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10 **Water Use Strategy of Riparian Conifers Varies with Tree Size and Depends**
11 **on Coordination of Water Uptake Depth and Internal Tree Water Storage**

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31 **Abstract.** Trees employ mechanisms to maintain safe xylem water transport including variations in trunk
32 water storage and the depth of root water uptake. We tested the hypotheses that 1) trunk water storage is
33 correlated with root water uptake in Eastern hemlock, 2) and that water use strategy varies with tree size.
34 High spatiotemporal sampling of soil and hemlock xylem (30 trees) water isotopic ratios (^2H , ^{18}O) and tree
35 tissue Relative Water Content (RWC) was conducted across seven months. Hemlock accessing more
36 evaporatively enriched water from shallow soils stored less water within their trunks during dry periods,
37 and more during wet periods. Soil and xylem water isotopic compositions revealed older and lower
38 elevation hemlock primarily sourced water uptake from the upper 10 cm of soils, whereas younger and
39 higher elevation trees sourced some water uptake from deeper soil layers. Larger diameter hemlock showed
40 significant temporal changes in trunk RWC. In contrast, smaller diameter trees exhibited more temporally
41 stable RWC. Observed species-level heterogeneity in xylem water isotope composition suggests the need
42 for reporting of tree ages and a standardization of field sampling protocols to support our understanding of
43 tree water use strategies. Our results inform the development of plant hydraulic strategies in
44 ecohydrological- and terrestrial biosphere-models to understand forest responses to external stressors.

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48 1. Introduction

49 Root water uptake of soil moisture and groundwater drives ecosystem primary production and influences
 50 the partitioning of precipitation between surface runoff (immediate streamflow), catchment stored water
 51 (e.g., soil moisture, groundwater), transpiration, and the fraction of available energy at the land surface that
 52 is latent heat transfer (Fan et al., 2017; Good et al., 2015). Trees, slowly generating organisms that remain
 53 fixed in place with limited dispersal capabilities, are particularly threatened by shifting climate conditions
 54 (Ammer, 2019; Bonan, 2008; Brodribb et al., 2020; Trugman et al., 2020). A stronger understanding of
 55 how foundational tree species are adapted to survive periods of subsurface water limitation would help to
 56 understand forest responses to external stressors, the design of forest management practices (King & Keim.,
 57 2019), and support the development of more accurate simulations of forested ecosystems (Anderegg et al.,
 58 2022; Knighton et al., 2021).

59 How plant water use strategies are defined is an evolving concept that connects the dimensions of stomatal
 60 regulation in response to vapor pressure deficits, xylem resistance to embolism, trunk water storage, root
 61 access to subsurface water sources, foliar water uptake, and carbon investments during periods of stress
 62 (Carminati & Javaux, 2020; Kannenberg et al., 2022). Investment in deeper or denser rooting systems can
 63 provide trees access to more temporally stable water sources (Chitra-Tarak et al., 2018; Fan et al., 2017;
 64 Knighton et al., 2021; Mackay et al., 2020). Xylem resistance to cavitation and subsequent embolism can
 65 allow trees to survive periods of water pressure deficits between soil moisture potentials at plant roots and
 66 the atmospheric water demand at leaves (Cardoso et al., 2019). Field studies have also provided empirical
 67 evidence that transpiration rates can be sustained during periods of soil moisture limitation by depleting the
 68 volume of water stored within trunks (Čermák et al., 2007; Z. Liu et al., 2021; Phillips et al., 2003; Preisler
 69 et al., 2021). The similar effects of these mechanisms in regulating stem water potentials allows for varied
 70 strategies for surviving periods of drought across forest trees.

71 Xylem resistance to cavitation and access to stable subsurface water sources are two closely related
 72 mechanisms that allow plants to maintain safe xylem water transport. There is evidence that these
 73 mechanisms are related to species identity, driving shifts in tree survival under shifting climate conditions
 74 (Anderegg et al., 2022; Knighton et al., 2021; Skelton et al., 2021). Global analysis shows that conifer root
 75 systems are closely correlated with the local water table depth (Knighton et al., 2021). There is also evidence
 76 that some conifers are well adapted to trunk water loss across the growing season and rely on seasonal
 77 refilling of xylem water during months when competition for water uptake is reduced (Mayr et al., 2014),
 78 whereas other trees require daily refilling of xylem tissues to maintain higher tree conductance (Yi et al.,
 79 2017). A study of Norway spruce during drought demonstrated that there is a safety range for conifer xylem
 80 pressure loss with minimal reductions in conductance, and opportunity for conductance recovery (Arend et



81 al., 2021). The buffering volume of internal tree water storage within conifers in a temperate forest was
 82 estimated to be 40 mm (rainfall water equivalent), a hydrologically significant reservoir (Knighton, Kuppel,
 83 et al., 2020). Given this empirical evidence and the hydraulic relationships between rooting systems and
 84 stem water potential, we hypothesize that trunk water storage is correlated with the depth of water uptake
 85 in conifers.

86 Recent studies have shown biome-scale correlations between rooting depths, stomatal regulation of
 87 transpiration and climate, and demonstrated their importance for understanding global hydrology (Canadell
 88 et al., 1996; Evaristo & McDonnell, 2017; Fan et al., 2017, 2019; Hodge, 2004; Jing et al., 2021; Knighton
 89 et al., 2021; Yaling Liu et al., 2021; van Oorschot et al., 2021; Schenk & Jackson, 2005). There is also
 90 evidence that some species have flexible water use strategies in that they vary sources of root water uptake
 91 and stomatal regulation across local environmental gradients (Allen et al., 2019; Juhlke et al., 2021;
 92 Knighton, Souter-Kline, et al., 2019; Link et al., 2014; Martin et al., 2018; Mumbanza et al., 2021).
 93 Empirical studies provide evidence that drought tolerance is related to diversity, species identity, climate,
 94 and water availability (Bhuyan et al., 2017; Harley et al., 2020; Kannenberg et al., 2019; Yanlan Liu et al.,
 95 2021; Lopez et al., 2021; Schoppach et al., 2021; Vitali et al., 2018; Vitasse et al., 2019), yet we lack an
 96 understanding of the relative importance of these variables.

97 Tree water requirements change with age (Delzon & Loustau, 2005; Wu et al., 2019). The significance of
 98 within-species variations in water use strategy have received relatively little attention compared to
 99 variations between species. Prior rooting studies using water isotopic evidence focus heavily on individuals
 100 of a single species in controlled settings (Nehemy et al., 2021; Seeger & Weiler, 2021; Vargas et al., 2017)
 101 or the responses of multiple individuals of a species in mixed-species plots across environmental gradients
 102 (Brinkmann et al., 2018; Evaristo et al., 2019; Knighton, Souter-Kline, et al., 2019; Link et al., 2014;
 103 Volkmann et al., 2016). Studies of age-varied rooting strategies suggest that the depth of water uptake
 104 increases with tree age, possibly related to increasing maximum rooting depths with tree growth (Song et
 105 al., 2018; Tao et al., 2021; Wu et al., 2019). Within-species variations in age, size, and topographic position
 106 are likely critical considerations given the close relationship with plant rooting depth and physiological
 107 function (Gaines et al., 2016). Based on this empirical evidence, we hypothesize that the rooting systems
 108 of older trees are deeper than those of younger trees, necessitating changes in water use strategy with growth
 109 stage.

110 We test these hypotheses by observing within-species variations in water use across a monoculture stand
 111 of riparian Eastern Hemlock (*Tsuga canadensis*) through high spatio-temporal sampling of soil and xylem
 112 isotopic ratios and tree core Relative Water Content (RWC).



113 2. Materials and Methods

114 2.1 Focus Species: *Tsuga Canadensis*

115 *Tsuga canadensis* (Eastern Hemlock) is a regionally threatened tree species due to infestation by the
 116 Hemlock Woolly Adelgid. Infestations drive loss of needles and death of hemlock trees. There is
 117 observational (Brantley et al., 2013; Kim et al., 2017) and process-based model derived evidence (Knighton,
 118 Conneely, et al., 2019; Singh et al., 2020) that the loss of hemlock will cause substantial changes in the
 119 regional hydrologic cycle of Northeastern US forests including wetter soils, increased groundwater
 120 recharge, surface runoff, and flooding. Prior research suggested that hemlock trees possibly vary sources
 121 of water uptake along hillslopes and by season and can rely on both soil moisture held under tension and
 122 groundwater (Knighton, Souter-Kline, et al., 2019).

123 2.2 Field Data Collection

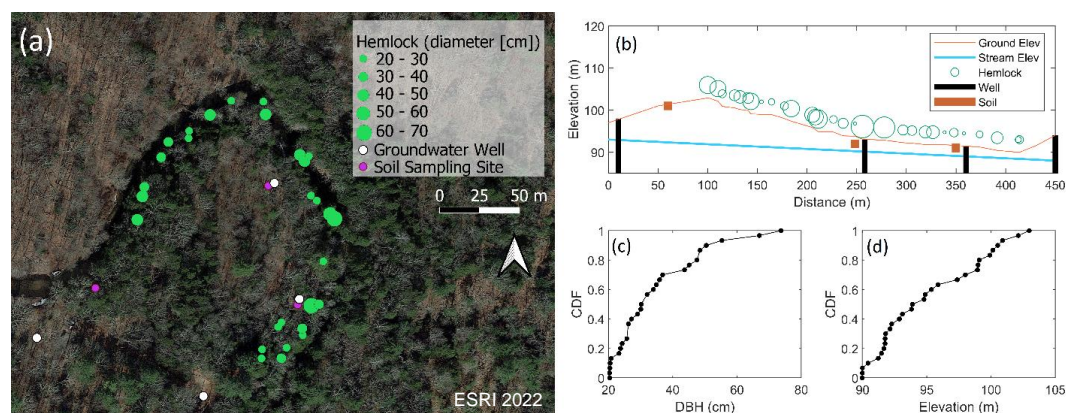
124 This experiment was conducted in the University of Connecticut Forest (CT, USA) (41.825, -72.233).
 125 Measurements were made along a north-facing 300 m riparian corridor bordering the Fenton River. The
 126 sample area in this study is largely monospecific, dominated by riparian Eastern Hemlock (hemlock basal
 127 area of 1.03 m²ha⁻¹), but also including mixed deciduous cultures of *Quercus sp.*, and *Acer sp.* situated
 128 further upslope. The climate is characterized by a mean annual temperature of 9.41°C and an average
 129 precipitation of 1,264 mm¹year⁻¹. The soil texture at this site is fine sandy loam (Miller & White 1998).

130 We cored 30 individual hemlock trees at a monthly interval from March through September 2021 (n = 210
 131 cores). Cores were collected at breast height with an increment borer to a depth of approximately 7.5 cm.
 132 The diameter at breast height (DBH), elevation, and horizontal distance from the stream were measured for
 133 each individual tree. There is no significant relationship between DBH and distance from the stream (Fig.
 134 S1a) or DBH and elevation (Fig. S1b). Tree elevation and horizontal distance are strongly correlated (Fig.
 135 S1c). Dry root mass per unit mass of soil was measured at the three soil sampling locations. Samples for
 136 root mass analysis were collected with an auger at depths of 5, 10, 20, 30, 40, 50, 75, and 100 cm. Collected
 137 roots and soils were oven dried at 100 °C. Roots were removed from dried samples by sieving and then by
 138 visual identification.

139 Soils were sampled monthly for bulk water isotopic analysis (²H, ¹⁸O) and Gravimetric Water Content
 140 (GWC) with an auger at depths of 5, 10, 20, 30, 40, and 50 cm at three locations (n = 132 soil samples).
 141 Soil Volumetric Water Content (VWC) was measured at a monthly interval (HS2 HydroSense) at three soil
 142 sampling locations across the top 12 cm (Fig. 1a, b). Each VWC measurement was the average of 5
 143 individual readings taken within a 1 m² quadrant. Groundwater samples were collected monthly from four



144 wells spanning the stand. Stream water samples were collected monthly near the midpoint of the sampling
 145 plot. Groundwater elevations were recorded at a 15-minute interval with a pressure transducer at a well
 146 located near the midpoint of the stand. Stream depth measurements were recorded at a 15-minute interval
 147 at a USGS station located 1.2 km upstream (USGS, 2022). Daily precipitation and air temperatures were
 148 collected at a weather station located 3.2 km from the study site (NCEI, 2022). Precipitation samples were
 149 collected daily (when present) for isotopic analysis.



150

151 **Figure 1 – Field site show a) relative locations of hemlock and hydrologic sampling, b) elevation profile of**
 152 **hemlock and sampling, c) CDFs of hemlock DBH, and d) horizontal distance from the stream.**

153 2.3 Lab Processing and Analysis of Soils and Tree Cores

154 Soil and hemlock core samples were stored frozen until Cryogenic Vacuum Extraction (CVE) of water. All
 155 CVE was performed at a pressure of 0.2 kPa and a temperature differential of 200 °C for a minimum of 60
 156 minutes. Water recovery data for CVE of soils and stems are presented in supplemental Fig. S2. Gravimetric
 157 Water Content (GWC) of both soils and cores were measured by weighing samples before and after CVE.
 158 Precipitation, groundwater, stream water, and extracted soil and tree core water were analyzed for $\delta^2\text{H}$ and
 159 $\delta^{18}\text{O}$ on a Picarro L2130-i. All samples were analyzed with three water standards spanning -16‰ to +5‰
 160 $\delta^{18}\text{O}$.

161 Hemlock xylem water $\delta^2\text{H}$ ratios were corrected for the effects of CVE discrimination using a proposed
 162 methodology based on Relative Water Content (RWC) (Chen et al., 2020). A subset of hemlock cores
 163 ($n=55$) was rehydrated after CVE in tap water for a period of 48 hours and then weighed to determine the
 164 average turgid GWC of hemlock tissue (1.89 g water / g dry tissue) (Fig. S3a). The average turgid GWC
 165 along with measured fresh and dried GWC for each core were used to compute RWC and xylem water $\delta^2\text{H}$
 166 corrections for each sample (Fig. S3b, c).



167 2.4 Statistical Analysis of Xylem and Soil Samples

168 We tested for significant monotonic relationships between xylem water isotopic ratios ($\delta^{18}\text{O}$, $\delta^2\text{H}$, and lc-
 169 excess) and tree RWC for each sampling period. Significance of relationships were tested with the non-
 170 parametric Kendall's τ . For this and all hypothesis tests, we discuss significance at the α thresholds of 0.1,
 171 0.05, and 0.01.

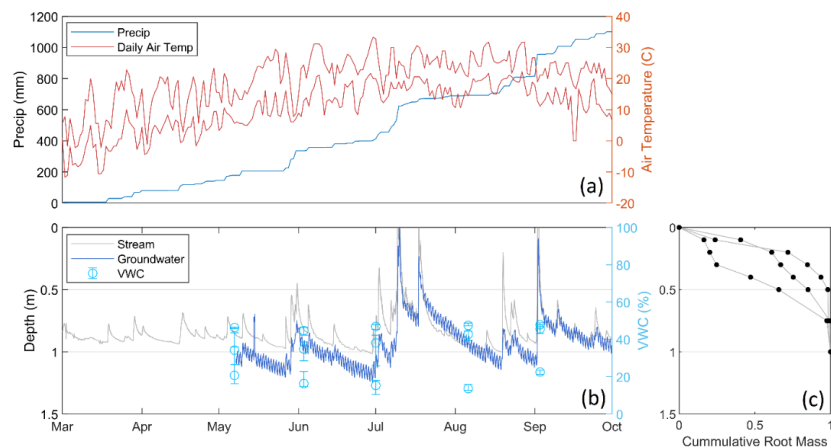
172 We tested for linear correlations between xylem water isotopic ratios ($\delta^{18}\text{O}$, $\delta^2\text{H}$) and DBH and elevation
 173 at base of tree for each collection period via multivariate linear regression. A second model was constructed
 174 that included horizontal distance from the stream despite this variable being significantly correlated with
 175 elevation (Fig. S1c). We present the coefficient of determination for each linear model to indicate the
 176 strength of isotopic predictions from tree characteristics. We tested the hypotheses that the coefficient of
 177 each tree characteristic (β_{DBH} and β_{ELEV}) was significantly non-zero (i.e., a predictor of isotopic variability).

178 We tested for significant differences between the growing season minimum and end of season tree RWC
 179 using a two-sample Kolmogorov Smirnov test. We tested for significant differences across all trees, tree
 180 DBH (divided into two groups by the median DBH value 31 cm), and tree elevation (divided into two
 181 groups by the median tree elevation of 94 m).

182 3. Results

183 3.1 Ecohydrologic Field Conditions

184 The study site received 1,100 mm of precipitation during sampling period (Fig. 2a). The groundwater
 185 surface elevation was consistently below the rooting zone except for three periods following tropical storm
 186 rainfall events occurring between July and September where the Fenton River flowed out of bank (Fig. 2b).
 187 Observed root mass was approximately uniformly distributed across the top 0.75 m of soil, with trace root
 188 mass found at 1 m (Fig. 2c). Shallow soil VWC varied with ground elevation, where shallow soils (top 12
 189 cm) at elevation 95 and 93 m were consistently wetted to approximately 45%. Soils at elevation 103 m were
 190 substantially drier throughout the growing season (Fig. 2b).



191

192 **Figure 2 – Hydrometeorological conditions during experiment a) daily minimum, maximum air temperatures**
193 **and cumulative precipitation, b) stream depth, groundwater depth, and soil Volumetric Water Content**
194 **(VWC), and c) observed root profiles.**

195 The isotopic composition of soils exhibited a more negative lc-excess than the local meteoric water line
196 (LMWL) indicating evaporative fractionation in all months (Fig. 3). Soils below 10 cm were more
197 isotopically depleted and showed less effects of fractionation. Groundwater and streamwater isotopic
198 compositions were stable and exhibited no evidence of evaporative fractionation across all months (Fig. 3).
199 Soil moisture was more strongly depth stratified for $\delta^2\text{H}$ than for $\delta^{18}\text{O}$ or lc-excess and is likely a stronger
200 predictor in xylem water of water uptake depths (Fig. S4).

201 The standard deviation of hemlock xylem water isotopic compositions in each month was small relative to
202 measured isotopic variation in subsurface waters and precipitation, with a minimum of 0.378‰ for $\delta^{18}\text{O}$ in
203 May and a maximum of 0.764‰ in July (Fig. 3). In March, prior to the growing season, the isotopic
204 composition of hemlock xylem water in all sampled trees did not overlap with any measured potential water
205 sources. From April through June, hemlock xylem water overlapped the bulk isotopic composition of the
206 upper 10 cm of soils. In July, hemlock xylem water overlapped with soil water across the upper 35 cm,
207 indicating uptake of deeper soil water within the stand. From August and September, hemlock xylem water
208 did not overlap isotopically with any measured soils, groundwater, or streamwater. During this end of
209 growing season period, the median of xylem water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ enriched by +0.73 ‰ and +8.303 ‰
210 respectively. In contrast, lc-excess increased by +1.737 ‰, indicating less evaporative fractionation of
211 stored xylem water.

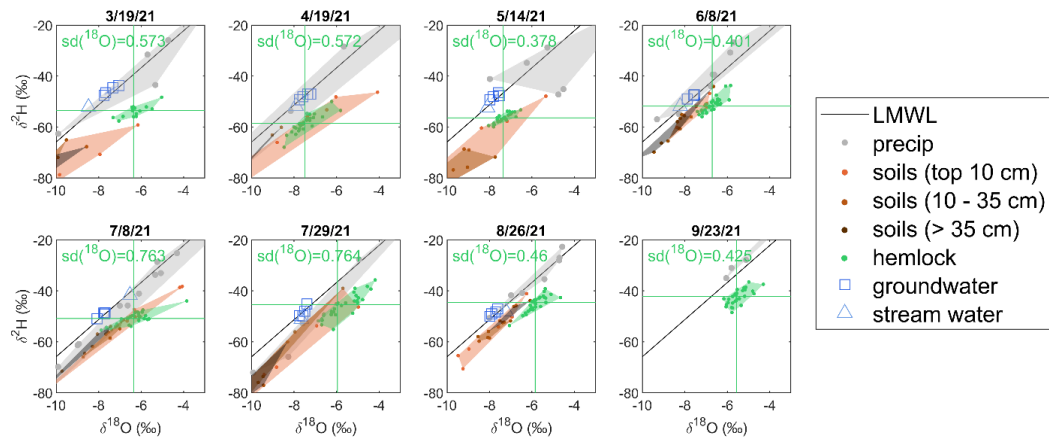


Figure 3 – Dual isotope plot of hemlock xylem water, soils, groundwater, stream water, and precipitation.
Convex hulls of all measurement types are shown as shaded regions.

4.2 Relationships between xylem water isotopic composition and Trunk RWC

Tree xylem water $\delta^2\text{H}$ and tree core RWC were significantly negatively rank correlated at the $\alpha < 0.05$ threshold in May and June and significant at the $\alpha < 0.1$ threshold in July (Fig. 4). Xylem $\delta^{18}\text{O}$ and RWC were significantly negatively correlated in June. Significant negative correlation between RWC and xylem water isotopic ratios in these months indicated that hemlock accessing more evaporatively enriched water sources tended to store less mass of water per unit mass of tree tissue. In early July, following a period of heavy rainfall (Fig. 2), xylem $\delta^{18}\text{O}$ and RWC were positively correlated (and lc-excess negatively correlated) indicating higher water contents in trees reliant on enriched water sources. Correlations were not significant in March, April, August or September.

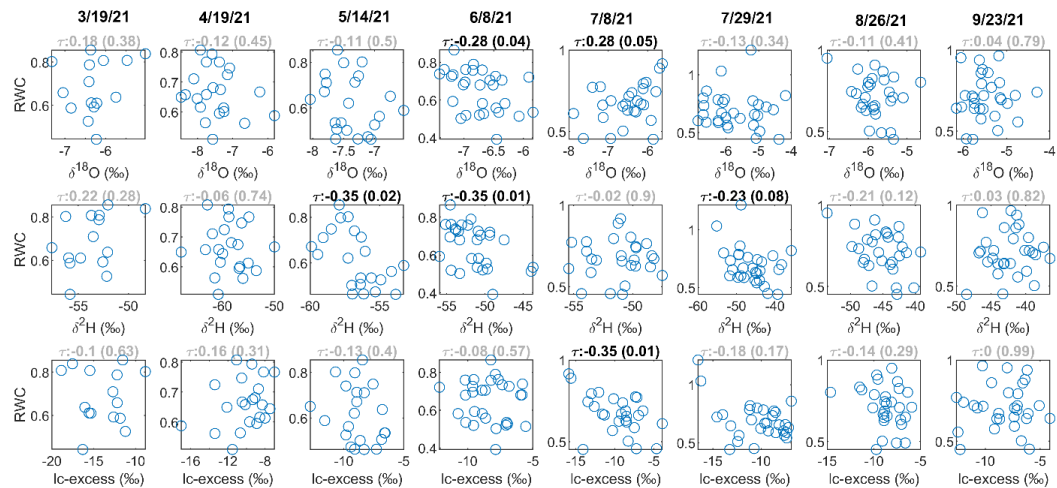
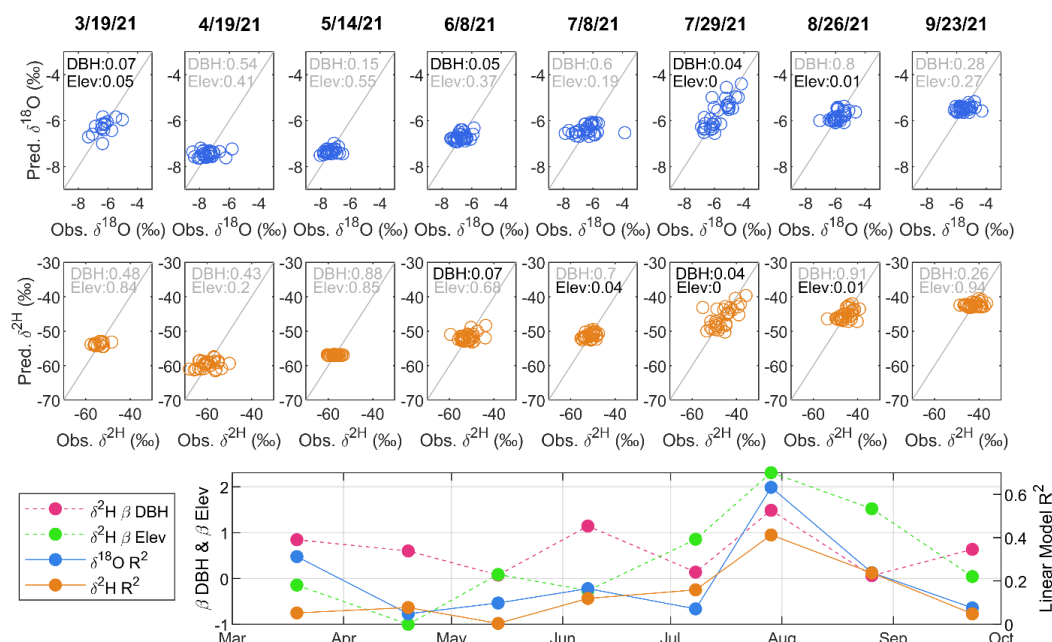


Figure 4 – Correlations between xylem water isotopic ratios and tree core Relative Water Content (RWC) showing Kendall’s correlation coefficient (τ) and p-values in parenthesis.

4.3 Relationships between xylem water isotopic composition and tree characteristics

In March, June, and August, xylem water $\delta^{18}\text{O}$ was significantly positively correlated with DBH, indicating larger diameter trees uptake more enriched water sources (Fig. 5). March $\delta^{18}\text{O}$ was significantly negatively correlated with elevation, but positively correlated in August. Correlations between tree characteristics and $\delta^2\text{H}$ were similar. Inclusion of the horizontal distance from the stream as a variable did not substantially improve model prediction skill (Fig. S5). Multivariate linear models predicting xylem water isotopic ratios from tree characteristics showed temporal variations in skill (Fig. 5), where R^2 for both models generally increased from May to late July and then decreased to September, suggesting changes in the partitioning of water across trees of differing characteristics throughout the growing season. Non-parametric univariate correlation tests on marginal distributions similarly indicated that larger diameter (Fig. S6) and higher elevation (Fig. S7) trees relied on more evaporatively enriched shallow soil moisture in July and August.

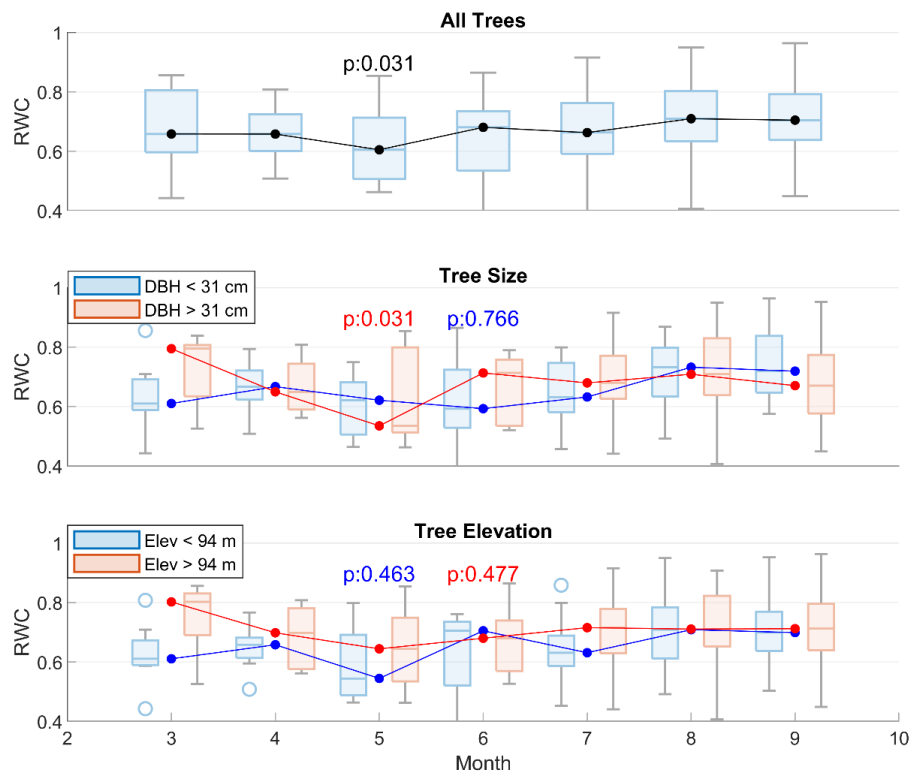


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239 **Figure 5 – Multivariate linear regression prediction of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ from tree diameter at breast height**
 240 **(DBH) and tree elevation for each sampling period. The bottom panel shows model coefficients and R^2**
 241 **through time.**

242 4.4 Relationships between tree characteristics and temporal variations in trunk water storage

243 The median tree core RWC of all trees reached a minimum in May (RWC = 60.5%) and gradually refilled
 244 throughout the growing season, reaching a maximum in September (RWC = 70.7%) (Fig. 6a). The median
 245 RWC was significantly lower in May than in September (Fig. 6a, p-value = 0.031). Temporal variations in
 246 core RWC were only significant in larger diameter trees (Fig. 6b, p-value: 0.031). Smaller diameter trees
 247 (p-value: 0.766) did not show significant differences in core RWC between May and September. Tree RWC
 248 varied between higher and lower elevation trees at the start of the growing season, with higher elevation
 249 trees showing greater RWC in most months. In neither cluster was the minimum month of GWC
 250 significantly lower than the maximum in September (Fig. 6c, p-values: 0.463, 0.477).



251

252 **Figure 6 – Temporal changes in tree core Gravimetric Water Content (GWC) for a) all trees, b) trees**
253 **partitioned by diameter, and c) trees partitioned by elevation**

254 **5. Discussion**

255 **5.1 Water Use Strategy Based on Coordination of Rooting Uptake Depth and Water Storage**

256 We hypothesized that there is a coordination between the quantity of water stored in trunks and rooting
257 strategies for water uptake in hemlock. Observation of significant correlations between tree RWC and
258 xylem water isotopic ratios across the growing season provides supporting evidence for this hypothesis
259 (Fig. 4), where trees reliant on shallow soil moisture also tended to store less water within their trunks
260 during drier periods and more during wetter periods. We note that soils were more strongly depth stratified
261 for $\delta^2\text{H}$ than $\delta^{18}\text{O}$ across most months (Fig. S4), which indicates that $\delta^2\text{H}$ is likely a stronger predictor of
262 the depth of water uptake than $\delta^{18}\text{O}$. This may explain why RWC was more strongly correlated with $\delta^2\text{H}$
263 than $\delta^{18}\text{O}$ or lc-excess. The lack of significant correlation in between RWC and $\delta^2\text{H}$ early July is potentially



264 related to substantial isotopic overlap between shallow (top 10 cm) and deeper soil layers (Figs. 3, and S4)
 265 caused by precipitation.

266 Differentials between the volumes of root water uptake and transpiration drive changes in tissue water
 267 content (Chitra-Tarak et al., 2018; Dralle et al., 2020; Nehemy et al., 2021). Reductions in tree stored water
 268 across the growing season have been described as the temporal integration of soil water stress occurring
 269 over weeks to months (Kannenberget al., 2022). In contrast with this concept, we observed that RWC in
 270 higher elevation hemlock (experiencing substantially lower soil water availability) tended to have higher
 271 RWC from March through May and similar RWC to lower elevation trees from June through September
 272 (Fig. 6c). This suggests that the observed variations in hemlock RWC were not the result of soil water
 273 limitations, but possibly a coordinated strategy for water use. Prior research suggests that, rather than an
 274 indicator of stress, trees can rely on internally stored water to buffer against brief periods of soil water
 275 limitations and sustain plant transpiration (Čermák et al., 2007; Z. Liu et al., 2021; Phillips et al., 2003;
 276 Preisler et al., 2021).

277 Soil and xylem water isotopic compositions indicated that all hemlock trees in the stand relied primarily on
 278 shallow (top 10 cm) soil moisture at the beginning and end of the growing season (Fig. 3). In July and
 279 August, trees showed evidence of a partitioning of water resources with elevation and DBH (Fig. 5). In
 280 contrast with our second hypothesis, older and higher elevation hemlock sourced a substantial proportion
 281 of water uptake from the upper 10 cm of soils, whereas younger and lower elevation trees sourced some
 282 water uptake from deeper soil layers (Fig. 5, S6, and S7). Partitioning of subsurface waters has been
 283 commonly observed across neighboring species (Brum et al., 2019; Cabal et al., 2020; De Deurwaerder et
 284 al., 2018; Fabiani et al., n.d.; Knighton, Souter-Kline, et al., 2019; Silvertown et al., 2015). Studies that
 285 specifically investigated age-related water uptake depths reached the contrasting conclusion that older trees
 286 relied more on deeper water sources (Song et al., 2018; Wu et al., 2019). Deeper water uptake by younger
 287 hemlock may represent within-species competition. Lateral root spread by mature trees can force deeper
 288 stand water uptake during periods of shallow water limitation (Agee et al., 2021). Younger hemlock may
 289 also have a greater need for sustained transpiration owing to growth requirements and less potential for
 290 internal water storage. Access to deeper water sources may allow younger hemlock to maintain higher stem
 291 water potentials and therefore greater plant conductance and transpiration.

292 Our observation of temporal variations RWC (Fig 6a) agreed with prior studies that transpiration rates can
 293 exceed water uptake in some months by depleting trunk-stored water (Čermák et al., 2007; Z. Liu et al.,
 294 2021; Phillips et al., 2003; Preisler et al., 2021); however, we did not observe this behavior across all
 295 hemlock. Larger hemlock trees (DBH > 31 cm) exhibited a 50% decrease in median RWC from March to
 296 June, and then refilled from June to September (Fig 6b). In contrast, smaller diameter trees showed no



statistically significant seasonality in tissue RWC. One possible explanation is that larger diameter hemlock trees occupy a higher canopy position than smaller trees. Larger trees likely received a greater proportion of incoming radiation, supporting greater growth rates early in the growing season (Coomes & Allen 2007). Greater available energy at the canopy could drive increased water needs to sustain transpiration and photosynthesis. This explanation may be less likely, as a greater water need might also drive larger diameter hemlock to rely on a greater fraction deeper soil layers with more temporally stable water availability.

Another potential explanation is that stem water storage and xylem capacitance requirements vary with hemlock age. Reductions in water storage and the capacity to survive lower xylem water potentials may represent both integrated water deficits and also a viable strategy for maintaining transpiration while minimizing investments in deep roots (Arend et al., 2021; Mayr et al., 2014). Older hemlock relied on shallow soil moisture, a rapidly depleted and replenished water store. These trees transpired a substantial amount of water that was stored in the trunk at the start of the growing season, and then rapidly refilled (Figs. 4 & 6b) following heavy precipitation in July (Fig. 2). In contrast, younger trees used a more stable water source (below 10 cm) during shallow soil moisture limitation (similar to previous observations of smaller diameter hemlock (Knighton, Souter-Kline, et al., 2019)) and maintained stable trunk water storage throughout the growing season. Deeper rooting oak trees were similarly observed to better maintain temporally stable trunk water storage and transpiration than shallow rooted neighboring maple trees (Matheny et al., 2017). Laterally extensive rooting systems may efficiently uptake recent precipitation into shallow soils; however, this rooting strategy may also expose trees to periods of shallow soil moisture limitation (Agee et al., 2021). Trees can rely on internally stored water to source transpiration (Čermák et al., 2007; Matheny et al., 2015) potentially providing a buffer against such short-duration soil water limitations, a strategy potentially employed by mature hemlock observed in this study.

5.2 Xylem Water Isotopic Spatio-temporal Heterogeneity

Significant open questions remain concerning field sampling of trees for isotopic analysis to estimate root water uptake. Water isotopic heterogeneity in the environment (i.e., soils, xylem, groundwater) has been identified as a primary complicating factor in the study of plant water uptake, storage, and transpiration (Barbeta et al., 2020; Beyer & Penna, 2021; Freyberg et al., 2020; Goldsmith et al., 2019; Oerter & Bowen, 2019). Across all months, the observed standard deviation of hemlock $\delta^{18}\text{O}$ (Fig. 3) was similar to that reported by prior studies (Allen et al., 2019; Freyberg et al., 2020; Goldsmith et al., 2019). Controlling for sampling month, the standard deviations of hemlock xylem water were substantially lower (Fig. 3), potentially suggesting that within-species heterogeneity is species specific or possibly less than previously suggested.



329 A global compilation of 531 species-level estimates of plant rooting strategies with isotopic observations
 330 shows that 67% of conclusions are based on 6 or fewer xylem samples (Evaristo & McDonnell, 2017). This
 331 disparity across studies potentially highlights a need for standardized vegetation isotopic sampling
 332 protocols; however, observation of significant phylogenetic signals across this compiled data possibly
 333 supports the concept that limited xylem isotopic sampling is sufficient to characterize tree water strategies
 334 (Knighton et al., 2021). Further, agreement between rooting estimates based on isotopic techniques and
 335 those derived from spatially-integrated datasets through ecohydrological modeling lends some support to
 336 the conclusions of past studies (Knighton, Singh, et al., 2020).

337 Rather than discussing xylem isotopic variability as a purely stochastic process, our research contributes to
 338 the growing understanding that sampling date, tree physical characteristics (Couvreur et al., 2020), micro-
 339 topography (Goldsmith et al., 2019; Oerter & Bowen, 2019), catchment-scale flowpaths (Knighton, Kuppel,
 340 et al., 2020; Knighton, Souter-Kline, et al., 2019), and elevation (Allen et al., 2019; Tetzlaff et al., 2021)
 341 can explain a significant amount of xylem isotopic variability during the growing season.

342 **5.3 Isotopic Offsets between Xylem and Subsurface Waters**

343 Prior studies have observed an isotopic separation between Eastern hemlock xylem water and measured
 344 end members (Knighton, Kuppel, et al., 2020; Snelgrove et al., 2021). Isotopic differences between xylem
 345 and soil water have been attributed to potential isotopic fractionation at the soil-root interface (Barbeta et
 346 al., 2020; Snelgrove et al., 2021), evaporative enrichment through bark (Snelgrove et al., 2021; Tetzlaff et
 347 al., 2021), lags in xylem isotopic composition due to internal storage and mixing (Knighton, Kuppel, et al.,
 348 2020), and artefacts of CVE (Allen & Kirchner, 2021; Chen et al., 2020). After correcting xylem for ^2H
 349 with stem RWC of each individual sample (Fig. S3), xylem water overlapped with measured soil end
 350 members during the peak growing season (Fig. 3), supporting the necessity of ^2H corrections after CVE.

351 Outside of the peak growing season, we observed hemlock xylem water isotopic compositions that did not
 352 overlap measured subsurface sources of water (Fig. 3). We posit that deviations between xylem water and
 353 measured subsurface water sources in March and August are due to an isotopic time lag induced by tree
 354 water storage. Xylem isotopic compositions at the end of the growing season were like those of soils in July
 355 when uptake would be highest (Fig. 3). Between July 29th – September 23rd, hemlock xylem water enriched
 356 slightly for both ^2H and ^{18}O but exhibited a less negative lc-excess (Fig. 3). This suggests that stored xylem
 357 water was not undergoing substantial evaporative enrichment via evaporation through the bark, as this
 358 process would have caused xylem lc-excess to gradually become more negative. A possible explanation
 359 that is physically consistent with these observations is the continual uptake of small volumes of water
 360 through roots or branches that mixed into a substantially larger reservoir of stored trunk water. Though no



measured soils would cause the observed isotopic change, we note that precipitation isotopic compositions could explain the gradual change in xylem isotopic composition (Fig. 3), potentially as branch water uptake (Losso et al., 2021).

5.4 Representations of Vegetation in Ecohydrological Models

Observations noted within this study suggest a fundamental need for the refinement of the currently used ecosystem and catchment models designed to simulate vegetation water uptake dynamics. Ecohydrological models commonly rely upon plant functional types to simulate spatiotemporal variations in water uptake, ignoring possible significant across- and within-species variability. Frequently employed simulations of plant ecohydrological responses assume species-level plasticity is minimal, and this remains largely uncharacterized within relevant ecohydrological modelling frameworks. This is likely a considerable oversight given observed correlations between species-level features/functional characteristics and water uptake strategies, as well as the sensitivity of the land surface water balance to complex rooting strategies, stem water storage, and plant conductance (Kennedy et al., 2019; Li et al., 2021; Mirfenderesgi et al., 2016; Sakschewski et al., 2021).

Past modelling approaches to simulating plant hydraulics have used GPP (Gross primary production) -based plant water stress parameterizations, root dynamics optimizations, and novel tree scale hydrodynamics to optimize leaf, stem, and root growth parameters (Mirfenderesgi et al., 2016; Wang et al., 2018; Kennedy et al., 2019; Li et al., 2021). Modern modelling approaches can identify differences in water uptake strategy across species and simulate plant hydraulics well, however water uptake heterogeneity is often averaged over across- and within-species variations (Mirfenderesgi et al., 2016; Wang et al., 2018; Kennedy et al., 2019; Li et al., 2021). Many ecosystem models assume physiologic and hydraulic parameters are not contingent upon species-level variations but are properties of plant functional type, hydraulic functional types, or genus-specific hydraulic attributes (Mirfenderesgi et al., 2016; Li et al., 2021). These methods are advantageous for simulating global-scale spatio-temporal water fluxes; however, they are not sufficient in reflecting the ecohydrological minutiae that drive plant survival over a broader range of conditions and at smaller scales. Observations of within-species variability in rooting depth, trunk volume storage suggest there is still need for the integration of new parameters in plant hydraulic models. Incorporating species-level distinctions in root water uptake and trunk storage can lead to improvements in mimicking transpiration from plants and other key environmental processes important to ecohydrological simulation. Granted, we also note that this new approach inherently requires a need for high spatio-temporal sampling and tighter constraints on model vegetation parameters, both of which can outpace the data or resources available. In brief, we suggest that current ecohydrological models should not overlook, but rather consider



393 integrating species-level variability as a feature to improve the accuracy of forestry and plant growth
 394 dynamics.

395 **6. Conclusions**

396 Current knowledge of tree water use strategy relies heavily on the assumption that the influence of species-
 397 level characteristics is minimal. Our results suggest that Eastern hemlock trees exhibit changes in water use
 398 strategy with growth stage, and there is a coordination between the trunk water storage and root water
 399 uptake strategy. We observed that older and higher elevation hemlock relied on shallow soil moisture,
 400 whereas younger and lower elevation trees often sourced water uptake from deeper soil layers. Younger
 401 trees employed a more stable water source during periods of shallow soil moisture limitation, maintaining
 402 stable trunk water storage throughout the growing season. Conversely, older hemlock trees exhibited
 403 significant seasonality in trunk water storage, hence, the trees reliant on shallow soil moisture tend to store
 404 less water within their trunks during drier periods and more during wetter periods. Notably, we observed
 405 enrichment of hemlock xylem water isotopic compositions at the end of the growing season coupled with
 406 less negative $\delta^{13}C$ -excess, possibly explained by recent precipitation taken up through roots or branches.

407 Understanding the species-level heterogeneity in plant water uptake and storage mechanisms is essential to
 408 answering fundamental questions surrounding plant water partitioning and will help to elucidate patterns
 409 of forest cover change and water availability under future climate conditions. This research demonstrates
 410 the need for reporting of species-level characteristics and development of a standardized methodology for
 411 field sampling protocols. Ultimately, these advances support our understanding of hydrology and help to
 412 refine modern process-based ecohydrological models through improved simulation of plant hydraulics and
 413 critical zone water partitioning.

414 **7. Data availability**

415 All data for this project are publicly available online (Knighton, J. (2022). Fenton Tract Research Forest -
 416 Hydrologic Data, HydroShare,
 417 <http://www.hydroshare.org/resource/8996065d3ba34907a018be9b4369c1d3>).

418 **8. Author contributions**

419 JK conceived and designed the study. KL and JK collected the data. KL and JK analyzed the data. JK
 420 created figures and led result discussion. KL drafted the manuscript. KL and JK provided edits. JK revised
 421 the manuscript.

422



423 9. Competing interests

424 The authors declare that they have no conflict of interest.

425 10. Acknowledgements

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