

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

Jing Yan et al.

jyan235@ucmerced.edu

Received and published: 23 July 2020

C1

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

Response to Reviewer Comments #1

July 23, 2020

General Comment

The manuscript entitled “Root uptake under mismatched distributions of water and nutrients in the root zone” aims to test how mismatched distribution of water and nutrient influence nitrogen acquisition and plant growth. The authors further investigate how hydraulic redistribution and changes in root morphology can explain their results. While the objective of the study is very relevant and rather clearly defined and justified, the rest of the manuscript (material and methods, results, discussion and conclusion) is hard to follow, with crucial elements lacking from the material and methods. It makes it difficult to understand why the authors did some measurements and what they really measured. In the discussion and conclusion, I found some part too speculative. For example, how could you conclude so strongly about the crucial role of root hairs and production of mucilage, only based on non-quantitative microscopic observations? I suggest you better describe what you really demonstrated and what your results only

C2

suggest. Overall, I think that the data provided here are of good quality, that the design was well thought, but the manuscript is poorly written. See specific comments to help you to improve it.

Response: The criticism of this reviewer was shared by the other two reviewers as well. The manuscript has been revised with this in mind. Details that were previously included in the supplemental materials are now added to the materials and methods section. As Figure 1, we added a schematic diagram that describes the treatments and placement of sensors. Details were added to captions, and the results are explained thoroughly. Finally, we revised the results and discussion sections to avoid over-interpretation. Specific suggestions and comments from all reviewers were helpful in making these edits.

Specifically, in the conclusion section, we synthesize the observations from study and offer a conceptual model of what we believe is a depiction of nutrient and water dynamics in natural environments where nutrients and water may exist in a distributed fashion. Some of these statements are hypotheses and require further testing. We make it clear when we have direct evidence, and when we are speculating. For example, the role of HR in nutrient cycling was not observed in this study. In fact, we intentionally avoided conditions that would complicate the interpretation of where the plants acquired nutrients from. However, it is very likely that HR plays a crucial role in mineralization when nutrients are locked in organic matter in the dry region. This hypothesis is, in part, supported by previous studies that documented HR in arid regions, where the soils are coarse-grained and of low nutrient content. The synthesis of our knowledge is presented in Figure 8a and 8b. We believe this synthesis is an important contribution that can serve as a launching point for further studies.

C3

Responses to specific comments are provided below each comment. To facilitate the 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.

1 Specific Comments

Abstract

Please precise which plant (or at least type of plant) you grew as I am not sure that trees, herbs and grass shows the same adaptations to mismatches. At least, it should be proven before concluding it. We lack the experimental design (at least briefly mentioned) in the abstract

Response: We revised the abstract by adding the plant species, i.e., tomato plants, and a brief description of experimental design.

L.13 – 15 : It is too strong from my perspective. You did not quantify root hair density, neither production of root mucilage.

Response: Yes, we agree with the reviewer that SEM and confocal microscopic images did not provide quantitative information on root hair density and mucilage content. However, we believe the indirect and qualitative evidence gleaned from these observations is essential in deciphering how the plant root functions under mismatched conditions. Reviewer #2 commented on the role of root hairs at L145 and suggested presenting and discussing the observation of root hair enrichment in dry compartments of treatment **C1** and **D**. Therefore, we removed the description of root hair and mucilage

C4

from the abstract and conclusion while emphasizing this topic's discussion. At L220, we revised the discussion: “..two possible pathways might have allowed roots to modify rhizosphere hydraulic properties...”. Besides, we revised our description of the results of root hair. In brief, we reported that root hair density appears to be denser in the dry compartments of treatment **D** and **C1** and root hairs appear to be thicker in the nutrient-rich dry compartment (treatment **D**), compared to the nutrient-poor dry compartment (treatment **C1**).

Introduction

L. 28 – 31 : I was pleased to read that you mention the role of rhizospheric soil microbes to make nutrients available for plants. This could, and for my perspective should, be mentioned in the discussion too (although not too extensively as you did not measure any microbial parameter here). You mention specific adaptations of plants to water or nutrient deficiency (or heterogeneous distribution), namely: (i) Preferential growth in moist areas and modifications of root exudation (L.32-36) and hydraulic redistribution (L. 38 – 42). In these two paragraphs, you develop more adaptations to water scarcity or heterogeneity in fact. Adaptations to N deficiency or heterogeneity are less developed. For example, roots of a non-legume plant can forage toward the roots of a legume plant (Weidlich et al., 2018). Associations with soil microbes, such as N-fixing bacteria and mycorrhizae are as well strategies to enhance N acquisition and avoid growth limitations. Differences in root morphology (SRL, ratio root length/dry mass) of absorptive roots are typically used to describe foraging behavior of roots to acquire root N (a mobile nutrient). Proliferation of root hairs (which is not mentioned here, although it seems to be important for your article), or root clusters (highly branched roots) are more known to enhance acquisition of P, a less mobile element often found in patches (Lambers et al., 2011; Bates et al., 2001). With regards to adaptations of roots to water scarcity, see as well the recent article from Bristiel et al., (2019). The adaptations cited

C5

here do not sufficiently cover the topic.

1. Weidlich, E. W., Temperton, V. M., & Faget, M. (2018). Neighbourhood stories: role of neighbour identity, spatial location and order of arrival in legume and non-legume initial interactions. *Plant and Soil*, 424(1-2), 171-182.
2. Lambers, H., Finnegan, P. M., Laliberté, E., Pearse, S. J., Ryan, M. H., Shane, M.W., & Veneklaas, E. J. (2011). Phosphorus nutrition of Proteaceae in severely phosphorus impoverished soils: are there lessons to be learned for future crops?. *Plant Physiology*, 156(3), 1058-1066.
3. Bates, T. R., & Lynch, J. P. (2001). Root hairs confer a competitive advantage under low phosphorus availability. *Plant and Soil*, 236(2), 243-250.
4. Bristiel, P., Roumet, C., Violle, C., & Volaire, F. (2019). Coping with drought: root trait variability within the perennial grass *Dactylis glomerata* captures a trade-off between dehydration avoidance and dehydration tolerance. *Plant and soil*, 434(1-2), 327-342.

Response: Thank you for the references and additional adaptation mechanisms. We have provided a more extensive introduction and discussion regarding the role of root morphology, microbial activities, root nutrients in nutrient foraging.

In the introduction (L37), we added:

“Strategies of root foraging toward local soil nutrient deficiency or heterogeneity can be more divergent. Such strategies can involve the proliferation of root branches, root hairs. For example, the occurrence of hairy roots and root clusters has been reported enhancing phosphorus acquisition (Lambers et al., 2011; Bates and Lynch, 2001). Furthermore, the association with N-fixing bacteria and mycorrhizae has been found essential

C6

in root growth and N acquisition. The root interaction between neighboring plants further complicated our understanding. For example, a recent study showed that the roots of a non-legume plant forge toward the neighboring legume plant roots, where nitrogen is locally enriched (Weidlich et al., 2018).”

L. 50 – 53: While the objective was rather clearly described, I do not see the point with these last sentences.

Response: We moved this up in the introduction. It now appears at the end of the first paragraph and as
“In addition to natural systems, such adaptation likely plays a critical role in dry-land farming and rangelands. ”

Material and methods

In general, this section lack clarity and there is several important missing information. The methods are often described without explaining their aim. The subsection 2.1 (which could be renamed experimental design) lack to present the experimental design. Instead, the signification of treatment D, C1 and C2 is given at the beginning of the results! I can't find figure S1. I lack as well the number of replicates. The duration of the experiment should be given here too. The quantities of N, water, how are loss compensated, where it is added should be described: : : please report what was done with accuracy.

Response: We added a more detailed description of the methodology section. In addition, we moved Figure S1 from the supplemental materials to the main document and added a table that summarized water and nutrient application for each treatment.

C7

Revision included:

“Our experiments were conducted using laterally split soil compartments arranged in three treatments with three replicates of each treatment, as depicted in Figure 1. ... The experiment lasted for 140 days with a total application of the 653 – 676 *mm* water and 120 *mg* N. The compartment-specified application schemes were reported in Table 1.”

L.62 – 67: the measurement of water content and water potential belong to plant and soil characterization

Response: The revision of the materials and methods section has addressed this.

L. 80: Please define NUE, I guess this is nitrogen use efficiency, but this should be written.

Response: We now provide the definition of acronyms when they are first introduced.

L. 86: What do you mean by “further gravimetric measurements”?

Response: We clarified the procedure by changing it into “gravimetric quantification of root mass”.

L.88-93: It is not clear why you are doing these microscopic analyses. Why laser of two different wavelengths are used? What is gold coating for?

Response: The electron and confocal microscopic analysis provided complementary evidence about the morphological adaptations of roots and rhizosphere. Specifically, while SEM images provided detailed surface information with a higher spatial resolution, a confocal microscope differentiates

C8

the autofluorescent root compounds and non-fluorescent soil matrix. Gold coating (sputtering) is a standard technique in SEM imaging, especially when samples are non-conductive and sensitive to beam damage, including most biological samples. A conductive homogeneous layer of gold provides sharp images while maintaining the integrity of the sample morphology. We added references in the main document that provide these justifications (Kim et al., 2010; Golding et al., 2016). The confocal microscope shoots the incident light with shorter-wavelength (405 nm in this study) to excite the fluorescent emission from the plant root tissues and other organic compounds roots released. It then captures the emitted signals with longer-wavelength (488 nm in this study). We used this technique to distinguish autofluorescent compounds (roots and other organic compounds) from the non-fluorescent sand matrix. We added more details in the methodology to justify using these imaging techniques:

“405 nm and 488 nm lasers were used to excite and acquire autofluorescent compounds from the roots that distinguish from the non-fluorescent soil matrix. ... We then used SEM imaging to gain detailed surface information of the rhizosheath with a higher spatial resolution. ... A homogenized gold coating was used to provide a conductive layer of metal that enhances image quality by preventing charging and damage (Kim et al., 2010; Golding et al., 2016).”

Results

The subsections are confusing. Is plant water and nutrient uptake (3.3) not related to plant physiology (3.1)? Please reorganize. Moreover, some parts belong to material and methods, other to discussion. Focus on what you have observed here.

L.105 — 115: This belongs to material and methods.

C9

Response: Moved to the methodology section as suggested.

L. 118 – 120: This is your interpretation of the results. It should go to discussion.

Response: Moved to the discussion as suggested.

L.122 – 124: This belongs to introduction

Response: Moved to the introduction as suggested.

L. 127: How did you test that root density do not differ between the two compartments? By comparing root masses? If this is the case, it is thus not root density but root mass. Moreover, in table A2, the wet and dry compartments of the treatment D are significantly different.

Response: We agreed with the reviewer that statistically more root mass was found in the dry than wet compartment in treatment D. We changed the sentences to:

“Results highlighted that 60% of cumulative root mass grow into the dry compartment of the treatment D, despite the vast disparity in water availability.”

L. 127 – 128: this belongs to material and methods

Response: Moved to the methodology section as suggested.

L. 130-131: Belongs to material and methods

Response: Moved to the methodology section as suggested.

C10

L. 131 – 134: Please indicate what this higher root masses in the deeper part suggests in the discussion. Here you should describe the results.

Response: We moved the “... suggesting slight ...” to the discussion.

L.135: Again root density or root mass?

Response: We changed it to “root mass”.

L.136- 138: again, belong to discussion. Moreover, avoid detailing twice the same idea. An increase in root mass in the deeper layer is seen in the three treatments D, C1 and C2.

Response: We moved it to the dicussion and carefully removed the redundancy.

L. 138 – 140: This should be stated in material and method, not here.

Response: Moved to the methodology section as suggested.

L. 140: Did you measure root growth? Or are you indicating root mass? Root mass is not equal to root growth as the root mass at a given point depends on root growth, and root death (life span / root turnover).

Response: We changed the “root growth” to “net root mass increase”.

C11

L.143 – 145 from “which is : :” belongs to discussion.

Response: We moved it to the dicussion as suggested.

L.146: How did you measure root hair density? What test did you do to conclude for significant differences?

Response: We did not measure the root hair density quantitatively; instead, the results were based on the visual comparison. To avoid further confusion, we remove the word “significantly”, which implies qualitative comparison.

L. 147 – 148: This belongs to discussion

Response: Moved to discussion as suggested.

L.150: Avoid starting a new paragraph with “the above observations”. It suggest you are still developing previous ideas, so why starting a new subsection?

Response: Revised as suggested.

L. 151 – 153: Belongs to introduction

Response: Moved to introduction as suggested.

L.155: Did you describe the frequency of the irrigation?

C12

Response: After the plants become established over the first two weeks, the application of water or nutrient solutions in wet compartments of treatment **D** and **C1** were provided daily, while in the dry compartments, a small volume of water and nutrient solutions were provided once a week. For treatment **C2**, water was applied daily, while nutrient solutions were provided once a week. The total amounts of water and nutrient application are now presented in a new Table 1. Moreover, Figure S1 was revised added to the main body of the manuscript as Figure 1 and shows the irrigation pattern. We added descriptions of the frequency of irrigation and nutrient application events in the methods section.

L. 157: The information about the frequency of N addition should be given in material and method.

Response: See above response.

L. 161: How did you converted soil water potential data to rhizosphere water content?

Response: The soil water retention curve was determined independently using the same sand used in the experiment. We used dew-point potentiometry (WP4c, Decagon Devices, Pullman, WA) and nutrient solution identical to the irrigation water used in the dry-compartment of treatment **D**. Because the principles of measurement of WP4s and psychrometer are identical, we were able to convert the results of soil water potential measured from the psychrometers to soil water content by using the soil water retention curve. The description of the method, the fitting of the data and its use are now explained in the Methods section.

L. 163 – 164: Avoid opinion terms such as “closer inspection”.

C13

Response: Edited a suggested.

L. 166: Do not cite literature in the results, you should describe what you found here.

Response: Edited as suggested.

L. 168: What do you mean by “habitable environment”? For the roots? For rhizospheric microbes? Your focus here is not nutrient uptake, please stay stick to it.

Response: Although our main story is about nutrients, the questions we aim to address include understanding the mechanisms by which nutrient uptake from dry soils is possible. This includes understanding how roots are able to survive and grow in dry soils to the extent that was observed in this study. Our observations suggest that HR prevents the soil from progressively drying towards a stage that could hamper root function. In Figure 7a, notice that because of the contribution of HR, the water potential fluctuated between -800 kPa and -600 kPa, but did not dry further than that. Moreover, maintaining the soil water status above a detrimental threshold would permit soil microbes to carry out essential nutrient cycling functions in the rhizosphere. Therefore, we believe the function of HR in nutrient uptake is closely tied to the contribution of HR to the habitability of the rhizosphere to roots and microbes. We have made these linkages between HR, rhizosphere habitability, and nutrient uptake more clear and coherent in the revised manuscript.

L. 173 – 175: Again, this is not the description of the results.

Response: We removed the sentence as suggested.

L. 176 – 179: This belongs to discussion

C14

Response: We moved the sentence to the dicussion as suggested.

L. 180: Do you assume that the organic coating is root mucilage? How did you quantified it? What are the two fluorescent wavelength for?

Response: The microscopic image provides only qualitative information about the changes in root and rhizosphere morphology. The organic coating provided evidence that the modification of root hairs and rhizodeposition on rhizosphere soil properties, which has been reported in previous studies (Koebernick et al., 2017, 2019; Carminati et al., 2010; Ghezzehei and Al-balasmeh, 2015). The fluorescent wavelength distinguishes the fluorescent root compounds, including both root tissue or amorphous rhizodeposition from the non-fluorescent soil matrix. More details can be found in our reply to L88-93.

L. 181: keep suggestion to the discussion

Response: We moved the sentence to the discussion as suggested.

L. 181: This is an interpretation, not a result.

Response: We moved the sentence to the discussion as suggested.

Discussion

L. 184–186: This belongs to introduction

Response: We moved the sentence to the introduction as suggested.

C15

L.192: How could you confidently conclude that plant performance are less sensitive to localized scarcity in water and N if nutrient and water are sufficient in other locations where the roots forage. You did not tested it. To know it you should have a mismatched distribution of water and nutrients, with an overall limitation in water and N (compared to your treatment D).

Response: We clarified this statement. The experimental designed included compared localized nutrient deficiency in wet environment (treatment **D**) and localized water deficiency in nutrient poor environment (treatment **C1**) with non-deficient uniform resource availability (treatment **C2**). Our observations (see Figure 2 and 3 in the revised version) show no significant differences between these three treatments. This lead to our conclusion that under experimental conditions we tested localized deficiency of nutrients and water did not have measurable impact of overall plant performance. We think that the above ground performance (greenness, biomass, flowering, fruits, nutrient content) were indistinguishable despite the considerable differences in resource distributions is an important finding that is supported by multiple measurements. We did not intend to address overall nutrient and/water limitations in this study. All plants received equal amounts of water and nutrients. The added elaborations and reorganization of the sequence of presentation will make the objective and experimental design clearer and consistent with the conclusions that we came up with.

L. 194: I can't see what allow you to draw this conclusion here. Nothing written in the paragraph above allow to conclude it, although I think that you are right to point different plant strategies in case of mismatches.

Response: This conclusion was based on observed differences in root distribution between compartments and within compartment; qualitative observation of root-hairs; and presence of HR. Specifically, HR was induced and

C16

dense growth of thick root-hairs was observed only when the plants had to rely on nutrients that were concentrated in dry soil. This strategy absent in plants grown without such spatial of nutrients from water (in the same soil and under the same total nutrient and water availability).

L. 197–198 : Sentence not clear

Response: The paragraph is now rewritten with more elaboration.

L. 198 : You did not measure root proliferation as far as I have understood and what do you mean by this term: root growth? Root turnover?

Response: We changed “root proliferation” to “the extensive root mass distribution in the dry nutrient-rich soil compartment...”

L.199: What is multi-scale signaling and feedback? This is too vague.

Response: We added more elaboration and listed potential mechanisms. A recent study reported that the spatial availability of water is a key trigger for biosynthesis and transport of root-inductive signal compounds (Bao et al., 2014).

L. 200: You did not describe root allocation in the results. You surely want to say that this is the relative mass of roots in the two compartments? Or in the various depths? Please specify it. I can't see how it points a whole plant scale regulation of root growth. Please explain.

Response: We revised the paragraph to emphasize the differences in root mass distributions between the treatments. The revised results section

C17

and clearly show the differences between the final root mass distributions. We use consistent terminology throughout the revised manuscript and use cross-references to direct the reader to the data that is the basis of the discussions and conclusions.

L. 201: This confirms the foraging behavior of non legume roots to legume roots (Weidlich et al. 2018).

Response: We agree.

L. 204–205: This is one of the most interesting result of the study. Please detail more.

Response: We expanded our discussion, as suggested. We now include a discussion on how the root mass and root-hair density observed under the dry nutrient-rich environment were much higher than the nutrient-free environment subjected to similar water application regimen. Moreover, we reiterated that HR was observed only in the former case. These observations were the basis for concluding that HR plays an important role in supporting the growth and maintenance of roots in an otherwise non-conductive dry environment. It is also important to note that because of HR, the water potential did not progressively decline in the intervening period between the weekly irrigation with nutrient solution.

L. 206: What do you mean by “vigorous”? How did you measure it? It is not clear to me how drying after wetting event can indicate vigor.

Response: We replaced ‘vigorous’ with ‘more effective in water uptake’.

L. 214: This is an important result too.

C18

Response: We agree. This portion was further clarified and elaborated in response to comments from reviewer #2.

L. 218–219: What do the references refer to? You conclude here from your own results and cite the related figure. I guess the references indicate that this has been previously shown?

Response: We thanked the reviewer's critical review. Our results were consistent with previous evidence. Therefore, we added an additional discussion to distinguish them from our findings:

"This result was consistent with previous studies, where the loss of hydraulic conductance of soil-plant systems has been attributed to the decline in HR magnitude (Scholz et al., 2008; Ryel et al., 2002; Meinzer et al., 2004)."

L. 221: Need a reference

Response: We added a reference as suggested (Koebernick et al., 2017, 2019).

L. 226–229: Avoid finishing with limitations. Specify them either in the conclusion or in the discussion but not at the end as this is the last take home message for the reader.

Response: We moved the discussion of limitations and combined with our suggestion of future study to L192. Please also see our reply to L192.

Conclusion

L.231: "could" or "did"? Be clear with what you have demonstrated. In general, better differentiate what you showed and what your results suggests.

C19

Response: To clearly present our results, we changed "plants" to "tomato plants" and "could" to "can."

L. 243: How did you measure root activity?

Response: The changes in soil moisture reflected the root activity in terms of water and the associated nutrient uptake. We revised the sentence to emphasize the root function in water uptake that guides the audience at L206. Please see more details in our reply at L206.

L. 244: What is a vigorous nutrient cycling. Did you measure it?

Response: We removed the ambiguous word. In this synthesis part of the conclusion we are combining what we observed with that is previously known to suggest possible functions of HR beyond what we observed here.

L. 250-260: I enjoyed the final thought about application, but it makes the conclusion quite long and bring new ideas. This paragraph may be moved to the discussion.

Response: We moved the material that describes the potential application in agricultural context to the end of the discussion section. The revised conclusion now includes only one sentence that summarizes the agricultural implication.

Table A1: I would enjoy a graph or table with the values measured here. N uptake is central in your article (according to the objectives).

Response: We added the mean and standard deviation values of each variable and treatment reported in Table 2. See at the bottom of this document.

C20

Figure 3: What does the different color means? It would be better to rename treatments with an easy understandable name, instead of D, C1 and C2, which looks more a code for labeling pots.

Response: This Figure now appears as Figure 6. The treatments and abbreviations have been defined at the outset in the methods section and in Figure 1. The different shades of red and blue in these figures are used to distinguish between replicates. The revised caption addresses these differences.

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 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

C21

not produce fruits, resulting in larger deviations in fruit dry mass and N update 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 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

C22

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 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.

C23

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.
- Bates, T. R. and Lynch, J. P. (2001). Root hairs confer a competitive advantage under low phosphorus availability. *Plant and Soil*, 236(2):243–250.
- Carminati, A., Moradi, A. B., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Vogel, H.-J., and Oswald, S. E. (2010). Dynamics of soil water content in the rhizosphere. *Plant and Soil*, 332(1):163–176.
- Ghezzehei, T. A. and Albalasmeh, A. A. (2015). Spatial distribution of rhizodeposits provides built-in water potential gradient in the rhizosphere. *Ecological Modelling*, 298:53–63.
- Golding, C. G., Lamboo, L. L., Beniac, D. R., and Booth, T. F. (2016). The scanning electron microscope in microbiology and diagnosis of infectious disease. *Scientific reports*, 6:26516–26516. Publisher: Nature Publishing Group.
- Kim, K. H., Akase, Z., Suzuki, T., and Shindo, D. (2010). Charging Effects on SEM/SIM Contrast of Metal/Insulator System in Various Metallic Coating Conditions. *MATERIALS TRANSACTIONS*, 51(6):1080–1083.
- Koebnick, N., Daly, K. R., Keyes, S. D., Bengough, A. G., Brown, L. K., Cooper, L. J., George, T. S., Hallett, P. D., Naveed, M., Raffan, A., and Roose, T. (2019). Imaging microstructure of the barley rhizosphere: particle packing and root hair influences. *New Phytologist*, 221(4):1878–1889.
- Koebnick, N., Daly, K. R., Keyes, S. D., George, T. S., Brown, L. K., Raffan, A., Cooper, L. J., Naveed, M., Bengough, A. G., Sinclair, I., Hallett, P. D., and Roose, T. (2017). High-resolution synchrotron imaging shows that root hairs influence rhizosphere soil structure formation. *The New Phytologist*, 216(1):124–135.
- Lambers, H., Finnegan, P. M., Laliberté, E., Pearse, S. J., Ryan, M. H., Shane, M. W., and Veneklaas, E. J. (2011). Phosphorus Nutrition of Proteaceae in Severely Phosphorus-Impoverished Soils: Are There Lessons To Be Learned for Future Crops? *Plant Physiology*, 156(3):1058.
- Meinzer, F. C., Brooks, J. R., Bucci, S., Goldstein, G., Scholz, F. G., and Warren, J. M. (2004). Converging patterns of uptake and hydraulic redistribution of soil water in contrasting woody

C24

vegetation types. *Tree Physiology*, 24(8):919–928.

- Ryel, R., Caldwell, M., Yoder, C., Or, D., and Leffler, A. (2002). Hydraulic redistribution in a stand of *Artemisia tridentata*: evaluation of benefits to transpiration assessed with a simulation model. *Oecologia*, 130(2):173–184.
- Scholz, F. G., Bucci, S. J., Goldstein, G., Moreira, M. Z., Meinzer, F. C., Domec, J.-C., Villalobos-Vega, R., Franco, A. C., and Miralles-Wilhelm, F. (2008). Biophysical and life-history determinants of hydraulic lift in Neotropical savanna trees. *Functional Ecology* DOI: 10.1111/j.1365-2435.2008.01452.x, 22:773–786.
- Weidlich, E. W. A., Temperton, V. M., and Faget, M. (2018). Neighbourhood stories: role of neighbour identity, spatial location and order of arrival in legume and non-legume initial interactions. *Plant and Soil*, 424(1):171–182.

C25

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

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-109>, 2020.

C26

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

C27

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

C28

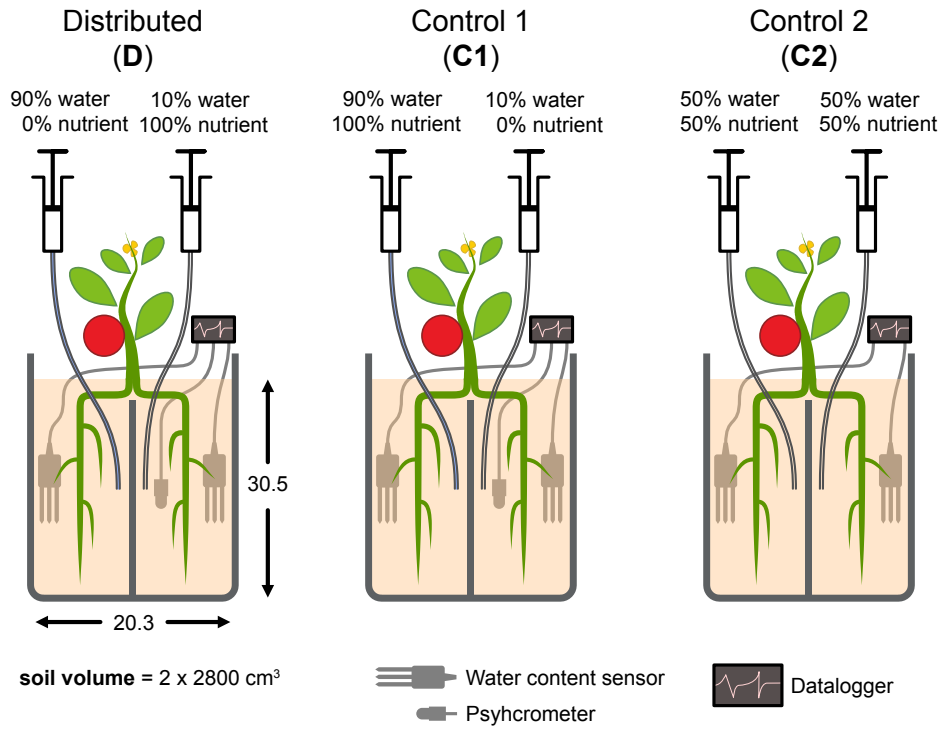


Fig. 1.

C29

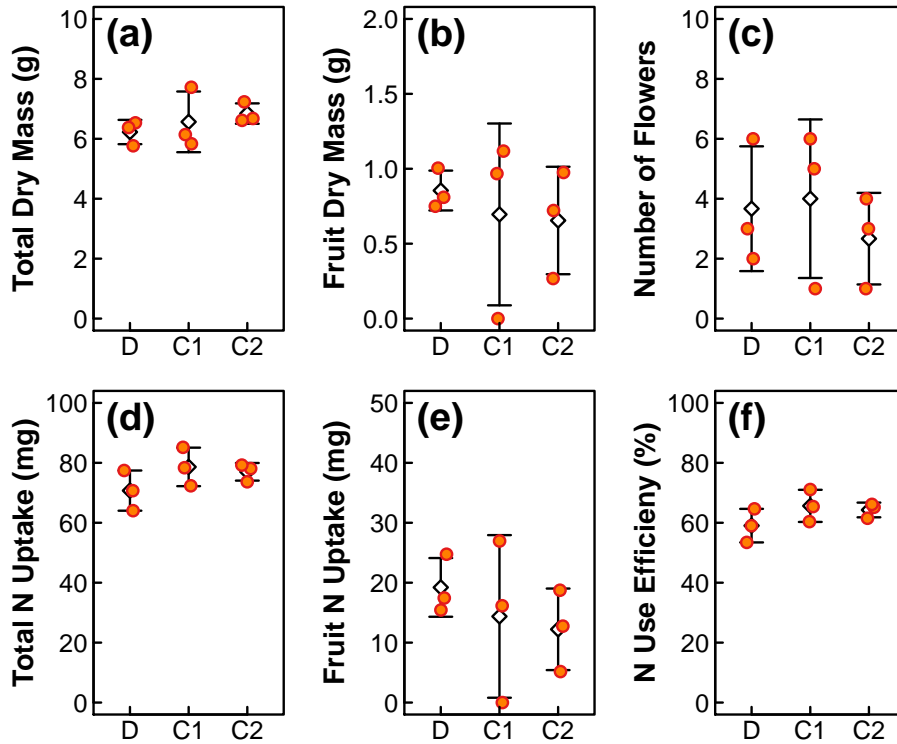


Fig. 2.

C30

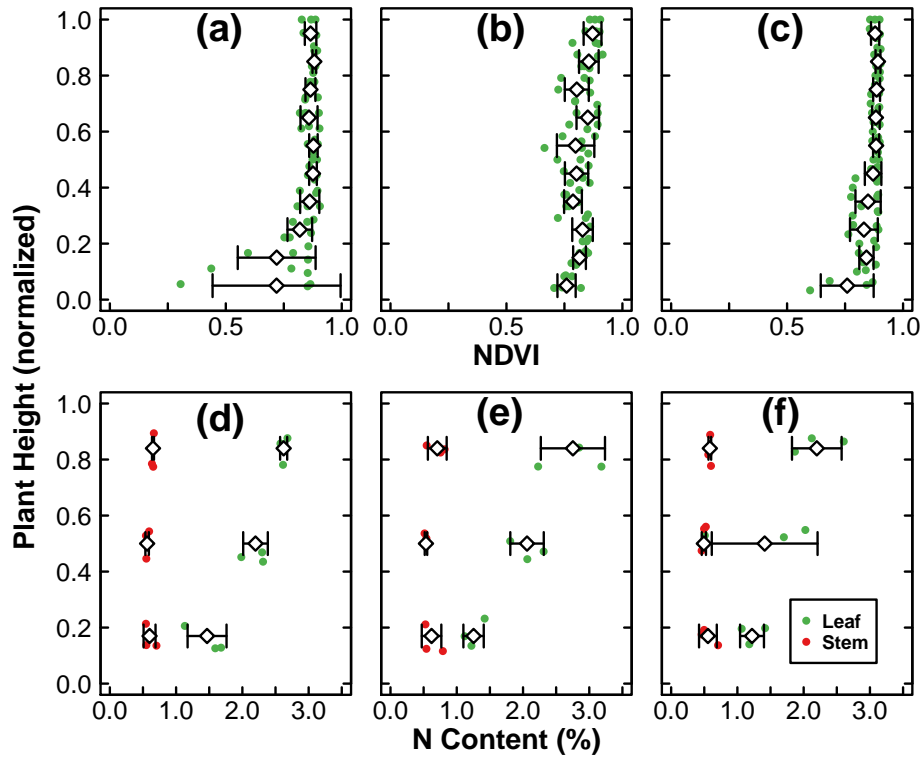


Fig. 3.

C31

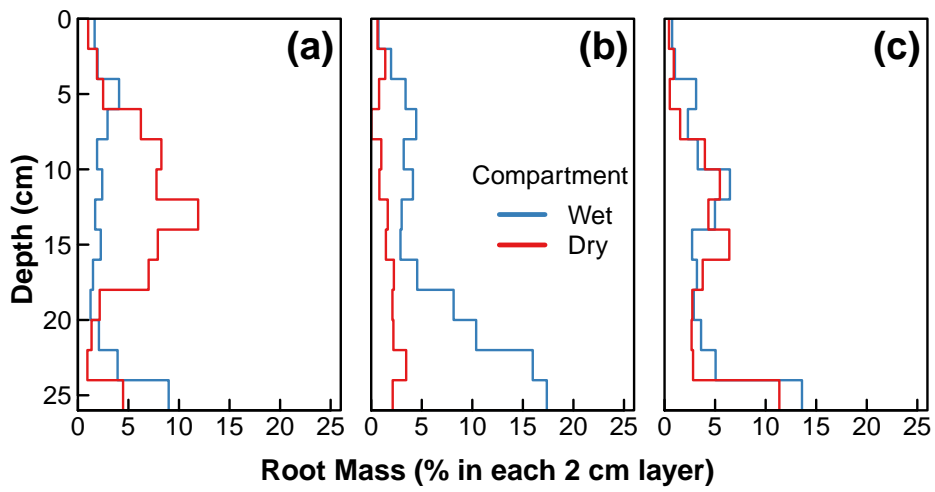


Fig. 4.

C32

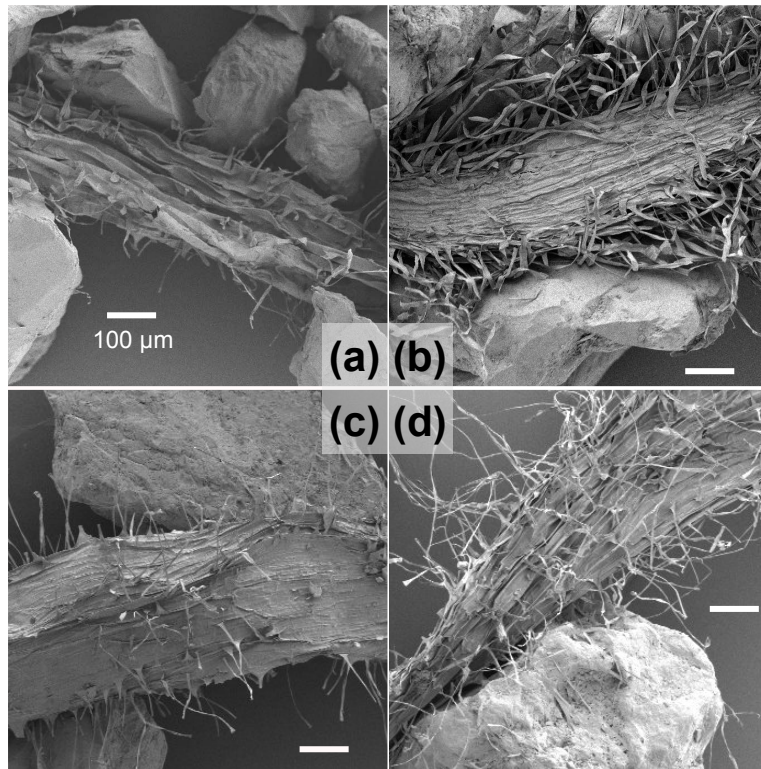


Fig. 5.

C33

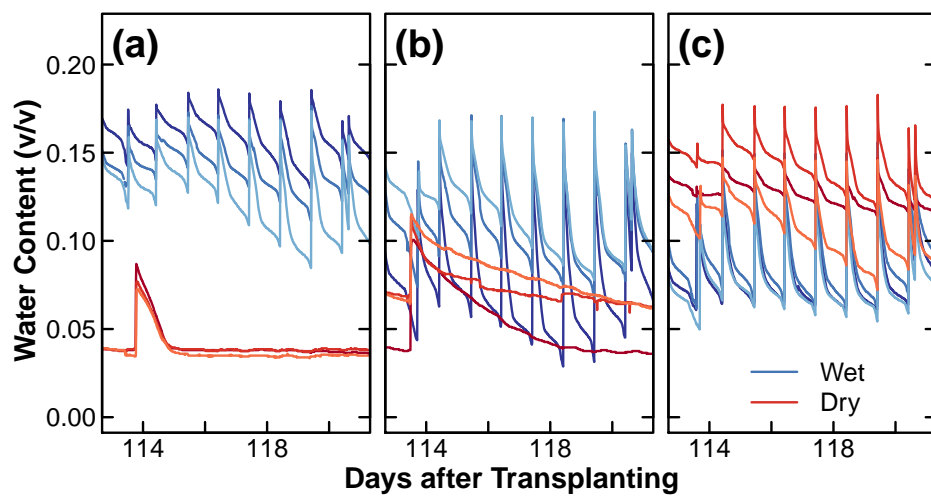


Fig. 6.

C34

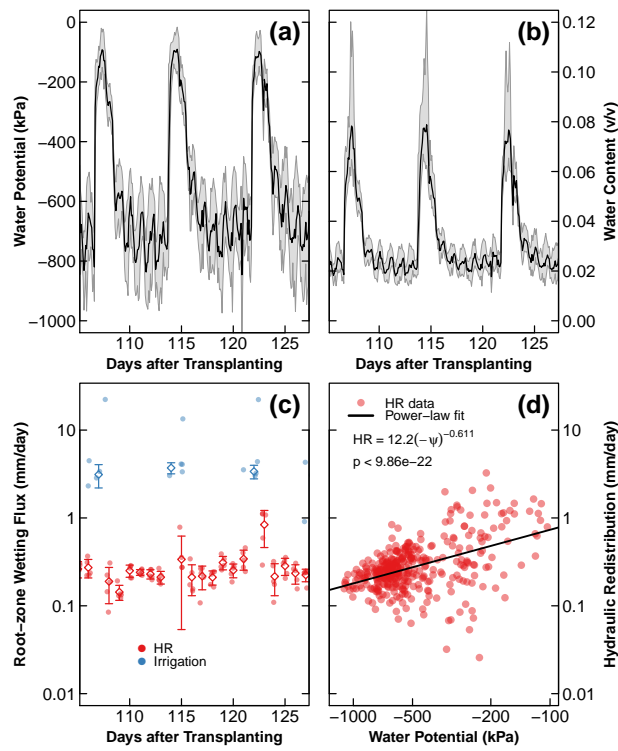


Fig. 7.

C35

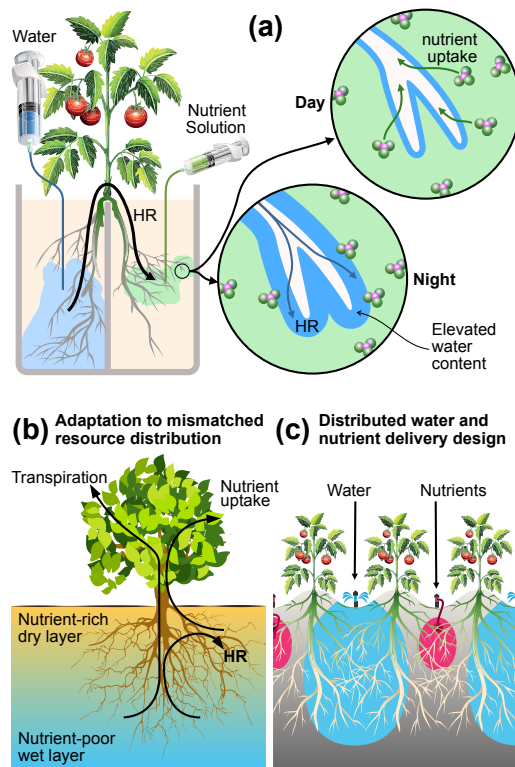


Fig. 8.

C36