

## **Reviewer:**

This manuscript examines responses of different genotypes of the same species to changes in inundation, thereby addressing an understudied area of potential plant responses to SLR. The results focus on plant responses to different flooding frequencies, highlighting differences in biomass, leaf and shoot, and rhizome growth between low-marsh and high-marsh genotypes of *E. athericus*. The revised version of the paper addresses most of the concerns raised during the first round of review by situating this work within the context of other studies examining population-level or genotypic differences in species' responses to global change factors, providing more information in the methods, and expanding the data presented in the results. The addition of these new data, however, raised some additional questions that should be addressed in a subsequent revision. I've noted these questions and some others below.

We appreciate the reviewer's comments. Below we address each of them separately. Line numbers refer to the newly revised manuscript. The author's reply is in blue font, and additions or changes to the original text are written in bold letters.

Hypotheses: consider expanding to include more than just the biomass response, as several variables deal with other growth responses (leaf and shoot length, rhizome length, etc.) and are subsequently discussed as important findings of the study.

We specified the hypothesis as follows:

Line 72: To test the hypothesis that the low-marsh genotype performs better at increased flooding frequencies than the high-marsh genotype, **which would be reflected in above- and belowground growth parameters like biomass production and shoot, leaf and rhizome lengths**, we conducted a factorial marsh organ experiment. We exposed both genotypes of *E. athericus* to three different flooding frequencies and quantified plant growth parameters to compare their performance and assess morphological adaptations of the low-marsh genotype.

Materials and methods: The revisions addressed most of the concerns raised previously, but more information on the field site from which plants were collected and how the experimental design relates to field-relevant conditions would be useful, as noted below for the different sections.

Section 2.1. Provide a brief description of the tides and elevation range of the site, the distribution of the genotypes along this gradient, and other environmental data (e.g., salinity), which would then allow the reader to understand how the experimental design corresponds to the field conditions.

In line 81, we would like to refer to the paper of Bockelmann et al. (2003) as it provides a very good description of the low- and high-elevation sites on Schiermonnikoog where the two genotypes were collected.

We added the available information accordingly:

Line 81: **On Schiermonnikoog, *Elymus athericus* can be found in higher and lower elevated sites, which are inundated 20 – 125 and 90 – 270 times per year, respectively (Bockelmann et al., 2003). Soil salinities range from 22 to 29 ppt (Bolhuis et al., 2013).**

Section 2.1, line 84: How did you quantify "similar size" of individual plants? Was it just initial shoot length and number as noted in the discussion? If so, this does not necessarily reflect potential differences in initial biomass. Are these variables correlated with biomass, and if so, are their allometric relationships to demonstrate this relationship? What about belowground biomass? It

would be helpful to more explicitly note the measures taken to document initial plant variables, which when combined with the random assignment of plants to treatments, minimizes potential size-based bias. If these data are not available, then anecdotal visual assessments of biomass (no obvious outliers), when combined with the shoot and leaf length data and random assignment of plants to treatments, would help reduce concerns about initial size bias in the study.

Additional comment:

Lines 180-185. Move this to the results or methods as evidence that any initial differences in plant size were insignificant.

It was not possible to measure other parameters like biomass at the beginning of the experiment as those measurements would have been destructive, but we will follow the reviewer's suggestion by adding the information regarding the visual assessment as follows:

Line 87: In July 2017, single plants of similar **biomass were selected based on visual assessment (no obvious outliers)**, transplanted to separate pots and randomly assigned to the flooding treatments. **Initial shoot length and shoot number was tested for differences between genotypes and flooding frequencies to ensure that results were not biased by unequal plant size at the beginning of the experiment. There were no significant differences regarding shoot length (genotype:  $F = 0.787$ ,  $p = 0.380$ ; flooding frequency:  $F = 0.127$ ,  $p = 0.881$ ; genotype\*flooding frequency:  $F = 0.231$ ,  $p = 0.795$ ) and number of shoots (genotype:  $Wald = 2.203$ ,  $p = 0.137$ ; flooding frequency:  $Wald = 0.357$ ,  $p = 0.837$ ; genotype\*flooding frequency:  $Wald = 0.005$ ,  $p = 0.997$ ).**

Section 2.3.1. The inclusion of root:shoot is a nice addition, but it raises some new questions. For root:shoot, rhizomes were excluded, yet it appears that rhizomes were included in the belowground biomass values reported in Fig. 1. Based on an estimated calculation using the biomass values in the figure, the root:shoot values do not appear to be derived from the values reported for above- and below-ground biomass in Fig. 1. If rhizomes had been included as belowground biomass for the calculations of root:shoot, it appears that a different pattern for root:shoot would emerge, especially for the high flooding treatment. This raises the question of where and when rhizomes were incorporated into measures of biomass and how this affects interpretations of results. To clarify this confusion, explain what material is included in measures of above- and below-ground biomass in the methods, justify the removal of rhizomes from calculations of root:shoot (or include rhizomes in the calculations and refer to it as belowground:aboveground ratio instead), and consider the implications of not including rhizomes in the calculation of root:shoot. For example, rhizomes would contribute to vertical resilience by contributing to soil volume, a mechanism noted in the paper, and may be an important component when considering potential allocation responses by the genotypes.

We agree with the reviewer that rhizomes also contribute to vertical accretion and should therefore be included in a ratio describing possible allocation patterns. Consequently, we will use the belowground:aboveground ratio instead of root:shoot. Please note, root and rhizome biomass will still be displayed separately (Figure 1d & 1e). Sentences in section 2.3.1 were changed as follows:

Line 116: **Belowground biomass was divided into rhizomes and roots and weighed. The length of rhizomes was also recorded. Additionally, above- and belowground biomass were used to calculate the belowground:aboveground ratio.**

Section 2.3.2. Provide additional information here about the differences in initial measures of plant growth, as noted above.

We added this information to section 2.1. (see comment above).

Results: Consider reorganizing the subsections so that all biomass measures are presented consecutively (Total biomass, above- and belowground biomass, root and rhizome biomass, ratio), followed by shoot and leaf length and number. The figures could be grouped similarly, rather than having the shoot and leaf data combined with the rhizome and root biomass.

In section 3.1, we merged all biomass measures into one paragraph following the suggested order. We also changed figures 1 and 2 accordingly.

Section 3.1. Include reference to Fig. 1 when presenting root:shoot results.

Addressed accordingly.

Section 3.2. Present these data in the order in which they are presented in figure 2 (or reorder the panels in figure 2).

Addressed accordingly.

Sections 3.2-3.3. The data for number of shoots and leaves and rhizome length are not shown anywhere, yet there are significant patterns of interest for leaves and rhizome length that are subsequently highlighted in the discussion. Add a table or figure to show the means for the different genotype x flooding combinations for these data so that all patterns are shown for variables with significant findings.

We added the new Table 2 showing mean and standard error of rhizome length, delta number of leaves and delta number of shoots for different genotype x flooding combinations.

Discussion:

Lines 179-180. What is the significance of low-marsh genotypes maintaining aboveground biomass regardless of flooding treatment?

We wanted to emphasise that in comparison to the high-marsh genotype, the low-marsh genotype did not show a stress response. We rephrased the original sentence to:

**Line 187: In contrast to the high-marsh genotype showing a marked reduction in aboveground biomass, the low-marsh genotype maintained aboveground biomass across all flooding treatments.**

Starting in line 208, we further describe the importance of aboveground biomass for vertical accretion and marsh resilience.

Lines 180-185. Move this to the results or methods as evidence that any initial differences in plant size were insignificant.

We moved it to section 2.1. (see above).

Update the discussion considering any changes in the presentation and interpretation of root:shoot data.

No changes required because rhizome and root biomass have been discussed irrespective of the belowground:aboveground- or root:shoot-ratio.

Some additional technical corrections are provided below:

Line 16: delete “a” prior to “higher aboveground biomass” - [done](#)

Line 41: add a space between “loss” and “(Chmura...” - [done](#)

Line 73: close the parentheses – [In the original pdf file \(bg-2020-manuscript-version3.pdf\) we see that the parenthesis is already closed. Or do you want us to remove the parentheses?](#)

Line 85: add “(described in 2.2)” after “flooding treatments” - [done](#)

Line 85: add “to facilitate drainage” at the end of the sentence. - [done](#)

Line 88: change “placed on each step” to “assigned to one of three flooding treatments” because steps are not explained until the next section. - [done](#)

Lines 143-145: cite Fig. 1 - [done](#)

Line 151: “with increasing flooding” - [done](#)

Line 152: Section numbering is repeated. This should be Section 3.3. - [done](#)

Line 156: Should be Section 3.4. - [done](#)

Line 178: include citations - [done](#)

Line 179: delete “a” before “high aboveground biomass” - [done](#)

Line 206: “have” instead of “has” - [done](#)

References: check formatting; some titles are capitalized throughout and others aren't; scientific names are not italicized - [done](#)

Figures: consider adding panel labels (A, B, C, D) and adding the specific panel reference to the text when figures are referenced. Also, check captions that suggest asterisks indicate significance, but no asterisks are included on the graphs.

[We removed the sentence regarding the asterisks in the captions of figure 2.](#)

[We added panel labels to both figures.](#)

For variables with main effects of flooding but no interactions, these differences can be shown on the graphs by adding letters or symbols denoting differences over the LM-HM pairs for each flooding treatment. Similarly, it would be useful to note variables with differences between genotypes (in the absence of interactive effects) somehow – perhaps by noting this in caption.

[Differences between genotypes within a given flooding treatment have already been indicated using asterisks, letters will be added to show showing differences between flooding treatments to figures 1 and 2.](#)

**Editor:**

Comments to the Author:

Dear authors,

Thank you for the revised manuscript and response letter, which have now been seen by one of the previous reviewers. The reviewer is positive about the changes that you made, but made some more comments and suggestions that need to be considered before this manuscript can be accepted for publication.

Especially the root data raised additional questions. Please clarify why you opted to exclude rhizomes from the belowground biomass for calculation of the root:shoot ratio and what the implications are. You can also consider showing the root:shoot ratio both with and without including rhizomes.

In addition, I would like you to rethink the use of ANCOVA for the statistics. This is not only a more powerful technique, but I think it is also a more correct one for your dataset.

Kind regards,

Sara Vicca

We appreciate the thoughtful comments provided by the Editor.

We agree with the reviewer that rhizomes could play an important role for vertical accretion in the studied system and should not be excluded from the calculated ratio. In addition to that, root and rhizome biomass remain separately displayed/discussed. We made changes to the text, figures and tables accordingly.

We compared ANCOVA and ANOVA results (Table 1 below). ANCOVA does not seem to provide more statistical power in our case. Specifically, we found for four parameters significant effects with ANOVA that were not significant with ANCOVA. Importantly, the opposite (i.e. non-significant with ANOVA but significant with ANCOVA) was never true. We believe that this is the case because ANCOVA assumes that the regression relationship between the dependent variable and covariate is linear. This linearity, however, is not given for all our parameters. In fact, the literature on plant-biomass responses to flooding in tidal wetlands usually shows non-linear, unimodal relationships between biomass parameters and flooding (Kirwan and Guntenspergen 2012, Langley et al. 2013, Redelstein et al. 2018, Mueller et al. 2016). We therefore did not hypothesize linearity and used ANOVA instead. As our study design only includes three flooding treatments, any model assuming a non-linear relationship between flooding and plant parameters is not useful either.

**Table 1:** Results (p-values and significance codes) generated by ANOVA and ANCOVA. Significance codes: \*\*\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \* =  $p < 0.05$ . Orange highlights effects that were detected significant by ANOVA but not ANCOVA.

	ANOVA						ANCOVA					
	ecotype		Flooding (category)		interaction		ecotype		Days of flooding (continuous)		interaction	
total biomass	0.000762	***	1.99E-06	***	0.044168	*	0.00136	**	7.72E-06	***	0.01863	*
aboveground biom	0.000585	***	0.003809	**	0.013577	*	0.000439	***	0.000736	***	0.002884	**
belowground biom	0.044	*	3.01E-07	***	0.594	ns	0.0805	ns	6.01E-05	***	0.3815	ns
root_shoot ratio	0.04064	*	0.00562	**	0.29874	ns	0.0537	ns	0.0672	ns	0.1632	ns
root biomass	0.860763	ns	0.000176	***	0.930845	ns	0.8698	ns	0.00153	**	0.90999	ns
rhizomes_weight	0.0146	*	0.0295	*	0.602	ns	0.0171	*	0.0526	ns	0.9563	ns
rhizomes_length	0.0177	*	0.2006	ns	0.6399	ns	0.0163	*	0.1034	ns	0.4672	ns
delta_leaf_length	0.0146	*	0.5877	ns	0.2493	ns	0.00346	**	0.31151	ns	0.14969	ns
delta_shoot_length	0.0125	*	0.7224	ns	0.3756	ns	0.0119	*	0.4446	ns	0.4485	ns

## References

Kirwan, M. L. and Guntenspergen, G. R.: Feedbacks between inundation, root production, and shoot growth in a rapidly submerging brackish marsh, *J. Ecol.*, 100(3), 764–770, doi:10.1111/j.1365-2745.2012.01957.x, 2012.

Langley, J. A., Mozdzer, T. J., Shepard, K. A., Hagerty, S. B. and Megonigal, J. P.: Tidal marsh plant responses to elevated CO<sub>2</sub>, nitrogen fertilization, and sea level rise, *Glob. Chang. Biol.*, 19, 1495–1503, doi:10.1111/gcb.12147, 2013.

Mueller, P., Jensen, K. and Megonigal, J. P.: Plants mediate soil organic matter decomposition in response to sea level rise, *Glob. Chang. Biol.*, 22(1), 404–414, doi:10.1111/gcb.13082, 2016.

Redelstein, R., Dinter, T., Hertel, D. and Leuschner, C.: Effects of inundation, nutrient availability and plant species diversity on fine root mass and morphology across a saltmarsh flooding gradient, *Front. Plant Sci.*, 9(February), 1–15, doi:10.3389/fpls.2018.00098, 2018.

# Plant genotype determines biomass response to flooding frequency in tidal wetlands

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10 **Abstract.** The persistence of tidal wetland ecosystems like salt marshes is threatened by human interventions and climate change. Particularly the threat of accelerated sea level rise (SLR) has recently gained increasing attention by the scientific community. However, studies investigating the effect of SLR on plants and vertical marsh accretion are usually restricted to the species or community level and do not consider phenotypic plasticity or genetic diversity. To investigate the response of genotypes within the same salt-marsh species to SLR, we used two known genotypes of *Elymus athericus* (Link) Kerguélen  
15 (low-marsh and high-marsh genotypes). In a factorial marsh organ experiment we exposed both genotypes to different flooding frequencies and quantified plant growth parameters. With increasing flooding frequency, the low-marsh genotype showed higher aboveground biomass production compared to the high-marsh genotype. Additionally, the low-marsh genotype generally formed longer rhizomes, shoots and leaves, regardless of flooding frequency. Belowground biomass of both  
20 genotypes decreased with increasing flooding frequency. We conclude that the low-marsh genotype is better adapted to higher flooding frequencies through its ability to allocate resources from below- to aboveground biomass. Given the strong control of plant biomass production on salt-marsh accretion, we argue that these findings yield important implications for our understanding of ecosystem resilience to SLR as well as plant-species distribution in salt marshes.

## 1 Introduction

25 Salt marshes are wetland ecosystems predominantly found along coastlines where they form a transition zone between the marine and the terrestrial environment. Salt marshes provide important ecosystem services like protection of coastlines against storm surges by wave attenuation (Möller et al., 2014), supply of nursery grounds for commercially important fish (Bolle et al., 2009) and mitigation of climate change by long-term carbon sequestration (McLeod et al., 2011). However, human interventions such as land reclamation, eutrophication and climate change threaten the persistence of salt marshes, causing loss rates of 1-2 % of the global area per year (Duarte et al., 2008). Particularly the threat of accelerated sea level rise (SLR)  
30 has recently gained increasing attention by the scientific community (Fitzgerald et al., 2008; Kirwan and Megonigal, 2013; Schuerch et al., 2018).

Generally, up to a certain locally varying threshold of SLR, salt marshes are able to keep up with rates of SLR through their ability to accrete vertically (Kirwan and Megonigal, 2013). During this process, salt-marsh plants act as ‘ecosystem engineers’ because their aboveground biomass reduces water flow velocity and hydrodynamic forces, which results in a decrease in the sediment-loading capacity of the water and an increase in sediment settlement (Morris et al., 2002; Yang, 1998). Furthermore, a high belowground biomass production and decreased decomposition rates in oxygen-deficient soils lead to an accumulation of organic matter and thereby expansion of soil volume. Whether above- or belowground processes predominantly contribute to vertical accretion, depends on the marsh type (i.e. minerogenic vs. organogenic) and often depends on local tidal amplitude (Allen, 2000; Kirwan and Megonigal, 2013; Nolte et al., 2013b). Yet, in both cases, biomass production of the marsh vegetation is an important driver of accretion. When accretion rates are too low and threshold values of SLR are exceeded, plant growth is negatively affected, leading to a negative feedback loop, which ensues marsh submergence and finally marsh loss (Chmura, 2013; Kirwan and Guntenspergen, 2012).

Recently, more studies have therefore been focusing on salt-marsh resilience to projected rates of SLR by studying the vegetation response. To examine how vegetation responds to changes in flooding regimes, so called ‘marsh organ’ experiments have been proven as convenient and insightful approaches. A marsh organ consists of several mesocosms arranged next to each other and along an elevational gradient. Kirwan and Guntenspergen (2012), for example, placed a marsh organ in a brackish marsh to examine the effect of varying sea levels on plant growth of two marsh species and what possible implications this may have on accretion processes. They observed that marsh elevation within the tidal frame determines whether root biomass increases or decreases with SLR. In contrast to belowground productivity, aboveground biomass response was species dependent. Numerous other studies confirmed that responses of marsh plants to sea level changes are highly species-specific (Eleuterius and Eleuterius, 1979; Kirwan and Guntenspergen, 2015; Langley et al., 2013; Morris et al., 2013). Therefore, the effect of SLR on plant biomass production, and thus the ability of the ecosystem to accrete vertically, can only be understood if SLR-induced shifts in plant species composition also are taken into account.

However, such studies on the effect of SLR on plants are usually restricted to the species or community level (Kirwan and Guntenspergen, 2015; Langley et al., 2013; Morris et al., 2013). They usually do not consider the high degree of phenotypic plasticity and genetic diversity within many species, as for instance evident between different locally adapted populations (Valladares et al., 2014). Yet, understanding a species’ adaptive genetic plasticity can be crucial to evaluate its response to environmental change (Razgour et al., 2019). Indeed, in some cases environmental change can in fact induce larger variability in plant biomass production within than between species (Beierkuhnlein et al., 2011). We therefore argue, that intraspecific differences in the biomass response to SLR of salt-marsh plants are likely relevant and require more attention by the scientific community to understand ecosystem resilience.

To investigate the response of genotypes within the same salt-marsh species to SLR, we used two known genotypes of the grass *Elymus athericus* (Link) Kerguélen. This species is widely distributed in NW European salt marshes and usually grows in high-elevated and therefore less-frequently flooded zones of the salt marsh (= high marsh) (Nolte et al., 2019). In the high marsh, the tall grass forms dense, monospecific stands with a low local plant species diversity (Bakker et al., 2003). Recently,



however, populations of *E. athericus* have been observed spreading into lower and thus more frequently flooded zones of the marsh (= low marsh) (Oloff et al., 1997; Veeneklaas et al., 2013). Low- and high-marsh genotypes are visually distinguishable, as the low-marsh genotype develops a specific phenotype different from the high-marsh genotype (Bockelmann et al., 2003). If genotypes respond differently to increased flooding frequencies, for instance in biomass productivity, it might affect salt-marsh responses to SLR.

The aim of this study was to investigate potential adaptations of the low-marsh genotype to increased flooding frequencies, to improve current evaluations of salt-marsh resilience to SLR. To test the hypothesis that the low-marsh genotype performs better at increased flooding frequencies than the high-marsh genotype, **which would be reflected in above- and belowground growth parameters like biomass production and shoot, leaf and rhizome lengths**, we conducted a factorial marsh organ experiment. We exposed both genotypes of *E. athericus* to three different flooding frequencies and quantified plant growth parameters to compare their performance and assess morphological adaptations of the low-marsh genotype.

## 2 Material and methods

### 2.1 Plant collection and culture

Plants were collected in April 2015 from a salt marsh on the Dutch Island Schiermonnikoog (53°30'N, 6°16'E) from stands that have previously been identified to be dominated by genetically distinct populations of *E. athericus*, i.e. high-marsh (HM) and low-marsh (LM) genotypes (Bockelmann et al., 2003). **On Schiermonnikoog, *Elymus athericus* can be found in higher and lower elevated sites, which are inundated 20 – 125 and 90 – 270 times per year, respectively (Bockelmann et al., 2003). Soil salinities range from 22 to 29 ppt (Bolhuis et al., 2013).** Plants and soil were extracted in the form of intact sods to keep them alive during transport. In Hamburg, soil was removed and roots were rinsed before both genotypes were planted separately in trays with standardised potting soil. Until the start of the experiment (i.e. for 24 months), plants were kept under identical environmental conditions in a common garden at the Institute of Plant Science and Microbiology. Ramets of these plants were used for this study. In July 2017, single plants of similar **biomass** were **selected based on visual assessment (no obvious outliers)**, transplanted to separate pots and randomly assigned to the flooding treatments (**described in 2.2**). **Initial shoot length and shoot number was tested for differences between genotypes and flooding frequencies to ensure that results were not biased by unequal plant size at the beginning of the experiment. There were no significant differences regarding shoot length (genotype:  $F = 0.787$ ,  $p = 0.380$ ; flooding frequency:  $F = 0.127$ ,  $p = 0.881$ ; genotype\*flooding frequency:  $F = 0.231$ ,  $p = 0.795$ ) and number of shoots (genotype:  $Wald = 2.203$ ,  $p = 0.137$ ; flooding frequency:  $Wald = 0.357$ ,  $p = 0.837$ ; genotype\*flooding frequency:  $Wald = 0.005$ ,  $p = 0.997$ ).** The pots were 15 cm in diameter, 17 cm in height and had holes in the bottom **to facilitate drainage**. They were filled with salt marsh soil taken from the salt marsh at Sönke-Nissen-Koog, Germany (54°36'N, 8°49'E) which was sieved (with a 1 cm mesh) and homogenised beforehand (see Nolte et al. (2013a) for soil properties). Eight replicates (i.e. single plants in separate pots) per genotype were **assigned to one of three flooding treatments**, so that a total number of 48 plants were used in this study.

## 2.2 Experimental set-up

Plants were placed onto three steps (step height: 20 cm) within a tidal-tank facility (Hanke et al., 2015), to create three different flooding frequencies. The tidal tank is located outdoors at the Institute of Plant Science and Microbiology and has a total volume of 6.75 m<sup>3</sup> (dimensions: 3 x 1.5 x 1.5 m). A pump was used to fill and empty the tidal tank at regular intervals to mimic tides by alternating between three different maximum water levels. Pots were fully drained between flooding events. Flooding with the respective maximum water level reached 3 cm above soil surface, lasted two hours and took place twice a day. The lowest step was flooded every day, which represented the highest frequency. The flooding of the middle step (moderate flooding frequency) happened weekly, while plants on the highest step were flooded only every two weeks (lowest flooding frequency). Highest and lowest flooding frequency reflect the natural flooding gradient between pioneer zone and high marsh in many NW European salt marshes, including the site where our plants were collected (Bockelmann et al., 2002). A CTD diver combined with a baro diver (Van Essen Instruments, Delft, The Netherlands) was used to monitor flooding cycles. Artificial sea salt (AB Aqua Medic GmbH, Germany) was suspended in tap water to create a salinity of about 20-22 ppt. To minimise the impact of other effects than flooding frequency and genotype, the pots were circulated on each step, other seedlings and algae were removed once a week. Concurrently, water level and salinity were checked as well. The experiment ran for approx. 12 weeks from mid-July to early October 2017.

## 2.3 Measurements

### 2.3.1 Biomass

At the end of the experiment (2nd of October 2017), the plants were harvested, separated into above- and belowground biomass, dried for two days at 70°C and weighed. **Belowground biomass was divided into rhizomes and roots and weighed. The length of rhizomes was also recorded. Additionally, above- and belowground biomass were used to calculate the belowground:aboveground ratio.**

### 2.3.2 Plant growth

At the beginning and the end of the experiment, plant shoot and leaf length as well as number of shoots and leaves were measured. The difference between both measurements was calculated and designated as  $\Delta$ . Only living plant material was taken into account and length measurements (leaf and shoot length) were carried out on the longest leaves or shoots.

## 2.4 Statistical analysis

Data were tested for normality by applying the Shapiro-Wilk-Test. Except count data (e.g. number of leaves), all parameters were normally distributed and therefore further analysed applying factorial ANOVAs. Due to the well-balanced study design, potential moderate deviations from homogeneity of variance between groups were considered unimportant for ANOVA testing (Box, 1954; McGuinness, 2002). Each analysis included genotype and flooding frequency as well as their interaction as

explanatory variables. To detect significant differences between treatments (flooding frequency and genotype), post-hoc tests (Tukey's HSD) were conducted. To analyse count data, i.e. number of shoots and number of leaves, generalized linear models (GLM) were applied assuming a Poisson distribution and including the explanatory variables genotype and flooding frequency, as well as two-way interaction effects. Each GLM was checked for overdispersion (Pearson Chi<sup>2</sup> dispersion parameter) and was refitted afterwards if necessary, using the standard procedure of the applied program. All statistical analyses were performed using STATISTICA 13 (StatSoft Inc., Tulsa, OK, USA).

### 3 Results

#### 135 3.1 Biomass

Total biomass, defined as the sum of dry above- and belowground plant biomass, differed significantly between genotypes and flooding frequencies (Table 1). In addition, the interaction of both factors showed a significant effect on total biomass. Total biomass production of the high-marsh genotype decreased steadily with increasing flooding frequency, whereas the total biomass of the low-marsh genotype decreased less distinctly (Fig. 1a). The difference between low-marsh and high-marsh genotypes was most pronounced at the highest flooding frequency. In fact, the low-marsh genotype produced almost twice as much total biomass at highest flooding frequency as the high-marsh genotype (LM:  $4.61 \pm 0.70$  g and HM:  $2.66 \pm 0.52$  g, mean  $\pm$  standard deviation).

Genotype and flooding frequency as well as their interaction had a significant effect on the aboveground biomass production. Aboveground biomass of the high-marsh genotype decreased with increasing flooding frequency from  $3.31 \pm 0.57$  g to  $2.03 \pm 0.38$  g (Fig. 1b). However, aboveground biomass production of the low-marsh genotype remained constant at about  $3.46 \pm 0.45$  g on all flooding frequencies.

In contrast to the genotype-specific aboveground biomass response to flooding, belowground biomass of both genotypes decreased with increasing flooding frequency (Fig. 1c). Results indicate a more pronounced effect of flooding frequency on belowground biomass production compared to the factor genotype (Table 1). Under all flooding frequencies, the low-marsh genotype produced slightly more belowground biomass than the high-marsh genotype (LM:  $1.63 \pm 0.78$  g, HM:  $1.30 \pm 0.76$  g).

Root biomass production (**belowground biomass without rhizomes**) was significantly affected by flooding frequency ( $F = 10.69$ ,  $p < 0.001$ ), but did not differ between genotypes. Root biomass decreased with increasing flooding frequency for both genotypes (Fig. 1d). In contrast, biomass of rhizomes was significantly affected by both genotype and flooding frequency (Table 1). Mean rhizome biomass of the low-marsh genotype was higher than of the high-marsh genotype (LM:  $0.55 \pm 0.50$  g, HM:  $0.26 \pm 0.28$  g) with the most pronounced differences on lowest and highest flooding frequency (Fig. 1e). In some cases, the low-marsh genotype formed very long rhizomes (up to 166 cm length, coiled around the soil). On average, rhizomes of the low-marsh genotype were nearly twice as long as those of the high-marsh genotype (LM:  $51.43 \pm 41.11$  cm, HM:  $26.63 \pm 27.23$  cm, **Table 2**). Genotypes significantly differed in the length of rhizomes ( $F = 6.102$ ,  $p < 0.05$ , **Table 1**).

160 Belowground:aboveground-ratio was significantly affected by flooding frequency (Table 1) and decreased with increasing flooding frequency (Fig. 1f).

### 3.2 $\Delta$ Leaf and shoot length

The increase in shoot and leaf length significantly differed between genotypes (Table 1). Regarding leaf length, the high-marsh genotype showed approximately the same increase on all flooding frequencies ( $1.9 \pm 4.45$  cm). The low-marsh genotype had similar increases of leaf lengths at the lowest flooding frequency but showed pronounced increases of leaf length with increasing flooding frequency ( $7.03 \pm 2.17$  cm) (Fig. 2a). Increase in shoot length of the low-marsh genotype was twice as high as that of the high-marsh genotype (LM:  $10.78 \pm 6.18$  cm, HM:  $5.57 \pm 6.58$  cm, Fig. 2b).

### 3.3 $\Delta$ Number of leaves and shoots

Neither genotype nor flooding frequency had a significant effect on the increase in number of shoots (Table 1). However, for the increase in number of leaves, a significant effect of flooding frequency was detected (Wald = 19.69,  $p < 0.001$ ). With highest flooding frequency, both genotypes produced the lowest number of new leaves (LM:  $10.4 \pm 4.0$ , HM:  $8.9 \pm 2.1$ ; Table 2).

## 4 Discussion

Assessments of plant responses to changed hydrological conditions (e.g. SLR) have thus far focused mainly on comparisons on species level. However, variability in plant responses within species can be considerably higher than between species (Beierkuhnlein et al., 2011). In this study, we therefore investigated differences in plant response between genotypes of the same species (*Elymus athericus*) to better understand the importance of intraspecific variability for evaluations of future ecosystem functionality and resilience. We found, in line with our hypothesis on biomass production, that the low-marsh genotype performs better than the high-marsh genotype under increased flooding frequency. Additionally, the low-marsh genotype generally formed longer rhizomes, shoots and leaves, regardless of flooding frequency (Fig. 1 & 2). We argue that these findings yield important implications for our understanding of ecosystem resilience to SLR as well as plant-species distribution in salt marshes.

We found a higher total biomass of the low-marsh genotype, which was particularly pronounced under high flooding frequency (Fig. 1a). This result indicates a better adaptation of the low-marsh genotype to lower elevated, more frequently flooded conditions. When separating above- and belowground biomass, the high-marsh genotype showed a decrease of both biomass parameters with increasing flooding frequency. Aboveground biomass is important for maintaining photosynthesis (Johnson, 2016), so that its reduction can be interpreted as reduction in performance as well. In contrast to the high-marsh genotype showing a marked reduction in aboveground biomass, the low-marsh genotype maintained aboveground biomass across all flooding treatments.

190 We also found a decrease of belowground biomass with increasing flooding frequency in both genotypes. This can be interpreted as an adaptive trait, because a reduction of belowground biomass reduces the number of respiring roots and thereby improves the diffusion of oxygen to the roots (Naidoo and Naidoo, 1992; Voesenek et al., 1988). In line with these results, an increased aboveground biomass production while belowground biomass decreased was found for other flooding adapted plant species such as *Taxodium distichum*, *Danthonia montevidensis* and *Paspalum dilatatum* (Megonigal and Day, 1992; Rubio et al., 1995).

The difference in aboveground biomass response between the two genotypes seems to be mainly explained by genotype-specific increases in leaf and shoot length, whereas the number of both remained similar (Fig. 2, Table 1). Likewise, Voesenek et al. (1988) found a marked increase in leaf length in the flooding adapted *Rumex palustris* under waterlogging, but no increase in number of leaves. The distinct increase in leaf and shoot length in addition to the simultaneous reduction of belowground biomass of the low-marsh genotype of *Elymus athericus* found in our study, indicate resource allocation as response to flooding. For other species it was found that reallocated resources fuel elongation of shoots and leaves to maintain gas exchange and avoid light dissipation through water (Blanch et al., 1999; Grace, 1989). Our results suggest that this response may be also present in *E. athericus*, which could improve its chances of survival under higher flooding frequencies e.g. due to accelerated SLR.

205 Vertical accretion in the minerogenic salt marshes of the Wadden Sea is primarily driven by sedimentation (Allen, 2000; Nolte et al., 2013b), which is strongly controlled by the sediment-trapping capacity of the aboveground biomass (Morris et al., 2002; Yang, 1998). The strong aboveground biomass response to increased flooding frequencies of the low-marsh genotype found in our study may therefore have a positive effect on vertical accretion rates and thereby marsh resilience to rising sea levels. *E. athericus* is not the only salt-marsh species characterised by a high degree of genetic diversity. In previous studies, genotypes of several salt-marsh grasses have been described and tested for intraspecific differences in plant response to changing environmental conditions, including *Puccinellia maritima*, *Phragmites australis* and *Spartina alterniflora* (Gray, 1985; Mozdzer and Megonigal, 2012; Proffitt et al., 2003; Seliskar et al., 2002). They showed high genotypic variations affecting colonisation success, species composition and even ecosystem function.

Compared to the root biomass of both genotypes, which responded similarly and decreased with increasing flooding frequency, rhizome length differed significantly between the genotypes (Table 1). The formation of longer rhizomes by the low-marsh genotype, especially under high flooding frequency, could serve as an escape strategy to expand into more favourable habitats (Hartnett and Bazzaz, 1983; Lovett-Doust, 1981). In previous studies, it was reported that *E. athericus* usually expands via a ‘phalanx’ growth strategy, which means that parental plants invest in many but rather short rhizomes to utilise resources in a favourable habitat (Bockelmann and Neuhaus, 1999). Field observations of the same authors and results of our study, however, indicate that *E. athericus* is able to alter its strategy to the ‘guerrilla’ form, by producing longer rhizomes. The ‘guerrilla’ strategy is usually found in plants characteristic for early successional stages as it enables plants to spread quickly and exploit new favourable areas (Lovett-Doust, 1981). However, overall rhizome length of our study should be interpreted with caution because of potential edge effects caused by the experimental mesocosms.

225 The change of expansion strategy together with a better adaptation to higher flooding frequencies may lead to a displacement of the high-marsh genotype under accelerated SLR. However, until now, the Wadden Sea salt marshes are able to cope with current rates of sea level rise due to high accretion rates (Esselink et al., 2017; Nolte et al., 2013a). If rates of SLR remain stable, the low-marsh genotype of the tall grass *E. athericus* has the potential to expand further into the low marsh and outcompete other species via light competition, potentially reducing local species diversity.

## 5 Methodological considerations

230 We suggest that the experimental setup including a tidal tank and steps proved suitable to investigate the effects of different flooding frequencies on salt-marsh vegetation. Nevertheless, we recommend repeating this experiment in situ, for example as a transplant experiment, to estimate actual effect size under more natural conditions, as drainage and plant-soil interactions might have been different in the tidal tank and could have affected biomass production.

## 6 Conclusion

235 The present work revealed marked differences in the plant biomass response to changes in flooding frequency between two genotypes of the dominant European salt-marsh grass *Elymus athericus*. Furthermore, we observed large differences in rhizome production between genotypes, which is interpreted as a change in growth strategy. The alteration of its growth strategy and the higher aboveground biomass productivity of the low-marsh genotype implies a larger potential of the low-marsh genotype to invade and establish at lower elevations of the tidal frame. Considering the generally low plant species  
240 diversity of salt marshes (e.g. Silliman, 2014; Wanner et al., 2014) and the strong feedbacks between plant growth and accelerated SLR (Kirwan and Megonigal, 2013), it is possible that intraspecific variation and adaptive capacity in salt-marsh plants acts as an important but overlooked mediator of ecosystem resilience.

## 7 Data availability

All data presented in this paper is available from the corresponding author upon request.

## 245 8 Author contribution

All authors contributed to the design of the experiments. SR and HT conducted the experiment and performed the measurements. SR analysed the data and wrote the manuscript. All authors contributed to the discussion of results and the final manuscript.

## 9 Competing interests

250 The authors declare that they have no conflict of interest.

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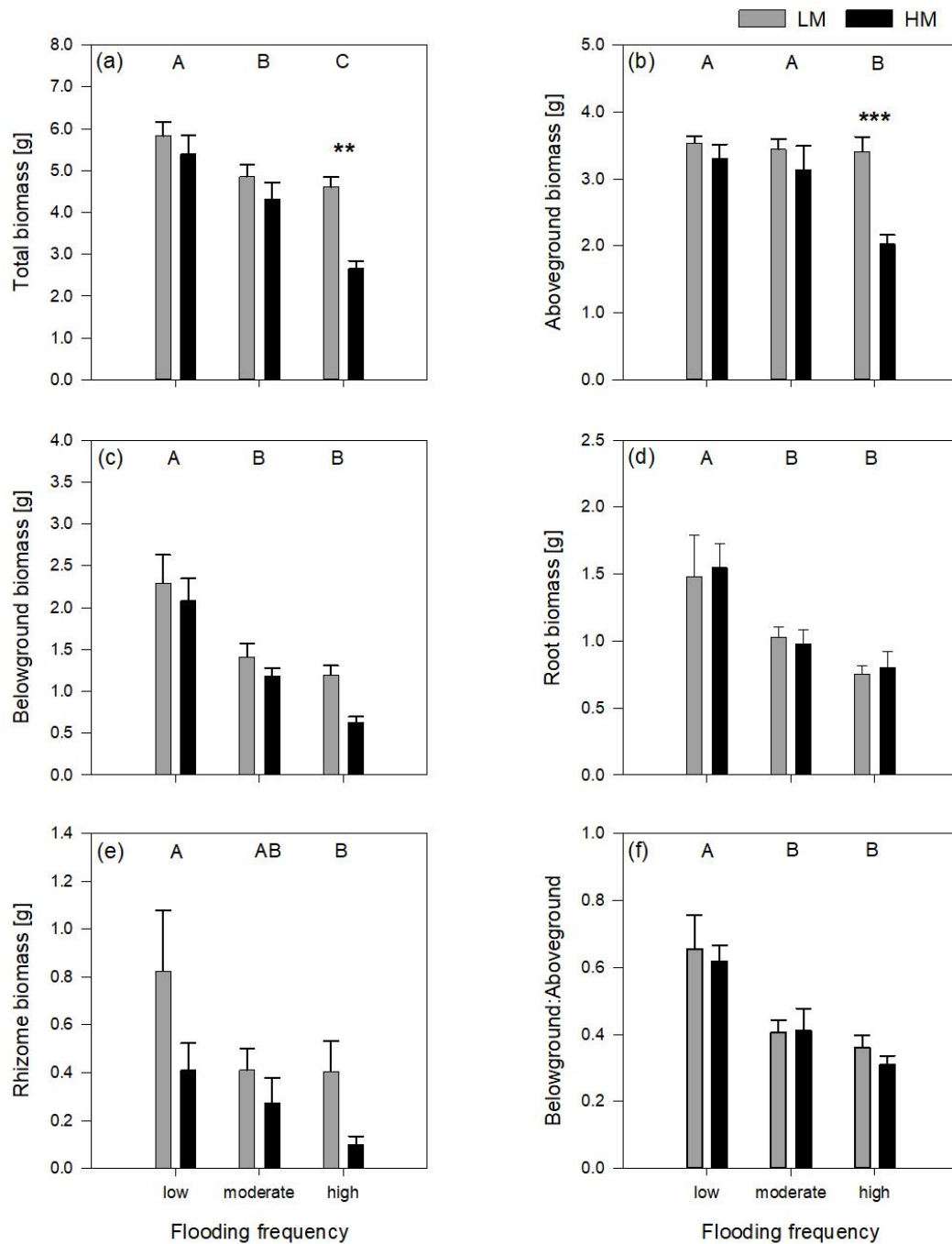
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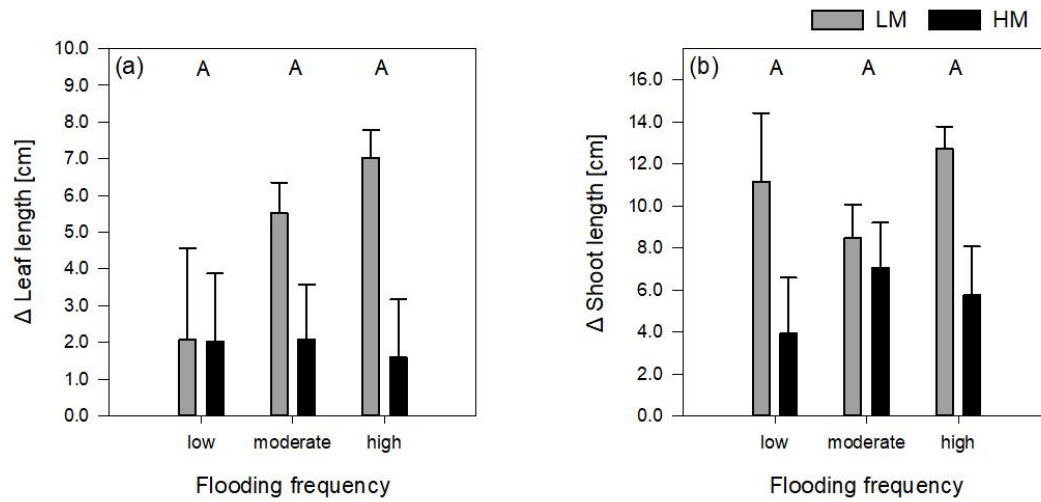
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**Figure 1: Total biomass (a), aboveground biomass (b), belowground biomass (c), root biomass (d), rhizome biomass (e) and belowground-aboveground ratio (f) of both genotypes on three different flooding frequencies (mean + standard errors). Stars show significant differences between low-marsh (LM) and high-marsh (HM) genotypes within the same flooding treatment based on**

365 Tukey's HSD post-hoc test (\*  $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Capital letters indicate significant differences between flooding frequencies.



370 **Figure 2: Delta leaf length (a) and delta shoot length (b) of both genotypes on three different flooding frequencies (mean + standard errors). Capital letters indicate significant differences between flooding frequencies.**

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**Table 1: Summary statistics of main response variables in the experiment testing for effects of flooding and genotype of *Elymus athericus* on its biomass and growth. Count variables (number of shoots, number of leaves) are analysed through GLM, all other variables by a two-way factorial ANOVA. Significant p-values are shown in bold letters.**

		genotype (df = 1)		flooding frequency (df = 2)		genotype * flooding frequency (df = 2)	
Response variable	statistical test	test statistic	p-value	test statistic	p-value	test statistic	p-value
<i>Total biomass [g]</i>	factorial ANOVA	F = 13.18	<b>0.001</b>	F = 18.24	<b>0.000</b>	F = 3.36	<b>0.044</b>
<i>Aboveground biomass [g]</i>	factorial ANOVA	F = 13.84	<b>0.001</b>	F = 6.38	<b>0.004</b>	F = 4.77	<b>0.014</b>
<i>Belowground biomass [g]</i>	factorial ANOVA	F = 4.31	<b>0.044</b>	F = 21.93	<b>0.000</b>	F = 0.53	0.594
$\Delta$ Shoot length [cm]	factorial ANOVA	F = 7.77	<b>0.008</b>	F = 0.32	0.725	F = 1.03	0.365
$\Delta$ Leaf length [cm]	factorial ANOVA	F = 5.09	<b>0.029</b>	F = 1.08	0.350	F = 1.42	0.252
$\Delta$ Number of shoots	GLM	Wald = 0.00	0.952	Wald = 5.87	0.053	Wald = 0.78	0.678
$\Delta$ Number of leaves	GLM	Wald = 0.44	0.507	Wald = 19.69	<b>0.000</b>	Wald = 1.60	0.449
<i>Root biomass [g]</i>	factorial ANOVA	F = 0.03	0.861	F = 10.69	<b>0.000</b>	F = 0.07	0.931
<i>Rhizome biomass [g]</i>	factorial ANOVA	F = 6.49	<b>0.015</b>	F = 3.84	<b>0.030</b>	F = 0.51	0.602
<i>Rhizome length [cm]</i>	factorial ANOVA	F = 6.10	<b>0.018</b>	F = 1.67	0.201	F = 0.45	0.640
<i>Belowground:Aboveground</i>	factorial ANOVA	F = 0.34	0.561	F = 14.56	<b>0.000</b>	F = 0.13	0.877

**Table 2: Mean and standard error (SE) of rhizome length [cm], delta number of leaves and delta number of shoots for different combinations of factors genotype and flooding frequency.**

			Rhizome length [cm]		Δ Number of leaves		Δ Number of shoots	
genotype	flooding frequency	N	mean	SE	mean	SE	mean	SE
LM	low	8	63.38	15.13	15.88	2.43	7.00	0.82
LM	moderate	8	45.28	10.35	21.75	2.45	9.50	0.78
LM	high	8	45.63	18.10	10.43	1.53	6.25	1.18
HM	low	8	37.13	9.85	18.00	3.19	8.13	1.52
HM	moderate	8	32.81	11.76	16.88	3.42	8.50	1.65
HM	high	8	9.94	2.91	8.86	0.80	5.88	1.08