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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

1 **ABSTRACT**

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

9 **Methods:** Seeds of three widely-distributed European coastal grassland species
10 (*Leontodon autumnalis* Asteraceae, *Plantago lanceolata* Plantaginaceae and
11 *Trifolium pratense* Fabaceae) were collected from a coastal grassland site in SW
12 England. In experiment 1 we quantified changes in leaf ion (K^+ , Na^+ , Cl^-)
13 concentrations as a response to short-duration (0-, 2-, 8- or 24-hrs) immersion in
14 seawater of the root-zone before monitoring longer-term effects on plant survival
15 and growth. In a second experiment we examined community-level responses by
16 subjecting mixed assemblages of all three species to seawater immersion for (0-, 12-
17 , 24-, or 96-hrs).

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

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

31

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

34

35 INTRODUCTION

36 The combined effects of sea level rise and increased likelihood of storm surge events
37 associated with anthropogenic climate change are likely to result in an increased
38 frequency of salt water inundation into low-lying coastal vegetation (Martin *et al.*
39 2011; Zappa *et al.* 2013; Feser *et al.* 2015). While ecosystems like salt marshes are
40 naturally susceptible to frequent saline water intrusion, supra-littoral habitats such as
41 sand dunes, grazing marshes, and pastures will likely be subject to periodic
42 immersion in seawater for the first time. Such habitats are both economically and
43 ecologically important since they provide a buffer against the sea for urban areas and
44 agro-ecosystems further in-land, while at the same time offering a refuge for many
45 plant and animal species excluded from intensive agriculture (Rhymer *et al.* 2010;
46 Fisher *et al.* 2011; Hanley *et al.* 2014). Consequently the response of plants in these
47 habitats to a likely increase in the frequency and duration of saline inundation may
48 have important ramifications for conservation and coastal land-use and management
49 (Hoggart *et al.* 2014; Hanley *et al.* 2014).

50 Although plants native to coastal vegetation may be naturally able to cope
51 with some degree of exposure to salinity, our understanding of salt stress and
52 tolerance in these ecosystems is limited. Even for coastal wetlands, relatively little is
53 known about how salt stress affects plant survival, growth, and reproduction beyond
54 a body of work focussed largely on the response of freshwater marsh species to
55 salinity pulses (Van Zandt and Mopper 2002; Van Zandt *et al.* 2003; Middleton
56 2009; Pathikonda *et al.* 2010). This contrasts markedly with a rich literature

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

61 Plant responses to soil flooding can be attributed to impeded gas exchange
62 resulting in oxygen deficiency and the toxic effects of chemically-reduced soils
63 (Colmer and Voesenek 2009; Voesenek and Bailey-Serres 2015), but when the
64 water is saline, this also imposes osmotic and ionic stresses. Osmotic stress can limit
65 the ability of plants to absorb water, while the ionic stress results from the toxic
66 effects of high Na^+ and Cl^- accumulated in tissues (Munns and Tester 2008). Many
67 species restrict the rate of entry of Na^+ and Cl^- to the shoots (termed ‘ion
68 exclusion’), but plants also need to cope with high concentrations of salt ions in cells
69 and re-establish homeostatic function which is generally achieved in three ways
70 (Maathuis and Amtmann 1999; Munns and Tester 2008). Firstly, via accumulation
71 of Na^+ and Cl^- in vacuoles to control concentrations in the cytoplasm; secondly, the
72 maintenance of cytoplasmic K^+ concentrations to retain a favourable K^+/Na^+ ratio
73 for enzyme function; and thirdly, the synthesis of organic solutes (e.g., sugars, sugar
74 alcohols, betaines and amino acids) and their accumulation in the cytoplasm to
75 retain osmotic balance with the ions in the vacuoles. Each of these processes can,
76 however, impose a cost on plant growth (Zhu 2001; Munns and Tester 2008; White
77 *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

101 Eurasian coastal grassland species; the first time that species-specific variation in
102 response to seawater soil flooding has been examined in controlled conditions for
103 more than one of these species. We did this in two ways; first by looking at how
104 each species responded to seawater soil immersion individually and linking this to
105 the accumulation in tissues of salt ions and organic solutes (Flowers and Colmer
106 2008; Munns and Tester 2008). In addition we also examined how short-duration
107 soil immersion in seawater affected the relative contribution of each species within
108 mixed assemblages; i.e. whether species-specific response to flooding could affect
109 plant-plant interactions and so impact on plant community composition.

110 **MATERIALS AND METHODS**

111 *Leontodon autumnalis* L., *Plantago lanceolata* L. and *Trifolium pratense* L. are
112 herbaceous perennials native to grassland habitats throughout Eurasia (Grime *et al.*
113 2007). The fact that each species is a common component of coastal grassland and sand
114 dune vegetation might indicate some potential for salt tolerance, but their absence from
115 habitats like salt marshes points to an inability to tolerate fully saline conditions and/or
116 sensitivity to inundation. Seeds of all three species were collected from a coastal
117 grassland at Wembury, south Devon, England (50°19'01"N 04°05'20"W) during
118 autumn 2011 (**experiment 1**) or autumn 2014 (**experiment 2**).

119 Experiment 1: Individual plant species response to soil immersion with
120 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.

162 The remaining leaf tissues were used for metabolite analysis; but for
163 *Leontodon* and *Trifolium* a lack of material ($n \leq 2$ for each soil immersion
164 treatment/species) limited sample replication and consequently we report only
165 results for *Plantago* (where $n = 4$). Tissue metabolites were extracted (minimum
166 sample mass = 45.5 mg *Plantago*) using cold (4 °C) 5% (w/v) perchloric acid and
167 then potassium carbonate was used to neutralise extracts (Fan *et al.* 1993) which
168 were filtered (0.22 µm) and stored at -80 °C until analysis by HPLC. The initial
169 HPLC analysis of glycinebetaine, proline, prolinebetaine and trigonelline, soluble
170 sugars (fructose, glucose, sucrose) and sugar alcohols (sorbitol, mannitol and
171 pinitol) was adapted from Slimestad and Vågen (2006). The HPLC system (Waters,
172 Milford, MA) consisted of a 600E pump, 717plus autosampler and a 996 photo-
173 diode array detector (PDA). As detection of fructose, glucose and sucrose with the
174 PDA at 195 nm is insensitive, an Alltech (Deerfield, IL, USA) evaporative light
175 scattering detector (ELSD) was also used to improve sensitivity by minimum 100
176 fold. Separation was achieved at 22 ± 1.0 °C on a Prevail ES Carbohydrate column
177 (250 x 4.6 mm i.d. with 5 µm packing; Alltech) using a gradient elution profile of
178 acetonitrile and water at 1 ml min⁻¹. Samples in the autoinjector were held at 10 °C,
179 the ELSD drift tube held at 85 °C and eluent nebulisation with high purity N₂ gas at
180 a flow rate of 2.6 l min⁻¹.

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

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

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

196 Plant survival and growth

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

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

208 Experiment 2: Post-immersion plant response in mixed assemblages

209 In October 2014, seeds of all three species were set to germinate as described above
210 and immediately following appearance of the radicle, seedlings were transferred to
211 110-mm diameter pots containing John Innes No.2 potting compost. Eight seedlings
212 of each species were planted together into a regular hexagonal array, such that each
213 was 20-mm away from its closest neighbour and seedlings positioned in the same
214 stratified, random configuration in each of 28 pots. By using this arrangement, we
215 ensured that patterns of association between the three species, which might
216 otherwise affect competitive interactions between neighbouring seedlings (Hanley
217 and Groves 2002), were held constant between treatments. The seedlings were
218 grown in greenhouse conditions (mean daily min temp = $7.1\text{ }^{\circ}\text{C} \pm 0.67$; max = 22.6
219 $^{\circ}\text{C} \pm 1.68$) for 63-d. At this time (mid-December 2014) each of the 28 pots was
220 subjected to immersion to pot level in seawater for 12-, 24-, or 96-hrs (in large
221 plastic tubs) collected from Plymouth Sound (electrical conductivity = 45.5 mS cm^{-1}
222 at $16.2\text{ }^{\circ}\text{C}$), with one group retained as an untreated control such that there were
223 seven replicates of each immersion treatment. In this way we simulated the average
224 1-d long seawater flooding event reported for low-lying UK coastline habitats, but
225 also extended the period to the maximum reported flood duration of 4-d
226 (Environment Agency 2014).

227 Pots removed from the immersion tank were allowed to drain fully before
228 being arranged randomly on a wire mesh-topped bench inside the greenhouse. These
229 pots were watered to pot capacity (with rain water) 48-hrs after seawater immersion
230 had been terminated, and then every 2-d thereafter for a further 130-d. Greenhouse
231 air temperatures during this phase of the experiment were: 4.5 °C (\pm 0.24) min and
232 22.6 °C (\pm 0.65) max. The plants were checked daily and mortality recorded. All
233 above-ground tissues of surviving plants were harvested at 193-d-old (late-April
234 2015), cleaned of any adhering compost and oven-dried at 50 °C for 24-hrs (roots
235 had become too inter-twined to separate reliably).

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

242 RESULTS

243 Experiment 1: Accumulation of salt ions and stress metabolites in leaves
244 There was substantial accumulation of Na⁺ and Cl⁻ in leaves of all three species as
245 root immersion time increased (‘Immersion Time’ for Na⁺ $F_{1,42} = 33.97$, $P < 0.001$:
246 for Cl⁻ $F_{1,42} = 15.66$, $P < 0.001$). Ion accumulation in *Trifolium* was particularly high
247 with increases of over 400% and 1000% for Na⁺ and Cl⁻ respectively, in all
248 treatment groups (Figure 1). While we found a significant ‘Species’ effect for Na⁺

249 ($F_{1,42} = 4.14$, $P = 0.023$: *Leontodon* had consistently higher concentrations), there
250 was no ‘Species’ effect for Cl^- ($F_{1,42} = 0.19$, $P = 0.828$) and no interaction between
251 ‘Species’ and ‘Immersion Time’ for either ion (Na^+ $F_{2,42} = 0.17$, $P = 0.845$; Cl^- $F_{2,42}$
252 $= 01.12$, $P = 0.337$) suggesting that responses to seawater immersion were broadly
253 consistent between species. There was no effect of ‘Immersion Time’ on K^+
254 concentrations ($F_{1,42} = 0.81$, $P = 0.373$); the significant ‘Species’ effect ($F_{1,42} =$
255 42.06 , $P < 0.001$) denoted the consistently higher K^+ concentrations in *Leontodon*.
256 Of the stress metabolites examined in *Plantago*, we found detectable amounts of
257 fructose and glucose, but these did not vary according to immersion time, whereas
258 sorbitol did increase in leaf tissue following the seawater immersion (Table 1).

259 Experiment 1: Plant survival and growth

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

267 Immersion of the root-zone in seawater, even up to 24-hrs, had no effect on
268 the survival of *Leontodon* or *Plantago* up until final harvest (Figure 2a). However,
269 *Trifolium* mortality increased with longer immersion time (logistic regression $\chi^2 =$
270 $8.675_{df=2}$, $P = 0.005$). For plants surviving to harvest at 106-d old, plant growth (dry

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

277 Experiment 2: Post-immersion plant response in mixed assemblages

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

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

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

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

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

316 **DISCUSSION**

317 Plant species and communities globally are expected to experience a number of
318 environmental pressures linked to anthropogenic climate change (Parmesan and
319 Hanley 2015). However, while the impacts of elevated CO₂ and associated shifts in
320 temperature and precipitation regimes on plants are well understood, relatively few
321 authors have considered the effect that increased sea-levels and storm surge events
322 are likely to have on coastal vegetation (for examples see: Middleton 2009; Tate and
323 Battaglia 2013; Hoggart *et al.* 2014; White *et al.* 2014). Our results evidenced
324 differences in grassland plant species responses to simulated seawater soil
325 immersion. While each species experienced salt ion accumulation in leaves
326 immediately after immersion of the roots, longer term impacts varied between
327 species, with *Trifolium pratense* showing particularly marked reductions in survival
328 and growth. The outcome was that final plant community composition in the mixed
329 assemblages shifted as a consequence of seawater soil immersion, a result
330 suggestive of potentially important ramifications of storm surges for low-lying
331 coastal grassland and dune ecosystems under future climate scenarios.

332 All species accumulated Na⁺ and Cl⁻ in their leaves as root immersion time
333 increased, something frequently observed when plants are subject to salt-stress
334 (Greenway and Munns 1980). By virtue of salt ion accumulation, seawater is widely
335 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 CO₂ 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**

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

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

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

381 soil flooding. Mortality again varied between species; although some *Leontodon* and
382 *Plantago* individuals died in the longer duration immersion treatments in mixed
383 assemblages, *Trifolium* mortality remained considerably higher. The magnitude of
384 post-immersion plant growth responses also varied. Although this did not translate
385 into major shifts in the relative contribution of each species to total pot biomass, the
386 dominance of *Plantago* in all treatments might explain why no large-scale changes
387 in pot composition developed. This situation is analogous to many studies on the
388 effect of elevated CO₂ on plant communities where dominance by one or two
389 species masked any major change in community biomass or even the relative
390 contribution of component species (Leadley *et al.* 1999; Niklaus *et al.* 2001; Hanley
391 *et al.* 2004).

392 As these studies highlight however, more subtle shifts in the performance of
393 subordinate species could be important. If the apparently greater susceptibility of
394 *Trifolium* to seawater soil immersion mortality found in our study is translated into a
395 field response, the loss of an N₂-fixing Fabaceae species could have long-term
396 impacts on nutrient cycling and community recovery following storm surge
397 flooding. Moreover, the relative decline in *Trifolium* biomass (mirrored as it was by
398 a similar increase in *Leontodon* contribution) is intriguing and suggestive of a need
399 to investigate community-level response to seawater flooding in more detail. **Indeed,**
400 **the role of salt-stress in the mediation of competition and structuring of coastal plant**
401 **communities is relatively well understood (Engels and Jensen 2010; Guo and**
402 **Pennings 2012), although these studies focus on the exclusion of competitively-**
403 **dominant freshwater plants from salinised environments. A recent study of tundra**

404 response to a major storm surge event in the Canadian Arctic (Lantz *et al.* 2015)
405 however, also shows that post-inundation recovery varies within terrestrial plant
406 communities, with graminoids exhibiting greater resilience than shrubs. In addition
407 recovery was also linked to soil salinity and the extent of subsequent leaching of soil
408 salts by freshwater inundation. This latter observation raises the possibility that
409 variation in seasonal rainfall might affect post-seawater inundation responses in
410 temperate coastal grasslands. Given the increased likelihood of storm surge events
411 over coming decades (Martin *et al.* 2011; Feser *et al.* 2015) and the importance of
412 low-lying coastal vegetation for conservation and coastal defence (Fisher *et al.*
413 2011; Hoggart *et al.* 2014), a more detailed understanding of the structural and
414 functional response of coastal vegetation to short-term seawater flooding is
415 particularly pressing.

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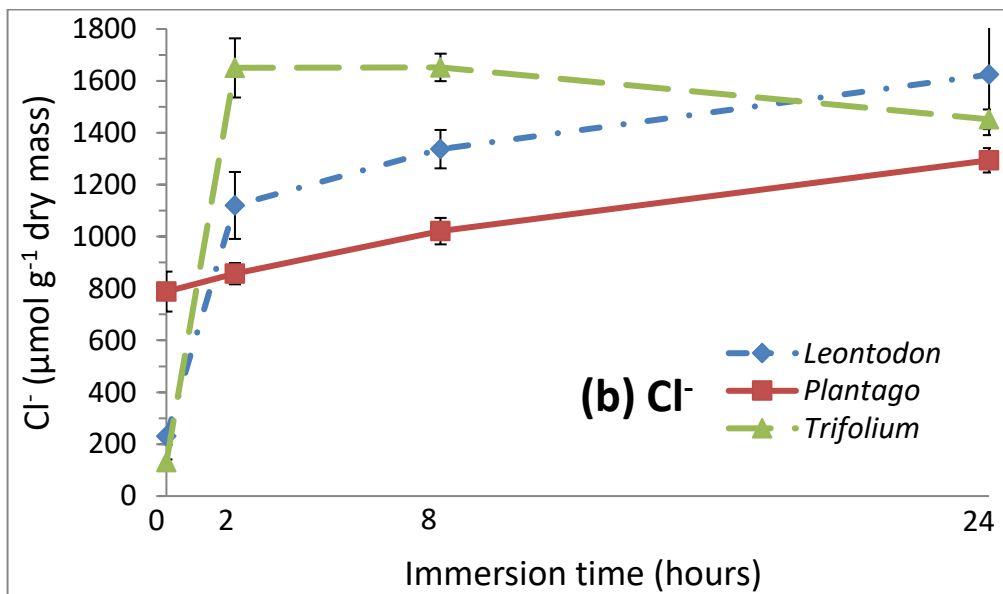
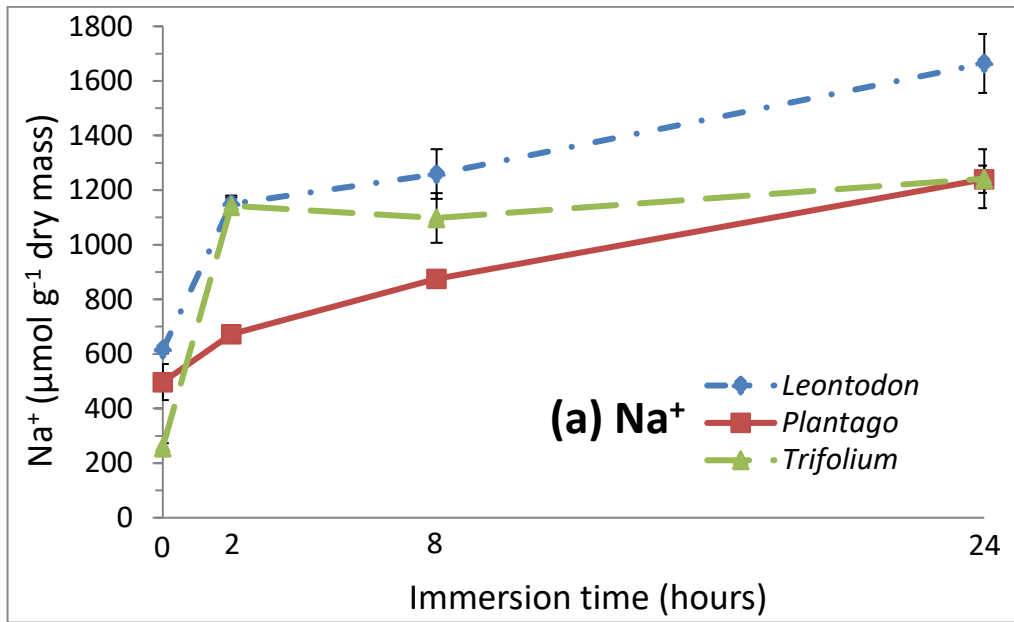
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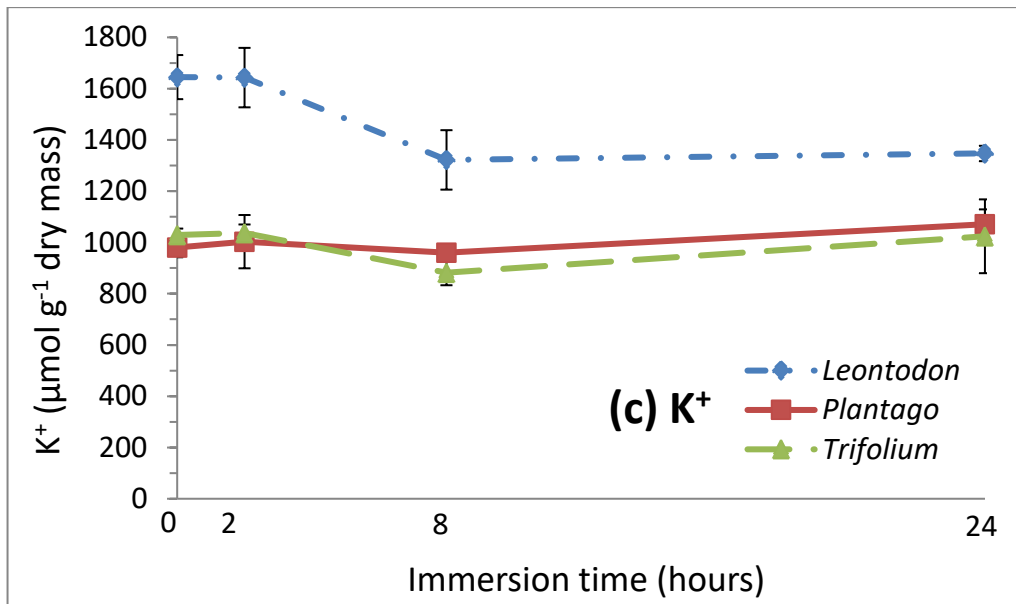


Figure 1. Ion concentrations ($\mu\text{mol g}^{-1}$ dry mass), (a) Na^+ , (b) Cl^- and (c) K^+ , in leaf tissues of three coastal grassland plant species (*Leontodon autumnalis*, *Plantago lanceolata* and *Trifolium pratense*) grown in monocultures and sampled 2-d after root-zone immersion in seawater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

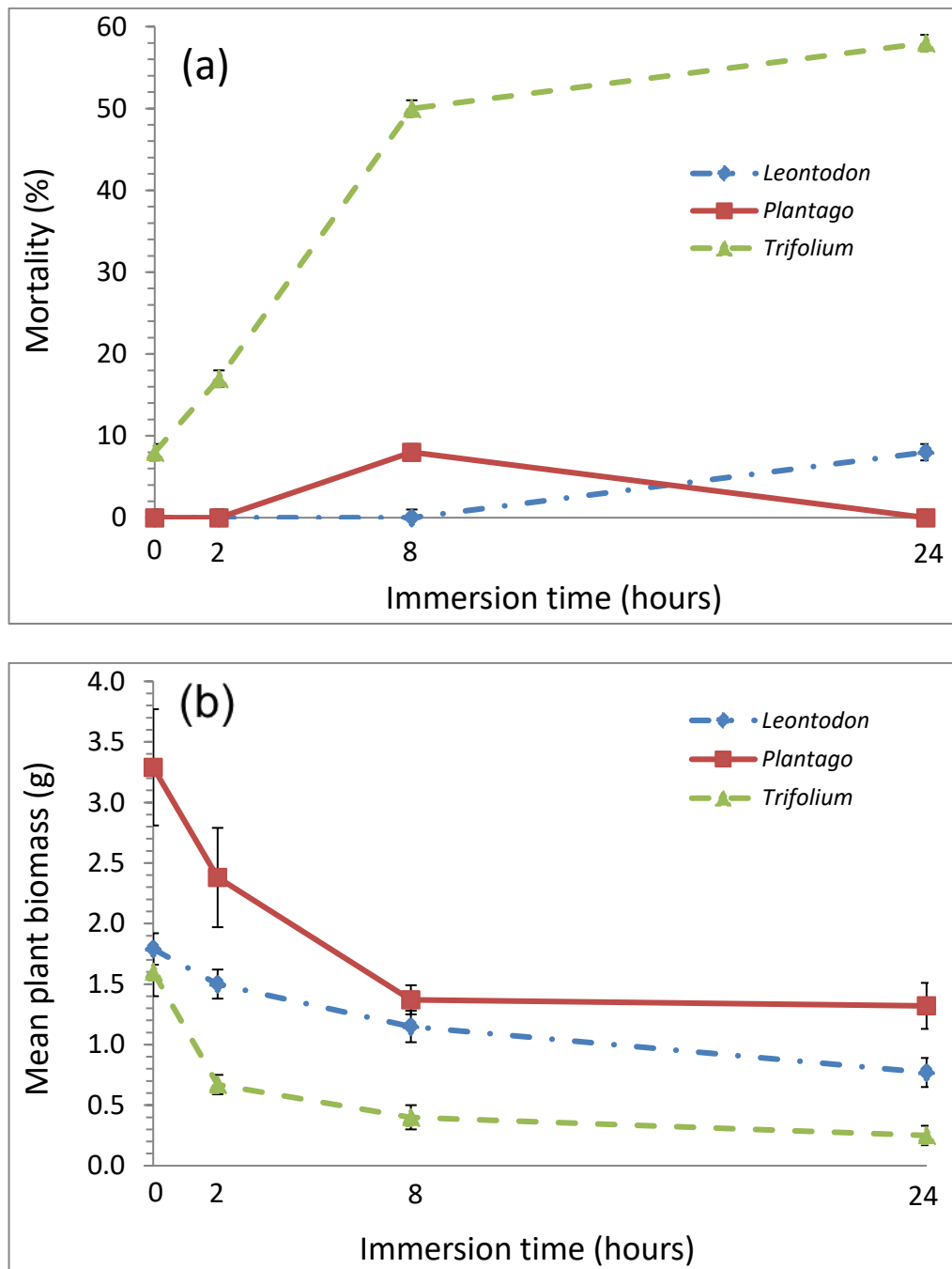
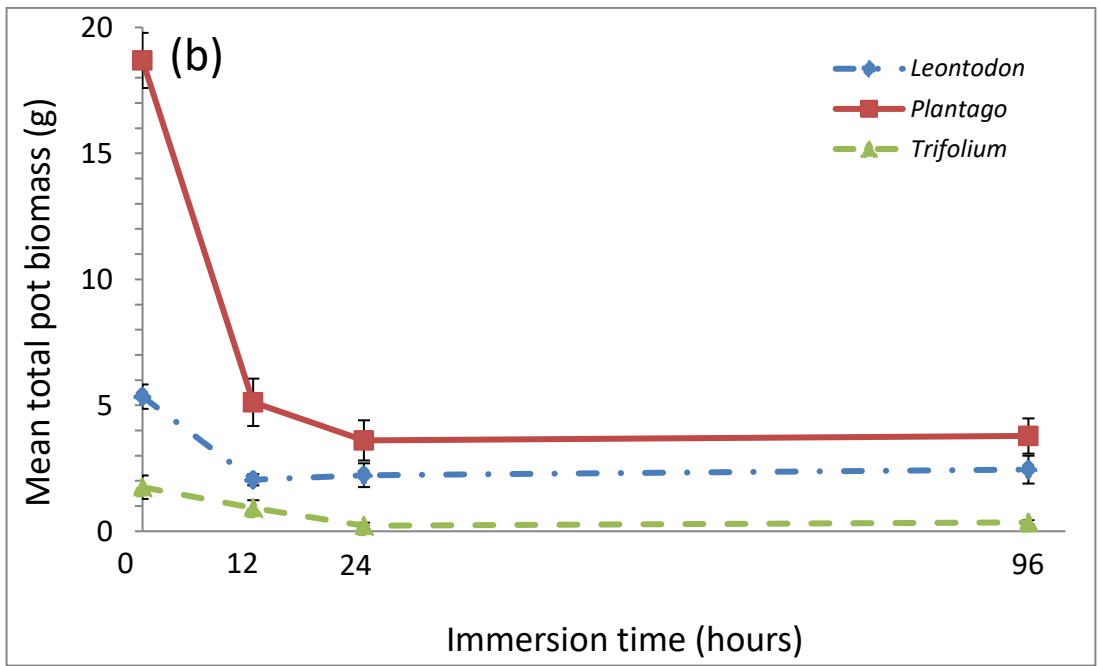
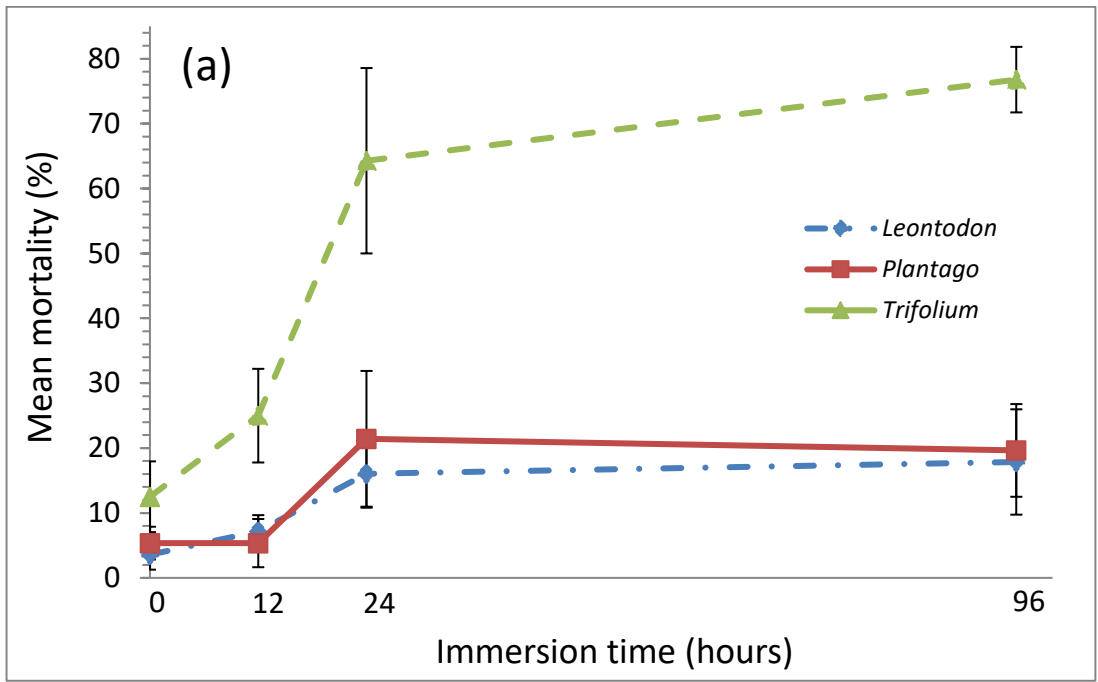


Figure 2. The effect of plant root-zone immersion in seawater on (a) total plant mortality and (b) mean individual dry mass of three coastal grassland plant species (*Leontodon autumnalis*, *Plantago lanceolata* and *Trifolium pratense*) grown in monocultures and sampled 56-d after transient immersion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



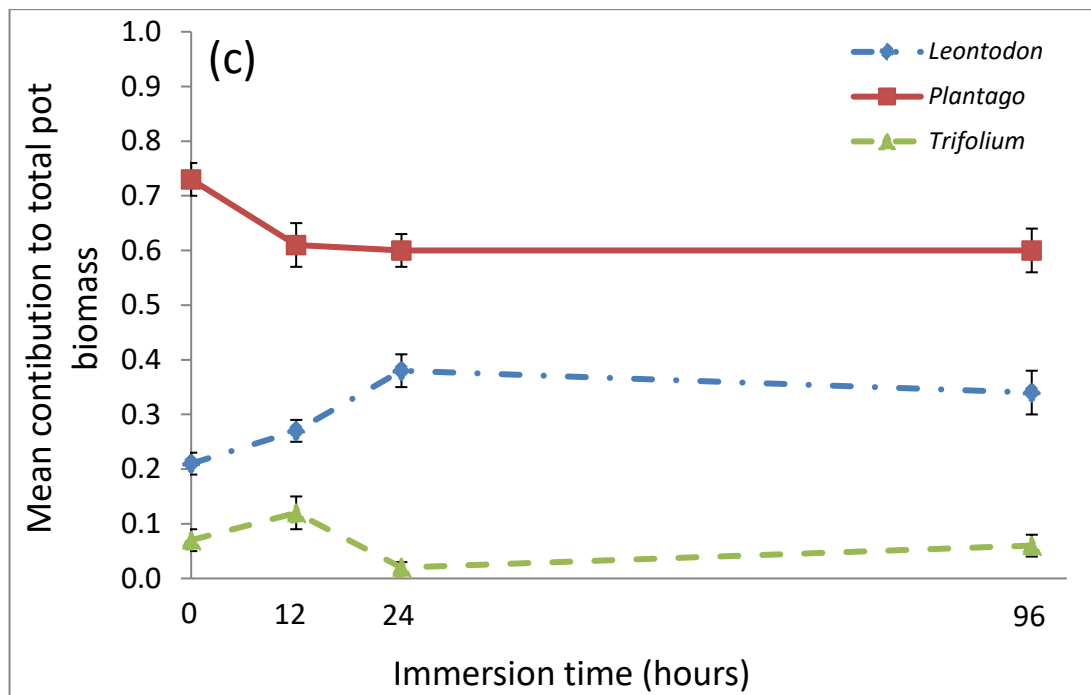


Figure 3. The effect of plant root-zone immersion in seawater on (a) plant mortality, (b) mean (\pm SE) total above-ground dry mass and (c) mean relative contribution to total above-ground dry mass of three coastal grassland plant species (*Leontodon autumnalis*, *Plantago lanceolata* and *Trifolium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

Immersion duration (hours)	<i>n</i>	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		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
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 $\mu\text{mol g}^{-1}$ Dry Mass), fructose (14 $\mu\text{mol g}^{-1}$ DM), glucose (20 $\mu\text{mol g}^{-1}$ DM), sucrose (6 $\mu\text{mol g}^{-1}$ DM), sorbitol (4 $\mu\text{mol g}^{-1}$ DM) and pinitol (36 $\mu\text{mol g}^{-1}$ DM).

Table 1. Organic solute concentrations ($\mu\text{mol g}^{-1}$ 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.