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Stress-resistance traits disrupt the plant economics - decomposition relationship across environmental gradients in salt marshes

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\textbf{Keywords:}
\textit{Spartina anglica}
\textit{Sporobolus anglicus}
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\textit{Orchestia gammarellus}

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\textbf{ABSTRACT}

Understanding how plants respond to environmental gradients and influence ecosystem functions remains a core challenge in ecology. Across species and ecosystems, plants have been shown to coordinate leaf, stem, and root traits along a gradient with optimal resource acquisition or conservation strategies at its extremes, termed the plant economic spectrum (PES), in turn driving ecosystem functioning. PES theory has been successfully applied in coastal wetlands to disentangle how the strong abiotic gradients affect ecosystem functions, such as litter decomposition. Yet, wetlands can be dominated by monospecific stands, and it remains unclear whether the PES applies within species. Here, focusing on a globally widespread salt marsh plant, \textit{Spartina anglica}, we investigated: a) if the PES holds at the intraspecific level along critical abiotic stress gradients (redox potential and soil salinity); b) how intraspecific changes in plant traits along the PES affect litter decomposition; and c) whether these changes in plant strategies influence the abundance of key macro-detritivores, mediating litter decomposition. We found remarkable variation in \textit{S. anglica} leaf and stem traits, coordinating along the PES and adopting a conservative strategy under stronger abiotic stress. Unexpectedly, leaves with a conservative strategy (higher leaf dry matter content and C/N ratio) attracted more macro-detritivores and decomposed faster. Other facets of litter quality beyond C/N seem to drive these counter-intuitive effects: leaves with a conservative strategy were likely more palatable because of higher protein content and lower toughness (low total carbohydrate content). Our study highlights that intraspecific trait variability can strongly drive litter decomposition, potentially impacting on the carbon storage capacity of salt marshes; and that specific stress-resistance traits can disrupt the PES - ecosystem function relationship.

1. Introduction

The plant economic spectrum describes a suite of correlated traits along a continuum of strategies, from resource conservation to resource acquisition (Freschet et al., 2010, 2012; Reich, 2014). These plant strategies have been successfully employed to understand community assembly processes and to scale up the effects that changes in plant traits have on ecosystem functions (Lavorel and Garnier, 2002; Suding et al., 2008). For instance, nutrient poor habitats select for plants with a conservative strategy, such as high tissue density, C to N ratio, and low nitrogen concentration (Freschet et al., 2010, 2012, 2013, 2012; Lin et al., 2020; Garnier et al., 2016), slowing down plant decomposition and nutrient cycling (Cornwell et al., 2008; Laughlin et al., 2015). Thus, the plant economic spectrum provides a strong approach for disentangling how environmental gradients (e.g. from rich to poor nutrient soil) affect ecosystem functions (e.g. decomposition).

Salt marshes are coastal ecosystems that provide a broad range of services to human populations, from water purification and fish nurseries to coastal protection and carbon storage (Barbier et al., 2011). In particular, marshes have attracted strong attention from researchers and managers for their contribution to carbon sequestration (e.g. Callaway et al., 2012; Ouyang and Lee, 2014). Coastal marshes, can accumulate large quantities of carbon (>240 g C m\textsuperscript{-2} yr\textsuperscript{-1}; Ouyang and Lee, 2014) as this highly productive ecosystem produces abundant plant litter which becomes buried in anoxic soil under the regular sedimentation associated with tides (Callaway et al., 2012; Canfield, 1994; Elsey-Quirk

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Various factors influence this carbon sequestration capacity (Davidson et al., 2017; Elsey-Quirk et al., 2019), but one key component is the after-life effects of vegetation litter, both in quality and quantity (Ford et al., 2016; Ouyang and Lee, 2014; Simoes et al., 2011). Therefore, understanding what influences plant litter (e.g. quality) is crucial for fully estimating and forecasting the capacity of salt marshes to act as carbon sinks.

In salt marshes, as in terrestrial systems, plant traits such as high leaf lignin content, and C to N ratio slow down litter decomposition rate (Klap et al., 1999; Simoes et al., 2011). Thus, according to the plant economic spectrum theory we would expect that also in salt marshes under more stressful conditions (i.e. low nutrients) plants would adopt a more conservative strategy, further reducing decomposition rates. Yet, the few studies that have tested this hypothesis in salt marshes (Minden et al., 2012) have found that, despite growing in poor nutrient soil, under strong abiotic pressure (e.g. inundation frequency, salinity, and low redox potential) plants adopted a more acquisitive strategy and not a conservative one, ultimately increasing decomposition. Thus, in salt marshes strong abiotic gradients can alter the response of plants economic traits, although the PES-ecosystem functioning relationship (i.e. decomposition) seems consistent with theory.

The plant economic spectrum-ecosystem functioning relationship has found strong support at the interspecific level across systems (Freschet et al., 2012; Garnier et al., 2016; Laughlin et al., 2015). However, intraspecific variability can play a key role in driving ecosystem functioning (Blanchet et al., 2020; Raffard et al., 2019). In particular, intraspecific variability will play a major role when the variation in traits within species is similar to that between species (Lecerf and Chauvet, 2008) or when monocultures of single species occupy large areas. For instance, in riparian systems intraspecific variation in leaf decomposition of alder (Alnus glutinosa) was similar to the variation in decomposition among co-occurring species (Lecerf and Chauvet, 2008).

Additionally, in US marshes Spartina alterniflora occupies vast areas and grows in either a tall or short form depending on marsh elevation; these eco-types have different cellulose content, which in turn affect their litter decomposition rates (Wilson, 1986). However, it is largely unknown if at the intraspecific level strong abiotic gradients disrupt plant strategies (i.e. acquisitive traits under high environmental stress) and the consequences for ecosystem functioning (i.e. lower decomposition with conservative plant litter). Overall, understanding how decomposition varies with intraspecific differences in plant traits under strong abiotic gradients is of great importance, both for generalising the PES-ecosystem functioning relationship as well as for understanding processes influencing the carbon storage capacity of salt marshes.

Besides plant traits, macro-detritivores play a crucial role in litter decomposition (Fuji et al., 2018) and thus nutrient cycles (Bohlen et al., 2004; Yeates, 1991). In European salt marshes, the amphipod Orchestia gammarellus has a predominant role (Sprung and Dias, 2003), feeding on micro-organisms living on plant litter (Lopez et al., 1977); its feeding and bioturbation activities increases litter fragmentation and sediment oxygenation, which increases litter decomposition (Schrama et al., 2015). Terrestrial macro-detritivore communities can interact with plant leaf and root traits, boosting decomposition in nutrient-rich litter (Fuji et al., 2018). Yet, in salt marshes we know little about how intraspecific variation in plant traits interact with macro-detritivores to modify litter decomposition processes.

We investigated whether changes in plant strategies under different abiotic conditions will vary affect litter decomposition of the widespread pioneer species S. anglica. Estuaries provides natural gradients in environmental factors due to the input of seawater and sand from marine sediments at the mouth of the estuary and freshwater input at the head. Therefore, we sampled leaf and stem traits in two salt marshes at the mouth and head of an estuary, to maximise the differences in abiotic factors, such as salinity, sand content, and sediment redox potential values (as recorded in De Battisti et al., 2019). We hypothesised that leaf and stem traits of S. anglica will comply with the PES theory and, therefore, we expected that plants from the mouth of the estuary, where abiotic conditions are more stressful, move towards a conservative strategy. In turn, we expected that litter of plants with a conservative strategy (at the estuary mouth) will have lower decomposition rates. Furthermore, we investigated if changes in plant strategies will influence the abundance of a key detritivore (O. gammarellus) feeding on plant litter and its effect on decomposition rates.

2. Materials and methods

2.1. Sampling design

We chose two sites, i.e., Pembrokeshire marsh (Lat = 51.678617, Long = −4.287275) and Loughor marsh (Lat = 51.682130, Long = −4.073385; Fig. 1A and B), at the mouth and the head of the Loughor estuary in South Wales (UK) respectively, in order to maximise the differences in abiotic factors. Previous investigation of the marshes from the two ends of the distribution range of S. anglica along the estuary (Fig. 1B) revealed that they strongly differed in sediment porewater salinity, redox potential, and sand content (De Battisti et al., 2019); in particular, the variability in abiotic factors within marsh was lower than the variability between marsh. In November 2017, within each marsh we established ten plots of 1 m² in the Spartina anglica zone (plots were ~2 m distance from each other at similar elevation), and, from each plot, we collected about ten S. anglica plants (~250 g) to measure leaf and stem traits and for performing the decomposition experiment (Table 1).

2.2. Plant traits measurements

Plant material was collected, transported to the laboratory in sealed plastic bags, and stored in a fridge until it was processed for trait measurements (within 24 h). After collection, each leaf of S. anglica was carefully washed with tap water by hand to ensure that leaves were free from sediment particles. Leaves and stems were taken randomly from each 1 m² and did not necessarily come from the same plant. However, given the small spatial scale of the plot, plant material likely came from the same clone. We measured six leaf traits and four stem traits (Table 1) to understand if plant traits coordinate along the plant economic spectrum and to unravel their effect on decomposition. We measured leaf dry matter content (leaf fresh mass over leaf dry mass) on three randomly selected leaves following Pérez-Harguindeguy et al. (2013). The average value of the three leaves was used for the statistical analysis. Similarly, 10 cm long stem segments were cut from the bottom part of three stems, weighed and dried at 70 °C per 48 h, after which the specific stem density (SSD) was calculated (stem dry weight over stem volume, considering the stem as a cylinder; Pérez-Harguindeguy et al., 2013). The three SSD values per plot were averaged and used for the statistical analysis. A sample of leaves and stems of ~3 g was oven-dried at 70 °C for 48 h, ground to a powder, and C and N content was analysed using a Carlo Elba Elemental Analyser. Afterwards, we calculated leaf C/N ratio.

Relationships between traits, the plant economic spectrum, and stress resistance are based on Freschet et al. (2012); Laughlin et al. (2015); Garnier et al. (2016); Minden and Kleyer, 2011, 2012; Minden and Kleyer (2015).

We used FTIR analysis (Fourier Transformed Infra-red Spectrometry) using a PerkinElmer Model Spectrum Two instrument for estimating leaf protein content and total carbohydrates. We chose these two traits because protein content in leaves has been related to resistance to salinity (Flowers and Colmer, 2008; Slama et al., 2015), while total carbohydrates has been related to structural compounds such as lignin and thus to resistance to detritivores (Cornwell et al., 2008; Garnier et al., 2016). The instrument was equipped with a diamond crystal ATR reflectance cell with a DTGS (Deuterated Tri Glycine Sulphate) detector scanning over the wavenumber range of 4000–450 cm⁻¹ at a resolution of 4 cm⁻¹. Background correction scans of ambient air were made prior to each sample scan. We measured the peak absorbance area...
corresponding to total carbohydrates (spectral region between 960 and 1130 cm\(^{-1}\)) and proteins (spectral region between 1580 and 1700 cm\(^{-1}\)) in three sub-samples of ground dried leaves from each plot. These areas were calculated using the proprietary FTIR software for each spectrum based on Ferro et al. (2019). The average value of the three sub-samples was used for statistical analysis.

2.3. Decomposition experiment

Oven dried leaves (5 g) were inserted into plastic litter bags of 15 × 7.5 cm with 1 mm mesh size. Separately, one oven dried stem of 10 cm in length was inserted in litter bags of the same type as for leaves. In each bag, we made 10 holes (5 per side) of ~0.5 cm in diameter to ensure that macro-detritivores could reach the leaf material (Schrama et al., 2015). Litter bags with leaves and stems from the Pembrey and Loughor marshes (200 litter bags in total) were randomly placed in a common garden at the landward of the Pembrey marsh only (Lat = 51.678617, Long = −4.287275). The common garden was above the S. anglica zone, in a mixed community mainly composed by Limonium vulgare, Plantago maritima, Triglochin maritima, ensuring that none of the samples had a site advantage. Ten blocks of 1 m\(^2\) were placed at the same elevation, 2–3 m from each other (Fig. 1C) and, within blocks, we placed 10 litter bags both for leaves and stems (20 bags per block in total); leaves and stem litter bags from the same plot were placed next to each other (same label colour in Fig. 1D). Litter bags were retrieved from the marsh after 3, 6, 13, 27, and 54 weeks, and washed to remove sediment with tap water. At this stage, the macro-detritivores inside the litter bags were collected, identified and counted. Leaves and stems were then dried at 70 °C per 48 h, weighed and then burnt into a furnace for 440 °C for 18 h to obtain ash-free dry weights. For the statistical analysis we used the ash-free dry weights of leaves and stems to avoid the inclusion of sediment material in measuring the loss in weight.

2.4. Statistical analysis

First, through t-tests we investigated differences in plant traits of S. anglica between sites. The Welch t-test was used as this is suitable when the homogeneity of variance assumption is not met. Stem C content and C/N ratio did not meet the normality assumption even after log transformation; therefore, for these traits we used the Wilcoxon rank sum test. Furthermore, we performed a principal component analysis using a promax rotation, both separately and together for leaf and stem traits known to be related to the plant economic spectrum (Table 1). We extracted the axes corresponding to the plant (PES), leaf (LES), and stem (SES) economic spectra (see Table 1) and we investigated with t-tests if plants from contrasting sites employed different economic strategies.

Second, to calculate decomposition rates, the ash-free dry weight mass loss was log transformed and used as a response variable in a linear mixed-effect model (separately for both leaves and stems), where time (week of litter bag collection, five levels) was considered both as a fixed factor and as a random slope. The random slope of week accounted for possible differences in decomposition rates among sampling plots. The hierarchical structure of the sampling collection (plot within marsh, two levels) was considered both as a fixed factor and as a random intercept and another random factor.
intercept was included to account for a block effect (ten levels). The random block effect was not significant (confidence intervals: 0–0.121 and 0–0.183 for leaves and stems, respectively) and thus excluded from the analysis. We extracted the coefficient of decomposition (k-values; fixed effect of week) for each plot and we used the k-values for further statistical analysis. Through ANOVA we investigated if k-values differed between sites, plant organs (leaf and stem), and the interaction between site and plant organ; k was the response variable, while site, plant organ, and the interaction of site with plant organ were the predictors. The response variable (k) was square-root transformed to meet the normality assumption. Using a linear regression we investigated the relationship between the PES and the k-values of leaves. Furthermore, we investigated the relationship between leaf protein and total carbohydrates with k-values through Pearson’s correlations.

Third, using negative binomial models we investigated if the number of *O. gammarellus* differed between leaf and stem litter bags and if they differed between litter bags from the head or the mouth of the estuary. Moreover, for leaves only (because there were almost no amphipods in stem litter bags), we investigated the relationship between the number of *O. gammarellus* with the PES through a linear regression and between *O. gammarellus* with the k-values, leaves protein content and total carbohydrates through Pearson’s correlations. All the analyses were carried out in R (R Core Team, 2018).

3. Results

3.1. Plant trait variables

Plant trait values of *S. anglica* strongly differed between sites (Fig. 2; Table 2). At the estuary mouth, plants had higher leaf dry matter content and stem C/N ratio, but lower leaf N and C content as well as lower stem N and C content. Furthermore, plants at the mouth of the estuary had higher protein content in leaves, but lower total carbohydrates, fewer leaves per stem, and lower aboveground biomass. No clear differences between marshes were found for stem specific density, plant height, or leaf C/N ratio.

3.2. Plant economic spectrum

We also observed intraspecific variability in the PES. The first PCA dimension considering all leaf and stem traits simultaneously, accounted for a high proportion of variance (0.53) and identified the presence of the PES (Fig. 3A, Table 3). Indeed, this PCA dimension discriminated between plants with leaf and stem traits related to a conservative
strategy (high LDMC, leaf and C/N) from plants with traits typical of an acquisitive strategy (high leaf and stem N content; Fig. 3A). Plants from the mouth of the estuary (Pembrey) adopted a conservative strategy (Fig. 3B; PES, t value = 5.356, p < 0.001), reflecting the higher leaf dry matter content and the lower leaf and stem nitrogen content (positive side of the x axes in Fig. 3A). Similar results were obtained when performing the PC analysis on leaf and stem traits separately: for leaf traits, the first PC axis identified the stem economic spectrum (SES, Fig. 3A, Table 3), with leaf traits displaying a conservative strategy (high leaf C/N and LDMC, positive side of the x axes in Fig. 3A) at the mouth of the estuary. This was also reflected by the relationship between the PES (first axes of the PC analysis with leaf and stem traits together) and decomposition (k-values): leaves decomposed faster when plants adopted a more conservative strategy (mouth of the estuary; Fig. 4B). Interestingly, leaf protein content and total carbohydrates were positively correlated with leaf protein content (Fig. 4E) but negatively with leaf total carbohydrates (Fig. 4F), suggesting that amphipods preferred nutrient rich leaves.

4. Discussion

This study found large variation in plant traits of S. anglica and demonstrated the existence of an intraspecific plant economic spectrum for this species. These differences in traits likely corresponded to the different environmental conditions that plants experienced at the two sites. The observed site differences in plant traits revealed the presence of the plant economic spectrum at the intraspecific level in S. anglica, with plants growing in more stressful conditions (i.e., mouth of the estuary) adopting a more conservative strategy in nutrient acquisition. In turn, these differences in plant strategies influenced decomposition rates; yet, in contrast to terrestrial studies, we observed that leaf litter from plants with a conservative strategy decomposed faster. Furthermore, our findings suggest that the macro-detritivore O. gammarellus played an important role in the decomposition of leaves but not stems.

4.1. Plant economic spectrum at the intraspecific level

We showed that leaf and stem traits of S. anglica correlated along the PES and this coordination among plant organs was further supported by the correlation between the LES and SES, indicating that plants adopting a more conservative strategy of nutrient acquisition in leaves (e.g., higher LDMC and leaf C/N) showed the same strategy in stems as well (e.g., higher stem C/N). This result is in accordance with plant economic theory which indicates that plants need to coordinate organs for an optimal resource acquisition or conservation (Reich, 2014; Reich et al., 2003). As an example, for fast resource acquisition plants invest in higher N with respect to C in leaves (high total leaf N and low leaf C/N) which allows higher photosynthetic rate (Wright et al., 2004; Reich, 2014); in turn, higher photosynthetic rate requires more water to be transpired and, therefore, plants need more and/or larger vessels in stems (higher stem production per unit of biomass; lower C/N ratio) for sustaining the leaf water demand (Reich, 2014). Our study adds to a growing body of evidence that traits are coordinated across organs for resource acquisition/conservation both across and within plant species (Freschet et al., 2010, 2012, 2012; Laughlin et al., 2010, 2015; Isaac et al., 2017; Jackson et al., 2013).

4.2. Effect of abiotic stress on economic spectra and plant traits

In line with our second hypothesis, site, underlying the abiotic environment, affected both single traits and economic spectra (LES, SES, and PES). Under increasing abiotic stress (e.g., lower redox potential, higher salinity, and higher sand content at the estuary mouth), S. anglica adopted a more conservative strategy (LES, SES, and PES) and produced

3.3. Decomposition coefficient k

The ANOVA analysis of the litter decomposition experiment revealed a significant interaction between plant organs and site (Table 4). Post-Hoc comparisons revealed that leaf litter decomposed faster than stem litter and, in particular, that leaf (but not stem) litter from the mouth of the estuary (conservative side of the plant economic spectrum) decomposed faster than leaf litter from the mouth of the estuary (acquisitive side of the plant economic spectrum; Fig. 4A; Table 4). This was also reflected by the relationship between the PES (first axes of the PC analysis with leaf and stem traits together) and decomposition (k-values): leaves decomposed faster when plants adopted a more conservative strategy (mouth of the estuary; Fig. 4B). Interestingly, leaf protein content and total carbohydrates were positively correlated with leaf decomposition k-values respectively, suggesting that these traits might have been involved in leaf decomposition. Furthermore, leaf k-values were positively correlated with amphipod number, indicating that decomposition increased at increasing amphipod abundance (Fig. 4C).
significantly lower biomass per unit of area (Fig. 2). It is also possible that abiotic factors did not directly influence plant traits, but they did indirectly through genetic differences between the two populations growing in different environmental conditions. Nevertheless, these results conform with studies in terrestrial systems, where different plant species growing in stressful environments displayed trait values characteristic of a conservative strategy (Garnier et al., 2018; Gross et al., 2013). However, our results contrast with previous studies in salt marshes, where, across species, plants shifted towards an acquisitive strategy (higher specific leaf area, lower LDMC and C/N ratio) under higher abiotic stress (Minden and Kleyer, 2011; 2012; Minden and Kleyer, 2015). Minden and Kleyer (2015) attributed such plants’ shift along the PES to specific adaptations to salinity, i.e. production of compounds (proteins and sugars) for maintaining cellular osmotic balance (Flower and Colmer, 2008; Slama et al., 2015). Here, plants experiencing higher salinity (mouth of the estuary) also produced higher leaf protein (Fig. 2), despite adopting a more conservative strategy (Fig. 3). Therefore, our study suggests that, at the intraspecific level, compounds for salinity resistance are not related to plant strategies. This discrepancy between our and previous salt marsh studies regarding trait shifts along the PES might relate to several factors. First, previous studies focused on changes at the community level (Minden and Kleyer, 2011; 2012; Minden and Kleyer, 2015), Minden and Kleyer (2015) attributed such plants’ shift along the PES to specific adaptations to salinity, i.e. production of compounds (proteins and sugars) for maintaining cellular osmotic balance (Flower and Colmer, 2008; Slama et al., 2015). Here, plants experiencing higher salinity (mouth of the estuary) also produced higher leaf protein (Fig. 2), despite adopting a more conservative strategy (Fig. 3). Therefore, our study suggests that, at the intraspecific level, compounds for salinity resistance are not related to plant strategies.

Fig. 3. Relationships between plant, leaf or stem economic spectra and abiotic variables. (A) Plots of the first two axes from the principal component analyses (PCA) with leaf and stem traits together (left), leaf traits only (middle), and stem traits only (right). Note, along the axes of the PCA plots traits are displayed according to their loading on that axis, e.g. on the PES axis negative values correspond to higher leaf N, leaf C, and stem N (see Table 3 for further details). (B) Plant differences along the PES, LES, and SES between marshes; stars indicate significant differences between marshes: ***p < 0.001, *p < 0.05. Dark green, samples from the mouth of the estuary (Pembrey marsh); Light green, samples from head of the estuary (Loughor marsh). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Principal component analysis of trait variability for Spartina anglica. For clarity, only axes with eigenvalues higher than one are reported. PCA trait loading values showing a significant difference from 0 are formatted in bold text (based on critical values for Pearson’s correlation coefficients at α = 0.05, N = 20).

<table>
<thead>
<tr>
<th>Spartina anglica</th>
<th>PES</th>
<th>LES</th>
<th>SES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First axis</td>
<td>Second axis</td>
<td>First axis</td>
</tr>
<tr>
<td>Proportion of Variance</td>
<td>0.53</td>
<td>0.19</td>
<td>0.58</td>
</tr>
<tr>
<td>Cumulative Variance</td>
<td>0.53</td>
<td>0.72</td>
<td>0.58</td>
</tr>
<tr>
<td>Trait loading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf dry matter content (mg/g)</td>
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<td>–</td>
<td>0.271</td>
</tr>
<tr>
<td>Leaf nitrogen content (%)</td>
<td>–0.887</td>
<td>–0.278</td>
<td>–0.951</td>
</tr>
<tr>
<td>Leaf carbon content (%)</td>
<td>–0.736</td>
<td>–</td>
<td>–0.729</td>
</tr>
<tr>
<td>Leaf C/N ratio</td>
<td>0.806</td>
<td>0.322</td>
<td>0.894</td>
</tr>
<tr>
<td>Stem specific density (mg/cm³)</td>
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<td>0.600</td>
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</tr>
<tr>
<td>Stem nitrogen content (%)</td>
<td>–0.869</td>
<td>0.412</td>
<td>–0.976</td>
</tr>
<tr>
<td>Stem carbon content (%)</td>
<td>–0.236</td>
<td>0.864</td>
<td>–0.504</td>
</tr>
<tr>
<td>Stem C/N ratio</td>
<td>0.842</td>
<td>–0.269</td>
<td>0.925</td>
</tr>
</tbody>
</table>
adopt a more conservative strategy. Second, differences in leaf structure might be responsible for the discrepancy between our and previous studies. We found that at the mouth of the estuary, where salinity stress is higher, leaves had higher protein content but, surprisingly, lower nitrogen concentration. Moreover, leaf nitrogen and protein content did not correlate either between (r = 0.44, p = 0.05) or within sites (Pembrey, r = -0.07, p = 0.841; Loughor, r = 0.123, p = 0.736), indicating that these two traits are not strongly linked. This lack of relationship between leaf N and protein content might have arisen because leaf N concentration accounts for all N present in a leaf and, thus also for non-protein components (e.g. nucleotides and lignin). Indeed, a study across 90 terrestrial species found that protein content does not account for all the nitrogen present in leaves (up to ~70%; Yeoh and Wee, 1994). Furthermore, another study showed that terrestrial evergreen plants invested more nitrogen in wall components for mechanical strength than in proteins for photosynthesis (Takashima et al., 2004). Thus, higher N concentration in leaves from the head of the estuary might be related to an higher requirement for mechanical strength, which seems also supported from the higher total carbohydrates content. On this regard, it is known that drag forces exerted by waves on marsh plants influence the plant C investment for increasing mechanical strength. For instance, in salt marsh plants can have soft or stiff stems (i.e., low and high mechanical strength respectively) for avoiding or resisting drag forces (Silinski et al., 2015; Heuner et al., 2015). Thus, in our study plants at the mouth of the estuary might have produced tougher leaves because they experienced higher wave/flow drag due to particular site characteristics. More studies are needed to fully elucidate how non-resource stressors, such as salinity or wave energy, affect plant traits expression and strategies.

Interestingly, we found a remarkable variability in S. anglica traits

<table>
<thead>
<tr>
<th>Predictor</th>
<th>df</th>
<th>MS</th>
<th>F-value</th>
<th>p</th>
<th>R^2</th>
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</thead>
<tbody>
<tr>
<td>Plant organ</td>
<td>1</td>
<td>5.892*10^-6</td>
<td>955.691</td>
<td>&lt;0.001</td>
<td>0.96</td>
</tr>
<tr>
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<td>6.119</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Plant organ*site</td>
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<td>2.800*10^-8</td>
<td>4.595</td>
<td>0.039</td>
<td></td>
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<tr>
<td>Tukey-HSD</td>
<td></td>
<td></td>
<td></td>
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Table 4
Two-way ANOVA of the effect of plant organ (leaf and stem) and site (Pembrey and Loughor) on the decomposition coefficient k. Pembrey, marsh at the mouth of the estuary; Loughor, marsh at the head of the estuary.

Fig. 4. Panel A, differences in decomposition k-values between leaf and stem both within and between marshes. Panel B, relationship between decomposition k-values with the PES (middle). Panel C, relationship between decomposition k-values and amphipod number (right). Panel D, relationship between amphipod number and the PES; Panel E, relationship between amphipod number with leaf protein. Panel F, relationship between amphipod number and leaf total carbohydrates. In plots B and D, toward positive values along the PES correspond plants with a more conservative strategy.
along environmental gradients (i.e., between the estuary mouth and head). In our study, variation in leaf N content corresponded to roughly 80% of the variation in the same trait found for alder (*Alnus glutinosa*) across Europe (Lecerf and Chauvet, 2008) and to 25% and 15% of the variation found across a wide range of species at ecosystems and global level respectively (Freschet et al., 2013; Diaz et al., 2016; Pietsch et al., 2014). Furthermore, leaf dry matter content (LDMC) variation here was ~20% of that found for LDMC across ecosystems (Freschet et al., 2013) and, specifically, both LDMC and C/N ratio variation were up to 45% of the variation found across species in salt marshes (Minden et al., 2012; Simões et al., 2011). Therefore, our study supports and highlights the key role that intraspecific variability can have in shaping ecosystem functioning (Blanchet et al., 2020).

### 4.3. The role of plant economic spectra, traits, and macro-detritivore abundance on litter decomposition

In our study, leaf and stem mean decomposition rate (k = 0.030 and k = 0.012, respectively) was in the range of decomposition for several Spartina species (Simões et al., 2011; Wilson, 1986; Wu et al., 2017). Leaves decomposed faster than stems (higher k-values), which is in accordance to terrestrial studies (Freschet et al., 2013; Simões et al., 2015); however, in contrast to these terrestrial studies, only leaf decomposition significantly differed between sites and leaves from plants with a more conservative strategy (mouth of estuary) decomposed faster than those with a more acquisitive strategy (head of estuary). In our study, despite shifting towards a more conservative strategy, at the mouth of the estuary plants produced leaves with higher protein content and lower total carbohydrates. Higher leaf protein content likely relates to resistance to salinity stress because proteins are involved in maintaining cellular osmotic balance (Colmer, 2003; Minden and Kleyer, 2015; Slama et al., 2015); while lower total carbohydrates possibly reflects the lower necessity for structural compounds, such as lignin, because *S. anglica* plants produce smaller leaves under stronger salinity (De Battisti et al., 2020). Therefore, the overall higher protein content and lower total carbohydrates might have boosted microbial activity and decomposition, despite the fact plants showed a conservative strategy.

We also suggest that, when placed under the same environmental conditions, the higher abundance of macro-detritivores was responsible for the higher decomposition rate in leaves from the mouth of the estuary compared to those from the head. Higher leaf palatability attracts macro-detritivores that increase litter decomposition both directly, feeding on leaves, and indirectly, boosting microbial growth (Heminga and Buth, 1991; Schrama et al., 2015). In this study, it is likely that the leaf litter at the mouth of the estuary had higher palatability due to its higher protein and lower total carbohydrates content, which attracted more *O. gammarellus* individuals and overall increased litter decomposition. Interestingly, this result would support the hypothesis proposed by Minden et al. (2012), where higher decomposition in plants growing at the seaward-side of the marsh is determined by specific adaptation to salinity stress (i.e. higher protein content). However, we found that decomposition is not directly related to changes in plant strategies, but it is likely that traits unrelated to the PES (e.g. protein content) mediate the effect of macro-detritivores on decomposition. Overall, our study highlights that it is crucial to consider the relationship between specific traits related to stress resistance and the detritivore community to fully understand variation in ecosystem functions.

Stems decomposed at similar rates in both sites, potentially related to the general high presence of structural compounds. High stem C/N ratio and low stem N content (compared to leaves) may have conferred stressed resistance to stem litter decomposition regardless of the economic strategy adopted by plants. This intrinsic low stem palatability could also explain the absence of *O. gammarellus* in stem litter bags, further reducing stem decomposition rates and, thus, minimising differences between sites. Yet, it is possible that the low abundance of *O. gammarellus* could be related to an experimental effect: we used one 10 cm stem per bag for standardising the material across litter bags; thus, the single stem per bag might have provided minimal coverage to detritivores (a habitat effect) which then avoided stem litter. It is also possible that due to the high stem structural compound (i.e. high stem C/N ratio) one year was insufficient to detect a significant difference in stem decomposition between sites.

Considering that a large portion of aboveground litter is retained within marshes and will not washed away with tides (Väikeäinen et al., 1982, 1985), our results can have important implications for carbon sequestration in salt marshes and coastal ecosystems in general. The high variability found in *S. anglica* traits within a single estuary indicates the strong role that intraspecific variability potentially plays in carbon storage in salt marshes. Considering that monocultures of single species can occupy vast areas in coastal systems around the world, such as *Posidonia oceanica* for seagrasses (Green and Short, 2003), *Avicennia germinans* in mangrove (Snedaker, 1982; Hodel et al., 2016), and *Spartina* species in salt marshes (Adams, 2002), intraspecific variability along abiotic gradients possibly plays a bigger role than currently thought for carbon and nutrient cycling. Moreover, our study indicates that under increasing salinity stress, *S. anglica* produces less aboveground biomass, which decomposes faster. Therefore, possible increments of salinity due to climate change (Watson and Byrne, 2009) could negatively impact the capacity of salt marshes to store carbon.

### 4.4. Conclusions

In this study, using the widespread *S. anglica* as a model species we showed that in salt marshes the abiotic environment can affect plant strategies at the intraspecific level and, consequently, litter decomposition rates. However, the effect of changes along the plant economic spectrum was in contrast with theory, with higher decomposition rates in the leaves of plants with a conservative strategy. This result likely arose from the interaction of specific adaptations (traits) to marsh salinity with the feeding activity of macro-detritivores. Thus, our study highlights that researchers should take specific plant adaptations to stress resistance into account in addition to plant economic strategies for fully understanding the effect of environmental gradients on ecosystem functions.

### CRediT author statement

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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