillas, and J. D. Thompson. 2000. The effects of population size limitation on fecundity in mosaic populations of the clonal macrophyte *Scirpus maritimus* (Cyperaceae). *Am. J. Bot.* **87**: 502–507.
- Charpentier, A., and J. F. Stuefer. 1999. Functional specialization of ramets in *Scirpus maritimus*. Splitting the tasks of sexual reproduction, vegetative growth, and resource storage. *Plant Ecol.* 129–136. doi:10.1023/A:1009825905117
- Coops, H., R. Boeters, and H. Smit. 1991. Direct and indirect effects of wave attack on helophytes. *Aquat. Bot.* **41**: 333–352.
- Coops, H., N. Geilen, and G. van der Velde. 1994. Distribution and growth of the helophyte species *Phragmites australis* and *Scirpus lacustris* in water depth gradients in relation to wave exposure. *Aquat. Bot.* **48**: 273–284.
- Coops, H., and G. Van der Velde. 1996. Effects of waves on helophyte stands: mechanical characteristics of stems of *Phragmites australis* and *Scirpus lacustris*. *Aquat. Bot.* **53**: 175–185. doi:10.1016/0304-3770(96)01026-1
- Cottenie, A., M. Verloo, L. Kiekens, G. Velghe, and R. Camerlynck. 1982. Chemical analysis of plant and soil laboratory of analytical and agrochemistry, State University Ghent [ed.].
- Cox, T. J. S. 2014. Tides: Quasi-Periodic Time Series Characteristics. R package version 1.1.

- 651 Dalrymple, R. A., and R. G. Dean. 1991. Water wave mechanics for engineers and scientists,
652 Prentice-Hall.
- 653 DeMaster, D. J. 1981. The supply and accumulation of silica in the marine environment. *Geochim.*
654 *Cosmochim. Acta* **45**: 1715–1732. doi:10.1016/0016-7037(81)90006-5
- 655 Denny, M. W. 2006. Ocean waves, nearshore ecology, and natural selection. *Aquat. Ecol.* **40**: 439–
656 461. doi:10.1007/s10452-004-5409-8
- 657 Doyle, R. D. 2001. Effects of waves on the early growth of *Vallisneria americana*. *Freshw. Biol.*
658 389–397. doi:10.1046/j.1365-2427.2001.00668.x
- 659 Friess, D. A., K. W. Krauss, E. M. Horstman, T. Balke, T. J. Bouma, D. Galli, and E. L. Webb.
660 2012. Are all intertidal wetlands naturally created equal? Bottlenecks, thresholds and
661 knowledge gaps to mangrove and saltmarsh ecosystems. *Biol. Rev. Camb. Philos. Soc.* **87**:
662 346–66. doi:10.1111/j.1469-185X.2011.00198.x
- 663 Henry, P.-Y., and D. Myrhaug. 2013. Wave-induced drag force on vegetation under shoaling
664 random waves. *Coast. Eng.* **78**: 13–20. doi:10.1016/j.coastaleng.2013.03.004
- 665 Heukels, H., and R. van der Meijden. 2005. Heukels' Flora van Nederland, 23rd ed. Wolters-
666 Noordhoff.
- 667 Heuner, M., A. Silinski, J. Schoelynck, and others. 2015. Ecosystem engineering by plants on wave-
668 exposed intertidal flats is governed by relationships between effect and response traits. *PLoS*
669 *One* **10**: 1–18. doi:10.1371/journal.pone.0138086
- 670 Houba, V. J. G., J. J. van der Lee, I. Novazamsky, and I. Walinga. 1989. Soil and plant analysis, a
671 series of syllabi, *In* Soil Analysis Procedures. Agricultural University, Wageningen.
- 672 Hroudová, Z., P. Zákavský, and M. Flegrová. 2013. The tolerance to salinity and nutrient supply in

- 673 four European *Bolboschoenus* species (*B. maritimus*, *B. laticarpus*, *B. planiculmis* and *B.*
674 *yagara*) affects their vulnerability or expansiveness. *Aquat. Bot.* **112**: 66–75.
675 doi:10.1016/j.aquabot.2013.07.012
- 676 Mariotti, G., and S. Fagherazzi. 2010. A numerical model for the coupled long-term evolution of salt
677 marshes and tidal flats. *J. Geophys. Res.* **115**: 1–15. doi:10.1029/2009JF001326
- 678 Maris, T., L. Oosterlee, and P. Meire, eds. 2013. Onderzoek naar de gevolgen van het Sigmaplan,
679 baggeractiviteiten en havenuitbreiding in de Zeeschelde op het milieu. Geïntegreerd
680 eindverslag van het onderzoek verricht in 2012.,.
- 681 Möller, I., M. Kudella, F. Rupprecht, and others. 2014. Wave attenuation over coastal salt marshes
682 under storm surge conditions. *Nat. Geosci.* **7**: 727–731. doi:10.1038/ngeo2251
- 683 Mullarney, J. C., and S. M. Henderson. 2010. Wave-forced motion of submerged single-stem
684 vegetation. *J. Geophys. Res. Ocean.* **115**: 1–14. doi:10.1029/2010JC006448
- 685 Niklas, K. J. 1992. Plant biomechanics: an engineering approach to plant form and function,
686 University of Chicago Press.
- 687 Paul, M., T. J. Bouma, and C. L. Amos. 2012. Wave attenuation by submerged vegetation:
688 Combining the effect of organism traits and tidal current. *Mar. Ecol. Prog. Ser.* **444**: 31–41.
689 doi:10.3354/meps09489
- 690 Puijalon, S., and G. Bornette. 2006. Phenotypic plasticity and mechanical stress: Biomass
691 partitioning and clonal growth of an aquatic plant species. *Am. J. Bot.* **93**: 1090–1099.
692 doi:10.3732/ajb.93.8.1090
- 693 Puijalon, S., G. Bornette, and P. Sagnes. 2005. Adaptations to increasing hydraulic stress:
694 morphology, hydrodynamics and fitness of two higher aquatic plant species. *J. Exp. Bot.* **56**:
695 777–86. doi:10.1093/jxb/eri063

- 696 Puijalon, S., T. J. Bouma, C. J. Douady, J. van Groenendael, N. P. R. Anten, E. Martel, and G.
697 Bornette. 2011. Plant resistance to mechanical stress: evidence of an avoidance-tolerance trade-
698 off. *New Phytol.* **191**: 1141–1149. doi:10.1111/j.1469-8137.2011.03763.x
- 699 Puijalon, S., T. J. Bouma, J. Van Groenendael, and G. Bornette. 2008. Clonal plasticity of aquatic
700 plant species submitted to mechanical stress: Escape versus resistance strategy. *Ann. Bot.* **102**:
701 989–996. doi:10.1093/aob/mcn190
- 702 R Core Team. 2014. R: A language and environment for statistical computing, R Foundation for
703 Statistical Computing [ed.].
- 704 de Roo, S., and P. Troch. 2013. Field Monitoring of Ship Wave Action on Environmentally Friendly
705 Bank Protection in a Confined Waterway. *J. Waterw. Port, Coastal, Ocean Eng.* 527–534.
706 doi:10.1061/(ASCE)WW.1943-5460.0000202.
- 707 Rupprecht, F., I. Möller, B. Evans, T. Spencer, and K. Jensen. 2015. Biophysical properties of salt
708 marsh canopies — Quantifying plant stem flexibility and above ground biomass. *Coast. Eng.*
709 **100**: 48–57. doi:10.1016/j.coastaleng.2015.03.009
- 710 Schaller, J., C. Brackhage, M. O. Gessner, E. Bäuker, and E. G. Dudel. 2012. Silicon supply
711 modifies C:N:P stoichiometry and growth of *Phragmites australis*. *Plant Biol.* **14**: 392–396.
712 doi:10.1111/j.1438-8677.2011.00537.x
- 713 Schoelynck, J., K. Bal, S. Puijalon, P. Meire, and E. Struyf. 2012. Hydrodynamically mediated
714 macrophyte silica dynamics. *Plant Biol.* **14**: 997–1005. doi:10.1111/j.1438-8677.2012.00583.x
- 715 Schoelynck, J., D. Meire, K. Bal, K. Buis, P. Troch, T. J. Bouma, P. Meire, and S. Temmerman.
716 2013. Submerged macrophytes avoiding a negative feedback in reaction to hydrodynamic
717 stress. *Limnol. - Ecol. Manag. Int. Waters* **43**: 371–380. doi:10.1016/j.limno.2013.05.003
- 718 Schoelynck, J., and E. Struyf. 2016. Silicon in aquatic vegetation. *Funct. Ecol.* 1323–1330.

doi:10.1111/1365-2435.12614

Silinski, A., M. Heuner, J. Schoelynck, and others. 2015. Effects of Wind Waves versus Ship Waves on Tidal Marsh Plants: A Flume Study on different Life Stages of *Scirpus maritimus*. PLoS One. doi:10.1371/journal.pone.0118687

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Agric. Chem. **46**: 829–835.

Struyf, E., S. Van Damme, B. Gribsholt, K. Bal, O. Beauchard, J. J. Middelburg, and P. Meire. 2007. *Phragmites australis* and silica cycling in tidal wetlands. Aquat. Bot. **87**: 134–140.

Struyf, E., S. Van Damme, B. Gribsholt, J. Middelburg, and P. Meire. 2005. Biogenic silica in tidal freshwater marsh sediments and vegetation (Schelde estuary, Belgium). Mar. Ecol. Prog. Ser. **303**: 51–60. doi:10.3354/meps303051

Sun, S. C., Y. L. Cai, and S. Q. An. 2002. Differences in morphology and biomass allocation of *Scirpus mariqueter* between creekside and inland communities in the Changjiang estuary, China. Wetlands **22**: 786–793.

Szmeja, J., and a Galka. 2008. Phenotypic responses to water flow and wave exposure in aquatic plants. Acta Soc. Bot. Pol. **77**: 59–65.

Temmerman, S., P. Meire, T. J. Bouma, P. M. J. Herman, T. Ysebaert, and H. J. De Vriend. 2013. Ecosystem-based coastal defence in the face of global change. Nature **504**: 79–83. doi:10.1038/nature12859

Tonelli, M., S. Fagherazzi, and M. Petti. 2010. Modeling wave impact on salt marsh boundaries. J. Geophys. Res. **115**: 1–17. doi:10.1029/2009JC006026

Turner, S. R., N. Taylor, and L. Jones. 2001. Mutations of the secondary cell wall. Plant Mol. Biol.

741 **47**: 209–219. doi:10.1023/A:1010695818416

742 Versluys, T., and P. Troch. 2010. Description of the conversion algorithm from pressure
743 measurements to surface elevations. Technical Report., Dept. of Civil Engineering, Ghent
744 University.

745 Walinga, I., W. Van Vark, V. J. G. Houba, and J. J. Van der Lee. 1989. Soil and plant analysis, a
746 series of syllabi, part 7: Plant analysis procedures, Wageningen Agricultural University,
747 Wageningen, Netherlands.

748 Wang, X., H. Ren, B. Zhang, B. Fei, and I. Burgert. 2012. Cell wall structure and formation of
749 maturing fibres of moso bamboo (*Phyllostachys pubescens*) increase buckling resistance. J. R.
750 Soc. Interface 988–996. doi:10.1098/rsif.2011.0462

751 Ysebaert, T., S. L. Yang, L. Zhang, Q. He, T. J. Bouma, and P. M. J. Herman. 2011. Wave
752 attenuation by two contrasting ecosystem engineering salt marsh macrophytes in the intertidal
753 pioneer zone. Wetlands **31**: 1043–1054. doi:10.1007/s13157-011-0240-1

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Figure legends

Figure 1: (a) Position of the Scheldt Estuary in the Dutch-Belgian border region (indicated by the black square). (b) Overview of the Scheldt Estuary with its tidal flats and marshes downstream of Antwerp to its mouth near Vlissingen. (c) Overview of the location of both monitoring transects (black stars) at both tidal marshes with indication of the breakwater and the shipping channel.

Figure 2: Monitoring field set-up indicating continuous, monthly (April to July for waves; April to September for surface and ground water measurements) and September sampling, along a distance gradient of 12 m from the marsh edge into the marsh.

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Figure 4: Average $\pm$ SE of morphological plant properties as quantified on 30 stems per plot and month (total length (cm), basal stem diameter (mm), shoot density ($\# \text{ m}^{-2}$) and dry biomass per m^2 (g m^{-2})). September campaign: significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.05 > * > 0.01 > ** > 0.001 > ***$).

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Figure 6: Strengthening compounds of shoots in $\text{mg g}^{-1} \pm \text{SE}$ ($n=3$ per plot and month), monthly for the sheltered and exposed site, and for the September sampling for both sites. September

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Figure 7: Biomechanical traits of shoots sampled in the September campaign ($n=20$). **Top:** tensile properties; **bottom:** bending properties. Significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.1 > \# > 0.05 > * > 0.01 > ** > 0.001 > ***$; significance was assumed at $p < 0.05$).

Figure 8: Morphological properties for the greenhouse control plants ($n=15$ per plot and site). Significant differences between sites at same distances into the marsh are indicated as resulting from t-tests ($p: 0.1 > \# > 0.05 > * > 0.01 > ** > 0.001 > ***$; significance was assumed at $p < 0.05$).

Figure 9: Schematic view of two marshes with contrasting wave exposure. Wave dynamics (1) induce direct mechanical stress affecting the morphology of the shoots (2A and 3A); indirect effects on the plants are the wave-induced effects on the sediment properties by determining the sediment texture and composition (2B, i.e. dissolved silica contents (DSi), organic matter contents (OM) and water contents (H_2O)). DSi content in the sediment is reflected in the biogenic silica contents of the shoots (BSi) (3B), which in turn is an important source for the DSi pool in the sediment after decomposition of the plant material. Both morphology and tissue composition determine the biomechanical strength of the shoots (4). More exposed shoots are more flexible₂ and smaller₂, which suggests a stress-avoidance strategy₂, whereas the increased belowground biomass at deeper depth might indicate a stress-tolerance strategy.

Tables

Table 1: Overview of significant wave height (H_s , cm), the mean over the highest 1 % of wave heights ($H_{1/100}$, cm), mean inundation depth (id_{mean} , cm), mean inundation time per tidal cycle (it_{mean} , min) and inundation frequency (if, %) for the three plots of the sheltered (shelt.) and exposed (exp.) site, respectively, during the growing season of 2014.

plot	H_s (cm)		$H_{1/100}$ (cm)		id_{mean} (cm)		it_{mean} (min)		if (%)	
	shelt.	exp.	shelt.	exp.	shelt.	exp.	shelt.	exp.	shelt.	exp.
0 m	2.8	5.7	9.7	20.3	81	77	138	127	100	100
4 m	2.7	5.2	9.2	18.0	71	67	125	115	99	99
12 m	2.1	3.5	6.9	12.6	51	47	99	87	94	94

Table 2: Physical sediment properties of Kopecky ring samples (top 5 cm of sediment), averaged over the entire transect, and core samples, averaged over depth at the marsh edge and 12 m plots: median grain size (d_{50} , $\mu\text{m} \pm \text{SE}$), organic matter contents (LOI, $\% \pm \text{SE}$), dry bulk density ($\text{g cm}^{-3} \pm \text{SE}$) and water contents (H_2O -contents, $\% \pm \text{SE}$); $n=12$ for Kopecky ring samples per transect, $n=3$ for core samples per plot.

sample	unit	Type/plot	site sheltered	exposed
d_{50}	μm	Kopecky		
		all	24 ± 7	135 ± 3
		core		
		Marsh edge	87 ± 21	121 ± 12
		12 m	31 ± 11	116 ± 15
LOI	$\%$	Kopecky		
		all	8.18 ± 0.87	1.15 ± 0.02
		core		
		Marsh edge	3.71 ± 1.00	1.70 ± 0.59
		12 m	7.87 ± 1.17	2.11 ± 0.81
dry bulk density	g cm^{-3}	Kopecky		
		all	0.76 ± 0.01	1.48 ± 0.01
H_2O -contents	$\%$	Kopecky		
		all	49.15 ± 0.01	23.92 ± 0.01

Table 3: Chemical sediment properties averaged over site (n=12 per site). Concentrations are given in $\text{mg g}^{-1} \pm \text{SE}$. p-value indicates significant differences between sites as obtained from a t-test. Significance was assumed at $p < 0.05$. ns: not significant.

sample	unit	site		p (t-test)
		sheltered	exposed	
$\text{PO}_4^{3-}\text{-P}$	mg g^{-1}	0.530 ± 0.07	0.030 ± 0.01	<0.001
$\text{NH}_4^+\text{-N}$	mg g^{-1}	0.002 ± 0.001	0.001 ± 0.0002	ns
total P	mg g^{-1}	1.020 ± 0.10	0.290 ± 0.02	<0.001
total N	mg g^{-1}	1.280 ± 0.16	0.340 ± 0.06	<0.001
N:P ratio	-	1.22 ± 0.08	1.13 ± 0.12	ns
pH, actual	-	8.20 ± 0.25	8.76 ± 0.16	<0.01
pH, potential	-	7.65 ± 0.20	8.30 ± 0.20	<0.01

Table 4: Correlation matrix for wave activity (waves: mean over the highest 1% of wave heights) and sediment variables, i.e. dissolved silica (DSi), median grain size (d_{50}), dry bulk density (BD), organic matter contents (OM) and water content (H_2O). Pearson's correlation coefficients are reported. The correlation of dry bulk density with water content is not reported (/) as water content results from the difference of fresh and dry bulk density.

	waves	DSi	d_{50}	BD	OM
DSi	-0.87				
d_{50}	0.86	-0.86			
BD	0.87	-0.96	0.88		
OM	-0.98	0.89	-0.97	-0.90	
H_2O	-0.86	0.96	-0.87	/	0.89

Table 5: Correlation matrix for wave activity (waves: mean over the highest 1 % of wave heights) and plant properties, i.e. plant length (length), basal stem diameter (diam), dry biomass per m² (BM), flexural stiffness (FS), Young’s modulus (YM), tensile strength (TS), breaking force (BF), cellulose (cell), lignin (lig) and biogenic silica (BSi). Pearson’s correlation coefficients are reported. The correlations of flexural stiffness with Young’s modulus and tensile strength with breaking force are not reported (/) as Young’s modulus and breaking force were used to calculate flexural stiffness and tensile strength, respectively.

	waves	length	diam	BM	FS	YM	TS	BF	cell	lig
length	-0.79									
diam	0.76	-0.57								
BM	-0.60	0.85	-0.45							
FS	-0.76	0.64	-0.35	0.51						
YM	-0.57	0.47	-0.35	0.48	/					
TS	-0.46	0.49	-0.31	0.43	0.56	0.37				
BF	-0.77	0.47	-0.33	0.32	0.58	0.26	/			
cell	-0.61	0.86	-0.86	0.89	0.69	0.83	0.65	0.50		
lig	-0.41	0.81	-0.91	0.86	0.55	0.78	0.57	0.36	0.93	
BSi	-0.81	0.88	-0.70	0.74	0.90	0.57	0.74	0.58	0.64	0.59

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210x296mm (300 x 300 DPI)

Figure 2: Monitoring field set-up indicating continuous, monthly (April to July for waves; April to September for surface and ground water measurements) and September sampling, along a distance gradient of 12 m from the marsh edge into the marsh.

266x355mm (96 x 96 DPI)

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146x199mm (300 x 300 DPI)

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249x299mm (300 x 300 DPI)

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194x259mm (300 x 300 DPI)

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249x249mm (300 x 300 DPI)

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249x179mm (300 x 300 DPI)

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219x79mm (300 x 300 DPI)

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355x266mm (96 x 96 DPI)