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Changes in cell size and tissue hydration ('succulence') cause curvilinear growth responses to salinity and watering treatments in euhalophytes

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Running title: Growth responses to salinity in euhalophytes

Highlights

Euhalophytes have a curvilinear growth response to increasing salinity

Euhalophytes have increased succulence (tissue hydration) with increasing salinity

We found a linear relationship between epidermal cell size and tissue hydration

There was also a linear relationship between tissue hydration and shoot dry mass

Variation in growth and succulence are therefore both linked to changes in cell size

Abstract

Our work focused on the widely recognised curvilinear growth response to salinity and the occurrence of succulence (increased ratio of tissue water/dry mass) in euhalophytes. We hypothesized that the curvilinear changes in growth with salinity were largely due to changes in cell size, confirmed by direct measures of epidermal cells and the ratio of tissue water/dry mass, an index of cell size at tissue scale. Two euhalophytes [*Salicornia europaea* L. and *Suaeda maritima* subsp. *salsa* (L.) Soó (syn. *Suaeda salsa* Pall.) were grown in soil at a range of salinities with water supplied at 40% or 80% field capacity. The salt and water treatments affected plant growth, cell size and tissue hydration. Both species had curvilinear growth responses to the solute potential of the soil solution, with a shoot dry mass optimum and cell size optimum occurring at about -0.6 MPa when watered to the equivalent of 80% field capacity, and about -1.2 MPa when watered to the equivalent of 40% field capacity. Tissue hydration was also affected in a curvilinear manner by the solute potential of the soil solution. For each species, there was a striking linear relationship between tissue hydration and shoot dry mass ($P < 0.001$), and between tissue

hydration and epidermal cell size ($P < 0.001$). It was concluded that the variation in growth of euhalophytes and their tissue hydration were both caused mostly by the same factor – variation in cell size with salinity.

Keywords

Soil solute potential, Osmotic adjustment, Sodium, Chloride, Plant water relations

Introduction

Euhalophytes from the family Amaranthaceae are salt accumulating plants that have a growth optimum in environments containing more than 0.5 percent sodium chloride (Chapman 1942). Euhalophytes typically have a 'curvilinear' growth response to external salinity, with peak growth at intermediate salinities (50–350 mM NaCl; Flowers and Colmer 2008; Rozema and Schat 2012; Song and Wang 2015). For example with 56 day old *Suaeda maritima*, when nutrient solutions were without NaCl, shoot dry mass (DM) was ~0.3 g, if they contained 170 mM NaCl shoot DM was ~67% higher, and with 680 mM NaCl the shoot DM was ~75% lower than the optimum (Yeo and Flowers, 1980). Similar growth responses to salinity are known to occur for *Salicornia rubra, Salicornia bigelovii, Salicornia dolichostachya, Sarcocornia natalensis, Suaeda aegyptiaca, Halosarcia pergranulata* and *Disphyma australe* (Tiku 1976; Ayala and O'Leary 1995; Katschnig et al. 2013; Naidoo and Rughunanan 1990; Eshel 1985; Short and Colmer 1999; Neales and Sharkey 1981).

Another feature of the growth of euhalophytes under saline conditions is the development of 'succulence'. Succulence is indicated by increases in water content per cell (Jennings 1968; Zhao et al. 2013), increases in shoot tissue hydration (e.g. the ratio of tissue water to DM) (Storey and Jones 1979; Zotz and Winter 1994; Inan et al. 2004; Qi et al. 2009; Han et al. 2013) and increases in leaf thickness (Black 1958; Aslam et al. 1986). It has been suggested that succulence is an adaptive trait in the stems and leaves of halophytes to dilute ions (e.g. Jennings 1968), however we argue that the phenomenon could simply be caused by differences in cell size associated with the changes in growth due to salinity. In general, plant cellular expansion occurs because cells behave as simple osmometers; their expansion is caused by increases in turgor within the cell and the cell wall behaves as a 'linear viscoelastic polymer', whose

thickness is maintained constant by the deposition of new materials (Lockhart 1965). If this is true, and if the cell walls constitute the bulk of the cellular organic DM, then tissue DM and the ratio of tissue water/DM (an indicator of succulence) would increase as cell size increased. Indeed the ratio of tissue water/DM can be thought of as an index of cell size, albeit at the whole tissue scale.

Why should there be changes in shoot DM growth and tissue hydration with changes in external salinity? Let us consider two alternative causes for the growth responses described above to increasing salinity. At one extreme, the changes might be because plants vary the number of cells in their tissues, increasing cell number as the external salinity increases from sub-optimal to optimal levels, and decreasing their number as salinities increase further from optimal to supra-optimal concentrations (Scenario 1). If this is so, then increases in growth would be accompanied by no change in directly measured cell size (where such measurements are possible to make), and with no change in the ratio of tissue water/tissue DM. At the other extreme, the changes in growth by euhalophytes with increasing salinity might be because plants vary in cell size, increasing as the external salinity increases from sub-optimal to optimal levels, and decreasing as salinities increase further from optimal to supra-optimal concentrations (Scenario 2). If this is so, then increases in growth would be accompanied by increases in directly measured cell size (where such measures are possible to make) and by increases in the ratio of tissue water/tissue DM.

Is there evidence for either of these scenarios occurring in euhalophytes? Scenario 2 (changes in cell size) provided a better explanation for the increase in growth with *Suaeda maritima*. In this species there was a 74% increase in FM as the external salinity increased from 0 to 340 mM (applied when plants

were 21 days old for a further 56 days) and the sum of cations in extracted leaf sap was 78% higher in plants grown under saline compared with non-saline conditions (Yeo and Flowers 1980). At 340 mM NaCl, the surface area of epidermal cells was more than twice that of plants grown under non-saline conditions (Yeo and Flowers 1980), the proportion of total shoot tissue volume composed of cell walls was 40% lower under saline than non-saline conditions (Hajibagheri et al. 1989) and the ratio of tissue water to tissue DM was 29% higher under saline than non-saline conditions (Yeo and Flowers 1980). That the increase in growth was accompanied by increased ion concentrations in the tissues was consistent with the view that better tissue osmotic adjustment increased cell size.

The cause of the decrease in tissue growth with further salinity (optimal to supraoptimal concentrations) is still subject to conjecture (Flowers and Colmer 2008). One suggestion is that at supra-optimal salinities there is an accumulation of ions in the apoplast which decreases turgor (c.f. Oertli 1968). Some support for this view comes from studies with the euhalophyte *Suaeda maritima:* when grown at 200 mM NaCl, concentrations of Na^+, K^+ and Cl^- in the apoplasm of root cortical cells (determined by X-ray spectrometry) were ~80-120 mM (Hajibagheri and Flowers 1989), and turgor pressures measured in the leaves of plants grown at 200-400 mM NaCl were quite low ($\leq$ 0.07 MPa) once those leaves had expanded (Clipson et al. 1985). Also, with the euhalophyte *Sarcobatus vermiculatus* grown in soil at 100-450 mM NaCl, direct measurements showed Na^+ and K^+ concentrations in the apoplasm of ~170-225 and ~50 mM respectively in leaves (James et al. 2006). Again, there could be two consequences of these changes. At one extreme, the decrease in tissue growth might be because the tissues produce fewer cells of similar size under supraoptimal conditions compared with optimal conditions (Scenario 1). If this

is so, then decreases in growth would be accompanied by increases in tissue Na^+ and/or Cl^-, but these would not be accompanied by a decrease in directly measured cell size or in the ratio of tissue water/DM. The alternative is that the decrease in tissue growth might be because the plants produced similar numbers of cells as under optimal conditions, but these were smaller than under optimal conditions (Scenario 2). If this was so, then decreases in growth would be accompanied by increases in tissue Na^+ and/or Cl^-, and by a decrease in directly measured cell size and in the ratio of tissue water/DM.

Euhalophytes often grow in situations where soils are saline due to the presence of shallow groundwater (Barrett-Lennard et al. 2013). Groundwater is therefore one of the major sources of water for halophytes in arid and semi-arid landscapes, which they access from the capillary fringe above the water-table (c.f. Barrett-Lennard and Malcolm 1999; Alharby et al. 2018).

Halophytes are widely found in landscapes affected by soil salinity and water deficit (Flowers 1985; Flowers et al. 1986; Glenn et al. 2013). To some degree these two factors can be different manifestations of the same stress, because the salinity of the soil solution (the major factor that affects their growth on saltland) is the ratio of salt concentration to water content in the soil. The salinity of the soil solution therefore becomes more adverse for growth as the soil becomes more saline and also as the soil becomes drier. The interaction between salinity and drought on euhalophyte growth has rarely been studied.

We conducted an experiment in pots of soil with two euhalophytic species [*Salicornia europaea* L. and *Suaeda maritima* subsp*. salsa* (L.) Soó (syn. *Suaeda salsa* Pall.] which were watered with saline water from the base of the pot (mimicking the situation of groundwater in the field) to two percentages of

field capacity. We hypothesised (H1) that if the increase in growth that occurs between sub-optimal and optimal salinity was due primarily to increases in cell size (Scenario 2), then as the external salinity (manipulated by combinations of soil water and salt) increased from non-saline to optimal concentrations we should be able to observe, simultaneously: (a) increases in shoot growth, (b) increases in internal Na^+ and Cl^- concentrations in the shoots, (c) increases in epidermal cell size, and (d) an increase in the shoots of the ratio of tissue water/DM. Furthermore (H2), if the decrease in growth as external salinities increase from optimal to supra-optimal levels is due to decreased osmotic adjustment (due to increased accumulation of Na^+ and Cl^- in the cell walls) and decreased cell size (also Scenario 2), then we should be able to observe simultaneously: (a) decreases in shoot growth, (b) further increases in internal Na^+ and Cl^- concentrations, (c) decreases in epidermal cell size, and (e) a decrease in the shoots of the ratio of tissue water/DM.

Materials and Methods

Plant material

Seeds of *Salicornia europaea* and *Suaeda maritima* subsp*. salsa* were collected from the halophyte botanic garden of the Fukang Desert Ecosystem Observation and Experiment Station (44.29°N, 87.93°E) in Xinjiang Province, in north-western China. They were stored in a refrigerator at ~4°C before use in experiments.

Plant culture and experimental procedure

Seeds of the two species were sown into drained plastic pots (33 cm high, 30 cm upper diameter, 19 cm lower diameter) filled with 12 kg of water-rinsed dry sandy soil. There were 20 seedlings in each pot. The plants were cultured in a

greenhouse under natural light conditions, at 28-34°C during the day and 17-23°C during the night. After one week, shoots had emerged, and 250 mL of a full-strength nutrient solution (composition given below) was applied at two-day intervals. At day 20, the plants were thinned to 5 plants of similar size per pot, and each pot was irrigated with sufficient water to wet this sandy soil to field capacity (FC), based on prior experience with this soil (Mao 2005).

The experiment had a completely randomized design with two plant species (*S. europaea* and *S. maritima*), four NaCl concentrations in the nutrient solution applied to the base of each pot (0, 170, 340 and 680 mM) and two water supply treatments (80% and 40% of FC). The experimental treatments commenced after 30 d of plant establishment. From this day forward, the pots were watered to 40% or 80% of the weight of water at field capacity every 2 days by adding nutrient solution of varying salinity to the tray that the pot was standing in. This solution was drawn into the soil through holes in the bottom of the pot and was redistributed through the soil by capillarity. To avoid osmotic shock, the salinity in the trays of nutrient solution was increased by $1/10^{th}$ of the final concentration per day over the next 10 days. After the final salinity concentrations were reached, the treatments were maintained for another 30 d.

The water that was added to the base of the pot each day was equal to that lost by evapotranspiration [determined by weighing the pot; Mao 2005; Wang 2008]. Every 2 days each pot received sufficient solution to restore pot weight to 40% or 80% FC treatment; this solution contained the required concentration of NaCl, plus (millimoles): 1.5 KNO_3, 1.0 $Ca(NO_3)_2{\cdot}4H_2O$, 0.50 $NH_4H_2PO_4$, 0.25 $MgSO_4{\cdot}7H_2O$, and (µmoles): 12.5 KCl, 6.25 H_3BO_3, 0.50 $MnSO_4{\cdot}H_2O$, 0.50 $ZnSO_4{\cdot}H_2O$, 0.125 $CuSO_4{\cdot}5H_2O$, 0.125 H_2MoO_4 , 0.125 $NiSO_4{\cdot}6H_2O$, 16 NaFeDTPA. The increase in pot weight due to the growth of the plants was ignored in making these water addition calculations.

Harvest and determination of plant shoot and soil samples

Each pot was harvested between 10 am and 3 pm, 48 h after the most recent watering. The days of harvest were without cloud cover and the order in which the treatments were harvested was random. The FM of the shoots was measured immediately. They were then placed in envelopes and oven-dried at 60°C for 72 h. They were then reweighed for determination of shoot DM and then ground into powder for chemical analysis. Ions were extracted from the powered shoot tissue shaken with 0.5 M HNO_3 for 48 h. Diluted extracts were analyzed for Na^+ (Flame Photometer, Model 2655-00 Digital Flame Analyzer; Cole-Parmer Instrument Company, Chicago, IL, USA) and Cl^- ($AgNO_3$ titration method - Song et al. 2006).

The soil in each pot was collected at three depths (0-10, 10-20 and 20-30 cm) using a small auger. The soil samples were weighed while wet, and they were then oven dried at 105 °C and weighed again. The difference in mass enabled the calculation of soil water content. The dried soils were extracted in DI water (1:5 dry soil: water) on a rotating shaker for 24 h. The extracts were then filtered and analyzed for Na^+ and Cl^- as outlined above. This enabled us to determine the concentrations of Na^+ and Cl^- in the soil solution at the three depths in each pot. The solute potential (MPa) of these solutions was calculated as:

$$\Psi_s = -iRTC_s$$

where *i* is the ratio of the osmolality/molality of the solution (NaCl i = 1.8), *R* = universal gas constant (8.314 x 10^{-3} kPa m^3/(mol K), T = temperature (^oK) and C_s is the concentration of NaCl (mol kg^{-1}).

Quantification of the degree of tissue hydration (succulence)

The degree of succulence was determined on recent completely expanded mature leaves of *S. maritima* and fleshy stems of *S. europaea* collected on the final harvest day. The collected leaves and stems were sealed in plastic bags and transported to the laboratory for determination of FM (to an

accuracy of 0.0001 g on a digital balance). The organs were then oven-dried at 60°C for 48 h and their DM was then determined.

Measurements of surface cells of leaves or stems

Similar leaves and stems as used for the tissue hydration measurements (above), were also collected for analysis of surface cell dimensions. The collected material was washed in distilled water to remove any dust and salt. Segments (5 mm long) were immersed in 3% glutaraldehyde, vacuum infiltrated, and then stored in a refrigerator at 4°C. The tissues were dehydrated using ascending concentrations of ethanol; critical point dried and finally surface-coated with gold (Lamont 1983) and the samples were then placed onto the stage of a Zeiss Supra 55VP Scanning Electron Microscope (Carl Zeiss, Jena, Germany) for observing and photographing. Measurements included the number of tissue surface cells (number per mm^2) and their area (μm^2). The data were derived from 3 plants per treatment. Leaf or stem tissue was selected from the same relative position on each plant. Cells were observed at about 20 locations for each replicate of each tissue. Typical images for the surface cells of *S. europaea* and *S. maritima* are provided in the Supplementary Materials (Fig. S1).

Statistical analyses of data

Regression analyses was conducted using Genstat 18^{th} Edition (VSN International Ltd). ANOVAs were conducted using Origin Pro 8.5.

Results

The use of soil in this trial, the introduction of NaCl through the base of the pot and the watering of the plants to two percentages of field capacity created great heterogeneity within the pot. We therefore describe: firstly, the effect of these conditions on the stratification of salt and water in the pot, secondly the effects

of this on the solute potential gradients between the soil and plants, thirdly the effects of the variation in solute potential on plant growth, cell size and tissue hydration, and how these factors were correlated with each other, and fourthly the effects of the variation in solute potential on Na^+ in the shoots. The impacts of each combination of NaCl and watering treatment on plant shoots (shoot DM, FM, water content) soil conditions (water content and Na^+), shoot ion concentrations, and epidermal cell size are reported in the Supplementary Materials (Figs S2 – S8).

Soil conditions – water content and ions

The addition of salt and water through the base of the pot created a heterogenous growth medium for the plants. The concentration of salt in the soil water (calculated from measurements of the soil water and salt concentrations in the soil) was affected by soil depth (highest at 0-10 cm), water supply treatment (lower with the watering treatment equivalent to 80% FC than 40% FC) and increased with NaCl treatment (see Supplemetary Materials; Fig. S1; statistical analyses Table S1).

Solute potential gradients between the soil and shoot

The most likely source of water for plant growth from within the pot is indicated by comparing the solute potential of the soil (at the 3 depths in the pot) and the solute potential of the shoots. Data on these variables at the time of harvest are reported in Fig. 1. In the soil, solute potentials were lowest (most negative) at the soil surface ($P < 0.001$), lower with the 40% FC than the 80% FC treatment, and decreased as the NaCl treatment increased ($P < 0.01$) (statistical analyses in Supplementary Materials Table S1 and Table S2). The data on solute

potential distribution indicate that water became more available for growth with increasing depth in the pot and with increasing water supply. At 20-30 cm, the solute potentials were all higher than -1.0 MPa for the 80% FC treatment and were higher than -1.5 MPa for the 40% FC treatment (Fig. 1 e, f). By contrast, at 0-10 cm depth, the solute potentials of the 80% FC treatment reached -2.0 MPa, and those of the 40% FC treatment reached -3.5 MPa (Fig. 1 a, b).

In the plants, solute potentials decreased with NaCl and the lower (40% FC) water supply treatment ($P < 0.01$) (statistical analyses are in Supplementary Materials Table S1). With both *S. maritima* and *S. europaea* grown at 680 mM NaCl and 80% FC, solute potentials were ~1.5 MPa lower (more negative) than with the 0 mM NaCl treatment (Fig. 1 g, h). There were also effects of water supply treatment. The shoot solute potentials of both species were ~1 MPa higher for the 80% FC than the 40% FC treatments. Solute potentials in the shoots were lowest (most negative) with the combination of salinity and low water supply (40% FC), -3.8 MPa for *S. europaea* and -3.5 MPa for *S. maritima* (Fig. 1 g and h).

Water can be expected to flow most readily across plant membranes from soil into plants down the largest solute potential gradient. The differences in solute potential between the soil and the plants, at 680 mM NaCl and 40% FC water supply treatment were between 0.03 and -0.26 MPa at 0-10 cm in the pots, between -0.65 and -0.85 MPa at 10-20 cm in the pots, and between -1.9 and -2.2 MPa at 20-30 cm in the pots of both species. The solute potential gradients were clearly greatest at 20-30 cm, and we have assumed that it is from this depth that most water was therefore taken up.

INSERT FIG. 1 NEAR HERE

Relationships between solute potential and growth, cell size and tissue hydration

Fig. 2 shows the relationship between the solute potential at 20-30 cm depth in the pot and shoot growth (g plant^{-1}; Fig. 2a, b), epidermal cell size (m^2 x 10^{-6}; Fig. 2c, d) and shoot water content (g g^{-1} DM; Fig. 2e and f) for the individual replicates of *S. europaea* and *S. maritima*. All of these datasets had significant curvilinear responses to the external solute potential (based on quadratic of best fit), although the curves for the higher watering rate (80% FC) were offset (falling over a higher, i.e. less negative) water potential range than the curves for the lower watering rate (40% FC) (Fig. 2). Interestingly, the optimal solute potential associated with highest shoot growth, highest shoot water content and highest epidermal cell size were similar for each of the three measured parameters, being at about -0.6 and -1.2 MPa for *S. europaea* at 80% and 40% FC respectively, and at about -0.5 and -1.1 MPa for *S. maritima* at 80% and 40% FC respectively (Fig. 2).

INSERT FIG. 2 NEAR HERE

The similarity in the shapes of the 12 fitted curves in Fig. 2 strongly suggested that the three parameters (shoot DM, epidermal cell size and the ratio of shoot water/shoot DM) were correlated with each other. This was confirmed in Fig. 3. For both *S. europaea* and *S. maritima*, shoot DM and epidermal cell size were significantly correlated with shoot water content, and irrespective of the level of watering, all points fell on the same lines (Fig. 3).

INSERT FIG. 3 NEAR HERE

Relationships between solute potential and Na^+ concentrations in the leaves

Fig. 4 shows the relationship between the solute potential at 20-30 cm depth in the pot and shoot Na^+ for *S. europaea* and *S. maritima*. For each species shoot Na^+ was correlated (simple line) with the solute potential at 20-30 cm depth, and irrespective of the level of watering, all points fell on the same lines (Fig. 4；The formulae for the lines of best fit are given in the Supplementary Materials Table S5). Given this, not surprisingly there were also curvilinear relationships between shoot Na^+ (tissue water basis) and shoot DM (Fig. 5 a, b), epidermal cell size (Fig. 5 c, d) and the ratio of shoot water/DM (Fig. 5 e, f). Lines of best fit showed that optimal shoot DM, optimal epidermal cell size and optimal shoot water/DM occurred at ~500 mM and ~600-700 mM for *S. europaea* at 80% and 40% FC respectively, and at ~400 mM and ~600 mM for *S. maritima* at 80% and 40% FC respectively (calculated from lines of best fit for curves in Fig. 5; see Supplementary Materials, Table S6).

INSERT FIGS.4 AND 5 NEAR HERE

Discussion

This experiment was conducted to test two hypotheses (H1, H2). In accord with H1, we confirmed earlier observations with *Suaeda maritima* (Yeo and Flowers 1980; Hajibagheri et al. 1984) that the increase in growth between suboptimal and optimal salinity was associated with increases in cell size (Scenario 2, Fig. 1). In both species in the present study there were simultaneous increases in shoot growth, increases in the size of the readily observable cells of the epidermis, and an increase in the ratio of tissue water/DM. That these increases

were associated with improved osmotic adjustment was implied by the increases in internal Na^+ and Cl^- in the shoots.

In accord with H2, we showed that the change in growth associated with increases from optimal to supra-optimal salinity was associated with decreased cell size: there were simultaneous decreases in shoot growth, decreases in the size of the readily observable cells of the epidermis, and decreases in the ratio of tissue water/DM. These changes were associated with further increases in internal Na^+ and Cl^- in the shoots. While we did not specifically measure Na^+ and Cl^- in the apoplasm, our data are nevertheless consistent with the view that decreases in growth can occur in euhalophytes at extreme salinities due to the accumulation of ions in cell walls (c.f. Hajibagheri and Flowers, 1989; James et al. 2006; Flowers et al.2015; Harvey 1981).

This discussion has two sections. Firstly, we discuss the layering of salt and water in the pots. Secondly, we discuss the relationships between external solute potential and shoot growth, cell size and tissue hydration at the range of salt and water contents that occurred in these pots.

Layering of salt and water in pots

Soil salinity can be highly heterogeneous in natural landscapes, varying within plant communities spatially over distances of 10-50 m (Davidson et al. 1996; Barrett-Lennard et al. 2013). However, it can also vary spatially and vertically in soil profiles at the scale of single plants (Barrett-Lennard and Malcolm 1999; Bazihizina et al. 2012a; 2012b; Alharby et al. 2014; 2018). In the present work, we simulated the vertical variation that can occur in root-zones of single plants by growing the plants in 30 cm pots of soil. Water containing 0–680 mM NaCl

was supplied to the base of these pots at rates sufficient to maintain soil hydration to an equivalent of 40% and 80% of field capacity. Based on the measured NaCl concentrations in the soil solution at the completion of the experiment, these conditions provided highly stratified variation in soil solute potential with depth, varying between about -1.2 and -3.7 MPa at the top of the pot (0-10 cm depth), and between about -0.2 and -1.5 MPa at the bottom of the pot (20-30 cm depth; Fig. 1). The differences in water potential between the soil solution and the inside of the roots was maximized at the base of the pots, so we therefore related shoot DM, epidermal cell size and tissue hydration to solute potentials at this depth (Fig. 2). Previous work with *Atriplex nummularia* growing in columns of soil suggested that halophytes typically access moisture mostly from the soil in the root-zone with the lowest salinity of the soil solution (Alharby et al. 2018; Marchesini et al. 2014)

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Relationships between external solute potential, shoot growth, cell size and tissue hydration

It is well known that in nutrient solution cultures increasing salinity causes curvilinear growth responses in dicotyledonous euhalophytes (Flowers and Colmer 2008). For example, *Cakile maritime* (at the vegetative stage), *Halosarcia pergranulata, Salicornia dolichostachya*, and *Suaeda maritima* had growth optima with external salinities of 100, 200, 300 and 340 mM NaCl respectively (Debez et al. 2004; Short and Colmer 1999; Katschnig et al. 2013; Yeo and Flowers 1980).

In the present investigation with two euhalophytes grown in soil watered to two percentages of FC, we saw curvilinear responses in shoot DM, epidermal cell

size and tissue hydration to the solute potential of the soil solution at 20-30 cm in the pot. Each parameter had suboptimal values at high solute potentials, greatest values at -0.6 MPa and -1.2 MPa (for soil water contents of 80% and 40% FC respectively; both species) and supraoptimal values at lower solute potentials. The curves for the plants growing at 80% and 40% FC (Fig. 2) did not overlap because shoot growth, epidermal cell size and tissue hydration at 40% FC were presumably also decreased by the lowering of soil matric potential. For both these species in which growth was affected by variation in salinity and soil water, there were impressive simple linear relationships between shoot DM, epidermal cell size and tissue hydration. This would occur if the changes in shoot DM due to variation in soil salinity and water content were caused mostly by variation in cell size, rather than cell number. We therefore conclude that Scenario 2 is substantially correct: the changes in tissue hydration associated with increased salinity are mostly a simple consequence of the changes in cell size as a consequence of improved osmotic adjustment.

Tissue hydration (or succulence) has been discussed as a physiologically important adaptation in halophytes, diluting the salt in the cells to decrease its harmful effects on metabolism (Jennings 1968; Storey and Jones 1979; Yeo 1983; Wang et al. 2012; Song and Wang 2015). This interpretation should be questioned. In the present experiments there was an increase in tissue hydration at moderate salinities (ψ_s values of -0 to -0.6 MPa) but at more extreme salinities (ψ_s values of -0.6 to -1.2 MPa), when it could be argued that cells had greatest need of protection from ion excess, tissue hydration actually decreased, so any effect of ion dilution through the increased uptake of water into cells was reversed.

As a final comment, we would observe that the link between tissue hydration and growth might be of some eco-physiological value as the rate of growth in halophytic shrubs is a relatively difficult factor to measure routinely in the field. Tissue hydration could therefore be a useful index of growth rate. Growth in such communities is likely to be highly episodic, varying depending on the availability of water in the root zone and the salinity of the soil solution. Methods for simply assessing the impacts for growth of such spatial and temporal variation are currently not available. Even at the level of measures of whole shoot biomass, methods are still relatively cumbersome. For example, some researchers estimate 'edible biomass' (for livestock) by removing all the leaves from plants, a slow process that may mix leaves with inedible twigs (Watson and Oleary 1993); others use the 'Adelaide technique' in which a branch of a shrub that is well-characterized in terms of leaf weight is visually compared with a broader range of other shrubs (Norman et al. 2010). An easily determined index of growth might therefore be of considerable value for field studies.

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Author Statement

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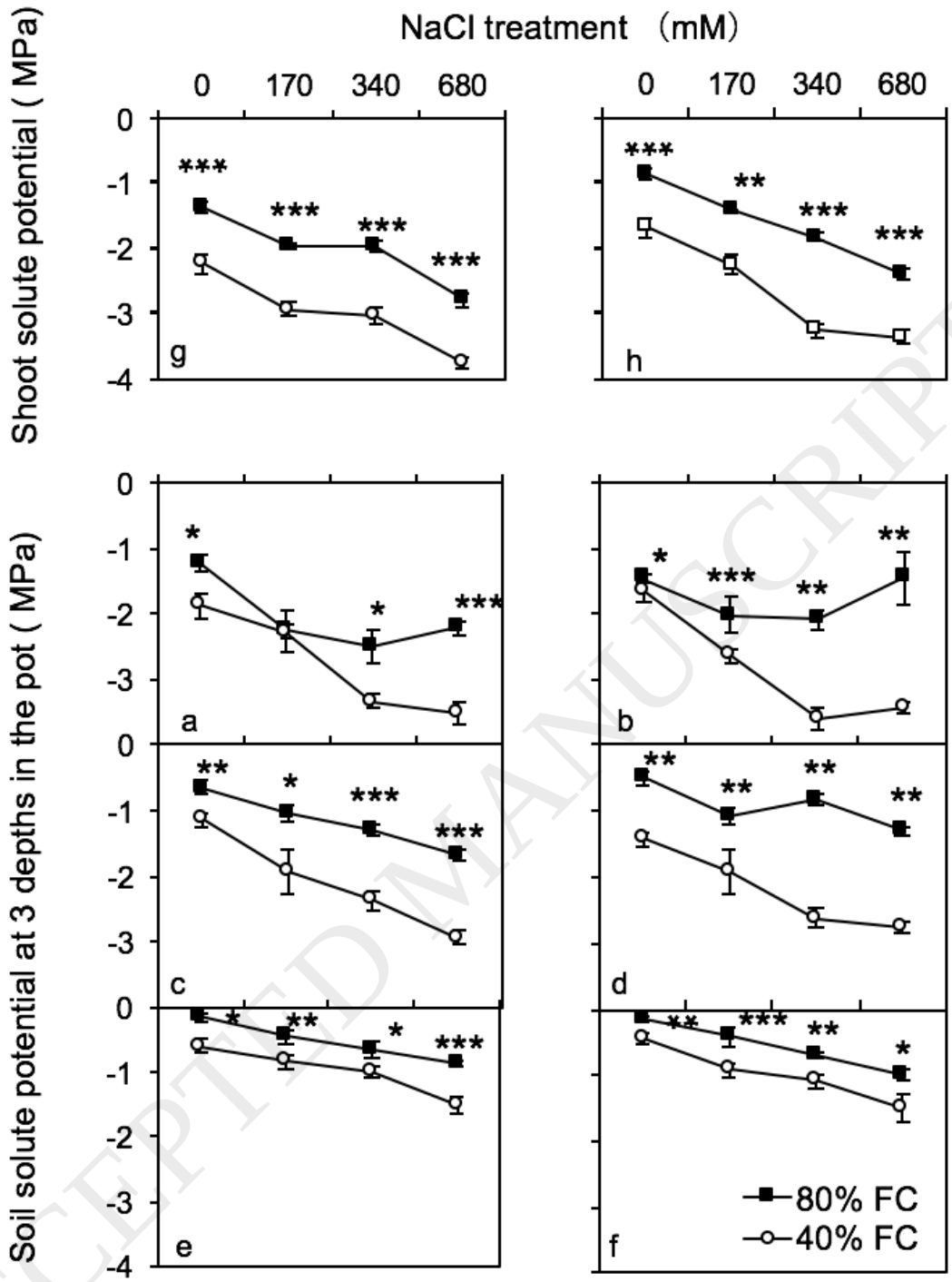

Fig. 1. Effects of NaCl and watering treatments on the solute potential of the soil at 3 depths in the pot [0-10 cm (a, b), 10-20 cm (c, d) and 20-30 cm (e, f)], and the solute potential of the shoots of *S. europaea* (g) and *S. maritima* (h). Parts (a), (c), (e) and (g) all refer to *S. europaea*. Parts (b), (d), (f) and (h) all refer to *S. maritima*. The pots were salinized by standing in trays with solutions of 0, 170, 340 or 680 mM NaCl, and were subsequently watered to the equivalent of 80% (■) or 40% of the weight of water at field capacity (○). Solute potentials were the calculated contributions of the measured Na^+ and Cl^- in the soil and plant tissue on a

water basis. Values are means (n = 3) ± SD. Asterisks above symbols indicate significant differences between watering treatments: *(P < 0.05); ** (P < 0.01); *** (P < 0.001)

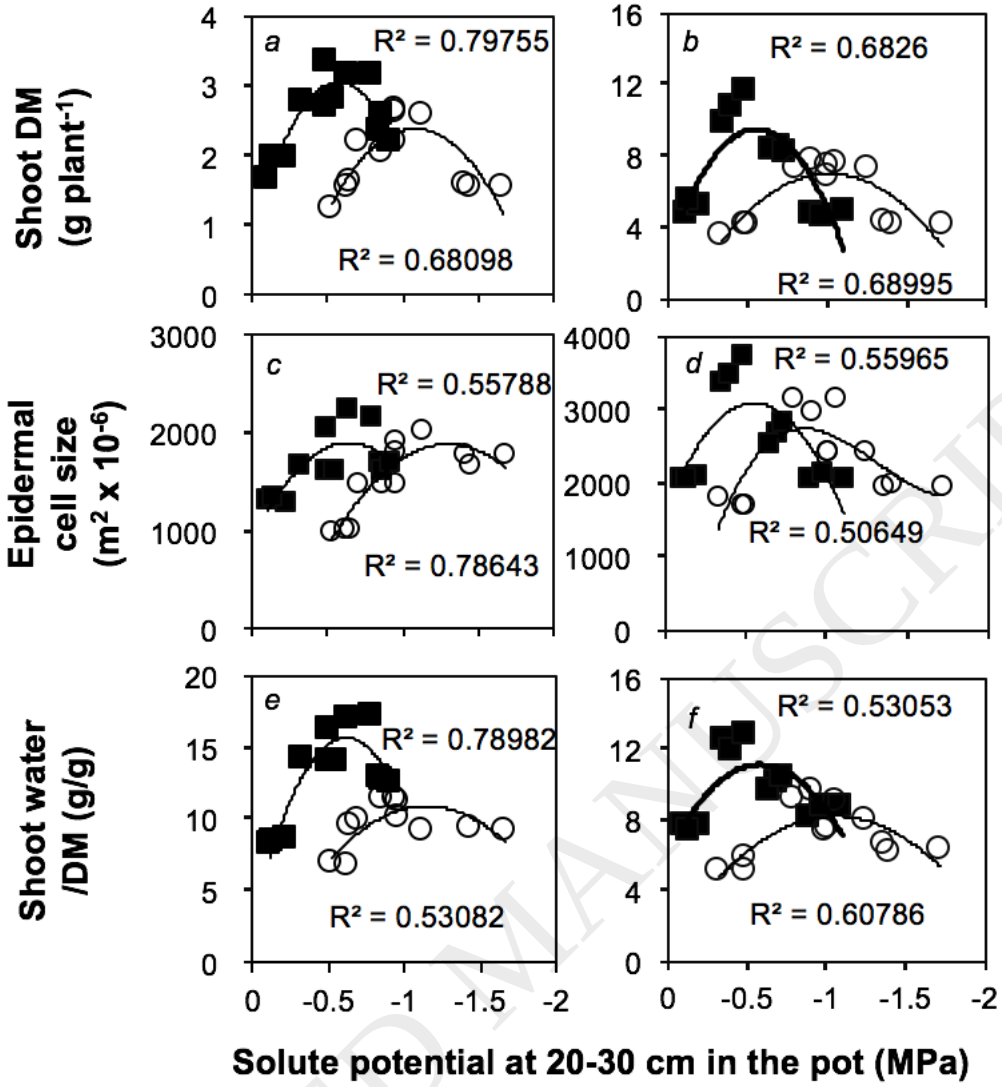

Fig. 2. Effects of variation in solute potential at 20-30 cm in the pot on the shoot DM (a, b), epidermal cell size (c,d), and ratio of shoot water / shoot DM (e, f), for *S. europaea* (a, c, & e) and *S. maritima* (b, d, & f) based on data from individual replicates. The pots were salinized by standing in trays with solutions of 0, 170, 340 or 680 mM NaCl, and were subsequently watered to the equivalent of 80% (■) or 40% (○) of the weight of water at field capacity. Soil solute potentials were calculated based on the Na^+ and Cl^- concentrations in the soil and the soil water content at 20-30 cm depth. The formulae for the lines of best fit are given in the Supplementary Materials – Table S3.

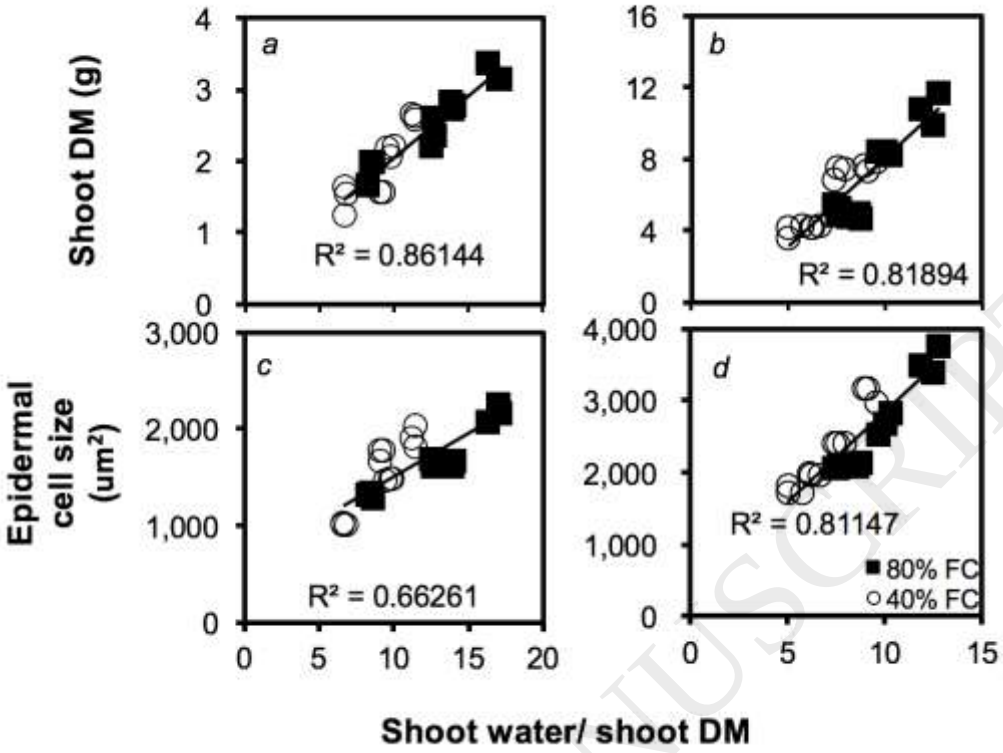

Fig. 3. Relationships between the ratio of shoot water / shoot DM and shoot DM (a, b), and size of epidermal cells (c, d) for *S. europaea* (a, c) and *S. maritima* (b, d) based on data from individual replicates. The formulae for the lines of best fit are given in the Supplementary Materials (Table S4). The pots were salinized by standing in trays with solutions of 0, 170, 340 or 680 mM NaCl, and were subsequently watered to the equivalent of 80% (■) or 40%(○) of the weight of water at field capacity

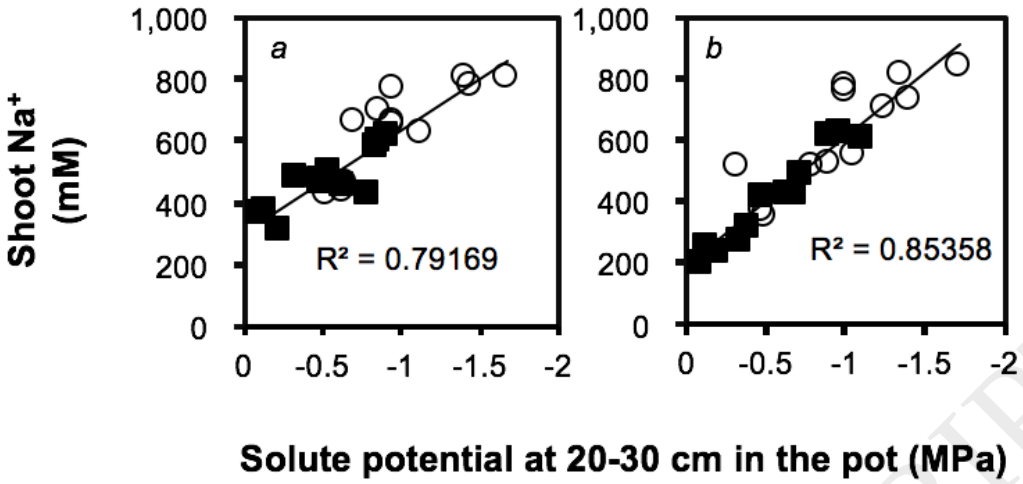

Fig. 4. Effects of variation in solute potential at 20-30 cm in the pot on the Na^+ concentration in the shoots of *S. europae* (a) and *S. maritima* (b) based on data from individual replicates. The formulae for the lines of best fit are given in the Supplementary Materials (Table S5). The pots were salinized by standing in trays with solutions of 0, 170, 340 or 680 mM NaCl, and were subsequently watered to of the equivalent of 80% (■) or 40%(○) of the weight of water at field capacity.

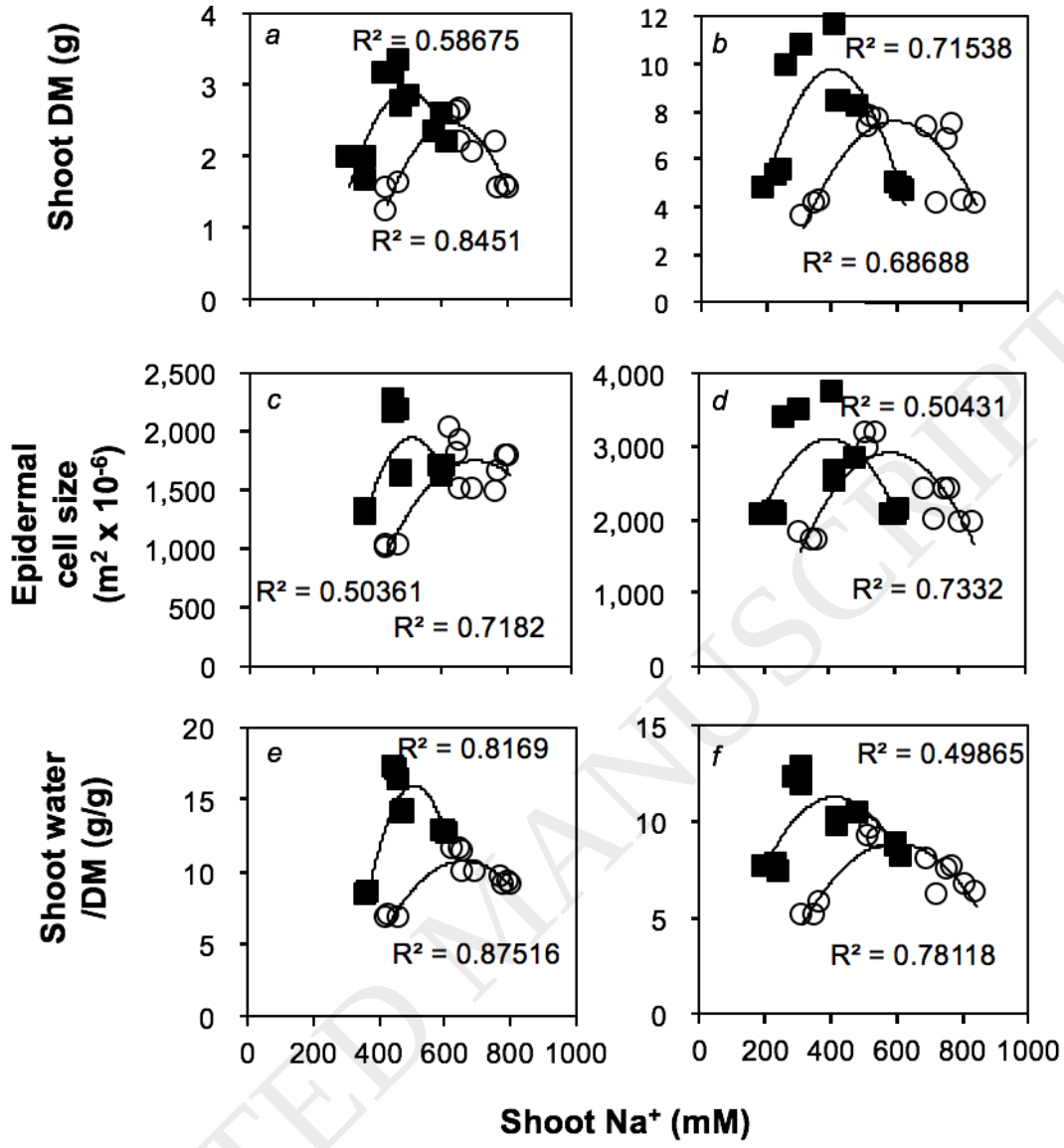

Fig. 5. Relationship between shoot Na^+ and shoot DM (a, b), shoot Na^+ and epidermal cell size (c, d), and shoot Na^+ and shoot water/DM (e, f) for *S. europae* (a, c, e) and *S. maritima* (b, d, f). The pots were salinized by standing in trays with solutions of 0, 170, 340 or 680 mM NaCl, and were subsequently watered to the equivalent of 80% (■) or 40%(○) of the weight of water at field capacity. The formulae for the lines of best fit are given in the Supplementary Materials (Table S6).