RESEARCH PAPER

Plant responses to heterogeneous salinity: growth of the halophyte Atriplex nummularia is determined by the root-weighted mean salinity of the root zone

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Abstract

Soil salinity is generally spatially heterogeneous, but our understanding of halophyte physiology under such conditions is limited. The growth and physiology of the dicotyledonous halophyte Atriplex nummularia was evaluated in split-root experiments to test whether growth is determined by: (i) the lowest; (ii) the highest; or (iii) the mean salinity of the root zone. In two experiments, plants were grown with uniform salinities or horizontally heterogeneous salinities (10–450 mM NaCl in the low-salt side and 670 mM in the high-salt side, or 10 mM NaCl in the low-salt side and 500–1500 mM in the high-salt side). The combined data showed that growth and gas exchange parameters responded most closely to the root-weighted mean salinity rather than to the lowest, mean, or highest salinity in the root zone. In contrast, midday shoot water potentials were determined by the lowest salinity in the root zone, consistent with most water being taken from the least negative water potential source. With uniform salinity, maximum shoot growth was at 120–230 mM NaCl; ~90% of maximum growth occurred at 10 mM and 450 mM NaCl. Exposure of part of the roots to 1500 mM NaCl resulted in an enhanced (+40%) root growth on the low-salt side, which lowered root-weighted mean salinity and enabled the maintenance of shoot growth. Atriplex nummularia grew even with extreme salinity in part of the roots, as long as the root-weighted mean salinity of the root zone was within the 10–450 mM range.

Key words: ion relations, root growth, split-root experiment, variable salinity, water relations, water uptake.

Introduction

Soil salinity in saline landscapes is rarely uniform (Bingham and Garber, 1970), and the ranges of soil salinities experienced by the roots of single plants can be large (Bazihizina et al., 2012). For example, within a 50 cm radius from the stem of the halophytic shrub Haloxylon ammodendron, the salinity of the soil solution varied from 20 dS m$^{-1}$ to ~85 dS m$^{-1}$ (Li et al., 2011). In field plots with Atriplex amnicola or A. nummularia, the salinities of the soil solution ranged from 40 dS m$^{-1}$ to 120 dS m$^{-1}$ or from 20 g Cl$^{-}$ l$^{-1}$ to >60 g Cl$^{-}$ l$^{-1}$ over distances of ~1 m (Davidson et al., 1996; Slavich et al., 1999). Although the high end of these salinity ranges is above the salinity level endured by most halophytes (cf. Flowers and Colmer, 2008), halophytic vegetation can be found in these hostile environments (e.g. Yakir and Yechieli, 1995), raising the question of how these plants
survive such levels of salinity. Better knowledge of halophytic adaptation to spatially heterogeneous salinities would be useful in understanding the limits to halophyte growth in natural and agricultural landscapes.

Based on split-root studies with non-halophytes, there are three main hypotheses (H) that can be put forward to explain how shoot growth responds to horizontally heterogeneous salinity. These are that shoot growth is determined by: (H1) the mean salinity of the root zone (Kirkham et al., 1969; Shani et al., 1993); (H2) the lowest salinity of the root zone (Flores et al., 2002; Zekri and Parsons, 1990); or (H3) the highest salinity of the root zone (Lycoskoufis et al., 2005). In contrast, halophyte growth responses under heterogeneous salinity have only been assessed in three studies (Messedi et al., 2004; Hamed et al., 2008; Bazihizina et al., 2009), and the data available do not allow testing of the three hypotheses. Data interpretations are complicated by the use of NaCl-free ‘control’ solutions in two of these studies considering halophytes (Messedi et al., 2004; Hamed et al., 2008). Growth stimulations in Sesuvium portulacastrum and Batis maritima under heterogeneous salinities compared with ‘control’ plants in an NaCl-free solution could well have been caused by suboptimal growth of the ‘controls’ owing to ion deficiencies in these low-salt plants (cf. Yeo and Flowers, 1980; Flowers and Colmer, 2008). Furthermore, these earlier studies of halophyte responses to heterogeneous salinities have only used a salinity range that still allowed growth for the evaluated halophytes [10–670 mM NaCl for A. nummularia (Bazihizina et al., 2009); 0–800 mM NaCl for B. maritima and S. portulacastrum (Hamed et al., 2008 and Messedi et al., 2004, respectively)]; thus H3, that the most saline area determines growth, has not yet been fully evaluated. Conceptually, however, if plant growth is indeed determined by the highest salinity of the root zone, then halophytes would not be able to acclimate to an extreme (i.e. toxic when uniform) salinity in part of the root system. H3 is therefore not expected to be supported for halophytes, as the few field data available indicate that extreme salinities can occur in spatially heterogeneous soils supporting halophytes (Bazihizina et al., 2012).

In a study where 10 mM NaCl was used in the ‘control’ nutrient solution, shoot growth of the halophyte A. nummularia exposed to heterogeneous salinities for 21 d was similar to that of the control plants grown with uniform 10 mM NaCl (Bazihizina et al., 2009). This result would at first sight appear to support H2 (i.e. that growth is best described by the lowest salinity in the root zone). However, A. nummularia has maximal shoot growth at 150–300 mM NaCl (Silviera et al., 2009), so the plants grown by Bazihizina et al. (2009) with heterogeneous salinities (10 mM NaCl in one root half and 230–670 mM NaCl in the other half) had mean salinities in the root zone close to this optimal salinity range. Therefore, with the data in Bazihizina et al. (2009), neither H1 nor H2 can be rejected. To test whether H1 or H2 best describes growth of halophytes in heterogeneous salinity, experiments are required where the mean salinity of the root zone is raised to values greater than those of the optimal salinity range for the evaluated species.

Shoot growth is tightly linked with root development patterns and, in heterogeneous saline soils, may depend upon increased root growth in the least saline areas. For non-halophytes, it has been found that most roots grow in the least saline/non-saline zones [e.g. Citrus aurantium (Zekri and Parsons, 1990) and Solanum lycopersicum (Flores et al., 2002)]. The halophyte A. nummularia, in contrast, had equal root growth in the low (10 mM NaCl) and high (670 mM NaCl) salt sides (Bazihizina et al., 2009). However, as 670 mM NaCl also did not affect root growth of A. nummularia when applied uniformly to the root system (Bazihizina et al., 2009), the effect of heterogeneous salinity on root growth patterns in halophytes still has to be evaluated using higher NaCl concentrations that do impede root growth.

The two experiments with heterogeneous salinity described here evaluated whether shoot growth and other physiological responses of the halophyte A. nummularia were determined by: (H1) the mean salinity, (H2) the lowest salinity, or (H3) the highest salinity, of the root zone. To test H1 and H2, a split-root experiment was conducted with A. nummularia in which the plants were grown with 10–450 mM NaCl on the low-salt side and 670 mM NaCl on the high-salt side. To test H3, as well as to obtain additional data to test H1 and H2 further, in an additional experiment A. nummularia was grown with 10 mM NaCl on the low-salt side and an extreme salinity of 1500 mM NaCl on the high-salt side. Measurements taken included: shoot and root ethanolic-insoluble dry mass (DM), stomatal conductance, leaf Na+ and Cl− concentrations, shoot water potential, and water uptake. These measurements provided a comprehensive analysis of the physiology and growth of this halophyte under conditions of heterogeneous root zone salinity when the concentrations were either within or above the optimal salinity range for growth.

Materials and methods

Plant material and culture

Rooted plants were established from cuttings of a commercial clone of A. nummularia Lindl. (‘Eyres’ Green’, Tamlin’s Nursery, South Australia). Cuttings were raised in a naturally lit phytotron (20/15 °C day/night) in pots of washed white sand irrigated with a gradually increasing concentration of nutrient solution, and, after 6 weeks, plants were transferred to an aerated full-strength nutrient solution. The full-strength nutrient solution consisted of (mM): 4.7 K2SO4, 9.3 CaCl2, 5.0 Na2SO4, 1.0 MgSO4, 0.7 Ca(NO3)2, 0.3 K2HPO4, 0.2 NH4H2PO4, and (µM): 80 Fe-EDDHA (‘Sequestrene 138’), 23 H3BO3, 2 MnSO4, 2 ZnSO4, 0.5 CuSO4, and 0.5 Na2MoO4. The nutrient solution was buffered with 1.0 mM MES and the pH was adjusted to 6.0, using KOH.

Six weeks after transferring the cuttings to the aerated full-strength nutrient solution, plants were selected for shoot and root uniformity, and transferred into split-root pots (one plant per split-root pot, with 0.6 litres of nutrient solution per side); a detailed description of these split-root pots has been given by Bazihizina et al. (2009). At this point, pots were moved to a controlled-environment room [20/15 °C day/night, 12 h day/12 h night, average relative humidity (RH) 56% and an average photosynthetically active radiation (PAR) at shoot height of 460 µmol m−2 s−1].

Experimental design

Responses of A. nummularia to heterogeneous salinities were studied in two experiments.

Experiment 1 (Expt 1) consisted of nine treatments with five replicates in a completely randomized block design and five additional plants for the initial harvest. In all treatments, the root system of a single plant was separated into two halves. In five treatments, both halves of the root system were exposed to the same NaCl concentrations (10, 120,
230, 450, or 670 mM) and in four treatments the two halves of the root system were exposed to two different NaCl concentrations; one side was exposed to 670 mM NaCl (high-salt side) and the other to 10, 120, 230, or 450 mM NaCl (low-salt side), all in the nutrient solution described above. For the 10 mM treatment, no additional NaCl was added as the basal solution already contained 10 mM Na+. On the fourth day after transferring the plants to the split-root pots, NaCl was increased on both sides of the split-root pots in increments of 55 mM every 12 h, until NaCl concentrations reached 670 mM on both sides of the root system. Three days after reaching 670 mM NaCl on both sides, all treatments were imposed with a single step down from 670 mM NaCl to the required concentration on each side (as in Bazihizina et al., 2009). This time was considered to be day 0 of treatment, and an initial harvest was taken (described below). Plants were all exposed to 670 mM NaCl before applying treatments to mimic seasonal dynamics in soil salinity in the field, where there can be salt accumulation after periods of high evapotranspirational demand in dry seasons, with rainfall rapidly leaching salts out of the upper soil in wet seasons (Mensforth and Walker, 1996). In plants exposed to heterogeneous salinity, leaf gas exchange parameters, shoot water potential, leaf ions, and total soluble sugars were measured on each side of the shoot, directly above each root side, but as there were no differences between sides these data were averaged for each replicate.

Experiment 2 (Expt 2) consisted of five treatments with four replicates in a completely randomized block design and four additional plants for the initial harvest. In three treatments, the two halves of the root system were both exposed to the same NaCl concentrations: 10, 50, or 1500 mM. The remaining two treatments had the two halves of the root system each exposed to different NaCl concentrations; one side was exposed to 10 mM NaCl (low-salt side) and the other to either 500 mM or 1500 mM NaCl (high-salt side). NaCl treatments were added to the basal nutrient solution, as described above for Expt 1. In contrast to Expt 1 with 670 mM NaCl as the highest NaCl level, the 1500 mM NaCl used in Expt 2 was expected to damage plants, and therefore not all plants were stepped up to this highest level and then stepped down to commence treatments; instead treatments commenced after stepping up to the highest concentration in the split-root compartments destined for the 1500 mM treatments. Seven days after plants were transferred into split-root pots, the NaCl concentration was increased in increments of 50 mM every 12 h until the required NaCl concentration for each treatment was reached. Twelve hours after reaching the highest salinity (1500 mM NaCl) was considered as day 0 of treatment, and an initial harvest was taken (described below). As for Expt 1, in plants exposed to heterogeneous salinity, leaf gas exchange parameters, shoot water potential, leaf ions, and total soluble sugars were measured on each side of the shoot, directly above each root side, but as there were no differences between sides these data were averaged for each replicate.

Leaf gas exchange
Leaf gas exchange measurements were taken on day 19 (Expt 1) or 20 (Expt 2) of treatment on three (Expt 2) or four (Expt 1) randomly chosen plants from each treatment. Measurements were taken between 11.00 h and 13.00 h. Measurements of net photosynthetic rate and stomatal conductance were determined on young fully expanded leaves using a LI-COR 6400 Photosynthesis System (LI-COR, Inc., Lincoln, NE, USA) at ambient RH (50–60%), reference CO₂ of 380 µmol mol⁻¹, flow rate of 400 µmol s⁻¹, and PAR of 1500 µmol m⁻² s⁻¹.

Water use measurements
On day 19 (Expt 2) or 20 (Expt 1) of treatment, water use was measured in all heterogeneous treatments on three (Expt 2) or four (Expt 1) randomly chosen plants from each treatment. As controls, water use in uniform 10/10 mM (Expt 2) or 670/670 mM (Expt 1) NaCl treatments were measured. Water use was not measured in other uniform treatments in Expt 1 (10, 120, 230, and 450 mM NaCl) and 2 (1500 mM NaCl) as there was no capacity to measure more plants. Plants were transferred into split-root pots designed for water use measurements, with water use being based on the precise re-filling of pots to the initial level ±10 µl (for details see Bazihizina et al., 2009); for these measurements, all pots were bubbled with pre-humidified air. Three (Expt 1) or four (Expt 2) blank pots (i.e. without plants) were used to determine background evaporative losses. Pots were initially filled with nutrient solution containing the relevant NaCl concentrations and then, on the day of water use measurements, each pot was topped back up to the pre-determined initial volume with deionized water at 06.00 h and then again at 18.00 h (i.e. at the commencement and end of the 12 h light period), and the volume added to each pot was recorded.

Stem extension rate and plant samplings
Plants were sampled on days 0 and 21 after the commencement of treatments for the determination of shoot and root DM. Stem length was measured with a ruler on days 0 and 21 to determine the extension rate during the treatment period. In order to assess any differences in root DM between sides, the two sides of each root system were harvested separately. Young fully expanded leaves (one for plants in uniform treatments and two for heterogeneous treatments, i.e. one leaf from each side of the plant) were collected between 11.00 h and 11.30 h for subsequent analyses of ions and total soluble sugars. These leaf tissues were snap-frozen in liquid N₂, stored at –80 °C, freeze-dried, and then stored at –20 °C. All remaining shoots were oven-dried at 60 °C. For root tissues, a subsample of the roots for each treatment was taken to determine root surface area per unit root DM, so that total root surface area could be estimated from total root DM. Roots were scanned for surface area using a WinRhizo root scanner (Regent Instruments Inc., Quebec, Canada) with a resolution that would have captured main and fine lateral roots but not root hairs. These root subsamples, and all remaining roots, were oven dried at 60 °C to determine DM.

Midday shoot water potential
In Expt 1 and 2, shoot water potential was measured after 21 d of treatment on excised shoots using a Scholander Pressure Chamber between 11.30 h and 12.30 h. In Expt 2, midday water potentials were not determined for plants growing in uniform 1500 mM NaCl as shoots were small and burst out of the chamber gasket when pressures >3.5 MPa were applied.

Ethanol-insoluble DM and measurement of total soluble sugars
To determine ethanol-insoluble DM, ground shoot and root tissues were extracted twice in boiling 80% ethanol, refluxed for 20 min, centrifuged for 10 min at 9335 g (IEC micromax ventilated micro-centrifuge OM3590, Needham Heights, MA, USA), the supernatant was poured off, and the insoluble fraction was then dried at 60 °C and weighed. Freeze-dried young fully expanded leaves were also extracted in boiling 80% ethanol, twice, and the supernatant was collected and used to measure total sugars using anthrone (Yemm and Willis, 1954). Total sugars (as hexose equivalents) were determined by measuring the absorbance of the samples at 620 nm in a UV-visible spectrophotometer (UV-1601, UV-visible spectrophotometer, Shimadzu, Kyoto, Japan), using a standard curve for glucose. The reliability of this method was verified by determining the recovery of known amounts of glucose added to additional tissue samples immediately prior to extraction, and also added to ethanol only. The recovery of glucose from these samples was 108%, so data presented here have not been adjusted.

Tissue ion concentrations
Ground young fully expanded leaf samples were extracted with 0.5 M HNO₃ by shaking in vials for 48 h. Diluted extracts were analysed...
for Na⁺ (Flame Photometer 410, Sherwood, Cambridge, UK) and Cl⁻ (Chloridometer 50CL, SLAMED ING GmbH, Frankfurt, Germany). The reliability of the methods was confirmed by analyses of a reference tissue (broccoli, ASPAC Plant number 85) taken through the same procedures. The recovery from the reference tissue was: Na⁺ 98% and Cl⁻ 102%, so no adjustments were made to the data presented.

Statistical analyses

Statistical analyses were conducted using Genstat for Windows 10th Edition (Genstat software, VSN International, Hemel Hempsted, UK). Analysis of variance (ANOVA) was used to identify overall significant differences between treatments and between sides within treatments, depending on the data set. When significant differences were found, mean separations were calculated using Duncan’s multiple range test. Unless otherwise stated, the significance level was \( P \leq 0.05 \). Linear or polynomial (quadratic or cubic) regression analyses were performed with SigmaPlot 11.0 (Systat Software Inc., Version 11.0, Chicago, IL, USA). Cubic regression curves were fitted to growth (stem extension rate and shoot ethanol-insoluble DM) and stomatal conductance data because these best describe the growth pattern expected for a dicotyledonous halophyte in response to increasing salinity, incorporating an optimum at moderate salinities (cf. Flowers and Colmer, 2008) with decreases in growth at salinities above the optimum (cf. the compound discount curve of Steppuhn et al., 2005). In contrast, visual inspection of the relationships between leaf Na⁺ or shoot water potential against external salinity showed that simple linear and quadratic relationships, respectively, could be fitted to these data (see the Results).

Results

Experiment 1

Shoot and root growth After 21 d of treatment, under uniform salinity, there was a growth enhancement in the 120–230 mM NaCl range, and at 450 mM NaCl growth was similar to that of plants exposed to 10 mM NaCl (stem extension rate, Fig. 1A; shoot ethanol-insoluble DM, Fig. 1B; leaf area, Supplementary Table S1 available at JXB online). However, with uniform 670 mM NaCl, the stem extension rate and shoot ethanol-insoluble DM were 58% and 71%, respectively, of those for plants with uniform 10 mM NaCl. In plants exposed to heterogeneous salinities, there were no significant differences in shoot ethanol-insoluble DM compared with the corresponding uniform salinity treatments (i.e. 10/670 versus 10/10, 120/670 versus 120/120; 230/670 versus 230/230, or 450/670 versus 450/450 mM NaCl). However, the stem extension rate was generally reduced compared with plants in the corresponding uniform treatments. The stem extension rate was 86% with 10/670, 66% with 120/670, and 72% with 230/670 mM of the respective values for plants in the uniform 10, 120, and 230 mM NaCl treatments. No significant difference (i.e. \( P > 0.05 \)) was found between plants of the 450/670 mM and uniform 450 mM NaCl treatments.

Fig. 1. Responses of shoot growth parameters of Atriplex nummularia after 21 d of treatment to uniform and heterogeneous NaCl in the root zone using a split-root pot system: (A) stem extension rate, and (B) shoot ethanol-insoluble dry mass (Expt 1). In (C) and (D), for the heterogeneous data set, shoot growth parameters are plotted against the lowest and the mean salinity of the root zone: (C) stem extension rate and (D) shoot ethanol-insoluble dry mass. In C and D, the arrows indicate the displacement of the heterogeneous data from when plotted against the lowest salinity to when plotted against the mean salinity of the root zone. In C and D, regression curves (cubic relationships) of best fit were found when the combined uniform and heterogeneous data were plotted against the mean salinity of the root zone, \( R^2 \) values are indicated for each curve and are significant \( (P \leq 0.05) \). Initial (day 0) shoot ethanol-insoluble dry mass (g) was 1.80 ± 0.22. Values are means \( (n=5) \) ±SE. Asterisks indicate significant differences between means: *\( P \leq 0.05 \); ***\( P \leq 0.001 \).
To assess whether the shoot parameters were determined by the mean salinity (H1) or lowest salinity (H2) of the root zone, regression curves (cubic relationships) were fitted to the combined uniform and heterogeneous data sets (stem extension rate, Fig. 1C; shoot ethanol-insoluble DM, Fig. 1D). The regression curves for these two parameters had the best fit when the heterogeneous data were plotted against the mean salinity, rather than the lowest salinity, of the root zone (see Table 1 for $R^2$ and $P$-values of the regression analyses).

In plants exposed to uniform salinity, root ethanol-insoluble DM was highest in the range 120–450 mM NaCl, although differences were not significant when compared with root ethanol-insoluble DM in uniform 10 mM NaCl. On the other hand, with 670 mM NaCl in the root zone, root ethanol-insoluble DM declined to 49–53% of that in the 120–450 mM NaCl range (Fig. 2A). In the heterogeneous treatments, with NaCl concentrations on the low-salt side $\geq$ 120 mM, there were declines in total root ethanol-insoluble DM compared with the corresponding uniform treatments (Fig. 2A). Total root ethanol-insoluble DM of the 120/670, 230/670, and 450/670 mM treatments was 66, 54, and 60% of the respective values in uniform 120, 230, and 450 mM NaCl (Fig. 2A). These reductions in total root ethanol-insoluble DM with heterogeneous salinities were caused by declines in ethanol-insoluble DM in the low-salt side compared with the ethanol-insoluble DM averaged across both sides of the corresponding uniform treatments. As examples, in the 230/670 mM and 450/670 mM treatments, the root ethanol-insoluble DM on the low-salt side was 59–66% of the ethanol-insoluble DM averaged across both sides of the uniform 230 mM and 450 mM NaCl treatments. These reductions in root ethanol-insoluble DM in heterogeneous treatments with NaCl concentrations on the low-salt side $\geq$120 mM caused a decrease in the root/shoot ratio compared with the corresponding uniform treatments: with 120/670, 230/670, and 450/670 mM the root/shoot ratio was 74, 60, and 65%, respectively of the ratio with uniform 120, 230, and 450 mM NaCl. Furthermore, the root/shoot ratios for these heterogeneous treatments were not different from that of the uniform 670 mM NaCl treatment.

**Water and ion relations**

In uniform treatments, stomatal conductance only declined at 450 mM and 670 mM NaCl, with conductance being 71% and 37%, respectively, of the value in the uniform 10 mM NaCl treatment (Fig. 3A). Net photosynthetic rate decreased as salinity in the medium increased, with a decline at 670 mM NaCl to only 49% of the rate for plants in uniform 10 mM NaCl (Supplementary Table S1 at JXB online). Compared with the corresponding uniform low-salt plants, stomatal conductance declined in heterogeneous treatments. For plants exposed to 120/670 mM and 230/670 mM, stomatal conductance rates were 70% of those of plants in uniform 120 mM and 230 mM NaCl.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean salinity in root zone</th>
<th>Lowest salinity in root zone</th>
<th>Figure</th>
<th>$R^2$</th>
<th>$P$</th>
<th>Figure</th>
<th>$R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem extension rate</td>
<td>0.8366</td>
<td>0.4302</td>
<td>1C</td>
<td>&lt;0.05</td>
<td>0.3825</td>
<td>NP</td>
<td>0.3825</td>
<td>NP</td>
</tr>
<tr>
<td>Shoot ethanol-insoluble mass</td>
<td>0.9233</td>
<td>0.6900</td>
<td>1D</td>
<td>&lt;0.05</td>
<td>0.0961</td>
<td>NP</td>
<td>0.0961</td>
<td>NP</td>
</tr>
<tr>
<td>Net photosynthetic rate</td>
<td>0.8721</td>
<td>0.8162</td>
<td>NP</td>
<td>&lt;0.05</td>
<td>0.1281</td>
<td>NP</td>
<td>0.1281</td>
<td>NP</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>0.9230</td>
<td>0.9801</td>
<td>NP</td>
<td>&lt;0.05</td>
<td>0.0961</td>
<td>3E</td>
<td>0.0961</td>
<td>NP</td>
</tr>
<tr>
<td>Midday shoot water potential</td>
<td>0.9176</td>
<td>0.8125</td>
<td>NP</td>
<td>&lt;0.05</td>
<td>0.0961</td>
<td>NP</td>
<td>0.0961</td>
<td>NP</td>
</tr>
<tr>
<td>Leaf Na+ concentration</td>
<td>0.9663</td>
<td>0.7744</td>
<td>NP</td>
<td>&lt;0.05</td>
<td>0.0961</td>
<td>NP</td>
<td>0.0961</td>
<td>NP</td>
</tr>
</tbody>
</table>

NP, not presented.
Differences in stomatal conductance between uniform and heterogeneous treatments were not caused by increases in total soluble sugars (Supplementary Table S1), ruling out possible negative feedback due to reduced growth (cf. Munns, 1993). Analyses on the combined uniform and heterogeneous data sets gave significant curves (cubic relationship) of best fit for stomatal conductance (Fig. 3D) and net photosynthetic rate when plotted against the mean salinity of the root zone (Table 1).

In uniform treatments, shoot midday water potential was approximately –2.0 MPa at 10–230 mM NaCl, but this decreased to –2.6 MPa at 450 mM and to –3.7 MPa at 670 mM (Fig. 3B). For plants in heterogeneous treatments, midday shoot water potentials were similar (within 0.1 MPa) of those in the corresponding uniform low-salt treatments (Fig. 3B). Analyses on the combined uniform and heterogeneous data sets gave a quadratic curve of best fit for shoot midday water potential (Fig. 3E) when plotted against the lowest salinity of the root zone.

Ion concentrations were calculated on a tissue water basis, which provides a more physiologically relevant interpretation of ion regulation in a succulent halophyte than expression on a DM basis (Short and Colmer, 1999). In uniform treatments, the concentration of Na⁺ in the young fully expanded leaves increased as the external NaCl concentration was raised (Fig. 3C), so that at 670 mM NaCl, leaf Na⁺ concentrations were 70% higher than at 10 mM NaCl. There were similar trends for leaf Cl⁻ concentrations in uniform treatments (Supplementary Table S1 at JXB online). Under heterogeneous salinity, leaf Na⁺ and Cl⁻ concentrations also increased as NaCl in the low-salt side was raised. With 10–230 mM NaCl in the low-salt side, leaf Na⁺ and Cl⁻ concentrations increased by 10–50% compared with the corresponding uniform low-salt treatment (Fig. 3C; Supplementary Table S1). At 450 mM NaCl, however, the concentrations did not differ between plants in the uniform low-salt and heterogeneous treatments. Analysis of the combined uniform and heterogeneous

![Fig. 3. Responses of Atriplex nummularia to uniform and heterogeneous NaCl treatments in the root zone using a split-root pot system: (A) stomatal conductance; (B) midday shoot water potential; and (C) Na⁺ concentrations in young fully expanded leaves (Expt 1). In (D), (E), and (F), for the heterogeneous data set, these parameters are plotted against the lowest and mean salinity of the root zone: (D) stomatal conductance; (E) midday shoot water potential; and (F) Na⁺ concentrations in young fully expanded leaves. In D, E, and F, the arrows indicate the displacement of the heterogeneous values from when plotted against the lowest salinity to when plotted against the mean salinity of the root zone. The regression line in F and the curve (cubic relationship) in D of best fit were found when the combination of uniform data and heterogeneous data were plotted against the mean salinity of the root zone. In E, the curve (quadratic relationship) of best fit occurred when the heterogeneous data were plotted against the lowest salinity in the root zone. R² values are indicated for each curve and are significant (P ≤ 0.05). In uniform treatments, both root halves were exposed to 10, 120, 230, 450, or 670 mM NaCl. In heterogeneous treatments, one root half was exposed to 10, 120, 230, or 450 mM NaCl (indicated on the x-axis) and the other root half was exposed to 670 mM NaCl. Values are means (n=4 in A and 5 in B and C) ±SE, with each replicate under heterogeneous treatment being the mean of two measurements per plant of opposing leaves (i.e. one leaf from above each split-root side). Asterisks indicate significant differences between means: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.](https://academic.oup.com/jxb/article-abstract/63/18/6347/501988)
Table 2. Effect of uniform and heterogeneous NaCl concentrations in the root zone on whole-plant water use and water uptake, measured over a 12 h light period, expressed on a root surface area basis.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Whole-plant water use (ml)</th>
<th>Water uptake on root surface area basis (µmol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low-salt side</td>
</tr>
<tr>
<td>10/670</td>
<td>23.2 ± 2.9 a</td>
<td>203.9 ± 21.3 a</td>
</tr>
<tr>
<td>120/670</td>
<td>21.6 ± 2.0 a</td>
<td>189.6 ± 7.8 a</td>
</tr>
<tr>
<td>230/670</td>
<td>19.9 ± 2.8 a</td>
<td>191.2 ± 28.4 a</td>
</tr>
<tr>
<td>450/670</td>
<td>13.8 ± 2.4 b</td>
<td>156.8 ± 49.0 a,b</td>
</tr>
<tr>
<td>670/670</td>
<td>8.9 ± 0.9 b</td>
<td>87.6 ± 10.8 b,c</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/10</td>
<td>31.3 ± 0.6 a</td>
<td>165.5 ± 32.0 a,b</td>
</tr>
<tr>
<td>10/500</td>
<td>22.8 ± 2.2 a</td>
<td>116.5 ± 12.8 a</td>
</tr>
<tr>
<td>10/1500</td>
<td>19.2 ± 1.3 a</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Water uptake measurements were taken from 06.00 h to 18.00 h, 20 d (Expt 1) or 19 d (Expt 2) after imposing treatments. In Expt 1, values are means (n=4) ±SE and in Expt 2 values they are means (n=3) ±SE. Differences in lowercase letters within each column and between data columns 2 and 3 in Expt 1 and 2 indicate significant differences (P ≤ 0.05).

data set showed simple linear fits for leaf Na⁺ (Fig. 3F) and for leaf Cl⁻ (data not shown) when plotted against the mean salinity of the root zone (see Table 1 for R² and P-values).

Water use

Under heterogeneous salinities, whole plant water use during the 12 h light period decreased with increasing salinity in the low-salt side (Table 2). At 450/670 mM NaCl, plant water use was similar to that measured with uniform 670 mM NaCl (8.9 ± 0.9 ml), and was ~60% of the water use in plants with 10/670 mM NaCl in the root zone. This reduction with 450/670 mM NaCl was in part due to a 20% decline in water uptake rate (on a root surface area basis), but was also caused by the above-mentioned decrease in root DM allocation to the low-salt side and small declines (although not significant) in leaf area (Fig. 1B, and for leaf area data see Supplementary Table S1 at JXB online). Nevertheless, in all heterogeneous treatments, most water (78–88% of the plant water uptake) was taken from the low-salt side.

Experiment 2

Shoot and root growth

With uniform salinity, stem extension rate and shoot ethanol-insoluble DM declined as salinity in the root zone increased (Fig. 4A, B; in contrast to Expt 1, in the figures for Expt 2, heterogeneous data have been plotted against the highest salinity in the root zone). With uniform 1500 mM NaCl, shoot ethanol-insoluble DM and stem elongation did not change between day 0 and 21. In the final measure of shoot ethanol-insoluble DM, dead leaves were not included. After 21 d, dead leaves in plants exposed to uniform 1500 mM NaCl were 15% of the shoot green DM while in all other treatments the DM of dead leaves was <3% of shoot green DM. With heterogeneous salinity, independently of the salinity in the high-salt side, after 21 d of treatment there were no significant differences in stem extension rate (Fig. 4A) or shoot ethanol-insoluble DM (Fig. 4B) compared with uniform 10 mM NaCl.

With uniform salinity, there was no significant reduction in total root ethanol-insoluble DM between low (10 mM NaCl) and moderate (500 mM NaCl) salinity after 21 d of treatments (Fig. 4C). However, the uniform 1500 mM NaCl treatment inhibited root growth; there was no increase in total root ethanol-insoluble DM between day 0 and 21. Twenty-one days after reaching the highest salinity, total root ethanol-insoluble DM with uniform 1500 mM NaCl was 41% of the root ethanol-insoluble DM of the uniform 10 mM NaCl treatment. Under both heterogeneous treatments, there was no significant reduction in total root ethanol-insoluble DM compared with uniform 10 mM NaCl (Fig. 4C). With heterogeneous 500 mM NaCl, this was because there was no reduction in the DM of roots on either side (Fig. 4D). On the other hand, with 1500 mM NaCl in one root portion, this was due to enhanced root growth in the low-salt side, resulting in 3.9 times the ethanol-insoluble DM on the low-salt side compared with that on the high-salt side compared with that on the high-salt side (Fig. 4D) and 1.4 times the ethanol-insoluble DM in each compartment in plants exposed to uniform 10 mM NaCl. Under heterogeneous 10/1500 mM NaCl, roots in the 1500 mM side did not increase in ethanol-insoluble DM during the 21 d of treatment, but these roots remained alive, demonstrated by the resumption of growth when plants were transferred back into 10 mM NaCl on both sides (a side experiment conducted only for plants grown in the 10/1500 mM NaCl treatment, results not shown).

Water and ion relations

In the uniform treatments, stomatal conductance (Fig. 5A) and net photosynthetic rate (Supplementary Table S2 at JXB online) declined as the salinity in the nutrient solution was raised. With uniform 1500 mM NaCl, net photosynthesis was almost completely inhibited (~1% of that for plants in uniform 10 mM NaCl, day 20) and stomata were almost closed (~10% of the stomatal conductance of plants in uniform 10 mM NaCl). Associated with this reduced photosynthesis and stomatal conductance, there was a substantial increase (1.8 times that of plants in uniform 10 mM NaCl) in the internal CO₂ concentration (Supplementary Table
S2) compared with plants grown with uniform 10 mM NaCl. Under heterogeneous salinities there were also declines in net photosynthetic rate and stomatal conductance compared with those for plants in uniform 10 mM NaCl. Stomatal conductance in plants exposed to 10/500 mM NaCl was 73%, and for plants in
10/1500 mM NaCl it was 61%, of those in uniform 10 mM NaCl. No differences were found between internal CO₂ concentrations within uniform 10 mM NaCl treatments and both heterogeneous treatments (Supplementary Table S2).

Twelve hours after reaching the highest salinity (1500 mM NaCl), the concentration of total soluble sugars in young fully expanded leaves under uniform extreme salinity (1500/1500 mM) increased to almost three times the concentration in plants in the uniform 10 mM NaCl (Supplementary Table S2 at JXB online). In contrast, there were no significant differences in total soluble sugars in young fully expanded leaves in any other treatments. After 21 d, no differences were found between treatments (Supplementary Table S2).

In uniform treatments, shoot midday water potential decreased as salinity in the root zone increased, and with uniform 500 mM NaCl the potential was 1.2 MPa more negative than with uniform 10 mM NaCl (Fig. 5B). It was not possible to complete the measurements of the shoot water potentials at uniform 1500 mM NaCl as shoots were small and burst out of the Scholander chamber gasket when pressures >3.5 MPa were applied; shoot potentials with uniform 1500 mM NaCl were therefore more negative than −3.5 MPa. In contrast, the midday water potentials of plants exposed to the heterogeneous salinity treatments (10/500 mM and 10/1500 mM), independently of the NaCl concentration on the high-salt side, were not different from those of plants exposed to uniform 10 mM NaCl.

In uniform treatments, the concentration of Na⁺ in young fully expanded leaves (Fig. 5C) increased almost linearly with the NaCl concentration in the root zone. With uniform 500 mM and 1500 mM NaCl, Na⁺ concentrations were 1.9 and 4.2 times, respectively, the concentrations in plants with uniform 10 mM NaCl. However, in heterogeneous treatments, concentrations of Na⁺ in leaves remained relatively constant between the 10/500 mM and the 10/1500 mM treatments, being only 1.5–1.7 times the concentrations in plants in uniform 10 mM NaCl. For Cl⁻, in the uniform 500 mM and 1500 mM NaCl treatments, the Cl⁻ concentrations in the young fully expanded leaves were 2.8 and 6.1 times, respectively, the concentrations in plants at 10 mM NaCl (Supplementary Table S2 at JXB online). However, in the heterogeneous treatments, the Cl⁻ concentrations were only 1.9–2.4 times the concentrations in plants at 10 mM NaCl (Supplementary Table S2).

Water use

In plants exposed to heterogeneous 10/500 mM and 10/1500 mM NaCl, whole plant water use was 73% and 61%, respectively, of that for plants exposed to uniform 10 mM NaCl (Table 2). In both heterogeneous treatments, most water (81–91%) was taken up from the 10 mM NaCl side. Nevertheless, in the 10/1500 mM treatment, despite the enhanced root growth in the low-salt side (Fig. 1C), there was no parallel increase in water uptake (ml) from the low-salt side compared with water uptake in the uniform 10 mM NaCl. Given the background evaporative losses, it was not clear whether any water had been taken up from the high-salt side of the heterogeneous 10/1500 mM treatment; there appeared to be a small amount of water uptake from two replicates but not the other replicate.

Water use efficiency was estimated from the estimated total plant water use [whole plant water use over the 21 d was estimated based on the water use measured in the 12 h light period on day 19 (see Table 2), the root dry mass on day 0 and 21, and assuming no water use at night] and the accumulated ethanol-insoluble dry mass, both over 21 d. Compared with the plants in uniform 10 mM NaCl, water use efficiency (g ethanol-insoluble DM ml⁻¹) doubled under extreme heterogeneous salinity, from 0.009 in the uniform 10 mM NaCl to 0.018 in the 10/1500 mM NaCl treatment. However, there were no significant differences between the heterogeneous treatments (10/500 mM and 10/1500 mM) and between the uniform 10 mM NaCl and the heterogeneous 10/500 mM treatment, where water use efficiency was 0.011 g ethanol-insoluble DM ml⁻¹. It is, however, important to note that water use measurements were only limited to a 12 h light period at the end of the experiment, and the estimated total plant water use may not be an accurate representation of water use over the experimental period.

Discussion

In many field situations roots of halophytes will probably experience spatially heterogeneous salinity (see the Introduction). Three hypotheses on how shoot growth responds to heterogeneous salinity were tested for the dicotyledonous halophyte A. nummularia: growth is determined by the (H1) mean salinity, (H2) lowest salinity, or (H3) highest salinity of the root zone (see the Introduction). In Expt 1, when A. nummularia was exposed to horizontally heterogeneous salinity, shoot growth parameters (shoot ethanol-insoluble DM and stem extension rate), leaf gas exchange, and leaf ion concentrations (Na⁺ and Cl⁻) all responded most closely to the mean salinity of the root zone, supporting H1. The only exception was shoot water potential, which was determined by the lowest salinity of the root zone, being consistent with most of the water being taken up from the low-salt side under heterogeneous salinity. Under uniform salinity (i.e. the same NaCl concentration in both root halves), A. nummularia was stimulated by salt and had maximal growth at 120–230 mM NaCl; ~90% maximal growth was obtained at 10 mM and 450 mM NaCl. Under heterogeneous salinity, further support for H1 was obtained by the finding that increasing the NaCl concentration on the low-salt side was progressively more damaging to the plants, as this increased the mean salinity in the root zone out of the 10–450 mM NaCl optimal range. In addition, data from Expt 2 enabled H3 to be rejected, as shoot growth of A. nummularia was maintained even with one root portion exposed to the extreme salinity of 1500 mM NaCl.

Heterogeneous salinities affect stomatal conductance and shoot ion relations, and the integrative approach of H1, namely that all salinities present in a plant’s root zone influence growth, does indeed seem the most appropriate. However, the results from Expt 2 indicate that calculation of the mean salinity of the root zone should take into account the relative root allocation on each side (i.e. the ‘root-weighted mean’ salinity). In Expt 1, root allocation between the low- and the high-salt sides was relatively similar, so mean salinity and ‘root-weighted mean’ salinity only differed by 3–50 mM NaCl (relatively small
concentrations for this halophyte). In contrast, in the extreme heterogeneous treatment (10/1500 mM NaCl) of Expt 2, root allocation between the low- and the high-salt sides differed significantly, with 79% of the root ethanol-insoluble DM occurring on the low-salt side; thus, in this case, the mean salinity of the root-zone (755 mM NaCl) was substantially higher than the ‘root-weighted mean’ salinity (316 mM NaCl). Drawing this together, data from both experiments are presented in the same figure for whole plant ethanol-insoluble DM (Fig. 6) and stomatal conductance (Supplementary Fig. S1 at JXB online).

The data were tested for relationships against: the lowest salinity (Fig. 6A; Supplementary Fig. S1A), the mean salinity (Fig. 6B; Supplementary Fig. S1B), or the ‘root-weighted mean’ salinity of the root zone (Fig. 6C; Supplementary Fig. S1C). Root ethanol-insoluble DM in Figs 2B and 4D were used to calculate the root-weighted mean salinities in Expt 1 and Expt 2, respectively (for root-weighted mean salinity values see Supplementary Table S3). Cubic regression curves were fitted to the combined uniform and heterogeneous data sets (Figs. 6A–C). Although R² values were slightly higher when whole plant ethanol-insoluble DM was plotted against mean salinity in the root zone (Fig. 6B), the fitted growth curve had an unrealistic trend, with an apparent increase at salinities >600 mM NaCl. The fitted curve in Fig. 6B was therefore considered to be unrealistic, and the curve in Fig. 6C was regarded as the best fit to these data. A similar argument can be used for the stomatal conductance data to choose the root-weighted mean salinity as the best predictor for stomatal conductance (Supplementary Fig. S1).

Under extreme heterogeneous salinity, enhanced root growth on the low-salt side (+40% ethanol-insoluble DM compared with plants with uniform 10 mM NaCl) was only observed when roots in the high-salt side were exposed to an extreme salinity (1500 mM NaCl) that completely inhibited growth on that side. In contrast, there was no preferential root growth on the low-salt side when 500 mM NaCl was applied to the high-salt side, as 500 mM NaCl did not affect root growth, being in the tolerated salinity range for this halophyte. Increased root growth on the low-salt side in the 10/1500 mM NaCl treatment probably contributed to the shoot growth, as the roots on the low-salt side provided most of the required water and were also likely to have provided most of the nutrients to sustain shoot growth.

In plants exposed to extreme heterogeneous salinity, stomatal conductance declined to 61% of that of plants in uniform 10 mM NaCl despite the root proliferation on the low-salt side. These declines in stomatal conductance were not associated with detectable increases in total soluble sugars (on both day 0 and 21) in leaf tissues, indicating that it is unlikely that sugars acted on stomata through negative feedback (Munns, 1993). Moreover shoot midday water potential of the extreme heterogeneous treatment was similar to that of plants in uniform 10 mM NaCl. Therefore, it could be possible that the reduced stomatal conductance was caused by non-hydraulic signals (e.g. abscisic acid, cytokinin, or changes in xylem pH; Pérez-Alfocea et al., 2010) from the high-salt side, as described for many plants with a root portion in dry soil (e.g. Khalil and Grace, 1993; Sobeih et al., 2004). Nonetheless the decline in stomatal conductance under heterogeneous extreme salinity was consistent with declines in whole plant water uptake and the apparent increased water use efficiency compared with plants in uniform 10 mM NaCl. Such
reduced stomatal conductance presumably also contributed to the water potential at midday, with heterogeneous salinity being similar to that of plants in uniform 10 mM NaCl. Interestingly in heterogeneous treatments, with \( \geq 500 \) mM NaCl in the high-salt side, despite midday shoot water potentials being similar to those in uniform 10 mM NaCl, plants took up water mostly from the low-salt side and, although the water potential gradient towards the high-salt side might have indicated the possibility of ‘reverse water flow’ towards the external high-salt solution, some water still was apparently absorbed from the high-salt side (albeit a small volume). Water uptake against an apparent water potential gradient has also been seen in wheat plants with root systems divided into solutions of different osmotic potentials, with one side exposed to -1.0 MPa polyethylene glycol (PEG) 4000 and the other side being without PEG (Lawlor, 1973). Furthermore, in an additional unpublished experiment with *A. nummularia* exposed to heterogeneous salinities with differences between high- and low-salt sides that might have enabled reverse water flow, it was not possible to detect any reverse water flow with deuterium label. Currently, it is unclear how plants maintain water uptake from the high-salt side, and this warrants further study.

Extreme heterogeneous salinity did not lead to toxic concentrations of Na\(^+\) and Cl\(^-\) in young fully expanded leaves. There was no significant difference in leaf Na\(^+\) and Cl\(^-\) concentrations between heterogeneous treatments in Expt 2; that is, even with 1500 mM NaCl on the high-salt side. This extends the findings of Bazihizina et al. (2009) where it was found that with 10 mM NaCl in the low-salt side, leaf Na\(^+\) and Cl\(^-\) concentrations remained relatively constant under heterogeneous treatments independently of the NaCl concentrations on the high-salt side. The results show that *A. nummularia* has a good regulatory mechanism for shoot ions, essential for salt tolerance in halophytes (Flowers and Colmer, 2008). Ion concentrations in leaves of plants in both heterogeneous saline treatments (i.e. 10/500 mM and 10/1500 mM NaCl) were, however, approximately double those in uniform 10 mM NaCl, but increases did not affect shoot growth, possibly as excess ions were likely to be sequestered in the leaf bladders (Mozaifar and Goodin, 1970; Aslam et al., 1986; Karimi and Ungar, 1986) and/or compartmentalized mainly in vacuoles, so that low concentrations of Na\(^+\) and Cl\(^-\) would probably have been maintained in the cytoplasm (Storey et al., 1983; Wyn Jones and Gorham, 2002; Flowers and Colmer, 2008). This tolerance of *A. nummularia* to high tissue ions may therefore indicate that the growth reductions observed in Expt 1, when NaCl in the low-salt side was raised under heterogeneous salinities to result in a mean root zone salinity exceeding the optimal range, were not likely to be caused by increases in tissue Na\(^+\) or Cl\(^-\), but perhaps were related to declines in water uptake from the low-salt side (Table 2).

Like most dicotyledonous halophytes, *A. nummularia* in the present study had a growth stimulation following the addition of NaCl, with maximal growth at 120–230 mM NaCl, and ~90% maximal growth in the 10–450 mM NaCl range. Therefore, plants maintained shoot growth under heterogeneous salinities if the ‘root-weighted mean’ salinity in the root zone was within this 10–450 mM NaCl range, even when salinity in one root half was at a concentration toxic to plants when applied in the uniform treatment. Given the intrinsic heterogeneity of saline landscapes, the preferential root growth and water uptake in least saline areas, combined with the high salinity tolerance of dicotyledonous halophytes, supports the hypothesis that these species can withstand large spatial variations in salinities in the root zone; these responses may be vital for the persistence of halophytic vegetation in heterogeneous saline landscapes that include areas with soil salinities above the known upper salinity tolerance limits from experiments in which salts were applied uniformly to the root zone.

### Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Use of different methods of expressing salinity in the root zone to explain responses to heterogeneous salinities of stomatal conductance of the halophyte *Atriplex nummularia*, expressed as % of the uniform 10 mM NaCl treatment.

Table S1. Response of leaf area, net photosynthetic rate (P\(_\text{n}\)), internal CO\(_2\) concentrations (C\(_i\)), total soluble sugars (expressed on a tissue water basis), and leaf Cl\(^-\) concentration of the halophyte *Atriplex nummularia* to uniform and heterogeneous NaCl concentrations in the root zone (Expt 1).

Table S2. Response of net photosynthetic rate (P\(_\text{n}\)), internal CO\(_2\) concentrations (C\(_i\)), total soluble sugars (expressed on a tissue water basis, measured in leaf tissues sampled on day 0 and 21), and leaf Cl\(^-\) concentration of the halophyte *Atriplex nummularia* to uniform and heterogeneous NaCl concentrations in the root zone (Expt 2).

Table S3. Mean and root-weighted mean salinities in the root zone for plants exposed to heterogeneous salinities in Expt 1 and 2 using a split-root system.

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### References


