

Driving forces behind latitudinal variations in plant–herbivore interactions in SW Atlantic salt marshes

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ABSTRACT: Despite long-standing interest in the hypothesis that the strength of plant–herbivore interactions decreases at higher latitude, supporting evidence is scarce and the data are conflicting. We conducted a field survey and 2 experiments to examine this hypothesis, focusing on herbivory by a stem-borer moth (*Haimbachia* sp. nov.) on the dominant SW Atlantic marsh grasses (*Spartina alterniflora* and *S. densiflora*). Field surveys indicated that herbivore abundances and damage, although tending to decrease simultaneously, are unrelated to latitude. Herbivore abundances were related to latitude-dependent variables such as day length and temperature, and also to latitude-independent variables such as precipitation, salinity, and tide amplitude. Abundances were indirectly related to the effects of these variables and sediment characteristics on plant traits like density, height, and tissue composition. After 33 mo, herbivore abundances and damage to high-latitude plants transplanted to low latitudes were 50 times greater than in plants transplanted from low- to high-latitude sites. In a common garden experiment (38° 56' S) without herbivore pressure, differences persisted in plant traits from high and low latitudes, suggesting a lack of herbivore-induced effects on these plant traits. The persisting conspecific differences in plant traits translocated along latitude suggest that these variations are under genetic control. Thus, our results provide evidence that although plant–herbivore interactions are more important at lower latitudes, many additional and contingent variables unrelated to latitude can interrupt this geographic pattern.

KEY WORDS: Plant–herbivore interactions · Latitude gradients · Coastal ecosystem ecology · Trophic interactions · Marsh · Salinity · *Spartina* · Stem-borer moth

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INTRODUCTION

Selective pressures on organisms vary throughout latitudinal gradients of abiotic factors (Schemske et al. 2009), as well as the nature of organism–abiotic factor interactions (Więski & Pennings 2014). Some evidence suggests that species interactions (e.g. herbivory, competition, and predation) become stronger toward the tropics (Schemske et al. 2009). In the case of plant–herbivore interactions, it is expected that herbivore pressure should increase at lower latitudes, and

correspondingly, plants should evolve more effective defenses (Coley & Barone 1996, Schemske et al. 2009, Więski & Pennings 2014, Hahn & Maron 2016). However, the generality of this hypothesis has been questioned, since it is supported only in the Northern Hemisphere (Zhang et al. 2016), and also due to the existence of opposite evidence, or lack of herbivory effects (e.g. Moles et al. 2011, Lim et al. 2015, Zhang et al. 2016). A possible explanation is the existence of many variables affecting plants or herbivores that are not controlled by latitude (Pennings & Silliman 2005,

McCall & Pennings 2012, Anstett et al 2016, Kooyers et al. 2017), which increase the context dependence of the observed pattern among different world regions. In addition, tests of this hypothesis are relatively scarce (e.g. Long et al. 2011, Więski & Pennings 2014), and deviations from this latitude–herbivory pattern could be due to comparisons of different species of plants and/or herbivores, or even changes in the communities along latitudinal gradients (Anstett et al. 2014). Given that physical factors are expected to change (Adam 2002, IPCC 2014), and that such factors also vary along latitude (e.g. De Frenne et al. 2013), understanding the effect of these geographic variations on the mechanisms driving species interactions is critical to understand ecosystem function and to predict responses to climate change.

Intertidal marshes are some of the most common coastal habitats (Adam 2002). These habitats are dominated by a few plant species, some of which are among the most widely distributed plants worldwide (e.g. *Spartina* species, Adam 2002). The herbivore pressure in marshes has been reported to be stronger at low than at high latitudes (e.g. Pennings & Silliman 2005). These species face very different environmental conditions along latitude (Pennings & Silliman 2005, Canepuccia et al. 2013), varying some of their traits constitutively (i.e. genetically based traits) or by induction (e.g. Więski & Pennings 2014) in relation to the prevailing local biotic and physical conditions. Southwest Atlantic (SWA) *Spartina* marshes are distributed along a latitudinal gradient (Isacch et al. 2006). These marshes are dominated by two *Spartina* grasses, *S. alterniflora* and *S. densiflora* (Isacch et al. 2006). Within these marshes, there is only 1 stem-boring moth (*Haimbachia* sp. nov.), which is a common *Spartina*-specific herbivore (Canepuccia et al. 2011). The moth imagoes emerge in early summer, and as a consequence of damage by larvae, the attacked stems die (Canepuccia et al. 2011). A single larva of this moth lives inside a *Spartina* stem, feeding on a large portion of the basal and central stem tissue. This particular herbivore–plant interaction allows easy identification of the herbivore damage as well as the variation in the number of herbivores per area (Cane-

puccia et al. 2011). Such characteristics, paired with the observed decrease in moth density with latitude (Canepuccia et al. 2011), make this plant–herbivore system extremely attractive to examine how changes in physical factors along latitudes modify plant–herbivore interactions. The use of this system precludes the existence of artifacts resulting from the comparison of different species of plants or herbivores, and different communities along latitudes.

By using the *Spartina*–moth interaction as a model, our goal was to test the hypotheses that: (1) the strength of plant–herbivore interactions decreases with latitude, driven by the variation in physical and environmental conditions, and (2) variations in plant traits that impact herbivory along latitudes are under genetic control. To test the first hypothesis, we describe the gradients in temperature, precipitation, tidal level, and salinity along 8° of latitude along the SWA coastline (Fig. 1). We then provide an overview

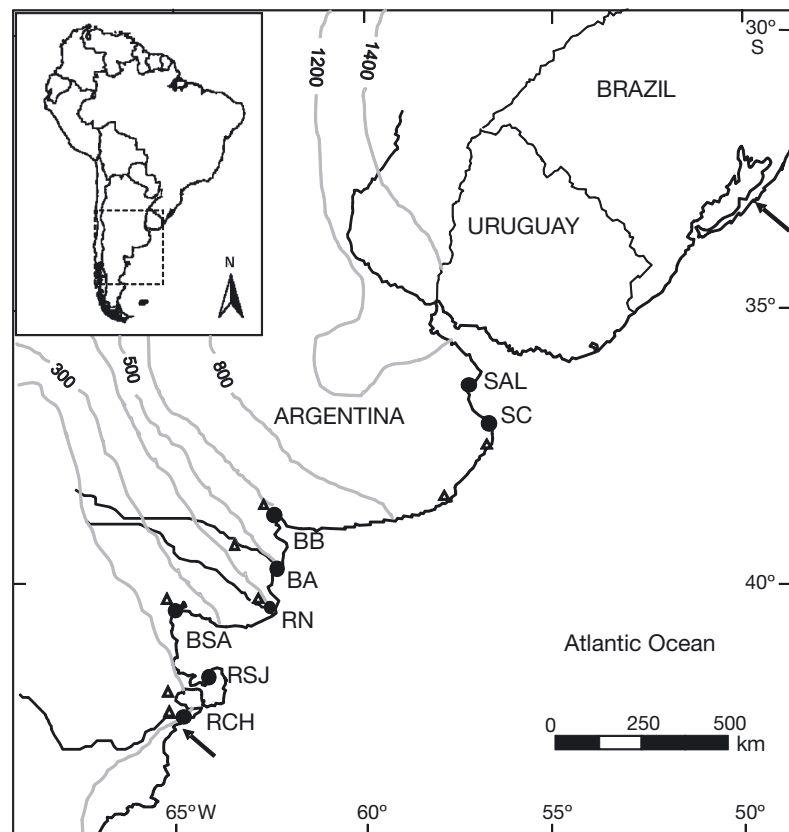


Fig. 1. Marshes sampled along the SW Atlantic coast: the outlet of Salado River (SAL); San Clemente (SC); Bahía Blanca (BB); Bahía Anegada (BA); the outlet of Rio Negro (RN); Bahía San Antonio (BSA); Riacho San Jose (RSJ); and the outlet of Rio Chubut (RCH). The coast between the arrows shows the area of development of tidal marshes dominated by *Spartina* spp. grasses. Triangles indicate meteorological stations. The isohyets for the study sites are gray lines with the rainfall values in millimeters

of the latitudinal variation in these variables, as well as their possible effects on herbivore abundances, plant traits, and plant–herbivore interactions. To test the second hypothesis, we experimentally explored if *Spartina* species transplanted to higher or lower latitudes from their origin, and therefore growing under different environmental conditions, suffer different herbivory pressure. Here, we were particularly interested in comparing the rates of moth attacks on plants with traits from high and low latitudes to determine if these plant trait variations were under genetic control or instead represented plastic responses induced by the variation in environmental cues. Thus, we evaluated the hypothesis that herbivory decreases with latitude, using natural gradients and performing a field survey and two experiments using the same species of plants and herbivore, within the same habitat.

MATERIALS AND METHODS

Study area

To evaluate the variation in plant–herbivore interactions, we selected 8 marshes dominated by *Spartina densiflora* and *S. alterniflora* along the SWA coast (Fig. 1). The sites were: the outlet of the Salado River (SAL: 35° 44' S, 57° 22' W); San Clemente (SC: 36° 19' S, 56° 46' W); Bahía Blanca (BB: 38° 51' S, 62° 07' W); Bahía Anegada (BA: 40° 31' S, 62° 19' O); the outlet of the river Rio Negro (RN: 41° 01' S, 62° 48' W); Bahía San Antonio (BSA: 40° 43' S, 64° 54' W); Riacho San Jose (RSJ: 42° 25' S, 64° 37' W); and the outlet of the Chubut River (RCH: 43° 19' S, 65° 04' W). These marshes are characterized by two main habitats: (1) the lower marsh, which develops at the maritime front and is occupied by a monoculture stand of the smooth cordgrass *S. alterniflora*; and (2) the high marsh, which develops at the upper intertidal height and is dominated by the dense-flowered cordgrass *S. densiflora* and the perennial glasswort *Sarcocornia perennis* (Isacch et al. 2006). Along SWA marshes, the larvae of the stem-boring moth *Haimbachia* sp. nov. (hereafter 'moth larvae') are common herbivores, and their abundance seems to decrease with latitude (Canepuccia et al. 2010a, 2011). This middle latitudinal range of marshes is distributed along a southward decreasing gradient in precipitation from 900 to 200 mm yr⁻¹ together with an increment of daily thermal amplitudes (Canepuccia et al. 2013). From the ocean side, the sampled marshes are distributed along a gradient of southward increases in

tidal amplitudes from a microtidal condition (0.75 m above sea level [a.s.l.]) to a meso-macrotidal condition (3 to 6 m a.s.l., Isacch et al. 2006, see Table S1 in the Supplement at www.int-res.com/articles/suppl/m603p093_supp.pdf).

Strength and significance of direct and indirect effects on plant–herbivore interactions

To estimate the frequencies of moth larvae, in spring of 2010 (full growing season of the moth larvae), we collected all mature plant stems from 10 randomly selected plots (25 × 25 cm) at the center of the spatial distribution of each *Spartina* species (*S. alterniflora* and *S. densiflora*) for each study site (Fig. 1). Hereafter sampling plots were at least 20 m apart to avoid collecting the same plant genotypes (e.g. Richards et al. 2004). All stems were taken to the laboratory and cut longitudinally. We only considered those stems with larvae inside as having been attacked, to prevent overestimating the attack rates by counting stems attacked in previous seasons. We then estimated the frequency of moth attacks as the ratio between the number of attacked stems and the total number of stems in each plot for each *Spartina* species.

We estimated plant traits that may be associated with their quality and quantity as food for marsh herbivores (e.g. Siska et al. 2002, Stiling & Moon 2005, Canepuccia et al. 2011), i.e. plant densities, heights of stems, fiber contents, and carbon:nitrogen (C:N) ratios, for both *Spartina* species for each study site. Densities of plants were measured by counting all mature stems in the same plots where moth frequencies were estimated. Plant height was estimated for a single randomly chosen plant within each plot. Total fiber (neutral detergent fiber, following Van Soest et al. 1991) and C:N ratios in plant tissue were estimated from a pool of 20 stems from 5 randomly chosen plots for each site and each plant species. All C and N samples were analyzed at the Stable Isotope Facility of the University of California, Davis (USA).

To characterize the prevailing environmental conditions at each study site, we estimated major physical variables associated with plant growth, such as precipitation (Canepuccia et al. 2010b, Eiserhardt et al. 2011), air temperature (Speziale et al. 2010), sediment organic matter, salinity, and moisture (Bertness et al. 1992, 2008, Dagar 2010), and particularly important for marsh vegetation, anoxic stress, sediment grain size, and tidal amplitude (Bertness et al. 1992, Pennings et al. 2005, Daleo & Iribarne 2009, Canepuc-

cia et al. 2010a). Averages of the last 5 yr of annual precipitation values and the daily maximum and minimum temperatures were calculated from the nearest Argentinean National Weather Service (Servicio Meteorológico Nacional Argentino) station for each study site (see Fig. 1) along the latitudinal gradient studied. This 5 yr average was calculated given that *Spartina* stems do not die in winter but survive almost 2 yr (stems survive on average 18 mo, Vera et al. 2009) while plant roots and plant stolons live for several years (A. Canepuccia pers. obs.). The mean of the maximum thermal amplitude was estimated for each year as the maximum daily difference between the maximum and minimum temperature for each site. For missing data, as well as for the BA site, which did not have a nearby meteorological station, the climate variables were estimated using the geostatistical kriging interpolation technique (e.g. Akkala et al. 2010), with latitude and longitude as independent variables.

Tidal amplitude, water salinity, sediment grain size distribution, sediment oxygen content (O_2), sediment moisture, and sediment percentage of organic matter (OM) were estimated for each study site. The averages of tidal amplitude were obtained from Isacch et al. (2006). Water salinity values were measured (precision of 1‰) from 10 water samples collected 20 m apart from each other along the coastline during high tides from each study site. The grain size distribution was estimated by collecting 5 samples of sediment (10 cm depth, 10 cm diameter) at each marsh habitat and each site. Sediment was sieved and fractions separated in $>1000 \mu\text{m}$ (cobble and pebble), $1000\text{--}125 \mu\text{m}$ (sand), and $<125 \mu\text{m}$ (silt and clay), dried (60°C , 72 h), and weighed. The OM content was estimated in sediment samples (10 cm depth, 10 cm diameter), by weight loss between dry (7 d at 60°C) and combusted (8 h at 500°C) sediment. Sediment oxygen content (O_2) was measured *in situ* by gently pushing a Clark-type glass microelectrode (500 μm tip, Unisense) 5 cm into the sediment. Analyses of variance (ANOVA) or Welch's test (adjusted for inequality of variances; Zar 1999), followed by Tukey's HSD (Zar 1999) were used to evaluate the null hypothesis of no differences in frequencies of herbivory by moth larvae, plant traits, and physical variables among locations. In each case, data were checked for normality using the Kolmogorov-Smirnov test (Lilliefors' correction) and for equal variance with the Levene median test.

Structural equation modeling (SEM, Grace 2006) was developed to evaluate direct and indirect (mediated by plant traits) effects of latitude and physical

variables on the frequencies of moth attacks on both *Spartina* species along the SWA coast. The physical variables were included as exogenous variables, and plant traits and moth attack frequencies were included as endogenous variables (see Fig. S1 in the Supplement). Because of the differences in the variable units (e.g. plant density, temperature), standardized coefficients were used to compare the magnitude of the paths. The links exhibiting high collinearity were eliminated to derive more stable path coefficients. To assess the fit of the models, we used the goodness-of-fit chi-square statistic (χ^2), the comparative fit index (CFI) best-fitting possible model, and the Tucker Lewis index (TLI) (Bentler & Chou 1987). The root mean square error of approximation (RMSEA), which is adjusted for degrees of freedom and is considered an index of parsimonious and well-fitting models, was also estimated (Browne & Cudeck 1992).

Given the relatively high complexity of the proposed models for the sample size, a bootstrapping procedure was used to evaluate model stability (Ievers-Landis et al. 2011). For that, a bootstrap procedure (10 000 iterations for each regression path in the model) of the initial model was run. After that, if the original sample weights did not differ from the mean of the weights obtained through the bootstrap, the models were considered to be unbiased (Ievers-Landis et al. 2011). To compare each regression path in the model, the pairwise differences with the original model values were calculated for each bootstrap iteration. When 0 fell outside the 95% confidence interval of the distribution of resampled differences (Manly 2006), the estimator did not differ between bootstrap models and original models. The parameter estimation and testing of model fit were performed using R (R Development Core Team 2012) with the Lavaan libraries (Rosseel 2012).

Transplant experiments

To analyze if variations in plant traits along latitudes are genetically based or are a plastic response to biotic and physical cues in each locality, 2 plant translocation experiments were conducted. In the first, both *Spartina* species were propagated clonally. We selected 40 plots (15 cm \times 15 cm) of each plant species from high- (SC), middle- (BB), and low-latitude sites (BSA, see Fig. 1). Attacked stems within each plot were removed. We then dug up the plant rhizomes, including sediment down to 25 cm depth. Ten random blocks of each plant from each geogra-

phic region were transplanted to the other sites (high-, middle-, and low-latitude sites), while 10 plots remained as controls. The field-collected clones were transplanted to the different sites within 24 h, to minimize the double transplant stress. To eliminate the possible effects of the maternal sediment characteristics, before growing each plant in the transplant locations, we removed the sediment containing the rhizomes in each individual plant. To evaluate the transplanting effects on plants, the last 10 blocks were transplanted within the same site (auto-transplants) as a treatment control.

A second experiment was performed to evaluate if in constant environmental conditions of plant growth, and without moth herbivory, there are variations in plant traits depending on their latitudinal origin. Ten experimental blocks of *S. densiflora* from each region (high, middle, and low latitudes) were transported to the laboratory and grown under the same experimental conditions in an open-air garden environment. Plants were planted (15 × 15 cm pot) in standardized substrate of a 50:50 mixture of commercial potting soil and sand (Pennings et al. 2009). To keep plants in intermediate conditions of growth of the latitudinal range studied, the experiment was performed in a coastal and mid-latitude site (Mar del Plata 38° 56' S) free of moths with natural photoperiod and temperature values. We checked to ensure that the experiment remained free of herbivores and watered the plants with fresh water twice a day until saturation.

Both experiments lasted 33 mo (3 consecutive growing periods) to be sure that all stems in each experimental unit had grown under the experimental conditions. At the end of this period, all stems were cut. The frequencies of moth larvae attacks in each block of each treatment in the field experiment, and plant heights (for each block), total fiber and C:N (for 5 random blocks) in each block of each treatment of both experiments were estimated as explained above. At the end of the field experiment, we only found the basal stem section of some plants in the transplant blocks (first 5 to 10 cm), presumably because larval feeding promoted stem breaking and dragging by tides (Canepuccia et al. 2011). To ensure the estimation of herbivory frequency, we discarded these samples, and only used those where the state of the plant allowed us to verify larval presence (larvae or signs of larvae, i.e. holes, tunnels, or sawdust). Considering the difference in sample numbers and the inequality of variances of data, to test for main effects (plant or site) and interaction effects (plant:site), we performed non-parametric statistical

tests (Anderson 2001). Permutation tests of the distributions of the *F*-statistics for main effects and interaction effects were used (Anderson 2001, Manly 2006). Permutation testing (10 000 permutations) was applied using R software (3.2.2; www.r-project.org) and the package 'asbio' (Aho 2017). The function 'perm.fact.test' was used to conduct permutation testing of the main effect and interaction effect. For pairwise comparisons, we performed Monte Carlo analysis (Manly 2006). For these analyses, we used random re-sampling with replacement (10 000 iterations) preserving the sample size. For each iteration, we recalculated the pairwise mean differences of variables (plant traits and moth attack frequencies) between treatments. Then, we considered that a variable differed significantly between treatments if 0 fell outside the 95% confidence interval of the distribution of resampled differences (following Manly 2006). ANOVAs followed by Tukey's HSD (Zar 1999) were used to evaluate the null hypothesis of no differences among plant traits from different locations in the moth-free environment experiment.

RESULTS

Strength and significance of direct and indirect effects on plant–herbivore interactions

Environmental variables (Table S1 in the Supplement), plant traits (Table S2), and herbivore densities changed with latitude (Fig. 2). The attack frequencies of moths in both plants varied along the SWA marshes (Welch's test of Box-Cox transformation of attack frequencies in *Spartina alterniflora*: $F_{6,27} = 109.45$, $p < 0.01$, $\theta = 0.7$; *S. densiflora*: $F_{7,30} = 21.77$, $p < 0.01$, $\theta = 0.8$), and was higher at lower-latitude sites (SAL and SC), decreasing towards higher-latitude sites (BB and BSA). However, attack frequencies increased again at the higher-latitude sites located at river outlets (RN for *S. alterniflora*, RCH for *S. densiflora*, Fig. 2).

SEM fitted models for plant–herbivore interactions for each marsh habitat explained variation in plant densities (53% for *S. alterniflora*, 85% for *S. densiflora*), plant heights (84% for *S. alterniflora*, 91% for *S. densiflora*), plant fiber content, and C:N ratios (100% for both plants) and moth attack frequencies (93% for *S. alterniflora*, 76% for *S. densiflora*, Fig. 3; Table S3 in the Supplement). The observed correlation matrix did not differ from the expected matrix ($\chi^2 = 13.68$, $df = 21$; $p = 0.88$ for *S. alterniflora*; $\chi^2 = 43.39$, $df = 51$; $p = 0.73$ for *S. densiflora*), and the low

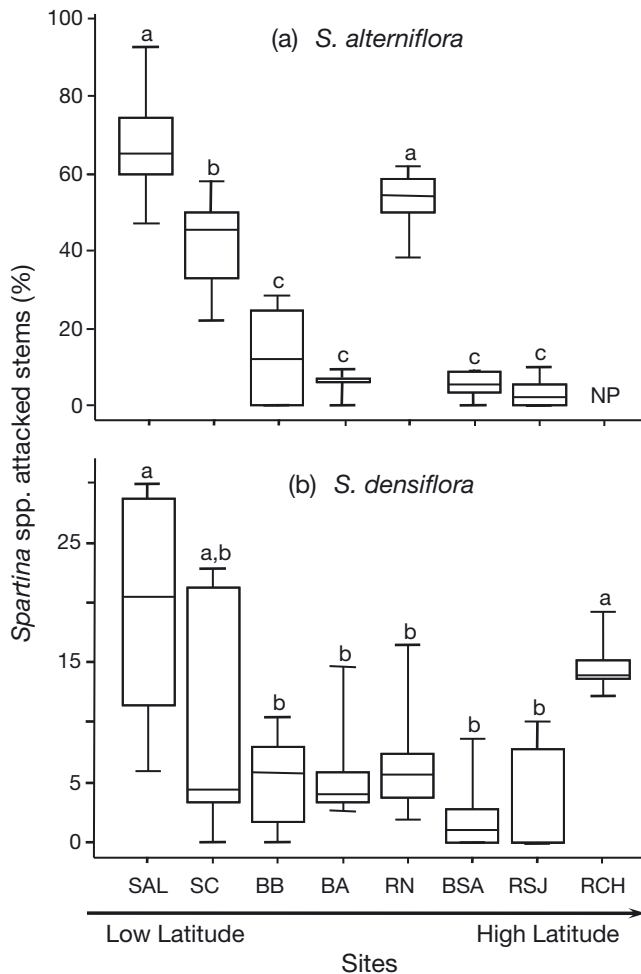


Fig. 2. Percentage of stems of (a) *Spartina alterniflora* and (b) *S. densiflora* attacked by the moth *Haimbachia* sp. nov. along 8 SW Atlantic coastal marshes (see Fig. 1 for full site names). Box limits are the 25th and 75th percentiles, whiskers are the minimum and maximum, and midlines inside the boxes represent the median values. Different letters indicate significant differences ($p < 0.05$) by Tukey's test after Welch's ANOVA; NP: absence of plants at that location

RMSEA (<0.0001 for both plant species) indicated a good model fit. The tests comparing the target model with the null model indicated parsimonious and well-fitting models (CFI = 1.00; TLI = 1.00 for both plant species). The non-standardized regression weights for the original models were no different from those of bootstrap samples, suggesting that all of the paths were unbiased for both plants (Fig. 3, Table S3), indicating parsimonious and well-fitting models.

According to the *S. alterniflora* model, the plant density increased with increasing O_2 but decreased with the increase of minimum temperature. Height of this plant increased with OM and decreased with

increasing salinity and tidal amplitudes (Fig. 3a, Table S3). Fiber content increased with tidal amplitudes and decreased with maximum and average temperatures, OM, O_2 , and water salinity. The C:N ratios increased with increasing average temperature, O_2 , and OM; and decreased with increasing minimum temperature and water salinity (Fig. 3a, Table S3). Finally, the attack frequencies of the moth on *S. alterniflora* increased with increased average temperatures and plant traits such as height, densities, C:N ratios, and fiber content; while they decreased with increased tidal amplitudes (Fig. 3a, Table S3).

According to the *S. densiflora* model, plant density increased with OM, O_2 , and sediment moisture and decreased with increasing maximum temperature, minimum temperature, and salinity. The height of *S. densiflora* increased with increasing precipitation and plant densities, and decreased with increasing salinity, tidal amplitude, and O_2 (Fig. 3b, Table S3 in the Supplement). The fiber content in plant tissues increased with decreasing day length and OM, and decreased with increasing maximum temperature, minimum temperature, salinity, and sediment moisture. The C:N ratios in *S. densiflora* tissue increased with increasing fiber, O_2 , and salinity, and decreased with the increasing day length and sediment moisture (Fig. 3b, Table S3). Finally, the attack frequencies of moths on *S. densiflora* stems increased with plant densities, plant height, C:N ratio, and tidal amplitudes, and decreased with increasing fiber and salinity (Fig. 3b, Table S3).

Transplant experiments

At the end of the field experiment, plants transplanted to a low-latitude site (SC) showed higher frequencies of moth attacks than plants transplanted to a mid-latitude site (BB) and a high-latitude site (BSA, Fig. 4). These attack ratios differed according to the plant origin (interaction affects, Fig. 4 and Table S4). For example, all stems of *S. alterniflora* from mid- and high latitudes showed signs of having been attacked by moth larvae (Fig. 4). For both plant species, there were no differences in the attack frequencies between controls of the transplant treatments (auto-transplants) and control plants at each experimental site.

At the end of the field transplant experiment, the heights of both plant species tended to be taller at low and middle latitudes than at high latitudes (Tables S4 & S5). The heights of plants originating from low latitudes tended to be taller than those

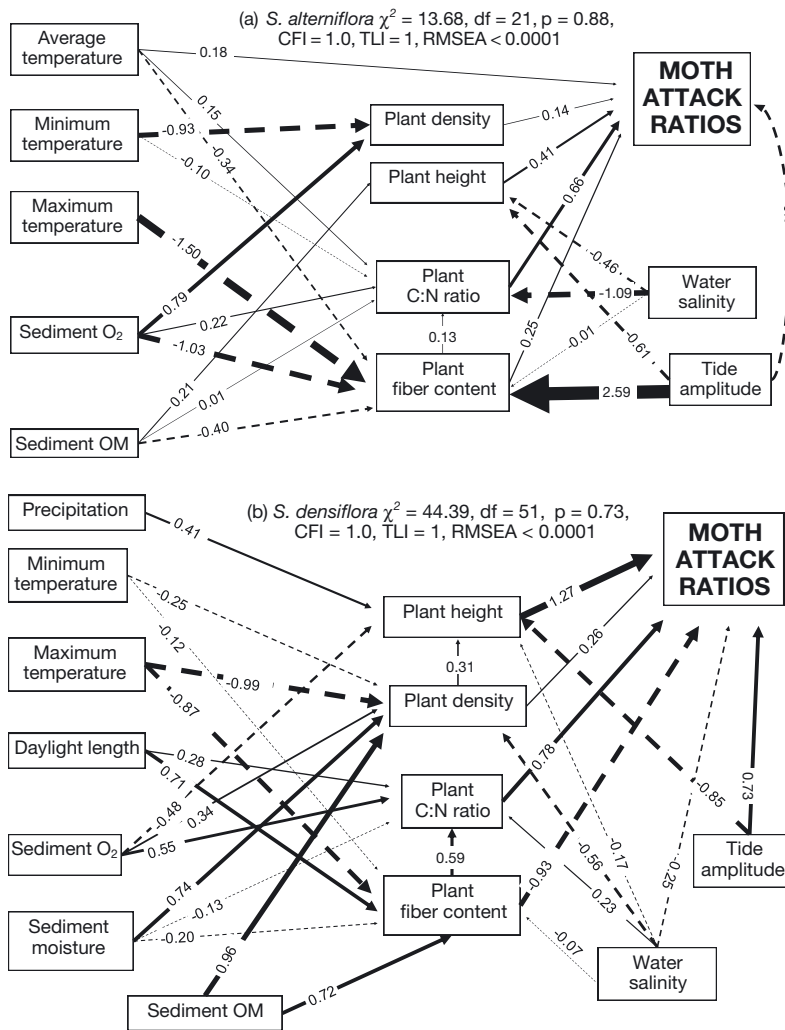


Fig. 3. Path diagrams of the structural equation model estimating the relative importance of direct and indirect effects of physical variables on plant traits and on the moth attack frequencies in (a) *Spartina alterniflora* and (b) *S. densiflora* across 8 SW Atlantic coastal marshes. Arrows designate the direction of causality; values are standardized coefficient estimates. Arrow widths are proportional to the size of the path coefficients. Solid (dashed) arrows represent positive (negative) relationships. Goodness-of-fit statistics are χ^2 : chi-squared, p = p -values of χ^2 , CFI: comparative fit index, TLI: Tucker Lewis index, and RMSEA: root mean square error of approximation

from mid- and high-latitude sites (interaction effect, Table S4). *Spartina alterniflora* at mid-latitudes and all high-latitude plants transplanted to low-latitude sites were killed by moths (broken stems with remnants of hollows made by larval feeding, see Canepuccia et al. 2011). Hence the measurements of plant traits could not be done. At mid- and high-latitude sites, plants coming from high latitudes showed the lowest heights, whereas at high-latitude sites, plants coming from low latitudes showed the greatest heights (Table S4 and S5 of the Supplement). There

were no differences in heights for both plant species between 'treatment controls' and 'controls' for each site (Table S5 in the Supplement).

Fiber content in *S. alterniflora* tissues showed the highest values in plants transplanted to high-latitude sites, while for *S. densiflora*, the lowest fiber content was found in plants transplanted to mid-latitude sites (site effect, Table S4). With the exception of *S. densiflora* transplanted to mid-latitudes, there were no differences in fiber content between control plants of transplant treatments and control plants coming from each site and each species (Table S5). The C:N ratios in *S. alterniflora* tissues showed lower values in plants transplanted to mid-latitudes (site effect, Table S4), while for *S. densiflora*, lower C:N ratios were found in plants transplanted to mid- and low-latitude sites (Table S5). There were no differences in C:N ratios of plants coming from different sites within low- and high-latitude sites (Tables S4 & S5).

In the experiment in the moth-free environment, we checked continuously and did not see tracks of other herbivores. *Spartina densiflora* from high, middle, and low latitudes growing at intermediate latitudes without moths ('common garden' outside the marsh) showed differences in their morphology and tissue composition. Plants from low latitudes showed greater heights than those from mid- and high latitudes (ANOVA: $F_{2,27} = 8.84$, $p < 0.001$, Table S6). The fiber content was lower in plants from low latitudes than in those from mid- and high latitudes (ANOVA: $F_{2,12} = 13.73$, $p < 0.001$, Table S6). There were no differences in C:N ratios in plant tissues from low-, mid-, and high-latitude sites (ANOVA: $F_{2,12} = 0.50$, $p = 0.62$, Table S6).

DISCUSSION

Our results document that along SWA marshes, herbivore abundances and plant damage were related to latitude-dependent and -independent variables.

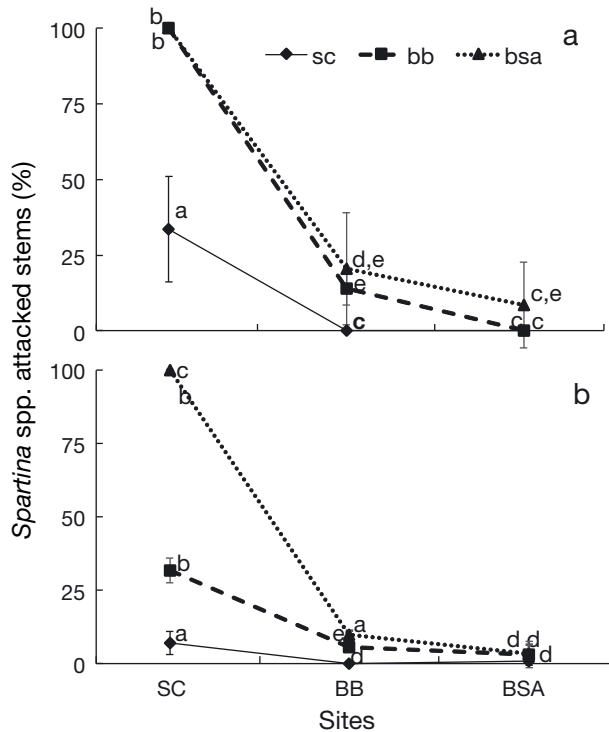


Fig. 4. Interaction plot of frequencies of moth attacks (means with SE) on low- (sc) vs. middle- (bb) and high-latitude (bsa) plants (each in separate lines) transplanted to high- (BSA) vs. middle- (BB) and low-latitude sites (SC) for (a) *Spartina alterniflora* and (b) *S. densiflora*. Different letters next to the datapoints indicate significant differences assessed by 10000 Monte Carlo simulations of the pairwise differences in the frequencies of moth attacks between treatments

Among latitude-dependent variables, the frequency of moths in *Spartina alterniflora* increased with increasing average temperature. This association is probably because cold affects the survival of herbivorous arthropods (Deutsch et al. 2008). In addition, herbivore abundances increased with plant densities, which also increased with average temperatures, minimal temperature, and low daily thermal amplitude. Within our study range, the minimum temperature consistently decreased toward higher latitudes, resulting in a markedly increased daily thermal amplitude at high latitudes. This decrease in weather stability can have negative effects on plant growth (e.g. Speziale et al. 2010), indirectly enhancing the temperature effects on herbivore abundances and plant damage.

Not only plant morphology was affected; C:N ratios in plant tissues also increased with decreasing daylight and minimum temperatures. Consistent with a widespread latitudinal increasing trend of leaf N content (Reich & Oleksyn 2004), plant N contents increased with latitude in salt marshes (Siska et al.

2002). This variation may be because plants can accumulate more N at higher latitudes (He & Silliman 2015) since cold temperatures limit photosynthetic C intake more than root N uptake (Reich & Oleksyn 2004). Moreover, a short growing season requires a higher N content to allow rapid growth in high-latitude marshes (Siska et al. 2002), which may change the trade-off of food preference with latitude.

Surprisingly, moth abundances increased with increasing C:N ratios in both plants, despite herbivores often preferring food with higher N content (Gratton & Denno 2003). This association could be because larvae may prefer stems with higher proportions of structural tissues like fiber, decreasing the likelihood that debilitated stems (burrowed by the larvae) break down due to tidal action (Canepuccia et al. 2011). Alternatively, this could be a result of plants facing lower herbivory rates, developing lower chemical defenses and maintaining higher N levels (Siska et al. 2002). Whatever the underlying cues, latitudinal proxy variables emerge as important determinants of latitudinal variations in plant–herbivore interactions in SWA marshes.

However, other factors varied geographically but were not latitude-dependent, which were also selected to assess differences in plant–herbivore interactions. For example, the abundance and height of plants increased with increasing OM content, and all tended to increase at lower-latitude sites (e.g. Moles et al. 2009). Also, there may be feedback between OM and the plant community, but most of the detritus produced by marsh plants is exported by tides (Montemayor et al. 2011), which play an important role in the dynamics of these environments. Plants also decreased their heights with increases in tidal amplitude, which can decrease the risk of stems breaking while tidal floods increase. Given the toxicity of Na^+ and Cl^- , plants also reduce their growth and heights as salinity increases (Canepuccia et al. 2010b). Indeed, as rain may dilute salt in the high marsh (Canepuccia et al. 2010b), the precipitation increment is positively associated with *S. densiflora* height. Since these variations in plant traits imply variation in the amount of food for moths, these variables also have indirect effects on moth abundances through their effects on plant traits.

Furthermore, in the low marsh, the C:N ratio in *S. alterniflora* tissues decreased with an increase in salinity, which could be a consequence of the production of osmolytes due to salt stress (e.g. Flowers et al. 1977, Rozema et al. 1985). This variation in tissue composition could have a negative impact on moths. Indeed, at high latitudes, in low marshes located at

river outlets, where salinity is lower, the incidence of herbivory increased again. At the same time, fiber content in plant tissues increased with increasing tidal amplitudes. This may happen due to an increased need for being resistant to higher tidal power. It is possible that the negative relationship between OM and fiber content in the low marsh was indirectly due to the loss of OM by outgoing tides (e.g. Tam & Wong 1998) when tidal amplitude increases. This higher resistance could therefore also be causing increased moth frequency in *S. alterniflora*. Indeed, moth abundances also decreased with increasing tidal amplitude. If moths select stems with higher C:N ratios and fiber content, and with lower risk of being broken by tides, then salinity and tides would indirectly be varying the moth selectivity.

However, in the high marsh, which is exposed to drier conditions, higher temperature changes between day and night (Bertness 1992), and salt accumulation (Fanjul et al. 2008), the C:N ratios in *S. densiflora* tissues increased with increasing salinity. These results that contrast with those from the low marsh may be because the dry conditions and excessive salinity levels in the high marsh increase plant woodiness (e.g. Stiling & Moon 2005). This may also explain why *S. densiflora* height decreased with the increase in sediment O₂ content. A greater degree of soil drainage and aeration in the high marsh may increase water evaporation, desiccation, and thus salt concentration in the sediment during longer periods between flooding tides. In fact, since high salinity levels negatively impair herbivory (e.g. Canepuccia et al. 2010b, Dagar 2010), moth abundances decreased directly with increasing salinity in the high marsh. In addition, fiber content in *S. densiflora* is about twice that in *S. alterniflora*, which may greatly reduce plant digestibility and palatability (Randolph et al. 1991) resulting in an inverse plant fiber–moth abundance relationship. In the high marsh, tidal effects are milder (weaker force and lower frequency) and thus, the stem rigidity (and plant fiber content) is not so critical for larvae to complete their cycle within stems. Salinity and tides may then indirectly change the trade-off of food preference between plant species. The geographic differences in plant–herbivore interactions thus are likely to respond idiosyncratically to the same environmental gradients in different habitat contexts, even between neighboring habitats.

After 33 mo, both plant species from high latitudes transplanted to low latitudes grew smaller, had higher relative fiber content, and suffered more herbivory than plants located at lower latitudes. The opposite result was observed for lower-latitude plants trans-

planted to higher-latitude sites, with herbivores strongly preferring high-latitude plants, suggesting, as in Northern Hemisphere marshes (e.g. Salgado & Pennings 2005, Więski & Pennings 2014), latitudinal differences in plant traits and palatability. Differences in plant traits still persisted after almost 3 yr following the transplant, which suggest that differences in plant traits among sites are under genetic control and thus that latitudinal variation in herbivory could be selected for the geographical variation in plant traits along latitudes (Salgado & Pennings 2005). However, since the differences were smaller than among plants separated geographically (i.e. controls), the results also suggest plant plastic responses to environmental cues.

Many of our observations suggest that Southern Hemisphere marshes undergo similar processes to those documented in Northern Hemisphere marshes (e.g. Więski & Pennings 2014). The suggested hemispheric asymmetries in the latitudinal herbivory hypothesis could be due to the fact that studies are biased towards the Northern Hemisphere (see Zhang et al. 2016). In addition, in the Zhang et al. 2016 review, only leaf-chewing insects were included, while other herbivores (e.g. sap-sucking, stem-boring; Canepuccia et al. 2011, Anstett et al. 2014) were excluded from the analysis. Moreover, since some high-latitude plants transplanted to low-latitude sites were completely eaten by the end of the experiment, we also found latitudinal differences in plant resistance to herbivory. After the experiment, larvae still preferred plants from high latitudes over those from low latitudes, supporting the idea that the variations in plant anti-herbivore defenses among sites are also genetically based traits. Our experiment did not allow us to determine whether there is an induced anti-herbivore defense in these plants. However, plants from different latitudes growing in a ‘common garden’ (without moth herbivory) maintained similar patterns of traits as those growing in the field transplant experiment (with moth herbivores). These results suggest that, at least in the assessed traits, the plant defenses are not mediated by herbivory pressure. Plants can allocate more resources to the constitutive defenses when the selective pressure of herbivores is predictably stronger (‘optimal defense hypothesis’, Stamp 2003). On an evolutionary scale, this can help them to cope with higher rates of herbivory at lower latitudes, driving in part the geographic differences in plant–herbivore interactions along SWA marshes. The herbivore traits could also be driven by plant trait variation in an evolutionary ‘arms race’ (Vermeij 1987). A moth–plant imbalance of armaments may vary the

strength of local selection, and physical conditions change the arms-race trajectory (e.g. Toju & Sota 2006) at different rates along the SWA coast. Additional research is needed to further understand the determining factors driving latitudinal gradients in herbivore traits (e.g. tolerance for plant defenses) along latitudinal gradients in plant traits.

Our study shows that plant–herbivore interactions in SWA marshes tend to be more intense at lower latitudes. This variation is caused by an increase in the abundance of herbivores and an increase in plant resistance to herbivore damage at low latitudes. However, there are several inconsistencies in the pattern of latitudinal variation in this interaction, which are related to variation in plant or herbivore traits not necessarily related to latitude. Indeed, the plant responses to variables not related to latitude (e.g. salinity, rainfall, tidal amplitude), indirectly affect traits important to consumers, further increasing the mechanisms responsible for plant–herbivore variations along latitude. Our results thus emphasize the value of considering multiple ways in which plants and consumers respond to ecological processes shaped by factors that operate differentially across geography when examining variation in organism interactions along latitudes.

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