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Differential responses of three coastal grassland species to seawater flooding

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Running Head – Species-specific response to seawater flooding of soil

ABSTRACT

Aims: Supra-tidal plant communities fulfil a vital role in coastal protection, but despite an increased likelihood of seawater flooding resulting from anthropogenic climate change, we understand little about how tidal inundation affects these habitats or interactions between their component species. Our aim was to determine how three common coastal grassland species responded to simulated seawater flooding and how subsequent changes to their ecophysiology, growth and survival might affect plant-plant interactions in mixed assemblages.

Methods: Seeds of three widely-distributed European coastal grassland species (*Leontodon autumnalis* Asteraceae, *Plantago lanceolata* Plantaginaceae and *Trifolium pratense* Fabaceae) were collected from a coastal grassland site in SW England. In experiment 1 we quantified changes in leaf ion (K^+, Na^+, Cl^-) concentrations as a response to short-duration (0-, 2-, 8- or 24-hrs) immersion in seawater of the root-zone before monitoring longer-term effects on plant survival and growth. In a second experiment we examined community-level responses by subjecting mixed assemblages of all three species to seawater immersion for (0-, 12-, 24-, or 96-hrs).

Important Findings: When grown individually, one species (*Trifolium*) had markedly reduced survival with increasing soil immersion time, but a consistent decline in plant growth for all species with flooding duration was most likely linked to osmotic and ionic stresses caused by salt ion accumulation. In mixed assemblages, all species suffered increased mortality and reduced growth following seawater flooding, although the relative contribution of one species (*Leontodon*) to

total biomass increased in flooded microcosms. We thus demonstrate a number of species-specific responses to simulated seawater flooding and show that when grown together, interactions between plants are altered as a consequence. We argue that variation in the responses of component plant species will dictate how coastal plant communities respond to, and recover from, expected changes in sea levels and transient floods following storm surge events. Such information is vital in order to predict future impacts of seawater floods on supra-tidal vegetation.

Key Words – Climate Change; Salinity; Sea-level rise; Soil waterlogging; Storm surge; Microcosm

INTRODUCTION

The combined effects of sea level rise and increased likelihood of storm surge events associated with anthropogenic climate change are likely to result in an increased frequency of salt water inundation into low-lying coastal vegetation (Martin *et al.* 2011; Zappa *et al.* 2013; Feser *et al*. 2015). While ecosystems like salt marshes are naturally susceptible to frequent saline water intrusion, supra-littoral habitats such as sand dunes, grazing marshes, and pastures will likely be subject to periodic immersion in seawater for the first time. Such habitats are both economically and ecologically important since they provide a buffer against the sea for urban areas and agro-ecosystems further in-land, while at the same time offering a refuge for many plant and animal species excluded from intensive agriculture (Rhymer *et al.* 2010; Fisher *et al*. 2011; Hanley *et al.* 2014). Consequently the response of plants in these habitats to a likely increase in the frequency and duration of saline inundation may have important ramifications for conservation and coastal land-use and management (Hoggart *et al.* 2014; Hanley *et al.* 2014).

Although plants native to coastal vegetation may be naturally able to cope with some degree of exposure to salinity, our understanding of salt stress and tolerance in these ecosystems is limited. Even for coastal wetlands, relatively little is known about how salt stress affects plant survival, growth, and reproduction beyond a body of work focussed largely on the response of freshwater marsh species to salinity pulses (Van Zandt and Mopper 2002; Van Zandt *et al*. 2003; Middleton 2009; Pathikonda *et al*. 2010). This contrasts markedly with a rich literature

documenting how increased salinity in agricultural systems affects plant growth and yield (Zhu 2001; Munns and Tester 2008; Bennett *et al*. 2009; Ventura *et al*. 2015) and our wider understanding of salinity tolerance in halophytes (Flowers and Colmer 2008, 2015; Bose *et al.* 2015; Nguyen *et al*. 2015).

Plant responses to soil flooding can be attributed to impeded gas exchange resulting in oxygen deficiency and the toxic effects of chemically-reduced soils (Colmer and Voesenek 2009; Voesenek and Bailey-Serres 2015), but when the water is saline, this also imposes osmotic and ionic stresses. Osmotic stress can limit the ability of plants to absorb water, while the ionic stress results from the toxic effects of high Na^+ and Cl^- accumulated in tissues (Munns and Tester 2008). Many species restrict the rate of entry of Na^+ and Cl^- to the shoots (termed 'ion exclusion'), but plants also need to cope with high concentrations of salt ions in cells and re-establish homeostatic function which is generally achieved in three ways (Maathuis and Amtmann 1999; Munns and Tester 2008). Firstly, via accumulation of Na^+ and Cl^- in vacuoles to control concentrations in the cytoplasm; secondly, the maintenance of cytoplasmic K^+ concentrations to retain a favourable K^+/Na^+ ratio for enzyme function; and thirdly, the synthesis of organic solutes (e.g., sugars, sugar alcohols, betaines and amino acids) and their accumulation in the cytoplasm to retain osmotic balance with the ions in the vacuoles. Each of these processes can, however, impose a cost on plant growth (Zhu 2001; Munns and Tester 2008; White *et al.* 2014).

78 The problem of storm surge flooding is likely to be particularly acute for
79 low-lying coastal vegetation (Middleton 2009; Hoggart *et al*. 2014), but the impact
80 of salt water inundation in these ecosystems is poorly studied. In one of the few
81 relevant field studies, Tate and Battaglia (2013) reported how the effects of artificial
82 flooding along a Floridian estuarine system imposed the greatest negative impact in
83 the most inland (pine savannah) habitat. However, while a wide taxonomic range of
84 component plant species were affected, the ecophysiological mechanisms
85 underpinning species responses, and the likely impacts on community composition,
86 were unclear beyond a suggestion that plants from more saline habitats would
87 colonise the pine savannah. Other studies (Hanley *et al*. 2013; White *et al*. 2014)
88 have examined plant ecophysiological responses for single-species in controlled
89 conditions and have linked reduced plant performance following seawater soil
90 immersion to the accumulation of salt ions and/or reallocation of plant resources to
91 osmotic or ionic adjustments. Interestingly White *et al.* (2014) show that for white
92 clover (*Trifolium repens*), response to seawater varied between ecotypes collected
93 from along a natural salinity gradient. This finding suggests that by virtue of
94 ecotype-specific variation in the ability to tolerate seawater soil immersion, salinity
95 and/or flooding stress could act as a selective filter, removing some ecotypes or
96 species from the ecosystem. Consequently, any change in the frequency and severity
97 of storm surge events might therefore be expected to influence greatly the
98 composition of supra-littoral coastal vegetation.

99 Our primary goal was to elucidate how exposure of roots to seawater
100 immersion affected plant survival and immediate onward growth for three common

Eurasian coastal grassland species; the first time that species-specific variation in response to seawater soil flooding has been examined in controlled conditions for more than one of these species. We did this in two ways; first by looking at how each species responded to seawater soil immersion individually and linking this to the accumulation in tissues of salt ions and organic solutes (Flowers and Colmer 2008; Munns and Tester 2008). In addition we also examined how short-duration soil immersion in seawater affected the relative contribution of each species within mixed assemblages; i.e. whether species-specific response to flooding could affect plant-plant interactions and so impact on plant community composition.

MATERIALS AND METHODS

Leontodon autumnalis L., *Plantago lanceolata* L. and *Trifolium pratense* L. are herbaceous perennials native to grassland habitats throughout Eurasia (Grime *et al*. 2007). The fact that each species is a common component of coastal grassland and sand dune vegetation might indicate some potential for salt tolerance, but their absence from habitats like salt marshes points to an inability to tolerate fully saline conditions and/or sensitivity to inundation. Seeds of all three species were collected from a coastal grassland at Wembury, south Devon, England (50º19'01"N 04º05'20"W) during autumn 2011 (experiment 1) or autumn 2014 (experiment 2).

Experiment 1: Individual plant species response to soil immersion with seawater

121 Seeds were set to germinate in 350 mm × 215 mm × 70 mm (deep) plastic seed trays
122 containing John Innes No.2 compost. One-week following germination (late October
123 2011), 96 seedlings per species were transferred to 70 mm × 70 mm × 80 mm (deep)
124 plastic pots (one seedling per pot) containing John Innes No.2 compost and were
125 grown on for a further 43-d in greenhouse conditions; mean daily temperatures
126 varying between 11.1 °C (± 0.4) min and 28.6 °C (± 1.1) max, with daily watering
127 and natural daylight. When the plants were 50-d-old (mid-December), they were
128 divided into 4 equal groups; *i.e.* 24 replicate plants per treatment, but for *Leontodon*
129 *autumnalis* we excluded a number of plants that appeared to be *L. hispidus*, leaving
130 18 plants per treatment group for this species.

131 Three groups were subjected to immersion to pot level in seawater for 2-, 8-,
132 or 24-hrs (in large plastic tubs) collected from Plymouth Sound (electrical
133 conductivity = 47.8 mS cm^{-1} at 19.3 °C), with one group retained as an untreated
134 control. By immersing to pot-level (in large plastic tubs) we simulated short-term
135 soil waterlogging. Although seawater inundation following a storm-surge event
136 would potentially also result in shoot submergence, our approach allowed us to
137 focus on the effect of ionic imbalance in the root-zone rather than the additional
138 impact on shoots of oxygen deficiency or direct salt contact to the leaves which
139 would occur by full submergence. In addition, while post-storm surge flooding can
140 persist longer than 24-hrs, records from the UK coast suggest a 1-d long seawater
141 flooding event is typical (Environment Agency 2014).

142 Immediately after pot immersion, the pots were allowed to drain fully before
143 being arranged randomly on a wire mesh-topped bench inside the greenhouse; the
144 wire mesh allowed free drainage and prevented cross-contamination by any
145 leachates from pots between treatment groups.

146 ## Leaf tissue ion and metabolite analyses

147 Eight plants per treatment per species for *Plantago* and *Trifolium* and 6 plants per
148 treatment for *Leontodon* were harvested for chemical analysis 2-d after immersion
149 (these plants were subsequently discarded). A single fully expanded, non-senescent
150 leaf free of adhering compost or other contamination was removed from each plant
151 and dipped in liquid nitrogen for 10-s before being freeze-dried. Samples were then
152 stored in sealed containers with desiccant in a freezer at -80 °C until analysis.

153 Samples were combined (by adding two samples together) to achieve
154 sufficient mass for analysis (n = 4 for each species/treatment combination). For ion
155 analysis, dried samples were extracted in 5 ml dilute (0.5 M) nitric acid while
156 suspended on a shaker in dark conditions for 2-d at room temperature prior to
157 determination by flame photometry of Na^+ and K^+ and by chloridometry of Cl^-
158 (Munns *et al.* 2010). This procedure was applied to 4 replicate samples per soil
159 immersion treatment/species (minimum sample mass = 20.4 mg *Leontodon*; 20.5 mg
160 *Plantago*; 20.0 mg *Trifolium*). A reference tissue taken through the procedures
161 confirmed the reliability of these measurements.

The remaining leaf tissues were used for metabolite analysis; but for *Leontodon* and *Trifolium* a lack of material ($n \leq 2$ for each soil immersion treatment/species) limited sample replication and consequently we report only results for *Plantago* (where $n = 4$). Tissue metabolites were extracted (minimum sample mass = 45.5 mg *Plantago*) using cold (4 °C) 5% (w/v) perchloric acid and then potassium carbonate was used to neutralise extracts (Fan *et al*. 1993) which were filtered (0.22 μm) and stored at -80 ºC until analysis by HPLC. The initial HPLC analysis of glycinebetaine, proline, prolinebetaine and trigonelline, soluble sugars (fructose, glucose, sucrose) and sugar alcohols (sorbitol, mannitol and pinitol) was adapted from Slimestad and Vågen (2006). The HPLC system (Waters, Milford, MA) consisted of a 600E pump, 717plus autosampler and a 996 photo-diode array detector (PDA). As detection of fructose, glucose and sucrose with the PDA at 195 nm is insensitive, an Alltech (Deerfield, IL, USA) evaporative light scattering detector (ELSD) was also used to improve sensitivity by minimum 100 fold. Separation was achieved at 22 ± 1.0 ºC on a Prevail ES Carbohydrate column (250 x 4.6 mm i.d. with 5 μm packing; Alltech) using a gradient elution profile of acetonitrile and water at 1 ml min^{-1}. Samples in the autoinjector were held at 10 ºC, the ELSD drift tube held at 85 ºC and eluent nebulisation with high purity N_2 gas at a flow rate of 2.6 l min^{-1}.

Quantification was based on PDA peak area for glycinebetaine, proline, prolinebetaine and trigonelline, and ELSD peak area for soluble sugars and sugar alcohols. Calibration curves were generated from peak area versus the mass of standard analyte injected, with linear relationship for the PDA and a power

relationship for the ELSD output. A standard was analysed every 10 samples to check for any instrument/detector drift. Retention times of standards were used to identify analytes in the sample extracts with the PDA spectral data and peak purity used to confirm glycinebetaine, proline, prolinebetaine and trigonelline. Typical sample injections were 20 µl and runtime was 20 min per sample with Empower™ 2 software (Waters) used for data acquisition and processing.

Following *ln* transformation, the effect of immersion on leaf tissue ion (Na^+, K^+ and Cl^-) concentrations was examined using general linear models including all three species with 'Immersion Time' and 'Species' as fixed factors. The effect of 'Immersion Time' on (*ln* transformed) organic metabolite concentrations in *Plantago* was also examined using a general linear model.

Plant survival and growth

The remaining plants were watered to pot capacity (with rainwater) 48-hrs after drainage following seawater immersion, and then every 2-d (with rainwater) thereafter for a further 54-d. Greenhouse air temperatures during this phase of the experiment were: min 10.9 °C ± 0.3 and max 29.8 °C ± 1.0. All surviving plants were harvested at 106-d-old (mid-February 2012 – 56-d after soil immersion), cleaned of any adhering compost and oven-dried at 50 °C for 24-hr. The total dry weight (roots and shoots combined) attained during the period after immersion was taken as a measure of plant growth. The effect of sea water soil immersion on plant survival at harvest was examined using logistic regression. The effect of immersion

on variation in total plant dry weight for each species was examined using general linear models with 'Immersion Time' and 'Species' as fixed factors.

Experiment 2: Post-immersion plant response in mixed assemblages

In October 2014, seeds of all three species were set to germinate as described above and immediately following appearance of the radicle, seedlings were transferred to 110-mm diameter pots containing John Innes No.2 potting compost. Eight seedlings of each species were planted together into a regular hexagonal array, such that each was 20-mm away from its closest neighbour and seedlings positioned in the same stratified, random configuration in each of 28 pots. By using this arrangement, we ensured that patterns of association between the three species, which might otherwise affect competitive interactions between neighbouring seedlings (Hanley and Groves 2002), were held constant between treatments. The seedlings were grown in greenhouse conditions (mean daily min temp = 7.1 ºC ± 0.67; max = 22.6 ºC ± 1.68) for 63-d. At this time (mid-December 2014) each of the 28 pots was subjected to immersion to pot level in seawater for 12-, 24-, or 96-hrs (in large plastic tubs) collected from Plymouth Sound (electrical conductivity = 45.5 mS cm^{-1} at 16.2 ºC), with one group retained as an untreated control such that there were seven replicates of each immersion treatment. In this way we simulated the average 1-d long seawater flooding event reported for low-lying UK coastline habitats, but also extended the period to the maximum reported flood duration of 4-d (Environment Agency 2014).

Pots removed from the immersion tank were allowed to drain fully before being arranged randomly on a wire mesh-topped bench inside the greenhouse. These pots were watered to pot capacity (with rain water) 48-hrs after seawater immersion had been terminated, and then every 2-d thereafter for a further 130-d. Greenhouse air temperatures during this phase of the experiment were: 4.5 °C (± 0.24) min and 22.6 °C (± 0.65) max. The plants were checked daily and mortality recorded. All above-ground tissues of surviving plants were harvested at 193-d-old (late-April 2015), cleaned of any adhering compost and oven-dried at 50 °C for 24-hrs (roots had become too inter-twined to separate reliably).

The effect of seawater immersion on plant survival, and mean total (per individual plant species) and mean proportion of total (all species combined) above-ground dry weight at harvest was examined using a general linear model with 'Species' as a fixed factor and 'Immersion Time' as a covariate. All responses, except mean total above-ground dry weight biomass, were subjected to arcsine transformation before analysis.

RESULTS

Experiment 1: Accumulation of salt ions and stress metabolites in leaves

There was substantial accumulation of Na^+ and Cl^- in leaves of all three species as root immersion time increased ('Immersion Time' for Na^+ $F_{1,42} = 33.97$, $P < 0.001$: for Cl^- $F_{1,42} = 15.66$, $P < 0.001$). Ion accumulation in *Trifolium* was particularly high with increases of over 400% and 1000% for Na^+ and Cl^- respectively, in all treatment groups (Figure 1). While we found a significant 'Species' effect for Na^+

($F_{1,42}$ = 4.14, P = 0.023: *Leontodon* had consistently higher concentrations), there was no 'Species' effect for Cl^- ($F_{1,42}$ = 0.19, P = 0.828) and no interaction between 'Species' and 'Immersion Time' for either ion (Na^+ $F_{2,42}$ = 0.17, P = 0.845; Cl^- $F_{2,42}$ = 01.12, P = 0.337) suggesting that responses to seawater immersion were broadly consistent between species. There was no effect of 'Immersion Time' on K^+ concentrations ($F_{1,42}$ = 0.81, P = 0.373); the significant 'Species' effect ($F_{1,42}$ = 42.06, P < 0.001) denoted the consistently higher K^+ concentrations in *Leontodon*. Of the stress metabolites examined in *Plantago*, we found detectable amounts of fructose and glucose, but these did not vary according to immersion time, whereas sorbitol did increase in leaf tissue following the seawater immersion (Table 1).

Experiment 1: Plant survival and growth

When assayed 2-d after soil immersion, leachates from pots immersed in seawater had substantially higher Electrical Conductivity (ECw) than control pots (ECw range 3.0 to 3.3 mS cm^{-1}) but were broadly similar for across species and immersion treatments (ECw range 29.3 to 39.7 mS cm^{-1}) (Supplementary material Appendix 1 Fig. S1a-1c). There was a steady decline in ECw until 35 days post immersion, when conductivity for all species/treatment combinations declined to levels similar to controls (ECw range 2.1 to 3.1 mS cm^{-1}).

Immersion of the root-zone in seawater, even up to 24-hrs, had no effect on the survival of *Leontodon* or *Plantago* up until final harvest (Figure 2a). However, *Trifolium* mortality increased with longer immersion time (logistic regression χ^2 = $8.675_{df=2}$, P = 0.005). For plants surviving to harvest at 106-d old, plant growth (dry

weight) for all species (Figure 2b) was consistently reduced by increasing immersion times ('Immersion Time' $F_{1,122} = 31.28$, $P < 0.001$). We also detected a significant 'Species' effect ($F_{2,122} = 28.93$, $P < 0.001$) due to the generally larger size of all *Plantago* individuals, but there was no species-specific variation in final dry weight response of plant species to immersion time ('Immersion Time' × 'Species' $F_{2,122} = 1.15$, $P = 0.226$).

Experiment 2: Post-immersion plant response in mixed assemblages

When assayed 2-d after immersion, conductivity of leachates from pots immersed in seawater was substantially higher (ECw range 34.6 to 38.3 mS cm^{-1}) than the control (ECw 2.1 mS cm^{-1}) (Supplementary material Appendix 1 Fig. S2). There was a steady decline in ECw until harvest at 106 days post immersion, although conductivity did not fall to similar levels of the controls (ECw 0.9 mS cm^{-1}) for any species/treatment combinations (ECw range 2.1 to 4.0 mS cm^{-1}).

Plant mortality (Figure 3a) increased with longer soil immersion times ('Immersion Time' effect $F_{3,72} = 9.73$, $P < 0.001$), and although the response varied between species ('Species' effect $F_{2,72} = 18.72$, $P < 0.001$), there was no interaction ('Immersion Time' × 'Species' $F_{6,72} = 1.15$, $P = 0.345$). Unlike Experiment 1, immersion in seawater caused *Leontodon* and *Plantago* mortality. However as in Experiment 1, *Trifolium* suffered most of the three species, with a comparable mortality (64%) in the 24-hrs treatment here to that in Experiment 1 (58%), but with over three quarters of all plants dying in the 96-hrs immersion treatment.

Similarly the negative effect of seawater soil flooding on total above-ground dry mass (Figure 3b) increased for each species with longer immersion durations ('Immersion Time' $F_{3,72} = 26.50$, $P < 0.001$). There was variation between species ('Species' effect $F_{2,72} = 51.60$, $P < 0.001$) and a significant interaction effect ('Immersion Time' × 'Species' $F_{6,72} = 11.71$, $P < 0.001$). All three species displayed reduced shoot dry mass, but the greatest effects were seen in *Plantago* (average shoot mass in the 12-hrs treatment was only 27% of controls), with *Leontodon* showing a smaller, but still marked, decline from the 0- to 12-hrs treatment (38%). The impact of 12-hrs immersion on *Trifolium* growth was less pronounced than on the other species (53%), although for longer durations *Trifolium* shoot dry mass was much reduced (at only 12.5% of the control, *Trifolium* in the 24-hrs treatment suffered the most severe relative decrease for any of the three species).

When we considered the relative contribution made by each species to above-ground dry mass (Figure 3c), the decline in absolute total above-ground *Plantago* biomass had very little impact on the species' relative contribution to the assemblage; *Plantago* dominated all treatments with over 60% above-ground biomass in each. Indeed increased immersion duration had no overall impact on relative biomass ('Immersion Time' $F_{3,72} = 0.58$, $P = 0.632$), and the variation between species ('Species' $F_{2,72} = 335.95$, $P < 0.001$) probably reflected *Plantago*'s dominance. However, *Leontodon* was the only species to display an increased relative contribution to total pot shoot dry mass following immersion (most apparent at 24-hrs duration) and this response mirrored the decline shown by *Trifolium*,

probably accounting for the significant interaction effect ('Immersion Time' × 'Species' $F_{6,72} = 6.22$, $P < 0.001$).

DISCUSSION

Plant species and communities globally are expected to experience a number of environmental pressures linked to anthropogenic climate change (Parmesan and Hanley 2015). However, while the impacts of elevated CO_2 and associated shifts in temperature and precipitation regimes on plants are well understood, relatively few authors have considered the effect that increased sea-levels and storm surge events are likely to have on coastal vegetation (for examples see: Middleton 2009; Tate and Battaglia 2013; Hoggart *et al*. 2014; White *et al*. 2014). Our results evidenced differences in grassland plant species responses to simulated seawater soil immersion. While each species experienced salt ion accumulation in leaves immediately after immersion of the roots, longer term impacts varied between species, with *Trifolium pratense* showing particularly marked reductions in survival and growth. The outcome was that final plant community composition in the mixed assemblages shifted as a consequence of seawater soil immersion, a result suggestive of potentially important ramifications of storm surges for low-lying coastal grassland and dune ecosystems under future climate scenarios.

All species accumulated Na^+ and Cl^- in their leaves as root immersion time increased, something frequently observed when plants are subject to salt-stress (Greenway and Munns 1980). By virtue of salt ion accumulation, seawater is widely expected to exert the kind of relatively rapid negative effect on plant growth and

336 survival seen here (see Munns and Tester 2008 for a review). Osmotic imbalance
337 leads to longer periods of stomatal closure and reduced CO_2 uptake with associated
338 impacts on photosynthesis, cell division, and cell expansion (Zhu 2001); ion toxicity
339 causes leaf necrosis also reduces photosynthetic area, further diminishing the plant
340 carbon budget (Munns *et al*. 1995). Increased mortality in *Trifolium*, but with
341 similar leaf ion concentrations as *Leontodon*, indicates lower tissue tolerance of the
342 potentially toxic Na^+ and/or Cl^- for this species; whereas *Plantago* leaf tissue
343 maintained lower Na^+ and Cl^- concentrations indicating a superior capacity for ion
344 'exclusion' from the leaves. In addition to potential ion toxicity, *Trifolium* appears to
345 have been more susceptible to the 'osmotic shock' imposed by seawater soil
346 inundation, as this species showed the highest mortality during the post-flooding
347 recovery phase. In addition, as a legume species capable of forming a symbiosis
348 with nitrogen-fixing *Rhizobium* bacteria, *Trifolium* may also be particularly
349 susceptible to seawater flooding, as both short-term anoxic conditions (James and
350 Crawford 1998), and the direct impact of salinity (Brunning *et al*. 2015) are known
351 to adversely affect rhizobia. Indeed, many herbaceous legumes, including *Trifolium*
352 species, are relatively intolerant of salinity (Rogers *et al*. 2005).

353 An ability to maintain K^+ in leaf tissues when challenged with excess Na^+
354 can enable a plant to retain a favourable cytoplasmic K^+/Na^+ ratio for enzyme
355 functioning (Greenway and Munns 1980; Maathuis and Amtmann 1999). In this
356 study, leaf tissue K^+ declined moderately in *Leontodon*, but was maintained in
357 *Trifolium* and *Plantago*, following saltwater immersion of the roots. Organic solutes
358 which accumulate in the cytoplasm in response to salinity stress are important for

cellular osmotic balance (Greenway and Munns 1980; Flowers and Colmer 2008; Slama *et al*. 2015) although our ability to detect trends in these metabolites was limited by low availability of leaf tissue samples for two of the three species under investigation. Nonetheless for *Plantago*, we did detect an increase in sorbitol with longer immersion durations, a result that mirrors the accumulation of this solute when the species has been challenged by drought or salinity (Lewis 1984). It is interesting to speculate that the accumulation of sorbitol would have contributed to the relative tolerance of *Plantago* under the conditions of transient seawater soil immersion.

In the relatively diverse vegetation of coastal ecosystems, it seems likely that by virtue of variation in ecophysiological responses, component species will differ in their tolerance of, and recovery from, the pulse of salinity and likely soil anoxia imposed by storm surges (Colmer and Flowers 2008; Munns and Tester 2008; Bennett *et al*. 2009). While we report consistent negative impacts on plant growth responses for all three species to 24-hr long seawater immersion (experiment 1), there was particularly high mortality in *Trifolium* and marked reductions in plant growth for survivors (*Trifolium* plants in the 24-hr treatment attained a mean mass only 15% of that recorded for controls, by contrast the same comparison for *Plantago* and *Leontodon* was 49% and 43% respectively). This result points to species-specific variation in plant response to simulated seawater flooding.

Our pot microcosm study demonstrated the potential for shifts in community composition as a result of variation in plant species response to transient seawater

soil flooding. Mortality again varied between species; although some *Leontodon* and *Plantago* individuals died in the longer duration immersion treatments in mixed assemblages, *Trifolium* mortality remained considerably higher. The magnitude of post-immersion plant growth responses also varied. Although this did not translate into major shifts in the relative contribution of each species to total pot biomass, the dominance of *Plantago* in all treatments might explain why no large-scale changes in pot composition developed. This situation is analogous to many studies on the effect of elevated CO_2 on plant communities where dominance by one or two species masked any major change in community biomass or even the relative contribution of component species (Leadley *et al*. 1999; Niklaus *et al*. 2001; Hanley *et al*. 2004).

As these studies highlight however, more subtle shifts in the performance of subordinate species could be important. If the apparently greater susceptibility of *Trifolium* to seawater soil immersion mortality found in our study is translated into a field response, the loss of an N_2-fixing Fabaceae species could have long-term impacts on nutrient cycling and community recovery following storm surge flooding. Moreover, the relative decline in *Trifolium* biomass (mirrored as it was by a similar increase in *Leontodon* contribution) is intriguing and suggestive of a need to investigate community-level response to seawater flooding in more detail. Indeed, the role of salt-stress in the mediation of competition and structuring of coastal plant communities is relatively well understood (Engels and Jensen 2010; Guo and Pennings 2012), although these studies focus on the exclusion of competitively-dominant freshwater plants from salinised environments. A recent study of tundra

response to a major storm surge event in the Canadian Arctic (Lantz *et al.* 2015) however, also shows that post-inundation recovery varies within terrestrial plant communities, with graminoids exhibiting greater resilience than shrubs. In addition recovery was also linked to soil salinity and the extent of subsequent leaching of soil salts by freshwater inundation. This latter observation raises the possibility that variation in seasonal rainfall might affect post-seawater inundation responses in temperate coastal grasslands. Given the increased likelihood of storm surge events over coming decades (Martin *et al.* 2011; Feser *et al.* 2015) and the importance of low-lying coastal vegetation for conservation and coastal defence (Fisher *et al.* 2011; Hoggart *et al.* 2014), a more detailed understanding of the structural and functional response of coastal vegetation to short-term seawater flooding is particularly pressing.

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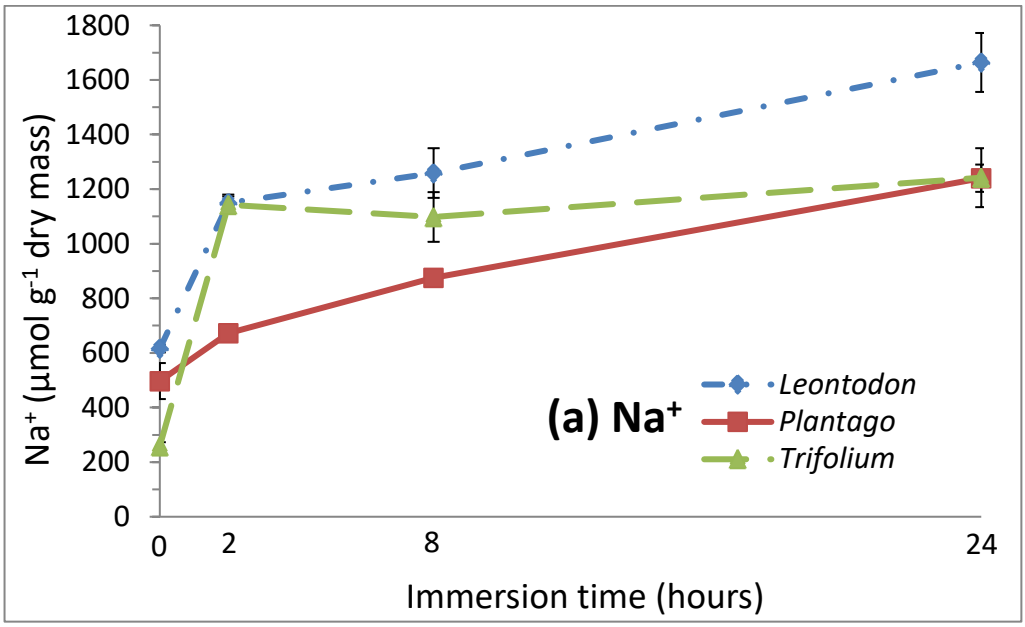
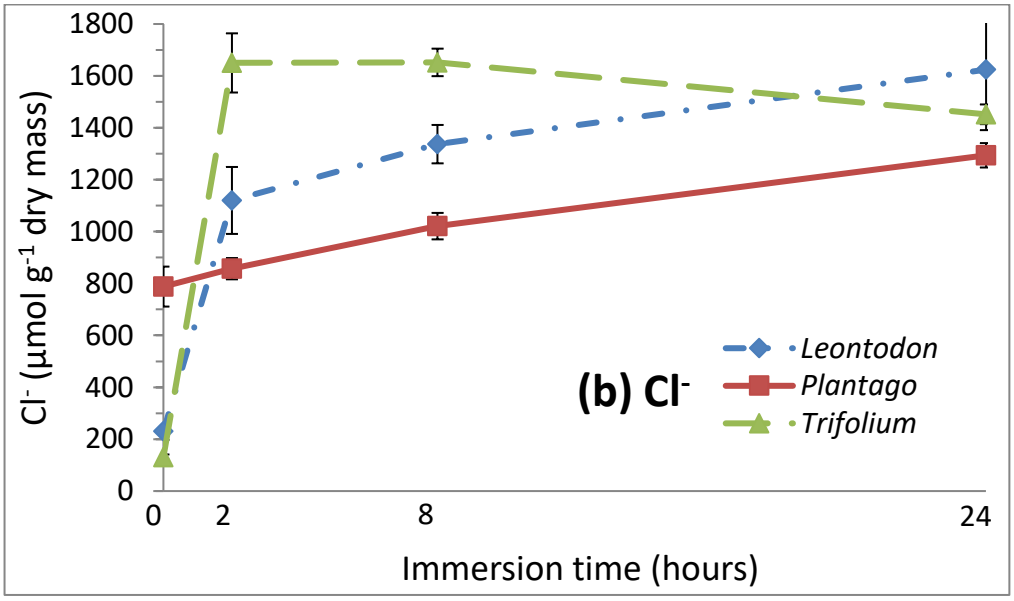

1800
1600
1400
1200
1000
800
600
400
200
0
Na+ (µmol g-1 dry mass)
0
2
8
24
Immersion time (hours)
(a) Na+
Leontodon
Plantago
Trifolium


1800
1600
1400
1200
1000
800
600
400
200
0
Cl- (µmol g-1 dry mass)
0
2
8
24
Immersion time (hours)
(b) Cl-
Leontodon
Plantago
Trifolium

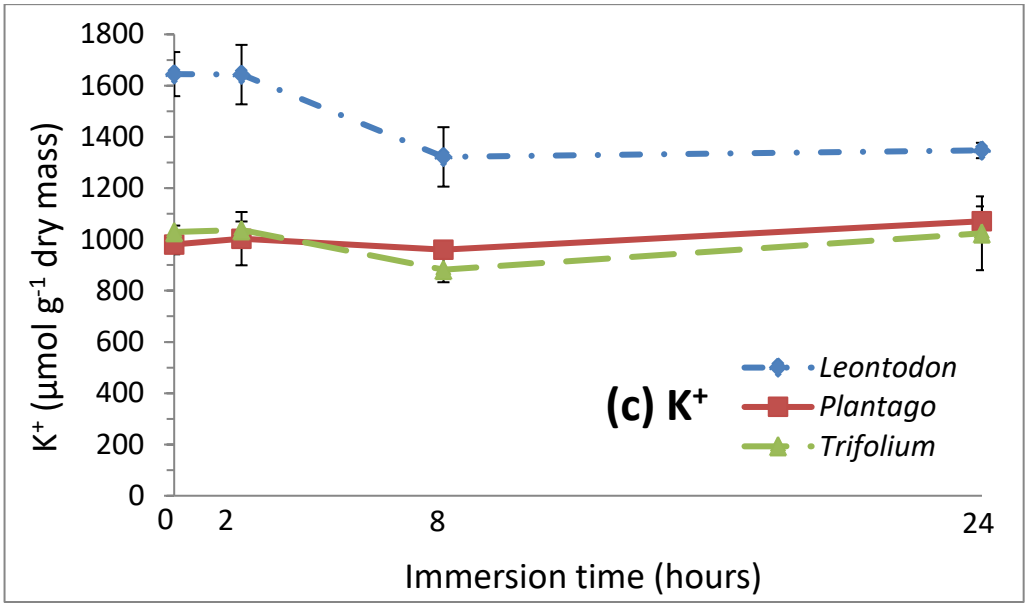


Figure 1. Ion concentrations (µmol g^{-1} dry mass), (a) Na^+, (b) Cl^- and (c) K^+, in leaf tissues of three coastal grassland plant species (*Leontodon autumnalis, Plantago lanceolata* and *Trifolium pratense*) grown in monocultures and sampled 2-d after root-zone immersion in seawater (2, 8, or 24 hours with a zero hour control).

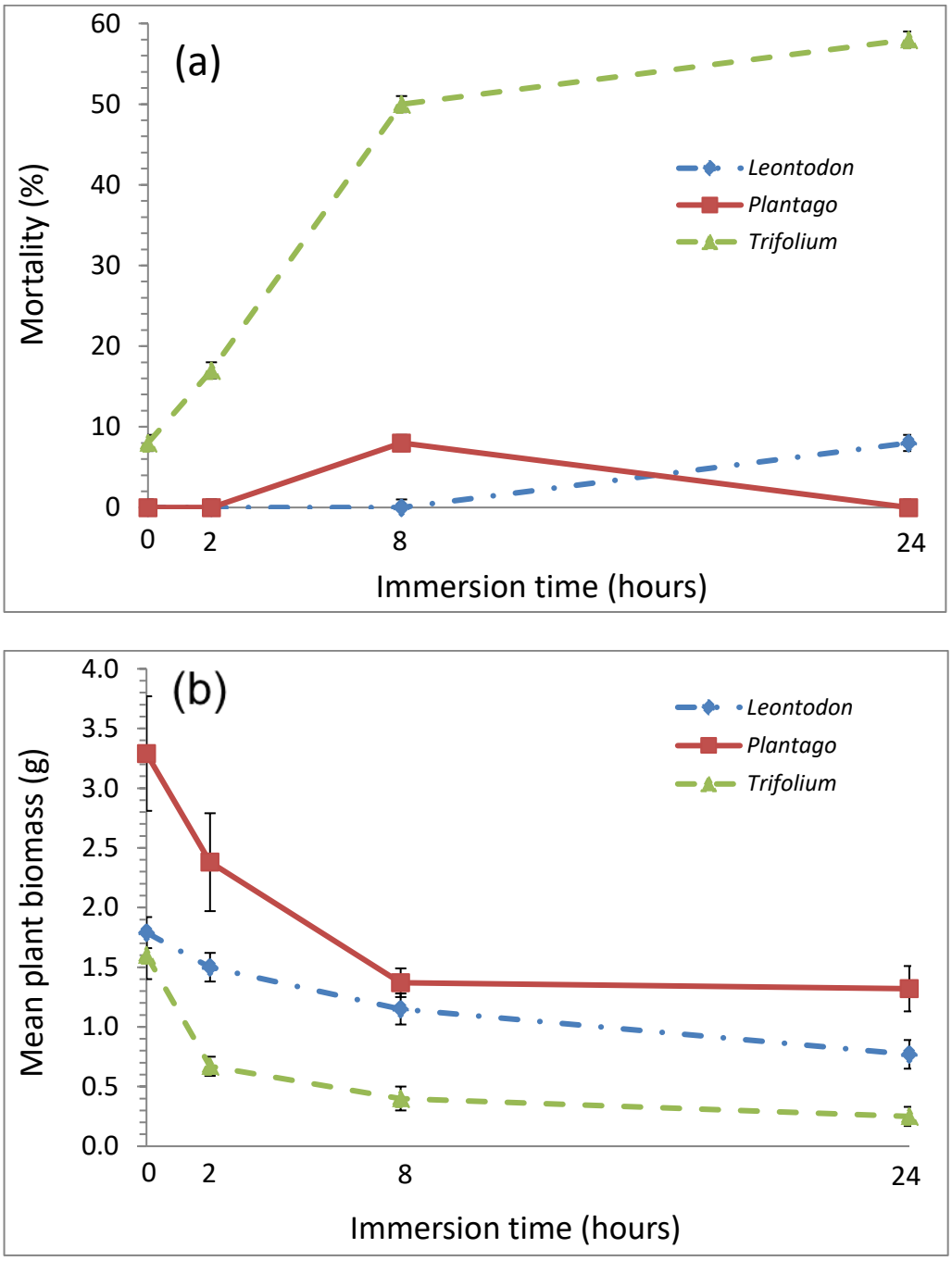
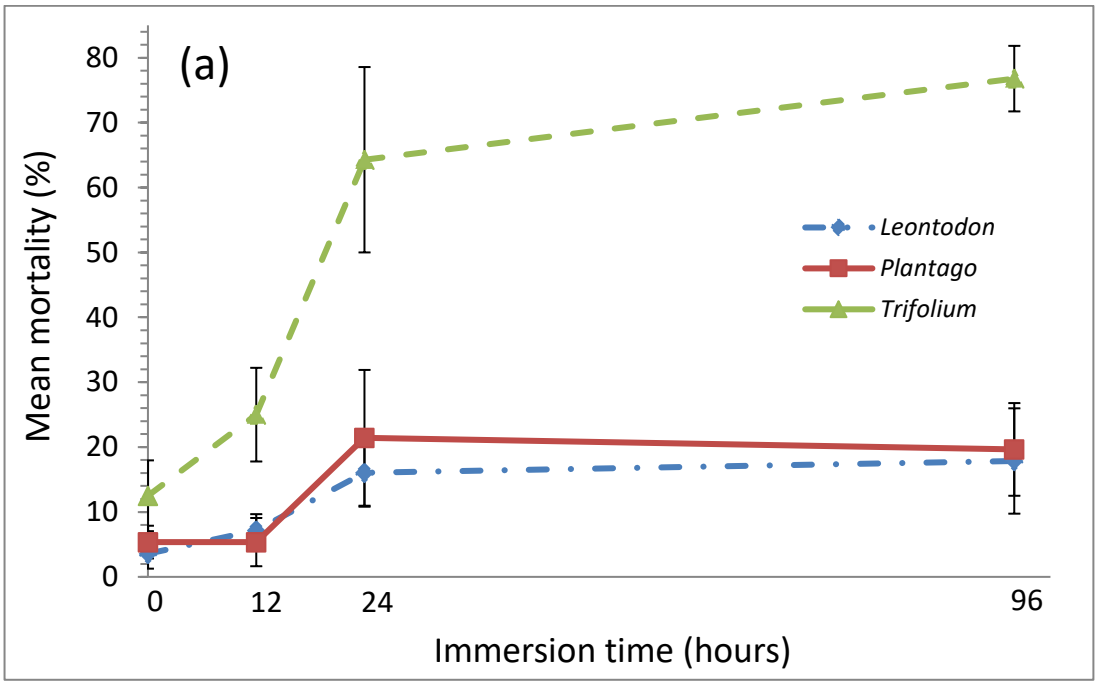
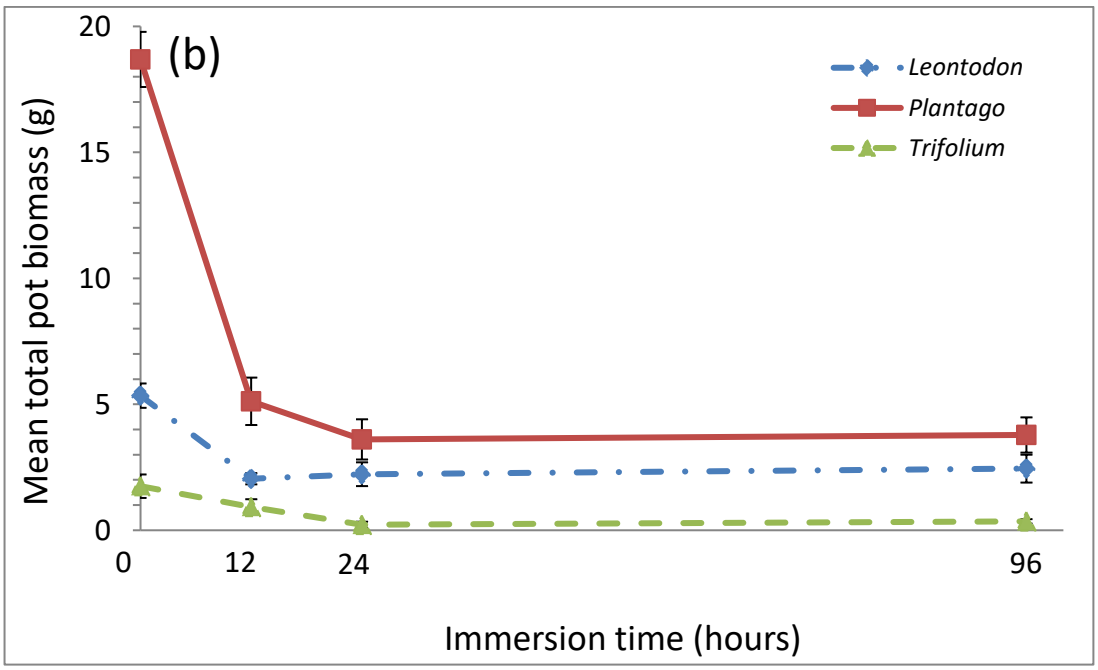


Figure 2. The effect of plant root-zone immersion in seawater on (a) total plant mortality and (b) mean individual dry mass of three coastal grassland plant species (*Leontodon autumnalis*, *Plantago lanceolata* and *Trifolium pratense*) grown in monocultures and sampled 56-d after transient immersion (2, 8, or 24 hours with a zero hour control).


(a)
Mean mortality (%)
80
70
60
50
40
30
20
10
0
0
12
24
96
Immersion time (hours)
Leontodon
Plantago
Trifolium


(b)
Mean total pot biomass (g)
20
15
10
5
0
0
12
24
96
Immersion time (hours)
Leontodon
Plantago
Trifolium

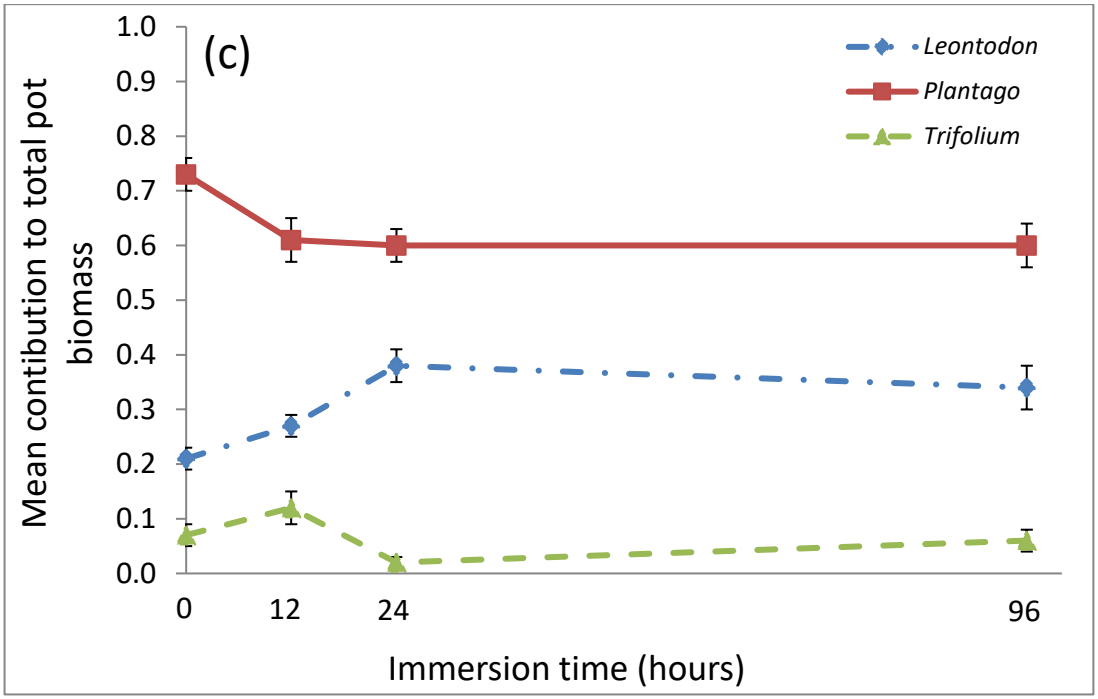


Figure 3. The effect of plant root-zone immersion in seawater on (a) plant mortality, (b) mean (± SE) total above-ground dry mass and (c) mean relative contribution to total above-ground dry mass of three coastal grassland plant species (*Leontodon autumnalis*, *Plantago lanceolata* and *Trifolium pratense*) grown in mixed assemblages 130-d after transient immersion (12, 24, or 96 hours with a zero hour control).

Immersion duration (hours)	*n*	Fructose		Glucose		Sorbitol	
		Mean	SE	Mean	SE	Mean	SE
0	4	15	5	74	4	64	4
2	4	13	5	78	10	98	6
8	4	24	7	83	12	124	15
24	4	ND		67	4	90	2
GLM Factor		***F***	***P***	***F***	***P***	***F***	***P***
Immersion Time$_{(DF = 3,12)}$		1.01	0.422	0.60	0.625	11.06	**0.009**

ND denotes 'not detected', a failure to detect any quantity above the detection limit which for the various compounds were: proline (12 µmol g^{-1} Dry Mass), fructose (14 µmol g^{-1} DM), glucose (20 µmol g^{-1} DM), sucrose (6 µmol g^{-1} DM), sorbitol (4 µmol g^{-1} DM) and pinitol (36 µmol g^{-1} DM).

Table 1. Organic solute concentrations (µmol g^{-1} dry mass) in leaf tissues of *Plantago lanceolata* when grown in monoculture and sampled 2-d after root-zone immersion in seawater (2, 8, or 24 hours with a zero hour control). The results of a general linear model showing how immersion duration affected solute concentrations are given with significant ($P < 0.05$) responses shown in bold.