

Masterthesis submitted to obtain the degree of Master in Biology,
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Response of morphology and tissue properties of tidal marsh plants to wave activity

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Abstract

The notion that coastal salt marsh plants attenuate waves and hence play a key role in ecosystem-based coastal defence becomes more and more accepted in recent literature. However, little is known about the morphological, biochemical and biomechanical response of vegetation to these incoming waves. With a field study we investigated whether observed differences in morphology, biochemical tissue composition and biomechanical properties are due to different wave exposure.

Two sites of contrasting wave exposure with *S. maritimus* as dominating pioneer were selected along the brackish part of the Scheldt Estuary (Belgium). From April to September 2014, we monitored plant morphology of *S. maritimus* at these two sites on three levels close to the marsh edge. Waves, ground and surface water levels were measured continuously from April to October 2014. In September, a more extensive field campaign including abiotic variables as well as flexibility of basal plant stems and belowground biomass was determined for plants of each plot. As control, tubers from both sites were sampled in March 2014 and grown under equal conditions in a greenhouse until June 2014.

Our results show that individual shoots of *S. maritimus* respond to wave exposure with a stress avoidance mechanism, e.g. smaller stature and more flexible plants at the most exposed plots (both between sites as well as within one clonal stand). We found that this might be the result of phenotypic plasticity. Moreover, the whole clonal stand responds by growing in higher densities at the exposed site, which is a stress tolerance mechanism, resulting in more effective wave attenuation. Therefore the morphological responses of both individual shoots and the whole clonal plant have important consequences for the wave attenuating capacity of wave exposed tidal marshes.

Key words: tidal marshes, wave exposure, plant morphology, biomechanical properties, strength molecules.

Samenvatting

De positieve invloed van schorrevegetatie op het afzwakken van golfenergie en de rol die het hierbij vervult als een op ecosysteem gebaseerde kustbescherming wordt meer en meer erkend in de literatuur. Het is echter onduidelijk hoe vegetatie met deze golfenergie omgaat. Met een veldexperiment gaan we na of de geobserveerde verschillen in morfologie, biochemie en biomechanica een gevolg zijn van de blootstelling aan golfenergie.

Twee schorren met contrasterende golfblootstelling waarin *S. maritimus* de dominante soort is, werden geselecteerd waarna de plantmorfologie van april tot september 2014 gemonitord werd over drie afstanden vanaf de rand van het schor. Golven, grond- en oppervlaktewater werden doorlopend gemeten van april tot oktober 2014. In september werden de metingen nog uitgebreid met abiotische variabelen, de biomechanische eigenschappen van de stengel en de ondergrondse biomassa. Een controle experiment werd opgesteld door knollen van beide schorren te verzamelen in maart 2014. Deze werden opgekweekt onder gelijke omstandigheden in een serre waarna de eigenschappen in juni 2014 opgemeten werden.

Onze resultaten tonen aan dat *S. maritimus* zich aanpast aan blootstelling aan golfenergie door middel van de stress te ontwijken, bv. kleinere en flexibele planten in de meest blootgestelde locaties (zowel tussen de twee schorren als binnen een kloon). Dit kan het gevolg zijn van fenotypische plasticiteit van de plant. Verder zien we ook dat de groei densiteit hoger is in het blootgestelde schor. Dit suggereert een stress tolerantie mechanisme op het niveau van de hele kloon welke voor een efficiëntere golf afzwakking zorgt. Dus zowel de eigenschappen van individuele stengels als van de volledige klonale plant hebben gevolgen voor de capaciteit om golven af te zwakken in sterk blootgestelde schorren.

Sleutelwoorden: Schorre, golf blootstelling, plantmorfologie, biomechanische eigenschappen, stevigheid moleculen.

Layman's abstract

Tidal marshes are vegetated areas along coasts and tidal rivers (estuaries), which are regularly inundated. The frequency of inundation depends on the tides and the elevation of the marsh. Vegetation in a marsh is exposed to incoming waves. Research shows that these plants can weaken the energy of these waves, which helps protecting the mainland. However, one can imagine that plants also encounter a stress from this wave activity. In this study we investigate the effect of wave exposure on plant properties.

Along two sites in the Scheldt Estuary, with very different wave exposure, we looked at the most common species, which was *Scirpus maritimus* (family of the sedges and rushes). From April to September 2014, we measured plant morphological, chemical and mechanical properties of *S. maritimus* at these two sites on three levels close to the marsh edge. Waves, ground and surface water levels were measured continuously from April to October 2014. In September, we included the measurements of some extra physical and chemical variables as well as the flexibility of stems and we collected the root material at each plot. As a control, root tubers from both sites were collected in March 2014 and grown under equal conditions in a greenhouse until June 2014.

Our results show that the shoot properties of *S. maritimus* are adapted to wave exposure in a way that they try to avoid the stress created by the waves, i.e. smaller and more flexible plants at the most exposed plots. We found that this might be the result of morphological adaptations in response to the environment without genetic changes. Moreover, the shoots of the exposed marsh grow in higher densities, this enables the marsh vegetation to tolerate wave exposure and enhance the dissipation of waves. Therefore both, the individual shoot properties as well as the properties of all shoots together are important determinants for the marsh's ability to weaken the incoming waves.

Keywords: tidal marshes, waves, plant properties.

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1 Introduction

1.1 Estuarine ecosystems

Estuaries form the highly dynamic transition zone where a river meets the sea. Although the main features of normal river valleys can also be found in estuaries (e.g. meanders and tributaries), several typical geomorphological features are formed additionally (Davidson et al., 1991). In middle to high latitudes, tidal motion creates intertidal areas such as mudflats and marshes along shorelines of estuaries. In the tropical and subtropical regions, mangroves are the counterpart of marshes. Both are characterized by vegetation that can cope with frequent inundations, variable salinity and temperature fluctuations since they are flooded daily down to several times a month, depending on their elevation relative to mean sea level and the daily (ebb and flood) and monthly (spring tides and neap tides) tidal cycles (Mccluskey et al., 2004). In regards to water salinity, estuaries are typically subdivided in a marine salt water part, a brackish part and further inland a fresh water part, each with its characteristic fauna and flora (Davidson et al., 1991). The salt water part, strongly influenced by the sea, is dominated by salt tolerant marine species. The fresh water part, mainly influenced by upstream tributaries, is dominated by fresh water species. Since the brackish part is affected by both marine and freshwater, it forms an extremely variable and dynamic environment challenging species even more. Brackish water species should be adaptive. Hence, biodiversity is relatively low compared to the other two parts (Cognetti and Maltagliati, 2000; Crain et al., 2004).

Vegetated intertidal areas, i.e. marshes in the temperate climate zones and mangroves in tropical areas, are known to provide a range of ecosystem services such as coastal protection, water quality improvement, carbon sequestration, fish nursery and thus benefits for fishery, breeding area for birds and recreation (Barbier et al., 2011; Costanza et al., 2008; Kirwan and Megonigal, 2013; Möller et al., 2014; Temmerman et al., 2013). Consequently they form one of the most important ecosystems on Earth and are therefore often protected by legislations (e.g. EU Habitat and Bird Directives).

Despite their importance and protected status, salt, brackish and freshwater tidal marshes are seriously threatened both by human and natural stressors and scientific knowledge is needed to conserve or restore these valuable ecosystems (Spalding et

al., 2014; Barbier et al., 2011). Intensive human activities such as land reclamation, harbour construction, dredging and tourism development impose great pressure on the structure and function of salt marshes all over the world. Global warming, relative sea-level rise, and extreme weather conditions will compound these problems (Li et al., 2013). For instance, sea level rise is predicted (using simulations of the IPCC) to transform 20-45% of the existing salt marshes (based on the salt marshes along the Georgia coast, USA) into open water by the end of this century (Craft et al., 2009) when assuming little dynamics (i.e. small tidal range and low sediment load) (Kirwan et al., 2010). Such losses induce substantial changes in current ecosystem services that are provided (Li et al., 2013).

Thoughtful management of estuarine ecosystems can help protecting whole coastal societies from the impacts of climate change (e.g. tidal marshes can act as adaptive buffer zones against sea level rise, they attenuate wind waves and storm surges, and temper shoreline erosion) (Temmerman et al., 2013; Möller et al., 2014). Nevertheless, applying management is only useful when we have a thorough understanding of the ecosystem, hence preventing unwanted side effects.

1.2 Pressures shaping the estuary

In estuaries like those of the Scheldt (Belgium and SW Netherlands), Elbe, Ems and Weser (Germany), situated in dense populated areas with high economic activity (i.e. harbours), the pressure on the ecosystem is very high. Organisms are not only exposed to natural dynamics (e.g. tides, wind waves) but also to additional dynamics originating from anthropogenic interventions often resulting in an even wider range of stress factors to cope with. For instance, the maintenance of accessibility for big ships to the harbour of Antwerp is ensured by dredging (deepening and widening) the naturally changing channels of the Scheldt Estuary which, hence, become more or less fixed in time and space (Meire et al., 2005). As a result of these human activities intertidal marshes are exposed to stronger hydrodynamic forces, i.e. stronger currents (Cox et al., 2003) and higher tidal range (Dam et al., 2013) due to dredging, and bigger waves due to passing ships (Houser, 2010). Lateral expansion or retreat of intertidal marshes is strongly affected by these hydrodynamics that form the driving force of sedimentation and erosion processes, which determine the elevation of the tidal flats and marshes (Fagherazzi et al., 2007).

As autogenic ecosystem engineers (*sensu* Jones et al., 1997, 1994), marsh vegetation reduces hydrodynamic energy promoting sedimentation and reducing erosion processes, hence stabilizing and elevating the soil (Bouma et al., 2005b; Kirwan and Temmerman, 2009; Morris et al., 2002). These biogeomorphological feedback processes are scale-dependent (i.e. local facilitation and large-scale inhibition of plant growth) and density-dependent (i.e. high-density facilitation and low-density inhibition of plant growth), hence creating dendritic marsh landscapes (Bouma et al., 2009; Van Wesenbeeck et al., 2008; Vandenbruwaene et al., 2011). When the load of sediment in the water column is high enough, the vegetation-induced sedimentation has a positive feedback on vegetation survival and development, which is an effective mechanism by which marshes can adapt to and keep up with sea level rise (Kirwan et al., 2010; Wang and Temmerman, 2013).

Regarding coastal defence, Temmerman et al. (2013) emphasised the need for alternatives to traditional coastal protection (e.g. dikes). Research increasingly confirms the attenuating effect of incoming waves by marsh vegetation and consequently the potential of tidal marshes as ecosystem-based coastal defence systems (Möller, 2006; Koch et al., 2009; Temmerman et al., 2013; Möller et al., 2014). The majority of wave energy entering a marsh is attenuated close to the marsh edge, hence wave attenuation (i.e. the relative reduction of wave height per distance travelled through the marsh vegetation) by marsh vegetation is a non-linear process (Yang, 1998; Möller et al., 2002; Bouma et al., 2005a; Koch et al., 2009; ; Ysebaert et al., 2011; Möller et al., 2014). For instance Ysebaert et al. (2011) found vegetation to attenuate wave heights (which is correlated to wave energy (Houser, 2010)) up to 80 % over the first 50 m of vegetation. Even under extreme water levels and wave heights (e.g. under storm surge conditions), when coastal defence is most important, marsh vegetation attenuates the incoming waves (Möller et al., 2014). However, the capacity of a marsh to attenuate waves depends on plant morphological traits. Flume experiments showed that stiffer shoots cause more wave attenuation under these strong hydrodynamics (Bouma et al., 2013; Bouma et al., 2010). Furthermore it was found that a high standing biomass, which is a better proxy for biomass density compared to the number of stems (Penning et al., 2009; Ysebaert et al., 2011), increases the attenuation of waves. Also under the extreme water levels as e.g. produced during a storm, taller shoots seem to facilitate wave attenuation (Ysebaert et

al., 2011). For this reason, intertidal marsh vegetation and its associated morphological plant characteristics are important determinants for both wave attenuation, thus the efficiency of the ecosystem-based coastal protection, and for shaping the estuarine landscape.

1.3 How marsh vegetation copes with hydrodynamics

Apart from the attenuating effect of vegetation, waves in their turn have also an effect on the marsh vegetation - both directly by drag and pulling force (challenging both, above and belowground vegetation parts) (Henry et al., 2013), as well as indirectly by determining the soil texture and composition (Coops et al., 1991). Since plants are able to change their tissue composition and allocation as a result of external stress (Sultan, 2003) it can be assumed that variation in wave exposure, e.g. small wind waves versus higher ship waves and heavily wave-exposed versus wave-sheltered locations has different effects on marsh vegetation. Hammond et al. (2002) showed that the intertidal macrophyte, *Spartina anglica*, was not even able to establish in conditions associated with longer (i.e. ship induced) waves. Since wavelength and celerity are positively correlated, longer waves are more likely to affect bed sediments and thus cause uprooting of plants (Silinski et al., 2015).

In order to cope with hydrodynamic forces macrophytes are thought to use different strategies as proposed by the tolerance-avoidance trade-off theory (Coops et al., 1994; Puijalon et al., 2011). Enhanced flexibility enables the shoot to minimize and thus avoid the impact of external mechanical forces on the shoot (e.g. by bending they experience a reduced drag force from the currents) while, to the contrary, investments in rigidity, allow the shoot to tolerate hydrodynamic stress (Puijalon et al., 2011). Macrophyte shoots get most of their structural properties, i.e. rigidity or flexibility from adjustments in the layer of sclerenchyma tissue. Unlike animal cells, plant cells are protected by an extra so-called cell wall. This wall consists of several layers of cellulose, which form long microfibrils surrounded by a matrix of hemicellulose and pectin. These cellulose fibrils provide the shoot with longitudinal elasticity. Rigidity of the shoot is enhanced when lignin and cellulose fibrils associate forming a secondary cell wall (Turner et al., 2001; Wang et al., 2012). Apart from lignin and cellulose, biogenic silica (BSi) can also provide support to the shoot as Schoelynck, et al. (2012) showed an increase of BSi accumulation when the plants had been exposed

to hydrodynamic stress. Since cellulose and lignin are molecules with high investment costs, Schoelynck et al. (2012) suggested a theory of alternative investment strategies where BSi could be a more cost-effective alternative. Therefore regarding the chemical tissue properties, we expect more wave exposed shoots to invest more in flexibility and thus to contain a higher amount of cellulose while higher silica and lignin concentrations are expected in the more sheltered locations (Schoelynck et al., 2012).

Aside from withstanding hydrodynamic induced drag and pulling forces on the aboveground biomass, below ground biomass is responsible for anchoring the shoot, leading to a stabilisation of the sediment. Shoot-root ratios of *Spartina sp.* have been shown to have different patterns depending on species and the environmental condition to which plants are exposed (Hemminga et al., 1996). However as a direct response to hydrodynamics, it was shown that seagrasses enlarge their root system when exposed to high flow velocity, thereby decreasing the shoot-root ratio (Peralta et al., 2006). Additionally, Puijalon and Bornette (2006) found higher shoot densities, smaller growth forms and biomass reallocation towards the roots for *Berula erecta* in response to increasing hydrodynamic stress. These adaptations suggest a stress avoidance strategy as stated by Puijalon et al. (2011). Since plant morphological properties differ between the stress avoidance and stress tolerance strategy, this has consequences for the plant's capacity to diminish hydrodynamics, which is important for the capacity of tidal marshes concerning coastal protection.

Because many macrophytes are clonal species, individual structural units have various functions such as nutrient uptake, reproduction or anchoring, often divided between the aboveground and belowground parts of the plants. This is the so-called division of labour (Charpentier and Stuefer, 1999). Therefore, clonal species are more tolerant to physical disturbances compared to non-clonal species (Szmeja and Galka, 2008). For instance, one single shoot at the edge of a clonal patch could encounter negative effects due to their exposure to hydrodynamics. However, the calmer conditions in the middle of the patch enhance local nutrient uptake, reproduction, growth and survival. Therefore, the whole clonal plant, with all individual shoots grouped into one patch, could benefit from division of labour among the different shoots (Bouma et al., 2005a; Charpentier and Stuefer, 1999). The responses of individual shoots to these

disturbances are generally manifested by plastic responses (Szmeja and Galka, 2008). Since marsh vegetation is exposed to a variety of location-specific conditions, these plastic responses could be essential for survival of the individual shoot and more important, for the whole clonal plant (Clevering and Hundscheid, 1998).

Consequently plant morphological adaptations are both important for the efficiency of wave attenuation by plants, and for plant establishment or survival under hydrodynamic forces; hence plant morphological traits are crucial for the ecosystem-based coastal protection value of marshes (Ysebaert et al., 2011). Previous research has focussed on current-induced hydrodynamic stresses and their effect on 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). Several studies compared species survival and morphology among water depth and differently wave-exposed marshes (Karagatzides and Hutchinson, 1991; Coops et al., 1994; Clevering and Hundscheid, 1998). However, little is known about the response of marsh vegetation (e.g. avoidance-tolerance trade-offs and tissue properties) to incoming waves within one species, neither about exposure gradients within a marsh and especially regarding an integration of wave attenuation.

1.4 Goals and hypothesis

The aim of this study is to investigate the correlation in morphological and tissue properties of marsh vegetation with differences in wave exposure. This was done in the brackish part of the Scheldt Estuary near the Dutch-Belgian border. The species under investigation was *Scirpus maritimus*, which is a clonal, dominant pioneer marsh plant along the brackish part of NW European estuaries. We investigated how *S. maritimus* shoots differ morphologically (e.g. aboveground and belowground biomass, tiller height, stem diameter) and chemically (contents of ‘strength molecules’ such as lignin, cellulose and silica) at two locations with contrasting wave exposure. Furthermore, we investigated the plant properties and environmental variables along a distance and exposure gradient through both marshes, i.e. from the marsh edge (lower elevation relative to mean sea level, highly exposed to hydrodynamics) to an increasing distance behind the marsh edge (higher elevation relative to mean sea level, less exposed to hydrodynamics due to energy dissipation by vegetation).

We test the hypotheses that:

- (i) At the end of the growing season, *Scirpus maritimus* has adapted its morphological, biochemical and biomechanical properties depending on wave exposure (varying with site and distance into the marsh), i.e. smaller and more flexible shoots when exposure is stronger;
- (ii) these adaptations develop progressively over the growing season;
- (iii) these adaptations are the result of phenotypic plasticity of this species.

2 Material and Methods

2.1 Studied species

In this study the brackish marsh plant *Scirpus maritimus* L. Palla (syn. *Bolboschoenus maritimus*) (fig. 1) is used to investigate the response of plant morphology and chemical and biomechanical tissue properties to different wave exposures. Once established, this species grows clonally by producing several underground rhizomes (with a rooting depth of approx. 20-30 cm), which then form erect aboveground shoots, which in turn have the ability to form new rhizomes. Throughout the growing season fleshy storage tubers are formed at the base of the shoots. In this way a complex network of sympodial rhizomes with increasing tuber biomass according to decreasing age is produced (Charpentier et al., 2012 and references therein). At the end of the summer this clonal expansion stops and the aboveground biomass first dries and then dies in autumn. In spring, new shoots emerge from the hibernated tubers in the soil.



Figure 1: *Scirpus maritimus* flowering

The shoots form a hard triangular stem with thin leaves (we used the height of this triangle as a proxy for stem diameter). Based on preliminary field measurements the (basal) stem diameter ranges from 5 mm to 15 mm. At peak biomass the size of a shoot can range between 0.5 m and 2 m in a natural environment. Under optimal conditions a shoot can grow up to 15 cm per day. For sexual reproduction hermaphrodite flowers are formed (fig. 1). The species prefers nutrient rich fresh to

brackish water and hence is typically found in intertidal marshes in the freshwater and brackish parts of estuaries. As pioneer species they form the dominant vegetation at the lower parts of marshes in Northwest European estuaries (e.g. Scheldt, Ems, Weser, Elbe) (Heukels and Meijden, 2005).

2.2 Study sites

Two hydrodynamically contrasting sites have been selected along the Scheldt Estuary: Groot Buitenschoor near Zandvliet (N 51°21'57.3", E 4°14'46.7", Belgium) and Rilland (N 51°23'50.8", E 4° 9'46.7", the Netherlands). Mean high water is 5.12 m TAW (with TAW being the Belgian reference level for elevation measurements) and mean low water is 0.08 m TAW. This results in a mean tidal range of around 5 m, hence forming a macrotidal system. Both sites are located in the brackish part of the Scheldt Estuary (salinity varies from 3.0 to 6.5 g Cl⁻¹ L⁻¹ over the yearly cycle with the lowest values in winter and the highest in summer (Meire et al., 2005) close to the Dutch-Belgian border, downstream of the harbour of Antwerp (fig. 2). *S. maritimus* is the dominant pioneer species. Both marshes have been expanding by clonal outgrowth of the pioneer vegetation over the adjacent bare tidal flats, locally in the order of 2 m year⁻¹, over the last decade.

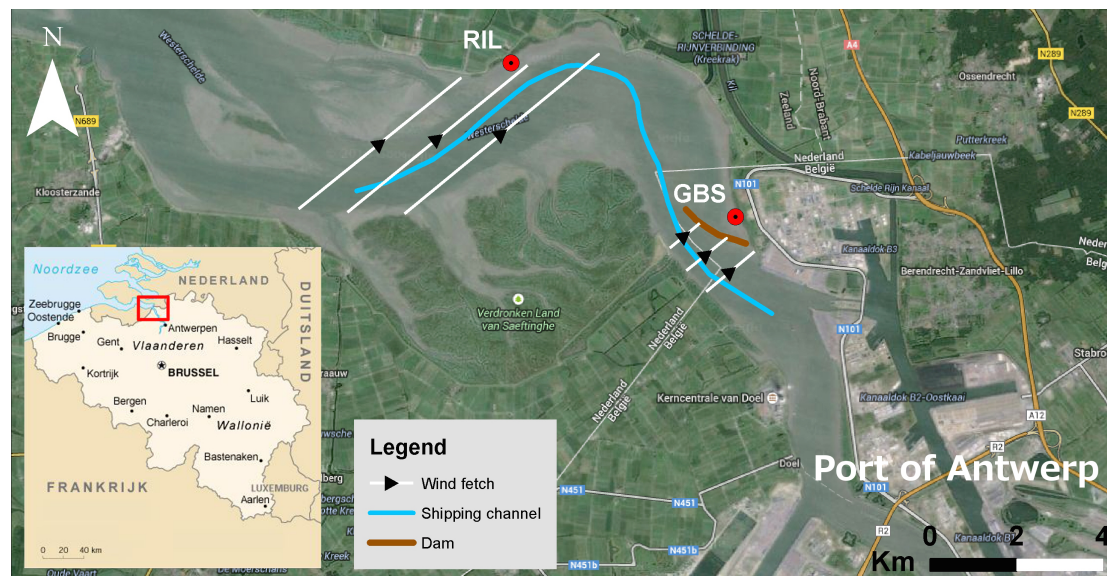


Figure 2: Study sites at the Dutch-Belgian border; RIL: Rilland, wave exposed; GBS: Groot Buitenschoor, wave sheltered. The prevailing wind direction is Southwest. To point out the differences in exposure between the two marshes the shipping channel (blue line) as well as the dam at Groot Buitenschoor (brown line) and the southwestern wind fetch (white lines with black arrows) is indicated.

Groot Buitenschoor is a wave sheltered site as wind fetch (distance of how far wind can blow over an open water surface) is limited here and ship and wind waves are blocked by a dam that lies between the marsh and the shipping channel (fig. 2). Rilland on the other hand is strongly exposed as the shipping channel lies close to the marsh (approximately 400 m) and ship waves hit the shore without attenuation. Due to its location and orientation, Rilland has also a larger wind fetch than Groot Buitenschoor (fig. 2) and therefore it is exposed to stronger wind wave activity.

As waves and water dynamics have an effect on the grainsize composition of the bottom sediment, sediment analysis (n=3) show a contrast between the two sites. Sediment at Rilland is typically dominated by sand and contains hardly any silt or clay ($2.4 \pm 1.6\%$ clay, $9.9 \pm 9.0\%$ silt, $87.8 \pm 10.5\%$ sand) while the sediment at Groot Buitenschoor contains much more silt ($11.5 \pm 4.1\%$ clay, $50.2 \pm 20.9\%$ silt, $39.3 \pm 24.9\%$ sand). Organic matter contents are consequently higher at Groot Buitenschoor than at Rilland ($5.9 \pm 2.3\%$ vs. $1.7 \pm 0.1\%$, respectively). Measurements of dry bulk density show that values for Rilland are higher than for Groot Buitenschoor ($1.48 \pm 0.02 \text{ g cm}^{-3}$ and $0.76 \pm 0.03 \text{ g cm}^{-3}$ respectively). See results for a more detailed comparison.

2.3 Experimental set-up

At both locations a transect starting at the marsh edge and reaching 12 m into the marsh was installed in March 2014 (fig. 3). Along these transects, plant development, as well as environmental parameters were monitored during the growing season of 2014, i.e. from April to September. At both locations, the marsh edge had the same elevation ($4.26 \pm 0.02 \text{ m TAW}$) at the beginning of the monitoring. The slopes within the vegetation were comparable between both sites ($2.5 \pm 0.19^\circ$). Equal elevation and similar slopes were important in order to have sites and transects of equal tidal inundation frequency, depth and duration as this has an influence on, for instance, the photosynthetic efficiency (Clevering et al., 1995). Since the marshes are close to each other, tides and tidal currents are expected to be similar as well. All these similarities are important due to their determinant role in vegetation development, so that differences in vegetation development between the two locations can be attributed as much as possible to the direct or indirect effects of wave exposure.

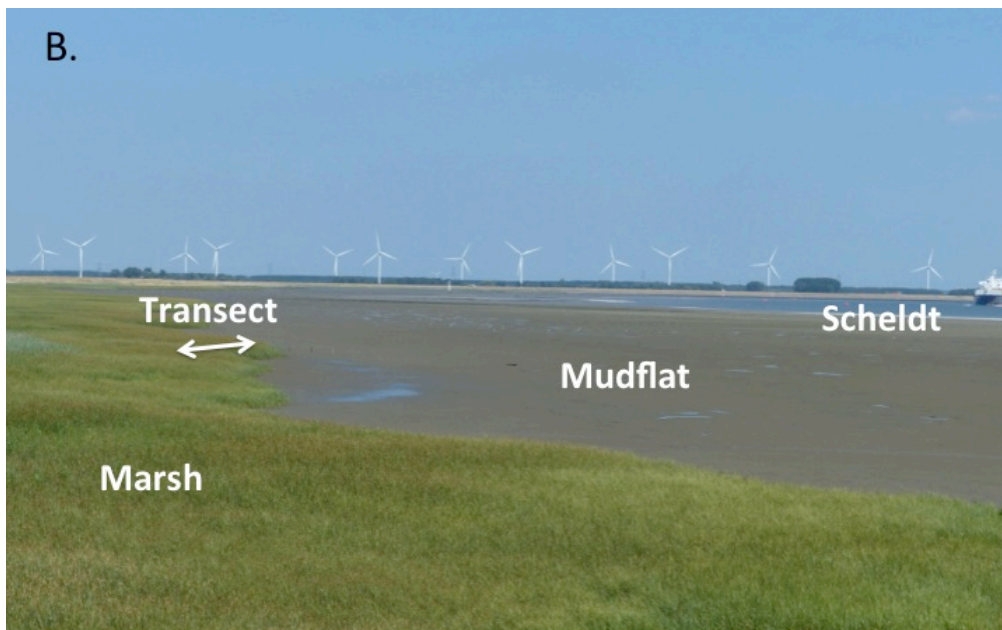
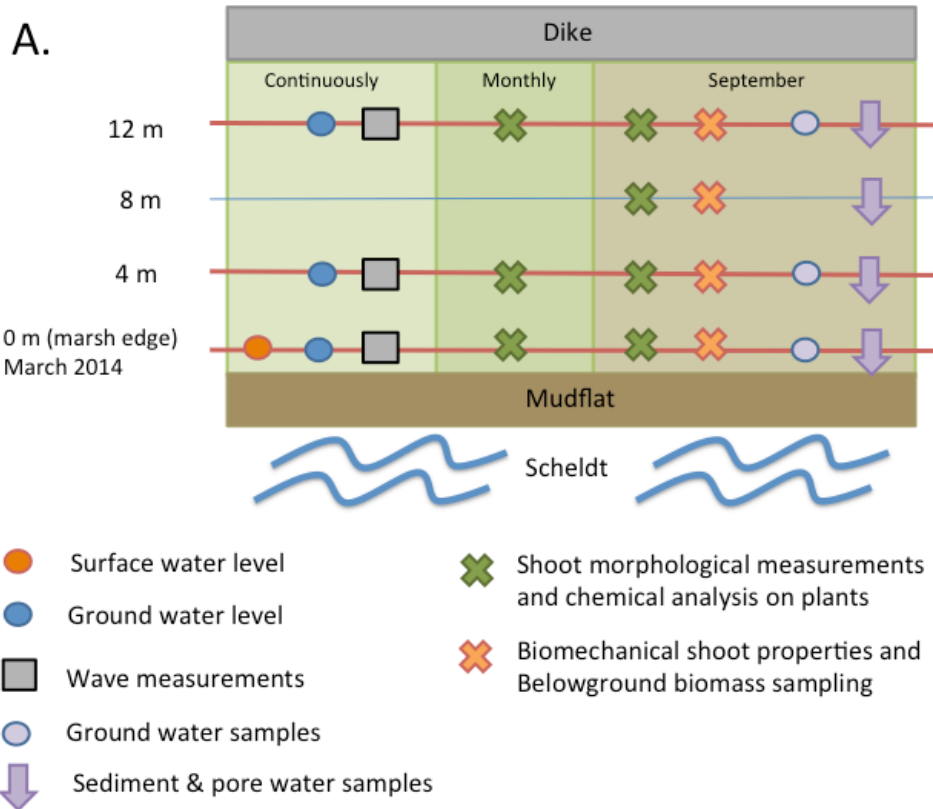


Figure 3: A. Schematic sketch of the experimental set-up. For the groundwater level and surface water level fluctuations, piezometers were installed. Shoot morphological measurements includes fresh and dry biomass, total shoot length, presence of flowers, stem diameter and vegetation density. With the chemical analysis on plants we determined silica, cellulose, lignin, N and P content (see 2.4. for more details).

B. Picture of the field situation at Rilland at low water (July 2014). The marsh is situated in front of the dike. The transect stretches over the first 12 m of the marsh. Between the main channel of the Scheldt and the marsh, a bare mudflat is formed.

Two distinct field campaigns were planned and conducted. The first one, started up in April 2014, consisted of monthly measurements of plant properties (e.g. shoot density, height, stem diameter, silica, cellulose and lignin contents) and continuous measurements of physical parameters (waves, groundwater level) (see 2.4.1. and 2.4.2. for details). These measurements were done at three different distances from the marsh edge along the established transects, i.e. at the marsh edge (0 m) as found at the beginning of the growing season in March 2014 (note: the marsh edge can further grow out during the growing season, i.e. in a range of 1 and 2 meters) and at 4 m and 12 m from that marsh edge. These distances correspond to elevations of 4.26, 4.40 and 4.56 ± 0.02 m TAW. In this way we were able to assess differences in shoot development and abiotic variables within a marsh along an elevation and exposure gradient. Furthermore this seasonal monitoring allowed us to clarify whether shoots start from a common physiological and biochemical point and then diverge depending on the conditions to which they are exposed or whether they already differ from the start of the growing season. Morphological variations among different field locations are often difficult to link with location-related conditions due to the differences in population genetics. Therefore a control experiment was conducted where shoots taken from differently exposed field locations were grown under equal controlled conditions in a greenhouse (see 2.5.).

The second field campaign was conducted at the peak of the growing season (i.e. peak biomass, September 2014) and was more detailed both spatially and in terms of monitored parameters: compared to the monthly measurements, we sampled at more plots along the transects (e.g. one plot every 4 m, i.e. 4 plots per transect instead of three plots per transect; fig. 3) and in addition to the monthly sampling, we also sampled pore water, groundwater, sediment, root biomass and measured biomechanical properties (see 2.4.3 for details). In this way a detailed set of parameters was obtained which can give more information on the observed differences and potential connections between shoot development and abiotic properties.

2.4 Performed measurements

2.4.1 Continuous measurements

- *Wave measurements*

Three wave-meters were installed alternatingly at both locations to measure one spring-neap-tidal cycle of 14 days during each month along each transect (with wave meters at the marsh edge, 4 m and 12 m into the marsh) in order to assess wave impact on the vegetation at the different distances from the marsh edge during the growing season (fig. 3). The devices were moved bi-weekly between the two locations. The monitoring was started in the second half of March 2014. Potential bi-weekly differences in for instance wind conditions and tidal water levels could be possible. Therefore we simultaneously placed one wave-meter at the marsh edge of both locations for one month at the end of the monitoring campaign in November 2014. The wave meters consist of sensors that record pressure and thereby water level above the sensor, at high frequency (16 Hz). Tom Versluys from UGent (Department of Civil Engineering, Ghent University, Belgium) who built the wave meters for us also provided a LabView stand-alone routine, which enabled us to extract wave heights and periods from the raw dataset. Hence, we assessed overall wave stress, as proxy for exposure, for the two locations as well as wave attenuation (i.e. reduction of wave height due to friction with the bottom and with the plants) along each of the transects. However, we encountered some technical problems, e.g. some wave meters were sometimes not measuring and two were broken in September 2014, which created gaps in the available dataset (appendix 1).

The extracted wave data were then processed in R (by A. Silinski) in order to obtain the significant wave heights, $H_{1/3}$ (the arithmetic mean of the highest 1/3 of waves recorded), which is usually considered as representative wave height for irregular wave fields, for each position along the transect and time period of 14 days. Relative wave heights, i.e. wave heights of the in-marsh-plots compared to the wave heights at the marsh edge, were deduced from these calculations in order to be able to compare wave propagation and wave attenuation through the two marshes. The wave attenuation rate was calculated as the reduction in wave height per meter and as the percentage height reduction per meter (Gedan et al., 2011; Möller and Spencer, 2002) for both sites over four time periods during the growing season.

- *Groundwater and tidal surface water level monitoring*

In order to gather information about groundwater level fluctuations linked to the tidal surface water cycle three piezometers (i.e. tubes in which groundwater level is measured) were installed along each transect, at the same three plots as the wave meters (marsh edge, 4 m and 12 m into the marsh) (fig. 3). The piezometers reach 2 m vertically into the ground and their lowest 1 m is perforated, in order to allow groundwater to enter the tube. The inner diameter of the tubes is 40 mm. At the initial marsh edge another piezometer with a total length of 1.5 m was installed, of which the perforated top of 0.5 m sticks out of the sediment, which allowed surface water to enter the tube for surface water level measurements. Divers (Cera and Mini-divers from Schlumberger), which are pressure sensors that measure water levels, were inserted to the bottom of the piezometers. They measured with a sampling frequency of 2 minutes. To correct for air pressure, a Baro-Diver (Schlumberger) was installed on the dike at Groot Buitenschoor. The monitoring started in April 2014 and ended in October 2014. The tidal data gathered with the surface water divers was analysed with the R package “Tides” (Cox, 2014) (executed by A. Sliniski).

2.4.2 Monthly measurements

2.4.2.1 Plant morphology

From April 2014 until September 2014, the plant evolution in the field was monitored along the transects. The shoot density (newly formed shoots) was counted on two rectangular surfaces of 40 x 40 cm for each plot. This measurement was always done in the same undisturbed squares to see differences in development over time. These counts were converted into stems per square meter. Furthermore, at each of the three levels, 30 stems were clipped close to the sediment surface and were collected. We then measured the following shoot characteristics: number of (living) leaves, length from stem base to the tip of the longest leaf of the shoot (total shoot length), stem diameter about 1 cm from the bottom of the shoot, presence of flowers, fresh weight and dry weight (biomass). Dry weight was determined after drying the shoots for 72 h at 70 °C in batches of 10. By applying the determined respective conversion factors, which indicate how much weight was lost after drying, we were able to determine individual dry weights.

2.4.2.2 Chemical analysis

The chemical analysis of ‘strength molecules’ gives information on how plants respond on wave activity by investments in rigidity or flexibility (see 2.4.3. — biomechanical properties). To prevent contamination from silica present in the sediment, the plant material was washed thoroughly. After the morphological measurements (see 2.4.2.1), the dried plant material was used for chemical analysis. Investigated properties are biogenic silica (BSi) (i.e. silica that is incorporated in an organism as amorphous silica), cellulose and lignin content together with NP contents. As a first step, the dried plant material was ground in batches of 10 shoots (i.e. 3 grouped samples per plot and time). The final grinding mesh was 1 mm (Retsch ZM2000). Then the ground material was stored in plastic tubes. In this way we ensured to have enough biomass for the different chemical analyses and we have three replicate measurements per plot and time. For silica analysis, the alkaline (0.1 M NaCO₃) extraction methodology by DeMaster (1981) was followed. Lignin and cellulose contents were quantified according to the Van Soest method (Van Soest, 1963). Furthermore NP analyses were done in order to show that differences in shoot morphology were not due to variable nutrient availability. On the one hand total NP concentrations were determined after acid (H₂SO₄) destruction with the Walinga et al. (1989) methodology. Finally, weight ratios for NP were calculated. As for the ‘strength molecules’ (silica, lignin and cellulose) 3 grouped samples per plot and time (10 shoots per sample) were used and measurements were done on the samples of April, June, August and September.

2.4.3 September campaign

- *Experimental plots*

The spatially extended field campaign was done in September 2014, when *S. maritimus* was theoretically at the peak of its growing season and had reached its peak biomass (originally planned for August 2014, however, due to logistical issues, we had to delay this field campaign a few weeks). Unfortunately due to an early spring the vegetation was already starting to deteriorate, and, as seen from the biomass measurements, the peak biomass was already passed. Compared to the monthly campaign, an extra plot was sampled (i.e. the marsh edge as it was in March 2014, 4 m, an additional 8 m plot and the 12 m plot). The shoot density was counted on three instead of two rectangular surfaces of 40 x 40 cm for each plot.

- *Belowground biomass*

Additionally to the monthly measurements more morphological properties were quantified. Root biomass was determined by taking cores of a diameter of 10 cm and of a depth of 45 cm (maximum depth of roots seen from preliminary field observations). For each level three replica cores were sampled and every core was divided into three parts (i.e. 0-15 cm depth, 15-30 cm depth, 30-45 cm depth) in order to quantify root biomass allocation in the ground. Belowground biomass was separated from the sediment by washing and sieving after which the samples were dried (72h, 70 °C) and weighed. A shoot-root ratio was calculated based on the conversion of both measurements (aboveground and belowground dry biomass) to mass per square meter.

- *Biomechanical properties*

Biomechanical properties of the 20 shoots were quantified for every plot. These 20 stems were sampled additionally to the monthly 30 stems. First morphological properties (total length, diameter, fresh weight, presence of flowers and count of leaves) were measured in order to make sure that these 20 shoots were representative for and similar to the 30 shoots based on which the chemical analyses were done. The lowest 30 cm of the stems was cut off and sent to Dr. Sara Puijalón at Université Lyon 1, CNRS, ENTPE who quantified bending and breaking strength, with a universal testing machine (Instron 5942, Canton, MA, USA).

Regarding the tensile tests, the sample was clamped into the jaws of the machine and a constant extension rate of 5 mm min⁻¹ was applied until it broke. We then calculated:

- the breaking force (N) which is defined as the maximum force that the sample can bear without suffering mechanical failure;
- the tensile strength (N m⁻²) which is calculated as the breaking force per cross-sectional area of the plant stem.

Regarding the bending tests, we performed 3-point bending tests. The plant stem is fixed at both ends where after a force is applied at a constant rate of 10 mm min⁻¹ to the midpoint of the sample. We then derived:

- the Young's modulus (E in Pa) which quantifies the sample stiffness, i.e. how

much force has to be applied to bend the stem to a defined displacement;

- the second moment of area (m^4) which quantifies the distribution of material around the axis of bending, i.e. the effect of stem morphology on its flexibility;
- the flexural stiffness ($N\ m^2$) which quantifies the stiffness of the fragment and was calculated by multiplying the obtained Young's modulus and the second moment of area.

- *Abiotic site characteristics*

Abiotic site characteristics were determined in order to obtain a more detailed site description. Both groundwater and pore water samples were taken to quantify the nutrient (NP), sulphur, chloride and dissolved silica (DSi) (i.e. water soluble silica, which is not incorporated in the organism) contents. Samples of groundwater were taken from the piezometers after clean flushing them three times to make sure that we sampled fresh groundwater. Directly in the field pH and temperature of the groundwater samples were determined after which the samples (one sample for each piezometer) were stored in cool conditions. Sediment pore water was sampled to quantify the available substances for plants. Therefore rhizons were installed into the top 10 cm layer of the soil and extraction was started 5 h after high water. For each level, 5 replicates were lumped into one sample each for lab analysis.

Concerning soil characteristics, both dry bulk density and water content were determined by using a Kopecki ring (defined volume of the top 5 cm of the sediment surface) to take samples of the surface sediment (samples were dried for 72 h by 105 °C). All these samples were taken with three replicates for all 4 levels. Furthermore sediments cores were taken and analysed for granulometry and chemical composition, and dried for loss on ignition (LOI) (dried for 6 h by 550 °C). A corer of 10 cm diameter was used to take 45 cm deep cores. As for the root samples, the sediment cores were divided into 3 depths (i.e. 0-15 cm, 15-30 cm, 30-45 cm) and stored for analysis. Granulometry was determined with a Malvern Mastersizer 2000 after a combined H_2O_2 and HCl treatment to remove organic parts and destroy compounds. Chemical soil analyses were conducted to obtain the potential and real pH, the content of phosphate, ammonium and nitrate.

2.4.4 Control experiment

As the field-based observations on differences in plant morphology between the marsh edge and the sheltered locations further into the marsh could result from genetically programmed adaptations or age effects rather than from the plasticity of one genotype (polyphenism) in the presence of different wave exposure, a control test in a greenhouse facility was set up. On March 12, in total 30 tubers were gathered from the plot 12 m into the marsh at both transects (exposed and sheltered site), i.e. 15 samples per plot and site. Additionally, on March 31, 15 newly formed ending tubers were collected from the marsh edges of both sites. The tubers were sampled in the field as soon as the shoots were visible above the sediment surface, which implies that they hardly experienced any location-dependent stress by waves or inundation times and should all be equal. The plant pieces were then transplanted into PVC pots of 24 cm height and 12 cm diameter. The sand that we transplanted the shoots into was natural sediment from the Scheldt Estuary ($d_{50}=0.32$ mm). Aside from practical reasons, this sand was used because of previous positive experiences for growing *S. maritimus*. It was mixed with Osmocote (Substral), a slow releasing (6 months) fertilizer, in order to obtain equal nutrient conditions.

The pots were randomly arranged in the greenhouse. Every day in the morning, they were watered by artificial rain (with 2.2 L m^{-2} before April 21 and 3.0 L m^{-2} after April 21 in order to compensate increasing evapotranspiration). The pots were placed in plastic boxes that function as water basins in which a water level of ± 7 cm of water was maintained in order to simulate groundwater and waterlogged conditions within the pots (the pots were open at the bottom). In this way the plants received water both from top and from below which more or less resembled natural field conditions where the plants are inundated by most of the tidal high water levels, and where soil conditions are mostly waterlogged. New small shoots were cut off to prevent differences in investments and morphology due to multiple shoots. This follow-up was done once per week. Since all conditions were the same for the different shoots, the propagation of similar morphological properties would prove the phenotypic plastic response to wave exposure (direct or indirect). On June 13, the same morphological properties as for the monthly measurements were determined for all plants (see section 2.4.2) since they had reached their peak biomass (i.e. they

started to show signs of deterioration). Plant material was grouped, dried and ground per plot (i.e. one ground sample of 15 plants per plot) to obtain BSi, lignin and cellulose as well as NP content according to the aforementioned methods (see section 2.4.2.2).

2.5 Statistics

Statistical analyses of the measured data were done with SPSS statistics 22.0 and the statistical package R (R Core Team, 2014). A two-way analysis of variance (ANOVA) was performed in order to compare measured variables between both marshes and the different distances from the marsh edge. Transformations were done when required in order to approach the assumptions (log transformation: belowground biomass, second moment of area and NP ratios; square root transformation: basal stem diameter, dry biomass, flexural stiffness, Young's modulus, breaking force and lignin content). Marsh (i.e. site) and distance from the marsh edge were the fixed factors. A t-test was performed to compare the site effect for one level at a time; e.g. significance symbols on the September and greenhouse control graphs (fig. 5, 9 and 10, 15) indicate these site effects (for interactions or distance effect, see text). The Pearson correlation coefficient was used to determine correlations between the measured variables. The significance levels used in these figures and the correlation tables are P: 0.1 > '#' > 0.05 > '*' > 0.01 > '**' > 0.001 > '***'.

3 Results

3.1 Site abiotics

- *Wave measurements*

Significant wave heights over the transects were lower at the sheltered site compared to the exposed site (table 1). The mean of the 99th percentile (i.e. the 99th percentile of the bi-weekly measurements averaged over four time periods during the growing season) (on average around 1700 waves per plot) were also lower at the sheltered site compared to the exposed site (table 1). Simultaneous wave height measurements at both marsh edges from November to December 2014 lead to significant wave heights of 3.19 cm and 5.25 cm for the sheltered and exposed site, respectively. The mean of 99th percentile (on average also around 1700 waves) was 12.05 cm and 19.41 cm, respectively. Furthermore, maximum wave heights of up to around 45 cm were recorded at the exposed site, while the highest waves at the sheltered site were of 32 cm in the simultaneous marsh edge monitoring, and of only 25 cm during the actual growing season. This shows that the wave exposure between sites differs especially for the highest wave events. When looking at absolute wave height and wave heights relative to the incoming wave heights at the marsh edge (fig. 4), there is a difference in absolute significant wave heights between sites, and also a trend of wave attenuation towards the back of the transect. It can also be seen that the shoots in the back of the marsh grow in an increasingly sheltered position over the course of the growing season. Furthermore, the exposed site shows higher wave attenuation rate during the whole season (fig. 4).

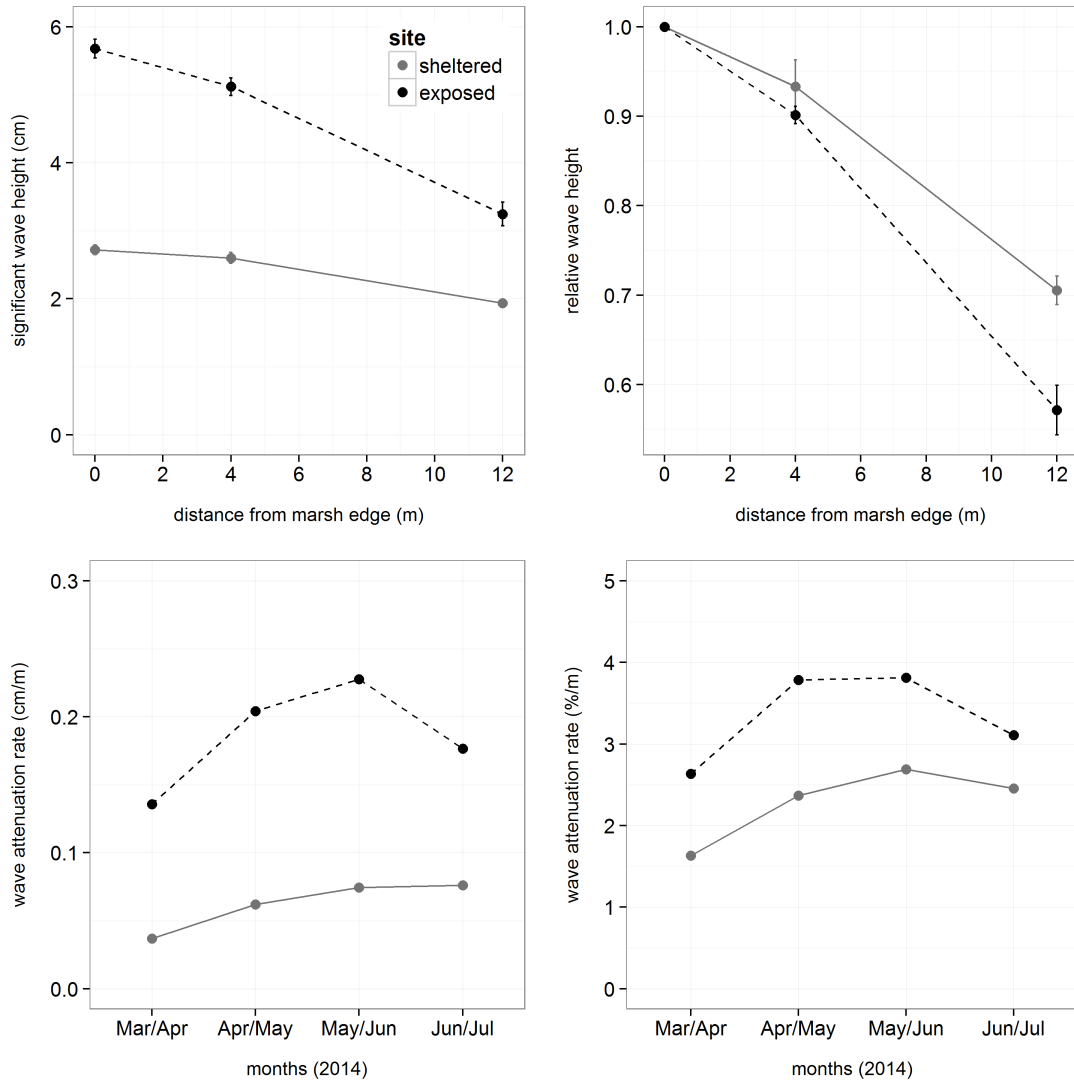


Figure 4: Significant wave height (cm) (n=4) and relative wave height (% relative to the wave height at the marsh edge) are shown for the whole season over a transect of increasing distance from the marsh edge (marsh edge, 4 m and 12 m) in both the exposed and sheltered site (above). The error bars for the average significant wave heights show the variation of four two-week periods of wave measurements (when the error bars are not visible, they are smaller than the point symbol). De seasonal changes in wave attenuation rates over the whole marsh transect are shown for both sheltered and exposed marsh (below). Left, the wave attenuation rate is in cm per meter and right, it is in percent per meter.

- *Surface water and soil water level fluctuations*

The analyses of the tidal surface water level fluctuations shows that the three plots along the transects at both sites experience similar inundation frequencies: the plots that lie 12 m into the marsh experience inundation frequencies of 97.0 % and 95.0 % at the sheltered and exposed site, respectively, meaning that over the total of 156 potential inundations over the measured period, 6 and 9 high waters did not reach the plots. The two other plots, at 4 m into the marsh and at the marsh edge itself, were

inundated at both sites with every tide. Mean high water levels over the observed period are of 4.92 m TAW (see table 1 for mean inundation depths). The most extreme high waters of the observed period (9th percentile) are around 5.48 m TAW at both sites (see table 1 for extreme water depths). Average inundation time increases from around 110 min for the highest plot to 130 min at the intermediate plot, to around 150 min at the marsh edge.

Regarding groundwater fluctuations, neither significant differences were found within a site, nor between sites.

Table 1: Significant wave heights (cm) (n= 60 000) and the mean 99th percentile (cm) (n=1700) for different distances from the marsh edge (0 m (marsh edge), 4 m and 12 m) at both, exposed and sheltered. The mean water depths (cm) and the most extreme water depths (9th percentile) (cm) for 0 m (marsh edge), 4 m and 12 m from the marsh edge, were similar in both sites.

Distance (m)	Sign. wave heights (cm)		Mean of 99th percentile (cm)		Mean water depth (cm)	Extreme water depths (cm)
	Exposed	Sheltered	Exposed	Sheltered		
0	5.67	2.80	20.26	9.67	66	122
4	5.22	2.69	18.02	9.21	54	120
12	3.49	2.10	12.57	6.86	32	88

- *Pore and groundwater composition*

Pore water and groundwater quality measurements (table 2) were done at the end of the growing season in order to describe the chemical growth conditions of the plants at both sites. From table 2 we see that no exceptional differences or trends are found, except for DSi, which is up to five times higher at the sheltered site ($t(6) = -8.38$; $P < 0.0001$). Overall the concentrations in groundwater are higher compared to the concentrations in the pore water. Nitrate and nitrite concentrations were below detection limit ($<0.01 \text{ mg L}^{-1}$).

Table 2: Ammonium (NH₄⁺-N), Phosphate (PO₄³⁻-P), Sulphate (SO₄²⁻), Chloride (Cl⁻) and Dissolved Silica (DSi) concentrations (mg L⁻¹) of pore water and groundwater for different distances from the marsh edge (0 m (marsh edge), 4 m, (8 m) and 12 m) at both, exposed and sheltered, marshes in September 2014 (n=1).

	Site	Distance (m)	NH ₄ ⁺ -N mg L ⁻¹	PO ₄ ³⁻ -P mg L ⁻¹	SO ₄ ²⁻ mg L ⁻¹	Cl ⁻ mg L ⁻¹	DSi mg L ⁻¹
Pore water	Exposed	0	1.29	0.95	828.5	5825.0	6.26
		4	0.24	0.50	803.9	5808.6	4.84
		8	0.45	0.66	757.8	5806.7	7.57
		12	0.59	1.34	715.7	5804.9	8.59
	Sheltered	0	0.26	1.71	782.6	5767.0	18.22
		4	0.22	3.57	546.9	5496.8	25.35
		8	0.16	0.04	540.3	4724.2	20.47
		12	0.77	0.03	548.6	4862.0	20.50
Groundwater	Exposed	0	1.38	1.63	820.4	6765.1	29.05
		4	5.39	3.64	630.2	7761.2	27.09
		12	4.33	2.52	879.6	8426.8	25.83
	Sheltered	0	0.64	6.09	888.9	6917.4	35.50
		4	0.28	6.24	903.8	6241.2	27.78
		8	0.45	10.32	567.6	6673.7	33.10
		12	0.45	10.32	567.6	6673.7	33.10

- *Soil measurements*

Soil sediment characteristics determined at the end of the growing season show that the exposed site has a larger median grainsize, higher bulk density, less relative water content and less organic matter contents compared to the sheltered site (table 3). Chemical soil analysis shows a lower actual pH at the sheltered site compared to the exposed site (8.2 ± 0.25 and 8.76 ± 0.16 respectively). The same pattern is found for the potential pH, although with slightly lower values (7.65 ± 0.20 and 8.30 ± 0.20 respectively). These are still close to the optimum pH of *S. maritimus*, i.e. 8.3 (Hroudová et al., 2014). Furthermore, nutrient content in the soil material does not differ a lot in depth or distance from the marsh edge. However, soil nutrient composition at the sheltered site has higher total N and P compared to the exposed site (average values: 1.276 ± 0.556 mg N g⁻¹ vs. 0.342 ± 0.209 mg N g⁻¹ and 1.024 ± 0.356 mg P g⁻¹ vs. 0.283 ± 0.057 mg P g⁻¹ respectively for sheltered and exposed site).

Table 3: Median grainsize (d_{50} , in μm), dry bulk density (dry DB, in g cm^{-3}), water content (%) and organic matter content measured by loss on ignition (LOI, in %), of soil samples for different distances from the marsh edge (0 m (marsh edge), 4 m, 8 m and 12 m) at both, exposed and sheltered, marshes in September 2014. Mean values with standard deviations are shown ($n=3$).

Marsh	Distance	d_{50} μm	Dry BD g cm^{-3}	Water content %	LOI %
Exposed	0	121 ± 21	1.47 ± 0.04	0.248 ± 0.005	0.017 ± 0.010
	4	122 ± 25	1.50 ± 0.02	0.239 ± 0.002	0.017 ± 0.008
	8	135 ± 7	1.49 ± 0.02	0.235 ± 0.003	0.012 ± 0.004
	12	116 ± 25	1.46 ± 0.02	0.235 ± 0.006	0.021 ± 0.014
Sheltered	0	87 ± 37	0.76 ± 0.02	0.502 ± 0.004	0.037 ± 0.017
	4	63 ± 49	0.75 ± 0.02	0.468 ± 0.020	0.058 ± 0.031
	8	23 ± 8	0.82 ± 0.02	0.501 ± 0.004	0.064 ± 0.025
	12	32 ± 19	0.72 ± 0.02	0.496 ± 0.003	0.079 ± 0.020

3.2 Plant responses to wave exposure

3.2.1 Plant morphology

In order to assess our hypothesis that there are differences in plant properties between sites with different wave exposure, we look for differences between two sites with contrasting wave exposure (sheltered and exposed). The results show the plant properties at the end of the growing season when plants are fully developed. Next, we look if these differences are also found over increasing distance from the marsh edge (i.e. marsh edge, 4 m, 8 m and 12 m from the marsh edge).

Looking at fig. 5, we see that plant morphology differs between wave exposure conditions and within the sites (i.e. in terms of the distance from the marsh edge, which also corresponds to a difference in wave exposure, see fig. 4). Shoot length is smaller at the exposed site and at the marsh edge (fig. 5: site \times distance interaction ANOVA $F_{4,290} = 24.838$; $P < 0.0001$). The basal stem diameter shows an interaction between site and distance (fig. 5: site \times distance interaction ANOVA $F_{4,290} = 18.347$; $P < 0.0001$). Diameters are bigger at the marsh edge and become smaller with increasing distance from the marsh edge. However, this effect is more pronounced at

the exposed site, where, compared to the sheltered site, diameters are bigger close to the marsh edge (fig. 5: $t(58) = 4.804$; $P < 0.0001$). The densities of individual shoots show an interaction between site and distance (fig. 5: site \times distance interaction ANOVA $F_{4,44} = 2.963$; $P = 0.045$). At the marsh edge, shoot densities are low and similar at both sites (fig. 5: $t(4) = 1.118$; $P > 0.05$). Deeper into the marsh, the shoot density increases rapidly until a constant density at both sites is reached. However, this increase is bigger at the exposed site where densities can be almost twice as high (fig. 5). Dry biomass per individual shoot also shows an interaction between sites and distance (fig. 5: site \times distance interaction ANOVA $F_{4,290} = 10.924$; $P < 0.0001$). However, at the marsh edge there is no difference between sites (fig. 5: $t(58) = 4.98$; $P > 0.05$). At the exposed site there is no change when going deeper into the marsh (fig. 5: exposed, distance ANOVA $F_{3,116} = 2.466$; $P > 0.05$). At the sheltered site, the biomass increases towards the back of the marsh, where the weight of shoots is almost twice as high as at the exposed site. In contrast, regarding dry biomass per m^2 (i.e. dry biomass per individual shoot multiplied with the number of shoots per m^2), the biomass also increases towards the back of the marsh at the exposed site. Therefore sites and distances differed, but no interaction was found (fig. 5: site ANOVA $F_{1,235} = 18.501$; $P < 0.0001$).

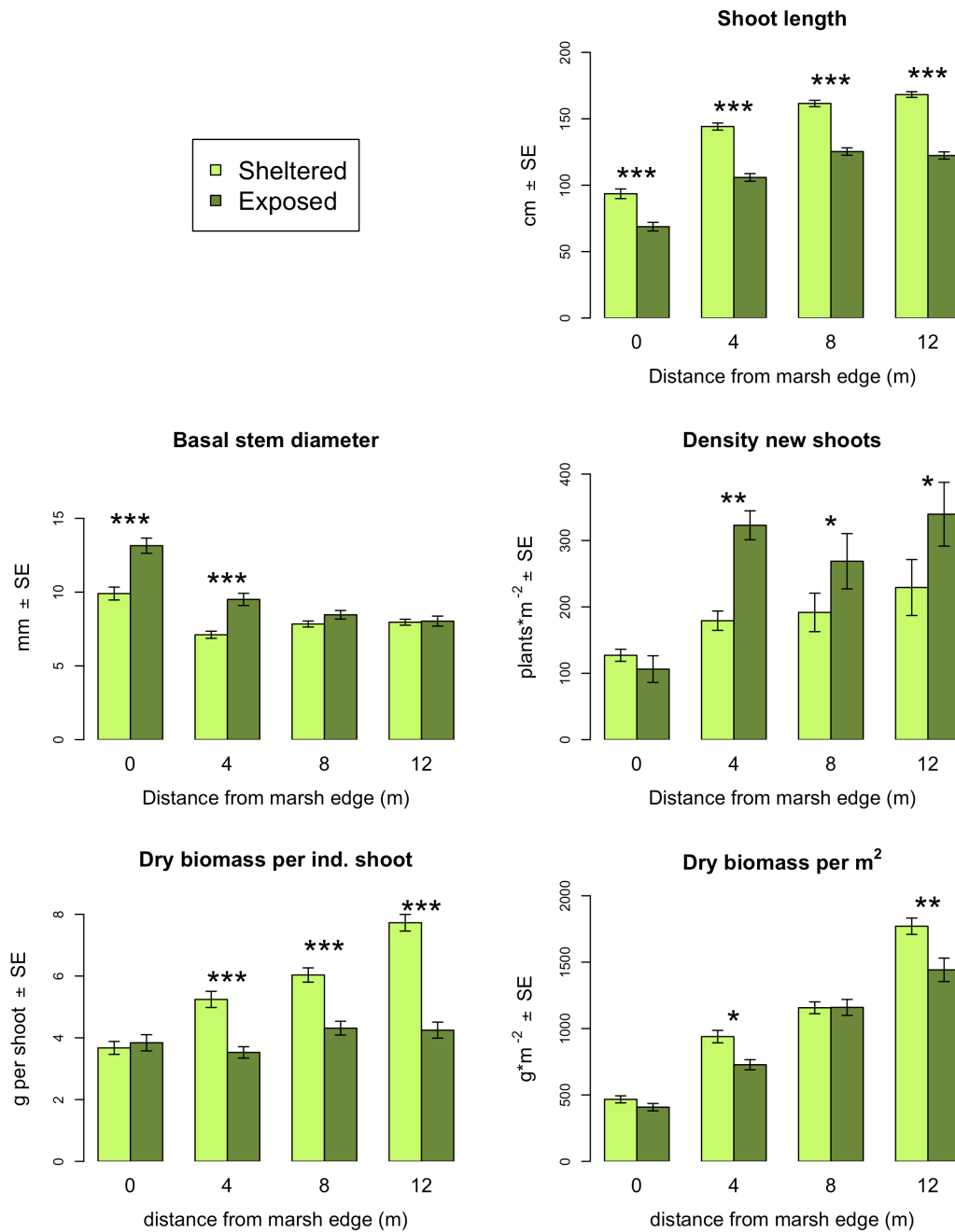


Figure 5: Spatial patterns over distance from the marsh edge of morphological properties at the end of the growing season compared between marshes with contrasting exposure. Total shoot length (cm), Basal stem diameter (mm), shoot density (shoots m^{-2}), dry biomass per individual shoot (g) and dry biomass per m^2 ($g\ m^{-2}$) are shown ($n=30$ except for density $n=3$). The symbols indicate the significant site differences between the two marshes per distance to the marsh edge (see text for distance effects and interaction effects). Significance levels for P: $0.1 > \# > 0.05 > * > 0.01 > *** > 0.001 > ****$.

Regarding total belowground biomasses, there are no differences between sites ($t(64) = 0.964$; $P > 0.05$). Total belowground biomass significantly increases with increasing distance from the marsh edge ($F_{3,61} = 13.235$; $P < 0.0001$). The distribution of biomass over depth differs between sites and distance, although in the back of the

marsh belowground biomass allocation is similar in both sites (i.e. difference for 0 m (marsh edge), 4 m and 8 m from the marsh edge; fig. 6: site \times depth interaction ANOVA $F_{2,42} = 5.026$; $P < 0.05$). Plants have more deep roots at the exposed site (absolute values: fig. 6; relative to total biomass fig. 7).

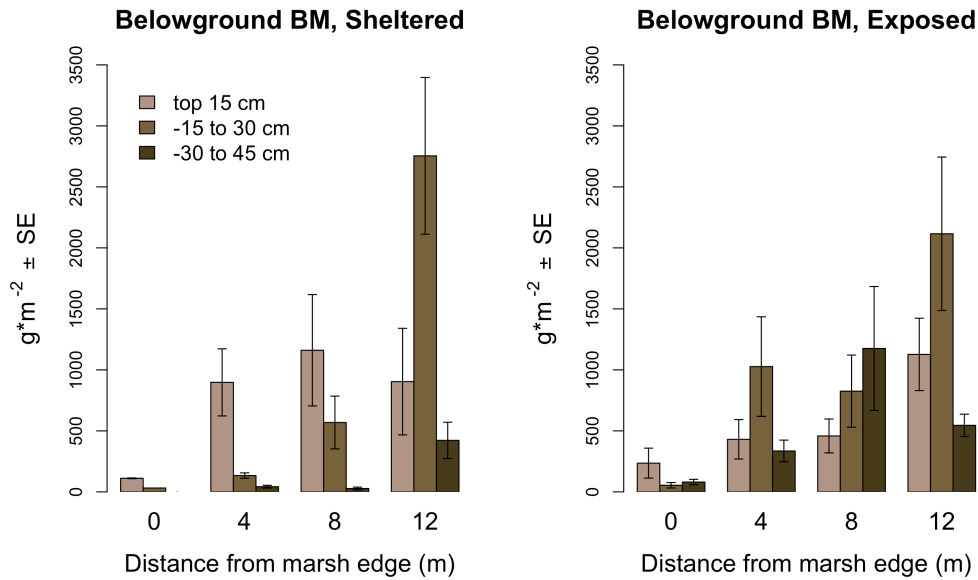


Figure 6: Spatial patterns in belowground biomass at the end of the growing season compared between marshes with contrasting wave exposure. Root biomass (g) is shown per depth and distance from the marsh edge (n=3).

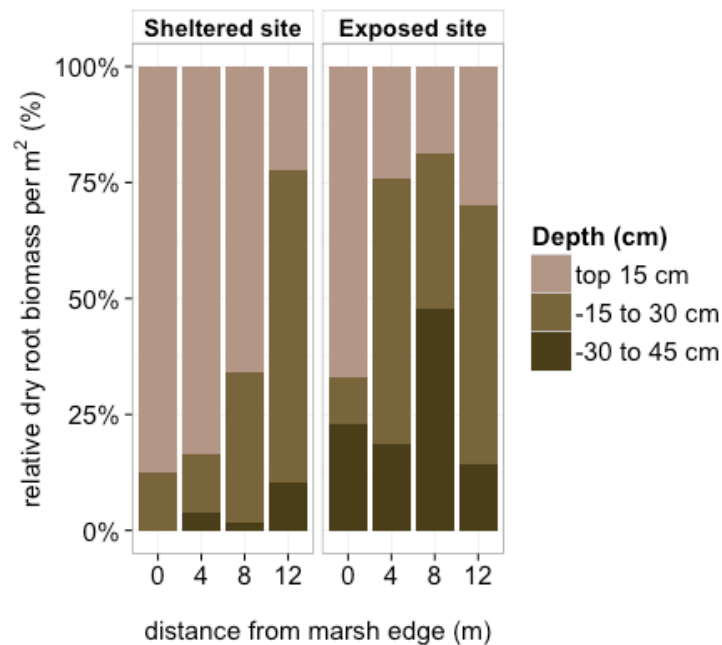


Figure 7: Distribution of belowground biomass over depth relative to the total belowground biomass compared between marshes with contrasting wave exposure as well as between distances from the marsh edge (i.e. marsh edge, 4 m, 8 m and 12 m) (n=3).

Shoot-root ratios show that there is more biomass allocation towards the belowground parts in the back of the marsh (e.g. for the exposed site: a decrease from 4.63 ± 1.18 at the marsh edge towards 1.52 ± 0.30 at the 12 m from the marsh edge). However, shoot biomass remains bigger than root biomass. A site effect (lower ratio at exposed site) seen close to the marsh edge diminishes deeper in the marsh until both sites reach similar ratios (fig. 8).

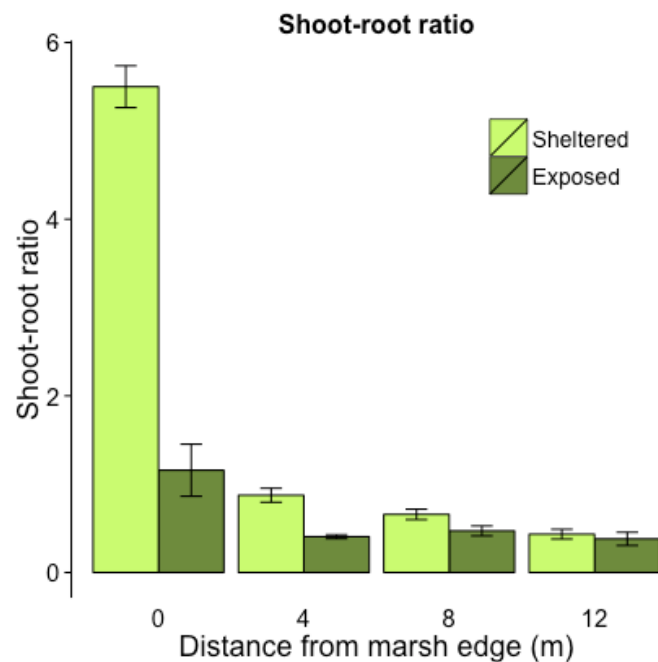


Figure 8: Spatial patterns over distance from the marsh edge in shoot-root ratios at the end of the growing season compared between marshes with contrasting exposure (n=3).

3.2.2 Chemical composition

Lignin contents of shoots are similar between sites although there is a significant difference between sites at the marsh edge (fig. 9: site \times distance interaction ANOVA $F_{4,16} = 3.833$; $P = 0.03$). The BSi contents differ between sites and between distances (fig. 9: site \times distance interaction ANOVA $F_{4,16} = 17.303$; $P < 0.0001$). Concentrations are up to three times higher at the sheltered site and they increase towards the back of the marshes. Cellulose contents, however, do not differ between sites (fig. 9: site ANOVA $F_{1,19} = 1.662$; $P > 0.05$). Over distance there is a difference, although it is small (fig. 9: distance ANOVA $F_{3,19} = 24.206$; $P < 0.0001$). Looking at the NP (weight) ratios, we found that nutrient uptake does not differ between sites (fig. 9: site ANOVA $F_{1,14} = 0.391$; $P > 0.05$).

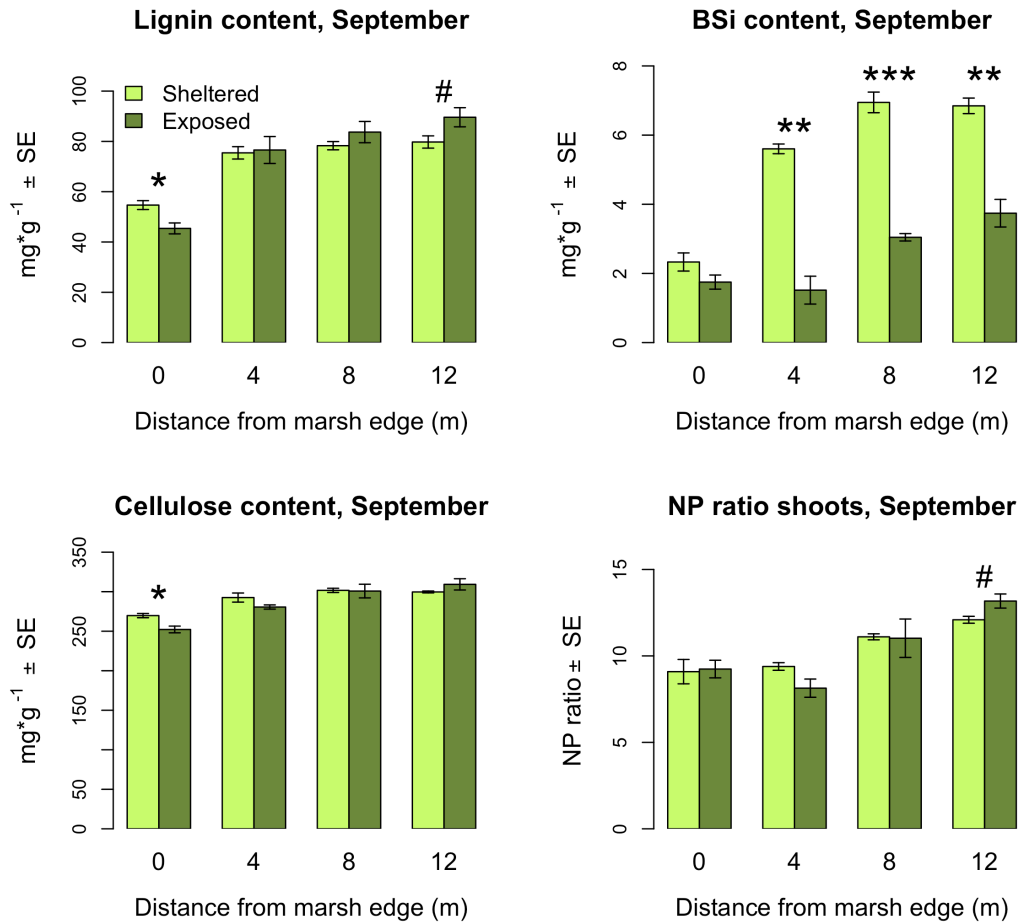


Figure 9: Spatial gradient over distance from the marsh edge in chemical tissue composition and shoot NP (weight) ratios at the end of the growing season compared between marshes with contrasting wave exposure (n=3). The symbols indicate the significant site differences between the two marshes per distance to the marsh edge (see text for distance effects and interaction effects). Significance levels for P: 0.1 > ‘#’ > 0.05 > ‘*’ > 0.01 > ‘**’ > 0.001 > ‘***’.

3.2.3 Biomechanical tissue properties

Biomechanical properties of macrophyte shoots form an important measure in order to resist hydrodynamic forces such as wave exposure. Our results indicate a differentiation of these properties between sites and distances from the marsh edge. Measurements on bending properties (flexural stiffness, Young’s modulus and second moment of area) show that shoots at the sheltered site are stiffer (fig. 10). Also with increasing distances from the marsh edge, shoots are stiffer (fig. 10: site × distance interaction ANOVA $P < 0.0001$). The marsh edge shows similarities between the sites, although not consistent for all bending properties. Breaking properties (breaking force and tensile strength) show similar results of stronger plant material at the sheltered site and in the back of the marsh (fig. 10: site × distance interaction ANOVA $P < 0.0001$).

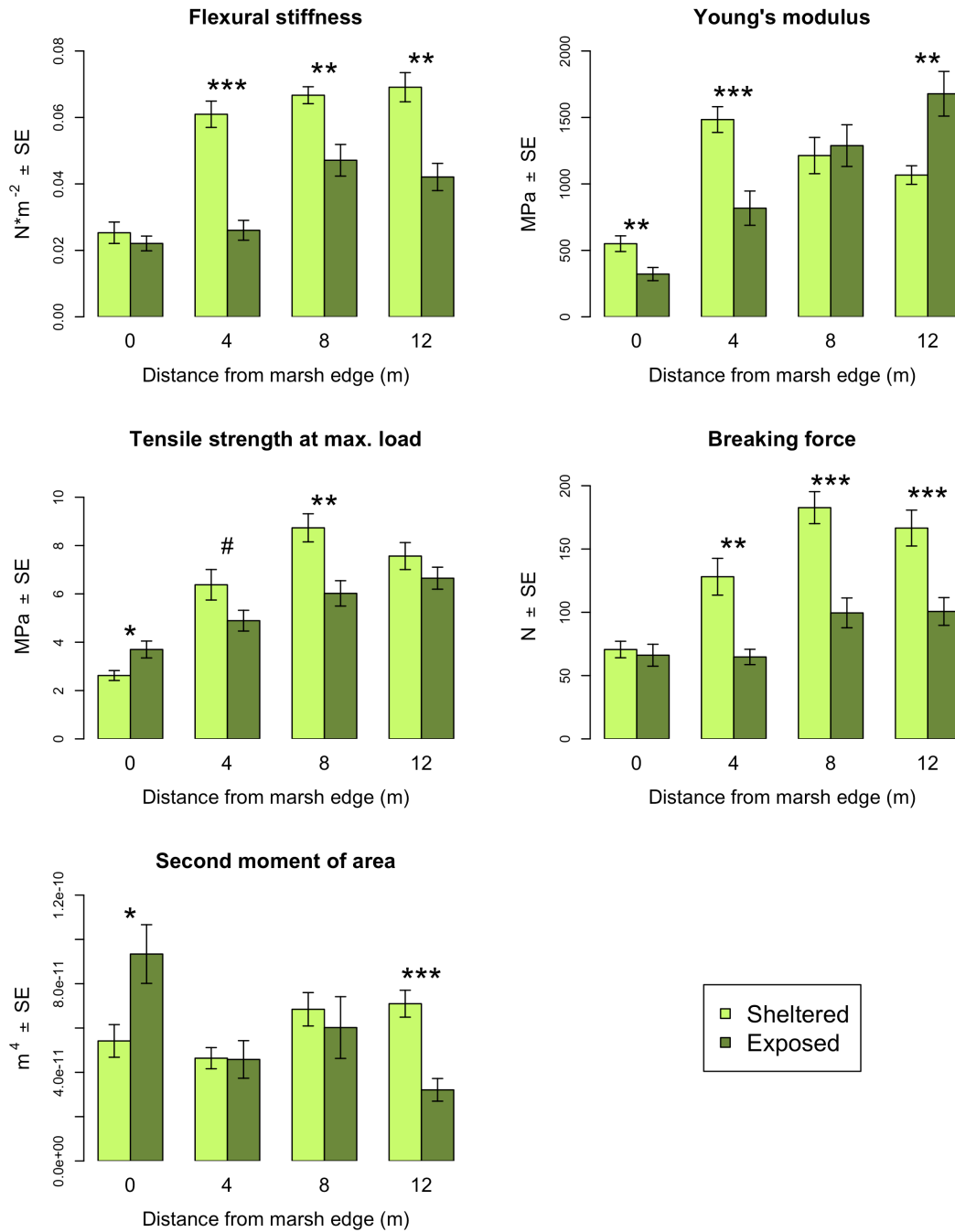


Figure 10: Spatial patterns in biomechanical properties at the end of the growing season compared between marshes with contrasting exposure. Flexural stiffness (N m⁻²), Young's modulus (MPa) and second moment of area (m⁴) are a proxy of bending strength whereas tensile strength at max. load (MPa) and breaking force (N) are a proxy of tensile strength (n=20). The symbols indicate the significant site differences between the two marshes per distance to the marsh edge (see text for distance effects and interaction effects). Significance levels for P: 0.1 > '#' > 0.05 > '*' > 0.01 > '**' > 0.001 > '***'.

3.2.4 Correlations

Pearson's correlation tests were done in order to explore the relationships between wave activity and soil characteristics and shoot BSi content (table 4A).

In addition the same test was done to determine the correlation of wave activity with the different plant properties (table 4B). Except for stem diameter, only negative correlations were found for wave activity. Positive correlations were found between length and biomechanical properties as well as with 'strength molecules'. Stem diameter was negatively correlated with shoot morphological parameters, biomechanical and 'strength molecules'. Furthermore, the biomechanical properties are naturally correlated to each other. A strong positive correlation was found between flexural stiffness and BSi. The second moment of area was not significantly correlated with 'strength molecules', nor with morphological properties (values not shown). Overall, biomechanical properties are stronger correlated to biochemical properties compared to the weaker correlations with morphological properties. Only six data point were used to correlate with wave activity. Therefore, the obtained correlations and significances should be considered carefully.

Table 4: 4A. Correlation matrix for wave activity (i.e. mean 99th percentile of the waves, W) and the abiotic variables, i.e. dissolved silica (DSi), median grainsize (GS), dry bulk density (BD), organic matter content (LOI) and water content (WC). 4B. Correlation matrix for wave activity (i.e. mean 99th percentile of the waves, W) and the most relevant plant properties, i.e. morphological properties: length (L), basal stem diameter (D) and dry biomass per m² (B); Biomechanical properties: flexural stiffness (F), Young's modulus (Y), tensile strength (T) and breaking force (BF); biochemical properties: cellulose (Cell), lignin (Lig) and BSi. Pearson's correlation coefficients are shown with indications of significance level (significance levels: 'ns' = not significant and P: 0.1 > '#' > 0.05 > '*' > 0.01 > '**' > 0.001 < '***'). The correlations of BD with WC, F with Y and T with BF are not relevant since BD is used to calculate WC; Y and BF were used to calculate F and T respectively (indicated with '/').

4A.	W	DSi	GS	BD	LOI	WC
DSi	-0.87*					
GS	0.86*	-0.86***				
BD	0.87*	-0.96***	0.88**			
LOI	-0.98***	0.89**	-0.97***	-0.90**		
WC	-0.86*	0.96***	-0.87***	/	0.89**	
BSi	-0.81*	0.78*	-0.89**	ns	0.89**	ns

4B.	W	L	D	B	F	Y	T	BF	Cell	Lig
L	-0.79 [#]									
D	0.76 [#]	-0.57***								
B	ns	0.85***	-0.45***							
F	-0.76 [#]	0.64***	-0.35***	0.51***						
Y	ns	0.47***	-0.35***	0.48***	/					
T	ns	0.49***	-0.31***	0.43***	0.56***	0.37***				
BF	-0.77 [#]	0.47***	-0.33***	0.32***	0.58***	0.26**	/			
Cell	ns	0.86***	-0.86***	0.89***	0.69***	0.83***	0.65***	0.50*		
Lig	ns	0.81***	-0.91***	0.86***	0.55***	0.78***	0.57***	ns	0.93***	
BSi	-0.81*	0.88***	-0.70***	0.74***	0.90***	0.57***	0.74***	0.58***	0.64***	0.59***

3.3 Seasonal development

3.3.1 Plant morphology

In the beginning of the growing season shoot properties are still similar. In this section we show how they develop further over the season. As shown in section 3.2.1, shoots are overall taller by the end of the growing season at the sheltered site. During development, the elongation of marsh edge shoots levels off half way in the growing season (June). Further into the marsh, elongation continues longer and only levels off at the end of the growing season in August-September. However at the exposed site, the elongation starts to level off earlier around June-July (fig. 11). In the back of the marsh, basal stem diameters do not differ over the season, nor between sites. However at the marsh edge, stems are thicker. Here the basal stem diameter increases until the maximum thickness is reached around June-July. At the sheltered site, the higher thickness in June is probably due to the limited amount of shoots at the marsh edge, hence limiting the possibilities of fully random sampling. Shoots at the exposed site produced more flowers at intermediate distance from the marsh edge with a peak around August (83% of all shoots were flowering at 4 m from the marsh edge; only 30% at the sheltered site). Also flowering shoots had less leaves compared to non-flowering shoots (data not shown).

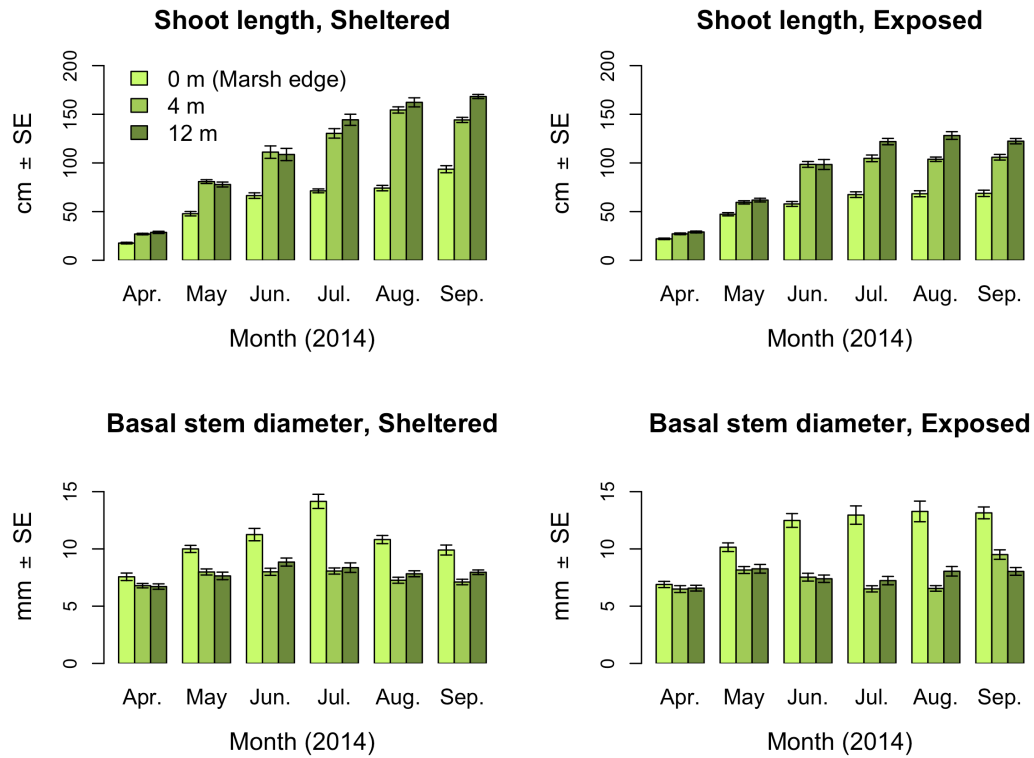


Figure 11: Plant development during the growing season (April-September) at both the sheltered and the exposed marsh in terms of total shoot length (cm) and basal stem diameter (mm) (n=30).

Individual shoots start their growing season with a similar weight at all sites and distances (two-way ANOVA site \times distance interaction $F_{2,174} = 0.186$; $P > 0.05$). Increase in individual shoot biomass at the sheltered site levels off at the marsh edge in June-July. Deeper in the marsh, the increase of individual shoot biomass continues longer and levels off later (fig. 12). At the exposed site all distances from the marsh edge start to level off in June-July. As a result, overall dry biomass per individual shoot becomes higher at the sheltered site, except for the marsh edge, which has similar weights for both sites (see section 3.2.1). Dry biomass per area shows the effect of the numbers of shoots (shoot-density). Densities at the marsh edge are similar for both sites and so is the individual shoot biomass. Therefore at the marsh edge, dry biomass per m^2 is the same in both sites. However deeper in the marsh, densities at the exposed site are higher, but constant. This compensates the lower individual shoot biomass and thus the dry biomass per m^2 is similar to the sheltered site. Although at the end of the growing season, when the increase in density levels off, the sheltered site exceeds the dry biomass per area of the exposed site.

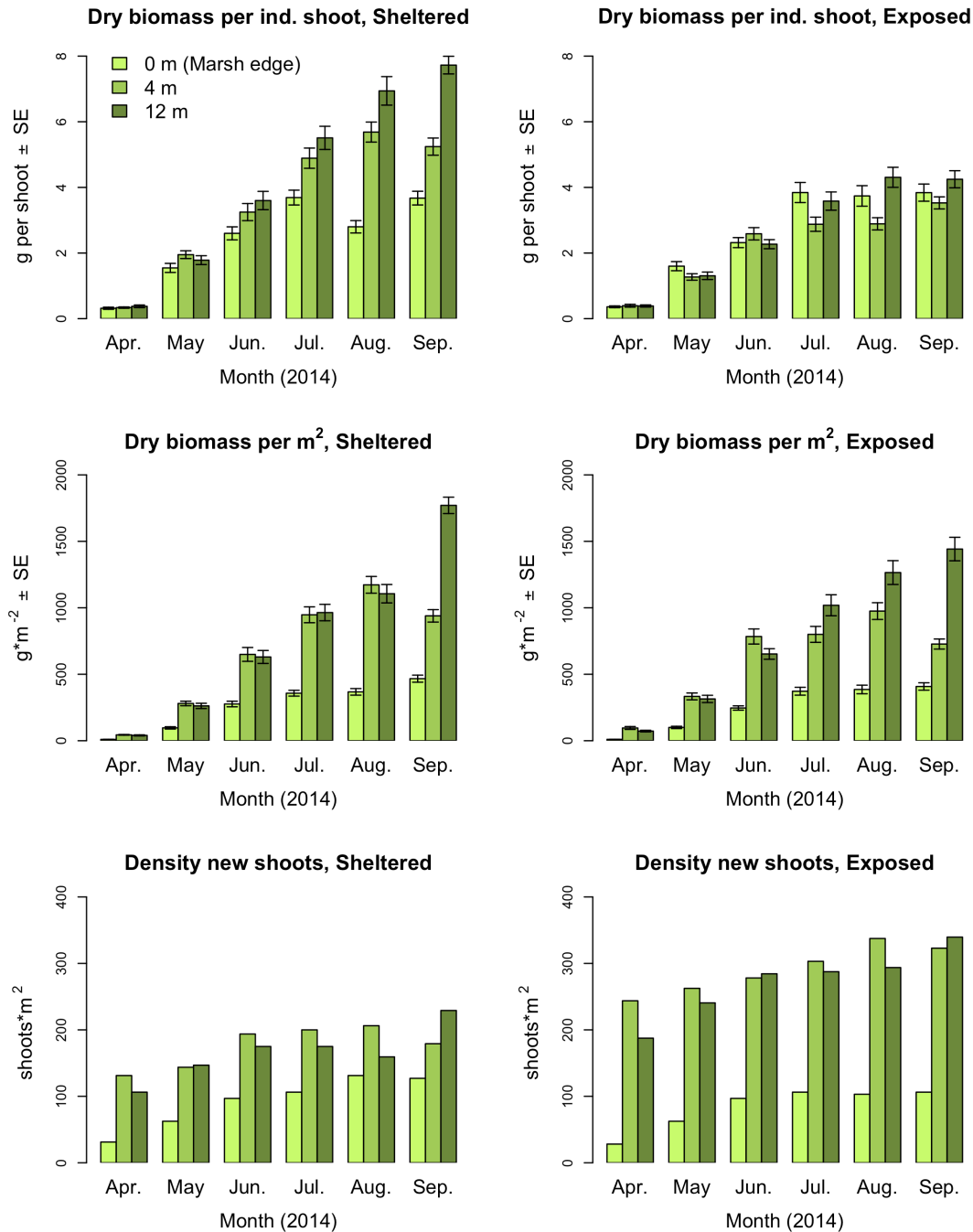


Figure 12: Plant development during the growing season (April-September) at both the sheltered and the exposed site in terms of dry aboveground biomass ($n=30$) (g per shoot and $g \cdot m^{-2}$) and shoot density ($n=2$) ($shoots \cdot m^{-2}$).

3.3.2 Biochemical stem composition

Chemical ‘strength molecules’ and nutrient contents of the plants were determined for the sampled stems of April, June, August and September in pooled samples of 10 stems each (i.e. 3 pooled replicates per plot and time). Cellulose contents increase a little bit from April to June but level off afterwards. There are no biologically relevant differences between sites or distances. Starting from similar concentrations, the lignin

contents increase over time. The same pattern is seen at both sites; the marsh edge shoots have lower lignin contents compared to the shoots deeper in the marsh. At the sheltered site, BSi contents at the back of the marsh increase over time. At the marsh edge, BSi contents level off in June. In contrast to the sheltered site, the BSi contents at the exposed site do not increase a lot over the season. After a small increase at the beginning of the season, BSi contents in all plants level off (fig. 13).

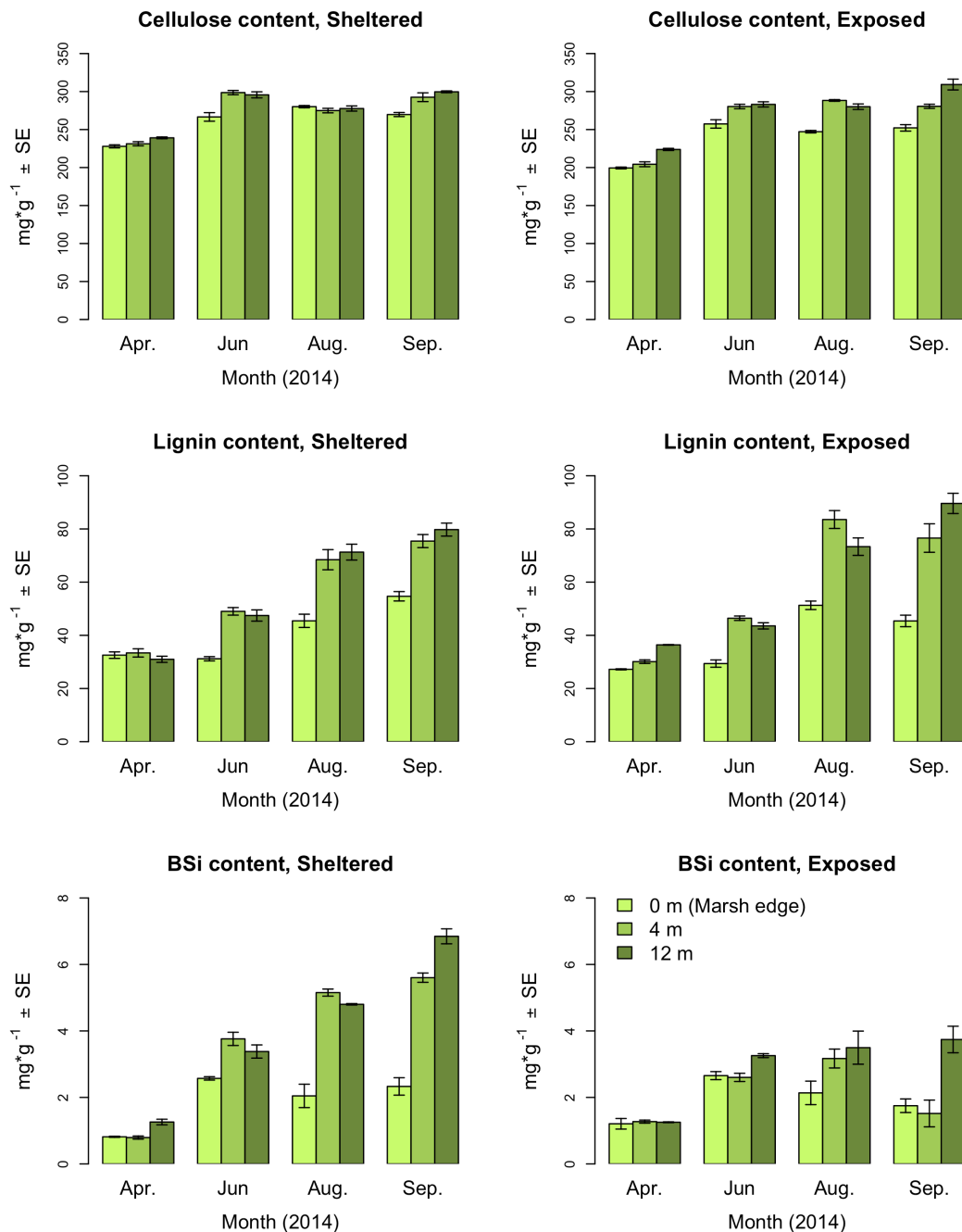


Figure 13: Development during the growing season (April-September) at both the sheltered and the exposed site in terms of chemical tissue composition (n=3).

Nutrient composition of the plant tissue shows similar seasonal patterns at both sites for the NP (weight) ratios (fig. 14). The ratio increases towards the end of the growing season.

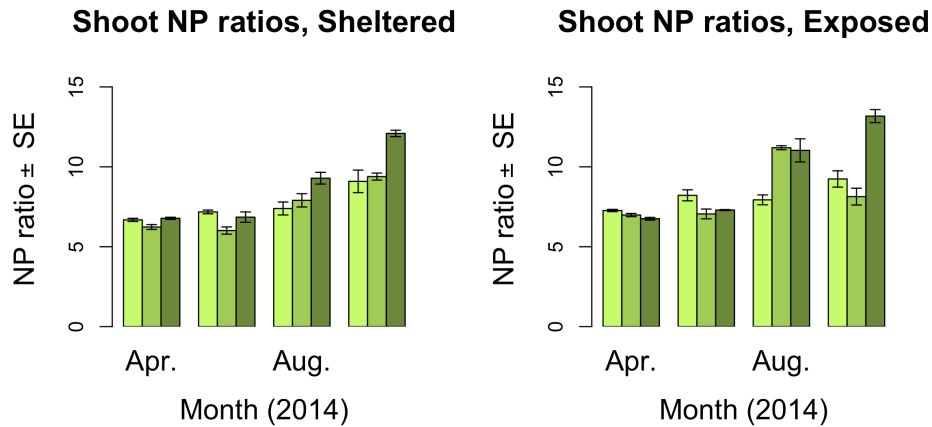


Figure 14: Development of NP (weight) ratios during the growing season (April-September 2014) at both the sheltered and the exposed site (n=3).

3.3.3 Greenhouse control

The greenhouse experiment shows hardly any differences between sites ($P > 0.05$ for all measurements, except for dry weight, which showed an interaction between site and distance) (fig. 15). Although there is a difference in shoot length of the old 12 m tubers between sites ($t(26) = -2.248$; $P = 0.033$), this difference is much smaller compared to the differences in the field. Between the new marsh edge tubers and the old 12 m tubers there is a difference for plant length where old 12 m tubers are bigger (distance ANOVA $F_{1,55} = 14.109$; $P < 0.0001$). An interaction between site and distance was found for dry biomass per individual shoot (site \times distance interaction ANOVA $F_{53,1} = 7.531$; $P < 0.05$). The shoots from the old 12 m tubers have a higher dry biomass at the sheltered site ($P < 0.0001$). At the exposed site there is no significant difference. Furthermore the amount of ‘strength molecules’ and NP contents differs much less compared to the field (appendix 2). However, no statistical tests were done due to the lack of replica samples.

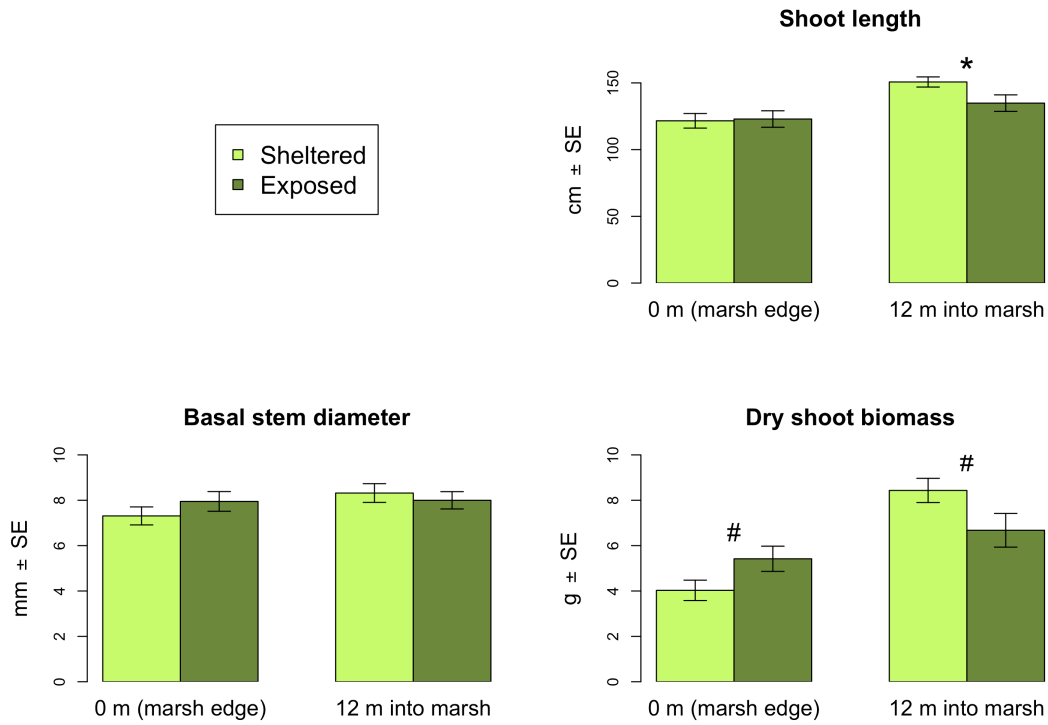


Figure 15: Results of greenhouse experiment of plants with different ages and origin grown under equal controlled conditions. The plant morphological traits shown are shoot length (cm), basal stem diameter (mm) and dry biomass per individual shoot (g) (n=15). Shoots at 0 m (marsh edge) are grown from newly formed tubers and shoots from 12 m into the marsh were grown from tubers, which were already formed in former years. The symbols indicate the significant site differences between the two marshes per distance to the marsh edge (see text for distance effects and interaction effects). Significance levels for P: 0.1 > ‘#’ > 0.05 > ‘*’ > 0.01 > ‘**’ > 0.001 > ‘***’.

4 Discussion

Wave attenuation by marsh vegetation is well studied, and the role of marsh vegetation for sustainable coastal protection against waves and wave-induced erosion is well recognised in high impact journal articles (e.g. Möller et al., 2014; Temmerman et al., 2013). It is known that plant morphological properties, such as aboveground biomass, stem density and stem stiffness, are key determinants of the plants' attenuating efficiency (Bouma et al., 2013; Paul et al., 2012; Penning et al., 2009; Peralta et al., 2008; Rupprecht et al., 2015; Ysebaert et al., 2011). However, little is known about the opposite relationship, i.e. morphological responses of marsh vegetation to wave exposure. In this study we focus on the feedback response of tidal marsh plants to wave exposure within one species in terms of plant morphological, biochemical and biomechanical properties. Summarizing our main findings, the differences of abiotic variables between sites can be attributed to the contrasting wave exposure (table 4A). We found that DSi availability could be an indirect variable that might play an important role in determining the shoot properties. The phenotypic plasticity of *S. maritimus*, confirmed by the greenhouse control experiment, shows that the responses in plant morphological traits result from direct and/or indirect wave exposure. The plant properties of *S. maritimus* showed differences linked to exposure as seen both between sites as well as within sites (i.e. along the exposure gradient with distance from the marsh edge within one clonal stand of *S. maritimus*) (fig. 5, 9 and 10 and table 4B). More exposed shoots are adapted to intense wave exposure by developing a smaller stature and enhanced flexibility, which suggests a stress avoidance mechanism, however the whole clonal stand grows in higher densities, which suggests a stress tolerance mechanism (fig. 16). These adaptations combined, result in an overall more efficient wave attenuation at the exposed site. In the following sections these statements are supported and argued.

4.1 Wave-induced abiotic variables

Wave exposure differs between sites and with distance from the marsh edge (fig. 4 and table 1). In the back of the marsh, wave heights become smaller at both sites and the difference between sites diminishes due to wave attenuation as a result of friction by the plants and by the sediment bottom. This confirms the strong wave attenuating effect close to the marsh edge (Ysebaert et al., 2011). Although absolute wave heights are higher at the exposed site, the efficiency of wave attenuation is also bigger at the

exposed site (fig 4) and of the same order of magnitude compared with other studies (Gedan et al., 2011). Distance from the marsh edge is correlated to an elevation gradient, however this gradient was similar at both sites, implying that inundation time and depth was similar at both sites at equal distances from the marsh edge (table 1). Comparing sites with equal elevations (relative to mean high water) is important because it is well known from literature that elevation differences within a site can be a determining variable, e.g. differences in root age, inundation time and photosynthetic period (Clevering and Hundscheid, 1998; Coops et al., 1994; Karagatzides and Hutchinson, 1991). Furthermore, once marsh edge shoots are inundated they are exposed to stronger hydrodynamics (e.g. no wave attenuation yet, deeper water column).

An indirect effect of wave exposure is differences in sediment composition due to hydrodynamic sorting (table 4A and table 3). The strong hydrodynamics at the exposed site resulted in coarse sandy sediments due to erosion or the lack of the deposition of fine sediments. The calmer hydrodynamics at the sheltered site resulted in sedimentation and less erosion, which explains the presence of finer sediments (i.e. silt and clay). Finer sediments (i.e. silt and clay) have a higher capacity to retain water, organic matter and nutrients compared to coarse sandy sediments. Therefore, water contents, organic matter and nutrient contents are lower at the sandy exposed site than at the silty sheltered site (table 3). As a result, although groundwater fluctuations are similar (section 3.1 surface water and soil water level fluctuations), the water content of the sediment does differ due to the different grainsizes. No remarkable patterns were found in both pore water and groundwater quality assessments, however, these results should be interpreted with caution since we only took one replica per level at only one day and at one moment in the tidal cycle. Additionally, the nutrient composition of the plants did not differ a lot between sites (fig. 9) and earlier findings suggest hardly any influence of nutrient availability on macrophytes in systems where nutrients are abundant (Hilton et al., 2006) such as the Scheldt Estuary (Meire et al., 2005; Soetaert et al., 2010). Nevertheless, differences between sites were found for DS_i concentrations in the pore water (and ground water) (a similar pattern might be present over the distance gradient, although this should be considered carefully due to the lack of replicates) (table 2). Higher DS_i contents at the sheltered site are expected to result from the calmer hydrodynamic conditions (table

4A), which would imply more deposition of silica out of the water column (e.g. diatom shells), more silica retention by fine particles, and more organic matter retention (i.e. organic matter is a source of silica after mineralisation and can enhance the silica cycle of the marsh (Jacobs et al., 2012; Struyf et al., 2005)). Wave exposure is thus the main variable between sites and creates directly (mechanical stress induced by the wave motion) and indirectly (sediment composition and DSi content) differences in abiotic site conditions. Therefore, shoot morphological, biochemical and biomechanical differences could be linked to wave exposure (table 4B).

4.2 Wave-induced adaptation in plant properties

4.2.1 Plant morphology

The patterns found in the wave induced abiotic differences are also found in the individual shoot morphological properties (table 3 and fig. 5). At the beginning of the growing season, shoots of both sites start to sprout between the remaining dead stem stumps of the previous year. Biomass growth stops earlier at the exposed site than at the sheltered site (fig. 12). Limited growth at the exposed site is probably the result of the environment (i.e. wave exposure in this case) rather than a later shoot initiation (i.a. Clevering & Hundscheid, 1998). At both sites, shoot densities are already close to their maximum (i.e. September densities) at the beginning of the growing season (fig. 12). That means that new sprouting shoots are rare during the growing season. This could be the result of a gradual change of investment priority from aboveground shoots to vegetative reproduction and storage tubers for hibernation.

At the end of the growing season the more exposed shoots have a smaller stature in both the exposed site as well as within a site, close to the marsh edge (fig. 5; table 1 and 4B). These results are similar to the results of previous studies on the morphological response of marsh vegetation to hydrodynamics, although they did not include the strong tidal dynamics (e.g. water level fluctuations) but only waves (Coops and Van der Velde, 1996; Coops et al., 1991). This might explain the negative correlation of the basal stem diameter and the shoot length found in our study for *Scirpus maritimus* (table 4B), which is in contrast with the positive correlation shown for *Scirpus lacustris* (Coops and Van der Velde, 1996). Therefore, the biochemical tissue composition is seems to be a more important driving force for the response of

the plant to wave exposure (stronger correlations with biomechanics, table 4B) than stem diameter. Furthermore, within-site differences along the transect could be attributed to tuber age (Clevering et al., 1995) while more inundation alone has been shown to result in taller shoots (Clevering and Hundscheid, 1998), which is the opposite in our case. This suggests that waves, instead of inundation, are the main cause of thicker and smaller shoots in our study. Marsh edge biomass was comparable with former studies (e.g. Karagatzides and Hutchinson, 1991), however in the back of the marsh we found up to three times more standing biomass. Individual shoot biomass was also higher in the back of the sheltered marsh. Therefore, according to our results, the distance gradient starting from the marsh edge, which is correlated to increased elevation and thus less inundation depth and time, might have less influence on shoot stature in the presence of waves compared to the situation without waves.

Apart from environmental sources, different offspring sizes can also arise from genetic variance between and within sites (Charpentier et al., 2012). Nevertheless, the greenhouse control experiment confirms the role of wave exposure. Although differences in shoot morphology were found, they were less pronounced than in the field (compare fig. 15 with fig. 5). The sampled tubers were formed under the field conditions of the previous year. Observed differences could still be a result of the conditions under which they have been produced in the previous year (Charpentier et al., 2012). However, the morphology of all greenhouse plants was remarkably similar to the field-shoots of the sheltered site. This suggests that the observed differences in the field are a response to wave-induced variation (i.e. phenotypic plasticity) (Karagatzides and Hutchinson, 1991).

In addition to a small shoot stature, high shoot densities could be an adaptation to exposure of the whole clonal plant in order to create a strong framework of shoots, distributing the hydrodynamic forces over the entire clonal entity (Coops and Van der Velde, 1996). The highly efficient wave dissipation at the exposed site (fig. 4) is probably the result of the higher shoot densities, resulting in comparable standing biomass per m^2 between both sites (fig. 5), which is an important trait in wave attenuating capacity (Ysebaert et al., 2011). Lower shoot densities at the marsh edge are explained by a younger and smaller root system (Charpentier and Stuefer, 1999). Differences in grazing pressure (e.g. by geese) at both sites could also be a possible

explanation for differences in shoot densities and even shoot length (Clevering and Van Gulik, 1997), however we do not have data on grazing densities, and moreover we did not observe any severe or obvious damage from geese (a potential grazer present in the wider study area) within our plots. Shoots of the wave exposed *S. maritimus* are thus smaller, thicker and have a smaller dry biomass per individual shoot, although the biomass per area is comparable in both sites due to high shoot densities at the exposed site.

4.2.2 Biochemical and biomechanical shoot properties

Together with morphological properties, the biochemical ‘strength molecules’ determine the biomechanical tissue properties (Mullarney and Henderson, 2010; Rupprecht et al., 2015; Schoelynck et al., 2012). In comparison with shoot morphology (fig. 5) and flexural stiffness (fig. 10), the second moment of area – which quantifies the contribution of stem shape – does not follow the same pattern regarding wave exposure (fig. 10). Although it is generally thought that long, thin shoots are more flexible (Mullarney and Henderson, 2010), our results show the opposite relationship (fig. 5 and fig. 10), as the shoots from the marsh edge – which are the thickest – are also the most flexible ones. In contrast, the Young’s modulus, which is a measure for the contribution of stem material, shows a similar pattern as seen for the flexural stiffness, which indicates a significant contribution of the biochemical tissue composition to the biomechanical strength (i.e. by ‘strength molecules’) (fig. 9 and fig. 10). Comparable results were found for tensile strength and breaking force, i.e. softer/more flexible tissue at the exposed locations. This ‘softer’ tissue could be an explanation for the thicker stems of more exposed shoots, i.e. to maintain an upward position in between tidal inundations, the softer shoots at the more exposed locations might increase their diameter. For instance, by changing the distribution of the softer parenchyma and the stiff sclerenchyma tissue (Niklas, 1995; Schulgasser and Witztum, 1997), plant could adapt to withstand mechanical stress.

Cellulose and lignin are the typical molecules that provide strength to the shoot. Nevertheless, no concentration differences of these molecules between sites were found that could explain the contrasting stiffness (fig. 9). We did find a trend for BSi contents between sites and distance (fig. 9), which hints to the fact that BSi plays an

important role in determining the shoots stiffness (Schoelynck et al., 2012, 2010). We show that the most exposed shoots have a higher flexibility and correspondingly they have lower BSi contents, compared to the sheltered shoots, both between sites as well as with increasing distance to the marsh edge (fig. 9 and fig. 10). Within sites, decreasing trends in stiffness towards the marsh edge can also be attributed to the lower lignin contents in shoots at the marsh edge. Therefore, the fact that the biochemical composition is the determining variable for flexibility (rather than stem morphology) and that there is no site effect for cellulose and lignin contents of shoots, but a clear site effect of BSi contents, strongly indicates that the more flexible shoots at the exposed site are due to lower BSi contents (fig. 9 and fig. 10). Interestingly, the observation of BSi contents are in line with the difference in DSi availability in the pore water (at least for the contrasting sites): Schaller et al. (2012) and Struyf et al. (2005) show that the BSi contents of shoot tissue reflects the DSi content of pore water. The retention of silica in the silica cycle of the marsh is higher at the sheltered site (see section 4.1), which likely leads to higher DSi uptake in plants and higher BSi concentrations. Increased silica uptake under hydrodynamic conditions as shown by Schoelynck et al. (2012) for freshwater species (i.e. *Egeria densa*, *Limnophila heterophylla* and *Nuphar lutea*) is not found here. This suggests that silica uptake in *S. maritimus* is mainly passive (Carey and Fulweiler, 2014). DSi contents of the pore water is likely an indirect consequence of wave exposure (table 4A), as it is likely to be linked to the prevailing sediment fractions present at both sites, i.e. higher DSi contents in finer silty sediments with higher organic matter contents typically for calm conditions (see discussion in 4.1 above). However, it has been suggested that the combined effect of mechanical stress and nutrient availability can have different outcomes in terms of morphological and biomechanical properties (Puijalon et al., 2007; Schaller et al., 2012). Furthermore, shoots grown under greenhouse conditions show a similar BSi content, comparable to the sheltered locations (appendix 2). This also confirms that BSi content is affected by environmental variability. Therefore, both mechanical stress through wave exposure and DSi availability – which is indirectly affected by wave exposure, too (table 4A) – are likely to have a significant effect on the biomechanical properties of *S. maritimus* (table 4B).

The maximum lignin content is reached at the end of the growing season (fig. 13), which shows that lignin contents of a shoot increase with age. The increasing BSi

content levels off earlier in the season (June) at the exposed site (fig. 13). This size dependent increase as a presumed result of passive accumulation of silica from the pore water, is already shown in other studies (Carey and Fulweiler, 2014). According to our biochemical data, we expect a trend of increasing stiffness with age as was found in *Scirpus lacustris* (Coops and Van der Velde, 1996), however, we did not measure biomechanical properties during the growing season.

4.2.3 Wave exposure determines root architecture

Regarding the belowground biomass, we did not find significant differences in total root biomass between sites, which is in agreement with earlier findings (Clevering and Hundscheid, 1998). Charpentier and Stuefer (1999) and Clevering and Hundscheid (1998) showed that in *S. maritimus* nutrient storage is done by tubers, which only increase in size when resources are sufficient, while in contrast to other clonal species, root expansion by rhizomes (spacers) is unresponsive to environmental variables. Therefore, Kiswara et al. (2009) suggested that hydrodynamics do not have a major impact on total root biomass.

Although there is no difference in total belowground biomass, it does show a trend of deeper rooting at the exposed site (fig. 6 and 7). The differences in rooting depth result from plant adaptation to site-specific conditions as for instance the abiotic soil conditions. Since nutrient availability does not differ remarkably between sites (table 2 and NP ratios fig. 9) and former research showed that root architecture of sea grasses is determined by hydrodynamics and sediment stability (Peralta et al., 2006), rather than differences in sediment type and nutrient availability (Kiswara et al., 2009), we conclude that the observed differences in rooting depth are the result of the contrasting wave exposure. For instance, the directly wave-induced drag force on the aboveground shoots (Henry and Myrhaug, 2013) forms a mechanical stress to which the plants respond by investing in deeper roots to avoid being pulled out of the sediment. Indirectly, wave exposure causes sediment bed mobility (erosion and sedimentation) to which the plants could respond by investing in deeper roots to avoid uprooting.

Aside from this overall tendency of deeper rooting at the exposed site, within sites, shoots close to the marsh edge are too young to have an extensive root system (fig. 6). Often only some tubers and a rhizome, which connects them with their mother shoot,

are present (Charpentier and Stuefer, 1999). Together with high sedimentation rates (inducing marsh expansion (Fagherazzi et al., 2007)), shoots deeper into the marsh are taller and originate from an older root system; therefore the root system in the back of the marsh is bigger at both sites. The same patterns were observed earlier (Charpentier and Stuefer, 1999; Charpentier et al., 2012; Clevering and Hundscheid, 1998; Minden and Kleyer, 2015), where the authors attributed this intra-clonal variation to ontogenetically fixed growth patterns. Different root architecture regarding distance from the marsh edge are mainly the result of the division of labour in *S. maritimus* where distinct clonal entities have different functions that benefit the whole clonal plant, i.e. wave attenuation, reproduction, anchoring and nutrient uptake (Bouma et al., 2010, 2005a; Charpentier and Stuefer, 1999; Puijalon et al., 2008). The importance of clonal facilitation is emphasised by a study that shows a low survival chance for individual seedlings (small and few roots) on the mudflat (comparable to the marsh edge situation) (Silinski et al., n.d.).

4.2.4 A wave-induced stress avoidance mechanism

This study shows that wave exposure induces direct and/or indirect morphological, biochemical and biomechanical responses of *S. maritimus*. Heavily exposed shoots experience mechanical stress by strong drag forces and thus potential uprooting (Henry and Myrhaug, 2013). In order to cope with these mechanical stressors, shoots may adapt their stature and tissue in a way that they avoid or tolerate this stress, referred to as the stress avoidance-tolerance theory (Puijalon et al., 2011). Shoots of *S. maritimus* have a higher flexibility when they are more exposed to waves. Increasing flexibility decreases the drag force on the shoot (Sand-Jensen, 2003). Together with a smaller shoot stature, clonal stands of *S. maritimus*, exposed to strong wave activity are able to avoid mechanical stress on individual shoots. Interestingly, within the same species and even within the same clonal stand, a stress tolerance mechanism of the individual shoots is found too at the more sheltered locations (both the sheltered site as well as in the back of the marsh). Furthermore, deeper rooting avoids uprooting by wave-induced motion of the sediment bed. The observed morphological, biochemical and biomechanical responses on the scale of individual wave-exposed shoots can be interpreted as a stress avoidance mechanism.

At a scale larger than individual shoots, the whole clonal plant grows in higher

densities at the exposed site. This creates a strong framework of shoots, which enhances the facilitation of wave energy over all the shoots. In contrast to individual shoots, this adaptation on the clonal scale suggests a stress tolerance mechanism. These hypothetical scale-dependent mechanisms are visualised in figure 16.

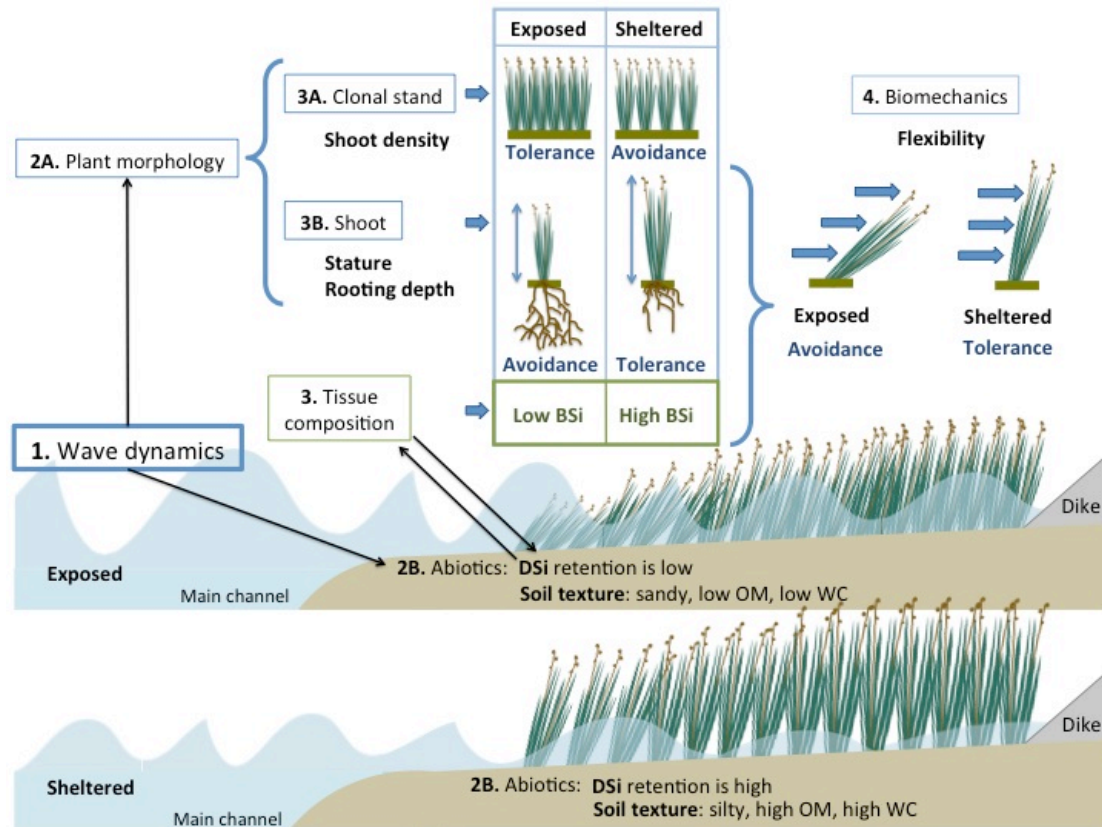


Figure 16: Schematic view of two marshes with contrasting exposure. Wave dynamics (1) induce direct mechanical stress affecting the morphology of the shoots (2A) and indirectly, dynamics on the sediment bed, which determines the soil texture and composition (2B), i.e. dissolved silica contents (DSi), organic matter contents (OM) and the water content (WC). The DSi content in the soil is reflected in the shoot's biogenic silica content (BSi) (3A), which in turn is an important source for the DSi pool in the soil. Both morphology and tissue composition determine the biomechanical strength of the shoots (4). More exposed shoots are more flexible, have a smaller stature and show a trend of deeper rooting suggesting a stress avoidance mechanism (3B), while sheltered shoots show the opposite characteristics and suggest a tolerance mechanism. Remarkably, the whole clonal stand grows in higher densities when exposed (3B). This suggests a stress tolerance mechanism on the clonal plant scale.

4.3 Future research

In this study we searched for correlations between plant properties and wave exposure, as a first indication of plant adaptations to wave exposure. These kinds of field measurements are a useful first step towards understanding the mechanisms underlying the system. However it is difficult to take into account all confounding

variables when comparing two different sites. Although the sites are close to each other, there could be differences in abiotic parameters that are not caused by wave exposure. For instance, local high concentrations of pollutants such as heavy metals in older sediment layers deposited by former wastewater drainage could be mobilised near the rhizosphere and consequently affect the shoot development (e.g. Du Laing et al., 2007; Padinha et al., 2000; Vandecasteele et al., 2002). However, due to low accumulation of heavy metals by *S. maritimus* (Teuchies et al., 2013), this negative impact seem unlikely in our case. We tried to take into account this kind of variation by performing a control experiment under equal conditions, and by measuring many environmental variables in the field. Nevertheless, our results need to be interpreted with caution since we only sampled one growing season. Considerations were made to decide what to measure and whether or not it was feasible to do (e.g. no heavy metals were measured, however we did measure the ‘strength molecules’ and pore water, groundwater, soil and shoot nutrients). Furthermore, since there could be an interaction between mechanical stress and nutrient availability in the development of vegetation, the interpretation of field data becomes even more complex. In our case it could be interesting to disentangle the individual contribution of silica availability and wave exposure to plant properties. Controlled experiments in laboratory conditions could provide better insights needed to understand the functioning of the ecosystem.

As an example of future research building further on this study, a flume transplantation experiment could be performed. At the beginning of the growing season, tubers from both sites could be transplanted into several flume basins. Within these basins, tidal inundation and wave activity could be simulated and plants from both sites could be grown under equal conditions, i.e. without any indirectly wave induced differences in field conditions such as sediment composition. In one set of basins, sheltered wave conditions could be simulated, in another set of basins exposed wave conditions could be simulated. Furthermore, transplantation into contrasting sediment types (indirect effect of wave exposure, e.g. in terms of available silica) could be interesting, too. In order to take into account the potential different growth conditions of previous years, tubers could be grown under equal conditions for several years. Therefore, flume experiments under different wave exposure could be done with plants of equal backgrounds.

4.4 Wider context: regarding coastal protection

The coastal protection value of marshes receives increasing attention in scientific studies and coastal management plans in the face of globally increasing coastal risks induced by sea level rise and increasing storm intensity (Cheong et al., 2013; Möller et al., 2014; Temmerman et al., 2013). Coastal protection by the wave attenuating effect of intertidal marshes depends on plant morphological traits: rates of wave attenuation (i.e. the relative reduction of wave height per distance travelled through the marsh vegetation) are found to be higher when marsh plants are stiffer, taller and when they have a higher biomass, when shoot density is higher (Bouma et al., 2013; Paul et al., 2012; Penning et al., 2009; Peralta et al., 2008; Rupprecht et al., 2015; Ysebaert et al., 2011). Plant morphological, biochemical and biomechanical adaptations induced by a stress avoidance mechanism could have a major impact on the wave attenuating capacity of a marsh (Bouma et al., 2013, 2010; Penning et al., 2009; Ysebaert et al., 2011), especially under extreme conditions (high water levels) (Möller et al., 2014). However, independently of shoot properties, water movement is strongly attenuated close to the sediment bed underneath the canopy. Furthermore, the clonal root systems stabilize the sediment, which in turn prevents erosion (Christianen et al., 2013). Although stress avoidance of individual shoots at the exposed site suggests lower wave attenuation capacities, the whole clonal entity grows with higher stem densities. This clonal plant property is known to have a strong effect on wave attenuation (Penning et al., 2009; Ysebaert et al., 2011). As seen in figure 4, indeed the exposed marsh attenuates the waves more efficiently compared to the sheltered marsh. The conservation and restoration of intertidal marshes is important for the estuarine ecosystem and among others for the protection of communities living behind dikes. Our results show that even at strongly exposed sites along estuaries or coasts, restoring or creating intertidal marsh areas can be an effective measure in coastal protection. In order to do so, initial calm conditions for shoot establishment are needed (Balke et al., 2013), and engineering measures could be used to create these temporary conditions. When densities are high enough, the marsh is able to sustain and to provide coastal protection (Bouma et al., 2009b). Therefore, a sustainable cooperation between ecologists, geologists and engineers is desirable and important.

5 Conclusion

We can conclude that shoot properties of *S. maritimus* differ between sites and between within-site locations with contrasting wave exposure. Furthermore these plant adaptations develop progressively over the growing season. At the exposed site development stops earlier after which plant properties diverge further between sites. With increasing distance from the marsh edge, however, exposure-specific adaptations diminish in general at both sites. In agreement with our wave measurements, this also confirms that waves are rapidly attenuated close to the marsh edge (Ysebaert et al., 2011). A smaller stature and higher flexibility in response to wave exposure looks like a stress avoidance mechanism, which has been suggested earlier between two species (Bouma et al., 2010; Puijalón et al., 2011), however we show the same relationship within one species, and within a few meters of one clonal stand. It remains difficult to determine whether the observed differences are a direct phenotypic adaptation to wave exposure or rather an indirect response as a result of the contrasting sediment composition; therefore, further research is needed. Since cellulose and lignin contents are similar between sites, biogenic silica is the ‘strength molecule’ that determines the observed differences in shoot biomechanical properties. Although this stress avoiding mechanism causes less wave attenuation by a single shoot, the higher densities of the whole clonal stand, suggesting a stress tolerance mechanism, results in a more effective wave attenuation at the exposed site. This suggests that wave attenuation by tidal marshes is a scale-dependent mechanism.

In summary an individual shoot of *Scirpus maritimus* develops morphological, biochemical and biomechanical adaptations suggesting a stress avoidance mechanism when exposed to waves. The whole clonal plant, however, is adapted by growing in higher densities, which indicates a stress tolerance mechanism resulting in more effective wave attenuation at exposed sites. Therefore the morphological responses of both individual shoots and the whole clonal plant have important consequences for the wave attenuating capacity of wave exposed tidal marshes, and in this way also coastal defence.

6 References

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7 Appendix

Appendix 1: Available data for each site obtained from the wave meters (green). The orange time periods were not used in the calculations due to the lack of a complete dataset. Gray represents a smaller dataset, nevertheless it could still be used (GBS: Groot Buitenschoor, sheltered; RIL: Rilland, exposed).

Location	17.3-31.3	31.3-16.4	16.4-6.5	6.5-22.5	22.5-5.6	5.6-18.6	18.6-3.7	3.7-18.7	18.7-30.7	30.7-10.9	10.9-16.12
GBS 12											
GBS 4									1 day ok		
GBS 0			2 days ok							No data	
RIL 12											
RIL 4				No data							
RIL 0		No data		3 days ok							

Appendix 2: Biochemical data from the greenhouse plants. Cellulose (Cell), lignin, biogenic silica (BSi) and NP ratios are shown (n=1).

Site	Distance	Cell mg g ⁻¹	Lignin mg g ⁻¹	BSi mg g ⁻¹	NP ratio
Exposed	0	230.94	32.63	5.42	12.60
	12	238.98	35.12	6.59	13.93
Sheltered	0	240.35	28.76	6.32	11.77
	12	248.31	39.00	4.91	12.69