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A Survey of the Effects of Nutrient Spatial Distribution under Heterogeneous Rootzone
Salinity in Tomato

By

DANTE LEVENTINI
THESIS

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DAVIS

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Beyond this experience, I carry the vivid memory of John Vigil, who supported and reassured me as I applied years ago, but who will not see either of us graduate. His memory and family continue to be a spring of love, inspiration, and gratitude.

Abstract

This thesis explores the broad physiological responses of tomato (*Solanum lycopersicum)* in solution culture to various spatial potassium (K) distributions under heterogeneous rootzone salinity (NaCl). Chapter 1 is a collaborative review of heterogeneous soil salinity, introducing how management and environment influence salt distribution patterns, and reviews ensuing physiological responses. The review also summarizes the limited research on interactions between heterogeneous salinity and nutrient distribution, particularly split-root experiments, a line of inquiry which this research seeks to enrich. Chapter 2 outlines an original experiment where tomato plants were grown in solution culture with roots evenly divided between two compartments. Except for a salt-free control group (Treatment 0), the same overall amount of salt (NaCl) was either provided to the plant uniformly across the entire root zone (treatment 1) or provided to only one half of the root system (treatments 2, 3, and 4). Treatments 2, 3, and 4 feature an increase in the share of the K budget which is supplemented in the saline compartment compared to the non-saline compartment. Treatment 2 provides nutrients including K to one side and NaCl to the other. Treatments 3 and 4 increase K in the saline compartment to 40% and 80% of the K budget, respectively. The impacts on biomass accumulation, biomass partitioning, water uptake, sodium uptake, and potassium uptake were measured and analyzed for statistical significance. There was no difference in the total biomass, overall water uptake rate, or root distribution between root halves across uniform treatment groups (0 and 1) despite a major difference in overall solution NaCl concentration (0 mM and 20 mM average, respectively). In all treatments where supplemented sodium (40 mM) was confined to half of the root zone (2, 3, and 4), plant water uptake was restricted almost completely to the non-saline compartment demonstrating a remarkable plasticity of root response to local saline conditions. Whole plant water uptake rates were generally comparable irrespective of saline distribution. Saline compartments of treatments 2, 3, and 4 did not show sodium or potassium uptake, regardless of potassium richness. Across all treatments, there was a strong tendency for water, and potassium uptake, as well as root growth, to occur in the Na-free compartment.

The only instance of plants utilizing solution K in the presence of NaCl occurred in treatment 1, where K was supplemented along with all other nutrients uniformly in an overall saline root environment. Interestingly, this treatment was also the only clear instance of sodium uptake to occur among any treatment groups.

The results of these experiments suggest a salt-avoidant response, whereby the presence of any salt-free and nutrient-rich root zone will result in preferential water uptake from that zone. Research (summarized in Chapter 1) also demonstrates that the provision of a full nutrient supply exclusively to the saline side of a split root system will result in considerable water uptake from the saline compartment and increase whole plant salt uptake. This research was conducted to determine which of the nutrients in the nutrient-rich zone was responsible for plant activity in the saline zone that may otherwise have been avoided. The research performed here demonstrates that the driving dynamic for this plant response is not K alone. The goals of minimizing the incidence of salinity stress and maximizing nutrient use efficiency are inextricable in the agronomic system. Understanding the relationship between nutrient use and salt localization is important if we are to optimize management systems under the heterogeneous ion distributions that are commonplace in irrigated agriculture.

Chapter 1: Collaborative Review of Heterogeneous Salinity Physiology and Research Perspectives

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INVITED REVIEW

Plant responses to heterogeneous salinity: agronomic relevance and research priorities

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• **Background** Soil salinity, in both natural and managed environments, is highly heterogeneous, and understanding how plants respond to this spatiotemporal heterogeneity is increasingly important for sustainable agriculture in the era of global climate change. While the vast majority of research on crop response to salinity utilizes homogeneous saline conditions, a much smaller, but important, effort has been made in the past decade to understand plant molecular and physiological responses to heterogeneous salinity mainly by using split-root studies. These studies have begun to unravel how plants compensate for water/nutrient deprivation and limit salt stress by optimizing root-foraging in the most favourable parts of the soil.
• **Scope** This paper provides an overview of the patterns of salinity heterogeneity in rain-fed and irrigated systems. We then discuss results from split-root studies and the recent progress in understanding the physiological and molecular mechanisms regulating plant responses to heterogeneous root-zone salinity and nutrient conditions. We focus on mechanisms by which plants (salt/nutrient sensing, root-shoot signalling and water uptake) could optimize the use of less-saline patches within the root-zone, thereby enhancing growth under heterogeneous soil salinity conditions. Finally, we place these findings in the context of defining future research priorities, possible irrigation management and crop breeding opportunities to improve productivity from salt-affected lands.

Key words: Irrigation, nutrient heterogeneity, phytohormones, root foraging, root-to-shoot signalling, salt sensing, stomatal conductance, water uptake.

INTRODUCTION

The world's population is expected to increase to 9.9 billion by 2050 (Liu *et al.*, 2020*a*), prompting a need to double annual food production within the next 30 years (Razzaq *et al.*, 2021). To achieve this production goal, agriculture will inevitably expand further into marginal lands (Pancaldi and Trindade, 2020; Ahmadzai *et al.*, 2021; Khanna *et al.*, 2021; Razzaq *et al.*, 2021), which often suffer from poor soil structure and low fertility (Mantovani *et al.*, 2015; Shukla *et al.*, 2017). Many of the marginal areas are also affected by soil salinity.

Soil salinity, both naturally occurring (i.e. primary salinization) and as a consequence of human activities (i.e. secondary salinization), is a threat to agriculture and a major limitation to food production. The salinization of agricultural land commonly occurs as a result of using irrigation water containing elevated levels of ions (e.g. Na^+ and Cl^-) without adequate, periodic leaching of the accumulated salts from the soil. Secondary dryland salinization can also occur in non-irrigated areas due to changes in the hydrological balance of a landscape and rising water tables (e.g. as a result of replacing deep-rooted vegetation with annual crop and pasture species, which results in a higher proportion of incoming rain entering the groundwater; Pannell and Ewing, 2006; McFarlane *et al.*, 2016). The problem is often exacerbated by decreases in soil permeability caused by sodicity (i.e. when the accumulation of Na^+ exceeds that of other cations) and over-exploitation of groundwater, which exhaust high-quality water resources, resulting in water extraction from less favourable groundwater that may be brackish or saline (Ruto *et al.*, 2021). Increases in salinity can also be expected in low-lying coastal areas associated with sea-level rise

due to climate change and salinization of groundwater due to salt water intrusion into depleted aquifers (Vellinga and Barrett-Lennard, 2021).

While the vast majority of research on crop responses to salinity has been conducted under homogeneous saline conditions, root-zones of plants in both natural and managed environments can commonly experience spatial and temporal heterogeneity in soil salinity (Yakir and Yechieli, 1995; Bleby *et al.*, 1997; Davidson *et al.*, 1996; Silvestri *et al.*, 2005; Xing *et al.*, 2019; Zhang *et al.*, 2019). The nature of soils and irrigation practice, crop type and phenology, climate-type and seasonal weather, and the duration of crop exposure, together determine the extent and impact of salinity and its heterogeneity on plant growth and crop productivity (Bazihizina *et al.*, 2012*a*; Northey *et al.*, 2006; Bogunovic *et al.*, 2017; Xing *et al.*, 2019; Xu *et al.*, 2019). Despite this complexity, most experiments on the impacts of salinity on plants have imposed homogeneous root-zone salinity, which does not represent saline agricultural settings. The extent of the temporal heterogeneity in soil salinity in the field is illustrated within an irrigated wheat trial in China (Fig. 1A) and a rain-fed wheat trial in Western Australia (Fig. 1B). These patterns of saline heterogeneity can be contrasted with the near homogeneous conditions commonly imposed in controlled-environment research trials (Fig. 1C). Since complex multi-faceted traits (developmental, physiological, anatomical, morphological and biochemical) are involved in plant tolerance of salinity, this raises the question of whether trials conducted under near-uniform soil (or root-zone) salinity are indeed optimal for identifying and selecting traits of most value to increasing plant tolerance to the common reality of heterogeneous salinity.

The generic guidelines used to predict crop response to soil and water salinity, produced under near homogeneous conditions, are generally described by crop yield curves that consist of a threshold value at which salinity induced damage first occurs, and a linear percentage yield reduction with every increment in the electrical conductivity of the saturated soil extracts (EC_e) thereafter (Maas and Hoffman, 1977; Maas and Grattan, 1999). Such static diagnostic criteria do not reflect soil salinity under realistic field conditions that are highly spatially and temporally heterogeneous (Chen *et al.*, 2019). Thus, the prevailing standard of describing plant response to salinity is both inadequate and generally overestimates crop response (Tanji & Kielen, 2002), is not relevant nor easily interpreted under field reality, and may not adequately inform irrigation practice, crop selection or salinity mitigation strategies (Ayars, 2012). In the following, we summarize the pattern of occurrence of salinity heterogeneity in rain-fed and irrigated systems, and discuss results from studies of plant responses to heterogeneous root-zone salinity. We then discuss the mechanistic understanding of root physiological and morphological adaptations to heterogeneous conditions, and place these findings in the context of defining future research priorities and possible management and crop breeding opportunities to improve productivity in saline lands.

SOIL SALINITY HETEROGENEITY

In naturally saline environments, within the rooting zone of a single plant, non-saline patches can coexist with nearby saline ones, ranging from a few millimolar to several times seawater (Bazihizina *et al.*, 2012*a*). The magnitude of this heterogeneity varies in time and space depending upon soil parent material, landscape position (Aldabaa *et al.*, 2015), soil physical and chemical characteristics (e.g. texture, sodicity and alkalinity; Hillel, 1980; Robbins *et al.*, 1980), surface runoff and subsurface lateral flow of water, intrusion by saline groundwaters or seawater (Tiggeloven *et al.*, 2020; Choukr-Allah, 2021), and root water extraction (Heuperman, 1995; Barrett-Lennard and Malcolm, 2000; Alharby *et al*., 2014, 2018). Climatic conditions also affect the temporal and spatial heterogeneity of soil salinity, with rainfall leading to soil leaching events while droughts and heatwaves concentrate solutes depending on soil features and topography, and differences in radiation and resulting evaporation due to aspect and slope (Schwantes *et al.*, 2018).

Irrigation also dramatically influences soil salinity (Figs 2 and 3). Irrigation-induced heterogeneity can commonly result in differences in soil EC_e greater than 10-fold (Bernstein *et al.*, 1955; Bernstein and Fireman, 1957; Bernstein, 1975). An extremely heterogeneous distribution of salinity in irrigated systems makes it difficult to design a soil sampling regime to determine the truly effective root-zone salinity (Bernstein *et al.*, 1955). This effect is highly relevant to modern drip and micro-irrigated agriculture (see Box 1 for definition of terms) in arid regions (Fig. 2), which are the most common irrigation strategies in many areas of the world. While micro-irrigation is generally considered a valuable way to improve water use efficiency and allows controlled fertigation strategies, these systems may complicate salinity management, generating highly non-uniform salt and disparate nutrient deposition patterns below the irrigation emitter (Bar-Yosef, 1999). These salt/nutrient deposition patterns below the micro-irrigation emitter directly impact root growth, root activity, and nutrient and salt movement in the soil within the root zone, with effects strongly determined by crop placement, soil preparation, irrigation design and management.

Bar-Yosef (1999) further discussed the risk of salt accumulation in the root-zone under drip irrigation, suggesting that salts are not efficiently displaced to the periphery of the wetted soil volume as might occur under a full surface irrigation system. Under drip irrigation, salts can accumulate in the wetting front after several irrigation cycles but this wetting front will shrink and swell with subsequent irrigation events and root water consumption. Varying the frequency and volumes of irrigation events can manipulate this salt displacement and represents a management strategy. Salts can also accumulate at the upper margin of the wetted soil volume (close to the soil surface) due to capillarity driven by soil evaporation. This effect is particularly marked with buried drip irrigation systems where the depth of irrigation tubes, shape of the furrow, plant and environmental water use patterns (Fig. 3) influence the ultimate salt distribution.

Based upon current understanding, irrigation system placement and operation could theoretically be managed to ensure that the deposition of salinity is largely restricted to the outer margins of the wetted root-zone, thereby providing a zone of lower inner salinity with abundant plant root activity. A better understanding of soil processes and plant responses under heterogeneous conditions may therefore allow us to mitigate the adverse effects of salinity (Lycoskoufis *et al.*, 2005). Although
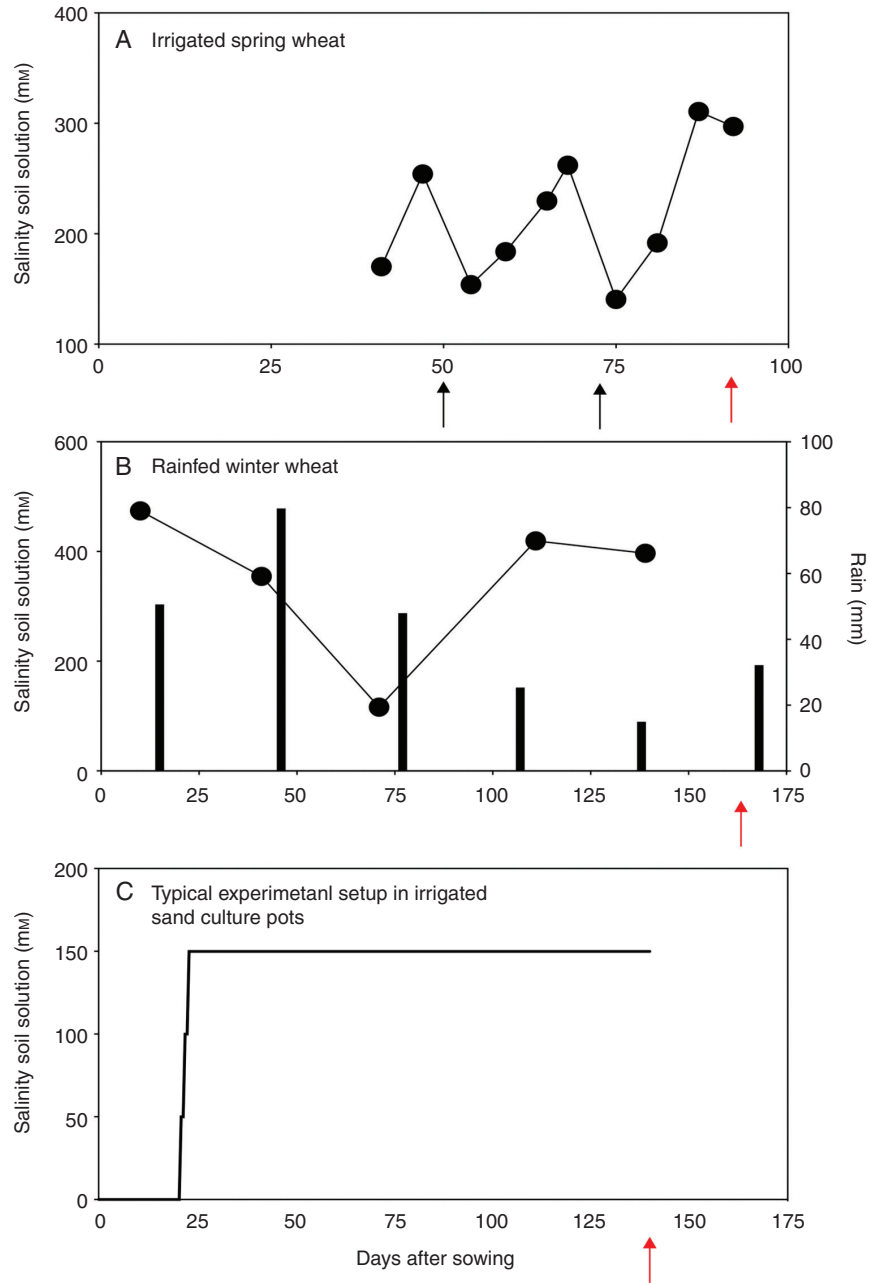
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FIG. 1. Temporal variations in soil salinity measured in irrigated and rain-fed wheat in saline land compared with the typical experimental setup used to assess salt tolerance. In (A) the crop was irrigated with water diverted from the Yellow River that had an average electrical conductivity of 0.75 ds m⁻¹. Crops were planted in a field with shallow saline groundwater. The electrical conductivity (EC) and depth of the groundwater varied from 0.5 to 3 ds m⁻¹ and 80 to 200 cm, respectively. Irrigation events are indicated with black arrows. Red arrows indicate the crop harvest (date of crop harvest is assumed based on the maturation days generally required for spring wheat). Data modified from Xu *et al.* (2013). In (B) field trials to evaluate the salinity tolerance of wheat accessions under rain-fed conditions were conducted on saline sites in Western Australia. Data modified from Setter *et al.* (2016). (C) Diagram showing a typical experimental protocol used to assess salt tolerance in irrigated sand culture in pots, where the salinity of the soil solution is increased gradually to achieve the desired concentration, which then remains constant throughout the experimental period (Hussain *et al.*, 2021). After an initial trial in hydroponics to evaluate salt tolerance at the seedlings stage, the protocol shown in (C) was used to screen the salt tolerance in different wheat germplasm grown in sand irrigated with a saline Hoagland solution. Depending on the irrigation schedule (not indicated), the salinity of the soil solution is expected to have varied depending on the evapo-transpiration and the decline in water content in the pots.

very few field experiments have tested this theory that drip irrigation can be optimized to minimize the impacts of salinity, several studies of split-root plants showed greater plant growth under heterogeneous salinities than uniform salinity, at the same average root-zone salinity (Sonneveld and Voogt, 1990; Zekri and Parsons, 1990; Flores *et al.*, 2002; Mulholland *et al.*, 2002; Tabatabaei *et al.*, 2004; Attia *et al.*, 2009; Kong *et al.*, 2012; Sun *et al.*, 2016; Zhang *et al.*, 2019). This suggests that
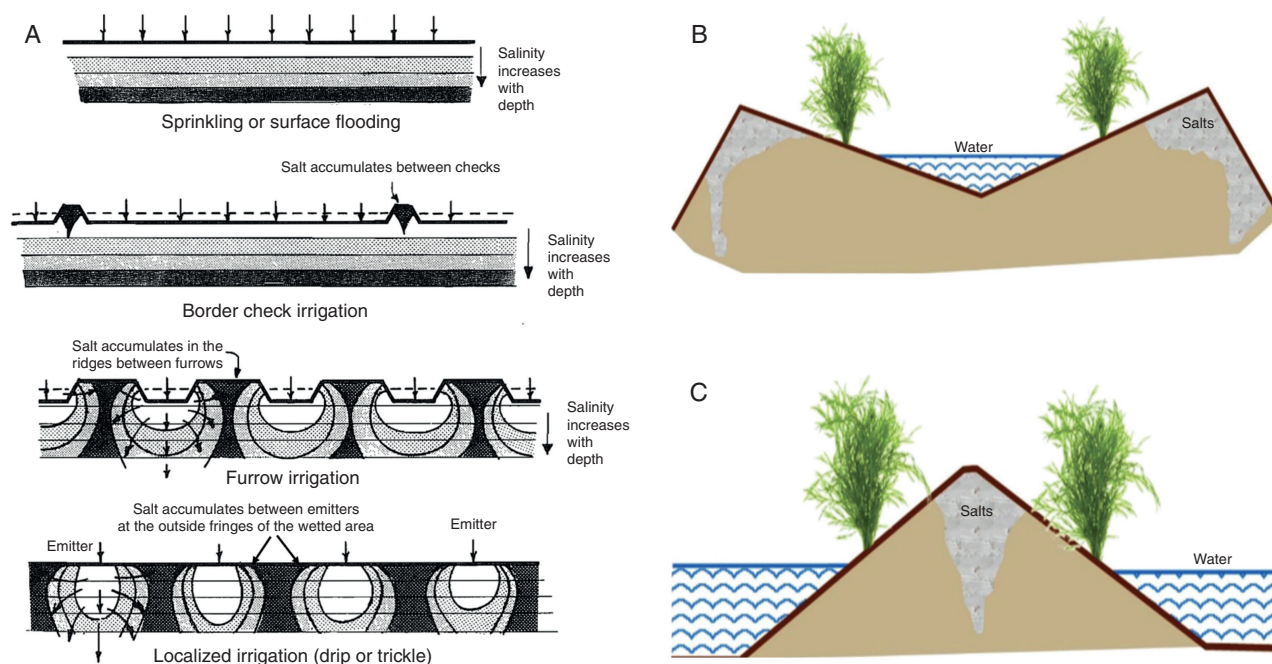
FIG. 2. Irrigation induced heterogeneity in root-zone salinity. (A) Typical salt accumulation patterns in surface soils for various methods of water application. Salinity ranges from low (unshaded) to high (darkened). Arrows indicate the direction of soil water flow. Reproduced with permission from Ayers and Westcot (1985). (B, C) Examples of sloping bed irrigation style and their impact on localized salt deposition around sloped furrow irrigation. Reproduced with permission from Zaman *et al.* (2018).

further developing these irrigation strategies should be fruitful. Understanding the nature of plant responses to heterogeneous salinity is therefore essential to develop and implement improved irrigation practices for saline systems. In particular, this opens an excellent opportunity to improve production by manipulating the heterogeneity in the salinity of the soil solution, thereby harnessing the abilities of plants to make optimum use of less-saline patches within root-zones.

ROOT RESPONSES TO HETEROGENEOUS SOILS

Under naturally occurring and agriculturally induced salinity, plant growth is affected by the salinity of the soil solution or the ratio of salt (of which the EC_e or $EC_{1:5}$ are measures) and the water content of the soil. Soil salinities vary on spatial scales of micrometres to metres, and on temporal scales ranging from seconds to seasonal changes (Bazihizina *et al.*, 2012a; Rellán-Álvarez *et al.*, 2016; Dinneny, 2019). Thus, roots of a single plant will be exposed to a range of soil water salinity levels that vary temporally and spatially, with differential effects depending upon the stage of plant growth. Nevertheless, while heterogeneous salinities typically occur in salt-affected soils, experiments have almost exclusively imposed homogeneous salinity or highly manipulated experimental conditions such as split-root systems, which expose a portion of a root system to salinity while the remainder receives non-saline conditions. Although split-root experiments may not adequately mimic a complex field condition, these have provided valuable insights by demonstrating how plant responses to heterogeneous conditions differ markedly from those of homogeneous saline conditions.

Split-root experiments indicate a more nuanced plant response to saline environments than commonly recognized. As summarized in Bazihizina *et al.* (2012a), key features of plants exposed to heterogeneous salinities are: (1) shoot water potentials are determined by the salinity level of the low-salinity zone, (2) water uptake occurs predominantly from the low-salinity medium and (3) greater maintenance of shoot growth even when a large proportion of the root system is exposed to high NaCl concentrations that would greatly inhibit growth if applied uniformly to the roots. Transcriptome profiling of plants exposed for 6–9 h to heterogeneous salinities indicated that improved performance under heterogeneous conditions compared to uniform salinities is related to the rapid activation of salt resistance genes and crosstalk between the non-saline and high-saline root sides (Kong *et al.*, 2016; Xiong *et al.*, 2018; Zhang *et al.*, 2019). This suggests that roots operate as the central hub that control: (1) how stress is perceived, (2) long-distance communication with the shoots and (3) the integration of long-distance systemic signals with local root-based ones. Furthermore, salinity heterogeneity is inevitably linked to temporal and spatial variation in the distribution and biological availability of water, essential nutrients and soil pH (Li *et al.* 2011; Feng *et al.*, 2018; Zhang *et al.*, 2019), with the latter having a major impact on root membrane potential, thus affecting both a plant's ability to acquire essential nutrients and exclude toxic Na^+ and Cl^- ions (Babourina *et al.*, 2001; Zhang *et al.*, 2018). Thus, responses at the root level also play a critical role in: (4) how plants compensate for water/nutrient deprivation and limit salt stress by optimizing root-foraging in the most favourable part of the soil. Understanding how roots respond to heterogeneous salinities is therefore of utmost importance and is needed to develop
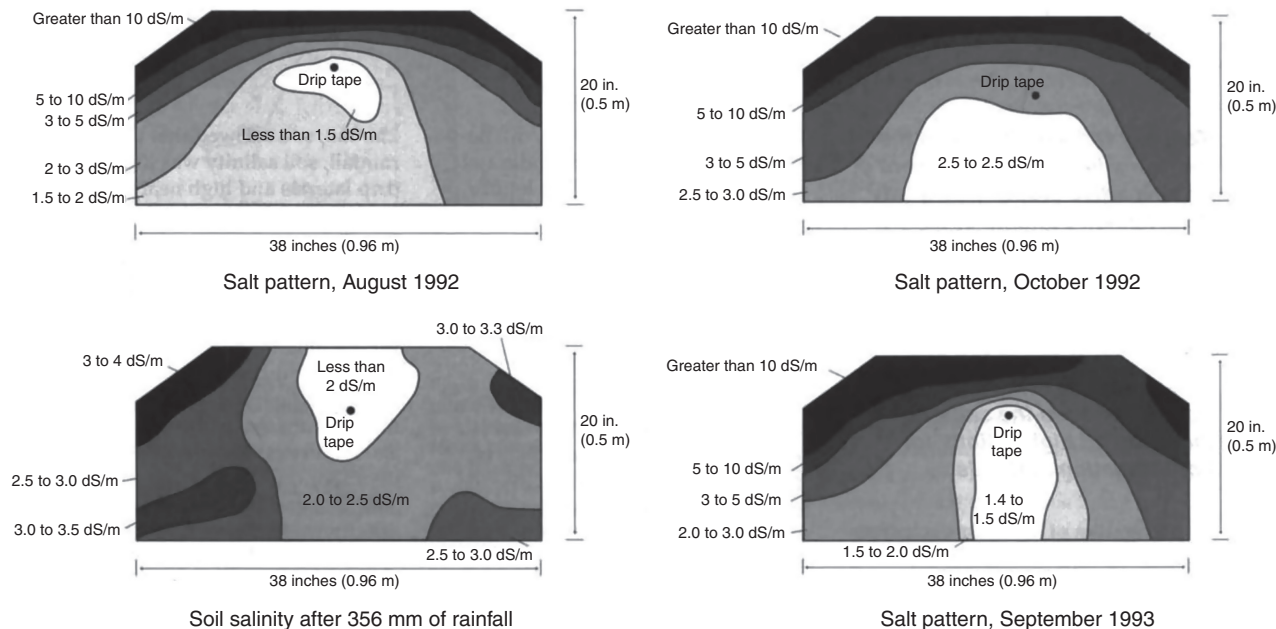
FIG. 3. Depiction of salt accumulation patterns from subsurface irrigation in a lettuce crop in the Santa Maria Valley (California, USA). While a low EC zone is formed beneath the emitter, salts can accumulate above and require rain or other surface irrigation to percolate salts beneath the emitter for leaching. Soil types were a clay loam and a fine sand. Reproduced with permission from Hanson and Bendixen (1995).

Box 1. Irrigation system terminology

Term explanation

Surface Irrigation

All formats of irrigation which rely on gravity (rather than pressurized conveyance systems) to distribute water across a field. Examples include flood and furrow irrigation. It is important to note that while these systems are collectively referred to as surface irrigation, they are not the only irrigation systems which apply water to the soil surface; overhead sprinkler, micro-sprinkler and surface drip also irrigate the soil surface but are not generally categorized as traditional 'surface irrigation'.

Drip

Highly local, small volumes of water emanating from points along a pressurized plastic pipe ('drip line') which is installed either on the soil surface (surface drip) or buried beneath the crop ('subsurface drip'/'subirrigation") with very little loss to evaporation.

Micro-sprinkler

Micro-irrigation

Any small, stationary sprinklers which are installed near the soil's surface and irrigate the soil's surface without applying water to the canopy. Micro-sprinkler irrigation is more targeted than overhead sprinkler irrigation, particularly with orchard crops which are widely spaced and have high canopies which can lose large fractions of overhead irrigated water to evaporation.

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management strategies to optimize resource use and crop productivity in saline soils. Different processes enable roots to integrate fluctuating soil conditions into appropriate developmental and physiological responses that ultimately determine how efficiently resources are captured. These are fundamentally controlled at variable spatial scales, from the single cell to the entire organ.

Salinity sensing

Local patches of high salinity are sensed in individual cells, and then integrated into organ-scale processes. After salinity increases, plants experience multiple constraints ranging from reduced water availability, disturbance to cytosolic ion homeostasis, and dramatic increases in reactive oxygen species (ROS) accumulation. The emerging picture suggests that more than one sensory mechanism may operate in the same cell at the same time, with some common downstream signalling pathway(s) (Shabala *et al.*, 2015; Feng *et al.*, 2018; Niu *et al.*, 2018; Fichman and Mittler, 2020, 2021; Peck and Mittler, 2020).

Calcium and ROS signals are amongst the first signals commonly evoked upon biotic and abiotic stressors. Ca^{2+} and ROS signals are established second messengers involved in most (local) stress responses, and increasing evidence suggests that these act in tandem, interacting and amplifying each other during root salt sensing (Dodd *et al.*, 2010; Choi *et al.*, 2014; Shabala *et al.*, 2015; Evans *et al.*, 2016; Niu *et al.*, 2018; Pottosin and Zepeda-Jazo, 2018). Several molecular components underlying Ca^{2+} and ROS signalling (including *MOCA1*, *OSCA1* and *RBOHs*) have been identified and are currently being considered as potential salt sensors (Yuan *et al.*, 2014; Jiang *et al.*, 2019; Liu *et al.*, 2020b). Interestingly, local salt stress at the root apex triggers immediate cytosolic Ca^{2+} increases at the point of application, leading to propagation of

a *TPC1*- ('two-pore channel 1') dependent Ca^{2+} wave to distal shoot tissues, passing through cortical and endodermal cell layers (Choi *et al.*, 2014). By combining experimental analyses and mathematical modelling, Evans *et al.* (2016) also clearly linked the $[Ca^{2+}]_{cyt}$ wave triggered by a localized salt application with systemic ROS waves. Additional salt sensors (extensively reviewed in Shabala *et al.*, 2015, 2016; Byrt *et al.*, 2018; Rui and Dinneny, 2020; Gigli-Bisceglia *et al.*, 2020) include: (1) cell walls, and in particular the salt-induced alterations in cell wall integrity and composition that are sensed by the receptor-like kinase, FERONIA (*FER*) (Feng *et al.*, 2018); (2) mechanosensory channels and transporters (e.g. *OSCA1*, *MSLs*, *MCAs*) that sense the mechanical force exerted on the plasma membrane due to the osmotic component of salinity and translate hydraulic cues into chemical signals (Yuan *et al.*, 2014; Yoshimura *et al.*, 2021); and (3) Na^+ transport systems and proteins with regulatory Na^+ binding sites (e.g. *MOCA1*; Jiang *et al.*, 2019).

While it is becoming increasingly clear that plant cells sense and respond to salinity stress by activating multiple sensing networks, much of our knowledge on root salt sensing and signalling has utilized uniform conditions, with no such studies attempted for heterogeneous salinities. Such experiments will generate valuable information on how salt sensing at the single cell level is integrated into organ-scale processes, revealing how the signal propagates and its effects on root system architecture, developmental trade-offs and root plasticity.

the non-saline areas was associated with increased lateral root growth, which doubled compared to plants with both root halves in non-saline conditions (Feng *et al.*, 2017). However, root proliferation in the non-saline compartment does not always occur, with several studies showing no differences, or even a decline, in root growth compared to measurements under uniform non-saline conditions (see references in Table 1). This highlights the complexity of interpreting how heterogeneous conditions alter root growth, because responses depend on timescale, salt concentration and species sensitivity to salinity.

To understand root foraging it will be necessary to determine whether heterogeneous salinities (and the associated variability in water availability and nutrient distribution) affect root anatomical features, in addition to any effects on root morphology. The section below considers suberin deposition in root cell walls. Even when heterogeneous salinities do not alter root architecture, it remains possible that traits that reduce the metabolic cost of soil exploration, such as cortical cell enlargement and cortical senescence, could be beneficial. This has yet to be tested. Nevertheless, the anatomical traits that reduce the metabolic cost of root soil exploration are currently considered an advantage in water-, nitrogen- and O_2-limited soils as these improve water and nutrient uptake per unit investment in roots (Lynch, 2018, 2019; Schneider and Lynch, 2020; Colombi *et al.*, 2022). This topic therefore merits greater research efforts to identify key root traits that maximize soil resource capture under heterogeneous salinity.

Root foraging

Scaling up to the whole root level, the root system is a highly dynamic physical network that enables a plant to forage for resources and rapidly explore favourable soil patches. Under spatially heterogeneous soil salinities, preferential root growth can occur in the least (or non-) saline compartment, compensating to different degrees for root growth inhibition in the saline patches (Bazihizina *et al.*, 2009, 2012*b*; Feng *et al.*, 2017; Sun *et al.*, 2016; Xiong *et al.*, 2018; Table 1, Fig. 4). A split-root experiment that closely examined root morphology under heterogeneous salinities revealed that compensatory root growth in

Water uptake

Irrespective of environmental heterogeneity within the root-zone, plant water uptake is essential to maintain photosynthesis. Typically, water uptake from the non-saline side of the root system increases significantly, which is not always accompanied by increased root biomass (Fig. 4A). Roots can dynamically alter their water transport capacity to acclimate to the ever-changing soil conditions and rapidly explore favourable soil patches. Under heterogeneous salinity, preferential water uptake from the regions with the least negative water potentials are mediated by changes in root hydraulic conductivity that

TABLE 1. *Root distribution in different species under horizontally heterogeneous salinities as percentage of dry mass in control plants with low or no-salt media*

| Species	Salinity (mM NaCl)	Duration	Root biomass (% control)		Ratio L : H	Reference
			L	H		
Atriplex nummularia	10/500	3 weeks	88	87	1.0	Bazihizina *et al.* (2012b)
Atriplex nummularia	10/1500	3 weeks	153	32	4.8	Bazihizina *et al.* (2012b)
Hibiscus moscheutos	0/200	2 months	72	57	1.3	Feng *et al.* (2021)
Hibiscus moscheutos	0/200	2 months	79	20	4.0	Feng *et al.* (2021)
Medicago sativa	0/200	15 d	140	56	2.5	Xiong *et al.* (2018)
Medicago sativa	50/200	15 d	131	61	2.1	Xiong *et al.* (2018)
Medicago sativa	0/75	9 d	99	90	1.1	Sun *et al.* (2016)
Medicago sativa	0/150	9 d	113	82	1.4	Sun *et al.* (2016)
Medicago sativa	0/225	9 d	118	56	2.1	Sun *et al.* (2016)
Medicago sativa	75/150	9 d	82	60	1.4	Sun *et al.* (2016)
Medicago sativa	75/250	9 d	72	55	1.3	Sun *et al.* (2016)
Sorghum bicolor	0/200	2 weeks	100	49	2.0	Zhang *et al.* (2019)
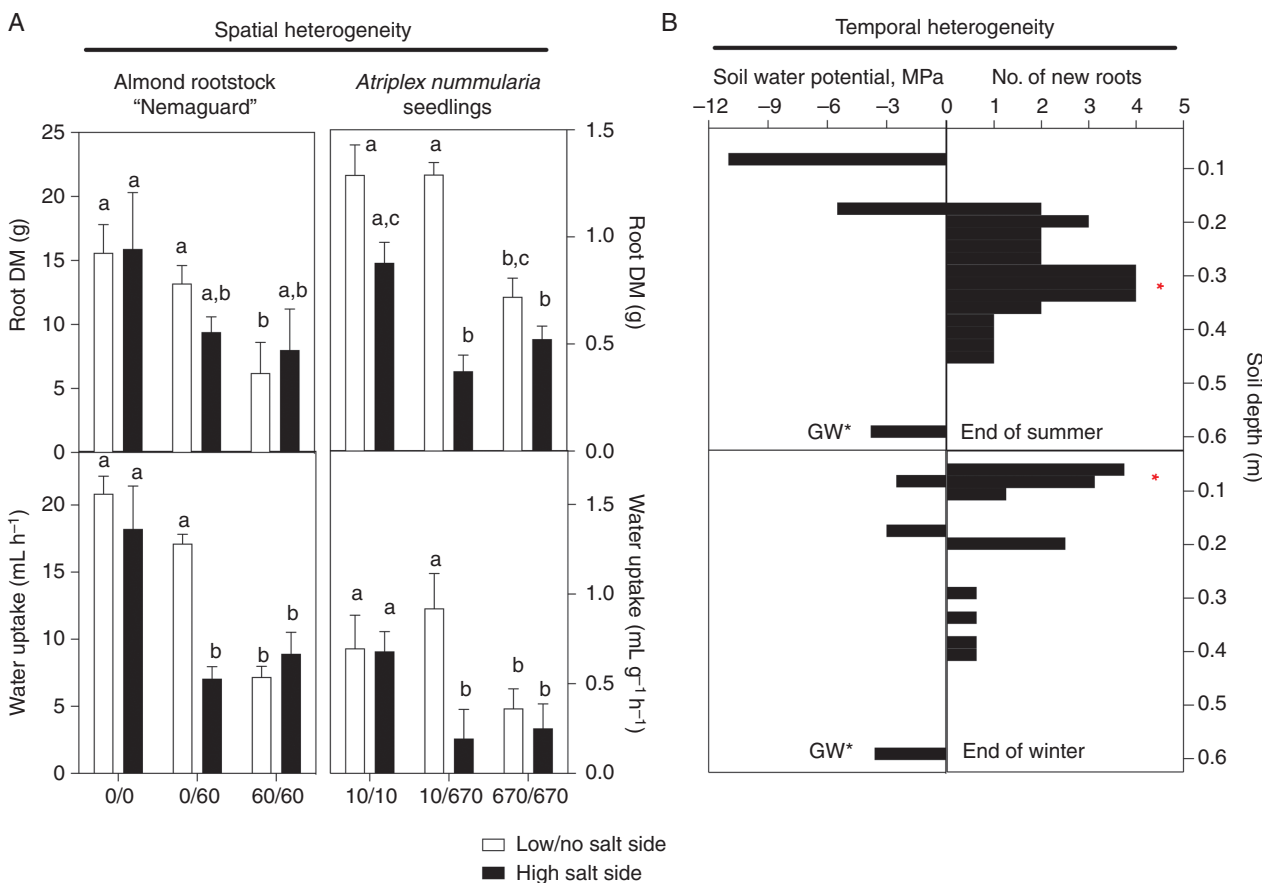
FIG. 4. Taking advantage of the patch. Plants use the most accessible water source under spatially and temporally variable salinity. The datasets in (A) show water uptake and root growth in the non-halophytic almond rootstock 'Nemaguard' (*Prunus persica* × *P. davidiana*) and the halophyte *Atriplex nummularia*, after 28 and 21 d of heterogeneous salinity treatment respectively. The data shown for almond rootstock and *Atriplex nummularia* are modified from Valenzuela (2018) and Bazihizina *et al.* (2009), respectively. Values are mean ($n = 4$) ± SE and different letters indicate a significant difference ($P < 0.05$) between treatments and root sides. The dataset in (B) shows temporal changes in soil water potentials and number of new roots in *Melaleuca halmaturorum* in response to fluctuating saline groundwater. The data shown in the upper two panels refer to soil water potentials and new roots observed at the end of summer, while those shown in the bottom two panels refer to soil water potentials and new roots at the end of winter. The red asterisks indicate the location of main water uptake (based on the uptake of stable isotope data). The figure is modified from Mensforth and Walker (1996).

occur within hours of salt exposure (Kong *et al.*, 2016). These are achieved through changes in the abundance or activity of water channel proteins named aquaporins that facilitate water diffusion across cell membranes (Maurel *et al.*, 2008; Gambetta *et al.*, 2017; Kong *et al.*, 2017; Maurel and Nacry, 2020). The activity of aquaporins is regulated at many levels, including altered transcription levels, channel gating between an open/closed state by various mechanisms including phosphorylation, pH or Ca^{2+}, and changed cellular trafficking (Maurel *et al.*, 2008; Gambetta *et al.*, 2017; Maurel and Nacry, 2020). Under heterogeneous conditions, increased water uptake from the non-saline roots has largely been attributed to changes in aquaporin expression levels (Kong *et al.*, 2017). After applying 200 mM NaCl to one root half in split-root cotton seedlings (with 0 mM NaCl to the other half), gene expression profiling revealed several aquaporin genes were up-regulated within 3 h in the non-salinized root half, resulting in 16 % higher root hydraulic conductivity when measured against NaCl-free controls (Kong *et al.*, 2017). By contrast, both root hydraulic conductivity and most of the differentially expressed aquaporin genes

were largely inhibited in the high-salinity side (Kong *et al.*, 2017).

The deposition of hydrophobic lignin and suberin in the cell walls of the exo- and endodermis also alters root hydraulic conductivity and restricts the free diffusion of solutes and water, including restricting entry of Na^+ and Cl^- from the soil into the vascular stream with high root-zone salinity (Krishnamurthy *et al.*, 2011; Barberon *et al.*, 2016; Wang *et al.*, 2019; Cui *et al.*, 2021). Accordingly, dynamic regulation of root hydraulic conductivity under heterogeneous salinities was also associated with altered expression of genes associated with cutin, suberin and wax biosynthesis in the salinized root portions (Xiong *et al.*, 2020). This could potentially explain decreased endodermal and exodermal permeabilities, which limit water and solute transport from the highly saline areas.

The ability of plants to acquire and transport water from the roots to the leaves also depends on root anatomy and architecture, and the combined hydraulic conductivities among root types and along the root length (Meunier *et al.*, 2017; Ahmed *et al.*, 2018). Thus, over the longer term (days), increases in new

root growth and altered root architecture (i.e. root proliferation and increased lateral root formation) and anatomy may have a more significant effect than localized changes in root hydraulic conductivity at the single root level. Nevertheless, our understanding of the timescale and concentration-dependent drivers of the long- and short-term responses of roots to localized salinity is inadequate. As highlighted in the following sections and above, responses are expected to become increasingly complex when heterogeneous salinity interacts with other environmental factors, such as heterogeneous nutrients as discussed below, and their impacts on plant nutrient and water acquisition.

Phytohormone root–shoot communication

Heterogeneous salinity can induce variable degrees of stomatal closure, with stomatal conductance similar to uniform salinity in some studies (Lycoskoufis *et al.*, 2005; Fig. 5). However, most split-root studies indicate greater plant water use under heterogeneous than uniform salinity, at the same average root-zone salinity. This is mostly because plant water uptake from the non-salinized part of the root system substantially increases, even exceeding water uptake from roots of non-salinized plants (see section above). Long-distance signalling *in planta* is implicated in regulating these plant water relationships under heterogeneous salinity by modulating root hydraulic conductivity and stomatal conductance.

Although leaf water status is regarded as an important regulator of stomatal responses (Christmann *et al.*, 2007), it is generally determined by the non-salinized part of the root-zone under heterogeneous salinities (Bazihizina *et al.*, 2009, 2012*a*, b; Feng *et al.*, 2021). Considerable stomatal closure of these plants (Fig. 5) suggests non-hydraulic mechanisms of stomatal closure. Homogeneous salinity induced multiple phytohormonal changes in salinized roots, according to the duration of exposure, with phytohormones such as abscisic acid (ABA), auxin and cytokinins (Albacete *et al.*, 2008), and their crosstalk, mediating the balance between growth and salinity stress responses (Yu *et al.*, 2020). The same applies to heterogeneous salinities. In cotton grown with heterogeneous salinity (0/200 mM NaCl), 200 mM NaCl induced only transient (within 3–12 h of treatment) increases in root ABA concentration. Root ABA levels were similar to controls after 24 h, presumably as sustained up-regulation of ABA catabolism (*CYP707A*) genes influenced root ABA concentrations more than concurrent up-regulation of ABA biosynthesis (*NCED*) genes (Kong *et al.*, 2016). Paradoxically, root ABA concentrations of the non-salinized roots exceeded those of salinized roots throughout the experiment, despite a limited and transient (3–6 h) up-regulation of (*NCED*) genes, implying considerable ABA transport into these non-salinized roots. Further studies are needed to elucidate the source of this additional ABA, since the shoot can regulate root ABA concentration (Manzi *et al.*, 2015; McAdam *et al.*, 2016), which in turn upregulates root hydraulic conductance (Thompson *et al.*, 2007).

Heterogeneous salinity also altered the concentrations of other phytohormones in the non-salinized portion of split-root cotton plants: with indole acetic acid, isopentenyladenine and
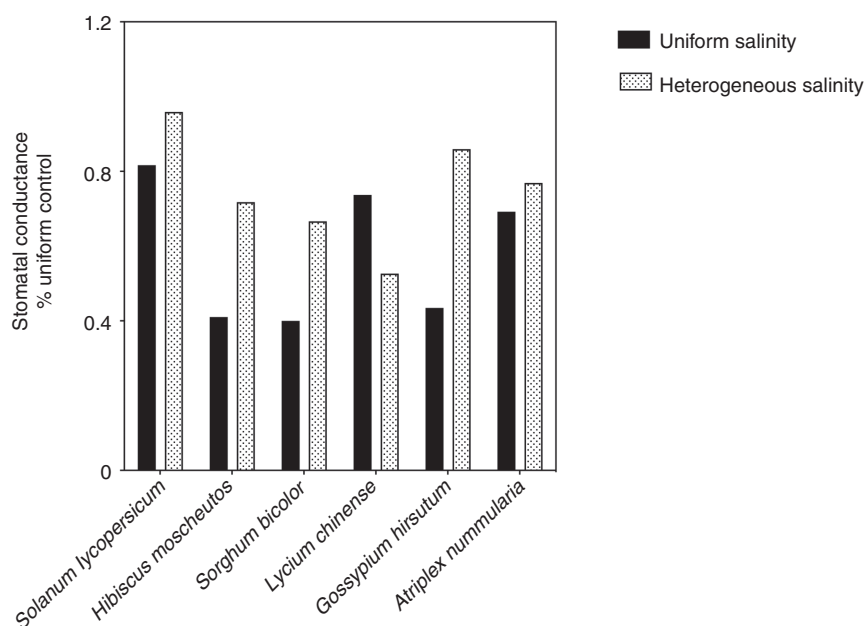

FIG. 5. Stomatal conductance of salinized plants (expressed as a percentage of non-salinized controls) exposed to heterogeneous (hollow symbols) and homogeneous (filled symbols) salinity at the same average root-zone salinity. Original papers were from: *Solanum lycopersicum* (Wang *et al.*, 2021), with plants grown with uniform (0 and 50 mM NaCl) and heterogeneous salinity (17/85 mM NaCl); *Hibiscus moscheutos* (Feng *et al.*, 2021), with plants grown with uniform (0 and 200 mM NaCl) and heterogeneous salinity (0/400 mM NaCl); *Sorghum bicolor* (Zhang *et al.*, 2019), with plants grown with uniform (0 and 100 mM NaCl) and heterogeneous salinity (0/200 mM NaCl); *Lycium chinense* (Feng *et al.*, 2017), with plants grown with uniform (0 and 170 mM NaCl) and heterogeneous salinity (0/340 mM NaCl); *Gossypium hirsutum* (Kong *et al.* 2012), with plants grown with uniform (0 and 100 mM NaCl) and heterogeneous salinity (0/200 mM NaCl); and *Atriplex nummularia* (Bazihizina *et al.*, 2009), with plants grown with uniform (10 and 230 mM NaCl) and heterogeneous salinity (10/450 mM NaCl).

zeatin riboside concentrations increasing compared to their concentrations in plants that were not exposed to salinity (Kong *et al.*, 2016). In this case, increased root cytokinin concentrations correlated with increased expression of *IPT* genes, which were maximal 3 h after salinizing the other part of the root system. Measuring root water potential in a transpiring plant (Adeoye and Rawlins, 1981) may help determine whether this was a transient response to altered root water relationships in the non-salinized roots. Such measurements (along with root gene expression) are required in girdled and non-girdled plants (since girdling at the root–shoot junction blocks phloem transport to the roots) to determine whether local root water relationships and/or a cumulative message from other parts of the plant regulate gene expression. Under heterogeneous salinity, Na^+ accumulation in the non-salinized portion of the root system doubled compared to roots from non-salinized controls. Such Na^+ accumulation depended on phloem transport from the salinized roots, as girdling prevented Na^+ transport to these roots (Kong *et al.*, 2012). Whether girdling eliminates changes in root phytohormone concentration in non-salinized roots, when the other part of the root system is exposed to salinity, needs to be addressed.

Irrespective of whether changes in root phytohormone concentration occur, it is uncertain whether they actually affect shoot phytohormone concentrations and physiological responses, since root-to-shoot signalling under heterogeneous soil conditions depends on relative sap flow from different parts of the root system (Dodd *et al.*, 2008). Under heterogeneous salinity, changes in root phytohormone concentration in the salinized root system may have little impact on shoot physiology since these roots contribute relatively little to total transpirational flow (Kong *et al.*, 2012). Interestingly, changes in root phytohormone concentration in the non-salinized roots may have a greater influence on shoot physiology, since these roots contribute most of the total water flux. Grafting techniques allow the relative contribution of different parts of the root system to root phytohormone export to be evaluated (Dodd *et al.*, 2008), but to date this has only been attempted in plants exposed to different soil moisture levels and such experiments should be applied to plants with heterogeneous root-zone salinity.

HETEROGENEOUS SALINITY AND NUTRIENT DISTRIBUTION: THE MISSING LINK?

In both natural and managed systems, a variable distribution of nutrients (and salinity) in soils is inevitable. This is associated with differential ion mobility and solubility, localized decomposition of organic matter, or applying soluble nutrients through a fertigation system. Furthermore, considerable evidence suggests that nutrient 'patches' can influence root foraging, lateral root formation and root hair formation. Thus, the mutual effects of salinity and nutrient heterogeneity are relevant.

Root physiological responses to nutrient heterogeneity in fertigated crops

Though probably a common occurrence in drip irrigated crops, very few studies have simultaneously varied both salinity

and nutrient distribution. The following discussion first considers experiments with only nutrient heterogeneity, before discussing the integration of nutrients with salinity heterogeneity.

In tomato, preferential nitrate (NO_3^-) uptake was found to occur from areas of the root-zone with higher (1.6- to 3.3-fold greater, with 10 ds m^{-1} being the highest EC) electrical conductivity (or more negative osmotic potential) generated by locally high nutrient concentrations (Sonneveld and Voogt, 1990), suggesting a local response of roots exposed to high concentrations probably due to their enhanced NO_3^- uptake kinetics. Mathematical simulations of nutrient uptake under heterogeneous conditions of NO_3^- and phosphate (PO_4^{3-}) using the Barber–Cushman model found a greater impact of soil heterogeneity and root plasticity, with NO_3^- uptake increasing 7–20 times under heterogeneous conditions (Jackson and Caldwell, 1996). Root proliferation and increased uptake kinetics from the enriched root-zones accounted for up to 75 % of NO_3^- supply of a plant and over 50 % of PO_4^{3-} acquired from enriched soil patches. Simulations demonstrated that plants lacking plasticity of root growth or uptake always acquired less nutrients under heterogeneous NO_3^- and PO_4^{3-} distributions.

In a split-root solution culture experiment on *Lolium multiflorum*, less than 24 h after depriving NO_3^- from half the root volume, net NO_3^- influx to roots in the nitrate-rich area increased, with root growth increments observed only after 1 week (Lainé *et al.*, 1998). *Brassica napus* responded similarly (Lainé *et al.*, 1995). This rapid variation in NO_3^- uptake was strongly associated with altered root hydraulic conductivities, with a sudden increase in NO_3^- concentration around the roots almost simultaneously increasing root hydraulic conductivity and preferential water uptake from the nitrate-rich patch (Gorska *et al.*, 2008). Split-root experiments applying NO_3^- to a portion of the root system demonstrated a localized and reversible response, with N starvation on one side of the root system leading to compensatory and enhanced NO_3^- uptake in the other root portion (Tabata *et al.*, 2014).

Heterogeneous NO_3^- distribution to split-root *Acer rubrum* and *Betula papyrifera* plants demonstrated a species-dependent response, with two-fold more fine roots measured for *B. papyrifera* in the high NO_3^- portion than *A. rubrum*, yet similar total NO_3^- uptake rate (Gloser *et al.*, 2008). Under heterogeneous conditions, *A. rubrum* had smaller leaves and N deficiency symptoms in the shoot portion directly above the nutrient-deficient root portion, while *B. papyrifera* had regular leaves with no visible deficiency symptoms. Vascular system architecture may explain this differential response (Orians and Jones, 2001). In species with sectored vascular systems (e.g. *A. rubrum*), in which contiguous and largely exclusive vascular traces occur from a specific root to a specific branch, N deficiencies occurring in isolated parts of the canopy reflect the nutritional status of the specific root that feeds that branch. In contrast, other species (*B. papyrifera*) have an integrated vascular system allowing nutrient transfer from an individual root to the canopy as a whole, avoiding the consequences of patchy nutritional deficiencies.

Root morphological responses to nutrient heterogeneity

In *Betula pendula*, dry matter allocation to roots can be modified in three different ways when the availability of

10

mineral nutrients is limited: (1) increased root growth in N-, P- or S-limited soils; (2) decreased root growth when K^+, Mg^{2+} and Mn^{2+} is limited; and (3) no effect on root growth when Ca^{2+}, Fe^{2+} and Zn^+ are limited (Ericsson, 1995). Root growth plasticity in patchy soil enhances the ability of plants to fill the soil volume rich in nutrients and was the most important trait influencing species success (Hodge, 2006; Rajaniemi, 2007). The ability of a plant to 'find' the nutrient-rich patch is essential if morphological/physiological root responses are to be expressed. For instance, while nutrient (N) heterogeneity in *Lolium perenne* did not lead to preferential root growth in the nutrient-rich soil patches (suggesting the patch was not explored to any greater extent than the bulk soil), there were overall increases in specific root length (length/biomass) and root elongation throughout the entire soil profile compared to the uniform N treatment (Nakamura *et al.*, 2008). This suggests that the overall plant N deficiency induced root elongation and

not the patchiness per se. The differential response of roots to nutritional patchiness is probably a consequence of complex nutrient-specific signal transduction pathways (López-Bucio *et al.*, 2003).

Impacts of simultaneous salinity and nutrient heterogeneity

To investigate the effects of heterogeneous root salinity and nutrient conditions, several split-root tomato experiments were conducted (Fig. 6; Valenzuela *et al.*, 2021). Water uptake from the saline root-zone dramatically decreased within 8 h of treatment (Fig. 6A, B) in contrast to the non-saline root-zone, with a more pronounced effect when nutrients were provided only to the non-salinized root-zone (Fig. 6A, B). This reduction in water uptake did not correlate with decreased root growth (which was maintained during Days 1–3), with the saline root-zone only
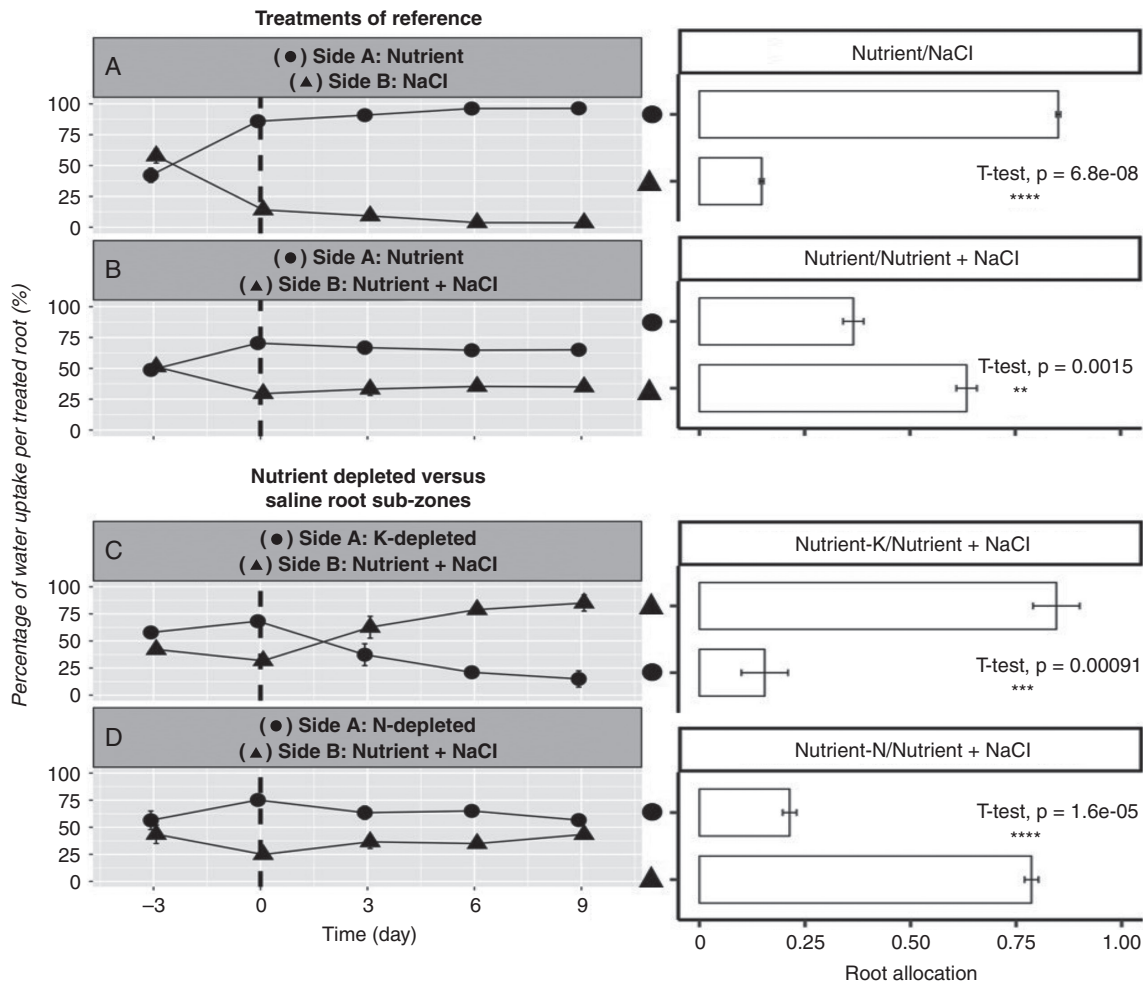

Fig. 6. Daily measurement of the percentage of water consumption per root side in a split-root system under hydroponics. The vertical dashed line shows the time when salinity application was initiated. Dots represent treatments applied to side A and triangles to side B. The saline agent was NaCl with a concentration of 50 mm. Bar graphs, at the right, show the root biomass allocation for each treatment. In these experiments, tomato (*Lycopersicon esculentum*) seedlings were grown in a hydroponic split-root method for 9 d under heterogeneous saline and nutritional conditions applied separately and in combination. Root activity was monitored by directly measuring root uptake of water and nutrients, biomass allocation and total nutrient uptake. The short experimental time frame and modest salinity concentrations were selected to minimize ionic toxicity and plant growth effects. Reproduced with permission from Valenzuela *et al.* (2021).

showing significantly less root growth towards the end of the experiment (Day 9). The rapidity and consistency of decreased water uptake by roots in the saline zone, from treatment imposition through to Day 9, suggests that a primary physiological response (possibly due to reduced aquaporin activity) was followed by a morphological response.

To further explore the role of heterogeneous nutrient provision on root activity, complete nutrient solutions were selectively depleted of either N or K^+ in the non-saline root half while the other root half received a saline, complete nutrient solution (Fig. 6C, D). These treatments provoked a 'two-phase-response'. Immediately upon treatment application, the saline conditions given to one side of the roots dominated, immediately decreasing water uptake of those roots. Subsequently, water uptake from the saline-treated, nutrient-supplied roots proportionally increased, probably in response to the nutrient deficiency induced by the omission of the nutrient on the non-saline side. This effect was marked when K^+ was only present in the saline root half and slight in the case of N. The presence of K^+ in the nutrient solution was the most important determinant of root activity even when coinciding with salinity, resulting in a notably higher shoot tissue Na^+ and Cl^- concentration when the sole source of K^+ was to the saline root volume (Valenzuela *et al.*, 2021).

This experiment and others described herein suggests that interpreting root responses to heterogeneous conditions depends markedly on context, time, salinity concentrations and plant nutrient status:

- Immediate (within hours) reductions in water uptake in salt-exposed root-zones commonly occur and are expected to be determined by the relative difference in salinity between root parts. Subsequently, relative nutrient availability affects root activity, with responses to K^+ depletion perceived within days. In the longer term, ionic stress and changes in relative root growth may further alter relative root activity in each root-zone.
- Plant responses to the relative distribution of nutrients in the root-zone probably also depend on plant nutrient status and the absolute concentrations of nutrients and salinity present in each root-zone. Thus, a K^+-replete plant, provided low levels of K^+ in the non-saline root-zone, or the presence of extreme salinity (>100 mM), would probably diminish the dramatic response seen here (Fig. 6).
- All split-root systems or otherwise manipulated heterogeneous root-zone experiments do not reflect the complexity of natural ecosystems, where soil heterogeneity is probably significantly more complex in space and time. However, certain agricultural conditions may closely resemble split-root studies, such as in substrate hydroponic systems, raised bed vegetable production and micro-irrigated arid zone crops.

While these simple split-root experimental approaches cannot explain all potential nutrient interactions, they do illustrate the rapidity and plasticity of plant responses and the importance of considering nutrients when studying heterogeneous salinity. Furthermore, this raises several important questions on the signalling pathways underlying root system architecture and functions under heterogeneous saline conditions: (1) What is the relative importance of the signal(s) under heterogeneous saline conditions (salt ions vs. nutrients vs. water vs. hormones)? (2) How does variation in salt tolerance affect this response (e.g. halophytes vs. non-halophytes)? (3) How do the local conditions (salinity vs. nutrient vs. water availability) and whole plant status (e.g. shoot Na^+, Cl^- and/or nutrient concentrations) modulate the response? Interestingly nutrient availability alters the endodermal specific ABA signalling in roots that modulates lateral root formation and root system architecture in response to salinity stress (Duan *et al.*, 2013). As demonstrated above, this suggests that the 'nutrient signal' might eventually override, or at least affect, the 'salinity signal' in regulating root growth and functions under heterogeneous conditions.

IMPLICATIONS FOR CROP MANAGEMENT

When considering crop responses to salinity, two parameters define salt tolerance: (1) the threshold salinity that causes the initial significant reduction in the maximum expected yield, and (2) the rate of yield decline as salinity increases beyond the threshold (i.e. slope; Maas and Hoffman, 1977; Maas and Grattan, 1999). However, the important underlying assumption of the threshold model by Maas and Hoffman (1977) is that steady-state conditions exist in the root-zone, with water content and salt concentration remaining constant in time and space. Since these conditions rarely exist in crop root-zones in the field (e.g. Fig. 1), conclusions from steady-state analyses can be questionable (Letey and Feng, 2007). This has critical repercussions when defining the salinity experienced by the roots when identifying breeding targets and soil management practices.

Re-defining soil salinity

One valuable tool in categorizing and quantifying genetic variation in salt tolerance has been to define crop relative yield responses in terms of threshold salinities up to which yields are unaffected and linear decreases in relative yield with increasing salinity thereafter (cf. Maas and Hoffman, 1977, and their successors). However, it is critical to recognize that these relationships have generally always been presented in terms of variation in parameters such as EC_e (the electrical conductivity of the soil saturation extract) or more occasionally in terms of variation in $EC_{1:5}$ (the electrical conductivity of a 1 : 5 soil : water slurry) that relate to the salinity of the soil. However, it is not the salinity of the soil (a parameter that does not account for variations in soil water content) that affects plant growth but the salinity of the soil solution, and thus the ratio of salt to water in the soil. This means that the salinity stress on a plant can be doubled by doubling the salt concentration in a soil or by halving the water concentration of the soil. Furthermore, as soils become drier, plant growth becomes affected by the increasingly negative matrix potentials (Ψ_m values) that develop in soils because of the adhesion of water by soil pores.

This view profoundly affects the whole idea of the heterogeneity of salinity stress in soils, because heterogeneity arises because of variable: (1) leaching effects of irrigation or rainfall on salt concentrations in soil, (2) hydrating effects of irrigation or rainfall on soil water contents, (3) effects of surface soil evaporation increasing salt concentrations by capillarity and decreasing water contents in the soil, and/or (4) water extraction

rates of roots and the ion uptake/exclusion capacity, which over time also influence ion and water abundances near the roots.

One variable that captures variation in both salt and water concentrations in soil is solute potential (Ψ_s; units MPa). For soils salinized with NaCl, this can be calculated as:

$$\Psi_s = -22.75 \times EC_{1:5}/W \tag{1}$$

where the $EC_{1:5}$ of the bulk soil is in units of ds m^{-1}, and soil water content of the bulk soil (W) has units of % dry mass.

Water potential of the soil (Ψ_{soil}) can be calculated (Slatyer, 1967) as:

$$\Psi_{soil} = \Psi_m + \Psi_s \tag{2}$$

Furthermore, a leaf's transpiration rate (E_t) can be related to its water potential (Ψ_{leaf}), the water potential of the soil (Ψ_{soil}) and the resistance to flow (R) as follows (Nulsen and Thurtell, 1980):

$$E_t = (\Psi_{soil} - \Psi_{leaf})/R \tag{3}$$

While theoretically sound, plants change these simple mathematical relationships. First, salt accumulates in the root-zone making Ψ_s more negative than can be calculated using the $EC_{1:5}$ and W measurements of the bulk soil. Passioura and Frere (1967) define the variable U as the factor by which Ψ_s at the root surface is more negative than in the bulk soil. Experimental approaches suggest that U can be around 2 in well-hydrated soils (e.g. Sinha and Singh 1974, 1976), but modelling approaches suggest that U could increase to values around 10 as the soil becomes drier (Passioura and Frere, 1967). Given this, eqn (2) can be modified to:

$$\Psi_{soil} = \Psi_m + U\Psi_s \tag{4}$$

Second, the resistance of water flow to the surface of the root increases as the soil pores around the root become depleted of water (Stirzaker and Passioura, 1996). This increases the variable R in eqn (3).

Given this background, how should heterogeneity of salinity in the soil solution in the root-zone be viewed? Unfortunately, very few of the critical experiments have been done under uniform conditions in the root-zone, let alone variable ones. To our knowledge, no split-root experiments have ever attempted to compare the effects of different (or even the same) Ψ_{soil} values by manipulating the salt and water concentrations on each side of the root-zone of a plant, even though the components of Ψ_{soil} (Ψ_m, Ψ_s and U) can all be determined experimentally. Maintaining such treatments is technically challenging, requiring new experimental protocols to be developed. In such experiments, there could be considerable rewards by comparing plants of different salt tolerance.

Opportunities to better manage irrigated agriculture

All irrigation water introduces salts to the system (Hanson and Bendixen, 1995) and in regions with high evapotranspiration and low rainfall, traditional salinity management emphasizes deliberate leaching of salts away from the root-zone while avoiding elevation of the water table to prevent damage to crops (Hopmans et al., 2021). Leaching is usually achieved by applying irrigation water in excess of crop evapo-transpirational demands. The fraction of applied water that drains below the root-zone is referred to as the 'leaching fraction' and this value is used to coarsely gauge the extent of leaching (Hanson et al., 2009). Larger leaching fractions generally result in larger zones with a low soil water salinity but may necessitate disposal of large volumes of saline drainage water and may cause additional salinization through capillary rise of saline water by raising the water table (Grismer et al., 1988; Corwin, 2021), as well as environmental impacts of drainage water disposal.

Designing the appropriate leaching fractions needed to avoid yield loss is context-specific and will depend on the crop, soil texture, climate, irrigation system and irrigation schedule, and the salinity of irrigation water being used (Ayers and Westcot, 1985; Hanson and Bendixen, 1995; Assouline et al., 2015). Ayers and Westcot (1985) developed a simple approach to calculate the leaching requirement based on salt mass balance calculations. This approach estimates the leaching fraction required to keep the average root-zone salinity below the salinity threshold of the crop, assuming a specific root distribution and a strictly vertical, continual water flow. Approaches like this neglect the spatial non-uniformity of irrigation water application as well as the temporal dynamics of irrigation and water uptake during the season (Letey et al., 2011) and assume that the average root-zone salinity determines the impact of salinity on the crop (Letey and Feng, 2007).

While the physical principles underlying salinity management have not changed since Ayers and Westcott developed these leaching guidelines, management goals have shifted over time to better recognize environmental impacts of nutrient and salinity losses and develop more advanced micro-irrigation and fertigation systems. This has given rise to both new challenges and new opportunities in managing salinity.

Challenge 1: Managing salinity under micro-irrigation systems. Spatial patterns of salt accumulation are diverse and differ by irrigation system (Riaz et al., 2018; Wallender and Tanji, 2011), with each irrigation system having specific challenges to salinity management. In the simplest case, flood irrigation applies water uniformly across the whole surface (although local topography and soil heterogeneity can cause spatially heterogeneous infiltration). In this case, salinity distribution is approximately uniform in the horizontal direction, but a salinity gradient exists vertically (Figs 2 and 3). Assuming sufficient leaching, salinity increases with depth in these systems (Ayers and Westcot, 1985) and uniform leaching of salts below the root-zone causes the salinity within it to be relatively homogeneous.

In contrast, applying water to only part of the surface causes strong horizontal salinity heterogeneity, as in furrow irrigation and more advanced micro-irrigation systems. Micro-irrigation aims to target water application to the root-zone, thereby improving water use efficiency by applying less water to regions with low root density and providing an opportunity to deliver water at a rate which matches crop demand. Flood and overhead sprinkler irrigation manage soil moisture and salt content at the field scale, while micro-irrigation approaches management at the root-zone scale. Targeted water application results in targeted leaching, with micro-irrigation leaching salts in zones which are rich with plant roots, while flood irrigation

requires additional water to also leach salts from field zones between plants with low root density, making micro-irrigation more efficient than furrow/sprinkler irrigation for managing salinity (Hanson *et al.*, 2009). When drip and furrow irrigation were compared, drip irrigation sustained higher yields of salt-sensitive crops compared to furrow irrigation when saline groundwater is shallow, while using less water than furrow irrigation (Hanson *et al.*, 2009).

The economic incentive to install micro-irrigation systems is context-dependent, with the advantage of micro-irrigation over conventional irrigation becoming less clear when growing salt-tolerant crops or when irrigation water is abundant. Despite its potential to accumulate salts in the root-zone, even subsurface drip can have advantages over salinity management with traditional irrigation. While higher tomato yields justified the expense of installing a subsurface drip irrigation system in California, the same was not true of cotton, which remained lucrative with furrow irrigation (Hoffmann and Johnsson, 2000; Hanson *et al.*, 2009), as such salt-tolerant crops tend to tolerate flood irrigation without yield loss provided that irrigation is applied pre-planting to avoid stand establishment losses (Ayars *et al.*, 1993; Hanson *et al.*, 2009).

In drip irrigation systems with strongly localized water application, salt is not only leached downwards, but significant lateral water movement away from the drip emitter also leaches salt horizontally (Raine *et al.*, 2007) resulting in salt accumulation in the fringes of the wetted volume (Fig. 2A). This leads to a strongly heterogeneous small-scale salt distribution where soil salinity levels in the top 20 cm can vary by a factor of more than five within only 40 cm of horizontal distance (e.g. May and Hanson, 2006). Although the extent of horizontal salt movement depends on the soil texture and can be partially controlled by emitter spacing, under micro-irrigation, salts concentrated between emitters near the surface generally have little opportunity to intrude into the root-zone without precipitation, due to surface evaporation and irrigation (Hanson and Bendixen, 1995; Hanson and May, 2011). It is therefore recommended that crops be arranged close to emitters where salinity is low and that new lines be installed as close as possible to where old lines existed to avoid the need for pre-season reclamation leaching (Hanson and May, 2011).

Subsurface drip irrigation results in a different pattern of water flow and salinity accumulation. While water application at the soil surface causes salts to leach downward and outward from the water source, subsurface irrigation causes resident and irrigated salts to flow upward through advection and accumulate above the dripline where plants are present (Hanson and Bendixen, 1995; Hopmans *et al.*, 2021). This accumulation pattern antagonizes the establishment of many row crops because germination is relatively sensitive to salt stress (Bernstein *et al.*, 1955). Such production systems rely on pre-season rain, sprinkler or surface irrigation to leach salts below the drip line where they may be leached downward by subsurface irrigation (Hanson and Bendixen, 1995). Shallow installation of subsurface drip lines is advantageous where sufficient pre-season rains are present as irrigating the soil surface may be avoided altogether (Hanson and Bendixen, 1995). This issue can be mechanically managed in processing tomato by adding soil to planting beds (Hanson *et al.*, 1995), followed by irrigation to accumulate salts into the uppermost zone of the bed, which is subsequently removed and placed in the furrow between rows, where very little horizontal salt movement occurs (Hanson and Bendixen, 1995).

The strong localization of water application in drip irrigation questions the applicability of historical steady-state leaching models to micro-irrigation systems (Letey and Feng, 2007). These models insufficiently account for the highly local nature of micro-irrigation and underestimate both the local leaching fraction experienced by plants and the tolerable EC of irrigation water (Wallender and Tanji, 2011). Adequate management of heterogeneous salinity patterns and localized leaching under drip or micro-sprinkler may allow sustainable crop production in soils that would otherwise be deemed too saline for that species. Using transient models such as the HYDRUS model has been suggested as an alternative (Letey *et al.*, 2011). These models account for localized application of water and changes in flow rates over time by explicitly simulating two-dimensional (or even three-dimensional) water and solute transport in the root-zone by numerically solving mechanistic models. However, although these models are very strong in depicting physical transport processes, they often oversimplify the description of plant physiological processes governing water and solute uptake. For example, the HYDRUS model neglects that the distribution of water uptake is also affected by nutrient concentrations. Moreover, even if it was possible to perfectly simulate the water, nutrient and salinity dynamics for a given scenario, it would still be unclear how the calculated heterogeneous salinity distribution would translate into plant performance. Incorporating current knowledge of plant responses to heterogeneous conditions (both salinity and nutrient) might make these models more suitable for evaluating salinity management practices.

Challenge 2: How to simultaneously optimize N efficiency and minimize the impact of salinity. The necessity of a leaching fraction for long-term salinity management is coupled with the issue of nutrient loss, especially for nitrate (NO_3^-), which exhibits similar leaching potential as Cl^-. Any practice designed to remove Na^+ or Cl^- from the root-zone probably also leaches NO_3^- (Assouline *et al.*, 2015; Vaughan and Letey, 2015; Libutti and Monteleone, 2017). Although a common problem, few studies have addressed the integrated nature of salinity and nutrient management (Libutti and Monteleone, 2017). While NO_3^- and Cl^- are subject to very similar transport mechanisms and rates in the soil, their distribution in the soil can nevertheless be quite different, and high Na^+ and Cl^- concentrations do not necessarily coincide with high NO_3^- concentrations. This is because: (1) in contrast to Na^+ and Cl^-, NO_3^- is preferentially taken up by plant roots; and (2) nitrogen fertilizer is deliberately added to the irrigation water during fertigation and is to some degree independent of water (and therefore salt) application. Understanding crop nitrogen demands and responses to spatially localized nutrients and salinity may help manage fertigation systems to achieve the simultaneous goal of salinity leaching and minimal nitrate loss.

By providing nutrients through fertigation in a manner (rate, duration and timing during a fertigation event) that retains nutrients in the low-salinity zone adjacent to the drip-emitter, roots can avoid exploring the saline fringes of the wetted zones,

thus reducing salt exposure. HYDRUS-based modelling suggests that high-frequency applications of small amounts of nitrate, timed toward the end of a fertigation event, can help retain NO_3^- in the root-zone adjacent to the irrigation source while allowing salt (i.e. Na^+ and Cl^-) to be leached to the peripheral root-zone. Scheduling low but frequent NO_3^- applications, attuned to crop demand, allows the crop to take up most of the NO_3^- before it passes through the low-salinity zone into the saline fringes. Figure 7 simulates continuous NO_3^- application and a scenario which applies NO_3^- only every 10 d, while the total amount of NO_3^- applied is the same for both simulations. High-frequency applications of NO_3^- using drip irrigation increased N uptake efficiency in some cases (e.g. Scholberg *et al.*, 2002; Quiñones *et al.*, 2007).

Breeding targets

Challenging as they are, modern irrigation systems provide some leeway for controlling water and nutrient supply, to match plant demands. Nevertheless, genetic approaches to enhance salt tolerance are also needed.

Avenue 1: Breeding for root traits that facilitate water and nutrient uptake. Do specific root traits facilitate resource foraging (water and nutrients) under heterogeneous soil salinities? With the current (or lack of) knowledge it is very difficult to address this question. As advocated for other marginal environments (Lynch, 2018, 2019; Schneider and Lynch, 2020; Colombi *et al.*, 2022), root architectural traits and anatomical plasticity that reduce the metabolic cost of soil exploration might be beneficial in saline environments. Since heterogeneous salt distribution is probably associated with non-uniform water and nutrient distribution, careful consideration is necessary. For example, under saline conditions plants modify their root system architecture to reduce salt uptake (Julkowska *et al.*, 2014) by reducing the length and density of root hairs and thus the overall absorption surface area (Shabala *et al.*, 2003). However, marginal soils can also be highly deficient in phosphorus (P), and root hairs would be critical to allow root exploration beyond the root depletion zones and acquire P (and also other nutrients and water) from impoverished soil (Lynch, 2018, 2019; Rongsawat *et al.*, 2021). Our ability to understand how plants could resolve such dilemma, and identify which root traits might be more favourable under heterogeneous salinities, is hindered by the simplicity of the experimental systems employed to date.

Avenue 2: Breeding for tissue tolerance. Traditionally, crop breeding for salinity tolerance has targeted Na^+ exclusion traits (Genc *et al.*, 2010; Munns *et al.*, 2012; Wu *et al.*, 2019). This
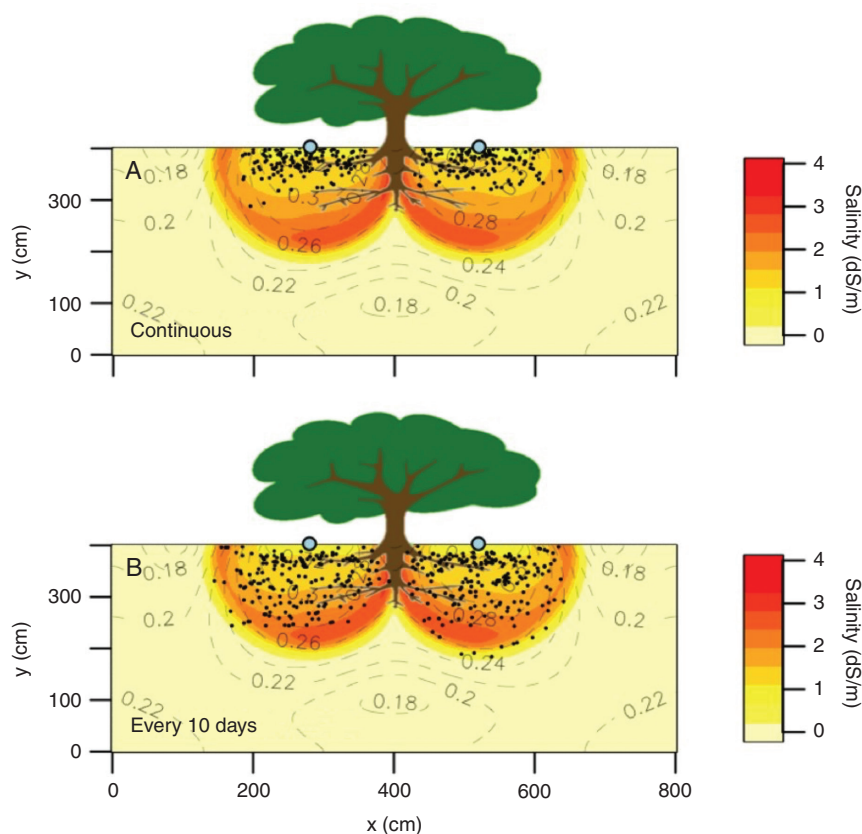

Fig. 7. Simulated spatial distributions of salinity and nitrate following a growing season with an equal amount of nitrate applied (A) continuously and (B) once every 10 d for 8 h. The density of black dots represents the concentration of nitrate in the soil and the isolines indicate volumetric water content (- -). This simulation accounts for plant uptake of nitrate and water over the growing season. The simulation was done using the software HYDRUS 2D (Šimunek *et al.*, 2012) assuming a constant transpiration rate of 8 mm d^{-1} and no surface evaporation over a period of 75 d (D. Reineke and P. H. Brown, pers. comm.)

strategy comes with a caveat of a progressive build-up of Na^+ in a root-zone (Liu *et al.*, 2020*a*), thus further exacerbating heterogeneity of Na^+ distribution profiles in the rhizosphere and affecting water uptake and ultimately growth (Alharby *et al.*, 2014, 2018). Furthermore, this strategy requires a heavy reliance on energetically expensive *de novo* synthesis of compatible solutes for osmotic adjustment (Munns *et al.*, 2020). A viable alternative may be to target crop halophytism, e.g. a set of anatomical and physiological traits that allow plants to include significant amounts of Na^+ in their tissues, without compromising their metabolic activity (Flowers and Colmer, 2015; Munns *et al.*, 2016). Amongst key traits conferring crop halophytism, vacuolar Na^+ sequestration, ROS desensitization, tissue succulence and salt deposition in trichomes are considered as promising targets in breeding programmes (Liu *et al.*, 2020*a*).

Avenue 3: Understanding the nature of root to shoot signals. Plant biomass is ultimately proportional to the amount of CO_2 assimilated by the shoot that, in turn, is determined by the efficiency of stomata in balancing CO_2 gain and water loss via leaf transpiration. Root-borne signals play a critical role in coordinating plant gas exchange and optimizing plant water use efficiency. The signalling between roots and shoots integrates various signals (from electrical and hydraulic signals, Ca^{2+} and ROS waves to hormones, peptides and RNA; Gilroy *et al.*, 2016; Shabala *et al.*, 2016; Li *et al.*, 2021) that ultimately determine a plant's ability to adapt to saline conditions. Stress-induced elevations in ROS levels is accelerated in halophytes compared to glycophytes (Ellouzi *et al.*, 2011), and NADPH oxidase-mediated root-borne ROS signals induce early stomatal closure in salt-tolerant species (Niu *et al.*, 2018). Although shoot ABA levels increase within 30 min of salinity exposure, the magnitude of this increase appears to be species-specific (Geilfus *et al.*, 2015; Hedrich and Shabala, 2018). While xylem sap ABA concentrations also increase (Albacete *et al.*, 2008), grafting experiments with ABA-deficient mutants indicate this is shoot-mediated (Li *et al.*, 2018). Nevertheless, grafting wild-type tomato scions onto an ABA-overproducing rootstock enhanced salt tolerance, even if it was difficult to establish consistent evidence of root-to-shoot ABA signalling (Martínez-Andújar *et al.*, 2021). As discussed above, it remains uncertain how plants exposed to heterogeneous salinity integrate signals from exposed and non-exposed roots to regulate stomatal aperture. Since a multitude of signals interact during root-to-shoot communication, it is critical to understand how they confer plant stress tolerance.

Avenue 4: Understanding how water and ion transport are coupled. Another emerging topic is the possibility of water and ion flow coupling by aquaporins. Initially described as water and neutral solute channels, aquaporins can also transport ions across various cellular membranes (Byrt *et al.*, 2017; Qiu *et al.*, 2020). This discovery challenges current concepts that water and solutes move across membranes via separate pathways and may account for situations where water movement into the xylem goes against an apparent water potential gradient (Wegner, 2017; Tyerman *et al.*, 2021). Such coupling may be especially crucial for roots exposed to heterogeneous salinity as transcriptional changes are probably too slow to account for the highly dynamic external ionic environment. In this context, a phosphorylation-dependent switch between ion

and water permeation in aquaporins (and, specifically, *PIP2;1*) might enable plant cells to rapidly adjust to altered ionic conditions in the rhizosphere and optimize ion transport at minimal energy cost. This option implies that plants that rely on Na^+ accumulation for osmotic adjustment and thus water uptake under hypersaline soil conditions can tolerate tissue Na^+ loads, thereby avoiding cytotoxicity, requiring that halophytic traits be incorporated into modern elite varieties.

CONCLUDING REMARKS

There are several constraints in conducting experiments or genotypic selection of plants under conditions that do not reflect real agricultural conditions of heterogeneous soil environments. Homogeneous conditions limit more complex interactions between salinity prevalence and plant responses such as exclusion of saline ions, nutrient and water uptake, root architecture, or adjustment to varying pH within the root-zone. As highlighted above, fundamental questions remain on plant responses to heterogeneous salinities and how this is affected by the associated variations in water and nutrient distribution. We call for a greater focus on understanding plant responses to heterogeneous soil salinity, which should be considered as the next frontier for salinity research and land management. Understanding responses to heterogeneous saline conditions holds significant promise for identifying new breeding targets for crop salt tolerance and adequate management practices of saline environments, which will accelerate the implementation of solutions to improve the productive use of saline land.

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Chapter 2: Broad Physiological Impacts of Sodium/Potassium Colocalization on Tomato in Solution Culture

Introduction

Salinization Background

The biosphere is becoming saltier, affecting both soil and freshwater so greatly that it will be a likely hallmark of the Anthropocene (Kaushal et al. 2021; 2023; van Vliet et al. 2023; Galella et al. 2023). This is exacerbated both directly and indirectly by climate change (J. Hopmans and Maurer 2008; Dennis L. Corwin 2020). Local changes in the hydrologic cycles can alter precipitation quantity and timing, reducing its utility to agriculture (Pathak et al. 2018; J. Hopmans and Maurer 2008; Schoups et al. 2005; López-Moreno et al. 2011; Notaro et al. 2014). In addition to declining overall quantity and quality of irrigation water, reductions in surface water reliability in California have resulted in farmers increasingly depending on groundwater for irrigation, and elsewhere in the world, even industrial effluent, both of which are generally more saline (Pulido-Bosch et al. 2018).

Soil salinization is a common consequence of irrigation (Ayers and Westcot 1985; Pulido-Bosch et al. 2018), and water use efficiency (WUE) goals focused solely on growing "more crop per crop" may exacerbate the issue (Amer et al. 2020). Efforts to increase WUE generally seek to minimize the amount of water that flows into and then beyond the rootzone known as the "leaching fraction" (Rhoades, Kandiah, and Mashali 1992). Leaching fractions are essential to the conventional framework of salinity management, as they can dissolve salts within the root zone and translocate them beyond the reach of roots (Ayers and Westcot 1985). These established approaches to salinity management emphasize field-scale salt balance and while actionable they

do not consider the diversity of physiological outcomes at a given field salinity level due to differences in plant-level salt distribution, let alone its relation to nutrient distributions (Dennis L. Corwin, Rhoades, and Šimůnek 2007).

Plant Adaptive Responses

Plants exhibit adaptive responses to heterogeneous distribution of nutrients in the rootzone, altering morphology, permeability, and modulating root ion channel/transporter activity and richness (Rengel, Cakmak, and White 2022). These traits have presumably been advantageous in an evolutionary timescale for their capitalization of nutrient patches, and energetic thrift in zones with few resources. Plants also exhibit adaptive responses to zones of high salinity, reducing water uptake not only as a consequence of the water potential gradient but also due to reversible changes in aquaporin expression and increased suberin deposition which both increase root radial hydraulic resistance (Zhang et al. 2021; Shao et al. 2021; Barberon et al. 2016).

In addition to these stress-avoidant adaptations, stress-tolerant mechanisms may also be deployed to manage sodium uptake and maintain ion homeostasis. Depending on genotype, sodium may be actively transported into vacuoles via the Salt Overly Sensitive pathway (SOS) pathway for long-term sequestration, or compartmentalized within the plant by similar means (Drakakaki et al. 2017; Wu et al. 2018; Böhm et al. 2018). Not only does this tolerance approach incur an energetic cost, but its efficacy varies greatly according to genotype (Negrão, Schmöckel, and Tester 2017; Munns 2002; Munns et al. 2020; Zhang et al. 2021). Unchecked sodium uptake into the plant may result in a disruption of ion homeostasis, namely the ratio of Na:K, where Na+ may competitively inhibit K+'s function as an enzyme cofactor, decreasing enzymatic efficiency, at greatest expense in

photosynthetic cellular compartments (Assaha et al. 2017; Pessarakli 2019; Stefanov, Rashkov, and Apostolova 2022).

Current Understanding of Salinity/Nutrient Interactions

Soil salinity, particularly in irrigated agriculture, is highly heterogeneous across both space and time (Flowers and Yeo 1995; Wadleigh and Fireman 1949; D. L. Corwin and Lesch 2005). While it is known that plants respond very differently to rootzone salinity based on its localization, existing salinity physiology research overwhelmingly imposes uniform salinity treatments (Bazihizina, Barrett-Lennard, and Colmer 2012; Bazihizina, Colmer, and Barrett-Lennard 2009; Negrão, Schmöckel, and Tester 2017; J. W. Hopmans et al. 2021). While the physiology of nutrient uptake and salt stress have been well studied respectively, it is scarcely understood how these phenomena interact or their synergistic implications for plant health. Meanwhile, present-day irrigation and nutrient management dictate the extent to which nutrients are colocalized with soil sodium, to a poorly understood effect (F. J. Valenzuela et al. 2022). Early investigations into these interactions have suggested that plants may tolerate salt exposure better when NaCl is spatially segregated from nutritive ions (F. Valenzuela, Fuentes, and Brown 2022; Bazihizina, Barrett-Lennard, and Colmer 2012). The means and relative importance of which ions are being sensed by plants to determine root growth under heterogeneous rootzone salinity are poorly understood at a nuanced or even an empirical level (Cees Sonneveld and Wim Voogt 2009; Bazihizina, Barrett-Lennard, and Colmer 2012; F. J. Valenzuela et al. 2022). This lack of heterogony salinity study is partly the result of scientific incentives to uniformly apply NaCl treatments, and partly due to the copious abiotic cues, including specific nutrients, which likely function in concert to influence physiology under heterogeneous salinity (Muchate et al. 2016). Interplay between Na

and nutritive ion uptake has been reported under many conditions, though it follows diverse patterns according to genotype and ionic environment (Bolat et al. 2006; Cees Sonneveld and Wim Voogt 2009; Tavallali, Rahemi, and Panahi 2008). Even within a single genotype, the significance of ion uptake can only be understood in the context of other elemental statuses and tissue concentrations, an additional challenge of interpretation and translating findings into specific guidelines.

Prior split-root solution culture experiments from Francisco Acevedo (F. Valenzuela, Fuentes, and Brown 2022) surveyed the relative importance of K and N on root water and NaCl uptake under heterogeneous salinity in tomato. With or without NaCl, plants showed a relative decrease in water uptake and root biomass accumulation in compartments following the removal of either K or N, with K showing a particularly strong and rapid response. Even with an ample and uniform supply of all other nutrients, root growth, and water uptake occurred preferentially in compartments which contained potassium. This effect held even when the K-rich compartment was supplemented with 40 mM of NaCl. Notably, this report shows instances where plant water uptake patterns were not predictable by water potential gradients alone: plants preferentially consumed water from the nutrient-rich saline compartment, despite its lower solute potential, when the non-saline compartment lacked K. This work underscores the importance of considering changes in root resistance in response to not only solute potential but also ion content. All necessary nutrients together were unable to increase root activity in a saline compartment when K was not present, suggesting that K is requisite for root activity in salt-rich zones. Given its apparent significance, perhaps the presence of K alone may increase root activity, even in salt-rich zones, with unknown consequences. The following research seeks to advance this line of questioning.

Outline of Original Research

Tomato is a global crop with recognizable culinary importance to much of the world's population (Silva et al. 2017). California is an important hub of tomato production and is among the arid climates affected by soil salinization (Costa and Heuvelink 2018; Schoups et al. 2005). Tomato's wide and accessible genetic base, with many short-generation, semi-dwarf genotypes to select from, make it amenable to experimentation and a sensible starting point for developing this nascent body of research. These experiments investigate the degree to which potassium localization may affect salinity tolerance under non-uniform rootzone salinity.

Table 2.1 Experimental Design

treatment	root side A	root side B
0 (n=5)	nutrients (0.5x)	nutrients (0.5x)
1 (n=5)	nutrients (0.5x) + salt (0.5x)	nutrients (0.5x) + salt (0.5x)
2 (n=3)	nutrients	salt
3 (n=5)	nutrients - 40% K	salt + 40% K
4 (n=5)	nutrients - 80% K	salt + 80% K

Table 2.1 The experiment included a total of 23 plants (46 buckets). Treatments 0, 1, 3, and 4 were each assigned 5 replicates, while treatment 2 was assigned only 3 replicates due to two plants not satisfying pre-treatment water uptake uniformity between root halves. All plants were exposed to overall equal amounts of potassium, nitrogen, phosphorous, and sodium (in the case of Na-containing treatments). Complete nutrients (N), potassium (K), and sodium (Na) allocations within each treatment were as follows: treatment 0, salt-free control (0.5N / 0.5N); treatment 1, uniform nutrients and salt (0.5N + 0.5Na / 0.5N + 0.5Na); treatment 2, complete nutrient and salt

segregation (1.0N / 1.0Na); treatment 3, 40% K/Na colocalization (1.0N - 0.4K / 1.0Na + 0.4K); treatment 4, 80% K/Na colocalization (1.0N - 0.8K / 1.0Na + 0.8K).

As is common in nutritional physiology research, some necessary counterions were supplemented along with ions of treatment (e.g. K has to be supplemented as KCl). To the greatest degree that was practical, the same amount of each ion was supplemented across treatments. Realistic deviations from this ideal are disclosed in Table 2.2. While there is some off-target variation in Cl localization between treatments, the content is relatively small.

In addition to broad comparisons between plants with no salt exposure (treatment 0), uniform salt exposure (treatment 1), and compartmentalized salt exposure (treatment 2), treatments 3 and 4 emulate environments with intermediate levels of potassium/sodium colocalization. While most treatments confine NaCl to one of each plant's two root fractions, these saline compartments were enriched with potassium (KCl) at the expense of the low-EC root half's potassium budget. This design is inspired by the shifting distributions of nutrients and salt which may occur in agricultural soils under varying management. Compensatory minerals were added to appropriate compartments to minimize changes in ionic composition due to "movement" of potassium to the saline compartment.

Hypotheses

Hypothesis I

As the extent of potassium/Na colocalization increases, so will salinity stress.

- o Evidence: terminal biomass

As the extent of potassium/Na colocalization increases, the rate of overall K uptake will decrease, and the rate of Na uptake will increase.

- o Evidence: measured solution Na and K concentration over time

Hypothesis III

When nutrients and Na are spatially segregated, plants will show a strong bias favoring water uptake from the Na-free zone, but this effect will be progressively weakened in treatments where K is colocalized with salinity, with the greatest weakening of this apparent salt avoidance occurring in treatments with the greatest extent of K/Na colocalization.

- o Evidence: changes in instantaneous water uptake bias according to treatment over time

Materials & Methods

Culture

The indeterminate tomato (*Solanum lycopersicum*) genotype, "VFNT cherry" (LA2705) was selected for its self-pruning trait and multi-R-gene TMV resistance, in an effort to reduce root variance due to viral introduction during manipulation. Dried seeds, obtained from the C.M. Rick Tomato Genetics Resource Center (Davis, CA 95616) were first disinfected with 2.7% sodium hypochlorite for 30 minutes and then rinsed in running deionized water for five minutes to improve germination (Charles and Bowman 1961).

Seeds were germinated on blue blotting paper (Anchor Paper Co., St. Paul MN), kept damp within a transparent plastic box and inside a growth chamber, maintained providing fluorescent light at a

16h light/8h dark interval at a constant 25°C for 5 days. Seedling taproots were severed with a sterile razor blade to a length of 0.5 cm before transferring each to a 12" conical container of disinfected vermiculite in 3 cm of standing half-strength Hoagland solution. Elemental concentrations of the nutrient solution were as follows: In mM, N, P, K, Ca, Mg and S were 3, 0.5, 3, 2.5, 1, and 1, respectively. In μM B, Fe, Mn, Mo, and Cu were 23, 10, 4.5, 0.2, 0.19, and 0.16, respectively. Vermiculite cones and solution culture systems were maintained in a glass greenhouse supplemented with light to maintain a 16h light/8h dark interval using high-pressure sodium lamps, as necessary. Seedlings were allowed to acclimate and grow in vermiculite containers for 14 days before roots were washed, split into two approximately even halves, and transplanted into nutrient solution-filled HDPE buckets, with each plant's root junction sheltered by a PVC U-pipe straddling two buckets with independent nutrient solutions. Each bucket was filled with 3.5L of half-strength modified Hoagland solution (Fig. 2.1A). All buckets were covered in aluminum foil to mitigate diurnal variations in rootzone temperature and evaporation, and each compartment was continually aerated to maintain oxygen saturation. Care must be taken to not aerate the solution beyond the point of oxygen saturation as this can result in unnecessary evaporative water loss. Before treatment imposition, plants were allowed to acclimate to nutrient solution culture in 0.5x strength Hoagland solution for 10 days (Fig. 2.1B). During this period, all root halves were subjected to the same nutrient composition and strength with no supplemented NaCl. Following 10 days of acclamation, all plants whose water uptake was approximately even between each root side were randomly assigned a treatment. Treatments and respective replicate numbers are found in Table 2.1. Plants were not permitted to flower, and buds were excised at the bud stage in an effort to control phenological influences on nutrient and ion partitioning between treatments (Hurd, Gay, and Mountifield 1979; Gautier, Guichard, and Tchamitchian 2001).

Figure 2.1 A) Early-stage tomato plant following root division and transplant into nutrient

solution. B) Mid-treatment tomato plants in the greenhouse with late-day supplemental lighting.

Table 2.2 Concentration of Potassium, Sodium and Chloride by Solution Compartment

	K (mM)		Na (mM)		Cl (mM)	
treatment	side A	side B	side A	side B	side A	side B
0	1.4	1.4	0	0	0	0
1	1.4	1.4	20	20	20	20
2	2.8	0	0	40	0	40
3	1.68	1.12	1.12	40	0	41.12
4	0.56	2.24	2.24	40	0	42.24

Table 2.2 The idealized experimental ion concentrations differ from the actual ion concentrations

due to limitations of available mineral salts and methods for supplying a specific ion of interest.

Ion concentrations based on salts used for nutrient solutions are presented here. Where relevant,

"side B" represents the salinized root compartment. Root half sub-treatments were assigned randomly following whole plant treatment assignment.

Measurement

Buckets were weighed daily to measure approximate water uptake and refilled to initial volume with deionized water 3, 5, and 7 days after treatment imposition.

EC and pH measurements were taken *in situ* daily using a Mettler Toledo portable pH/EC meter (model SG23). All starting treatment solutions were adjusted to pH 5.5. Initial pH adjustments were made using compatible acids/bases for each treatment (e.g., KOH, NaOH, nitric acid, and HCl), with only minor effects on relative ion content.

10 mL of solution was sampled 0, 3, 5, and 7 days into treatment and frozen for later ion quantitation using inductively coupled plasma atomic emission spectroscopy (ICP-OES; Thermo Fisher ICAP 7000). Samples were acidified and analyzed as a 3% nitric acid matrix, diluted by a factor of 3.33 with deionized water to achieve measurable analyte concentrations. Select solution ion quantitation was calculated based on standards of reference of both K and Na at concentrations of 0, 0.305, 30.5, 305, and 610 ppm, as well as a 1x National Institute of Standards and Technology standard reference (NIST 1643f multi-element standard; [Na] = 18.830 μg/L; [K] = 1932.6 μg/L), and Yttrium as an internal standard. Sample emission intensity values were collected at 589.592 {57} nm (Radial) for Na, and 769.896 {44} nm (Radial) for K. These wavelengths were found to yield the most consistent accord with internal standards and minimal interference from other solution constituents. It is a fair inference that extinction of potassium or sodium from the solution

is due primarily to plant uptake, but interpretation of similar data for an analyte like nitrogen would require significant control, as it features numerous routes of volatilization in situ (Pitton et al. 2022).

Biomass values for each plant segment were collected following fresh harvest and plant disassembly, followed by one week in a 37ºC drying oven.

Statistical Analysis

Comparisons across treatment groups and across time (DAT) were made using analysis of variance (ANOVA), making multiple pairwise comparisons according to Tukey's method at a 95% confidence interval (CI) unless otherwise stated. All analysis and figures were produced in R (version 4.3.1) using RStudio (Version 2023.06.1+524 (2023.06.1+524), Copyright (C) 2022 by Posit Software, PBC).

Results

Biomass

Total plant biomass (Fig. 2.2) was not significantly different between treatment groups receiving uniform ion distribution to each side (1 and 2). Treatment group 1 (even distribution of nutrients and NaCl to each side) had the highest average plant biomass, with treatment group 4 having the lowest. Only treatment 4 had an average mass that was significantly lower than both uniform control groups at a 95% CI. Treatment group 3 had a mass that was lower than treatment 1 but was not distinguishable from treatment 4. Total root biomass (Fig. 2.3) was not statistically different between treatment groups.

Canopy biomass (Fig. 2.4) followed a nearly identical distribution as total plant biomass, with no significant differences in mass between uniform control groups. Uniform treatments 0 and 1, as well as treatment 2 (complete separation of NaCl/nutrients), had significantly greater canopy biomasses than treatment 4 at a 95% CI.

Root biomass bias (Fig. 2.5) numerically represents the degree of biomass unevenness between root halves of the same plant. It is a useful derivative value which allows for comparisons of root evenness between plants irrespective of absolute mass. Uniform control groups (0 and 1) had similar averages, and clearly possessed the most even root mass distribution between compartments. While treatment groups 0 and 1 were indistinguishable from one another, they were distinct from treatment groups 2, 3, and 4, all of which featured greater levels of root biomass bias. Treatment groups 2, 3, and 4 were indistinguishable from one another at a 95% CI, aside from notably high variance within treatment 4.

Canopy:root biomass ratio (Fig. 2.6) follows a similar distribution as what was seen in total plant biomass and canopy biomass. This ratio was similar between uniform control groups (0 and 1) at a 95% CI. Treatment group 4 was significantly lower than groups 0 and 1, and treatment group 3 was lower than treatment 1, but not treatment 0. Treatment group 2 had a comparable average to both groups 0 and 1. Treatment groups which had a larger overall biomass featured a higher canopy:root biomass ratio.

Biomass of roots in the non-saline compartment (Fig. 2.7) was statistically comparable between uniform treatment groups (0 and 1). All non-uniform treatment groups also showed uniform root

biomass (2, 3, and 4). While groups 2, 3, and 4 feature nominally more root mass in their non-saline compartments than compartments belonging to treatment 0 or 1, only treatment group 4 was statistically distinguishable from both uniform controls at a 95% CI, but with notably high variance. Treatment group 3 had significantly more root mass in the non-saline compartment compared to the salt-free treatment (0), while treatment 2 was indistinguishable from either treatment 0 or 1 at a 95% CI.

Biomass of roots in the salinized compartment (Fig. 2.8) was greatly reduced in all non-uniform treatment groups (2, 3, and 4) compared to the root biomass of either compartment in uniform treatment groups 0 and 1. There were no significant differences in salinized root biomass within treatment groups 2, 3, or 4. Similarly, uniform treatment groups (0 and 1) were comparable to one another, despite differences in overall solution NaCl content.
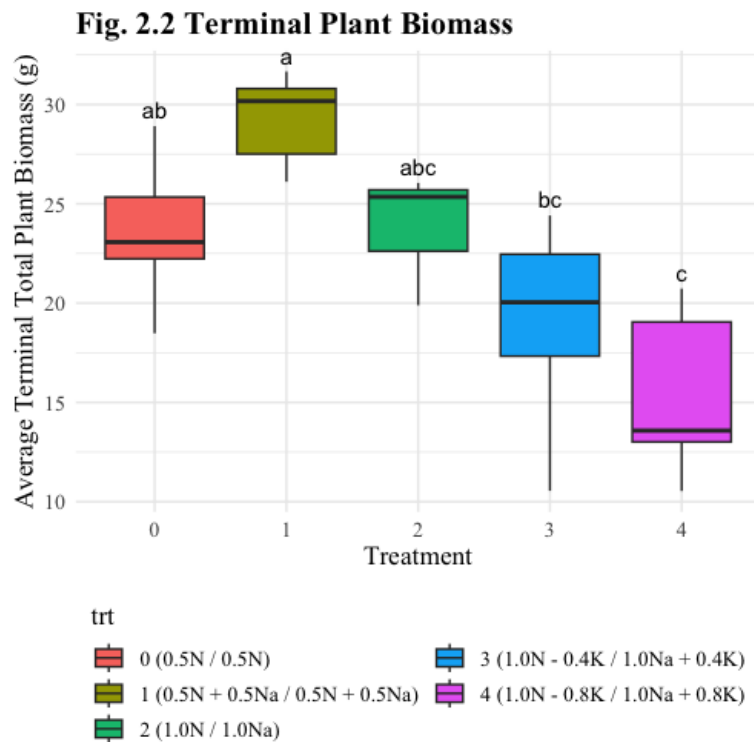
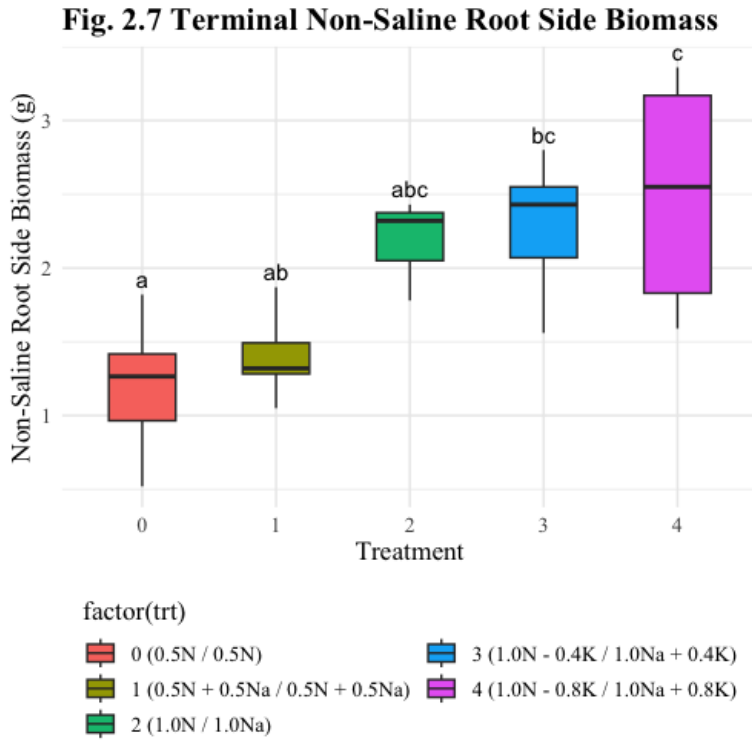**Figure 2.2** Boxplot of terminal whole plant biomass in grams, harvested on the seventh and final day of treatment (DAT 7). Biomass values were collected after sample drying.
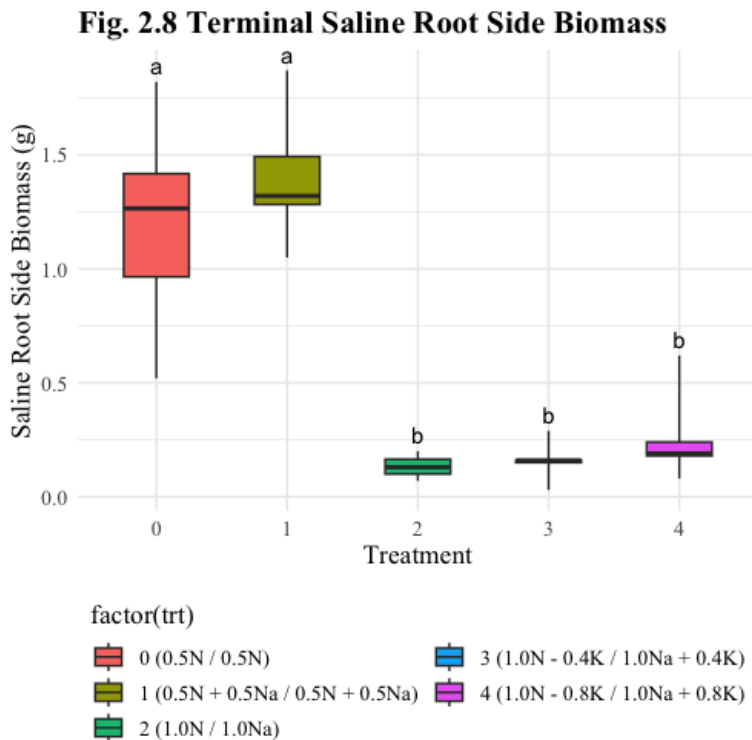
Fig. 2.3 Terminal Total Root Biomass
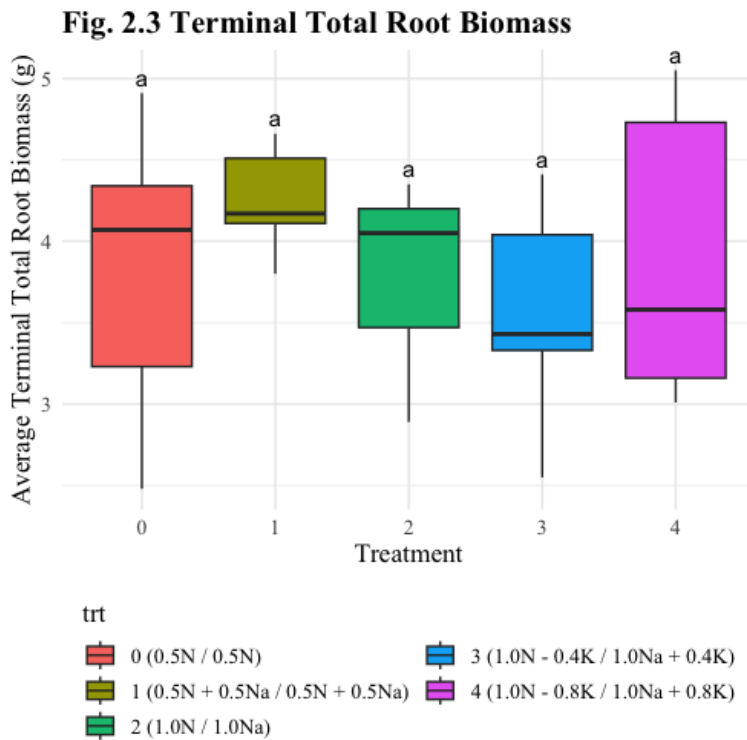
Figure 2.3 Boxplot of terminal root system biomass in grams, harvested on the seventh and final day of treatment (DAT 7). Biomass values were collected after sample drying.

Fig. 2.4 Terminal Canopy Biomass
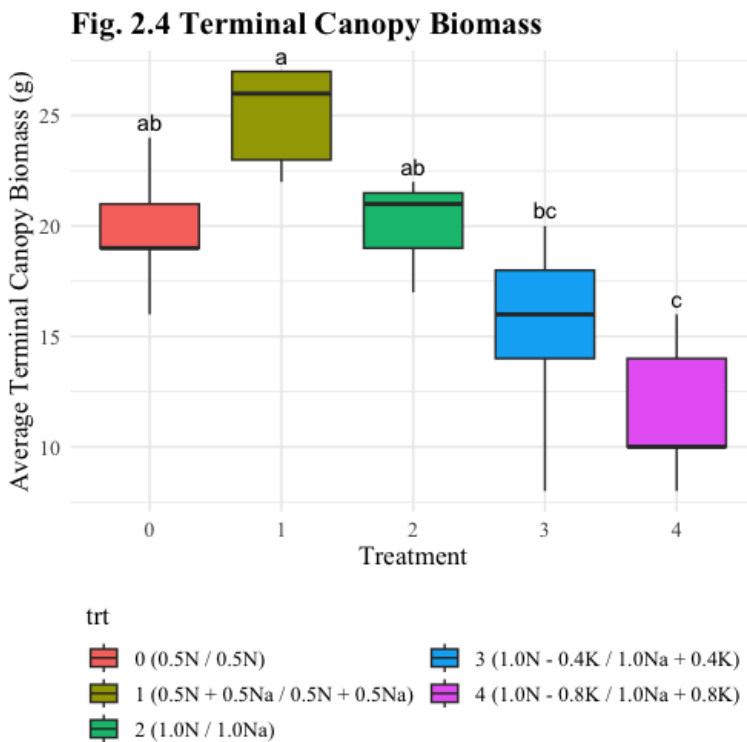
Figure 2.4 Boxplot of terminal canopy biomass (leaf and stem tissue) in grams, harvested on the seventh and final day of treatment (DAT 7). Biomass values were collected after sample drying.

Fig. 2.5 Terminal Root Biomass Bias
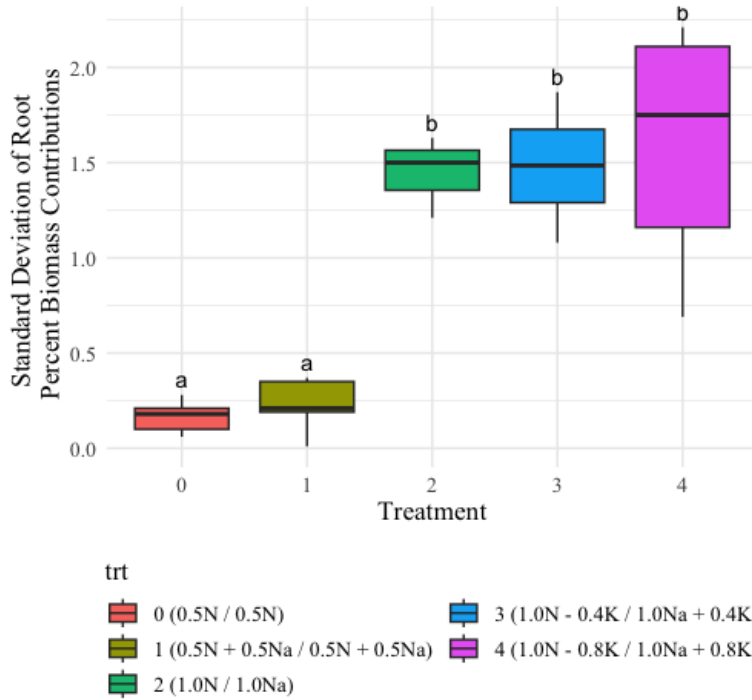
Figure 2.5 Boxplot of terminal root biomass bias in grams. Root biomass bias was calculated as the standard deviation of the percentage contribution of each root half to the total root mass of a given plant.

Fig. 2.6 Terminal Canopy:Root Biomass Ratio
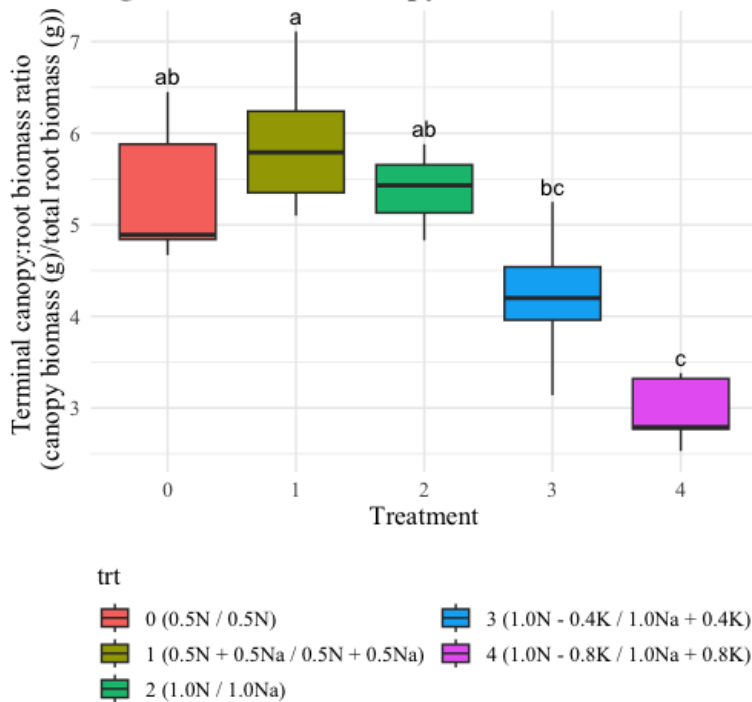
Figure 2.6 Boxplot of terminal canopy:root biomass ratio in grams, calculated on a per-plant basis as the canopy biomass divided by the total root biomass. Across all treatments, greater bias represented greater root water uptake and biomass accumulation in the non-saline compartment.

Fig. 2.7 Terminal Non-Saline Root Side Biomass

Figure 2.7 Boxplot of terminal non-salinized root biomass in grams, harvested on the seventh and final day of treatment (DAT 7). Biomass values were collected only after sample drying. Treatment 0 and 1 values were calculated as the average root biomass of all compartments within the treatment group since they lack a "saline" compartment.

Fig. 2.8 Terminal Saline Root Side Biomass

Figure 2.8 Boxplot of terminal salinized root biomass in grams, harvested on the seventh and final day of treatment (DAT 7). Biomass values were collected after sample drying. Treatment 0 and 1 values were calculated as the average root biomass of all compartments within the treatment group since they lack a "saline" compartment.

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Hydraulics

Whole Plant Marginal Water Uptake

Before treatment imposition, water uptake between groups did not differ significantly. In the 24 hours following treatment imposition, treatment group 0, which had no supplemented NaCl, nominally consumed the most water but varied significantly from only treatment 4 at a 95% CI. This lead was diminished significantly in the following 48 hours as non-uniform treatments adjusted to take up greater proportions of water from the salt-free compartment. Differences in total water uptake were statistically comparable from DAT 3 to DAT 6. On the seventh and final day of treatment, treatment group 4 depleted about 30% less water than either uniform treatment group (0, 1), reaching a statistically significant threshold with 95% confidence.

Marginal Water Uptake Root Side Bias

Before treatment imposition, treatment group 2 featured the greatest differences in marginal water uptake between root halves of each plant, but on no day was this difference statistically significant at a 95% CI. After 24 hours of treatment, clear biases developed, with treatment groups 2, 3, and 4 showing elevated bias compared to uniform control groups (0 and 1), and this pattern remained unchanged throughout the experiment. Across all seven treatment days, the collective level of water uptake bias between uniform treatment groups (0 and 1) and non-uniform treatment groups (2, 3, 4) was graphically and statistically clear, but there were no distinguishable differences between treatments within either of these groupings. Across all treatments, greater bias represented greater root water uptake in the non-saline compartment.

Fig. 2.9 Average Whole Plant Marginal Water Uptake

Treatment

- 0 (0.5N / 0.5N)
- 1 (0.5N + 0.5Na / 0.5N + 0.5Na)
- 2 (1.0N / 1.0Na)
- 3 (1.0N - 0.4K / 1.0Na + 0.4K)
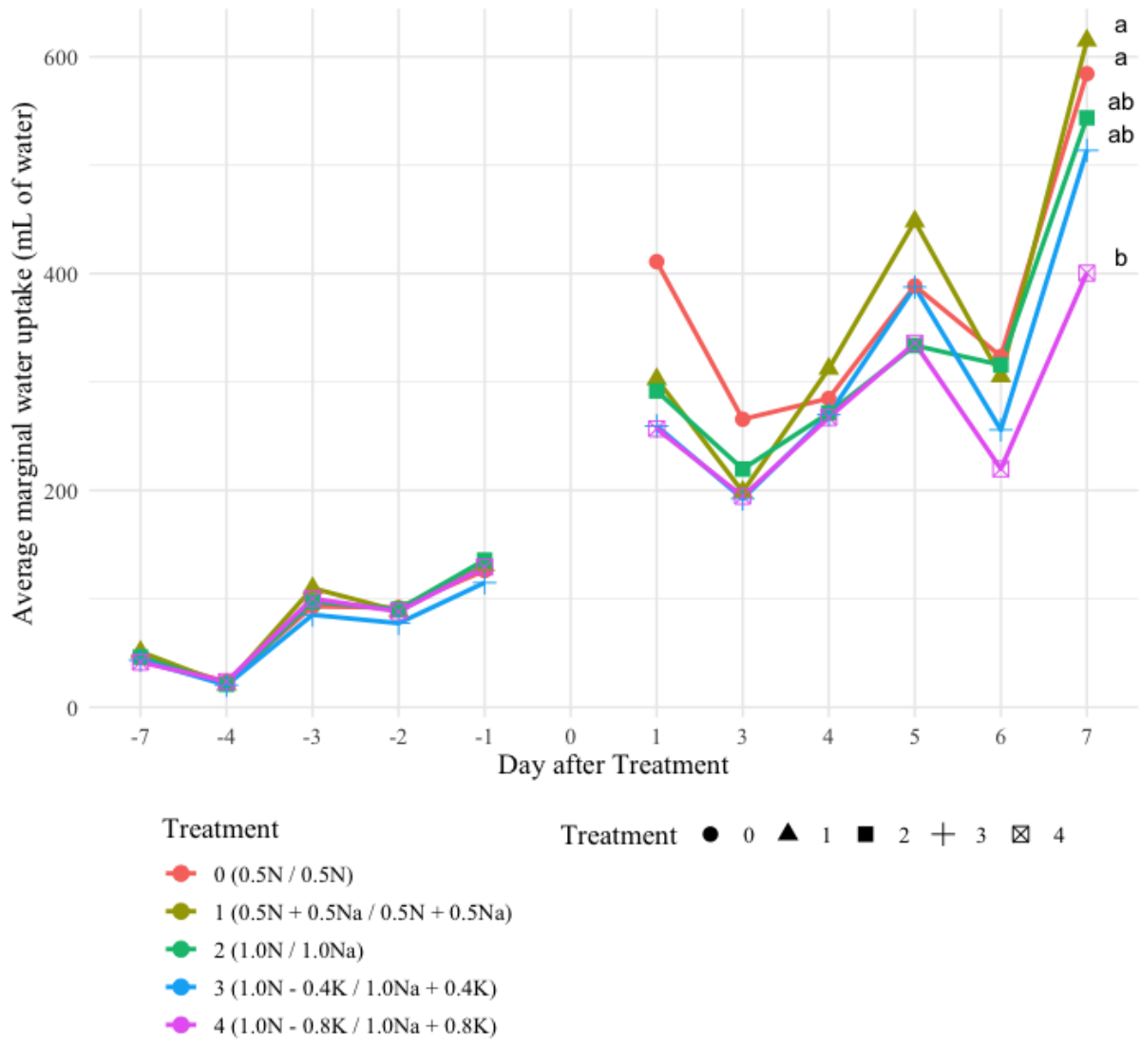- 4 (1.0N - 0.8K / 1.0Na + 0.8K)

Treatment ● 0 ▲ 1 ■ 2 + 3 ⊠ 4

Figure 2.9 Line graph of marginal water uptake over time, with statistical groupings provided comparing treatment groups on the final day of treatment. Marginal water uptake was calculated for each root compartment on each measurement day. It is equal to the amount of water that has been depleted since the most recent measurement, divided by the number of days since the last measurement. This is a proxy measure of average daily water uptake in grams. Each plant yielded two marginal uptake measurements, the summation of which equaled the whole plant marginal water uptake.
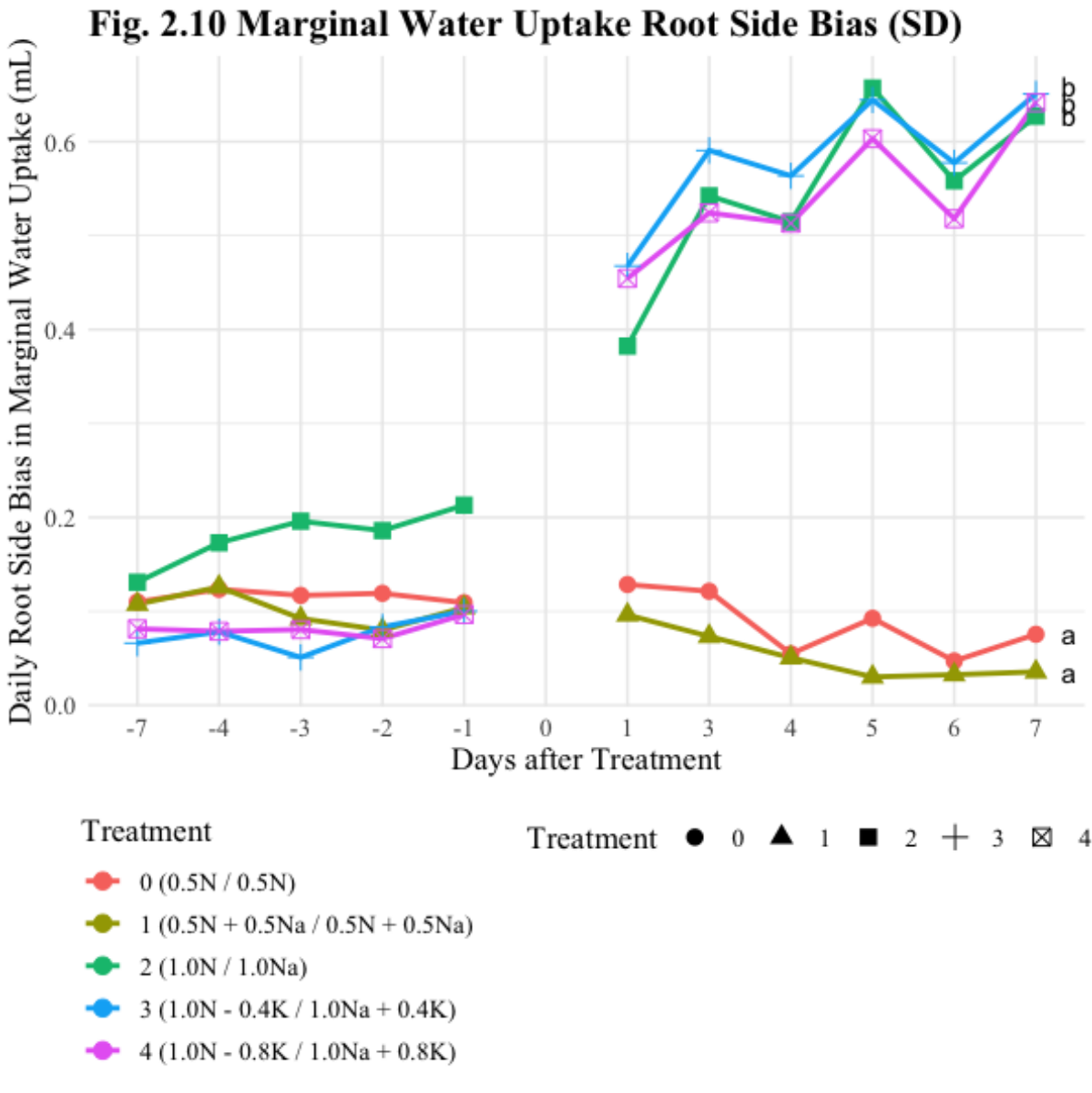
Fig. 2.10 Line graph showing the evenness of water uptake between root compartments with statistical groupings provided comparing treatment groups on the final day of treatment. Marginal water uptake root side bias was calculated as the standard deviation of the percentage contribution of each root half to the whole plant marginal water uptake. In this instance, higher values represent a greater degree of unevenness in marginal water uptake between root halves of the same plant. Across all treatments, greater bias represented greater root water uptake and biomass accumulation in the non-saline compartment.

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Ion Uptake

Changes in normalized ion concentration within each treatment group over time were graphed and analyzed for statistical significance, on both a whole plant and per-compartment basis. Not all figures display statistical groupings, but expanded figures and notes on statistical significance were included where necessary. Compartment <u>normalized ion concentrations</u> were calculated as the analyte concentration of a solution sample divided by the fractional fill level of its compartment at the time of sampling. This compensates for concentration/dilution in absolute ion concentration due to changes in water level over time, and more accurately reflects changes in concentration over time, despite fluctuating water levels with evaporation, root uptake, and refilling with deionized water.

<u>K Depletion</u>

There were stark differences in K uptake rates between compartments depending on nutrient/Na localization. Broad trends of solution K concentration shown in Fig. 2.11 reveal that in non-uniform treatments (2, 3, and 4) non-saline compartments were rapidly depleted of K, while their saline counterparts showed minimal or no change in K content over time. Interestingly, there was no apparent difference in K uptake pattern between uniform treatments 0 and 1, despite a large overall difference in NaCl content between treatments. All treatment compartments reached an asymptotically low K concentration by DAT 5 with the exception of saline compartments belonging to treatments 3 and 4 (Fig. 2.12 & Fig. 2.13). These compartments sustained their initial K concentrations for the duration of treatment, with the K-rich saline compartments of treatment 3 even increasing in K concentration to a statistically significant level by the final day of the experiment (Fig. 2.13).

Broad differences in plant-scale K utilization can be seen in Fig. 2.14. Despite initial variations in K content due to experimental error, differences in uptake patterns between treatments are clear. K that was supplied without other nutrients in NaCl-rich compartments was not depleted from solution over the course of the experiment. The apparent asymptotic decay of whole plant solution K concentration is due to depletion of K in the non-saline compartments, with values approaching the saline compartment's K concentration (Fig. 2.12 & Fig. 2.13).

Na Depletion

Broad trends of solution Na concentration shown in Fig. 2.15 revealed that only treatment 1 yielded a graphically apparent decrease in compartment Na over the duration of the experiment. At a 95% CI, the solution Na concentration of treatment 1 was measurably decreased by DAT 3 from initial levels, reaching the threshold of a statistically significant reduction again on the final day of treatment (Fig. 2.16). Treatment group 4 showed a significant reduction in solution Na from DAT 0 to DAT 3, but the concentration was stable beyond DAT 3. There was a notable variability in treatment groups 2 and 3, occasionally statistically significant but inconsistent as the experiment progressed. This is likely an artifact of variations in ICP measurements. The changes in Na concentration within treatment 1 follow a consistent downward trend with a greater magnitude than changes seen in other treatment groups. The cross-treatment difference in magnitude and directionality of these time series data may be seen in fig. 2.17, which includes linear regressions based on the changes in whole plant solution Na for each treatment group.

Whole plant solution Na concentration of treatment 0 remained at trace levels for the duration of the experiment. All remaining treatments show some decline in solution Na over time, with only

treatment 1 showing strong evidence of biological uptake. If biological uptake did occur in treatments 2, 3, or 4 it was at a rate below this experiment's sensitivity.
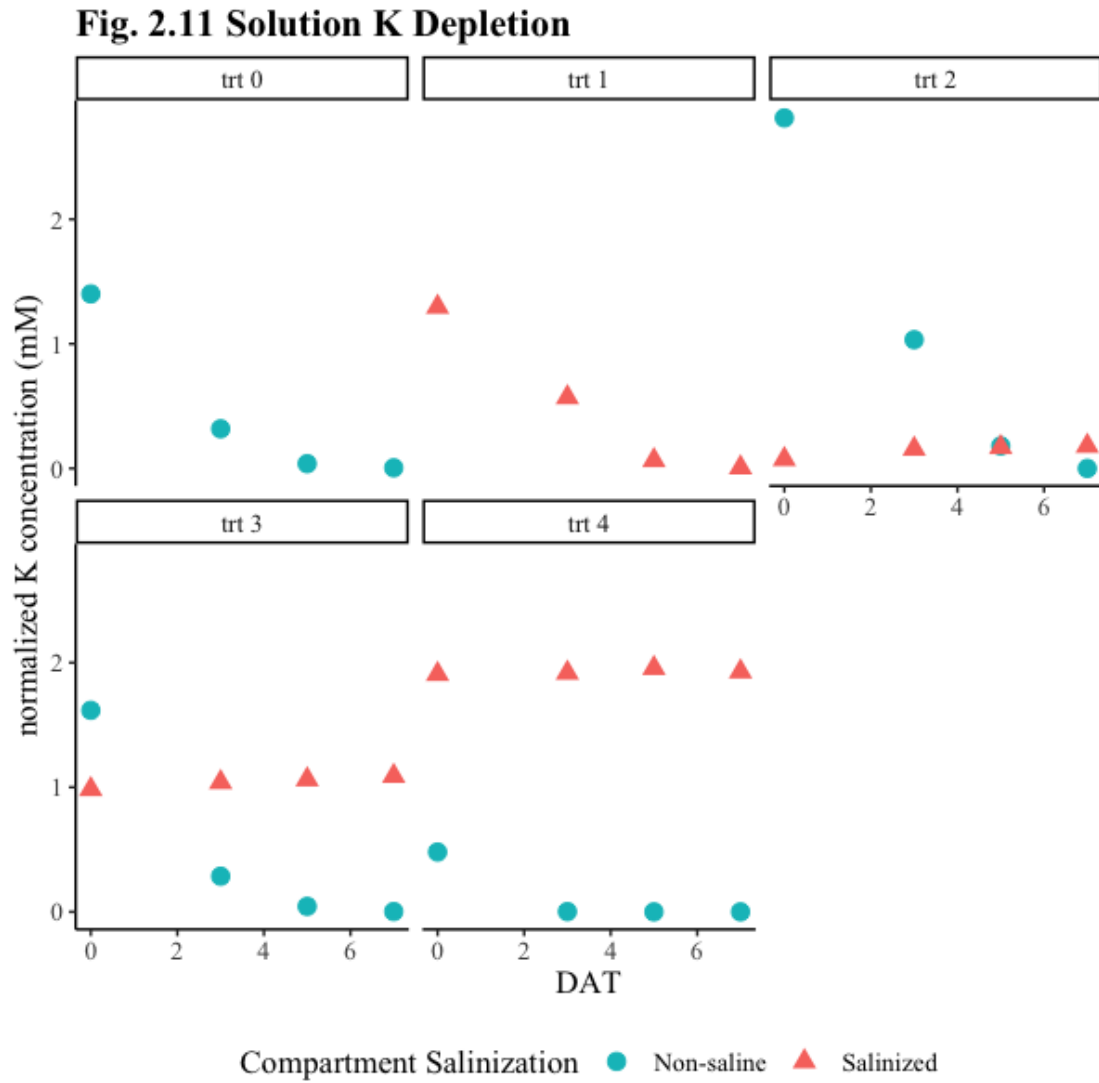

Fig. 2.11 Scatter plots of solution K concentration in saline and non-saline solutions by treatment.
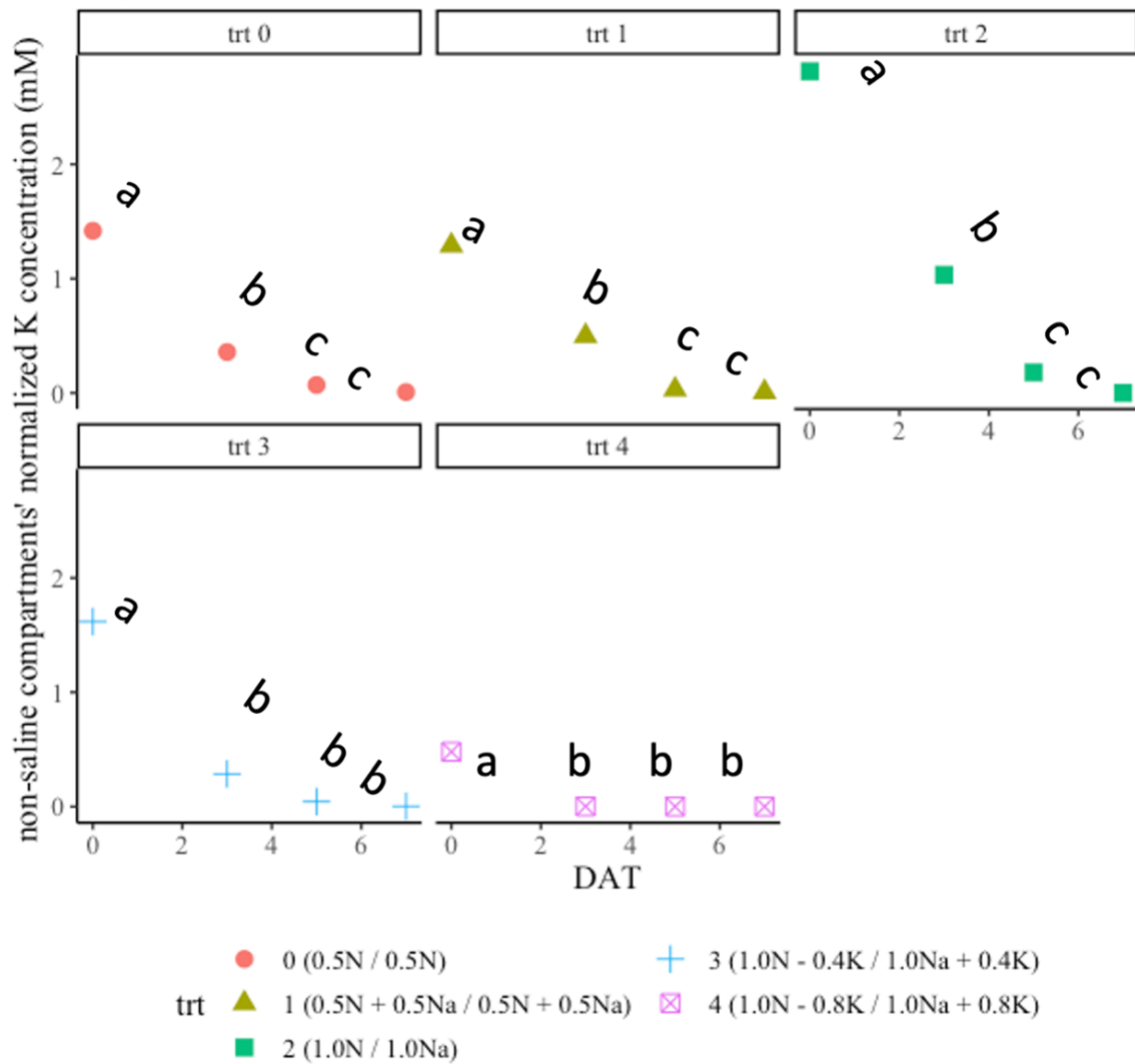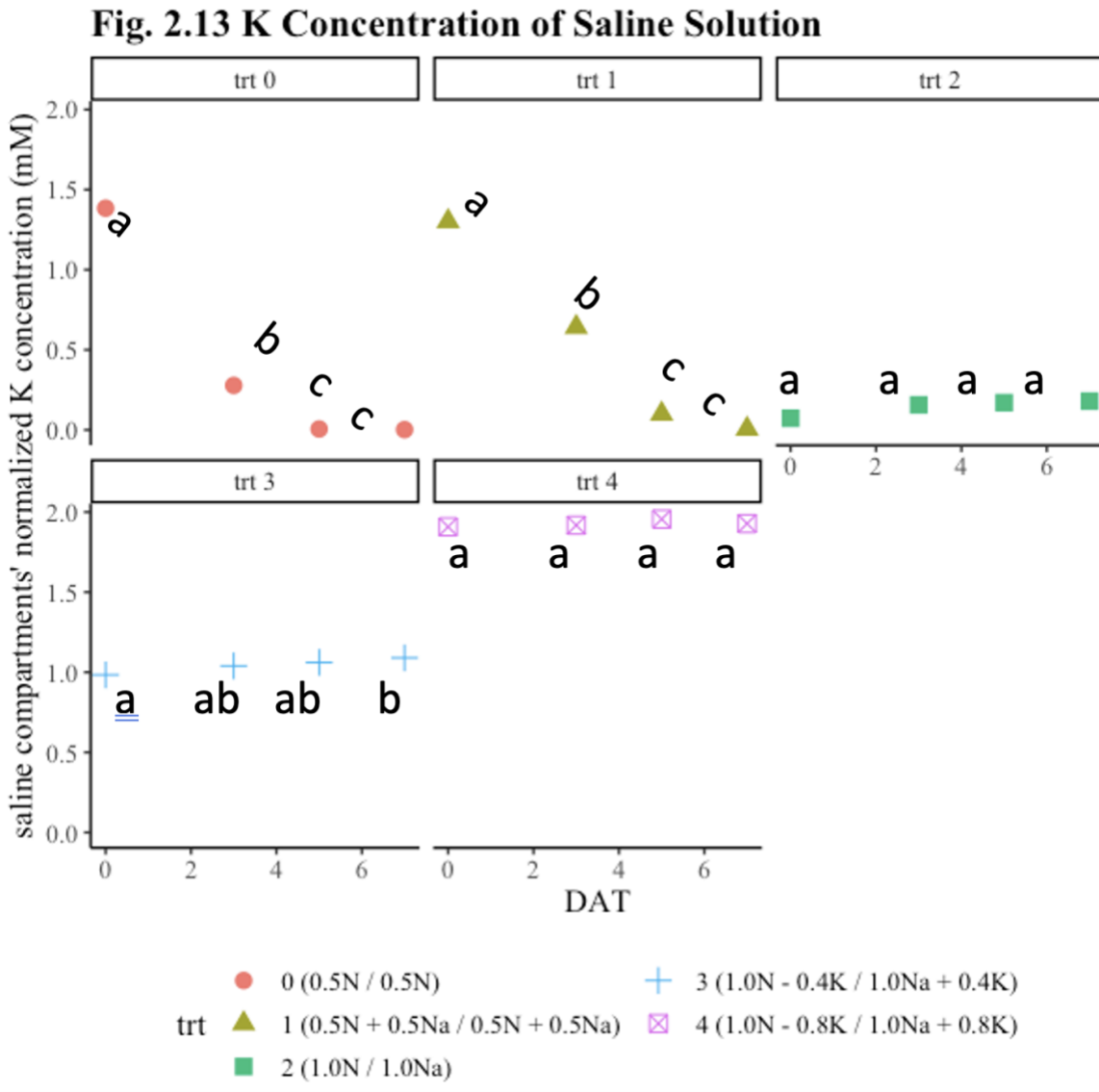
Fig. 2.12 Scatter plots of K content in non-saline compartments over time, with statistical analysis comparing changes across time within each treatment, not across each treatment. For comparison, treatment 0 and 1 averages across both root compartments are shown.

Fig. 2.13 Scatter plots of K content in saline compartments over time, with statistical analysis comparing changes across time within each treatment, not across each treatment. Treatment 0 and 1 averages across both root compartments are included for comparison.
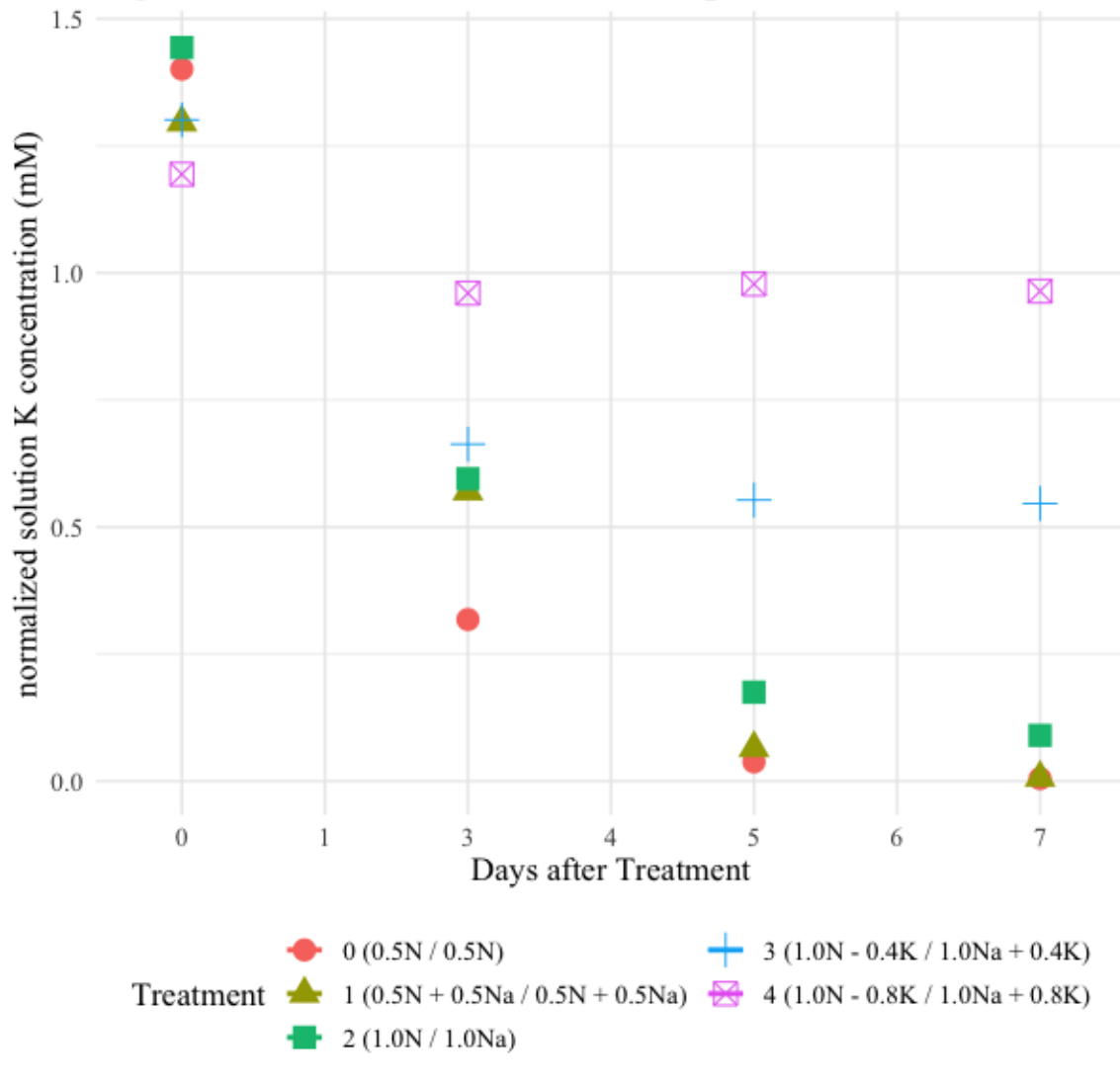
Fig. 2.14 A plot of solution K concentration averaged across both root compartments of each plant over time.
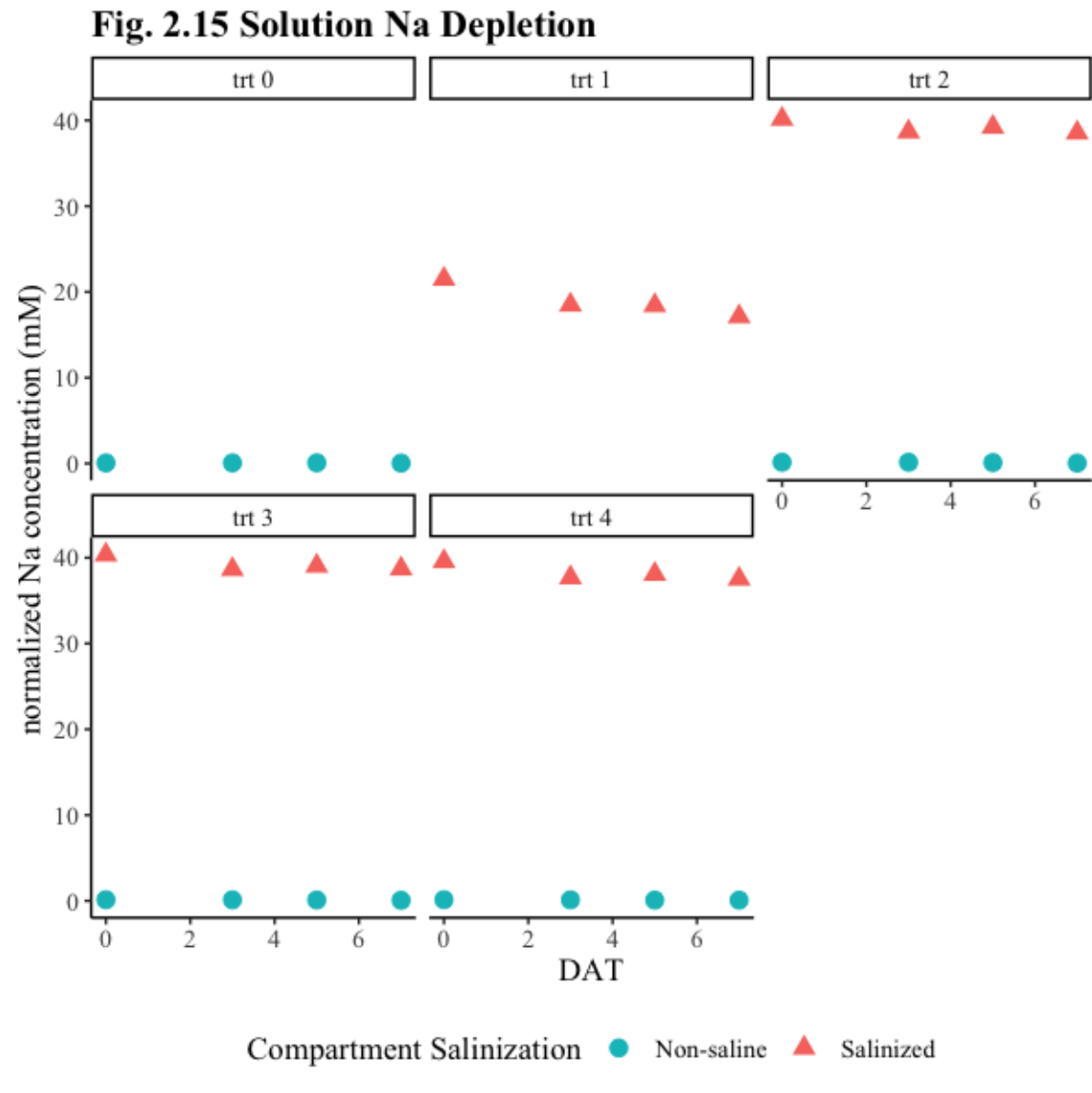
Fig. 2.15 Scatter plots of solution Na concentration in saline and non-saline solutions by treatment.

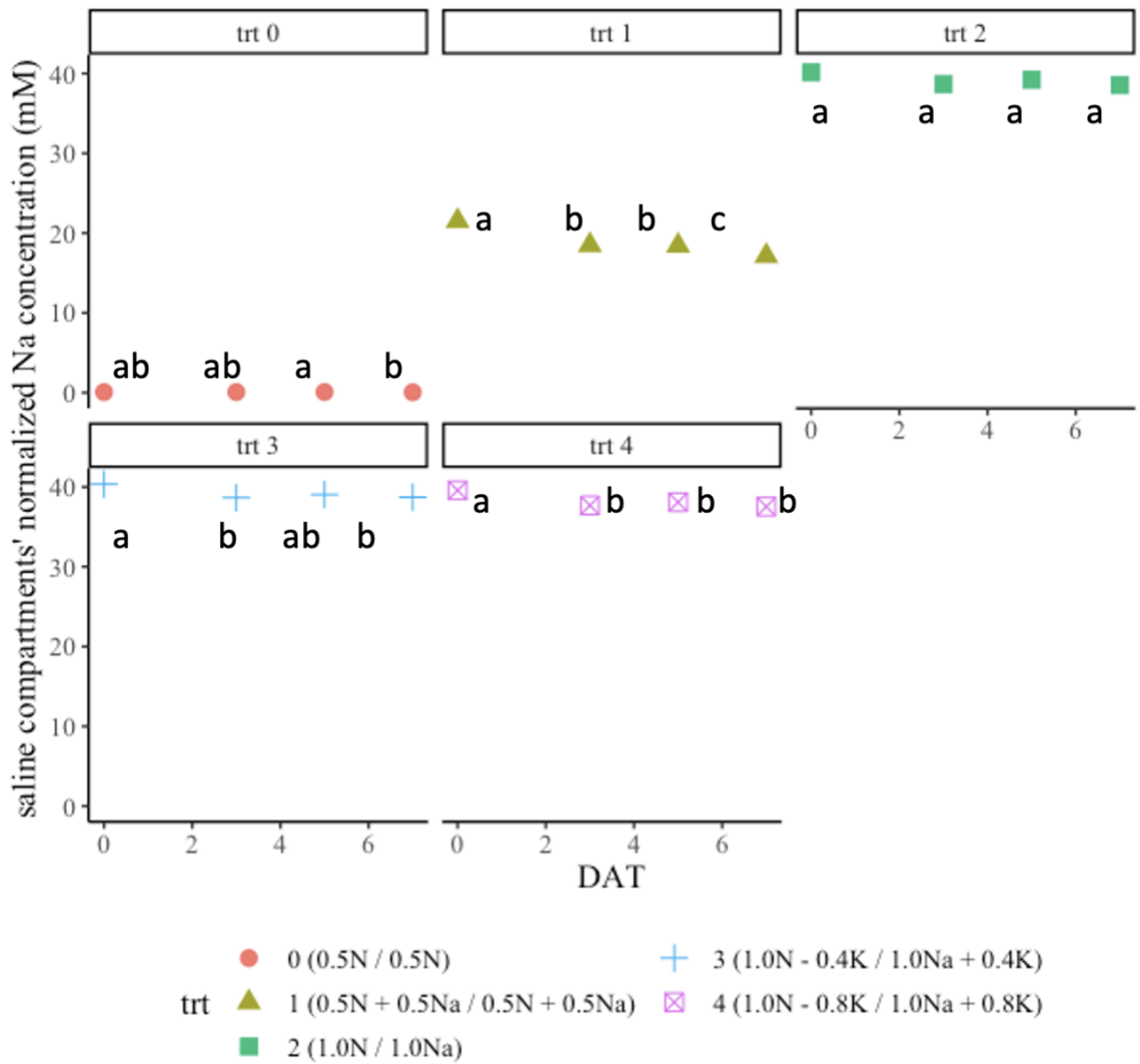# Fig. 2.16 Na Concentration of Saline Solution

Fig. 2.16 Scatter plots of Na concentration in saline compartments over time, with statistical analysis comparing changes across time within each treatment, not across each treatment. For comparison, treatment 0 and 1 averages across both root compartments are shown.
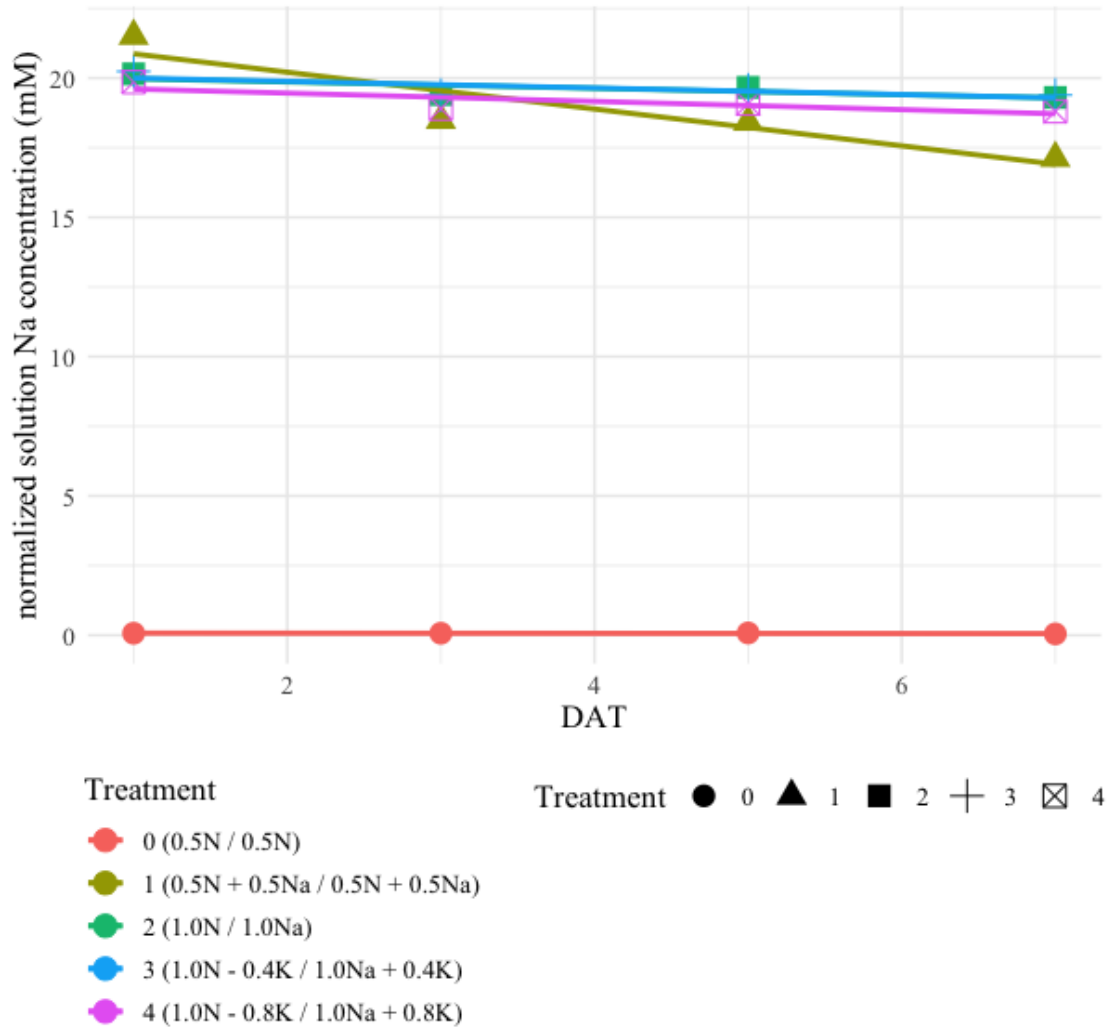
Fig. 2.17 A plot of solution Na concentration, averaged across both root compartments of each plant, including linear regressions by treatment.

Table 2.3 Slope and R-Squared Values of Linear Regression Modeling Changes in Solution Sodium over Time

treatment	slope	R-squared
0	-0.003	0.280
1	-0.661	0.844
2	-0.114	0.608
3	-0.118	0.567
4	-0.148	0.645

Table 2.3 Table displaying the relative fit and slope of linear regressions shown in Fig. 2.16. The notable rates of Na uptake within treatment 1 are underscored here by the dramatic slope and fitness of the regression showing changes in solution Na concentration over time.

Reappraisal of Hypotheses

Hypothesis III

    As the extent of potassium/Na colocalization increases, so will salinity stress.

Results were inconsistent. Compartments in which only K was colocalized with Na (treatment 3 and 4) (see Table 1 for treatment descriptions) had the lowest average total biomass values suggesting that the colocalization enhanced salinity stress and supporting the hypothesis. However, plants in which all nutrients where fully colocalized with Na (treatment 1) produced nominally more biomass than any other treatment group in apparent contradiction with the hypothesis.

Hypothesis II

    As the extent of potassium/Na colocalization increases, the rate of overall K uptake will decrease, and the rate of Na uptake will increase.

Na depletion from solution, very likely equating to Na uptake by the plant, was only observed in treatment 1, when Na and K, along with all other nutrients, were completely colocalized. While total solution K utilization decreased in response to increasing K/Na colocalization, this was not due to difference in K uptake rate from the saline compartment, rather, this trend is explained by an extreme inhibition of K uptake in the saline compartment, with overall K use efficiency then being dictated by the allocation of K supplied to the accessible, non-saline zone (i.e. treatment). Apparent root water, Na, and K uptake inhibition in the saline compartments of non-uniform treatments. Plants readily accessed K supplemented in the non-saline compartment and, even upon depletion, did not increase K uptake in the saline compartment as hypothesized.

The uniform salt/nutrient (treatment 1) were the sole instance of definitive K depletion in solution NaCl, this occurred only in the presence of all other plant nutrients. The only instance of definitive Na uptake occurred in the compartments of treatment 1, where K was being actively depleted from solution, along with other nutritive ions.

Hypothesis III

When nutrients and Na are spatially segregated, plants will show a strong bias favoring water uptake from the Na-free zone, but this effect will be progressively weakened in treatments where K is colocalized, with the greatest weakening of this apparent salt avoidance occurring in treatments with the greatest extent of K/Na colocalization.

No such effect was observed. In treatments where salt was compartmentalized, all plants showed a decisive tendency to draw water from the non-saline compartment regardless of potassium localization. There was no discernible difference in this effect between the relevant treatments (2,

3, and 4). Treatments with uniform ion distributions (0 and 1) showed not only a lack of preference between root compartments, but interestingly showed no lasting differences in whole plant water uptake rate despite the major difference in total NaCl concentration and solute potential between groups. In the first 6 days of treatment, despite dramatic changes in the location of water uptake, only the first day yielded significant differences in whole plant water uptake (between uniform controls and treatment 4). No statistically significant differences were present from DAT 3 to DAT 6. Only on the seventh and final day of treatment was treatment group 4 observed to take significantly less water than the other group, but at a level that was statistically discernible, once again, only form uniform treatments 0 and 1.

Discussion

Under heterogeneous root conditions, plants showed a tremendous capacity for plasticity in ion uptake, water uptake, and root growth, with significant changes clearly measurable 24 hours after treatment imposition in the case of water uptake, and 3 days in the case of ion uptake (possibly sooner, but not observed due to sampling interval). There were also significant differences in terminal root biomass despite treatment only occurring for the final ~20% of plants' lives. In the case of treatments 2, 3, and 4, where salinity was confined to half of the root zone, K was not taken up from the saline compartment. This inhibition was not weakened by increases in K richness of the saline compartment, or corresponding reductions in K concentration of the low-EC compartment, even when the active root zone's available potassium had been depleted. While the changes in root activity were dramatic, prior work has reported root manipulation in response to nutrient scarcity elsewhere in the rootzone (Tabata et al. 2014). Treatment group 1's seemingly unaffected biomass accumulation was unexpected, as was its seemingly unencumbered root

growth (Fig. 2.7). However, differences in total biomass accumulation are most meaningful in similar experiments over longer time periods, with effects like Na toxicity and K deficiency taking time to develop.

Within this experiment, there was a tradeoff to the salt-avoidant response when K is in the saline zone. Plants which utilized K in the presence of Na took up Na, and plants which avoided Na uptake were not able to utilize K in the same zone. Only treatment group 2, with complete separation of Na and all nutrients, was able to maintain high levels of K utilization while avoiding Na uptake.

The lower solute potential in sodium-rich compartments of treatments 2, 3, and 4 clearly alter root hydraulic and ionic resistance, regardless of K supplementation, resulting in no K or Na uptake (Karlova et al. 2021; Leal et al. 2022). Plants of treatment 1, where all compartments were rich in both nutrient and Na, were able to take up water and potassium at a comparable rate to other plants, but with apparent absorption of Na as well. Even though treatment groups 2, 3, and 4 were able to avoid Na uptake, they also failed to take up water or K from the saline compartment. These results represent a drawback to nutrient/Na colocalization where Na avoidance will also reduce nutrient acquisition in the saline region. This effect is seen here in root compartments of 40 mM NaCl enriched with KCl and no other nutritive ions. This effect being a consequence of an indiscriminate mechanism such as suberization may accommodate the observed continuation of water, nutrient, and sodium uptake seen in treatment 1 plants, which were exposed to an equivalent amount of sodium, but at half the concentration across the entire root zone and therefore with a less intensely negative solute potential at any one root location relative to the 1.0x NaCl treatment buckets. Even

if plants are successful in avoiding Na uptake, they may suffer an opportunity cost with biomass or overall productivity due to reduced nutrient uptake. Salinity is alos observed to reduce organic matter breakdown, microbial activity, and nutrient mineralization (Pecher et al. 2019; Yang et al. 2020).

One limitation of this research is that it fails to emulate the continuous in situ distributions or physio/chemical properties of ions present in agriculture. While distinct ion gradients are possible in soils (D. L. Corwin and Lesch 2005), such extreme compartmentalization, especially with potassium so isolated from other nutrients, is unrealistic. Soil-based experiments are a logical continuation of similar solution culture work in order to capture these distributions (Bazihizina, Barrett-Lennard, and Colmer 2012). This work also only evaluates a single per-plant sodium concentration, which would only reflect one possible salinity level out of many that are found in nature. Single concentration experiments may still be a practical value when exploring what is ultimately a highly multivariate and complex physiobiological response.

Future research should better differentiate the distinct effects causing this strong inhibition, a consideration of osmotic effects, utilizing lower NaCl concentrations, and changing ion distributions, or matching solute potential between compartments using biocompatible osmotica such as polyethylene glycol (PEG). The possibility of an "entourage effect" should also be investigated, where potassium may only stimulate root activity in the presence of other nutritive ions. These phenomena are no doubt both present, knowing that plant roots possess a potassium sensing niche (Wang et al. 2021) and changes in water uptake patterns may occur without biological intervention as a consequence of water potential alone (Caldwell, Dawson, and Richards

1998). And indeed, there is reason to believe that these phenomena are not entirely independent. Plants have been reported to utilize water from relatively low solute potential compartments when the alternative is a nutrient-void solution (F. Valenzuela, Fuentes, and Brown 2022).

Salinity management work has focused on field-scale models of salinity, and great strides have been made, but development of more precise agricultural practices will require supporting physiological investigation. There is potential for these benefits to carry favorably for perennial crops which accumulate salts in tissues across many growing seasons (Drakakaki et al. 2017; Walker, Torokfalvy, and Behboudian 1987; Boland, Jerie, and Maas 1997). In addition to input-intensive mechanisms of salinity resilience, there is a need for deeper physiological understanding of salt stress as it occurs in agricultural production so that it can be addressed in a resource-efficient, stress-avoidant manner. Unfavorable ions, their uptake and movement within plants, are mechanistically similar to nutrients, so it is sensible to consider their interactions if the issue of undesirable sodium uptake is to be addressed (Raddatz et al. 2020; Garcia and Charbaji 1993).

The two canonical phases of salinity stress, osmotic and ionic, are fundamental, but the distribution of salts in relation to nutritive ions may prove integral to the holistic understanding and management of salinity stress. With relatively little research connecting nutrient physiology with salt stress physiology, thoughtful investigation in this area can make great gains in understanding.

Conclusion

Plants have remarkably high morphological and functional plasticity under heterogeneous conditions. Consistent with many reports, plants grown in split root systems showed comparable

water uptake rates regardless of treatment with strong compensatory responses between root zones (Yan, Bogie, and Ghezzehei 2020; Bazihizina, Barrett-Lennard, and Colmer 2012). When salt was compartmentalized, nearly all water and ion uptake occurred in the non-saline compartment. When K was supplemented to these salinized compartments, it was not depleted from solution, even as the non-saline compartment became depleted of K. This apparent inhibition of root activity was not seen \here nutrients and NaCl were supplemented in all compartments. Importantly, plants provided uniform nutrient and no salt free root zone, took up far more sodium than any other group. While plants avoided Na uptake when an alternate low salt root zone occured, they also avoided uptake of K that was present in the saline compartment, resulting in greatly reduced K use efficiency.

It is impossible to conclude here if the effects reported above are due to ion-specific mechanisms or non-specific responses to solute potential. Much future work can be done to equalize the water potential across compartments to see what responses are attributable to ionic effects specifically. This interplay between osmotic and ion-specific effects, and its influences on ion uptake patterns offers a wealth of research opportunities. While these experiments were inspired by the ion gradients that exist in the natural world, a root compartment which is rich in water, Na, K, and Cl is not realistic. K likely functions in concert with other elements to modulate root activity in saline conditions. Given the value of irrigated agriculture, there is a need to determine what circumstances may stimulate root activity in the saline zone, and what the consequences for plant productivity may occur over a longer time scale than this experiment.

Prior work has shown a reduction in root activity upon the removal of K from nutrient-rich, saline compartments, suggesting that K plays a key role in modulating root activity in saline conditions (F. Valenzuela, Fuentes, and Brown 2022). In the context of this experiment, it is clear that K alone cannot stimulate water, Na, or K uptake in salinized zones when non-saline, nutrient-rich zones are available.

Funding Acknowledgement

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