

Interactive comment on “Root uptake under mismatched distributions of water and nutrients in the root zone” by Jing Yan et al.

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Response to Reviewer Comments #3

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General Comments

This manuscript presents experimental evidence that plants can satisfy their water and nutrient demand from mismatchingly distributed water and nutrient resources, if the overall available amount is sufficient. The plant adaptation strategies and regulating mechanisms related to this are discussed. Overall, this is a well-designed contribution of high interest. However, the methods in part lack clarity, and the results and discussion are in parts too speculative.

My first two points are about nomenclature:

Comment 1: The first is the definition of the term rhizosphere. There are different ways in literature how to use the term rhizosphere and thus I think it is important to

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define clearly what this term means in this paper. I think this paper rather means a part of soil which has a high root density, i.e. it is more used in the meaning of “root zone”. That could be confusing as a lot of other work understands the rhizosphere much more locally in form of gradients in the concentration of root-influenced solutes or other compounds extending from the root surface to the ‘bulk’ soil (Darrah et al., EJSS 57, 2006).

Response: We thanked the reviewer’s suggestions. We clarified the definition of the term “rhizosphere” in the methodology section. See more details in our reply to Comment 3.

Comment 2: The second is the term “exudates”. It is often used quite differently in different papers. I rather tend to distinguish “root exudates” as low-molecular weight organic carbon (such as citrate, sugars) and mucilage. An overarching term that includes both exudates and mucilage would be “rhizodeposition” (Oburger and Jones, Rhizosphere 6, 2018). I encourage the authors to also use this nomenclature.

Response: We thanked the reviewer for providing suggestions and relevant references. According to the suggestion, we carefully checked the terms throughout the manuscript and replaced “root exudation or mucilage” with “rhizodeposition.” The reference was added to the introduction when rhizodeposition was first mentioned in the manuscript.

Some methodological aspects were also not clear to me:

Comment 3: I could not find in which depths the water potential sensors were installed. I could also not infer in how far it is justified to call the resulting value a “rhizosphere” water potential. Is it not rather the water potential in the soil layer that has the highest root length density? One could understand this from your sentence on page 3, line 64:

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“: :to measure the water potential of the root zone”. Comparing the water content that was computed from the rhizosphere water potential (Fig. S5c) with the water content that was measured with the dielectric water content sensor that was installed in the middle of the compartment (Fig. S3a), I can hardly see a difference.

Response: We changed the term “rhizosphere” to “root zone” in the result section. We discussed our results that inferred rhizosphere activities in the discussion section. In terms of sensor locations, both psychrometric and dielectric sensors were installed at the same depth of 14 cm from the soil surface. We added more details to the methodology section about how and where the potential water sensors were installed accordingly. A new figure that illustrates experimental design was added for this revision (See Figure 1 below).

Comment 4: How can you be sure that the water increase in the root zone with highest root length density results from HR? Root water uptake and injections will create water potential gradients within one compartment that could result in redistribution of water in the soil.

Response: In principle, internal redistribution from moist soil to dry rhizosphere can result in a signature that looks like the trend we observed. However, we ruled out this process for several main reasons. First, the psychrometers were installed prior to root growth around them. If the source and sink for the redistribution were within the mid-section of the dry compartment, then we would have expected to see an out of phase fluctuation in at least one sensor. All the five sensors that functioned well during the experiment showed consistent nightly increase and daily decrease in water potential. Therefore, internal lateral redistribution could not have been the cause of the observed pattern. Second, vertical redistribution (that flow

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of water from above or below the densely rooted zone) is possible but not likely. In the treatment **C1**, root density was low in the mid-section. The water added at a weekly cycle was likely being redistributed up and down by capillarity and gravity. But the rate of this transfer is very slow as evidenced by the water content sensors, despite the water content being at a higher level than was observed in the dry compartment of treatment **D**. Therefore, we would expect vertical redistribution to be rather slow process and cannot explain the water potential fluctuation in the dry nutrient-rich compartment. Third, if you zoom in the dielectric water content sensor data, there appears a trace of fluctuation that is consistent with the water potential data. The dielectric sensors were installed vertically have effective volume of measurement that extends beyond the densely rooted zone. Therefore, if there was redistribution from above or below, these trends would not visible. That said, the level of fluctuations we observed is at the detection limit of the sensors. Finally, the water potential fluctuation pattern that we observed is consistent with our previous field observations (different plant, but similar textured soil and using the same sensors). In the previous study, we definitively concluded that HR was occurring using isotope tracing (Bogie et al., 2018). We supplied deuterium labeled water directly to deep roots from sealed vials, with no path other than the root for uptake. We detected the label in neighboring shallow-rooted plants within hours for several days, clearly showing that HR occurs in sufficient quantity to be able to be taken up by shallow rooted plants. When taken together, these arguments strongly support what we observed was indeed HR.

Comment 5: The structure of the paper needs attention. I suggest, for example, to move the paragraph lines 105-115 page 4 to the description of the split-root experiment in the Methods section. Then, the “D” and “C1” will be easier to understand in line 64 on page 3.

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Response: We moved the paragraphs, as the reviewer suggested. In addition, the criticism of manuscript structure and organization was well addressed according to all three reviewers. More details can also be found in our general replies to Reviewer #1 and #2.

Some claimed results seem a bit too speculative to me:

Comment 6: Comment 6: The reason for root accumulation at the bottom could also just be that the pot was too short. I.e., if almost all the carbon in C1 is invested in the wet and nutrient rich compartment, it may be possible that the roots would have grown much deeper than in the other treatments if they had been given the space.

Response: The reviewer was correct; we agreed that if the chamber had been open, the roots would grow deeper in the wet compartment of treatment C1. Due to the same reason, roots could grow deeper in both compartments of treatment C2. However, rather than understanding the constraints on rooting depth from the physical barrier, we tried to focus on how nutrient and water distribution drive the root distribution. The roots accumulated at the bottom of a close-end chamber, or alternatively, roots grew deeper in an open-end chamber; both would suggest that, presumably, roots extract the water and nutrients leached to the deeper soil layers given higher soil moisture conditions.

Comment 7: Comment 6: “Moreover, multi-scale signalling and feedbacks appear to be involved”: How could you support this statement with your results?

Response: We added a potential mechanism and reference after the sentence:
 “A recent study reported that spatial availability of water is a key trigger for biosynthesis and transport of root-inductive signal compounds (Bao et al., 2014).”

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Comment 8: While it is known that hormonal signaling may regulate the transpiration demand at the leaves, the water flow into our out (HR) of the roots follows (passively) local hydraulic gradients between xylem and soil (e.g. Rothfuss and Javaux, Biogeosciences 14, 2017). What regulation mechanisms exactly do you mean by your statement “HR is biologically-mediated”? Would that be regulation of root hydraulic properties? How could you support that with your results?

Response: We agreed with the reviewer that HR magnitude is biophysically regulated by the water potential gradients and conductance of plant-soil systems. However, our results suggested that the occurrence of HR correlated with nutrient availability because HR was observed only in the dry nutrient-rich patches but not nutrient-deficient ones. The nutrient enrichment drove the root growth, which builds a conductive bridge between the wet and dry compartments and eventually allows the occurrence of HR. Therefore, there is a plant-scale decision making, or biologically-driven decision-making that regulates the occurrence of HR, driven by the plant nutrient demands.

We expanded the discussion to clarify and emphasize the importance of biological regulation of HR occurrence by adding a sentence:

“As opposed to nutrient-deficient dry soil patch, the apparent occurrence of HR in nutrient-rich dry soil patches was probably a consequence of plant nutrient demands and extensive root distribution.”

Responses to minor comments are provided below each comment. To facilitate review of our responses, we added all the figures at the end of this document. We added three figures during this revision. Most figures have been revised and the captions have been expanded and clarified.

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Minor comments

1. I could not see that the number of replicates was mentioned in the Methods section.

Response: For each treatment, there were three replicates. We changed the sentence at L56 to

“Our experiments were conducted using laterally split soil compartments arranged in three treatments with three replicates of each treatment, as depicted in Figure 1.”

2. P3 L57: How long did it take the plants to reach that height?

Response: We changed the sentence to

“Tomato seedlings were germinated in potting mix and grown for 3 weeks until they reached 5 – 10 cm in height.”

3. P3 L59: How many roots were there at this stage? Was the tap root recognizable and was there a strategy to place it into a specific compartment?

Response: We provided a photo of the seedlings the root mass at the time of transplantation. This is added to the supplemental material as Figure S7. We did not differentiate the taproots from the other roots for the split-root experiments. We divided and the roots without consideration of which side was going to be in specific compartment. Any possible influence of initial root mass differences to be small and randomly distributed between treatments and compartments. The description now includes clarifications. To assist the review of our response, we show Figure S7 as Figure 9 in the response (see below). In the manuscript, Figure S7 will be presented in the supplemental materials.

4. P3 L85: When you scooped out the soil, did you cut the roots within these 2cm intervals?

Response: Yes, we cut the roots within each interval to obtain the root mass

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distribution in each soil layer. To clarify that, we changed the sentence to “The coarse root pieces in each interval were cut and removed ...”

5. Fig. 2a: I do not see the relevance of Fig. 2a. I also suggest to split the rhizosphere and root mass distribution to two separate figures. Fig. 2h: dry and wet labels are confusing for this treatment.

Response: We moved previous Figure 2a to the supplemental materials and split subfigures of root mass distribution and SEM images into two single figures.

6. P8 L165: “taken up by the roots”.

Response: Changed as suggested.

7. P11 L211: The absence of HR in C1 was not mentioned in the Results section.

Response: We added a sentence to the result section at L161 as

“The soil water potential in the dry compartment of treatment **D** fluctuated in a diurnal pattern with daytime decrease and nighttime increase. The fluctuation magnitude ranged between -100 to -1000 *kPa*, which was above the permanent wilting point -1500 *kPa*. In contrast to that, no nocturnal increase in soil water potential was observed in the dry compartment of treatment **C1**.”

Figure Captions

1. Schematic illustration of the experimental design. Each pot consists of two isolated compartments that are fused together by glue. Roots of seedlings were roughly divided half and a half during transplantation. The experiment consisted of one treatment in which the bulk quantity of water and nutrients were distributed separately (treatment **D**) and two control treatments in which nutrients were applied with most of the water. In Control 1 (**C1**) water was applied non-uniformly as in **D**, whereas in Control 2 (**C2**), water and nutrients were applied uniformly to

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both compartments. Placement of sensors and water and nutrient delivery tubes are illustrated. The diagram is not to scale.

2. Comparison of plant physiological indicators (a) total dry biomass, (b) fruit dry mass, (c) number of flowers, (d) total N uptake, (e) N uptake in Fruits, and (f) N use efficiency in treatment **D**, **C1**, and **C2**. The orange dots represent values of individual replicates. The white diamonds and whiskers represent the mean and standard deviation within each treatment. Distribution of N content along the canopy length is shown in Figure 3. One of the replicates in treatment **C1** did not produce fruits, resulting in larger deviations in fruit dry mass and N uptake in treatment **C1**.
3. Leaf NDVI as a function of normalized plant height at the end of the experiments in treatment **D** (a), **C1** (b), and **C2** (c); N content (%) of stem and leaf samples across canopy at the end of the experiments in treatment **D** (d), **C1** (e), and **C2** (f). The green dots represent leaf samples, while the red dots represent stem samples. The dots include three replicates within each treatment. The diamonds and whiskers represent the mean and standard deviation of replicates at the normalized plant height. Note: mean and standard deviation of leaf NDVI was calculated within an incremental height of 0.1; N content (%) of stem and leaf samples were separated into three portions across the canopy and thus reported as the normalized height of 0.17, 0.5 and 0.84.
4. Incremental root mass distribution along the soil profile in treatment **D** (a), **C1** (b) and **C2** (c). The coarse root pieces in the 2 cm interval were cut and removed for gravimetric measurement. The root mass within each interval was normalized to the total root mass from the two isolated compartments. Therefore, each step in the plot represents the normalized root mass within the 2 cm soil depth. Note: “Wet” and “Dry” compartments (compartments with 90% versus 10% water, respectively in Figure 1) were defined operationally to distinguish water supply for

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treatment **D** and **C1** mainly; in treatment **C2**, the water was supplied uniformly in the disconnected compartments. Detailed schemes of water and nutrient supply were provided in Figure 1.

5. SEM images of representative rhizosheaths collected from the “Wet” and “Dry” compartments of treatment **D** (a and b, respectively) and **C1** (c and d, respectively). All the SEM images have identical magnification (all four subfigures used a 100 μm scale bar) that permits visual qualitative comparison.
6. Changes in dielectric soil volumetric water content (v/v) during days of 113 to 121 after transplantation in “Wet” and “Dry” compartments of treatment **D**, **C1** and **C2** (a, b, c). The different shades of red and blue in these figures are used to distinguish between replicates. Note that the “Wet” compartments were irrigated daily, while the “Dry” compartments were irrigated once a week for the majority of the experiments (days of 40 to 140 after transplantation). The results plotted represent a short-term overview of the reoccurring cycles of soil water content changes. The long-term results of dielectric soil volumetric water content were provided in the supplemental materials.
7. Changes in soil water potential (a), water content converted from soil water potential (b), and root-zone wetting flux (c) from HR and irrigation as a function of time in “Dry” compartment of treatment **D** during days of 113 to 121 after transplantation; HR outflow magnitude as a function of water potential (ψ): HR described by a power-law model is shown in solid line (d). In (a) and (b), solid black lines and grey shade represented the average and the standard deviation of soil water potential and converted water content from five sensors distributed in three replicate compartments. Similarly, in (c), solid dots represent the calculated water flux from five sensors, and the diamonds and whiskers show the average and standard deviation of the water flux. In (d), water flux from HR during the whole experiment was used. The long-term results of soil water potential and converted

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water content were provided in the supplemental materials.

8. Mechanisms, functions, and applications of root uptake under mismatched distributions of water and nutrients in the root zone; (a) schematic representation of how HR supports nutrient uptake under our experimental condition; (b) hypothesized function of HR as an adaptation mechanism in natural systems, where nutrients are concentrated in shallow layers that are prone to frequent drying; and (c) a proposed management practice that can reduce nutrient leaching from irrigated agriculture by capitalizing on the mechanisms elucidated in this study.
9. Figure S7. Transplanting of seedlings to split pots. Pre-installed sensors and irrigation tubes are visible.

References

- Bao, Y., Aggarwal, P., Robbins, N. E., Sturrock, C. J., Thompson, M. C., Tan, H. Q., Tham, C., Duan, L., Rodriguez, P. L., Vernoux, T., Mooney, S. J., Bennett, M. J., and Dinneny, J. R. (2014). Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proceedings of the National Academy of Sciences*, 111(25):9319–9324.
- Bogie, N. A., Bayala, R., Diedhiou, I., Conklin, M. H., Fogel, M. L., Dick, R. P., and Ghezzehei, T. A. (2018). Hydraulic redistribution by native sahelian shrubs: bioirrigation to resist in-season drought. *Frontiers in Environmental Science*, 6:98. bogie2018.

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Table 1. Table 1. Total quantity of water and N applied to each compartments of the three treatments. Note that the nutrient applied nutrient solution includes other macro and macro nutrients. The composition of the nutrient solution is provided in Table A3.

Treatment	Code	Applied Water (mm)		Applied N (mgN)	
		Wet	Dry	Wet	Dry
Distributed	D	588	77	0	120
Control 1	C1	580	73	120	0
Control 2	C2	338	338	60	60

Table 2. Table A1. The mean, standard deviation of physiological indicators, and the p-value of Welch's ANOVA test across treatments. Note: comparison of Leaf NDVI was performed both at the 3rd to 6th branches (equivalent to the normalized plant height of 0.8 to 0.9) and the whole plant scale. Values with different letters indicate significant difference ($p < 0.05$).

Variables	Treatments			p value
	D	C1	C2	
Total dry mass (g)	6.23 ± 0.41	6.57 ± 1.01	6.84 ± 0.34	0.30
Shoot dry mass (g)	5.37 ± 0.54	5.87 ± 0.87	6.19 ± 0.43	0.28
Initial dry mass (g)	1.43 ± 0.34	1.26 ± 0.02	1.05 ± 0.12	0.16
Flower no.	3.67 ± 2.08	4.00 ± 2.65	2.67 ± 1.53	0.73
Fruit no.	2.00 ± 1.00	1.67 ± 1.53	2.00 ± 1.00	0.95
Fruit dry mass (g)	0.85 ± 0.13	0.70 ± 0.61	0.65 ± 0.36	0.70
Fruit N content (%)	2.23 ± 0.21	1.36 ± 1.23	1.87 ± 0.09	0.28
Fruit N uptake (mgN)	19.21 ± 4.9	14.37 ± 13.57	12.22 ± 6.8	0.48
Shoot N content (%)	1.35 ± 0.10	1.32 ± 0.11	1.16 ± 0.21	0.21
Shoot N uptake (mgN)	69.67 ± 6.11	80.11 ± 6.65	76.32 ± 8.15	0.28
Total N uptake (mgN)	70.73 ± 6.71	78.63 ± 6.42	77.01 ± 2.94	0.43
N usage efficiency (%)	59.04 ± 5.60	65.63 ± 5.36	64.28 ± 2.45	0.43
Leaf NDVI (0.8 – 0.9)	0.88 ± 0.01	0.86 ± 0.04	0.89 ± 0.01	0.10
Leaf NDVI (whole plant)	0.84 ± 0.10 ab	0.82 ± 0.06 b	0.86 ± 0.05 a	< 0.05

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Table 3. Table A3. The elemental composition of essential macro- and micro-nutrients in the irrigating nutrient solution. Note: the elemental concentration was reported as the normalized concentration to the nitrogen level. The calculated results were based on the information from the product manufacture label.

Macro- and Micro-Nutrients	Normalized Concentration
Nitrogen	1.00
Phosphorus	0.46
Potassium	1.45
Calcium	0.55
Magnesium	0.15
Sulfur	0.18
Boron	< 0.01
Copper	< 0.01
Iron	0.01
Manganese	0.01
Molybdenum	< 0.01
Zinc	< 0.01

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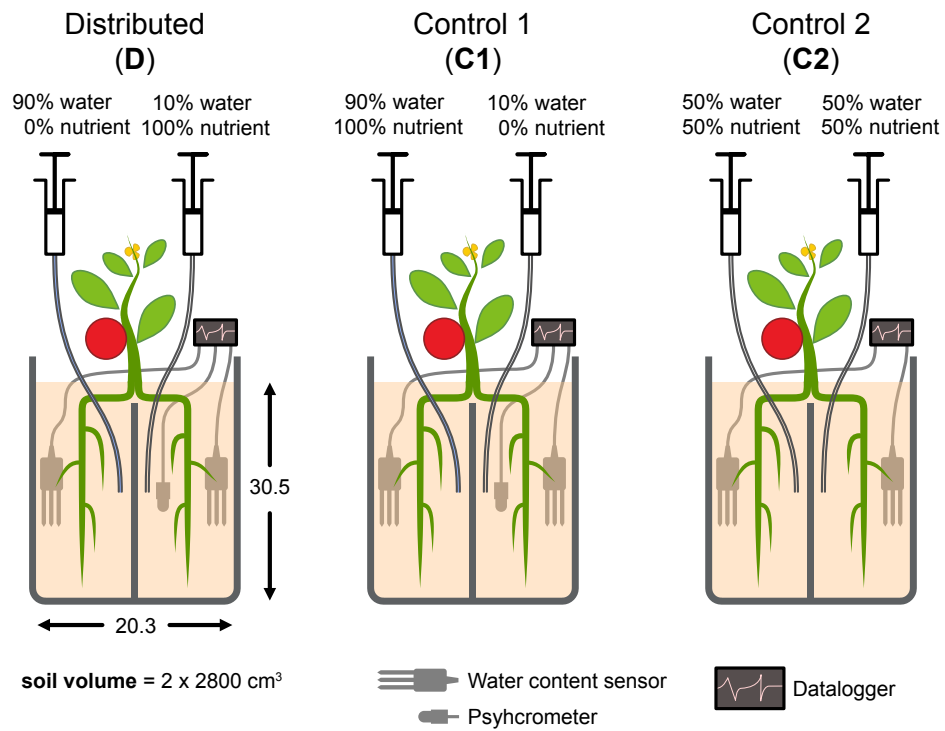


Fig. 1.

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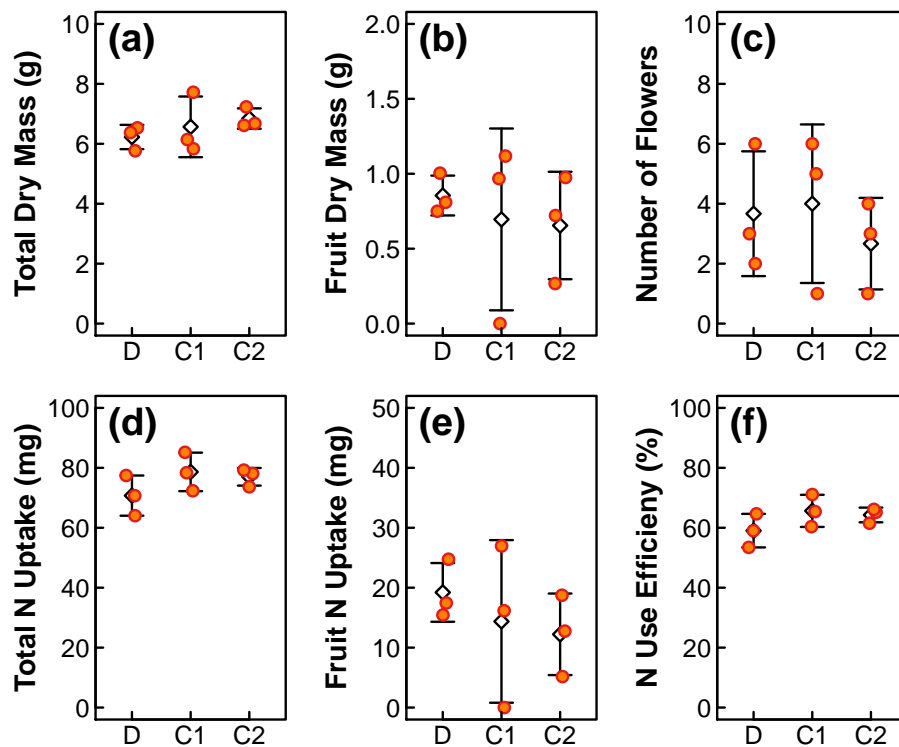


Fig. 2.

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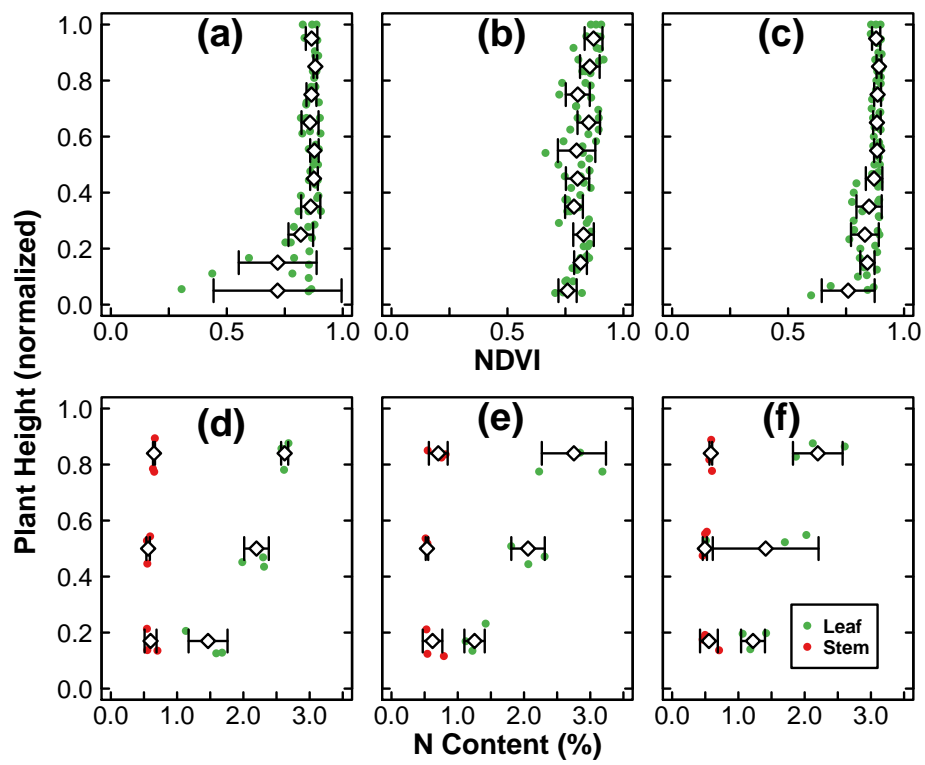


Fig. 3.

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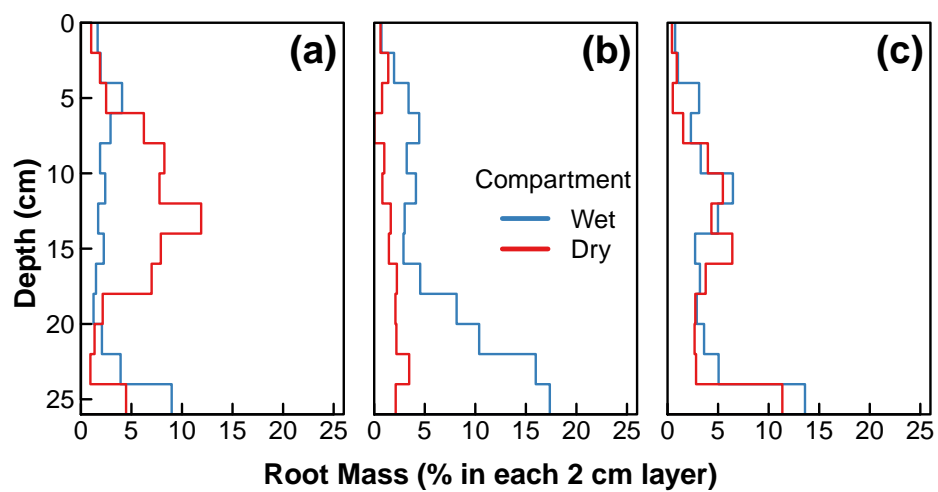


Fig. 4.

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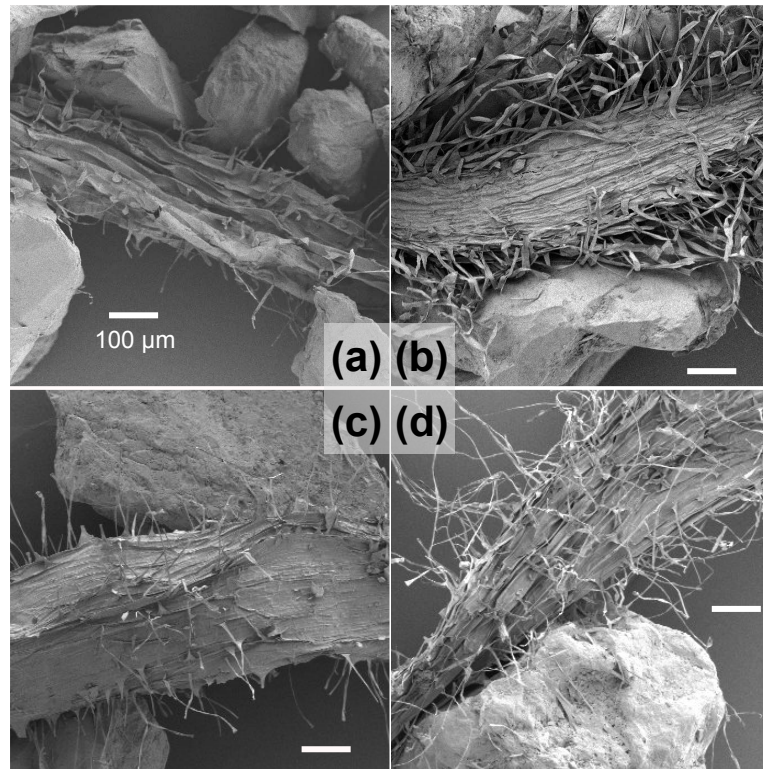


Fig. 5.

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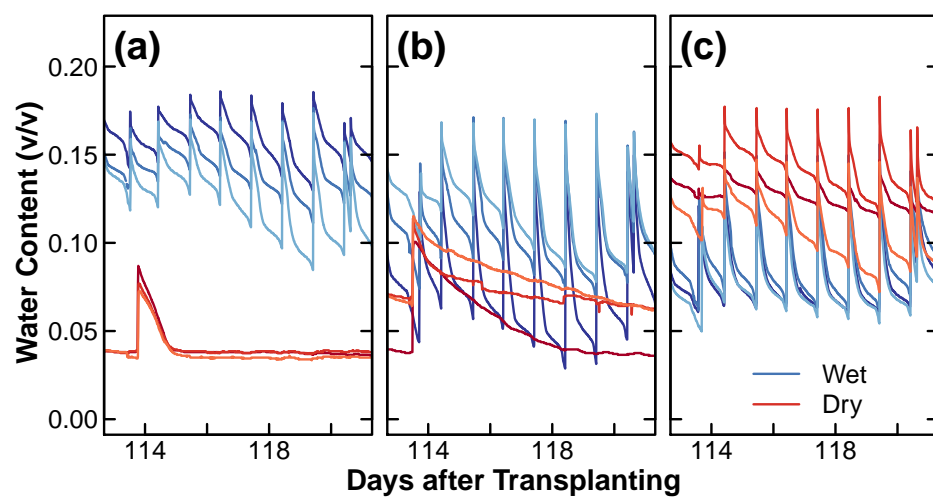


Fig. 6.

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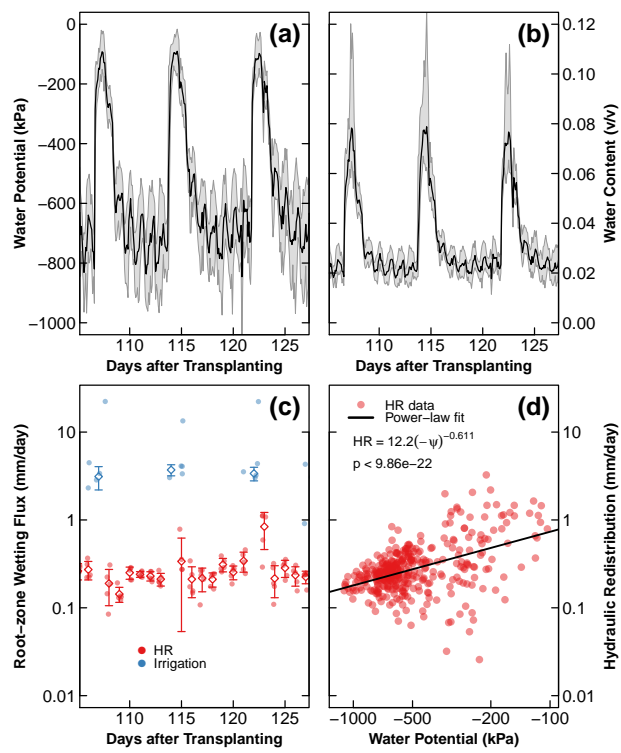


Fig. 7.

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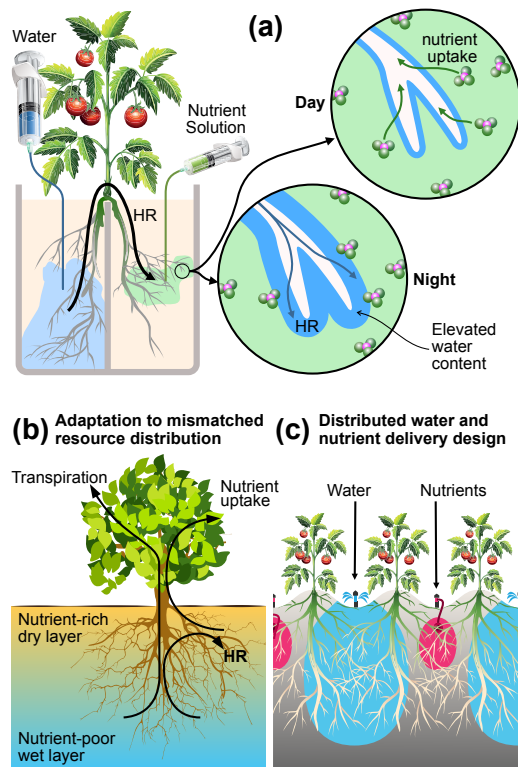


Fig. 8.

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Fig. 9.

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