ANNALS OF BOTANY
Founded 1887

PART OF A SPECIAL ISSUE ON COASTAL FLOODING AND STORM RISKS

Physiological and biochemical responses of the salt-marsh plant *Spartina alterniflora* to long-term wave exposure

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• **Background and Aims** Ecosystem-based flood defence including salt-marsh as a key component is increasingly applied worldwide due to its multifunctionality and cost-effectiveness. While numerous experiments have explored the wave-attenuation effects of salt-marsh plants critical to flood protection, little is known about the physiological and biochemical responses of these species to continuous wave exposure.
• **Methods** To address this knowledge gap, we developed a shallow-water wave simulator to expose individual *Spartina alterniflora* plants to waves in a greenhouse for 8 weeks. *S. alterniflora* individuals were partially submerged and experienced horizontal sinusoidal motion to mimic plant exposure to shallow water waves. A factorial experiment was used to test the effects of three wave heights (4.1 cm, 5.5 cm and a no-wave control) and two wave periods (2 s and 3 s) on the following key physiological and biochemical plant parameters: plant growth, antioxidant defence and photosynthetic capacity.
• **Key Results** Comparison of wave treatment and control groups supported our hypotheses that wave exposure leads to oxidative stress in plants and suppresses plant photosynthetic capacity and thereby growth. In response, the wave-exposed plants exhibited activated antioxidant enzymes. Comparison between the different wave treatment groups suggested the wave effects to be generally correlated positively with wave height and negatively with wave period, i.e. waves with greater height and frequency imposed more stress on plants. In addition, wave-exposed plants tended to allocate more biomass to their roots. Such allocation is favourable because it enhances root anchorage against the wave impact.
• **Conclusions** Simulated wave exposure systems such as the one used here are an effective tool for studying the response of salt-marsh plants to long-term wave exposure, and so help inform ecosystem-based flood defence projects in terms of plant selection, suitable transplantation locations and timing, etc. Given the projected variability of the global wave environment due to climate change, understanding plant response to long-term wave exposure has important implications for salt-marsh conservation and its central role in natural flood defence.

Key words: Ecosystem-based flood defence, salt-marsh, wave stress, response, *Spartina alterniflora*.

INTRODUCTION

Salt-marshes are an important pathway for mass and energy transfer between land and ocean and provide numerous valuable ecosystem services, including carbon sequestration, food production, habitat provisioning, flood protection and water purification (Barbier *et al.*, 2011). Due to sea level rise and increasing extreme weather events caused by climate change (IPCC, 2014), coastal communities worldwide are facing an increasing threat from flooding, and the function of salt-marshes as natural buffer zones protecting coastal areas from waves, tides and storms has drawn increasing attention (Gedan *et al.*, 2011; Shepard *et al.*, 2011). At the same time, purpose-built ecosystem-based flood defences, i.e. eco-shorelines or living shorelines, that are more cost-effective than conventional engineering infrastructures such as sea walls and levees and concurrently provide crucial habitat in intertidal zones, are now implemented on a large scale (Temmerman *et al.*, 2013). In both natural and man-made systems, salt-marsh vegetation plays a key role in flood and wave attenuation (Moller, 2006; Temmerman *et al.*, 2012), even under extreme conditions, such as storm surges (Moller *et al.*, 2014). Importantly, the establishment, survival and growth of salt-marsh plants are subject to hydrodynamic forcing, including wave conditions (Bouma *et al.*, 2009, 2016; Callaghan *et al.*, 2010; Friess *et al.*, 2012) and consequently, understanding the response of salt-marsh plants to wave stress is thus imperative within the context of ecosystem-based coastal management (Barbier *et al.*, 2008).

Numerous short-term experimental studies have quantified the wave-attenuation effects of plants (Augustin *et al.*, 2009; Anderson and Smith, 2014; Ozeren *et al.*, 2014). The specific

effect was found to depend on hydrodynamic parameters, such as wave height (Anderson and Smith, 2014), wave period (Jadhav *et al.*, 2013) and water depth (Paquier *et al.*, 2017), as well as plant traits, such as stem height, diameter, density and flexibility (Bouma *et al.*, 2005, 2010; Marsooli and Wu, 2014; Paul *et al.*, 2016). Furthermore, a number of short-term studies have explored the response of salt-marsh plants to waves. Such flume studies identified drag forces and scouring of sediment around plant stems as the two primary mechanisms that limit seedling establishment and may cause plant failure (Bouma *et al.*, 2009; Henry and Myrhaug, 2013). In another flume study, Silinski *et al.* (2015) explored the effects of wind (short) and ship (long) waves on seedlings and adult shoots of *Scirpus maritimus*, reporting that plants were well adapted to 'wind' waves during both life stages, whereas 'ship' waves tended to strongly bend their stems and potentially limited their survival. Short-term flume studies have also shown severe stem breakage and loss of above-ground biomass in response to extreme wave conditions under storm conditions (Rupprecht *et al.*, 2017).

Long-term studies focusing on the impact of waves on vegetation are, however, much rarer than short-term experiments. Coops and Van der Velde (1996) studied the effects of wave exposure on the mean stem height and density of *Phragmites australis* and *Scirpus lacustris* stands. By measuring the mechanical properties of both species, they demonstrated that *P. australis* stands withstand wave exposure better than *S. lacustris* due to the higher stem stiffness and lower susceptibility to breakage. Using wave mesocosms, La Nafie *et al.* (2012) showed that the survival of seagrasses was reduced by a combination of waves and nutrients, whereas waves reduced growth and nutrient loading reduced leaf strength. By comparing *S. maritimus* stands from areas with different wave exposures in the Scheldt Estuary (south-west Netherlands), Silinski *et al.* (2018) reported that shoots from more wave-exposed conditions developed significantly shorter, more flexible and thicker stems than those from more sheltered conditions, indicating plasticity in response to wave exposure following a stress-avoidance strategy. So far, however, the very few existing long-term studies on the impacts of waves have mainly focused on the growth, morphological and biomechanical properties of salt-marsh plants, whereas plant physiological and biochemical responses to long-term wave exposure remain elusive. Given that hydrodynamic forces such as flow and turbulence cause oxidative stress in plants and suppress photosynthetic capacity and growth (Kankanamge *et al.*, 2011; Ellawala *et al.*, 2013), long-term wave exposure may cause additional negative effects.

Under abiotic stress, plants typically exhibit oxidative stress (Mittler, 2002), which typically manifests as an elevated tissue concentration of reactive oxygen species (ROS), such as the superoxide anion (O_2^-), H_2O_2 and the hydroxyl radical (OH^-) (Navrot *et al.*, 2007). The over-accumulation of ROS induces oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage, resulting in cell injury and even cell death (Moller *et al.*, 2007). The content of malondialdehyde (MDA), as a secondary breakdown product of lipid peroxidation, has often been used as a tool to assess the severity of oxidative damage in plants (Canalejo *et al.*, 2014). As protection from oxidative damage, plants have evolved defence mechanisms that comprise the activation of ROS-scavenging antioxidant enzymes such as

ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD) and glutathione peroxidase (GPX), as well as the synthesis of non-enzymatic antioxidants such as ascorbate and glutathione (GSH) (Arora *et al.*, 2002; Gill and Tuteja, 2010). Hence, the activities of antioxidant enzymes such as APX, SOD, CAT, POD and GPX are commonly measured to examine the stress responses of plants (Duarte *et al.*, 2016; Asaeda *et al.*, 2017). In addition, stress often adversely affects the photosynthetic machinery, which is typically reflected by reduced photochemical quenching (q_p), indicating reduction in the proportion of open photosystem II reaction centres, as well as increased non-photochemical quenching (NPQ), indicating reduced demand for products of electron transport and hence increased energy dissipation as heat (Moradi and Ismail, 2007). The damaged plant photosynthetic machinery would result in reduced photosynthetic capacity (typically indicated by the maximum quantum efficiency of photosystem II (F_v/F_m)) and further suppress plant growth (Ellawala *et al.*, 2013; Atapaththu and Asaeda, 2015).

Spartina alterniflora is a perennial grass native to the east coast of North America and is spreading rapidly to estuaries and coastal salt-marshes in other parts of the world, including the Pacific coast of North America, Europe, New Zealand and China. In addition to its wide geographical distribution, *S. alterniflora* is often employed in eco-shoreline projects due to its tolerance of seawater inundation, ability to colonize and grow in a variety of wave environments, and effective erosion-mitigation and wave-attenuation abilities (Manis *et al.*, 2015). In order to better understand the suitability of *S. alterniflora* for use in eco-shoreline projects, we investigate the hypotheses that (1) long-term wave exposure causes oxidative stress in the salt-marsh plant *S. alterniflora* and suppresses its photosynthetic capacity and thereby growth; (2) wave-exposed *S. alfterniflora* activates antioxidant enzymes in response to wave-induced oxidative stress; and (3) the wave effects depend on wave parameters such as height and period. These questions were addressed by quantifying the effects of oscillatory currents induced by shallow-water waves on *Spartina* growth and ecophysiology, specifically (a) antioxidant defence via enzyme activities as indicators of plant response to oxidative stress induced by wave exposure, (b) lipid peroxidation as an indicator of oxidative damage to the plant, and (c) chlorophyll fluorescence as an indicator of the photosynthetic capacity of the plant. To translate current findings into what it means with respect to predicted shifts in wave environments experienced by natural salt-marshes (Hemer *et al.*, 2013; IPCC, 2014), we discuss the potential impacts of projected variability of the global wave environment due to climate change on the *S. alterniflora* communities in its primary geographical distribution areas (i.e. including its native and invasive ranges across the globe) and the relevant management implications.

MATERIALS AND METHODS

Shallow-water wave simulator

The remarkable lack of long-term wave-exposure studies is most likely attributable to technical constraints. Traditional wave flumes are not designed for long-term plant exposure

experiments, as they do not allow the continuous operation for extended periods necessary for physiological experiments, not to mention their additional requirement of simultaneous replications (La Nafie *et al.*, 2012). To resolve this issue, we adapted a slider-crank mechanism that has been previously used to study the discharge of jets into an oscillatory cross-flow (Lam and Xia, 2001) to create a system by which plants can be exposed under controlled conditions to contrasting wave regimes. In essence, the plants were grown on a swing system that moves the plants continuously back and forth through the water (Fig. 1) to mimic exposure to long-term shallow-water waves (for a full description of wave simulator design, see Supplementary Data Appendix A). The system was placed in a 45-m² purpose-built greenhouse located at the State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University.

The plants were exposed to four field-relevant types of shallow-water waves: two wave heights (4.1 and 5.5 cm) and two wave periods (2 and 3 s) were tested in a full factorial experiment, with the treatments coded as T2H4, T2H5, T3H4 and T3H5, respectively. A no-wave condition was also tested as a control (coded T0H0). Notably, the wave force (stress) acting directly on the plant is correlated positively with wave height *H* and negatively with wave period *T*. Therefore, T2H5 corresponded to the condition with the strongest wave stress and T3H4 the weakest. For full details of the selection of wave parameters and a basic explanation of the functional relationship between wave stress, wave height and wave period, see Supplementary Data Appendix B.

Four treatment tanks were installed, one for each wave treatment, plus another control tank. The control tank had a similar setup and management except for the absence of any water motion inside the tank. The tanks were all filled with a 31-cm water column, and the submergence depth above the containers was maintained at 10 cm. To reduce the effect of wave reflection, 10-cm-thick coil mats were placed inside the tank to absorb the waves, and the wave parameters were carefully checked to avoid creating standing waves, i.e. the generated wave length was equal to twice the length of the tank.

Plant material and growth conditions

A total of 100 soil cores (length 15 cm, width 15 cm, height 25 cm), each containing ten intact *S. alterniflora* individuals, were collected from salt-marsh in the Yellow River Estuary, Shandong Province, China, in October 2016 and transported to the laboratory. The soil cores were transplanted in pots (diameter 20 cm, height 30 cm) in the greenhouse and irrigated with fresh water in the first week and 5 % Hoagland nutrient solution prepared using 5 ‰ artificial seawater from the second week onward. At the end of the growing season, when the plants had senesced, the above-ground parts were harvested and the below-ground parts were continuously cultured. In April 2017, soil cores with healthy individuals, ~35 cm in height, were randomly selected and fitted into the plant culture zone of the containers. The soil surface was flush with the edge of the containers, and excess stems were trimmed to leave approximately
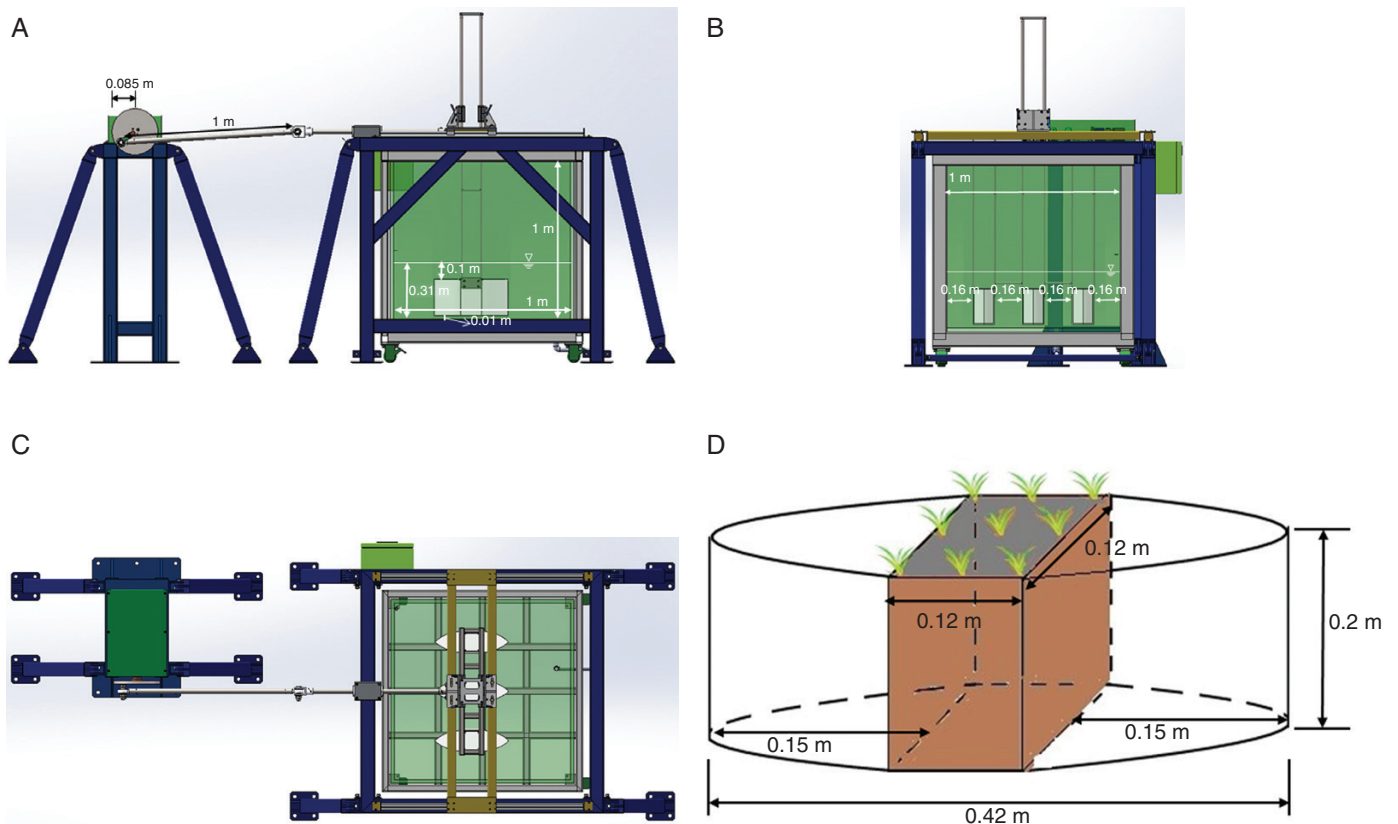

Fig. 1. Schematic diagram of the experimental setup. (A) Front view. (B) Side view. (C) Top view. (D) Streamlined container.

nine individuals per container, i.e. a shoot density equivalent to 400 stems m^{-2}, representative of natural *S. alterniflora* marshes (Knutson *et al.*, 1982). The same culture solution was used to fill the water tank and was renewed weekly. The temperature was controlled between 24 and 30 °C throughout the experiments in an unheated, naturally lit greenhouse. After being acclimatized for 1 week, plants were exposed to the four wave treatments and the no-wave control for 8 weeks. For each treatment, the three containers served as triplicates.

Growth measurements

At the end of each experiment, plant growth (individual stem height) was measured and plants were harvested and rinsed to remove soil and inorganic material. The above- and below-ground live materials were separated and oven-dried at 60 °C to a constant weight to estimate biomasses.

Antioxidant enzyme activities as markers of stress

Leaf samples were randomly extracted on the first day of every week. Plant tissue was homogenized with phosphate buffer solution (0.1 M, pH 7.4) at a 1:9 ratio (g FW:mL), and the homogenate was centrifuged at 3500 rpm for 15 min. Antioxidant enzyme activities were assayed using commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Specifically, SOD activity was assayed using the nitro blue tetrazolium (NBT) method, whereby one unit of SOD activity was defined as the amount of SOD required to inhibit 50 % of the reduction in NBT dye per millilitre of reaction solution per milligram of tissue protein. CAT activity was assayed using the hydrogen peroxide (H_2O_2) oxidation method, whereby one unit of CAT activity was defined as the amount of 1 μM H_2O_2 decomposed per milligram of tissue protein per second. GPX activity was assayed using 5,5-dithiobis-2-nitrobenzoic acid (DTNB) spectrophotometry, whereby one unit of GPX activity was defined as the amount of enzyme in 1 mL of reaction solution that catalyses the oxidation of 1 μM of GSH per minute at 37 °C (pH 7.4). POD activity was assayed using the guaiacol method, whereby one unit of POD activity was defined as the amount of enzyme that catalyses the production of 1 μg of substrate per milligram of tissue protein per minute at 37 °C.

Lipid peroxidation as a marker of cell damage

The MDA content of the plant samples was used as a marker of lipid peroxidation using a commercial kit (Nanjing Jiancheng Bioengineering Institute, China). The MDA content was measured through the absorbance of thiobarbituric acid-reactive substances (TBARS) at 532 nm and expressed as nanomoles of MDA per milligram of tissue protein.

Chlorophyll fluorescence as a marker of photosynthetic capacity

Chlorophyll fluorescence measurement was conducted using a FluorPen (FP 100, Photon Systems Instruments, Czech Republic) on the first day of every week. The third and fourth completely unfolded and undamaged leaves of randomly selected healthy *S. alterniflora* individuals were dark-adapted for 20 min before detection, and the saturation pulse of the FluorPen was set to 6000 μM m^{-2} to detect the chlorophyll fluorescence. Key chlorophyll fluorescence parameters, such as $F_\sqrt{}/F_m$, q_P, fluorescence decay rate (R_{fd}) and NPQ, were recorded.

Statistical analysis

The data were tested for normality and homogeneity of variance using a Shapiro–Wilk test and Levene's test, respectively, before statistical analyses. The results are presented as mean ± s.d. ($n = 27$ for stem height and biomass, $n = 6$ for enzyme assays and $n = 5$ for chlorophyll fluorescence assays). Differences in the temporal variation in parameters measured during the experimental period among the different wave treatments were analysed by repeated measures ANOVA (RM-ANOVA) to detect significant differences ($P < 0.05$), with Bonferroni correction for pairwise comparisons. When the assumption of sphericity was violated, Greenhouse–Geisser correction was applied. The parameters measured only at the end of the experimental period were analysed using one-way ANOVA followed by a *post hoc* Tukey test. Statistical analysis was performed using SPSS (v22.0) software.

RESULTS

Plant growth and biomass

Spartina stem height in the wave treatment groups was generally lower than in the control group (Table 1), indicating that waves had negative effects on plant growth. For the comparison among the different treatments, the 2-s wave treatments (T2 treatments; Table 1), which presumably corresponded to more stressful conditions for identical wave heights, yielded lower stem heights than the control ($P < 0.05$). Stem heights within the different treatment groups did not differ. The same was true of the 3-s wave treatments (T3 treatments) and the control group.

Consistent with the negative effects of wave treatment on plant elongation, total biomass was reduced by wave exposure (Table 1). Although no significant difference in the total biomass was evident between the T3H4 treatment (weakest wave stress among all wave treatment groups) and the control group, total biomass was reduced in other wave treatment groups with stronger wave stress. Significant decreases in above-ground biomass and the above-ground/below-ground biomass ratio were also apparent between the individual wave treatment and control groups. Notably, significant difference were consistently found between the T3H4 (weakest wave stress) and T2H5 (strongest wave stress) treatments, except for the above-ground/below-ground biomass ratio.

Antioxidant enzyme activities as markers of stress

Wave treatment stimulated elevated SOD (RM-ANOVA, $F_{28,175} = 10.116$, $P < 0.01$), CAT (RM-ANOVA, $F_{18.596,116.222} = 1.739$, $P < 0.05$), GPX (RM-ANOVA,

$F_{28,175}$ = 9.342, $P < 0.01$) and POD (RM-ANOVA, $F_{28,175}$ = 4.772, $P < 0.01$) activities compared with the control throughout the experimental period (Fig. 2). Among the different treatments (Fig. 2), the highest SOD activity was found in the plants subjected to the T2 treatments (i.e. stronger wave stress), whereas the plants grown in the T3H4 treatment (weakest wave stress among all wave treatment groups) and control exhibited the lowest SOD activities. CAT activities did not vary throughout most of the experiment, except in the T2H5 treatment (strongest wave stress among all wave treatment groups), which from the second week onward exhibited higher CAT activity than the control group. GPX activities in the wave treatment groups were higher than in the control group throughout most of the experiment, and higher activities were observed in treatments with more stress. POD activities in the T2 and T3H5 treatments were significantly higher than those in the T3H4 treatment and the control group throughout.

TABLE 1. *Plant height and biomass*

Responses	Treatment					$F_{(4,130)}$	P
	Control	T3H4	T3H5	T2H4	T2H5		
Plant height (cm)	79.31 ± 8.55[a]	76.89 ± 6.76[ab]	72.06 ± 3.55[ab]	59.76 ± 2.33[b]	53.81 ± 2.83[b]	4.67	<0.01
Total biomass (g)	2.42 ± 0.12[a]	2.29 ± 0.12[ab]	2.16 ± 0.08[bc]	2.02 ± 0.06[c]	1.83 ± 0.05[d]	46.29	<0.01
Above-ground biomass (g)	1.65 ± 0.04[a]	1.42 ± 0.02[b]	1.27 ± 0.02[c]	1.21 ± 0.02[c]	1.06 ± 0.03[d]	230.57	<0.01
Below-ground biomass (g)	0.77 ± 0.04[a]	0.88 ± 0.02[b]	0.88 ± 0.02[b]	0.81 ± 0.02[a]	0.77 ± 0.03[a]	14.19	<0.01
Above-ground/below-ground biomass ratio	2.15 ± 0.16[a]	1.62 ± 0.06[b]	1.44 ± 0.05[b]	1.51 ± 0.05[b]	1.37 ± 0.08[b]	33.28	<0.01

Data are mean ± s.d., $n = 27$.

Different superscripts in the same row indicate a significant difference between different wave treatment groups ($P < 0.05$).
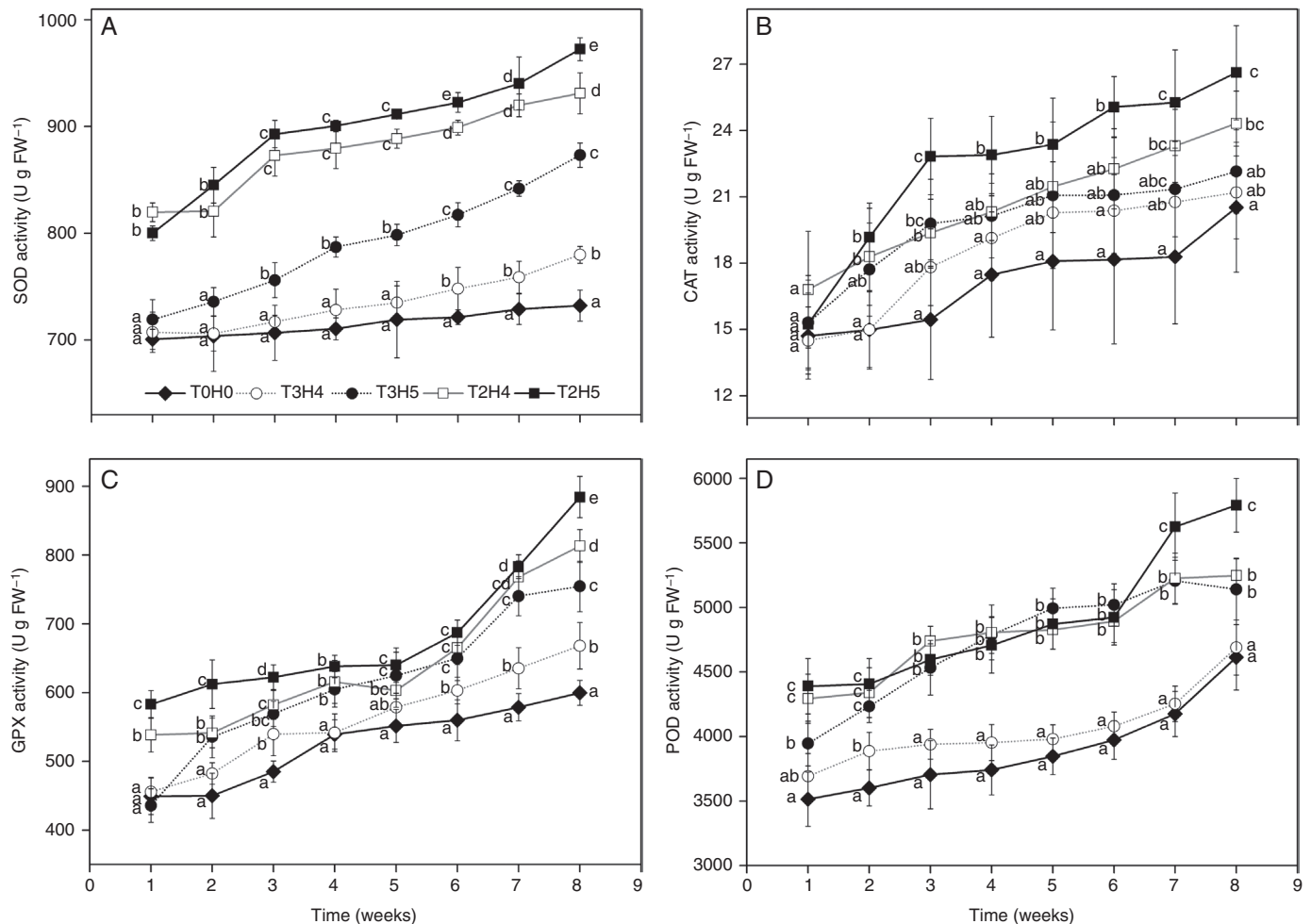

FIG. 2. Temporal variation in antioxidant enzyme activities in leaves of *S. alterniflora* during the experimental period when exposed to different wave treatments. Data are mean ± s.d., $n = 6$. Different letters at the same time point indicate a significant difference between different wave treatment groups ($P < 0.05$).
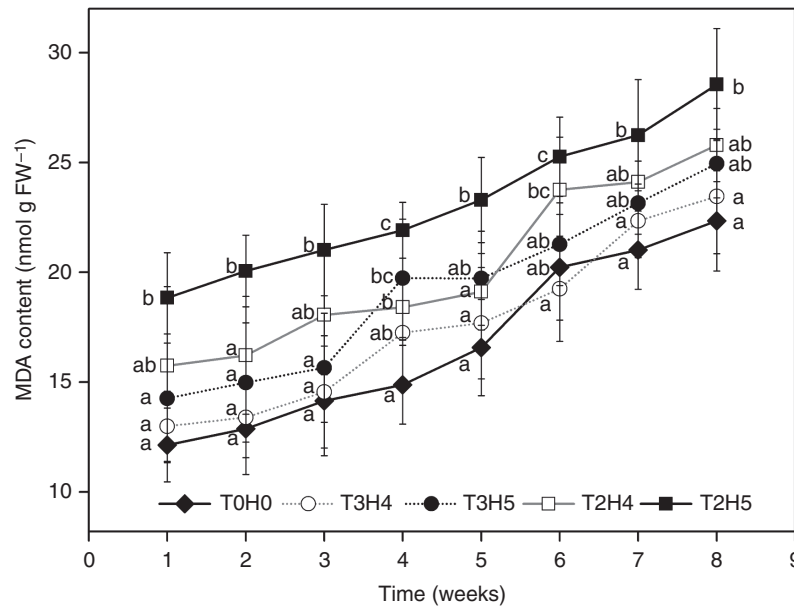
FIG. 3. Temporal variation in MDA contents in leaves of *S. alterniflora* during the experimental period when exposed to different wave treatments. Data are mean ± s.d., $n = 6$. Different letters at the same time point indicate a significant difference between different wave treatment groups ($P < 0.05$).

Lipid peroxidation as a marker of cell damage

The MDA content (Fig. 3) increased in the wave treatment groups compared with the control throughout the experiment (RM-ANOVA, $F_{12.838,80.238} = 2.604$, $P < 0.01$), regardless of the high POD activities. Among the different treatments, the MDA contents were similar among all groups throughout most of the experiment, except in the T2H5 treatment, which had a significantly higher MDA content than all other groups.

Chlorophyll fluorescence as a marker of photosynthetic capacity

Chlorophyll fluorescence parameters indicative of the plant's ability to produce carbohydrates, such as F_v/F_m (RM-ANOVA, $F_{28,140} = 2.539, P < 0.01$) q_P (RM-ANOVA, $F_{28,140} = 1.561, P < 0.05$) and R_{fd} (RM-ANOVA, $F_{28,140} = 2.567$, $P < 0.01$), were generally lower in the wave treatment groups than in the control (Fig. 4). At the same time, NPQ (RM-ANOVA, $F_{12.572,62.859} = 2.162$, $P < 0.05$) increased in the wave treatment groups. Among the different treatments, the q_P value was more sensitive to wave stress than the other chlorophyll fluorescence parameters, and all wave treatment groups exhibited significantly lower q_P values than the control group throughout most of the experiment.

DISCUSSION

In accordance with our hypotheses, we observed that wave exposure subjected salt-marsh plants to oxidative stress with concomitant negative effects on photosynthetic capacity and growth. In response, wave-exposed *Spartina* exhibited activated antioxidant enzymes and tended to allocate more biomass to roots to enhance anchorage. Also, the wave effects were found to depend on wave height and frequency such that waves with greater height and frequency generally imposed more stress on

plants. Overall, the results of this study indicate that long-term wave exposure has a major influence on the performance of this key temperate salt-marsh species, with possible repercussions for the response of the habitat to storm events.

Growth and ecophysiological responses

The reduction in *S. alterniflora* stem height caused by wave stress supports the hypothesis that long-term wave exposure suppresses its growth, which is consistent with previous laboratory and field investigations on wave exposure of the salt-marsh plants *S. lacustris* (Coops and Van der Velde, 1996) and *S. maritimus* (Silinski *et al.*, 2018). In previous studies on the effects of mechanical stresses induced by flow and turbulence on submerged freshwater macrophytes (Ellawala *et al.*, 2013; Atapaththu and Asaeda, 2015), reduced shoot height was attributed to the retardation of tissue elongation, i.e. thigmomorphogenesis (Biddington, 1986). Physiologically, this effect is also related to reduced photosynthetic capacity under wave stress. This response allows the plant to conserve energy and initiate the appropriate defence mechanisms necessary for survival (Van Breusegem *et al.*, 2001).

The decreased above-ground/below-ground biomass ratio indicated that plants allocated more biomass to their roots to enhance their anchorage against wave impact. Similar shifts in biomass allocation have been observed in seagrasses (Peralta *et al.*, 2006) and submerged freshwater macrophytes (Ellawala *et al.*, 2013). The gradual scouring of the surface substrate and hence the exposure of the top roots due to wave impacts make enhanced anchorage imperative for plant survival. Notably, however, previous studies on freshwater plant responses to increased flow regimes (Puijalon *et al.*, 2007) and seagrass responses to waves (La Nafie *et al.*, 2012) also reported contrasting effects of reduced below-ground growth.
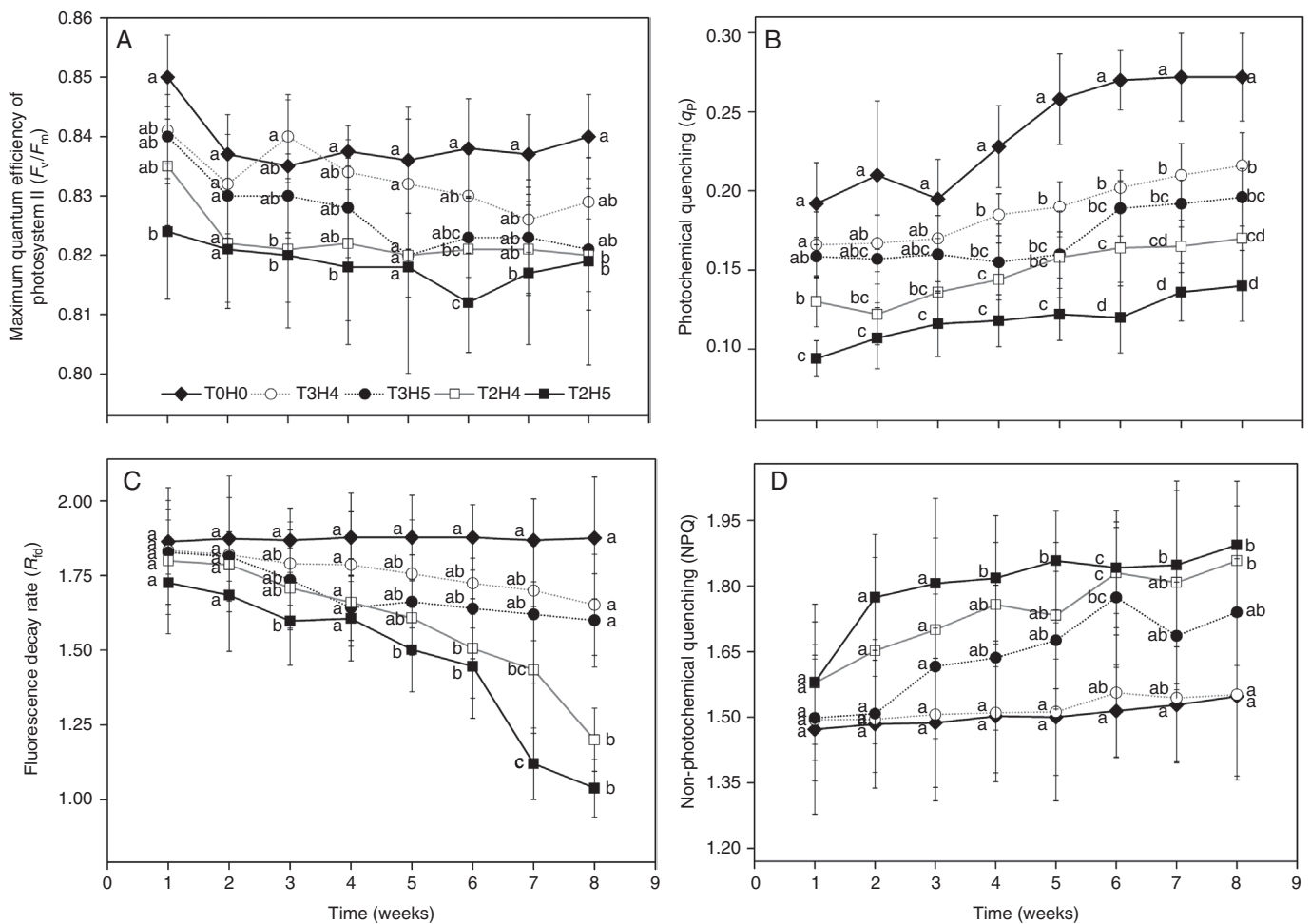
FIG. 4. Temporal variation in chlorophyll fluorescence parameters in leaves of *S. alterniflora* during the experimental period when exposed to different wave treatments. Data are mean ± s.d., *n* = 5. Different letters at the same time point indicate a significant difference between different wave treatment groups ($P < 0.05$).

It is well established that moderate water stirring reduces the thickness of the diffusive boundary layer and leads to enhanced nutrient and gas fluxes, and hence increased photosynthesis and plant growth (Bornette and Puijalon, 2011; Asaeda *et al.*, 2017). Therefore, the above-ground/below-ground biomass ratio may depend on the trade-off between the positive effects of wave exposure in the reduced diffusive boundary layer (especially for leafy plants) and negative effects, including scouring, which is presumably dependent on wave height and the frequency of wave exposure, with greater wave height and frequency likely leading to more negative effects and vice versa. Thus, the contrasting effects of reduced below-ground growth reported in La Nafie *et al.* (2012) may be attributed to the longer wave period (~50 s) and hence lower frequency in their experiment.

The elevated antioxidant enzyme activities in wave-exposed plants support the hypothesis that the ROS-scavenging system is activated when salt-marsh plants are exposed to waves. ROS levels in plants increase in response to hydrodynamic stresses and consequently trigger increases in antioxidant enzyme activities. For instance, previous studies reported the accumulation of H_2O_2 and elevated activity levels of antioxidant enzymes, including CAT, APX, POD and GPX, in some

aquatic macrophytes subjected to turbulence (Ellawala *et al.*, 2011*b*; Atapaththu and Asaeda, 2015). Indole acetic acid (IAA) is the principal form of auxin involved in the stimulation of plant elongation by inducing cell division. High POD activities are known to catabolize IAA and reduce auxin-regulated growth (Siegel, 1993), which may lead to reduced plant growth. Notably, POD is one of the ROS-scavenging enzymes with a high affinity and is activated in short-term responses (Dominguez *et al.*, 2010). Therefore, a decrease in POD activity may follow long-term exposure to mechanical stress (Kankanamge *et al.*, 2011; Ellawala *et al.*, 2013). However, POD activity measured in our study remained in the range of steady increase, albeit at varying rate.

Increased MDA content supports the hypothesis that long-term wave exposure causes oxidative damage to *S. alterniflora*. Previous studies regarding the effect of flow turbulence on aquatic plants also showed that with increasing turbulence the MDA content increased (Asaeda and Rashid, 2017). Observation of chlorophyll fluorescence parameters supports the hypothesis that the photosynthetic capacity of wave-stressed plants is reduced. This finding corroborates Ellawala *et al.* (2011*a*), who reported a reduction in photosynthetic capacity (reflected in reduced q_P and increased NPQ values)

in plants exposed to turbulence. Trends similar to the reduced F_v/F_m ratio detected in this study were observed by Ellawala *et al.* (2013) and Atapaththu and Asaeda (2015).

As mentioned above, plant response to water motion depends on the trade-off between positive and negative effects and thus may vary with the magnitude of the motion (Bornette and Puijalon, 2011; Asaeda *et al.*, 2017). Indeed, comparison of the present findings with the results of previous studies indicates that the biomass allocation of wave-exposed plants depends on the trade-off and thus wave height and frequency, with greater wave height and frequency likely leading to increased below-ground biomass. In principle, this trade-off between positive and negative wave effects should apply equally to all the growth and ecophysiological responses we examined here. However, the results obtained in our study suggested a general positive correlation between wave effects and wave force (stress), which is further correlated positively with wave height and negatively with wave period. This suggests that the wave parameters adopted in our study fell mostly within the more stressful range, and thus the negative wave effects dominated.

Projected changes in wave climate: impacts on Spartina-*dominated marshes*

Our experimental findings imply that the successful adaptation of the salt-marsh plant *S. alterniflora* to long-term wave exposure may be largely attributed to its response mechanisms, such as its antioxidant defence system and shifted biomass allocation. Long-term wave exposure also caused notable negative effects on the plants, as indicated by lipid peroxidation and reduced photosynthetic capacity and plant growth. Given the dependence of the wave effects on wave parameters, it is of interest to assess how the projected changes in global wave environment due to climate change would impact the stress on *S. alterniflora* communities and hence their resilience.

According to a recent study on the projected changes in wave environment due to climate change (Hemer *et al.*, 2013; note that these wave–climate projections are used throughout the discussion), we would foresee more wave-induced stressful conditions for the species to grow along the southern part of the east coast of North America and the Gulf coast, as a result of projected increase in wave height plus decrease in wave period (Supplementary Data Fig. S1b, c). In contrast, along the Pacific coast of North America the wave climate is predicted to be less stressful due to projected decrease in wave height plus increase in wave period, whereas for the rest of the primary geographical distribution areas of the species (e.g. the northern part of the east coast of North America, the coast of China and the European Atlantic coast; Supplementary Data Fig. S1a), the net effects are hard to assess due to the counteracting effects of concurrently decreasing wave height and period. Current findings imply that extra care should be taken to preserve *S. alterniflora* marshes in those areas of its native range where wave conditions are expected to become more stressful, i.e. the southern part of the east coast of North America and the Gulf coast, as plant growth performance will suffer. This may imply that extra management measures are needed in these areas to preserve the extant marshes and

to be able to use *S. alterniflora* in eco-shoreline projects. Wave-attenuating reefs fronting the marshes may offer solutions (e.g. Bouma *et al.*, 2014). Whereas for the invasive range of the Pacific coast of North America the foreseeable less stressful conditions would mean more prevention and mitigation efforts. Finally, it should be noted that the above discussion is restricted to wave stress. Further studies should hence focus on the interaction between wave stress and other climate-change and anthropogenic stress factors, such as sea-level rise, increasing temperature and eutrophication.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Appendix A: shallow-water wave simulator. Appendix B: selection of wave parameters and functional relationship between wave stress, wave height and wave period. Figure S1: (a) global distribution of *Spartina alterniflora*; (b) (left panel) averaged multi-model annual significant wave height for the time-slice representing the present climate (∼1979–2009); (right panel) projected future changes in multi-model averaged annual significant wave height for the future time-slice (∼2070–2100) relative to the present climate time-slice (∼1979–2009); (c) (left panel) averaged multi-model annual mean wave period for the time-slice representing the present climate (∼1979–2009); (right panel) projected future changes in multi-model averaged annual mean wave period for the future time-slice (∼2070–2100) relative to the present climate time-slice (∼1979–2009).

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