The work by Kevin Li and James Knighton describes an interesting investigation on tree water use across riparian trees of different diameters. The authors explored the relationship between patterns in tree water use, DBH and relative water content (RWC)

The investigation focused on eastern hemlock and the authors used xylem water and potential sources (i.e., soil water from different depths, groundwater, and stream) to understand patterns in tree water during the growing season. The authors sampled xylem water by coring trees and obtaining 7.5 cm cores. Those same samples were used to compute RWC from samples. Xylem isotopic data were compared against soil water distribution in dual-isotope space. Correlations between xylem water isotopic ratios and tree core RWC were computed. The authors also used multivariate models to understand the influence of DBH and elevation on xylem water isotope ratios. All xylem water isotope ratios were corrected based on RWC.

While I agree with the motivation of the work and the investigation is interesting, I see major issues with the methodology and conclusions drawn from the analysis. Additionally, some points need to be further clarified for a complete understanding of the work. Please see the main concerns below, followed by specific comments.

1) The hypothesis in the introduction (L107-109) does not reflect the study design or analysis.

The hypothesis will be rewritten to more clearly represent the study design and analysis. Changes are documented below where specific comments are made.

2) The study compared xylem water isotopic composition from trees of different DBH to identify patterns in tree water sources. When using xylem water to identify sources, the use of sapwood is usually the sampled portion of the tree. The sapwood depth of trees usually varies with the diameter (i.e., the larger the DBH, the larger the sapwood depth) and this also applies to Tsuga canadensis (e.g., Daley et al., 2007). Thus, the sampling depth (i.e., core length) should reflect the sapwood depth. However, here the authors collected a core of 7.5 cm for all trees (L 131) independent of the DBH. This results in trees of larger diameter having a sample that represents more sapwood, while trees of smaller diameter with a sample that is mostly composed of heartwood (e.g., Meinzer et al., (2013) *T. canadensis* of DBH ~ 35 cm, sapwood depth < 2 cm).

L245-246: By sampling 7.5 cm core from trees of different diameters (<31 and >31 cm in DBH) the authors likely sampled a different mix of sapwood vs heartwood between larger and smaller trees. It is very likely that the 7.5 cm core covered a larger portion of sapwood in relation to heartwood in larger trees (>31 cm), but a larger portion of heartwood in trees of smaller diameter (<31 cm). Heartwood water is shown to contribute to transpiration during periods of water stress, but it is less likely to contribute to transpiration in periods where soil water content meets transpiration demands. Additionally, heartwood water content is more stable over time. Thus, it is likely that the observed more considerable temporal variability in RWC in xylem water of larger trees is more representative of sapwood water content. In contrast, smaller trees would be seen as more stable in this study because of the more significant portion of heartwood in the sample.

We address the general statement #2 and the following specific comment below:

First, we note a minor misunderstanding. Our reporting of the average core depth seems to be interpreted by the reviewer as the depth beyond the start of the sapwood. Sapwood started at an average depth of 1 cm across all hemlock trees that we sampled. This 1 cm of phloem and cambium is included in the reported core depth. We will make this point clearer in a revision.

Second, we were also somewhat unclear in our choice of words. Our initial submission read "*Cores were collected at breast height with an increment borer to a depth of approximately 7.5 cm*." The depth of 7.5 cm was approximately the average distance from the outer edge of the tree to the heartwood across all trees (Fig R1). This depth does vary between trees, and we will present the observed sapwood, phloem, and cambium depth for all sampled trees in supplemental in a revision. We have also revised this sentence as: "*Cores were collected at breast height with a 152.4 mm increment borer to a depth that spanned the sapwood depth of each tree (Table S1).*"

Third, we disagree with the interpretation of the relationship derived from the data presented in Daley et al (2007). Reported DBH and sapwood depths are correlated with $R^2 = 0.99$ in Daley et al. (2007), which suggests a scaling relationship was used to compute sapwood depths from DBH (Fig R1). Daley et al (2007) does not specify how these data were generated, but they appear to have assumed that sapwood is always exactly 25% of the DBH (Fig R1). This assumption seems reasonable when compared to our observations of hemlock trees with DBH > 40 cm but greatly underestimates the sapwood depth of the smaller hemlock that we sampled (Fig R1). We have reviewed the Meinzer et al (2013) paper and do not see a description of hemlock with sapwood areas of 2 cm.

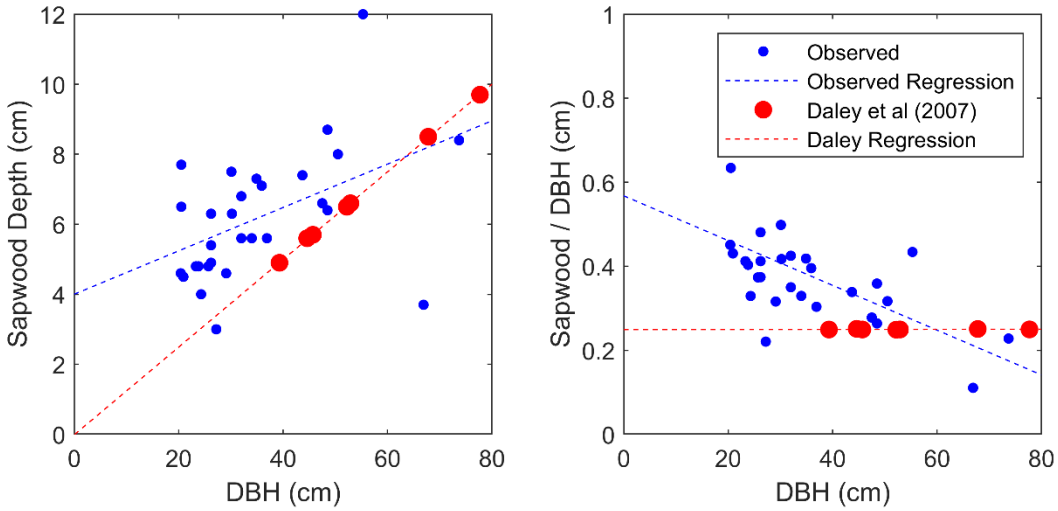

Figure R1 – Correlations between DBH and Sapwood depth

Finally, we understand that sapwood and heartwood may have different isotopic compositions or RWC. We posit that if there was a significant bias resulting from our sampling procedure, that this bias would appear consistently across all sampling periods. We note that there is no significant correlation between DBH and RWC or DBH and xylem water isotopic composition in most months.

Additionally, heartwood and sapwood have a distinct isotopic composition (Treydte et al., 2021). Thus, the comparison between sources across species of different diameters (a major point of this study) could be simply an artifact of the proportional sapwood sampled. Additionally, sapwood and heartwood have different RWC, which again, can affect another major conclusion of this study.

We do not believe that we have a consistent bias in our data resulting from our sampling procedure (see response above). We have rewritten a misleading sentence in the methodology and now provide more information on the trees that we sampled. We acknowledge that identification of the transition from sapwood to heartwood is somewhat challenging in conifers, which may have allowed for some heartwood to be analyzed. Further, it is difficult to know if core-extracted water is truly reflective of root water uptake

due to large radial differences in water fluxes over short distances in hemlock. We will include a more detailed discussion of these potential sources of uncertainty in our discussion.

3) The authors correct xylem water based on RWC. There is a lack of evidence that supports this approach to this study/species or at least data that justifies it to be applied to all samples. Further evidence is necessary to justify this broadly applied correction to the data.

In this review period, we conducted a hemlock tissue rehydration experiment to test ^{2}H enrichment of plant extracted water due the CVE process. We collected 30 hemlock cores in April 2022. These cores were immersed in tap water for a period of 48 hours and weighed to determine turgid weights. We then used the CVE to dehydrate the cores at a temperature of 60 C and then weighed them to determine dry weights. We then divided the cores into 3 groups of 10. The three groups were rehydrated for 2 seconds, 10 minutes, and 4 days, respectively, in a water isotopic standard (-47.35 per mil for δ^2H). We then extracted and analyzed the water from all cores for ^{2}H and RWC (using the same methodology as presented in the manuscript).

Results from four cores were discarded because of insufficient percent recovery during CVE. Two samples failed to be analyzed on the Picarro (needle failure) and were discarded after sitting at room temperature for more than 24 hours. Two core samples were flagged for organic contamination and returned water isotopic ratios very close to the standard spike. Finally, one sample returned a value that seems to be erroneous (+6 per mille ^{2}H bias). This value was likely the result of an unseen lab error, but we have no objective reason to discard this observation, so we are including this value for transparency. If we discard the 2 samples that were flagged for organic contamination and keep all other samples, the hemlock tissue experiment demonstrated a ^{2}H bias with RWC approximately equal to that observed by Chen et al (2020) (Fig. R2 blue).

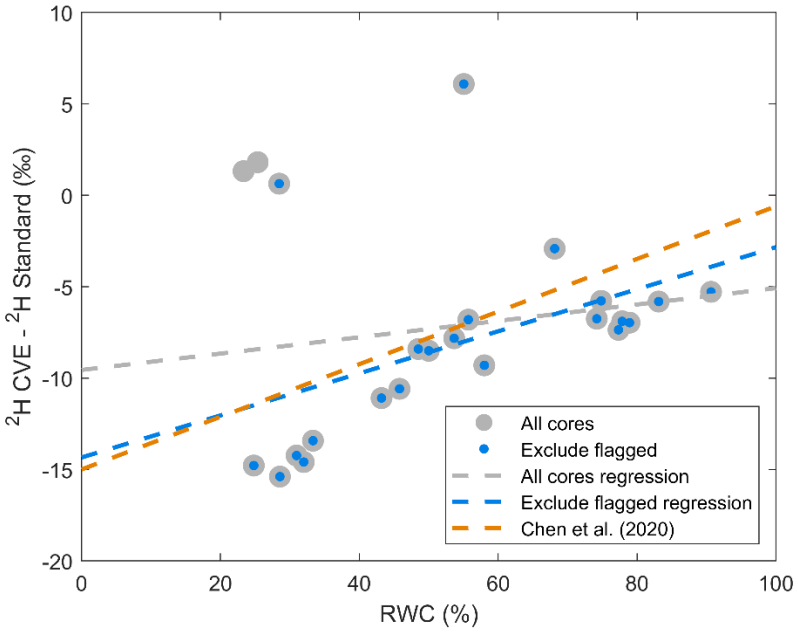

Figure 2 – Results of hemlock rehydration experiment

We will make the following changes to the manuscript. 1) We will include the results of the stem rehydration experiment in the supplemental material 2) We will include a general section describing methodological uncertainties (requested by reviewer 1), and 3) We have modified the discussion to include more detail on possible uncertainties resulting from this correction. Finally, we note that all raw data was provided as well as the corrected ^{2}H data so that readers can revisit and reanalyze our experimental results if new isotopic corrections are proposed.

L161-162: Since this is an area of large uncertainty in the field, especially because the mechanisms that drive observed fractionation are unclear and still in debate, caution is necessary. Therefore, additional information is required when describing the method and underlying assumptions.

More importantly, how did this correction affect the results? The authors use RWC to correct the samples and use the same data (RWC) to analyze patterns in tree water use. Later in the results, the relationship between xylem δ2H and core RWC is not always present. How does it affect the interpretations?

This point should be further explained in the methodology and later included in the discussion of the uncertainty of this analysis.

The original work by Chen et al., (2020) observed a relationship between δ2H (offset) and RWC. In this work, a clear relationship between xylem and RWC was not always evident, at least to the corrected presented xylem water. One would expect to see it consistently if the correction was necessary throughout the entire period. More information is necessary to evaluate this approach. See detailed comment below with additional concerns.

We agree with the reviewer on this point; however, we note that we would not expect to see consistent relationships between ^{2}H and RWC if a bias correction had been applied appropriately. This would only be evident in the uncorrected data (which we made available but did not discuss). Using all uncorrected ^{2}H observations, a consistent and significant positive correlation between lc-excess and RWC is observed across all months except March and July (Fig R3), suggesting more ^{2}H enrichment at lower RWC. This is the same direction and approximately the same magnitude of the generalized bias described in Chen et al (2020). In March this relationship is close to significant, and likely not only because we recovered fewer water samples (n = 14) from the CVE process. July is the month that we received heavy tropical precipitation and saw an inversion of the isotopic relationships observed in all other months. Upon reviewing these initial results, we decided that it was necessary to use the generalized RWC correction approach described in Chen et al (2020). When we make the correction using RWC, this relationship is no longer significant (except in July when the correlation is significantly negative, which we discuss in detail in the manuscript). We believe that these results (and the rehydration experiment described above) support using the correction proposed in Chen et al., (2020).

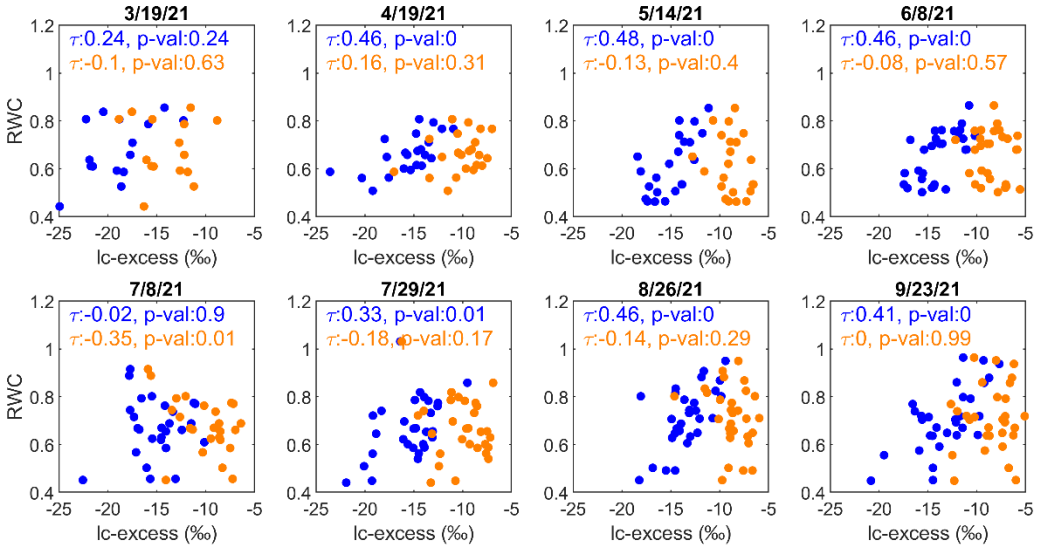

Figure R3 – Correlations between lc-excess and RWC using corrected (orange) and uncorrected (blue) datasets

4) There are contradicting results within the study. For example, in the dual-isotope analysis, the authors showed that xylem water does not overlap with any source in certain months (e.g., March, August, September) and when it does overlap, the overlap is with shallow soil layers (<10 cm), and rarely few xylem samples overlap with deeper layers (e.g. July). Overall, there is no indication at all that the trees at the site use deep soil water. The following analysis using correlation and multivariate models suggests that trees of distinct diameters are using different sources (e.g., deeper layers), or that there is a dynamic water use at the site. The data in the study does not support it.

First, we comment on the temporal variations in water use. Our hypothesis and experimental design were guided in part by our prior work with hemlock which showed that small diameter (<30 cm DBH) trees relied on shallow moisture at the beginning and end of the growing season but exhibited an ephemeral shift to deeper soil water (20 - 40 cm) in June, July, and August in the nearby Hammond Hill Research Catchment (Knighton et al., 2019). We were expecting to observe changes in water uptake depths only from June through August, and not necessarily in the other months. We sampled the other months to ensure we were covering the growing season in full. The mismatch between xylem isotopic signatures of hemlock and those at the beginning/end of the growing season we have attributed to time lags induced by tree xylem water storage (Knighton et al., 2020). We discussed this in detail in the manuscript (see specific comment below).

Second, we comment on the depth variations in water uptake. There may have been a misunderstanding with respect to our use of the word “deep.” We agree that all hemlock at this site are likely only using water from the upper 20 cm. We previously binned soils into 0 – 10 cm and deeper layers in Figure 2, which was not fully informative. The partitioning occurs between water use from the upper 5 cm and other trees using soil moisture 5-20 cm. This can be more clearly seen on Figure S6, which binned soils 0 – 5, and then 5 – 20 cm. We will make this change to Figure 2 so that the depth variations are clear to the reader. We will also rewrite so that we no longer use the word “deep” but rather discuss the actual soil depths.

With respect to the reviewer's assertion that there is no partitioning: The soils that we measured all have water isotopic ratios in the upper 30 cm are negatively correlated with depth (i.e., more depleted in deeper layers). This signal, if reflected in the xylem, should indicate differing depths of water uptake. We note that we did formally test the hypothesis that DBH is predictive of xylem water isotopic composition which show significant correlations in both the multivariate linear regression (Fig 4) and a ranked correlation of only DBH against xylem isotopes (Fig S6). MVLR showed significant results in June and July (and only July in ranked correlation analysis), which supports the concept that trees of different sizes were accessing different water sources at the time of sampling.

To address this comment, we will make the following changes: 1) Figure 2 will be updated to plot small and large diameter hemlock with different symbols so size-related isotopic variations in July can be seen clearly. We will also change the soil bins to disaggregate the upper 10 cm into two bins. 2) We will modify the abstract, results, and discussion to describe the subsurface water use partitioning as ephemeral and only in the upper 20 cm. We did not intend to imply that the partitioning was constant through time or that hemlock trees were using water at the deep end of the rooting zone.

Specific comments:

The first paragraph of the introduction contains many different ideas (e.g. subsurface water partitioning, latent heat transfer, tree dispersal, foundational species, external stressors) and is not cohesive. Consider re-writing it.
We agree with the reviewer that the first paragraph of the introduction is not cohesive. We will address this issue by rewriting the paragraph to introduce each idea in a cohesive manner. Please see specific responses to reviewer 1.

The third and fourth paragraphs of the introduction could be summarized as this goes beyond the scope of this study and distracts the reader.
We agree with the reviewer that the third paragraph requires revision, and that the fourth paragraph goes beyond the scope of the study. We will considerably rework the third paragraph of the introduction. In addition, we will remove the fourth paragraph (stomatal regulation) as this topic is not further addressed in this study.

L34: High spatiotemporal sampling resolution
We will add 'resolution' to this phrase.

L49-52: This sentence is too long and hard to follow. It starts with subsurface water partitioning and ends with latent heat transfer. I would suggest breaking this down. How does root water uptake influence surface runoff?
We agree with the reviewer and will break down this sentence. We will reword to more concisely state how root water uptake influences surface runoff.

L52: generating? Consider substituting by growing.
We will substitute 'generating' with 'slow-growing.'

L64-66: This sentence is not very clear. Rephrase it.
We will rephrase this sentence for clarity.

L72: unclear what safe xylem water transport means in this context.
By 'safe' we are meaning to imply that xylem transport is stable when these key mechanisms are allowed to function properly. We will replace 'safe' with 'reliable.'

L76: what does "well adapted to trunk water loss" mean? Clarify and re-phrase.
We will re-phrase this sentence for clarity.

L83-84: "the hydraulic relationships between rooting systems and stem water potential" this idea is not well illustrated in the text above with references. L87-89: Revise reference list. Not all the work here shows "biome-scale correlations between rooting depths, stomatal regulation of transpiration and climate".

Because we do not specifically investigate differences in stomatal conductance. We will remove this sentence in question and rewrite the introduction to make this clear.

L118-120: Why the loss of hemlock specifically would cause it? And not only any tree species? This is not clear within the text.
The first sentence in the paragraph will be rewritten to imply the regional abundance of eastern hemlock.

L120-122: Be more specific. This is a quite generic sentence in a paragraph that describes the species.
We will rewrite this section on hemlock to be more concise and introduce this facet in a manner that is clear to the reader.

L128-129: Where the climate data was obtained from? What is the period in consideration?
We will address this by removing this statistic and deriving climate data directly from NOAA rather than a third-party source.

L131: What kind of increment borer? What is the diameter?
We will specify the brand and diameter of the increment borer.

L131: Why did the authors use a 7.5 cm depth? The sapwood depth of T. canadensis in the literature for trees with a similar diameter to the ones in the study is smaller than the sampled depth in this study for water extraction. It is likely that the authors also collected heartwood water, which is shown to have different isotopic composition than sapwood (Treydte et al., 2021). What is the implication of this approach in the results of this study?
We agree with the validity of this implication for this study and address this topic under the main concerns listed by the reviewer.

L131: How were the cores stored in the field?
We will add a sentence to this section detailing how core and soil samples were stored in the field.

L131: How many trees per DBH class? Why did the authors later define 31 cm as the threshold between larger and smaller trees?
We provide a distribution of tree DBH sampled is depicted in an empirical CDF (Fig. 1c). Also, we provided the raw data that includes a detailed table of tree characteristics (https://www.hydroshare.org/resource/8996065d3ba34907a018be9b4369c1d3/)

31 cm is approximately the median DBH across all 30 trees sampled. This value is used to separate the tree DBH into two groups for statistical analysis.

L135: What the dry root mass per unit mass of soil can provide? A more standard practice in the literature is to report dry root mass per soil volume (root density).
We recognize that standard practice is to report root density per unit volume of soil. We sampled soils and roots with a 5 cm diameter soil and root auger. We could present root density results per unit volume; however, we feel that there was too much uncertainty in the volume of soils sampled with this common approach. We have more confidence presenting these results as dry root mass per soil dry mass. We have concerns about over-representing the precision in this measurement.

L140: Why the soil sampling depth was limited to the first 50 cm?
Most of the plant root mass in this study site was limited to the upper 50 cm of soil, with some between 50-75 cm and trace amounts between 75-1m depth. For this reason, we believe that soil isotope composition derived from the upper 50 cm is sufficient in capturing root water uptake. Further, we observed minimal depth variations in soil moisture isotopic compositions below 40 cm.

L139: missing delta
We will add the missing delta symbols.

L155: What kind of CVE system was used? Provide reference.

The CVE system we custom built based on the design and part specifications presented in Orlowski et al., (2013). The system was constructed by Swagelok (design part ALBNY-DG25225). Design CAD drawings of the CVE are available from Swagelok on request. The system was pressure tested to 8000 torr after construction.

L159: Plant water extracted via CVE is known to contain other co-extracted organic compounds (e.g., Millar et al., 2018) and result in spectral contamination in laser spectrometry which requires identification and correction (e.g., Martínâ€• Gómez et al., 2015). How did the authors deal with spectral contamination or identified it?

Picarro isotope analyzer software (ChemCorrect) will flag samples for CH4 and alcohol contamination in post-processing analysis. Flagged samples were identified and the extraction process (CVE) was repeated, and sample was re-analyzed. All other flagged samples were discarded. If the repeated sample still showed contamination, it was discarded.

This was repeated for samples flagged for other reasons including missing analyses (low water ppm/needle malfunction) and high/low relative deviations.

We have included these flags with the raw data which are publicly available.

L161-162: How did the authors define when correction was necessary using RWC? Or was it applied to all samples?

Corrections were applied to all samples. We answer is given in more detail in the general comment above.

Was there a relationship observed within the collected samples that justified this correction (e.g. Chen et al., 2020)?

We answer this in the general comment above.

Additionally, how much water was obtained per extracted core/sample?
On average we extracted ~ 0.7 g of water from each core sample. The volumes of water that were extracted for each sample are publicly available in our raw data: https://www.hydroshare.org/resource/8996065d3ba34907a018be9b4369c1d3/

How did the authors differentiate spectral contamination from VWC correction?

Contaminated samples were flagged by the Picarro analyzer. All contaminated samples were discarded. See comment above.

L165: When/how did the authors measure the fresh weight of the core? Describe this step in more detail as this plays an important role in this study.

The fresh weight of the core is referring to the wet weight of the core/sample weight or the weight of the core prior to drying. We weighed all cores with an Ohaus PX3202/E Pioneer Analytical Balance. We will add this detail to the manuscript.

167: Which software/ programs were used to conduct the analysis?
Analysis was conducted using MATLAB (R2022a). We will add this detail to the manuscript.

L178: How was the end of the season and growing season defined?

We approximate the beginning and end of the growing season as the months where mean daily max temperatures cross the threshold of 15 degrees C.

L179: Why was two-sample Kolmogorov Smirnov test applied? An additional sentence would be helpful to the reader.

We chose the KS test because it is a non-parametric test (requiring no assumptions) and measures any differences in the distributions (and not just a test of means or medians).

An additional sentence will be added to this line for clarification on why we used this test.

L185: How deep is the rooting zone? How was it defined? This information is not previously described in the manuscript. Previously, the authors presented a methodology to define root mass per soil mass (up to 100 cm soil depth) but method/results from root zone depth were not presented.

We note that the rooting zone is not defined in this text prior to it being mentioned in this line. We define the rooting zone as the upper 100 cm of soil. Where the groundwater table consistently lies below 100 cm depth and increases above 100 cm during and after tropical storm event. We confirm this assumption in the root mass/soil unit mass experiment where we find that the majority of observed root mass is distributed above 75 cm depth.

We will rewrite this section of the text to more clearly define the rooting zone.

L190: The text refers to soil water content in relation to elevation. This information is not presented in figure 2, referenced in the text. It would be helpful to show the temporal variation in SWC across the topographic locations.

We will revise figure 2 to use different symbols for different elevations.

L201-203: It would be interesting to show xylem water isotopic composition in dual-isotope space regarding the DBH since this is a key investigated aspect in this study.

We will update this figure to show different symbols indicated different DBH classes. Also see our response to a related comment from reviewer 1.

L211: The word stored here does not make sense. Not because it is erroneous, but because previously it was defined as xylem water to define transpiration sources, and at this point of the results is referred to as "stored". It would be useful to the reader that the authors establish their assumptions earlier in the paper (e.g., xylem water is a representation of bulk water, stored water and transpiration source, or something in this vein).

We agree with the reviewer that the word 'stored' is misplaced. We will remove the word and establish our assumptions surrounding 'xylem water' later in this study.

L218-220: But in May and June, all the hemlocks seem to be using shallow soil water (top 10 cm) (Figure 3). How is this possible? The two analyses do not seem to be supportive of one another.

We believe this comment reflects a miscommunication on our part. The observed variations in ^{18}O and ^{2}H across all tree cores is approximately 1.5 and 14 per mil, respectively, in May and June, which is a small amount of variation with respect to the observed variation across the soil profile. The absolute values overlap the top 20 cm of measured soils. The depth-partitioning that we observed is likely only across the top 20 cm, where some hemlock relied on the upper 5 cm, and others used slightly deeper water sources. We did not mean to imply the use of water at the lower end of the rooting zone (or groundwater).

In June, DBH was identified as a significant predictor of ^{18}O (p-value = 0.08; and close to significant for ^{2}H, p-value = 0.12) (Fig 4), which does support our conclusion. We do acknowledge that ranked correlations between the marginal distributions of xylem water isotopes and DBH were not significant at the 0.1 threshold in these months (p-values = 0.16 and 0.12) (Fig S6). We will rewrite the conclusions to make it clear that the partitioning is occurring over a short distance across soil depths and that this result carries some uncertainty.

How is xylem water correction using RWC affecting this result itself?

Please see our prior responses which support using this correction. We will now include these results in the manuscript, and we will also include a methodology discussion where we describe how these corrections could impact results.

L228: In March there was no overlap between xylem and available water sources (L204-205). How does the author see this follow-up analysis in March being valid?

We noted in the manuscript that xylem water did not overlap any measured sources: "*In March, prior to the growing season, the isotopic composition of hemlock xylem water in all sampled trees did not overlap with any measured potential water sources."*

In section 5.3 "Isotopic Offsets between Xylem and Subsurface Waters" we specifically said: "*We posit that deviations between xylem water and measured subsurface water sources in March and August are due to an isotopic time lag induced by tree water storage.*"

We agree that the MVLR (and ranked correlation) analyses are not well supported in March (for the reasons that we described in Section 5.3). We will add a discussion of this where those results are reviewed.

L235: Or simply, a change in the ability of the model to explain xylem water? Perhaps other parameters would be more relevant throughout the growing season.

We agree with the reviewer that other parameters such as maximum rooting depths (which are not easily measured) could be more relevant. We will address this gap in a new discussion section about uncertainties in our study and analysis.

L256-257: This wasn't earlier hypothesized in the paper. This adds to an earlier comment on the need for clear hypotheses in the introduction.

We will rewrite the hypothesis in the introduction to more comprehensively reflect the study design and analysis.

L272-273: How would the authors explain these results? What would be this strategy? Is there any evidence in the literature that supports higher stomata control in hemlocks?

We will revise this section to provide a more detailed description of the coordinated strategies. Ford & Vose (2007) showed that hemlock leaf-scale transpiration rates increased linearly with VPD (Fig. 4; Ford & Vose 2007). Based on the Penman Monteith equation (assuming no stomatal closure with increasing VPD), transpiration rates should increase exponentially with increasing VPD (as the VPD term appears in both the turbulent flux term and surface resistance terms). The observed linear relationship between transpiration and VPD could be replicated if we assumed that stomatal resistance in hemlock increased with increasing VPD. This concept was explored using the numerical model EcH2O-iso model (Kuppel et al 2018) calibrated to hemlock (Knighton et al., 2020), though we did not explicitly discuss this result in that paper.

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