Received: 11 August 2022 | Accepted: 3 September 2022

DOI: 10.1002/jpln.202200279

RESEARCH ARTICLE

Local and systemic metabolic adjustments to drought in maize: Hydraulic redistribution in a split-root system

Lena Maria Werner[1] | Roman Paul Hartwig[1] | Isabelle Engel[1] | Bastian Leander Franzisky[2] | Stefanie Wienkoop[3] | Martin Brenner[3,4] | Julian Preiner[3] | Dagmar Repper[1] | Jens Hartung[5] | Christian Zörb[1] | Monika Andrea Wimmer[1]

[1]Institute of Crop Science, Department Quality of Plant Products, University of Hohenheim, Stuttgart, Germany

[2]Department Soil Science and Plant Nutrition, Hochschule Geisenheim University, Geisenheim, Germany

[3]Unit of Molecular Systems Biology, Department Functional and Evolution Ecology, University of Vienna, Vienna, Austria

[4]Department of Pharmaceutical Sciences, University of Vienna, Vienna, Austria

[5]Institute of Crop Science, Department Biostatistics, University of Hohenheim, Stuttgart, Germany

Correspondence
Monika A. Wimmer, Institute of Crop Science, Department Quality of Plant Products, University of Hohenheim (340e), Emil-Wolff-Str. 25, 70599 Stuttgart, Germany.
Email: m.wimmer@uni-hohenheim.de

This article has been edited by Klaus Dittert.

Funding information
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)

Abstract

Background: It is yet unknown how maize plants respond to a partial root drying under conditions of a limited total water supply, and which adaptation mechanisms are triggered under these conditions.

Aims: The aims of this study were to assess whether partial root drying results in distinguishable local and systemic physiological and metabolic drought responses, and whether compensatory water uptake and/or alteration of root architecture occurs under these conditions.

Methods: Maize plants were grown in a split-root system. When plants were 20 days old, the treatments 'well-watered', 'local drought' and 'full drought' were established for a period of 10 days. Shoot length and gas exchange were measured non-destructively, root exudates were collected using a filter system and biomass, relative water content, osmolality and proline content were determined destructively at final harvest.

Results: Local drought triggered stress responses such as reduced biomass, shoot length, relative water content and increased osmolality. Maintained root growth was systemically achieved by hydraulic redistribution rather than by altering root architecture. Local and systemic osmolyte adjustments contributed to this hydraulic redistribution.

Conclusions: Both local and systemic metabolic responses helped the plants to induce hydraulic redistribution, enhance water availability and in consequence plant water relations. This resulted in a surprisingly well-maintained root growth even in the drought stressed root compartment.

KEYWORDS
exudate collection, local drought, partial root drying, rhizosphere, root exudates

1 | INTRODUCTION

A consequence of climate change is water scarcity in many agricultural regions. To cope with drought, plants use several strategies, including stomatal closure to reduce transpiration, thereby limiting water loss and stabilizing the carbon status (Tardieu et al., 2018). Another strategy is osmotic adjustment by accumulation of compatible solutes in order to maintain a gradient in water potential between the bulk soil and the plant, thus upholding water movement and cell turgor (Blum, 2017), which helps in preserving root growth and reorganizing root architecture to allow access to water in deeper soil layers (Dietz et al., 2021). However, the synthesis of compatible solutes is energy-consuming and can lead to suboptimal plant growth especially in case of a quickly restored water supply (Poorter et al., 2012). Thus, maintaining water relations by other mechanisms would represent a less costly adaptation strategy. One option is hydraulic redistribution (HR), which is the passive movement of water from moister to drier soil regions following a gradient in the soil water potential, using the roots as a conduit (Burgess et al., 1998). Such HR includes hydraulic lift, that is, the uplift of water from deeper to shallower soil layers (Caldwell et al., 1998), but also refers to lateral or downward water movement (Hafner et al., 2017).

Drought responses are triggered locally at the cellular level (*e.g.*, production of osmolytes), but systemic signalling results in responses of the whole plant. For example, drought perception in roots stimulates the production of abscisic acid (ABA), which is transported to the shoot and induces stomatal closure (Tardieu et al., 2018). Depending on plant species, other signalling components include brassinosteroids, strigolactones, ethylene, sap pH or the small peptide CLE25, which moves through the vasculature to plant leaves where it activates ABA production via NCED3 (Gupta et al., 2020). It is to our knowledge not yet clear whether partial root drying results only in local metabolic responses, or also sets off measurable systemic adaptations of the whole plant.

The quantity and composition of root exudates can also vary in response to water deficit (Gargallo-Garriga et al., 2018). Carbon skeletons derived from photosynthetic CO_2 fixation serve as precursors for synthesis of compound classes detected in exudates, such as sugars, amino acids, organic acids, fatty acids, sterols, vitamins, growth factors, enzymes, flavonoids, nucleotides and purines (Vives-Peris et al., 2020). The mechanism of CO_2 concentration also affects plant exudate production, as C_4 plants release more amino acids compared to C_3 plants (Vranova et al., 2013).

Drought stress alters not only the amount but also the composition of root exudates (Chen et al., 2022). This may improve the contact of the root movement through and the nutrient uptake from the soil (Gargallo-Garriga et al., 2018). Following osmotic adjustment of the plant, concentrations of metabolites in exudates can increase (Gargallo-Garriga et al., 2018), thus lowering the water potential of the rhizosphere and improving water flow to the root. Since exudates also attract beneficial microorganisms promoting plant recovery after a stress event (Munoz-Ucros et al., 2022), a better understanding of root exudation in response to drought could contribute to secure crop production. Despite known effects of root exudates in the rhizosphere, it is—to our knowledge—not clear if changes in exudate composition can be triggered under local stress in roots. Given that roots directly sense the water content in soil (Schachtman & Goodger, 2008), such a local response might be feasible.

Split-root settings are suitable to induce partial root-zone drying by irrigating just half of the root system. They are excellent systems to assess local responses to drought and were used in several studies, for example, to demonstrate increases in the water use efficiency under partial drought for several species (reviewed in Schachtman & Goodger, 2008). For maize, partial root-zone drying induced a compensatory increase in the total water uptake from the irrigated root half (Hu et al., 2011). However, all these studies provided unlimited water to maintain an optimum soil water content in the irrigated root compartment. Here, using a split-root system, the overall aim of this study was to assess whether local and locally induced systemic physiological drought responses can be distinguished under conditions of partial root drying combined with limited total water supply. Specifically, we addressed two hypotheses: (1) plants exposed to partial root drying respond locally by compensatory increased water uptake from the watered root half and/or compensatory root growth; (2) partial root drying induces local and systemic acclimation strategies which act synergistically to improve water relations in the plant.

2 | MATERIALS AND METHODS

2.1 | Plant growth conditions

The *Zea mays* line B73 was used in all experiments. All seeds were provided by the group Crop Functional Genomics of the University of Bonn. Seeds were sterilized with 10% H_2O_2, rinsed in water and soaked for 4 h in saturated $CaSO_4$ solution. Seeds were then germinated between layers of filter paper (REF 150010; MN: 710; Macherey-Nagel, Germany) and imbibed with 4 mM $CaSO_4$ solution in a dark climate chamber (24°C, 65% relative humidity [rH]; WeißTechnik Fitotron HGC 0714). When primary roots reached a minimum length of 1 cm, they were cut off, and seedlings were kept between filter paper soaked with 2 mM $CaSO_4$ solution until the shoot emerged (day 4 after sowing [DAS]), then they were exposed to light (350 μM m^{-2} s^{-1} PAR; 12 h per day), with roots covered. On DAS 7, seedlings were transferred to soil filled split-root rhizoboxes (Figure 1B). Soil sieving, filling and fertilization were performed as described in Vetterlein et al. (2021). Emerging lateral roots were evenly distributed between the root compartments. Boxes were covered with a black sheet and placed at a 52° angle. The experiment was conducted in a greenhouse at the University of Hohenheim (48°42′39.2"N, 9°11′53.0"E) with an average temperature of 24.6°C, rH of 51% and LED lights adjusted to 400 μM m^{-2} s^{-1} PAR for 12 h per day. Beneficial insects were used for pest control.

2.2 | Experimental setup and watering regime

Until DAS 19, all plants were watered daily with filtered rainwater (120 mesh/130 micron, Netafim, Germany) to a volumetric soil water content (VWC) of 22% (v/v), determined by weighing the rhizoboxes. On DAS 20 (day of treatment [DOT] 0), the three treatments, well-watered (WW), full drought (FD), and local drought (LD), were established. WW plants were kept at 22% VWC in both root compartments. In LD, one root compartment (LD_{wet}) was supplied with half of the amount of water of WW, while the other compartment did not receive any water (LD_{dry}). In FD, water supply was completely stopped. Plants were harvested at DOT 10.

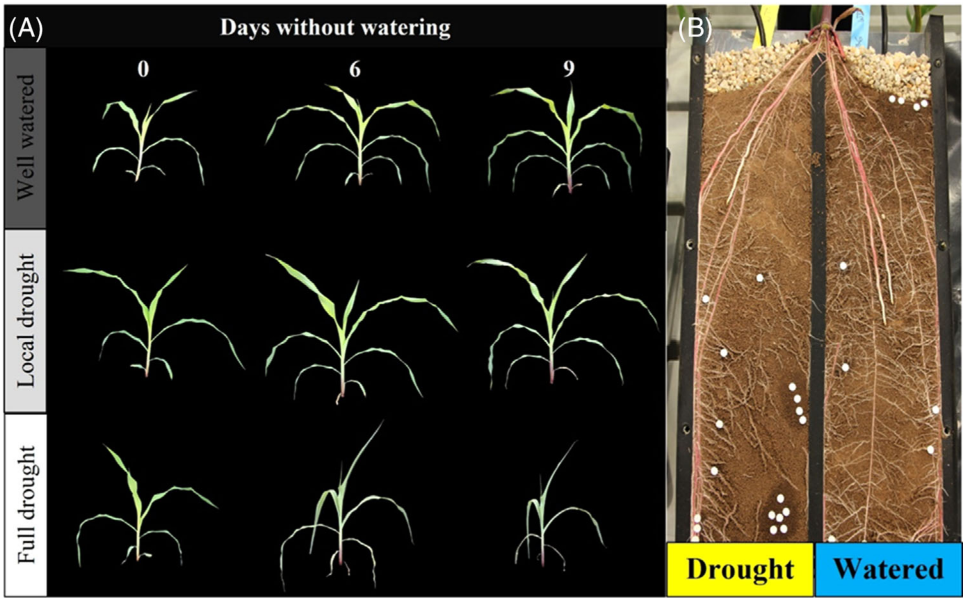

FIGURE 1 Shoots of *Zea mays* at 0, 6 and 9 days of the treatments well-watered, local and full drought (A) and roots after 9 days of local drought treatment with the watered right side and the non-watered left side of the rhizobox (B). Filters for exudate collection were placed consistently on larger roots (50%) and on smaller roots (50%), and additionally on root-free bulk soil in the lower half of the rhizobox.

One-third of the water was always supplied from the top, and two thirds via angle arrow droppers (Netafim) fixed to the centre of each root compartment and connected to an irrigation system (multi-control duo, Gardena, Germany). Preliminary tests validated a consistent water supply accuracy and even water distribution.

2.3 | Non-destructive measurements of shoot length and gas exchange

Shoot length and gas exchange were determined daily 2 h after turning the lights on. Photosynthetic rate (A) and transpiration rate (E) were measured on the second youngest fully elongated leaf (FEL) (20 cm from the leaf tip) using a leaf chamber/soil respiration analysis system (L.MAN-LCI; ADC BioScientific Ltd. Hoddesdon, Herts, EN11 0DB).

2.4 | Sampling and analysis of root exudates and rhizosphere compounds

Root exudates and rhizosphere metabolites were collected on DOT 9 with filter papers according to Neumann et al. (2014). Sampling started 2.5 h after lights were turned on and lasted for 3 h. Briefly, 10 sorption filters (diameter 0.5 cm; MN818; Macherey-Nagel, pre-rinsed in 80% methanol and MilliQ water) per root compartment were placed on exposed roots in the lower rhizobox half to ensure that sampled roots had indeed grown during (and not before) the treatment period (Figure 1B). Filters were kept moist, and roots were protected from light by a black foil. After removal, they were immediately frozen (−80°C) and freeze-dried before analysis. Rhizoboxes were returned to their positions for 24 h before harvest on DOT 10.

Metabolites were extracted from filters in two steps by incubating in 80% methanol and 80% ethanol (each at 95°C for 30 min). Derivatization was carried out according to Mehmeti et al. (2013). In brief, 20 μL of methoxyamin hydrochlorid in pyridine (40 g L^{-1}) was added to each sample/standard, the pellet completely dissolved and samples were incubated at 30°C for 90 min in a shaker (700 rpm). Subsequently, they were silylated by adding 80 μL MSTFA (95%–100%, Macherey-Nagel) and incubating at 37°C for 30 min (700 rpm).

GC-MS measurement was performed as described in Turetscheck et al. (2017), with an Agilent 7890B GC coupled to a LECO Pegasus® BT GC-TOFMS (LECO Corporation, Michigan, USA). Raw data were processed with the LECO Chroma-TOF® software (LECO® Corporation, Michigan, USA).

Metabolites were identified using MS-Dial (ver. 4.60) (Tsugawa et al., 2020). Data were exported as centroid and nominal masses and converted utilizing Reifycs Abf Converter. Settings were chosen as follows: smoothing level 3, average peak width 20, minimum peak height 1 × 10e4, mass slice width 0.5 and mass accuracy 0.5. Measured alkanes were used for retention index calculation, with a retention index tolerance set to 20. Gap filling by compulsion was activated and sample max/blank average filter was set to 5. All metabolites were normalized to the internal standards phenyl ß-D-glucopyranoside and pentaerythritol, according to their minimum distance of retention index (Weiszmann et al., 2020). Confirmation of level 1 identification (Schymanski et al., 2014) was given by measuring a mixture of standard compounds in different concentrations within each batch. Relative quantification of metabolites was done by normalized peak intensities of the quant masses of all target metabolites. These relative values were utilized for further statistical data analysis.

Even though the filter collection method is established for sampling of root exudates (Neumann et al., 2014), some shortcomings should be considered. First, because the volume of collected solution cannot be determined, absolute quantification of metabolites is not possible. To assess treatment effects, the sum of all detected peak intensities was calculated, and each compound was expressed in percent of the total sum. For construction of the heatmap, mean values of the treatments were further expressed as fold-change relative to the WW conditions. Secondly, collected samples represent a mixture of root exudates and metabolites present in the rhizosphere before the sampling (*e.g.*, possibly also produced by microorganisms), and the contribution of each source cannot be distinguished. For reasons of simplicity, we consciously use the term 'exudate' throughout this study even though strictly speaking it should be 'exudates and surrounding rhizosphere compounds'.

2.5 | Final destructive harvest

On DOT 10, all plants were harvested to determine root and shoot biomass, relative water content (RWC), osmolality and proline. Additionally, soil water content was determined. The harvest started 2 h after turning the lights on and was performed within 3 h to avoid bias resulting from diurnal variance (Hachez et al., 2008). Each parameter was determined in defined parts of the plants, that is, the second FEL (distal 25 cm) was used for osmolality, the third FEL for proline extraction and the fourth FEL (distal 15 cm) for RWC. In addition, approximately 1 g of roots from the lower half of the rhizobox were also used for proline extraction. Fresh weight of all sampled material was determined.

Leaf RWC was determined according to Wedeking et al. (2016), using two 4 cm long leaf segments without midrib. Osmolality was determined in cell sap collected by repeated freezing/thawing of leaves and centrifuging (5 min, 3600 × *g*). Duplicates were analyzed using a vapour pressure osmometer (Vapro, Model 5600, ELITech). For proline extraction, leaves and roots were cut, washed twice in deionized water (roots) and ground in liquid N_2. Extraction was performed with 30 mg FW in 1.5 mL 70% (v/v) ethanol (80°C, 20 min) and centrifugation (RT, 5 min, 18,800 × *g*). The supernatant was mixed (1:1) with a ninhydrin solution (1% [w/v] ninhydrin in 60% [v/v] acetic acid and 20% [v/v] ethanol), heated (95°C, 20 min), cooled on ice and measured photometrically (TECAN infinite M nano) at 520 nm (modified from Chinard, 1952). Remaining roots were washed, blotted and oven-dried (65°C) together with the remaining shoot for dry weight (DW) determination.

Soil samples from three different depths (0–10, 10–20, and 20–30 cm) of each root compartment were mixed, weighed (FW), dried at 105°C and reweighed (DW) to determine gravimetric soil water content (GWC).

2.6 | Experimental design and statistics

Treatments were randomized to pots/plants according to a resolvable row-column design with six replicates, two rows and six columns per replicate. Additionally, columns of two subsequent replicates were latinized, resulting in additional complete blocks. Replicates were allocated side-by-side, forming two rows and 36 columns. Thus, units of 1 × 12 and 2 × 6 form complete blocks.

Data were analyzed according to the design with the following mixed model:

$$y_{ijklmn} = \mu + b_k + d_l + r_{lk} + c_{mk} + p_{lmk} + \tau_i + \varphi_j + (\tau\varphi)_{ij} + e_{ijklmn}, \qquad (1)$$

where y_{ijklmn} is the observation of genotype i treated with watering treatment level j in side n of row k, column m with the complete blocks k and l; μ is the intercept; b_k and d_l are the fixed effects of complete block k and l; r_{lk}, c_{mk} and p_{lmk} are the random effects of row l, column m and pot lm within replicate k; τ_i, φ_j and $(\tau\varphi)_{ij}$ are the fixed main and interaction effects of genotype i and treatment j; and e_{ijklmn} is the error of y_{ijklmn} associated with the side. The error variance was allowed to be genotype, treatment or genotype-by-treatment specific if this increased model fit was measured via AIC (Wolfinger, 1993). Note that WW, LD and FD treatment resulted in compartments treated with four treatment levels: WW, LD_{wet}, LD_{dry}, and FD. Normal distributed and homogeneous variance of residuals were checked graphically via residual plots. If necessary, data were square-root, log or logit transformed prior to analysis to fulfil these pre-requirements. Adjusted means were back-transformed for presentation purpose only. Standard errors were back-transformed using the delta method.

The current study considers a single genotype and data from pots harvested at DAS 40, even though the experiment included another genotype and harvest time. All available data were used to adjust means and to estimate variances. Afterwards, results were limited to the genotype and harvest of interest.

For gas exchange measurements, only two-thirds of the pots were randomly measured. As information about row and column effects per day is sparse and can cause convergence problems, both effects were dropped from the model. Additionally, blocks are incomplete now and thus were fitted as random.

3 | RESULTS

3.1 | Plant growth and water uptake

Ten days of LD did not lead to visual drought stress symptoms, while such symptoms (*e.g.*, wilting) were observed in the FD treatment after 6 DOT (Figure 1A). Growth was significantly reduced beginning after 5 DOT (shoot length, Figure 2A) and after 10 DOT (shoot dry weight, Figure 3A), respectively. At the final harvest, shoot DW was reduced by 26% (LD) and by 59% (FD) in comparison to WW, respectively (Figure 3A). The root dry weight did not differ between LD_{wet}, LD_{dry} and WW and was also similar in LD_{wet} and LD_{dry}, but was significantly reduced under FD (Figure 3B). The ratio between root and shoot dry weight was significantly increased in both LD and FD (Figure 3C).

Significant differences were observed in photosynthetic (A) and transpiration (E) rates between LD and FD (Figure 2B,C).

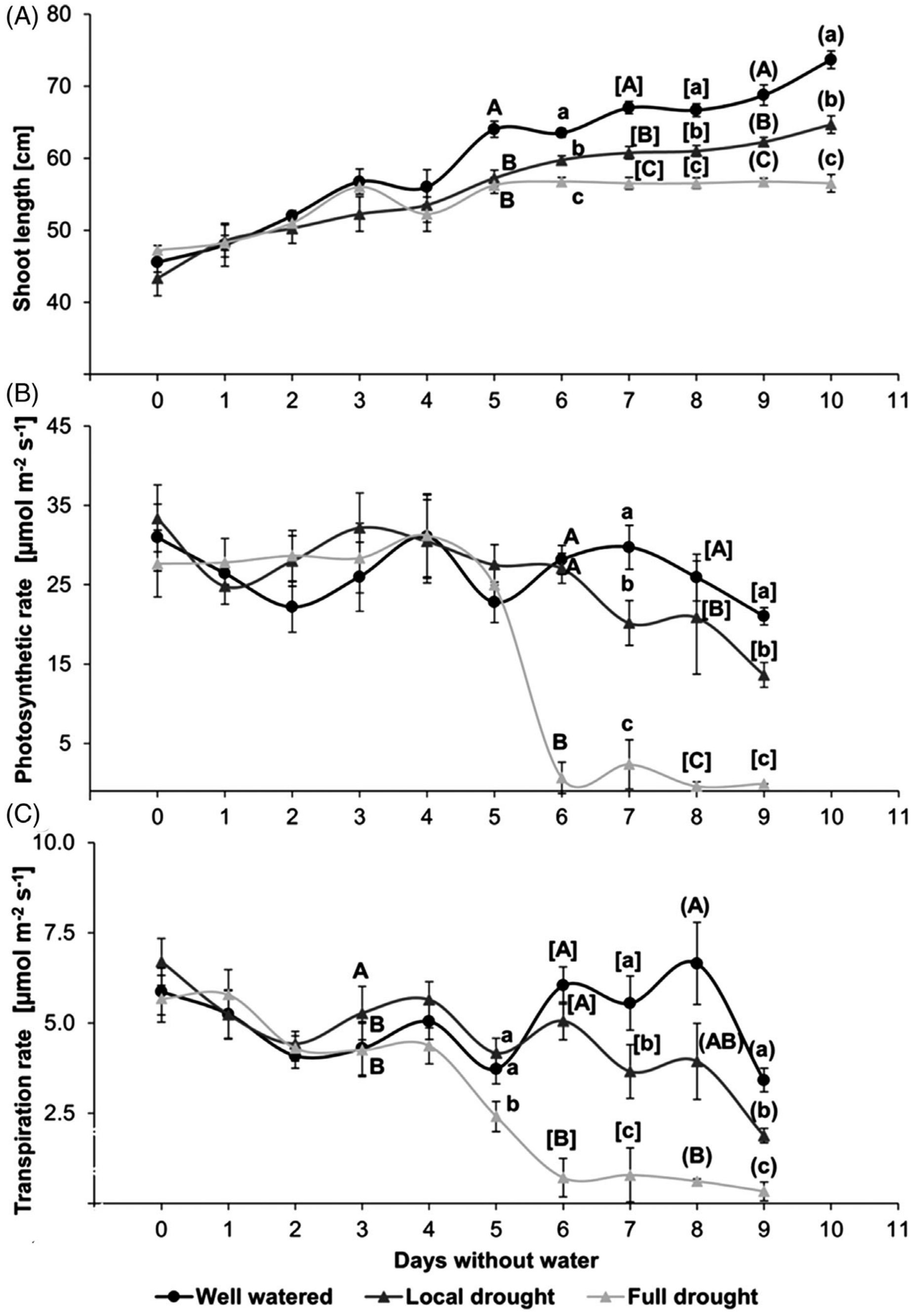

FIGURE 2 Shoot length (A), photosynthetic rate (B) and transpiration rate (C) of *Zea mays* during 10 days of well-watered (filled circles, black), local drought (triangles, dark grey) and full drought (triangles, light grey) treatments. Values represent adjusted means, and error bars indicate the corresponding standard errors ($n = 4$). Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test) between the treatments well-watered, local drought and full drought. Significance was tested for each individual day. Significance per day is indicated by capital and lowercase letters and non or differing brackets.

Under FD conditions, transpiration rate decreased steadily starting at DOT 5, while photosynthetic rate dropped drastically on DOT 6 (Figure 2B,C). Afterwards, both E and A were almost neglectable. On the other hand, LD resulted in a significant reduction of both E and A from DOT 7 onwards. However, both rates were maintained at a (at least partly) functional level, still reaching 65% (A) and 56% (E) of the WW plants on DOT 9 (Figure 2B,C).

At DOT 10, the soil in FD contained less water (6% GWC) than that of the WW treatment (15% GWC). Under LD conditions, the soil GWC was significantly reduced in both root compartments, but in addition $\mathrm{LD}_{\mathrm{wet}}$ was significantly wetter (10% GWC) than $\mathrm{LD}_{\mathrm{dry}}$ (7% GWC) (Figure 3D). Interestingly, the soil in $\mathrm{LD}_{\mathrm{dry}}$ contained slightly, but significantly, more water than FD soil. Root water content did not differ significantly between $\mathrm{LD}_{\mathrm{wet}}$ and $\mathrm{LD}_{\mathrm{dry}}$, between WW and $\mathrm{LD}_{\mathrm{wet}}$, as well as between $\mathrm{LD}_{\mathrm{dry}}$ and FD conditions (Figure 3E).

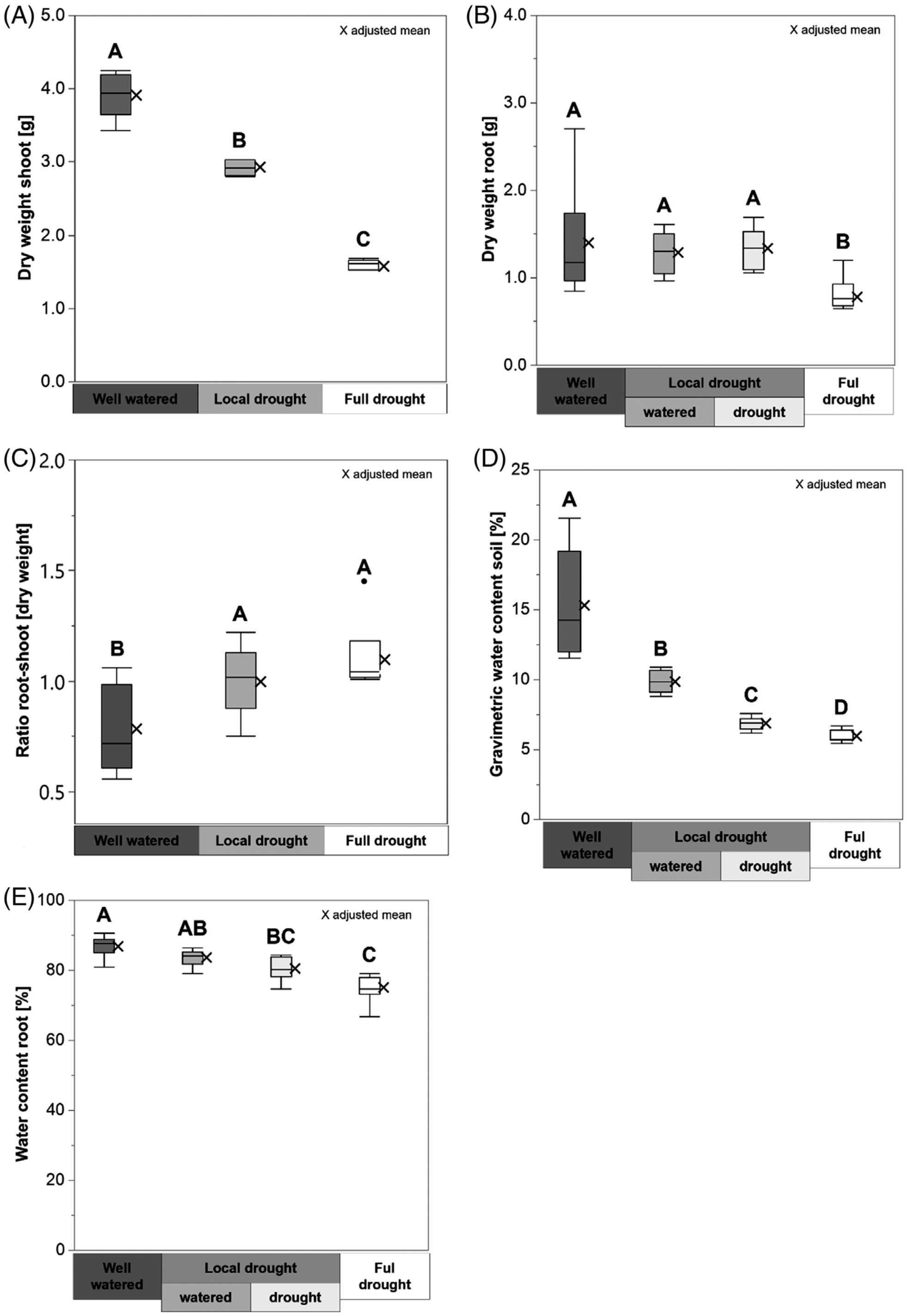

FIGURE 3 Boxplots of dry weight of roots (A) and shoots (B), gravimetric water content of the soil (C), root-shoot ratio (D) and root water content (E) of *Zea mays* after 10 days of well-watered, local drought and full drought treatments. The cross indicates the adjusted mean within the range ($n = 6$). Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test) between the treatments.

3.2 | Plant water relations and accumulation of osmotic solutes

Under WW conditions, the average RWC in the shoot at the final harvest was 98%. It was slightly but significantly reduced to 93% in LD and strongly reduced to 56% in FD (Figure 4A). Similarly, shoot osmolality was moderately increased by 25% compared to WW plants in LD, and by 113% in FD (Figure 4B), and shoot proline concentration was increased by 19% (though not significant) in LD, and by 244% in FD (Figure 4C). Root proline concentrations were significantly increased

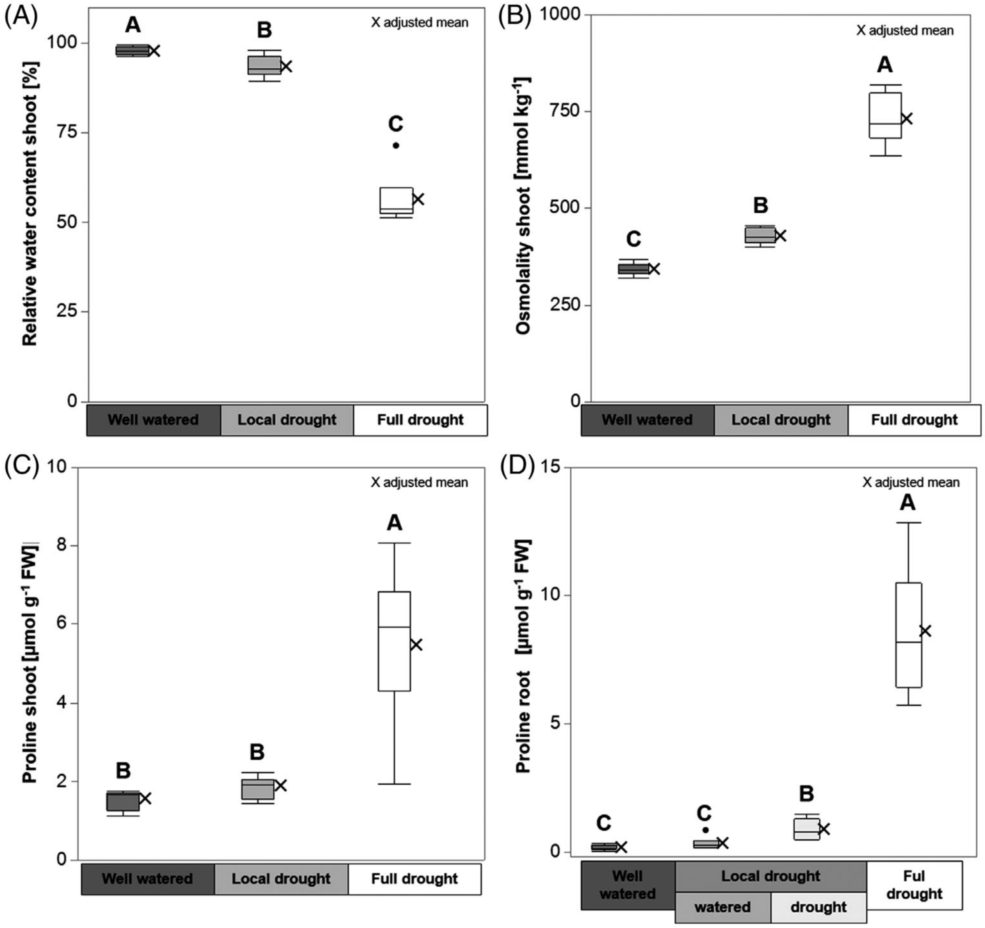

FIGURE 4 Boxplots of relative water content (A), osmolality (B), proline concentration in shoots (C) and roots (D) of *Zea mays* after 10 days of well-watered, local drought and full drought treatments. The cross indicates the adjusted mean within the range, and ($n = 6$). Means with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test) between the treatments.

in FD, as well as in LD_{dry}, even though the latter increase was less pronounced (Figure 4D).

3.3 | Composition of root exudates

In total, 39 metabolites were identified in root exudates and were classified into five categories (Figure 5). Irrespective of the treatment, sugars represented by far the largest fraction (on average 66% across all treatments), followed by organic acids (18%), amino acids (9%), sugar alcohols (1.6%) and amines (0.8%).

The largest impact on exudate fractions was triggered by FD. Compared to WW, fractions of proline, putrescine, maltose and trehalose significantly increased under FD (Figure 5). In LD_{dry}, these metabolites also showed a trend of higher values, but only putrescine was significantly increased compared to WW. A tendency towards higher values under drought was also observed for the organic acids fumaric acid, threonic acid, gluconic acid and the amine spermidine, as well as the amino acids leucine, glycine and proline. A significant reduction compared to WW conditions was observed in FD for malic acid and glucose, while tyrosine, phenylalanine, tryptophane, glutamate, lysine, ornithine, glutamine and fructose showed a trend towards lower values. Between the two sides of LD, fractions of 2-oxoglutaric acid and phenylalanine were lower in LD_{dry} than in LD_{wet}. However, a similar difference of these compounds was not observed between FD and WW (Figure 5).

4 | DISCUSSIONS

4.1 | Local maintenance of root water content is systemically achieved by hydraulic redistribution rather than by locally altered root architecture

One hypothesis of this study was that plants perceiving a local drought stress would respond by compensatory increased water uptake from the watered root half and/or compensatory root growth.

Hydraulic redistribution, that is, the movement of water from moist to drier soil using the plant roots as a conduit (Burgess et al., 1998), is a well-known process especially in arid or semi-arid ecosystems, and

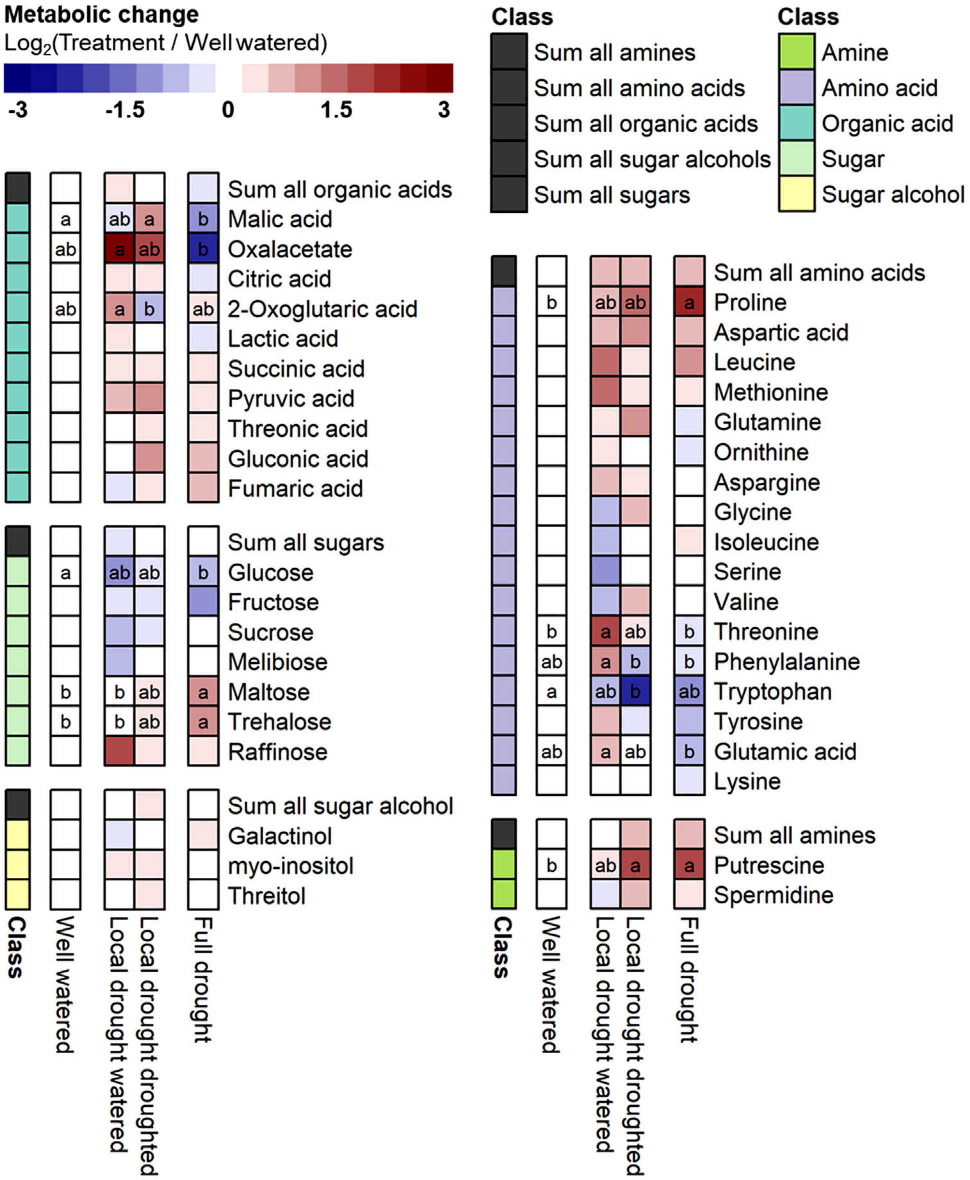

FIGURE 5 Metabolic changes in root exudates of *Zea mays* after 10 days of (left to right) well-watered, local drought watered, local drought drought stressed and full drought treatments. Metabolic changes are presented as means of each treatment, normalized to the well-watered control, in log2 scale. Colours indicate increases (red) and decreases (blue) compared to well-watered conditions. For each metabolite, values with at least one identical letter are non-significant from each other ($p < 0.05$; one-way analysis of variance (ANOVA); Fisher's LSD test). Metabolites without letters did not show significant differences between treatments (data provided in Table S1).

most common in trees and shrubs (Hafner et al., 2017), but was not yet described for annual crops. It has significant ecological implications not only by providing water to the stressed root parts, but also by releasing water into the dryer soil which can be used by neighbouring plants and root-associated microorganisms (Hafner et al., 2017). In the following discussion, we use the term 'hydraulic redistribution' in the strict sense, that is, only when referring to water movement via the roots, while we use the term 'water movement' for water flow within the soil but without root contribution. The significantly higher soil water content (GWC) in LD_{dry} compared to FD could be the result of HR, or alternatively of a lower water uptake in LD_{dry}, or both. However, in combination with the lower GWC in LD_{wet} compared to WW, and the similar root water content in both root halves of LD, a hydraulic redistribution of water from the watered to the drought stressed root side is more likely. This indicates that the locally stressed plants were able to replenish water in the stressed root half. It is important that no direct water movement via the soil was possible from one root compartment to the other. Aquaporins such as PIP1.2, PIP2.1 or PIP2.5 have an important role in water uptake regulation of maize (Hachez et al., 2006). However, gene expression levels of PIP1.2, PIP2.1, and PIP2.5 in root tissues were not significantly different between the drought conditions, with the exception of PIP2.5, which was expressed less in LD_{dry} compared to FD (Tables S2 and S3). Since it was, however, similarly low in LD_{wet}, this down-regulation is unlikely to explain the observed differences in GWC of the soil. It seems that enhanced water movement via osmotic adaptation in combination with HR was sufficient to maintain root growth, even though we cannot exclude the possibility of a change in water uptake via altered aquaporin activity rather than expression.

Reorganization of the root system is also important for fostering the water supply during water limitation (Dietz et al., 2021). At least under moderate drought, shoot growth is usually more rapidly reduced than root growth (Poorter et al., 2012), which can be even enhanced to access deeper soil layers (Dietz et al., 2021). On the other hand, in drying soils, root elongation can be limited by a reduced hydrostatic pressure in the root-tip cells (K. Jin et al., 2013). Thus, an increased root-shoot ratio is reported under moderate drought (Poorter et al., 2012). In the present study, a small but significant increase in root-shoot ratio was observed in both LD and FD, even though it was not different between the two drought treatments (Figure 2D). This is in line with the fact that in LD, shoot growth was significantly reduced, while root biomass was not altered (Figure 2A,B), resulting in a larger root–shoot ratio. The lack of a further increase in root–shoot ratio in FD can be explained by the onset of significant root growth inhibition. It is noteworthy that we did not observe any compensatory root growth in either root side of LD. Indeed, root growth was equally maintained in both root compartments despite a final GWC of only 7% in LD_{dry} (Figure 2A,C), which is close to the permanent wilting point for the loamy soil used in this study (6% GWC or 8% VWC: Vetterlein et al., 2021). This surprising lack of root growth inhibition would again be in line with the suggested HR described above.

Collectively, these data indicate that local maintenance of root water relations was achieved at least in part by HR between the watered and the stressed root halves, that is, a systemic response of the plants, rather than by a local compensatory change of root growth or architecture.

4.2 | Local and locally induced systemic adjustments of osmolytes in roots and exudates contribute to hydraulic redistribution

We also wanted to know whether the partial root drying resulted in local stress responses only in the drought stressed root half, or whether the whole plant responded systemically with metabolic and physiological acclimation strategies.

Maintaining water uptake in a drying soil depends on the ability of the plant to maintain a sufficient gradient in water potential between soil and plant, which can be achieved by osmotic adjustment (Dietz et al., 2021). In the present study, despite no visual drought symptoms under LD conditions (Figure 1A), plants clearly experienced drought stress as indicated by reduced transpiration and assimilation rates (Figure 2), inhibited shoot growth (Figure 3) and accumulation of osmolytes and proline in leaves and roots (Figure 4). The lack of wilting symptoms correlates well with the shoot RWC, indicating that water availability to one root half was sufficient to maintain almost normal water relations in the plants. Nevertheless, these plants responded with a metabolic adaptation of the whole plant, that is, a systemic metabolic response, as indicated by an increased osmolality in the shoot. Thus, partial root drying resulted in a systemic increase in shoot osmotic potential, thus likely enhancing water movement from the bulk soil to the root in both root compartments. Since half of the roots had access to water, this effect is likely to have a positive effect on the whole plant water relations.

Proline increases under drought stress (Ilahi & Dorffling, 1982), and not only serves as osmolyte but also protects cell membranes from damage by reactive oxygen species (Trovato et al., 2008). The strong observed increase in proline in both shoots and roots in FD indicated severe stress in the whole plant. To a much lower extent, but still significantly, proline concentration was also elevated in roots of LD_{dry} but not of LD_{wet}, indicating an additional local response (Figure 4D). In this setting, we were concerned that the determination of osmolality in roots would be flawed by the necessary extensive washing of the roots to remove adhering soil. We thus determined proline content in roots assuming that it would correlate with total osmolality. Indeed, in the shoot, proline and total osmolality correlated significantly ($R^2 = 0.9406$), even though proline, on average, represented less than 1% of the total osmolality (Figure 4B,C). We would thus suggest that other osmolytes (not determined here) were likely also increased locally in LD_{dry} roots.

This local metabolic adjustment to drought was further supported by changes of exudate composition in LD_{dry} and FD (Figure 5). Overall, more pronounced effects in FD compared to LD_{dry} (including a significant decrease of malic acid and glucose in FD) confirm that some metabolic changes occur at the transition from moderate to severe drought stress (Schneider et al., 2019) and correlate with shutdown of assimilation (Ulrich et al., 2022). The trend of increasing proline, maltose and trehalose, however, correlated with decreasing soil water content, supporting an effect of drought intensity on exudate composition and specifically osmolytes (Gargallo-Garriga et al., 2018). In addition to osmotic effects, especially carbohydrates may also contribute to a better movement of the roots through the drying soil (Gargallo-Garriga et al., 2018), and proline, putrescine, trehalose and maltose are known to enhance beneficial microorganisms under drought (Y. Jin et al., 2019; Kuiper et al., 2001; Vílchez et al., 2000; Vílchez et al., 2016). Whether their increasing trend has a measurable effect on microbiomes of locally stressed root parts still needs to be clarified. Many root exudates, particularly sugars, are released mainly by passive or facilitated diffusion (Li et al., 2018), and the composition of root exudates seems to reflect that of the root tissue (Gargallo-Garriga et al., 2018). It is thus likely that the observed increases in exudate metabolites in LD_{dry} roots reflect corresponding increases in the root tissue. Such local accumulation of osmolytes would lower the water potential in the drought stressed root half, with two possible consequences. First, this would further contribute to a steeper gradient in water potential between bulk soil and root and enhance water movement towards the roots. A local increase of osmolyte concentrations in root exudates would intensify this effect by lowering the water potential in the rhizosphere. Second, it would result in an increase in water potential gradient between the two root halves, and thus enhance RH from the wetter to the dryer root compartment via the roots.

Altogether, our data indicate that local and locally induced systemic osmolyte accumulation acts synergistically under partial root drying by inducing hydraulic redistribution, enhancing water availability and in consequence plant water relations and root growth under conditions of local drought stress.

5 | CONCLUSIONS

The locally drought stressed root side experienced a stress level sufficient to trigger both local (*e.g*., osmolyte accumulation) and locally induced systemic (*e.g*., osmolyte accumulation in the shoot, stomatal closure) responses, even though no difference in root growth was observed compared to the watered side. These adaptation mechanisms collectively helped the plants to not only improve water movement from soil to root but also to distribute water from the watered to the drought stressed side via the roots, resulting in a surprisingly well-maintained root growth even in the dried compartment despite a very low soil water content (similar to that of a full drought stress). Systemically, osmotic adjustment increased the water potential gradient between soil and plant to enhance water movement despite partial closure of stomata. Locally, osmotic adjustment and concurrent reduction in water potential of the drought stressed root half probably contributed to water movement from soil into roots as well as hydraulic redistribution from the watered to the drought stressed root compartment. Whole-plant water relations were little affected as long as parts of the root system still had access to water. Additional local and systemic changes in root exudate composition were observed and may possibly have lasting effects on the microbiome structure of the rhizosphere.

ACKNOWLEDGEMENTS

This study was conducted within the *priority program 2089*, funded by the *Deutsche Forschungsgemeinschaft* (*DFG, German Research Foundation*). Seeds of the maize genotype B73 were kindly provided by *Caroline Macron* and *Frank Hochholdinger* (University of Bonn). We thank *Prof. Asch* (University Hohenheim) for the Licor instrument, and gratefully acknowledge excellent technical support by *Christiane Beierle*, staff members of the Phytotechnikum and the TZ workshop of the University of Hohenheim.

Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Monika Andrea Wimmer https://orcid.org/0000-0002-7456-5667

REFERENCES

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment*, *40*(1), 4–10.

Burgess, S. S., Adams, M. A., Turner, N. C., & Ong, C. K. (1998). The redistribution of soil water by tree root systems. *Oecologia*, *115*(3), 306–311.

Caldwell, M. M., Dawson, T. E., & Richards, J. H. (1998). Hydraulic lift: Consequences of water efflux from the roots of plants. *Oecologia*, *113*(2), 151–161.

Chen, Y., Yao, Z., Sun, Y., Wang, E., Tian, C., Sun, Y., Liu, J., Sun, C., & Tian, L. (2022). Current studies of the effects of drought stress on root exudates and rhizosphere microbiomes of crop plant species. *International Journal of Molecular Sciences*, *23*(4), 2374. https://doi.org/10.3390/ijms23042374

Chinard, F. P. (1952). Photometric estimation of proline and ornithine. *Journal of Biological Chemistry*, *199*, 91–95.

Dietz, K. J., Zörb, C., & Geilfus, C. M. (2021). Drought and crop yield. *Plant Biology*, *23*(6), 881–893.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., & Peñuelas, J. (2018). Root exudate metabolomes change under drought and show limited capacity for recovery. *Scientific Reports*, *8*(1), 1–15.

Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, *368*(6488), 266–269.

Hachez, C., Heinen, R. B., Draye, X., & Chaumont, F. (2008). The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. *Plant Molecular Biology*, *68*(4), 337–353.

Hachez, C., Moshelion, M., Zelazny, E., Cavez, D., & Chaumont, F. (2006). Localization and quantification of plasma membrane aquaporin expression in maize primary root: A clue to understanding their role as cellular plumbers. *Plant Molecular Biology*, *62*(1), 305–323.

Hafner, B. D., Tomasella, M., Häberle, K. H., Goebel, M., Matyssek, R., & Grams, T. E. (2017). Hydraulic redistribution under moderate drought among English oak, European beech and Norway spruce determined by deuterium isotope labeling in a split-root experiment. *Tree Physiology*, *37*(7), 950–960.

Hu, T., Kang, S., Li, F., & Zhang, J. (2011). Effects of partial root-zone irrigation on hydraulic conductivity in the soil–root system of maize plants. *Journal of Experimental Botany*, *62*(12), 4163–4172.

Ilahi, I., & Dörffling, K. (1982). Changes in abscisic acid and proline levels in maize varieties of different drought resistance. *Physiologia Plantarum*, *55*(2), 129–135.

Jin, K., Shen, J., Ashton, R. W., Dodd, I. C., Parry, M. A., & Whalley, W. R. (2013). How do roots elongate in a structured soil? *Journal of Experimental Botany*, *64*(15), 4761–4777.

Jin, Y., Zhu, H., Luo, S., Yang, W., Zhang, L., Li, S., Jin, Q., Cao, Q., Sun, S., & Xiao, M. (2019). Role of maize root exudates in promotion of colonization of *Bacillus velezensis* strain S3–1 in rhizosphere soil and root tissue. *Current Microbiology*, *76*(7), 855–862.

Kuiper, I., Bloemberg, G. V., Noreen, S., Thomas-Oates, J. E., & Lugtenberg, B. J. (2001). Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by *Pseudomonas fluorescens* strain WCS365. *Molecular Plant-Microbe Interactions*, *14*(9), 1096–1104.

Li, X., Dong, J., Chu, W., Chen, Y., & Duan, Z. (2018). The relationship between root exudation properties and root morphological traits of cucumber grown under different nitrogen supplies and atmospheric CO2 concentrations. *Plant and Soil*, *425*(1), 415–432.

Mehmeti, V., Fragner, L., & Wienkoop, S. (2013). Medicago truncatula root and shoot metabolomics: protocol for the investigation of the primary carbon and nitrogen metabolism based on GC–MS. In W. Weckwerth & G. Kahl (Eds.), *The handbook of plant metabolomics* (pp. 111–123). Wiley.

Munoz-Ucros, J., Wilhelm, R. C., Buckley, D. H., & Bauerle, T. L. (2022). Drought legacy in rhizosphere bacterial communities alters subsequent plant performance. *Plant and Soil*, *471*(1), 443–461.

Neumann, G., Bott, S., Ohler, M. A., Mock, H. P., Lippmann, R., Grosch, R., & Smalla, K. (2014). Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as affected by different soils. *Frontiers in Microbiology*, *5*, 2. https://doi.org/10.3389/fmicb.2014.00002

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30–50.

Schachtman, D. P., & Goodger, J. Q. (2008). Chemical root to shoot signaling under drought. *Trends in Plant Science*, *13*(6), 281–287.

Schneider, S., Turetschek, R., Wedeking, R., Wimmer, M. A., & Wienkoop, S. (2019). A protein-linger strategy keeps the plant on-hold after rehydration of drought-stressed *Beta vulgaris*. *Frontiers in Plant Science*, *10*, 381. https://doi.org/10.3389/fpls.2019.00381

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environmental Science & Technology*, *48*, 2097–2098.

Tardieu, F., Simonneau, T., & Muller, B. (2018). The physiological basis of drought tolerance in crop plants: A scenario-dependent probabilistic approach. *Annual Review of Plant Biology*, *69*, 733–759.

Trovato, M., Mattioli, R., & Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei*, *19*(4), 325–346.

Tsugawa, H., Ikeda, K., Takahashi, M., Satoh, A., Mori, Y., Uchino, H., Okahashi, N., Yamada, Y., Tada, I., Bonini, P., Higashi, Y., Okazaki, Y., Zhou, Z., Zhu, Z.-J., Koelmel, J., Cajka, T., Fiehn, O., Saito, K., Arita, M., & Arita, M. (2020). A lipidome atlas in MS-DIAL 4. *Nature Biotechnology*, *38*(10), 1159–1163.

Turetschek, R., Desalegn, G., Epple, T., Kaul, H. P., & Wienkoop, S. (2017). Key metabolic traits of Pisum sativum maintain cell vitality during *Didymella pinodes* infection: Cultivar resistance and the microsymbionts' influence. *Journal of Proteomics*, *169*, 189–201.

Ulrich, D. E., Clendinen, C. S., Alongi, F., Mueller, R. C., Chu, R. K., Toyoda, J., Gallegos-Graves, V., Goemann, H. M., Peyton, B., Sevanto, S., & Dunbar, J. (2022). Root exudate composition reflects drought severity gradient in blue grama (*Bouteloua gracilis*). *Scientific Reports*, *12*(1), 1–16.

Vetterlein, D., Lippold, E., Schreiter, S., Phalempin, M., Fahrenkampf, T., Hochholdinger, F., Marcon, C., Tarkka, M., Oburger, E., Ahmed, M., Javaux, M., & Schlüter, S. (2021). Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale. *Journal of Plant Nutrition and Soil Science*, *184*(1), 35–50.

Vílchez, J. I., García-Fontana, C., Román-Naranjo, D., González-López, J., & Manzanera, M. (2016). Plant drought tolerance enhancement by trehalose production of desiccation-tolerant microorganisms. *Frontiers in Microbiology*, *7*, 1577. https://doi.org/10.3389/fmicb.2016.01577

Vílchez, S., Molina, L., Ramos, C., & Ramos, J. L. (2000). Proline catabolism by *Pseudomonas putida*: Cloning, characterization, and expression of the put genes in the presence of root exudates. *Journal of Bacteriology*, *182*(1), 91–99.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., & Pérez-Clemente, R. M. (2020). Root exudates: from plant to rhizosphere and beyond. *Plant Cell Reports*, *39*(1), 3–17.

Vranova, V., Rejsek, K., Skene, K. R., Janous, D., & Formanek, P. (2013). Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. *Journal of Plant Nutrition and Soil Science*, *176*(2), 175–199.

Wedeking, R., Mahlein, A. K., Steiner, U., Oerke, E. C., Goldbach, H. E., & Wimmer, M. A. (2016). Osmotic adjustment of young sugar beets (*Beta vulgaris*) under progressive drought stress and subsequent rewatering assessed by metabolite analysis and infrared thermography. *Functional Plant Biology*, *44*(1), 119–133.

Weiszmann, J., Clauw, P., Jagoda, J., Reichardt-Gomez, I., Koemeda, S., Jez, J., Nordborg, M., Walther, D., Nägele, T., & Weckwerth, W. (2020). Plasticity of the primary metabolome in 241 cold grown *Arabidopsis thaliana* accessions and its relation to natural habitat temperature. *bioRxiv*, https://doi.org/10.1101/2020.09.24.311092

Wolfinger, R. (1993). Covariance structure selection in general mixed models. *Communications in Statistics-Simulation and Computation*, *22*(4), 1079–1106.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Werner, L. M., Hartwig, R. P., Engel, I., Franzisky, B. L., Wienkoop, S., Brenner, M., Preiner, J., Repper, D., Hartung, J., Zörb, C., & Wimmer, M. A. (2022). Local and systemic metabolic adjustments to drought in maize: Hydraulic redistribution in a split-root system. *Journal of Plant Nutrition and Soil Science*, 1–11. https://doi.org/10.1002/jpln.202200279