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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

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Response to Reviewer Comments #2

July 21, 2020

General Comment

I was generally pleased to read through the manuscript titled Root uptake under mismatched distributions of water and nutrients in the root zone. The manuscript sets out to demonstrate that plant roots are still able to operate well under conditions where water and nutrients are partitioned in segregated regions. The article presents a novel and imaginative set up well within the capacity to monitor a vast array of soil and plant physical and philological features. Results for the most part are clear and concise and the writing is very comprehendible. With the praise being said, there are a few critical points that need to be addressed in the manuscript.

Most of the points pertain to organization, but a few are on the science itself. The figures are in a strange order. I think the first figure that’s referenced is the last figure in the manuscript, and this makes no sense. There are a lot of subfigures that are never mentioned or mentioned in a strange order. I've gone through and make marks regarding these points and recommend that the authors make amendments accordingly. Just have the figures appear as they are mentioned in the text and make sure to mention all of the figures that you are presenting. That is pretty simple.

More pertinent is the matter of the science. In particular, the focus of the study somewhat diverges and tries to come back together towards the end of the manuscript. In the beginning, the authors are describing this split column root growth experiment in the context of nutrient acquisition and plant development. The authors then attempt to push the notion of hydraulic redistribution in the results later on. It comes off as a bit shoehorned in. My particular issue with this is that it is that your results might be suggesting a very subtle and highly local redistribution of water. The authors then demonstrate that HR is actually less effective under drier conditions when it would be most needed. Towards the end, I was almost convinced that HR wasn't a relevant topic matter. However, the authors did manage to sway me back in slightly when they were trying to make the argument that it was used for the nutrient uptake. I think the authors need to really focus on the subtlety that they are highlighting with their results and draw from some more fundamental principles to base their arguments. Consider that root nutrient acquisition relies on enzymatic reactions that may require a sufficient quantity of water to enact. I think something simple but fundamental would give this study a stronger foundation for its claims. The study already does a good job of illustrating that roots are not just passively behaving belowground. Their ability to actively take up water and nutrients is already interesting. The authors just have to better reconcile the results in figure 3 a and fgure4b. They appear contradictory.

Response: We thank the reviewer for the thorough and critical review. The criticism about order of figures and logical flow of content was shared by the other reviewers. In response, we have made substantial reorganization

of the content. We revised the figure that describes the experimental design and moved it to the main body of the manuscript as Figure 1. It now includes clear definition of the abbreviations, sensor placement and dimensions of the pot, which will make the results and discussions easy to follow. All the figures now appear in the order they are referred. All subfigures are now described in the body of the manuscript.

The revised manuscript is now more streamlined in a manner that the reader can easily follow to get to the main points of this paper. The first point we make is that nearly complete separation of water and nutrients did not significantly impact the overall performance of the tomato plants we investigated. This finding is the basis for the key question addressed in this paper: *Given that plants are not underperforming in this non-ideal resource availability, what are the adaptation mechanisms used by plants to survive and thrive?* Our answer has multiple related parts. First, there is a high-density root growth in the nutrient-rich dry soil (treatment **D**) compared to the nutrient-poor dry soil (treatment **C1**). Moreover, the roots in the nutrient-rich dry compartment were concentrated in the mid-section, where the nutrient pulses were applied. In contrast, when nutrients are added with the bulk water (in both versions of the control treatments), the highest root density was observed at the bottom of the pots, likely because the nutrients were leached down with the water. These observations support that (a) the roots can acquire nutrients from dry nutrient-rich soil and (b) root density is in part correlated with nutrient concentration. Moreover, the SEM images suggest that there is higher density of thicker root-hairs (qualitative observation) in the nutrient-rich dry soil compared to nutrient-rich-wet and nutrient-poor-dry compartments. This is consistent with the literature that describes the importance of root-hairs in nutrient acquisition (Bates, Lynch, 2001; Zhang et al., 2018).

The above observations lead to a more specific question: *how are these roots surviving and growing in the dry environment and how are they able to mobilize the nutrients?* Our psychrometer data suggests that a significant increase in water potential at night time. We interpreted this as hydraulic-redistribution from the wet compartment. We rued out internal redistribution of water within the dry compartment as none of the sensors placed there detected an out-of-phase dynamics. We argue that this wetting is responsible for supporting root proliferation and mobilization of nutrients. As the reviewer stated, the wetting can also be important in increasing microbial and enzymatic activity that is essential for nutrient acquisition. However, the latter role of hydraulic-redistribution was likely to be minimal under our experimental condition because (a) the soils lack organic matter and (b) all the nutrients were provided in plant-available form. That said, it is likely that in field conditions hydraulic-redistribution plays an important role in fueling enzymatic and microbial functions.

All the figures are reformatted for consistency and the key findings have been moved to the main body. The reviewer stated "...better reconcile the results in Figure 3a and Figure 4b. They appear contradictory." Both these figures show the water content dynamics in the nutrient-rich dry compartment. The data in Figure 4b (now appears as Figure 7b) was indirectly calculated from the matric potential data measured by thermocouple psychrometers. We used soil-specific water retention curve for the conversion. Because the psychrometer has much higher sensitivity to small changes in water potential (hence, water content), the data pattern reveals more pronounced dynamics. The data in Figure 3a (now appears as Figure 6a) is derived from direct measurement using dielectric moisture sensor. The sensitivity of the dielectric sensors is not sufficient to fully

reveal the hydraulic-redistribution dynamics.

Responses to specific comments are provided blow 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.

1 Specific Comments

https://www.biogeosciences-discuss.net/bg-2020-109/bg-2020-109-RC2-supplement.pdf The reviewer provided several comments on an annotated manuscript. All identified errors were fixed. Recommendations on wording were mostly accepted as suggested or replaced with alternative language that improved clarity. Substantial specific comments are addressed below. We used the line numbers of the original manuscript to refer to each question. The corresponding responses are provided under each comment.

1. L1-16: The reviewer added these comments and suggestions for clarifying the abstract 'Split in what way? Perhaps you could describe this here in a bit more detail', 'I think this sentence isn't needed. It's vague, unclear, and nicely explained in the subsequent text.', 'It almost reads like two abstracts. The relevance of HR seems like the specific phenomena that the manuscript is working on. Perhaps it's best to just focus more on this as to not distract the readers.', and 'Brief pulses of what?'
   Response: The abstract was rewritten to address these comments and the comments of the other reviewers: "Most plants derive their water and nutrient needs from soils, where the resources are often scarce, patchy, and ephemeral. It is

not uncommon for plant roots to encounter mismatched patches of water-rich and nutrient-rich regions in natural environments. Such an uneven distribution of resources necessitates plants to rely on strategies to explore and acquire nutrients from relatively dry patches. We conducted a laboratory study that elucidates the biophysical mechanisms that enable this adaptation. The roots of tomato seedlings were laterally split and grown in two adjacent, hydraulically-disconnected pots, which permitted precise control of water and nutrient applications to each zone. We observed that physical separation of water-rich and nutrient-rich zones does not significantly hamper plant productivity. Specifically, we showed that soil dryness does not reduce nutrient uptake, provided that the whole plant has access to sufficient water elsewhere in the root zone. We identified localized root proliferation in nutrient-rich dry soil patches as a critical strategy that enabled nutrient capture. Furthermore, high-frequency water potential measurements revealed nocturnal rewetting of the nutrient-rich but dry soil compartments. We interpreted this as a water-potential-gradient driven transfer of water from the wet to dry compartments, a process commonly known as hydraulic redistribution (HR). The occurrence of HR prevents the nutrient-rich soil from drying to the level of permanent wilting and subsequent decline of root functions. Moreover, cyclic rewetting of the rhizosphere likely increases nutrient mobility and uptake. It is also possible that roots facilitate HR by increasing root-hair density and length and deposition of organic coatings that increase water retention. Therefore, we conclude adaptation to mismatched resource distributions calls for plant-controlled biophysical regulation of soil and water dynamics in the rhizosphere. Our findings support a nature-inspired nutrient management strategy for significantly curtailing water pollution from intensive agricultural systems."

2. L24: In general, this does sound intuitively correct. However, doesn't this also rely on soil texture?
   Response: We agree with the reviewer that soil texture is an important factor that

regulates rate of evaporation and soil drying. In field conditions, plants are likely to experience mismatched distribution of resources when nutrient-rich surface layers dry faster and more often than nutrient-poor subsurface layers. This is likely to occur in coarse textured soils. In fact, most of the studies that reported field observation of hydraulic redistribution were carried out in areas with coarse soil texture (Neumann, Cardon, 2012). We added the following sentence. "This effect is likely to be pronounced in coarse textured soils that dominate most arid and semi-arid soil soils (Rodell et al., 2004)"

3. L33: "tracks" water infiltration patterns? Does this mean roots follow preferential paths of water infiltration?
   Response: We rephrased it to be consistent with the cited source: "In water-limited areas, rooting depth generally coincides with infiltration depth (Fan et al., 2017).

4. L34: Provide a bit of mechanistic detail. How are plants able to do this?
   Response: Changed to
   "Locally, roots can also respond by increasing the water retention capability of their immediate surroundings (the rhizosphere) by releasing a cocktail of organic compounds that sorb water and promote soil aggregation."

5. L36: Again, what are some of the mechanisms that facilitate this? McKay Fletcher et al. 2020 recently proposed citrate enhanced uptake on the basis of exudation.
   Response: We revised the sentence as
   "Furthermore, root exudation (release of low-molecular-weight rhizodeposits) can increase nutrient availability and accessibility by freeing tightly-bound nutrients (e.g., (McKay Fletcher et al., 2020)) and priming microbial mineralization of nutrients (e.g., (Keiluweit et al., 2015))."

6. L40: Be more specific as to where the claim is coming from. "Studies have found

that..." or "Reports have shown...". We don't want to just take suggestions from people in line at the grocery store (however wise they might be). :) I'm not quite sure if I'm following the rationale here. HR is water that is initially taken up by roots and subsequently released in drier regions of soil. How and/or why should the nutrients also be carried and released? I was of the understanding that plants take up nutrients via enzymatic reactions. I don't see how or why these nutrients would be re-located in the same way that water is. I could understand the water taken up and re-released in dry patches, which helps plants to take up nutrients in those dry patches, but I struggle to understand a sort of nutrient lift or nutrient redistribution. Perhaps I've just misunderstood the statement. If so, could you clarify what is meant by carrier for nutrients?
Response: This was indeed a vaguely written sentence. We revised it as "Studies have found that water released by HR can elevate ammonification, N mineralization, and plant inflorescence N uptake (Cardon et al., 2013) and enhance the overall nutrient mobility in dry soil patches (Matimati et al., 2014)."

7. L50: These sound like points that should come earlier in the the introduction and re-introduced in the discussion. They're both really nice points, but perhaps the authors can weave them into the text more smoothly.
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."

8. L56: You should make the first figure that you introduce appear as figure 1.
Response: We updated and moved Figure S1 to the main document as Figure 1 (see below). We also added new table (see Table 1) in which treatment descriptions and acronyms are defined and water and nutrient inputs are reported.

9. L64: C1? Why not just C? The different treatments should be stated explicitly before stating them.

Response: See above response. There are two different versions of control treatments. Now that these are defined in the text, Figure 1 and Table 1 at the beginning of the methods section it will be easier to follow.

10. L64: Perhaps you could state what the benefit of these redundancies. You have sensors for water content and matric potential. Do you have a SWC for your silica mixture? It could be useful for inferring impacts of rhizosphere features on soil water movement.
Response: The justification for using two different sensors is because the two approaches are well suited to different ranges of soil moisture. Psychrometers provide better sensitivity to small changes in soil moisture when the soil is very dry, with accurate measurement range of $-50\,\mathrm{kPa}$ to $-8000\,\mathrm{kPa}$. Whereas dielectric sensors have poor sensitivity to small changes in water content, particularly when the soil is dry. Therefore, we changed the sentences to
"Dielectric water content sensors (5TE of Meter, Pullman, WA) were placed at the center of each compartment at 14 cm below the surface to capture the bulk-scale soil moisture dynamics. At the same soil depth, the dry compartments of treatment **D** and **C1** were outfitted with pairs of thermocouple psychrometric water potential sensors (Psypro of Wescor Inc. Logan, UT) to measure the localized soil water potential with very high degree of sensitivity (Brown, Bartos, 1982; Andraski, Scanlon, 2002; Whalley et al., 2013). The combination of the two sensor types allows quantification of water dynamics with high degree of fidelity from the wet to dry moisture range.

11. L95: Please provide some details regarding what these tests are.
Response: In this study, Welch's ANOVA was used to test whether there are statistically significant differences between the means of plant performance indicators in the three treatment groups (treatment **C1**, **C2**, and **D**). A regular one-way ANOVA assumes homoscedasticity or homogeneous variance inside each test group; however, in our study, indicators showed different variances inside each

treatment. For example, the fruit dry mass of C2 showed much larger variances than the other treatments. The Welch's ANOVA allows the test for groups with heteroscedasticity or heterogeneous variances inside each testing group. The Games-Howell test was used to rank the means of plant performance indicators in three treatment groups. We changed the sentence to
"Plant physiological indicators were compared across treatments using a Welch's analysis of variance (ANOVA) to avoid interference from heteroscedasticity of those indicators (Welch, 1947) and posthoc Games-Howell test for multiple comparisons from R (Games, Howell, 1976)."

12. L101: This line isn't very clear. Could you elaborate in a few more sentences? Also, are you making a distinction between soil physical properties near and away from the rhizosphere? If so, it might be worth stating this explicitly and later highlighting these differences in a table.
Response: The "rhizosphere wetting" here was referred to as the "HR magnitude." We carefully checked the manuscript to replace different terms with a consistent term of HR magnitude throughout the manuscript. We performed the SWC measurement of pure sand with nutrient solutions with the same concentrations for the irrigation. According to Reviewer #3, we changed the "rhizosphere water" content to "root zone water content". More detail that was previously provided on the supplemental material is now added to the methods section.

13. L108: Your labeling system has to be clearly defined earlier in the text. It might be worth just making a short table that indicates the different experimental scenarios.
Response: We added a Figure 1 and a Table 1 to provide more details. Treatments are described in detail at the beginning of the methods section. See above responses.

14. L111: This statement sounds a bit redundant with the methods and materials

section. Just state what's being plotted in figure 1.
Response: Removed as suggested.

15. L114: It might be worth moving Figure S2 back in here. It seems to merit its place in the main text.
Response: We modified and moved Figure S2 to the main document as Figure 3.

16. Figure 1: Describe the figure a bit more. Perhaps state why some of these results have massive spreads. Also, what's the difference between the points and the points with the uncertainty ranges? I know you're trying to demonstrate that there are no discernible trends based on these results, but do not be cavalier when presenting this data. Also, if you really don't think it's worth plotting the data in Figure S2, then it might be worth stating it only briefly. Otherwise, I think it would be nice to integrate the information with this figure.
Response: We added more detail to the figure caption (now appears as Figure 2)
"Figure 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 update in treatment **C1**. "

17. L118: I would refrain from using language that is so astringent. You are still operating under an artificial set up with a select cultivar. Your data stands strong on its own, so there's no need for the hard sell.
Response: Removed "unequivocally" as suggested.

18. L122: This reads as though it belongs in the intro. I would suggest moving it there and rephrasing this intro.
Response: We moved and incorporated it into introduction as suggested.

19. L126: Remarkable has a connotation of suspense and surprise. Perhaps rephrase to,
"Results highlighted that the density of roots in the wet and dry compartments are indistinguishable, despite the vast disparity in water availability." Your results speak for themselves. They require no added shock value.
Response: Revised as suggested.

20. L127: This belongs in materials and methods.
Response: Moved to methodology as suggested.

21. L128: What does this mean? Should there have been a 3D root architecture? The figure illustrates the photo of the set up.
Response: Figure 4a shows the bulk distribution of roots in the dry and wet compartments. However, the spatial distribution of the roots prior to excavation is not visible in this photograph because the roots settled at the bottom after the soil was excavated. The paragraph was revised as follows. This photograph was not adding a significant information to the story of the paper, so has now been moved to supplemental material. It is now presented along with other photographs of experiments including the roots of the seedlings before transplantation to the split pots.

22. L131: None of the distributions in the figure look uniform. (f) and (h) even have slight bumps towards the centerline of the set up. I would re-phrase this to more accurately describe the results.
Response: The Stacked bar-chart format used previously was hiding the pattern. See revised Figure 4 below. We also add references to the specific part of each figure as we describe the different treatments.

23. L134: As stated before, both seem to exhibit this concentration in the midsection. Perhaps I'm misunderstanding the figure. If so, please try to elaborate in a way that is clear and consistent with the figures.
Response: See the above response.

24. L138: This is a generalization for specific soil conditions and nutrients. For instance, phosphorus is highly susceptible to binding on finer soil particles, so you have to be careful with these broad brush strokes and generalizations on the basis of your set up.
Response: We agree with the reviewer that chemical retention might have hindered the leaching of strongly sorbing nutrients in natural systems, such as phosphorus, as the reviewer mentioned. The statement is specific to the soil conditions used in this study. We added a sentence to the discussion section:
"The coarse silica sand used in this study likely facilitated leaching of reactive nutrients such as phosphorous."

25. L140: Is this shown in any of the figures or tables? Please cite the figure where this is highlighted.
Response: We added cross-reference to Figure 4b.

26. L145: Authors need to reference their figures more thoroughly and provide some explanation for what the reader's should be paying attention to. For instance, root hairs appear to be more prominent in the dry cases in both scenarios. Though I am skeptical as to what mechanisms are at hand (i.e. root hairs lack the vasculature for rapid water movement), they must be doing something in dry soils (perhaps the structures alone are at play). It's worth noting this. Furthermore, the ordering of your figures is chaotic. They're good figures, but the authors need to organize them how they appear.
Response: We splitted previous Figure 4 into Figure 4 and Figure 5. We then reorganized Figure 5, so that the parts are ordered in the sequence they are ref-

erenced. We added cross-references to each component as they are discussed. All the SEM images have identical magnification that permits visual qualitative comparison. We noticed two patterns. First, there are more root hairs in the dry compartment than the wet compartment of both treatment **D** and **C1**. Second, the nutrient-rich dry compartment appears to have much higher density and thicker root hairs than the nutrient-free dry compartment. Thus, our interpretation of these observation is that increase in the density and thickness of root hairs is a response to availability of nutrients in dry soil.This observation of increased root-hair growth as adaption to nutrient availability has been reported in (Bates, Lynch, 2001; Zhang et al., 2018). These points have been made clear in the revised manuscript.

27. L150: This again reads like a piece from the methods and materials. This should be moved accordingly.
Response: Moved as suggested.

28. L157-159: Please reference a figure that will illustrate this comparison. Also, this doesn't seem to suggest hydraulic redistribution, but it does imply a more sophisticated control over root water uptake. It would seem that the atmosphere doesn't have such a complete tyranny over transpiration. ... I don't understand this argument. Could you please elaborate as to why this should be the case?
Response: This sentence is supported by comparing the water content dynamics in the 'dry' compartments of treatment **D** and **C1**, which are depicted by the red lines in Figure 6a and 6b, respectively. Although the 'dry' compartments of both treatments received equal amounts of water, the rates of drying were vastly different. The water content in the nutrient-rich compartment (Figure 6a) dropped to pre-irrigation level within one day while the water content in the nutrient-poor compartment declined slowly over several days. Thus, water redistribution within the compartment was ruled out as a primary factor. We attributed the faster rate of soil moisture decline in the nutrient-rich compartment to root uptake, which is

also consistent with the higher density of roots in this compartment (Figure 4b). Later in the manuscript, we argue that HR prevented the soil in the nutrient-rich compartment from progressively drying towards permanent wilting point, thereby enabling maintenance of root function in the dry spells between irrigation.

29. L162: Were the SWC's determined on the rhizosphere soil? If so, please state this clearly in the methods.
Response: The SWC of the clean sand was determined using dew-point potentiometry (WP4c, Decagon Devices, Pullman, WA). We used nutrient solutions with the same concentrations as the the irrigation water used in the dry compartment of treatment **D**. Because the principles of measurement that apply to WP4c and the *in situ* psychrometers are identical, the resulting SWC can be used to reliably convert water potential to water content. Possible effects of rhizosphere structure development on SWC is not accounted for and this assumption is considered acceptable as the psychrometer placement does not target rhizosphere soil. To clarify our definition as suggested by Reviewr #3, we changed the term "rhizosphere" to "root zone". These statements have been added to the methods section in the main body.

30. L163: So there was little redistribution. Isn't this apparent in Figure 3?
Response: Yes, it was apparent in Figure 3 (now Figure 6) that the water content reaches and is maintained at a constant value one day after irrigation. The dielectric sensors are not able to resolve the minute changes in water content that result from HR. The water content dynamics derived from the psychrometer data Figure 7b shows this HR derived dynamics clearly. The point of this statement is that HR does not significantly contribute to the **net transpiration** by the total plant as water released by HR at night is taken up again the next day. We made this point to highlight the fact that function of HR is not necessarily to increase water uptake as has been postulated elsewhere (Ghezzehei, Albalasmeh, 2015; Carminati et al., 2016).

31. L166: However, your results do not clearly point to this. Thus why should this be the conclusion drawn?
    Response: Our results show (a) increased root proliferation in dry nutrient-rich compartments, (b) rapid uptake of nutrient-rich irrigation water, (c) HR only in the presence of nutrients in dry compartments, (d) more vigorous root-hair development in nutrient-rich dry compartments, and (e) no significant difference in nutrient uptake whether nutrients are applied in dry or wet soils. When taken together, these observations suggest that HR water is likely playing an important role in supporting the observed root and root-hair growth and in increasing nutrient mobility. The discussion was revised here and elsewhere to make these inferences clearer.

32. L167: You never mentioned root hair proliferation in dry regions (I think I did!). Please do this earlier on and reference the figure. Furthermore, it seemed that it was the case for both D and C1, so does it really matter if it's nutrient rich? Lastly, why should this imply that HR was essential? If anything, these results do not illustrate (at least not clearly) that HR is even happening.
    Response: This is now addressed to revisions to the methods section described above.

33. L170: It appears to be showing water fluxes. Make sure this is being cross referenced appropriately. Also, Figure 4 (c) needs a bit more detail. What are the different colors? Please include the text in the figure.
    Response: Cross-referencing was corrected. The caption and methods section were revised to explain the HR fluxes."

34. L171: You mean the inward flux? HR has not been defined very clearly.
    Response: HR magnitude was defined as the water flux released from the root surface to the soil (mm/day). The ambiguity was clarified in the revised methods section.

35. L174: It doesn't make sense to me. What's the benefit of HR if it's facilitated under wetter conditions? It doesn't even seem like redistribution if it moves water better when the regions are wetter. Perhaps the dynamics play a role in the redistribution? If you have SWC for the rhizosphere soil, then shouldn't you be capturing most of the effects of the exudation, root hairs, and rhizosheath? It is interesting to suggest that the plants are more actively moving water, but I don't think that this evidence is quite strong enough or developed to make those claims. I think the authors should focus on the water uptake that they presented earlier, as those were strong indicators that plants have the capacity to selectively control the how they're taking up water. The HR portion doesn't hold up as strong on the basis of their results.
    A similar comment also appears in the conclusion
    L216: The logic here isn't tight. Think about the infiltration experiments in unsaturated soil. The onset, water movement is rapid because of capillarity. If you have water being released from roots, that water should be pulled out more rapidly in the onset. It's not an intuitive problem, but the argument doesn't appear sound.
    Response: The observed pattern can be explained if we assume the water flux at the root-soil interface is governed by a flux law that is analogous to Buckingham-Darcy law

    $$q = \frac{\psi_r - \psi}{\delta} K(\psi)$$

    The first term denotes the water potential gradient, i.e., difference between the value at the root surface (ψ_r) and the rhizosphere (ψ). δ denotes the thickness of the rhizosphere and K is hydraulic conductivity of the rhizosphere. Therefore, the relationship between ψ and HR flux depends on both hydraulic conductivity and hydraulic gradient. Notice that drying of the rhizosphere soil affects these two factors in opposite directions–by increasing the gradient and decreasing the conductivity. Therefore, the net effect should be dependent on the relative magnitudes of these effects. For simplicity, if we assume that K is described by

$K = K_s e^{\alpha(\psi-\psi_o)}$. Then, the above flux law can be simplified as

$$\frac{\delta q}{K_s} = (\psi_r - \psi) e^{\alpha(\psi-\psi_o)}$$

It can be shown that the above scaled flux has a maxima at $\psi = (\alpha\psi_r - 1)/\alpha$. Above this threshold, rhizosphere drying would increase flux while below the threshold the opposite would occur. In the range of measurements observed in this study ($-1000\,\mathrm{kPa} \leq \psi \leq -100\,\mathrm{kPa}$), the latter appears to dominate. Moreover, we want to point out that the analogy with infiltration suggested by the reviewer does not fully apply to the rhizosphere condition. In a typical infiltration experiment, the water potential at the wetting front is at or near zero, whereas during HR, the water potential at the root surface is much lower than zero. Therefore, a more appropriate analog is internal redistribution, which generally slows down with soil drying.

36. L186: This is a strong result.

37. L188: First off, this is a monster sentence. Please break it up. Second, it wasn't clear to me throughout the entirety of this document what pulses were until here, so that needs to appear MUCH earlier. Lastly, make it clear to the reader why this should be the case and why this should be important. Why would plants need to briefly wet regions that have nutrients?
Response: We added more details in the introduction to describe the process and impacts of HR in nutrient uptake. In the methodology, we clarified the terms of intermittent irrigation, HR magnitude. We further carefully checked and replaced the terms that can be potentially confusing to readers with clearer and consistent terms.

38. L193: "...within arbitrary discontinuous subregions of the rooting zone." That's a bit wordy too, isn't it? Perhaps find a way to better emphasize that water and nutrients can be remote and disconnected.
Response: We changed to
"within the spatially segregated rooting zone."

39. L194: This is not a paragraph. Link it with the previous one.
Response: Revised as suggested.

40. L199: This is a pretty vague statement. Which signaling feedbacks are you describing in particular? After describing them, please explain how you came to those conclusions.
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)."

41. L200: There were equal amounts, but they were spatially distributed very differently. I think the stronger way to form this argument would be to stating again that the plants ability to acquire their nutrients and thrive despite having them segregated and disconnected highlight more complex mechanisms at play during root water/nutrient uptake. Try to emphasize the disconnection.
Response: We revised the sentence as suggested. The sentence we added was:
"The marked differences in root mass distribution between the two compartments of the three treatments demonstrated that plants' ability to acquire water and nutrients and thrive, despite having received them from spatially disconnected soil regions. This observation highlighted a complex whole-plant scale regulation of root growth and functions."

42. L202: I'm not sure that you're truly monitoring the signaling with your set up. However, I think it's fair that you report the proliferation that you see and cite studies that link this to signaling.
Response: We added a potential mechanism and reference after the sentence:

"This nutrient-driven response is consistent with Weidlich (2018), where roots of non-legume plants were found actively proliferate toward N-producing legume plants."

43. Figure 5: This is very unclear. What do you mean by elevated water retention? Is the soil retaining more water? What is the basis of this? Water release by diurnal cycles makes sense, but use the language correctly. Elevated water retention is out of context here.
Response: We changed the "elevated water retention" to "elevated water content".

44. L204: Those aren't your results. Can your results confirm this claim? Otherwise, I suggest leaving it out.
Response: We removed the reference and changed the sentence to
"... HR to maintain root function of effective water uptake in dry soil patches..."

45. L205: You never make this link clear in the results section, and I'm not sure if you actually can. You will need to argue for why this is necessarily the case. Maybe consider the kinds of enzymatic processes associated with nutrient uptake by plants. It's likely that these reactions require some degree of moisture in the soil.
Response: This was addressed in our previous responses to comments on L40 (introduction) and L166 (results).

46. L208: Make this clear when you present the result
Response: We added a sentence in the result section: "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**."

47. L211: I would remove this list. It doesn't seem to help the flow.
Response: Removed as suggested.

48. L216: The logic here isn't tight. Think about the infiltration experiments in unsaturated soil. The onset, water movement is rapid because of capillarity. If you have water being released from roots, that water should be pulled out more rapidly in the onset. It's not an intuitive problem, but the argument doesn't appear sound.
Response: The response to this comment was merged with response to comment on L174.

49. L224: So take the arguments and synthesize them in the text so when the reader gets to this point they will be able to agree with you.
Response: We followed the reviewer's suggestion and added one sentence that synthesized the connection between HR and root exudation. The sentence we added was,
"The elevation of water retention in the rhizosphere potentially increased the soil moisture compared to bare soils, which assisted the occurrence of HR through enhancing the soil hydraulic conductance."

50. L226: This is good to point out. Try and hypothesize some of the differences that might occur under less ideal conditions and provide a rebuttal as to why this is a sufficient set up to make the claims that your study is making.
Response: Some of the key differences from real conditions include (a) the bulk of plant nutrients are likely to exist in non-available form affixed to mineral surfaces and/or as part of organic matter. Therefore, the role of HR in facilitating nutrient uptake from dry soil patches will depend to the degree at which HR can alter sorption-desorption reactions and rates of mineralization. Therefore, the effective water potential range within which meaningful benefit of HR can be realized is likely to depend on the specific mineralogy, organic matter content and composition, and microbiome. In our experiments, nutrient was delivered with

weekly pulses of irrigation, albeit in small quantities. This was necessary because the medium we chose does not contain any nutrient. In real conditions, such pulses are likely going to be less frequent. Whether HR alone can sustain root functions for extended period of dry spell requires further investigation. These differences and their implications have been added to the paragraph.

51. L233: Remove “multiple lines of”
    Response: Removed as suggested.

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 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 $100\ \mu m$ 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 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.

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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

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

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

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

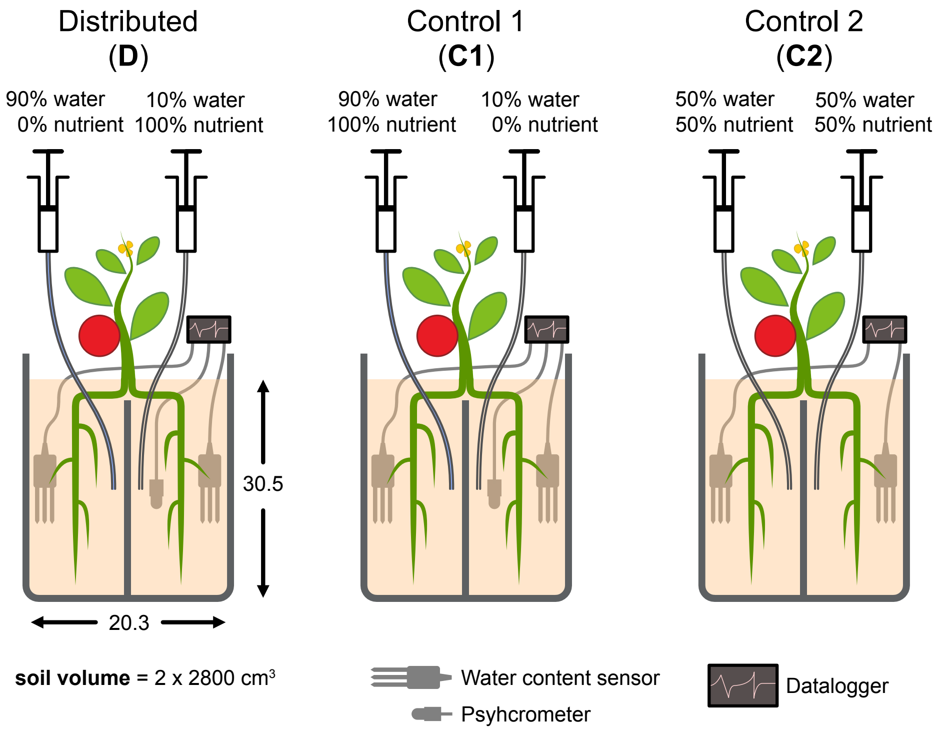
Distributed
(D)
90% water
0% nutrient
10% water
100% nutrient
Control 1
(C1)
90% water
100% nutrient
10% water
0% nutrient
Control 2
(C2)
50% water
50% nutrient
50% water
50% nutrient
30.5
20.3
soil volume = 2 x 2800 cm³
Water content sensor
Psyhcrometer
Datalogger

Fig. 1.

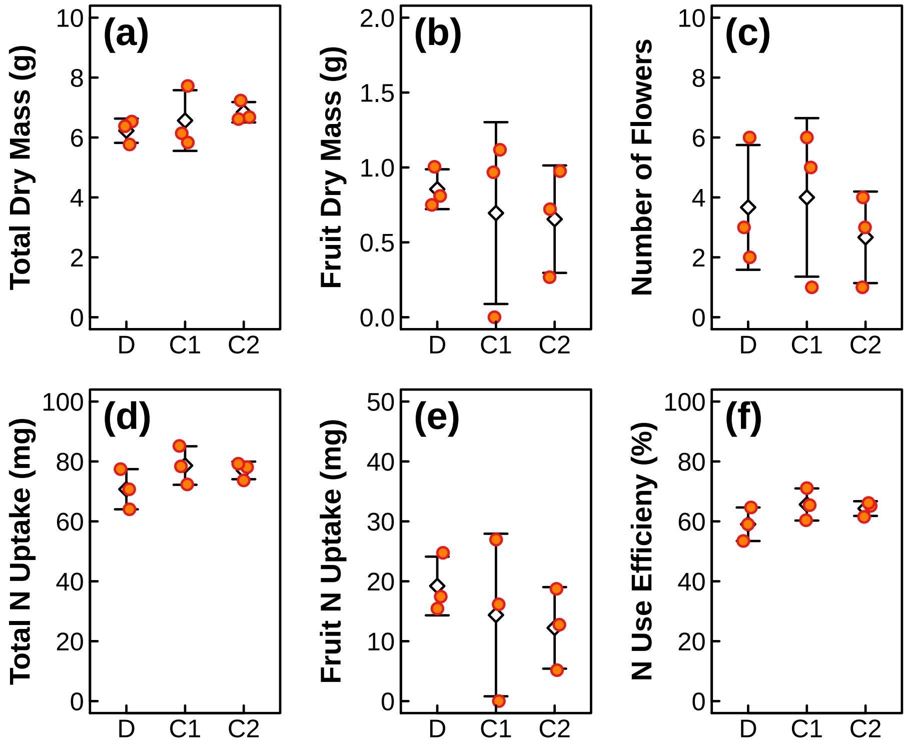
(a)
Total Dry Mass (g)
0
2
4
6
8
10
D
C1
C2
(b)
Fruit Dry Mass (g)
0.0
0.5
1.0
1.5
2.0
D
C1
C2
(c)
Number of Flowers
0
2
4
6
8
10
D
C1
C2
(d)
Total N Uptake (mg)
0
20
40
60
80
100
D
C1
C2
(e)
Fruit N Uptake (mg)
0
10
20
30
40
50
D
C1
C2
(f)
N Use Efficieny (%)
0
20
40
60
80
100
D
C1
C2

Fig. 2.

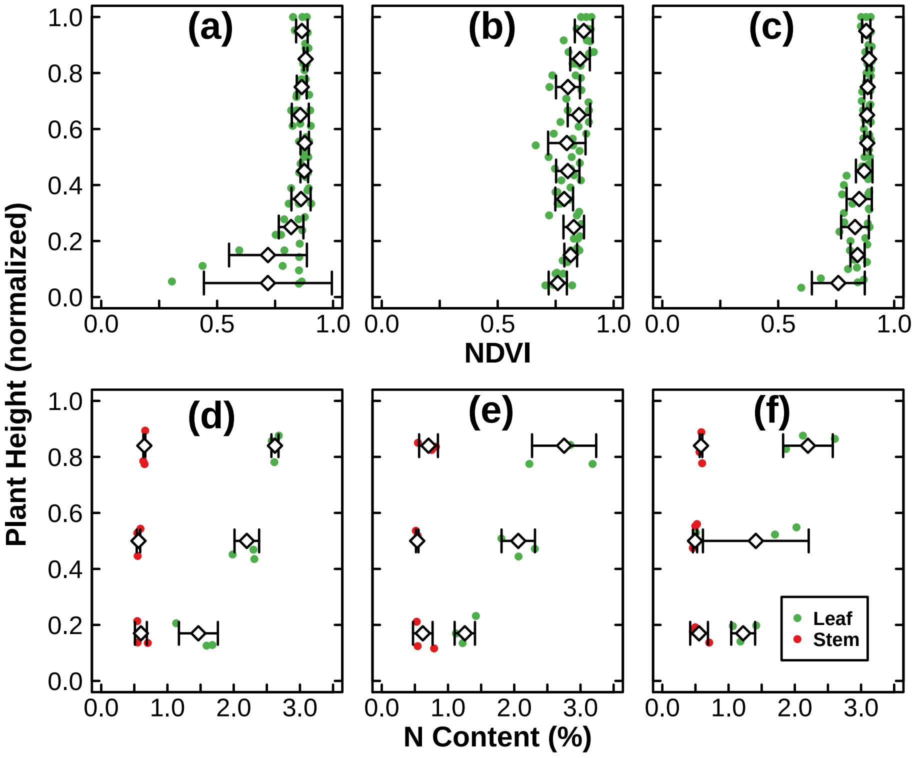
(a)
(b)
(c)
(d)
(e)
(f)
Plant Height (normalized)
1.0
0.8
0.6
0.4
0.2
0.0
NDVI
0.0
0.5
1.0
N Content (%)
0.0
1.0
2.0
3.0
Leaf
Stem

Fig. 3.

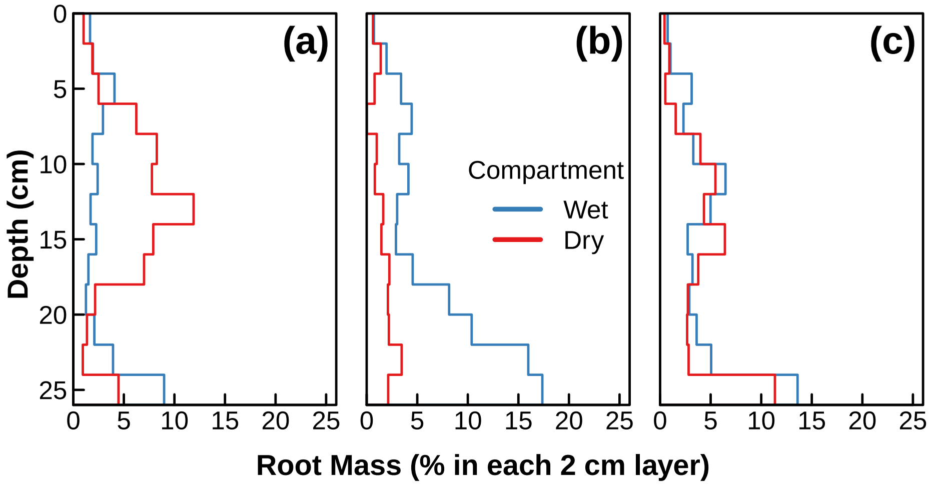
(a)
(b)
(c)
Depth (cm)
0
5
10
15
20
25
Compartment
Wet
Dry
Root Mass (% in each 2 cm layer)
0
5
10
15
20
25

Fig. 4.

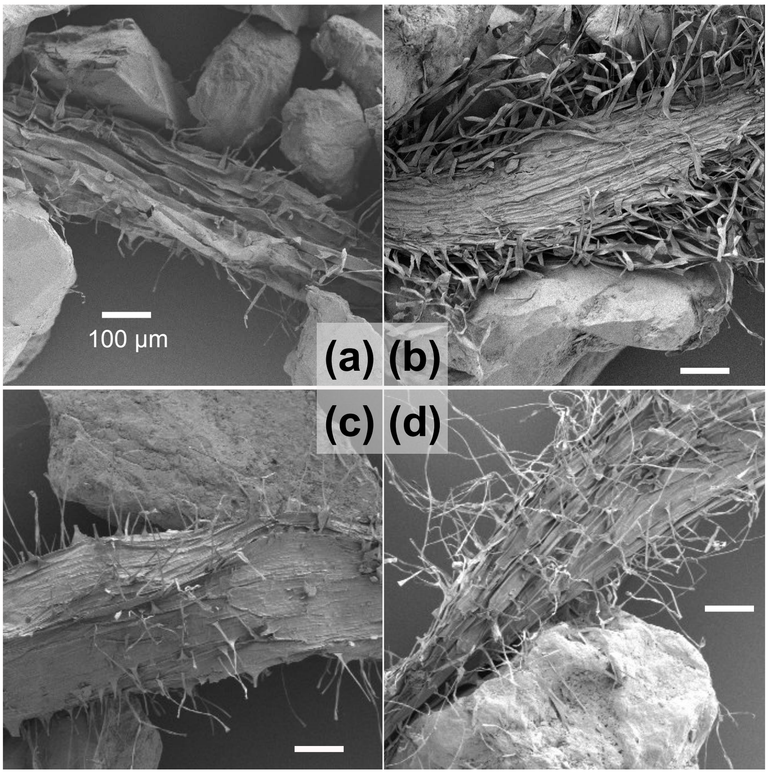
100 µm
(a)
(b)
(c)
(d)

Fig. 5.

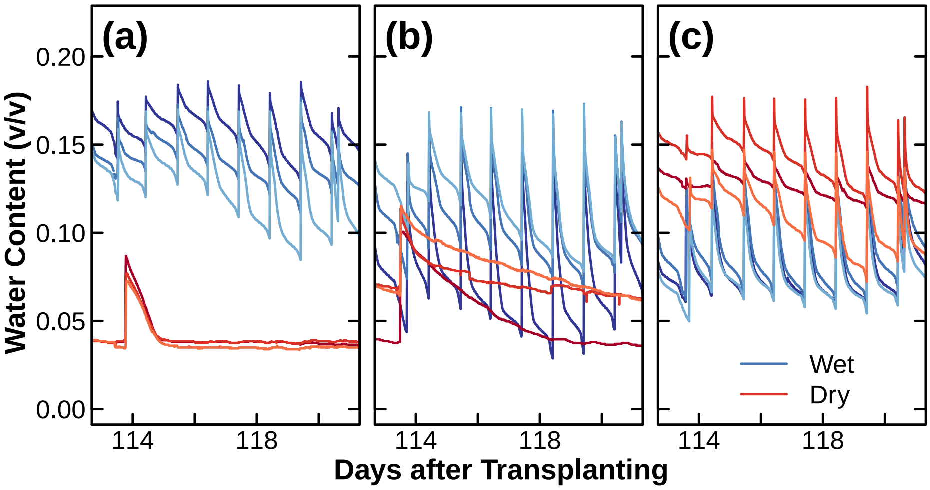
(a)
(b)
(c)
Water Content (v/v)
0.20
0.15
0.10
0.05
0.00
114
118
114
118
114
118
Days after Transplanting
Wet
Dry

Fig. 6.

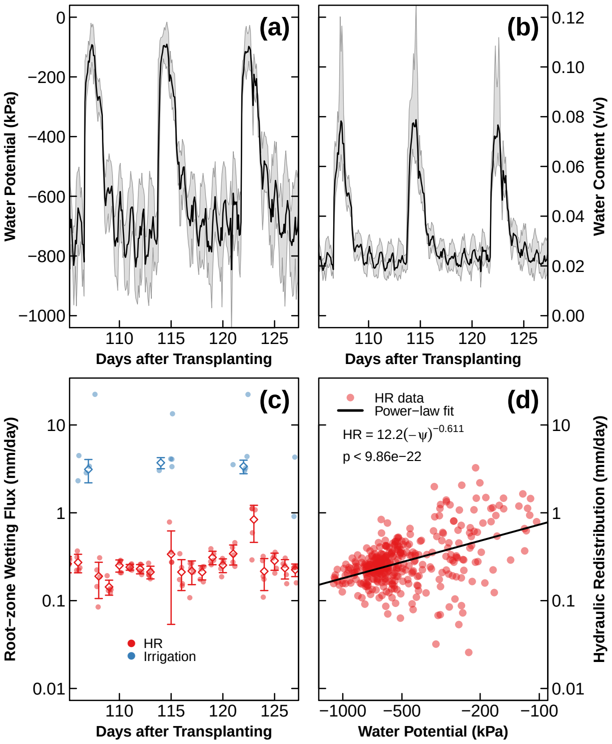
(a)
Water Potential (kPa)
0
−200
−400
−600
−800
−1000
110
115
120
125
Days after Transplanting
(b)
Water Content (v/v)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
110
115
120
125
Days after Transplanting
(c)
Root−zone Wetting Flux (mm/day)
10
1
0.1
0.01
HR
Irrigation
110
115
120
125
Days after Transplanting
(d)
HR data
Power−law fit
HR = 12.2(−ψ)^−0.611
p < 9.86e−22
Hydraulic Redistribution (mm/day)
10
1
0.1
0.01
−1000
−500
−200
−100
Water Potential (kPa)

Fig. 7.

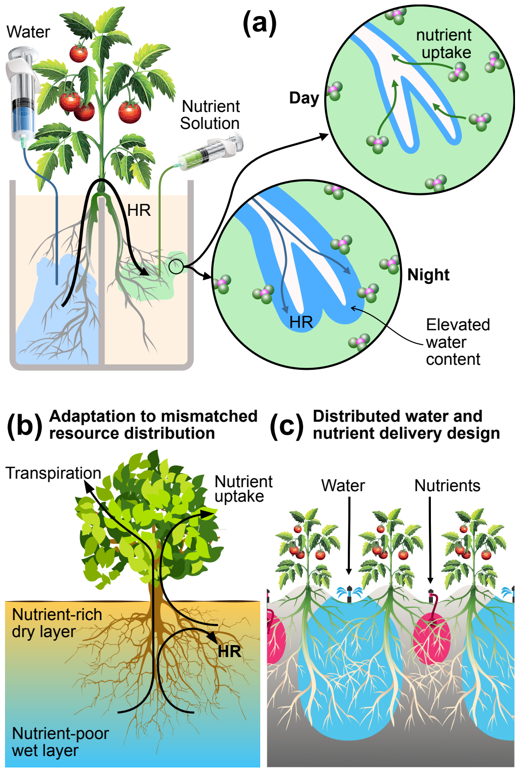
(a)
Water
Nutrient Solution
HR
Day
nutrient uptake
Night
HR
Elevated water content
(b) Adaptation to mismatched resource distribution
Transpiration
Nutrient uptake
Nutrient-rich dry layer
HR
Nutrient-poor wet layer
(c) Distributed water and nutrient delivery design
Water
Nutrients

Fig. 8.