Review for
MS No.: egusphere-2022-45
Title: Water Use Strategy of Riparian Conifers Varies with Tree Size and Depends on Coordination of Water Uptake Depth and Internal Tree Water Storage
Author(s): Kevin Li and James Knighton

Summary

In this work, authors collect data from 30 trees and soil (3 locations, 8 points along the depth), groundwater (4 locations) in Eastern hemlock over a period of 7 months, as follows:

1. **Xylem cores and truck storage from 30 trees on a monthly basis (a total of 210 xylem samples). 55 of these samples were also used to estimate trunk storage: xylem cores were rehydrated in tap water and used to estimate average turgid water content of hemlock tissues. This average turgid water content along with dried and fresh turgid water content were used to compute relative water content of trees**
2. **Root mass and distribution obtained from soil cores from 3 location at depths 5, 10, 20, 30, 40, 50, 75, and 100 cm (168 samples to evaluate the root mass). These soil core samples were dried and used to evaluate the rooting depth.**
3. **Bulk soil samples in these three locations at depth 5, 10, 20, 30, 40, and 50 cm on a monthly basis (132 soil water samples).**
4. **Ground water table has been observed every 15-minutes at four different locations (two sites were in close proximity of where soil samples were collected to evaluate water isotopic composition and root mass).**
5. **Other data: soil water content at depth of 12 cm was collected on a monthly basis at soil sampling sites. Precipitation isotopic composition data was collected on a daily and event triggered basis.**

The authors compared this soil and xylem isotopic composition data against tree tissue relative water content investigate the correlation between the depth of root water uptake and trunk water storage. They use similarity between xylem isotopic composition and rest of measurements as an indicate for the depth of root water. Their work highlights the need to consider physiological heterogeneity and call for a unified standardized field sampling too to improve understanding of tree water use strategies.

My comments are as follows, and I hope they help to improve the quality of your work.

General comments

While is good in this work, the following points can substantially be improved.

- **The introduction needs a very careful rewrite for a stronger expression of the motivations for this work. A much better statement of what are the knowledge gaps filled in this work, and why it is important to fill this gap. A good introduction for this conceptual data analysis should provide a deep and independent understanding of mechanisms that connects each element (subject to analysis) in this study and which other influential elements are left out due to various limitation and why their exclusion is not debilitating this study (in terms of filling its knowledge gaps). Also, a clear expression of the knowledge gaps filled by this study is missing.**

We will substantially rewrite the introduction with consideration for this comment. Changes that will be made with respect to the specific comments are detailed below.

- **The choices on type as well as spatial and temporal resolution of data collection is supporting this analysis and make the tools tailored to answer the research questions in this study. Figure 1 indicates you have made your choices with care, but it is not reflected in your text. I suggest addition of a separate section named “design of study” or something of this kind to address and justify in detail this topic (more in specific comments). And questions like, why a particular subset of xylem cores were chosen to account RWC can be answered.**

We will add a section to our discussion describing the uncertainties and limitations within our experiment.

- **Paper can improve on robustness of results and conclusions. Even though the approach applied in this research work is sensible, the analysis to challenge/state the limitations of the tools and inferences made from the analysis can be improved.**

We will substantially rewrite the results and discussion sections to include more detailed description of the limitations of tools and inferences made from the analysis.

Specific comments

Line 52: the term "organisms" to refer to tree may be a misleading terminology, I'd suggest using "species" instead.
We will replace "*organisms*" with "*species*"

Line 55: "subsurface" and "would" seems redundant.
We will delete "*subsurface*" and "*would*"

Line 56: Please, cite the research study that has concluded "understanding of how foundational tree species help to understand forest responses to external stressors" If no such work has shown this, please, remove this statement.
We agree with the reviewer that the previous citation did not clearly state how foundational tree species compositions can be affected by external stressors. We will add a new citation (Cleavitt et al., 2021), which provides an example of these potential effects in a temperate deciduous forest.

Line 71: I am not sure if xylem resistance and access to stable water sources are interrelated (the term "closely related" gives such an impression). Also, they are not the only important factors (you are excluding vapor pressure deficit and atmospheric demand). Also, I am not sure if stable access to subsurface water is what only matter as you are considering for instance tree water storage capacity in this study. So please, reword this part more carefully.
We agree with the reviewer that the term 'closely related' is misleading. We also agree that these are not the only factors worth mentioning. To address this, we will delete "*closely related.*"

In addition, we will reword the section to consider tree water storage capacity as a key mechanism. The phrase "among the key mechanisms" will be added to imply that there are other important factors (i.e., vpd, atmospheric demand) beyond what is listed: *"Xylem resistance to cavitation, access to tree water storage capacity, and access to stable subsurface water sources are among the key mechanisms that allow plants to maintain safe xylem water transport."*

For clarity, we will also change "*species identity"* to *"species-specific characteristics."*

Line 72: "there is evidence" and "shift" are repeated in two subsequent sentences, please, reword.
We will replace *"There is evidence"* with *"Past studies have shown."* We will also replace "*shifts*" with "*changes*"

Line 75: there is a gap, perhaps, make the link stronger by first addressing the relation between stable access to water sources and water table depths and adapting strategies in trees to improve.
We agree with the reviewer in that there is a gap in this section. To address this, we will add the following sentence to clarify the relation between access to stable water sources and adaptation to water table depth: *"Specifically, the maintenance of deeper rooting systems allows for access to more stable sources of water closer to the water table, possibly a competitive adaptation for survival during periods of low soil moisture availability."*

Line 76-83: same here. There is a gap, and you lose your audience here. As this information are not slightly disconnected from what has been previously said in your intro. Also, the link between this part and the last sentence in this paragraph is not immediate. I suggest rewrite.

We agree with the reviewer that the section is disconnected and that there is a gap here that needs to be addressed. To fix this issue we will revise this section (lines 76-89) to address the disconnect between the last sentence containing the hypothesis and first half of the paragraph. We will rewrite the section as follows:

"There is also evidence that some conifers are well adapted to trunk water loss across the growing season and rely on seasonal refilling of xylem water during months when competition for water uptake is reduced (Mayr et al., 2014), whereas other trees require daily refilling of xylem tissues to maintain higher tree conductance (Yi et al., 2017). A study of Norway spruce during drought demonstrated that there is a safety range for conifer xylem pressure loss with minimal reductions in conductance, and opportunity for conductance recovery (Arend et al., 2021). The buffering volume of internal tree water storage within conifers in a temperate forest was estimated to be a hydrologically significant reservoir (40 mm rainfall water equivalent) (Knighton, Kuppel, et al., 2020). Associations between management of internal tree water storage throughout the growing season, and the disposition for rooting systems to seek deeper and more stable sources of water are still unclear. Given the evidence of conifer rooting system adaptation to water table depth, and the observed hydraulic relationships between trunk water storage and tree conductance, we hypothesize that trunk water storage is correlated with the depth of water uptake in conifers."

Line 86-96: here you address stomatal regulation as an important mechanism to be understood. 1. Do you address or does your work create improved understanding of this mechanism? If not, why do you mention it in this paragraph. 2. How is it related to the two mechanisms stated in the previous paragraph? 3. Do you quantify "the relative importance of these variables" in this work? If not please, do not suggest it as a knowledge gap.

We agree with the reviewer, we do not specifically investigate differences in stomatal conductance. We will rewrite the introduction to make this clear.

Line 99: replace "between" with "among".

We will replace "*between*" with "*among*"

Line 110: please avoid using "these hypotheses" and in more detail your tools and avoid descriptive adjectives (such as high resolution) for improved clarity.

We will delete *"test these hypotheses by."* In addition, for clarification we will add the following: *"We observe within-species variations…"*

Line 129: you can add "creating a relatively wet condition" after stating the precipitation to let your reader develop better sense of the hydrological conditions.

We will add the following statement to this line: *"creating a relatively wet condition following annual average precipitation"*

Line 131: "breast height (1.37m aboveground)" instead of "breast height"

We will replace *'breast height'* with *"(1.37m aboveground)"*

Line 132: It would have been very beneficial if in the intro you would have explained the expected relation between DBH in trees with varied elevation and distance from stream.

And here you would have addressed why it is important to create a sampling population where no correlation between DBH and tree elevation or DBH and horizontal distance exists among these variables.

We agree with the reviewer in the point that the introduction should include explanation of expected relation between the DBH and topography variables. To address this gap, we will rewrite this section, to address the reviewer's concerns:

To the reviewer's second point, we add the following to line 136: "*To best characterize relationships between DBH and hemlock RWU…"* "*We specifically selected trees for sampling such that DBH and elevation were not correlated so that the effect of these variables could be studied independently*."

Line 132-149: if there is any particular reason for the choice on sampling location and resolution (for trees, soil, and groundwater level), please, mention it. Why two of soil sampling sites and two of the sites monitoring groundwater level coincide?

We will address the reviewer's concern by adding a section titled 'study design limitations.'

The experiment was conducted in the University of Connecticut Forest due to convenience (ease of access to researchers), access to multiple existing groundwater monitoring wells, and prevalence of riparian-situated eastern hemlock. Three soil sampling sites were chosen to span the 300-m riparian corridor at three different elevations. Two soil sampling locations were chosen to coincide with groundwater wells to simply field operations.

Why is soil sample collected at depth 12 cm?

We will clarify this, we did not collect a sample at 12 cm. This was the length of the soil moisture probe.

why soil isotopic composition has not been collected from same depth as you have collected samples for evaluating root mass?

The root mass sampling results were used to guide how we sampled for soil moisture water isotopic ratios. We observed that the majority of roots occurred in the upper 75 cm. We also observed via preliminary soil sampling that there were minimal isotopic variations with depth below 25 cm. We decided that our resources could be deployed as efficiently as possible if we only collected soil samples down to 50 cm.

Why is groundwater level studied at 15-minutes resolution?

An existing USGS streamflow gauge near this site records data at a 15-minute interval, which is appropriate to capture quick storm responses of the Fenton River. We set the recording interval of the pressure transducer to match this frequency. We will add a mention of this in the revised manuscript.

Why 100 cm is the deepest point you are collecting soil sample to evaluate the root depth? Is there an indication that trees of a certain age won't exceed this rooting depth?

Past research indicated that hemlock transpiration is strongly correlated with shallow soil moisture water contents, indicating shallow effective rooting depths (Meinzer et al., 2013). The mean depth of the water table at this location is approximately 100 cm. We therefore selected 100 cm as the maximum depth to investigate root mass as we did not anticipate finding hemlock roots in the saturated zone. We will add a mention of this in the revised manuscript.

If your choices of sample collected (location and time) are random, please specify it clearly and mention why you believe, these choices do not bear an impact on your analysis. You can address this topic under a separate section as "design of study".

The three soil sampling locations were not random but chosen to span the horizontal distance along the riparian corridor (Fig 1a). The trees were chosen with emphasis on sampling uniformly across elevations and DBH (Fig 1c) while maintaining no significant correlation between DBH and elevation. It was critical that we sampled trees in this way to properly evaluate our hypotheses that xylem isotopic ratios and tree characteristics were correlated (Fig 5). We will add a mention of this in the revised manuscript.

Line 154-160: please explain how you expect your method of storing sampling and time to analysis not affecting the isotopic compositions used in your analysis and shown in your results.

Soils and stems were collected in the field, placed in double seal plastic Ziplock bags under shade, and frozen within 3 hours. Soils were then stored frozen until CVE. Prior to CVE, soils were thawed for 1 hour at room temperature. Recent research on water loss during direct vapor equilibration in double seal plastic bags indicates that the mass of water lost (and the effect on soil water isotopic ratios) in our methodology would be negligible (Gralher et al., 2021).

Figure 1, part b: it is not clear how the size of circles representing hemlocks translates to their DBH or elevation. If circle size chosen here are consistent with what is shown in part (a) please specify it with a clear statement of which variable of hemlock trees they are presenting.
We will edit the figure, to include a legend showing how circles representing hemlock translate to DBH.

Figure 2:
panel (a), please distinguish the maximum and minimum of daily temperature by a light red and dark red color and add it to the legend. Also, I think the precipitation itself is better than its cumulative form as it enables better comparison and judgement of your results and analysis and inference in Figure 3. Also, read your own sentence on line 221
We will edit this figure, so precipitation is not cumulative and distinguish the minimum and maximum of daily temperature with color. We will split up the sentence on line 221.

Please add your key message for each panel, e.g., panel (c) measured root mass indicates uniform root distribution.
We will add more detailed information to the panel descriptions.

Panel (c), I think using root mass along depth will help you convey your key message for this panel better.
Panel c does show the cumulative root mass with depth. If we have misunderstood the comment, please let us know.

Line 196: rephrase for clarity and replace the word "below".
"*Below*" replaced with "*beneath.*" Sentence was rephrased to describe effect of fractionation as a lc-excess, as mentioned in the previous sentence: *"Soils beneath 10 cm were more isotopically depleted in δ^2H and $\delta^{18}O$ and showed more negative lc-excess than soils within 10-35 cm, thus indicating less fractionation."*
Figure S4: can you include your explanation on why on 3/12/21 the lc-excess is more negative compared to all other dates?
We agree with the reviewer in that isotopic differences exhibited in March require some explanation. We add the following line to our results: *"Soils in March exhibited more negative lc-excess compared to all other dates, most likely induced by evaporation of stored subsurface waters from the winter season (Fig. S4). This observation is possibly similar to observations made in the nearby Hammond Hill Research Catchment (Knighton et al., 2019). Water held in shallow soils at the end of the winter was estimated to be older water that had experienced substantial evaporative enrichment over the winter months. During the spring melt, the evaporatively enriched soil moisture was flushed out of the soils, lowering the lc-excess."*

Line 206-7: not necessarily, because you can see clearly the isotopic composition in precipitation is also more like that of soil in June-July.
We agree with the reviewer in that the isotopic composition of precipitation during our June-July sampling period is more like that of the soils. This is most likely because of heavy tropical storms occurring around the sampling period. We address this by adding a sentence to the paragraph (lines 230-242): *"In June through early-July, hemlock xylem water overlapped more with shallow soil within the upper 10 cm, indicating a temporary reliance on shallow soil water and precipitation occurring during and around the sampling period (Fig. 2)."*

Figure 4: is better to be rearranged, it is too much information and somehow hard to follow. Again, key message is missing in caption.
We will edit this figure for clarity and will add more information in the caption.

Line 216: rephrase for clarity. And avoid overusing the word significant.
In this context significant is a reserved word to indicate the exceedance of the alpha threshold in our hypothesis test. We understand it is repeated throughout, but we feel that this repetition is needed to convey the full meaning of the results. We will attempt to rewrite to avoid repetition.

Line 218: explain much better what does a negative and positive correlation mean and why.
We kept the word "*significant*" only where we list the α threshold in lines 247-248.

We rephrased lines 248-251 for clarity by elucidating what negative and positive correlations mean in the context of this study: *"Tree xylem water δ^2H and tree core RWC were significantly negatively rank correlated at the $\alpha < 0.05$ threshold in May through June and significant at the $\alpha < 0.1$ threshold in July (Fig. 4). Xylem $\delta^{18}O$ and RWC were negatively correlated in June through July which signified that increase in RWC paralleled with decrease in xylem isotopic ratios. More specifically, during these months the hemlock were accessing more evaporatively enriched water sources and tended to store less mass of water per unit mass of tree tissue."*

Line 242: I strongly recommend for this section to come much earlier and explain very well how RWC in each season may be correlated with precipitation (and water stored in soil), transpiration and root water uptake.
Details regarding RWC and its environmental controls are explained prior to Line 242, these details can be found at lines 285-295 and more throughout our discussion.

Line 284-286: would this not be an indication that classifying tree species based on age, may be a misleading approach to present physiological heterogeneity within or among tree species?
We agree with the reviewer's interpretation but temper our conclusions because the data presented in the studies we cited (Song et al., 2018; Wu et al., 2019) suggest the opposite result. We note these prior studies focused on trees that were younger, part of reforested plots, and not coniferous. Further, neither study sampled across the entire of the growing season. Therefore, we believe that our study does more to address this open question, but we do not discount these prior studies. We will rewrite this section to emphasize these points.

Discussion and Conclusion: I think more emphasis can be placed on the uncertainty in your findings and what needs to be done to add to the robustness of your results. For instance, how do you justify that your sample size is enough to add to the robustness of your conclusions. I suggest an emphasis on physiological heterogeneity rather than the emphasis on age in trees.
We agree with the reviewer's concerns on this point. We will substantially rewrite our discussion and conclusion with emphasis on physiological heterogeneity.

References

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