The seasonal origins and ages of water provisioning streams and trees in a tropical montane cloud forest

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Abstract

Determining the sources of water provisioning streams, soils, and vegetation can provide important insights into the water that sustains critical ecosystem functions now and how those functions may be expected to respond given projected changes in the global hydrologic cycle. We developed multi-year time series of water isotope ratios (δ^{18}O and δ^2H) based on twice monthly collections of precipitation, lysimeter, and tree branch xylem waters from a seasonally dry tropical montane cloud forest in the southeastern Andes mountains of Peru. We then used this information to determine indices of the seasonal origins, the young water fractions (F_{yw}), and the new water fractions (F_{new}) of soil, stream, and tree water. There was no evidence for intra-annual variation in the seasonal origins of lysimeter and stream waters, which were predominantly comprised of wet season precipitation, even during the dry seasons. However, branch xylem waters demonstrated an intra-annual shift in seasonal origin: xylem waters were comprised of wet season precipitation during the wet season, and dry season precipitation during the dry season. The young water fractions of lysimeter (<15 %) and stream (5 %) waters were lower than the young water fraction (37 %) in branch xylem waters. The new water fraction (an indicator of water ≤ 2 weeks old) was estimated to be 12 % for branch xylem waters, while there was no significant evidence for new water in streams. Our results indicate that the source of water for trees in this system varied seasonally, such that recent precipitation may be more immediately taken up by shallow tree roots. In comparison, the source of water for soils and streams did not vary seasonally, such that precipitation may mix and reside in soils and take longer to transit into the stream. Our insights into the seasonal origins and ages of water in soils, streams, and vegetation in this tropical montane cloud forest adds to understanding of the mechanisms that govern the partitioning of water moving through different ecosystems.

Introduction

Observations that the sources of precipitation that supply streamflow are not necessarily the same as the sources of precipitation that provision evapotranspiration (Brooks et al., 2010; Dawson & Ehleringer, 1991; Evaristo et al., 2015) raise questions about how precipitation entering an ecosystem will be partitioned into different fluxes over time (Kirchner & Allen, 2020a). One interesting implication of such observations is that the origins and ages of water in these fluxes differ from one another and that these differences are likely to arise from variation induced by the transport, mixing, and storage of water in the subsurface (Berghuijs & Allen, 2019; Berghuijs & Kirchner, 2017). Measuring the time it takes for water to transit through ecosystems can thus provide fundamental insights into ecohydrological processes, such as

the flood response of streams when there is significant precipitation (Gallart et al., 2020) or the drought response of plants when there is minimal precipitation (Harpold, 2016; Sprenger, Carroll, et al., 2022).

Much of what we know about the origin and ages of pools and fluxes of water in ecosystems originates from a long history of observations of the ages of water that sustain streamflow, particularly in relation to discrete precipitation events (Kirchner et al., 2023; Sprenger et al., 2019). Such research has consistently demonstrated that streamflow is comprised of waters that have been stored and mixed over long time periods in the subsurface, rather than waters from recent storms (McGuire & McDonnell, 2006). Nevertheless, stream water is generally known to range in age from days to decades (Jasechko, 2019; Sprenger et al., 2019). More recently, observations of the age of transpiration waters demonstrate that the water taken up by trees can originate from sources that are several months old (Allen et al., 2019a; Miguez-Macho & Fan, 2021). However, most of this information has come from comparatively dry seasonal climates (Berkelhammer et al., 2020; Brinkmann et al., 2018; Ehleringer et al., 1991; Hu et al., 2010; Kerhoulas et al., 2013; Martin et al., 2018), and there is evidence that total precipitation amount plays a role in the seasonal origin, or age, of water taken up by plants (Goldsmith et al., 2022). Even rarer are empirical studies that simultaneously measure ages of water provisioning streams and plants at the same location (Dai et al., 2022), limiting our ability to build a complete understanding of ecosystem hydrology.

Stable isotopes of water (δ^{18}O and δ^2H) provide a means for estimating the origins and ages of water that provision trees and streamflow, and exciting new data analysis approaches now support more robust estimates (Benettin et al., 2022). Three of these approaches are of particular interest. First, comparing the isotopic signature of water in streams or plants to the isotopic signature of precipitation falling in the wet or dry season can facilitate the study of the seasonal origins of water in streamflow and transpiration (the seasonal origin index *sensu* Allen et al., 2019a). One benefit of the seasonal origin index is that it can be calculated for relatively short time intervals, facilitating the examination of how seasonal origins of water leaving an ecosystem may change over time. Second, the amplitude of the precipitation isotope intra-annual cycle can be compared to the amplitude of the soil, stream, and plant branch xylem water isotope intra-annual cycles to understand the relative amounts of recent precipitation sustaining these pools and fluxes of water (the young water fraction *sensu* Kirchner, 2016). The young water fraction thus provides an approach for estimating the age of water in streams and taken up by plants. Third, a modified hydrograph separation framework can also be applied to stream and plant water, similar to techniques used for decades to understand the contribution of "new" water to streams during storms (e.g., Hooper et al., 1990). In this ensemble hydrograph separation approach, mixing end-members change with each sampling timestep to include the previous sample as the "old" water and the precipitation that fell between as the "new" water (Kirchner, 2019). The ensemble of hydrograph separations is then used to calculate a

100 representative new water fraction, or the fraction of water contained within streamflow or plant water that

fell as precipitation from the previous sample. Taken together, the seasonal origin index (order of 3–6

months), young water fraction (order of ≤ 2 months) and new water fraction (order of 2 weeks) provide

complementary approaches to estimating the origins and ages of water. When these approaches are applied

to soils, streams, and plants simultaneously, they can provide insights into the partitioning of water within

105 an ecosystem.

We studied the seasonal origins and ages of water within a tropical montane cloud forest watershed

and use these results to address whether streamflow and transpiration are supplied by the same sources of

water. While there has been considerable research on the origins and ages of soil water and streamflow,

comparatively little is known about plants. Information about these multiple hydrological compartments

110 from the same site can provide a more comprehensive understanding of how water flows through an

ecosystem but requires significant effort to collect all the necessary data over time. In this study, we

collected soil lysimeter, stream, and tree branch xylem waters approximately twice monthly over multiple

years and used these data to estimate the seasonal origin index, the young water fractions, and the new

water fractions of each compartment. In this seasonal wet tropical montane environment, we expected

115 shallow rooted trees to take up relatively young water and expected streamflow to be comprised of

relatively older water because of the permeable, fractured shale bedrock that increases the time it takes for

precipitation to move through the watershed (Burt et al., 2022).

Materials and methods

120 **Study Site**

This study was carried out in a tropical montane cloud forest within a small (0.33 km^2),

mountainous (3077 m asl, mean slope 33.8 °) watershed located in the Amazon Conservation Association's

Wayqecha reserve in the southeastern Peruvian Andes (-13.19255, -71.58795; Fig. 1). The dominant

lithology was the San José Group shale (Carlotto et al., 1996) and the soils were classified as umbrisols

125 (Asner et al., 2017). Mean annual precipitation, recorded via a tipping bucket rain gauge, is 4110 ± 418

mm, with a pronounced wet season from December to March, during which ca. 65 % of the annual

precipitation occurs. Mean annual temperature is ~ 11 ± 1 °C (Rapp & Silman, 2012). Previous studies in

this tropical montane cloud forest have used stable isotopes of water to establish paleohydrologic proxies

(Feakins et al., 2016) and as tools for fingerprinting organic carbon sources (Feakins et al., 2018; Ponton et

130 al., 2014). Another study used stable isotopes of water to create a hydrologic budget for a mesoscale (161

km^2) catchment (Clark et al., 2014). The small watershed studied here (0.33 km^2) is within the headwaters

of the mesoscale watershed (161 km^2) previously studied by other researchers.
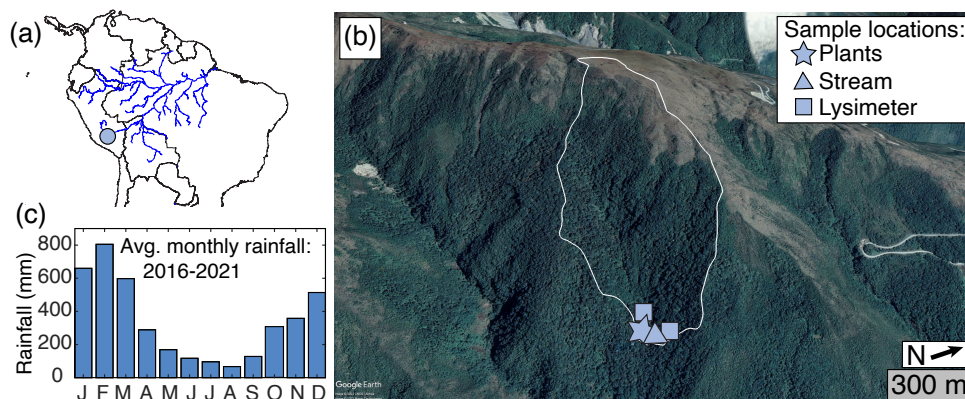
Figure 1. (a) Map of South America; the blue circle represents the approximate location of the tropical montane cloud forested watershed studied in this paper. (b) Google Earth imagery of the study watershed, outlined in white. Plant sampling location is represented by a blue star, stream sampling location by a blue triangle and the soil water lysimeters by blue squares. (c) Average monthly precipitation from 2016-2021.

Sample collection

From February 2018 to March 2020, we collected tree branch samples two times per month from two of the most common genera at the study site (Rapp et al., 2012): *Weinmannia* (16% of forest woody stems) and *Clusia* (13% of forest woody stems). During each sampling, we used a canopy bridge to access the crown of mature trees and collected one branch from one individual tree for three different species: *Weinmannia bangii* Rusby*, Weinmannia reticulata* Ruiz & Pav., and *Clusia alata* Planch. & Triana. Rather than sample the same three individual trees twice a month (which would quickly deplete available branches), we collected samples from a group of trees within ~ 100 m of a stream (see blue triangle in Fig. 1b). Each branch sample was collected by accessing a canopy bridge and cutting a mature branch sample > 10 cm away from the leaves and removing the bark. We then sealed the branch xylem samples into vials and immediately wrapped them with parafilm. The samples were frozen upon return from the field (< 24 h) and maintained frozen until water extraction.

From April 2016 to March 2020, we collected stream water, soil lysimeter water and precipitation samples twice per month. Stream water samples were filtered with a 0.2 μm diameter polyethersulfone filter in the field and sealed in vials. Soil water samples were collected from two suction lysimeters installed ca. 1 m below the soil surface: one in the valley bottom, near the stream riparian zone, and the other on the hillslope, near the tree sampling location (see Fig. 1b). The water in the lysimeters may thus reflect an integration of a two-week period: each sampling, the lysimeters were evacuated and left under

tension (-60 to -70 kPa) until the subsequent sampling, when the water was then collected. Upon collection,

160   lysimeter waters were filtered with a 0.2 μm diameter polyethersulfone filter in the field and sealed in vials.
Precipitation samples were also collected over two-week time periods in a bucket with a layer of vegetable
oil to prevent evaporation loss. On retrieval, precipitation samples were sealed in glass vials to prevent
evaporation and subsequently filtered with a 0.2 μm diameter polyethersulfone filter in the lab to remove
particulates. Precipitation was recorded using a tipping bucket gauge maintained by the Andes Biodiversity

165   and Ecosystem Research Group. Stream water level was logged every 15 min (WL16U Water Level
Logger; Global Water; College Station, TX, USA) beginning in January 2019 and through the end of the
study.

Stable isotope measurements

Water from branch xylem samples was extracted via cryogenic vacuum distillation at the Center for Stable

170   Isotope Biogeochemistry at UC Berkeley. Each branch xylem sample was placed under a vacuum of ≤ 60
mT and submerged in boiling water for at least 70 min while the water vapor collected in a liquid nitrogen
cold trap. Several studies have reported artifacts associated with processing and analyzing samples for
stable isotopes of water (von Freyberg et al., 2020), particularly with respect to cryogenic vacuum
extraction (Chen et al., 2020). Importantly, recent experiments suggest that the total volume of water

175   extracted from the plant sample is related to the magnitude of isotopic biases, such that extracting > 600 μL
of water minimizes methodological biases (Diao et al., 2022). Here, the volume of extraction water was ≥
600 μL for most samples. To assess completeness of the branch xylem water extraction, post-extraction
branches were weighed, dried for 72 h and reweighed. The post-extraction drying showed that cryogenic
extraction resulted in the recovery of > 90 % of the branch xylem water in nearly all branch samples (data

180   not shown). The extracted branch waters were analyzed for δ^{18}O and δ^2H via Isotope Ratio Mass
Spectrometry at the Berkeley Center for Stable Isotope Biogeochemistry. The standard deviation of an
independent quality control sample is 0.1 ‰ or better for δ^{18}O and 1.1 ‰ for δ^2H.

Lysimeter, stream and precipitation waters were analyzed for δ^{18}O and δ^2H via isotope ratio
infrared spectroscopy using a Picarro L2130i (Chapman University) and two Los Gatos Research Liquid

185   Water Isotope Analyzers (LGR) (Caltech and Lawrence Berkeley National Lab). For the Picarro, the long-
term standard deviation of an independent quality control sample was 0.1 ‰ for δ^{18}O and 0.5 ‰ for δ^2H.
For the LGR at Lawrence Berkeley National Lab, the standard deviation of an independent quality control
sample across runs was 0.1 ‰ for δ^{18}O and 0.5 ‰ for δ^2H. For the LGR at Caltech, the standard deviation
across runs of an independent quality control sample was 0.3 ‰ for δ^{18}O and 1 ‰ for δ^2H. All values are

190   represented as ‰ relative to V-SMOW.

Oxygen isotopes in precipitation display a strong relationship with elevation in this region (-1.7 ‰ km^{-1} elevation; Burt et al., 2022). Given that precipitation samples were collected at ~ 2900 m, and the watershed elevation ranges from ~2900 – 3500 m, we accounted for the effects of elevation on precipitation isotopes within the watershed by correcting the oxygen isotopes to a mean elevation of 3200 m.

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Stable isotope data analysis

To account for the isotopic effects of evaporation, branch xylem water isotopes (δ^{18}O and δ^2H) were corrected back to their source waters using the method described in Benettin et al. (2018). Water isotope evaporation lines were calculated for each month using long-term average relative humidity and

200   temperature (unpublished data). The evaporation-corrected branch xylem water isotope values were used to calculate an index of the seasonal origin of water and the young water fraction in stream, soil lysimeters and trees.

To calculate an index of the seasonal origin of water in different pools and fluxes, we followed Allen et al. (2019):

205    $$SOI = \frac{(\delta x - \delta annP)}{(\delta dryP - \delta annP)} \qquad \text{if } \delta_x > \delta_{annP} \qquad\qquad (1)$$

$$SOI = \frac{(\delta x - \delta annP)}{(\delta annP - \delta wetP)} \qquad \text{if } \delta_x < \delta_{annP} \qquad\qquad (2)$$

where δ_x is the oxygen isotope composition of individual stream, lysimeter, or tree water samples, δ_{annP} is the volume-weighted annual precipitation oxygen isotope composition, δ_{dryP} is the average dry season precipitation oxygen isotope composition, and δ_{wetP} is the average wet season precipitation oxygen isotope

210   composition. SOI near -1.0 indicates a sample of stream, lysimeter, or tree water mostly comprised of wet-season precipitation; a value of +1.0 indicates a sample of stream, lysimeter, or tree water mostly comprised of dry season precipitation.

The young water fraction requires estimating the amplitude of the seasonal differences in water isotopes. To do so, we fit a sinusoidal function to precipitation, lysimeter and stream water, as well as

215   evaporation-corrected branch xylem water isotope values to characterize the seasonal cycle in oxygen isotopes (Allen et al., 2018):

$$\delta^{18}O(t) = a_r \times \cos(2\pi f t) + b_r \times \sin(2\pi f t) + k \qquad\qquad (3)$$

where $\delta^{18}O(t)$ is the oxygen isotope ratio of precipitation, stream, or tree water at a given time, f is the frequency of the oxygen isotope seasonal cycle in each reservoir, a and b are the cosine and sine

220   coefficients, and k is the isotopic offset. The fit of Eqn. 3 to stream water and precipitation δ^{18}O was flow-weighted by stream discharge and precipitation amount, respectively. Coefficients a and b from Eqn. 3

were then used to calculate the δ^{18}O seasonal cycle amplitude (A) for precipitation, lysimeter, stream and branch xylem water as follows:

$$A_{\text{precip}} = \sqrt{a_{precip}^2 + b_{precip}^2} \tag{4}$$

225 $$A_x = \sqrt{a_x^2 + b_x^2} \tag{5}$$

where x is amplitude for the different pools or fluxes of water: lysimeter, stream or branch xylem water.

To calculate the young water fractions (F_{yw}) of lysimeter, stream or branch xylem waters, we followed Kirchner (2016 a, b):

$$F_{yw,x}\ (\%) = A_x/A_{\text{precip}} \tag{6}$$

230 where A is as defined by Eqns. 4 and 5 above.

To assess the uncertainty of the young water fraction estimates, we carried out a bootstrapped resampling of the precipitation, lysimeter, stream, and branch xylem water δ^{18}O datasets. To resample each dataset, we drew one sample at random from the complete dataset and then repeated this resampling with replacement until we had drawn the same number of random samples as the original dataset (e.g., for the

235 branch xylem water δ^{18}O dataset with 142 observations, we sampled 142 times, each time from the full dataset of tree water δ^{18}O values). We repeated this 10,000 times each for the precipitation, stream, and tree water δ^{18}O datasets. We then calculated Eqns. 4–7 for each of those 10,000 bootstrapped datasets. This allowed us to constrain the young water fraction as a distribution of values for the stream and tree water.

To calculate the new water fraction, the fraction of stream or branch xylem water comprised of

240 precipitation that has fallen since the previous sampling (Kirchner, 2019), we applied an ensemble hydrograph separation framework to the stream and tree water δ^{18}O datasets. In our study, the interval between samples is approximately 2 weeks, which means the new water fraction represents how much precipitation from ≤ 2 weeks ago is within the stream or tree water. We first calculated:

$$\delta^{18}\text{O}_{\text{p, j}} - \delta^{18}\text{O}_{\text{x, j-1}} \tag{7}$$

245 where $\delta^{18}O_{p,j}$ is the oxygen isotope composition of precipitation at a given sampling timepoint j and $\delta^{18}O_{x,j-1}$ is the oxygen isotope composition of stream or tree water at the previous sampling timepoint, j-1. We then calculated:

$$\delta^{18}\text{O}_{\text{x, j}} - \delta^{18}\text{O}_{\text{x, j-1}} \tag{8}$$

where $\delta^{18}O_{x,j}$ is the oxygen isotope composition of stream or tree water at a given sampling timepoint. We

250 calculated Eqns. 7 and 8 for all the stream and branch xylem water sampling timepoints and created a scatterplot with the results of Eqn. 7 on the x-axis and Eqn. 8 on the y-axis. The new water fraction is equivalent to the slope of the regression line between x and y. Finally, we performed analysis of covariance

to evaluate the relationship between $\delta^{18}O_{p, j} - \delta^{18}O_{x, j-1}$ and $\delta^{18}O_{x, j} - \delta^{18}O_{x, j-1}$ for stream or branch xylem water, with season as a covariate.

255   **Data availability**

All stable isotope and precipitation data used for the analysis, as well as the code for the young water fraction bootstrap resampling routine are available through HydroShare (Burt et al., 2023).

260   **Results**

Stable oxygen and hydrogen isotopes of water

Precipitation isotope ratios ranged from -20.1 to -0.5 ‰ for $\delta^{18}O$ and -153 to 18 ‰ for δ^2H and were used to calculate the local meteoric water line (LMWL), which had a slope of 8.49 and an intercept of 19.29 (Fig. 2). Cloud waters from Clark et al. (2014) were isotopically depleted compared to the LMWL.

265   Stream water isotope ratios ranged from -14.5 to -10.5 ‰ for $\delta^{18}O$ and -97 to -80 ‰ for δ^2H. Most stream water samples fell along the LMWL, with only a few falling to the left, indicating a minimal contribution of cloud water to the stream. In their study of the mesoscale 161 km^2 watershed, Clark et al. (2014) calculated that approximately 10 % of stream water originated from cloud water. Soil water isotope ratios ranged from -18.0 to -7.9 ‰ for $\delta^{18}O$ and -129 to -47 ‰ for δ^2H. Plant water isotope ratios ranged from -

270   14.8 to -4.6 ‰ for $\delta^{18}O$ and -114 to -37 ‰ for δ^2H. Plant water isotope ratios were generally enriched relative to the LMWL. Correction of plant water isotope ratios to account for evaporative enrichment yielded isotope ratios that ranged from -16.7 to -8.1 ‰ for $\delta^{18}O$ and -122 to -49 ‰ for δ^2H. We used the evaporation-corrected plant water isotope values for the seasonal origin index calculation and the young and new water fraction calculations.

275   Precipitation $\delta^{18}O$ demonstrated a strong seasonal cycle (Fig. 3a), with heavier $\delta^{18}O$ values during dry seasons (May-September) and lighter $\delta^{18}O$ values during wet seasons (December-March). In contrast, lysimeter and stream waters (Fig. 3b, c) demonstrated minimal seasonal variability in $\delta^{18}O$. Tree branch xylem water $\delta^{18}O$ (Fig. 3d) followed the precipitation isotope seasonal cycle more closely than stream or lysimeter water, though it was still attenuated compared to precipitation. The estimated amplitude of the

280     $\delta^{18}O$ seasonal cycle was 4.2 ± 0.5 ‰ (fit ± 1 *SE*) for precipitation, 0.7 ± 0.3 ‰ and 0.3 ± 0.1 ‰ for lysimeter waters, 0.2 ± 0.2 ‰ for stream water and 1.6 ± 0.1 ‰ for branch xylem water.
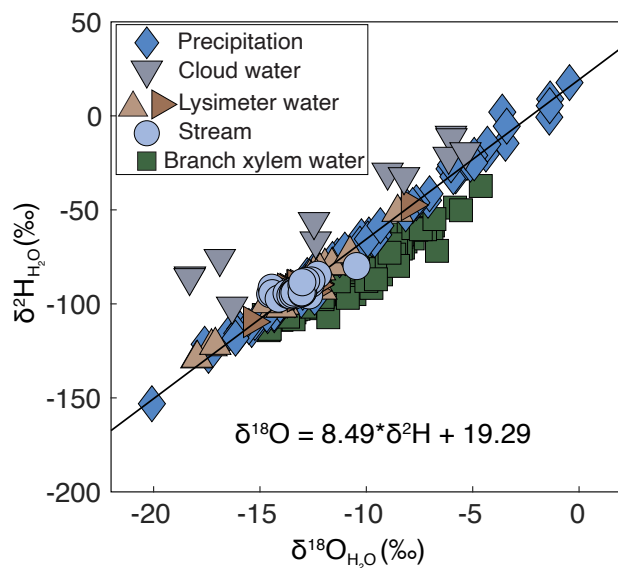
Figure 2. Precipitation, stream, soil, and plant water isotope ratios collected between 2016 and 2020 from a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains. Regression line and accompanying equation represents the local meteoric water line. Cloud water isotope ratios are from Clark et al. (2014).
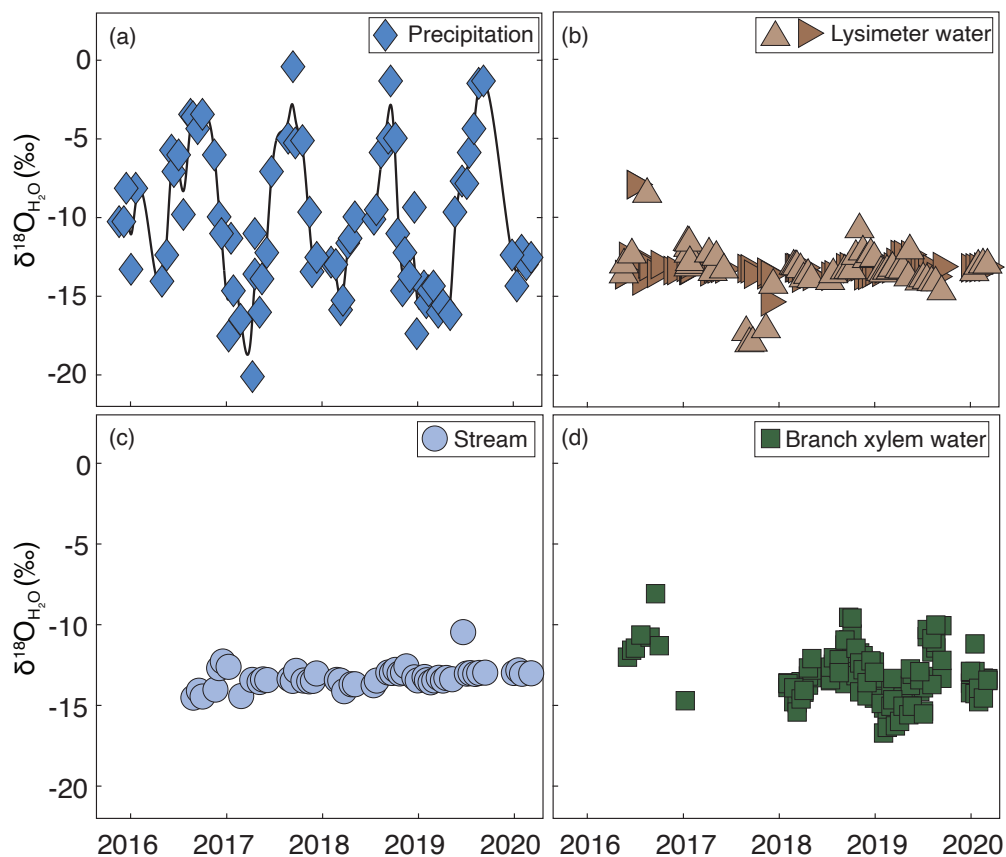
Figure 3. (a) δ¹⁸O of precipitation (solid line shows a smooth spline fit), (b) lysimeter waters, (c) stream water and (d) branch xylem water from a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains.

Seasonal origin indices

We calculated the intra-annual seasonal origin index (SOI) of lysimeter, stream and tree waters (Fig. 4). Lysimeter and stream water SOI values were consistently around zero or negative (Fig. 4 a, b). Neither lysimeter, nor stream, waters demonstrated intra-annual variation in SOI. Stream and lysimeter water SOI values both consistently indicate origin from wet season precipitation, even during the dry season (Fig. 4a, b). Branch xylem water SOI varied intra-annually, with low SOI during the wet season and wet-dry transition and high SOI during the dry season and dry-wet transition (Fig. 4c). To determine if different plant species use waters from different seasonal origins, we compared the SOI of different plant species collected on the same dates and found no evidence for systematic differences (Fig. S2).
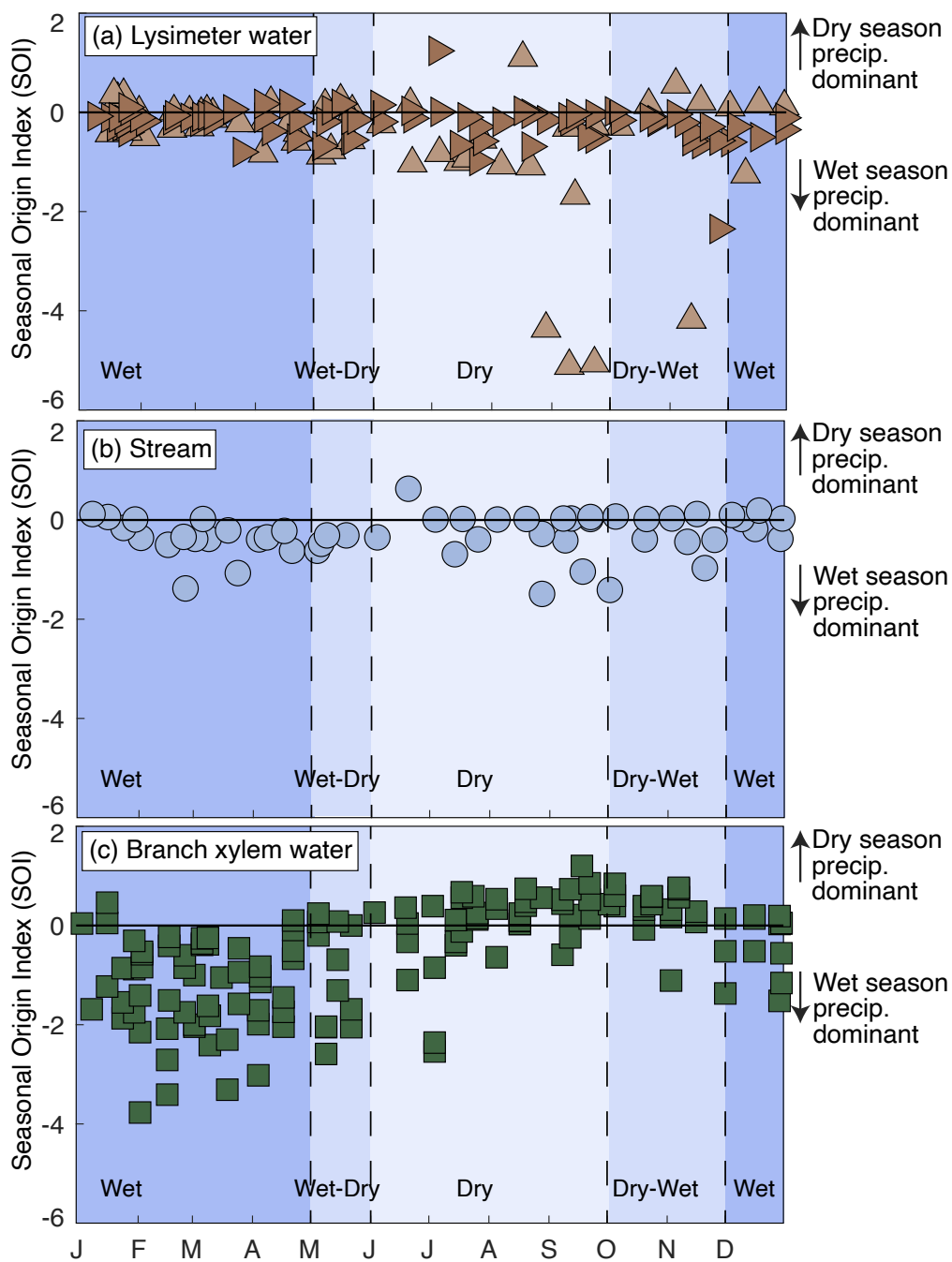
Figure 4. Intra-annual patterns of the seasonal origin of lysimeter, stream and branch xylem water from years 2016–2020 in a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains. Lysimeter water was collected from two lysimeters, one in the stream riparian area and one on the hillslope, distinguished by upwards-facing (hillslope) and sideways-facing

(riparian) triangles in different shades of brown. The x-axis shows month. Blue shading indicates the transitions between seasons.

Young water fractions

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Young water fractions (F_{yw}) were calculated for lysimeter, stream and branch xylem waters (Fig. 5). The lysimeter waters F_{yw}s and their Gaussian-propagated standard errors were 15 ± 43 % and 6 ± 47 %. The stream water F_{yw} was 5 ± 92 %. The high standard error of the stream water F_{yw} is a result of the very low amplitude of the seasonal cycle in $\delta^{18}O$. The branch xylem water F_{yw} was 37 ± 15 %. The non-

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overlapping and highly peaked distributions of F_{yw} for stream and branch xylem waters indicate that ages of water in the two fluxes are different from one another.
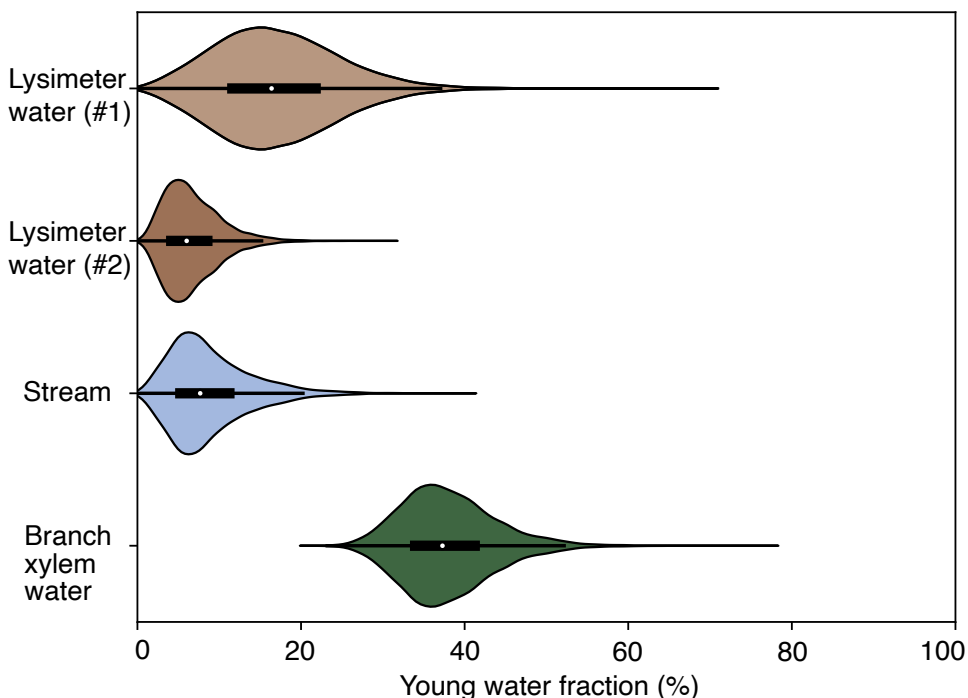
Figure 5. Violin and inset boxplots of the young water fractions (F_{yw}) for lysimeter, stream and

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branch xylem water, as determined from a bootstrap resampling routine. F_{yw} were calculated using water isotope data from years 2016–2020 in a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains.

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New water fractions

We calculated new water fractions (F_{new}) for stream and branch xylem water from the slope of the linear regression of data in Fig. 6. There was no evidence for a significant relationship between $\delta^{18}O_{p,j}-\delta^{18}O_{x,j-1}$ and $\delta^{18}O_{x,j}-\delta^{18}O_{x,j-1}$ for stream water ($f_{1,39}=0.7$, $p=0.4$), nor was there evidence for

335   differences between seasons ($f_{1,39}=1.1$, $p=0.3$), such that stream F_{new} was 0 %. This indicates that, on average, no measurable streamflow originated from precipitation that fell within the two weeks prior to sample collection. There was evidence for a significant relationship between $\delta^{18}O_{p,j}-\delta^{18}O_{x,j-1}$ and $\delta^{18}O_{x,j}-\delta^{18}O_{x,j-1}$ for branch xylem water ($f_{1,31}=8.1$, $p=0.008$); however, there was no evidence for differences between seasons ($f_{1,31}=0.2$, $p=0.3$). This indicates that, on average, 12 % of branch xylem water

340   originated from precipitation that fell within the two weeks prior to sample collection.
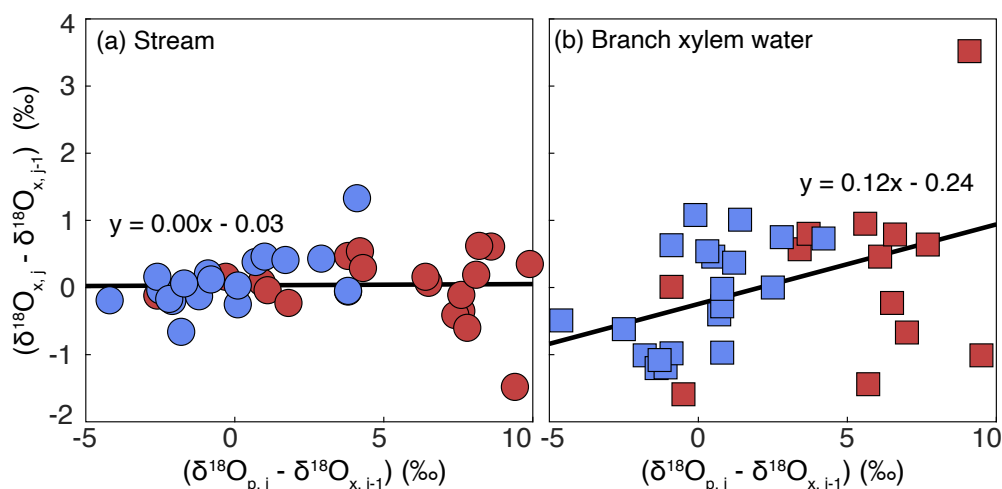
Figure 6. Ensemble hydrograph separation performed on (a) stream and (b) tree branch xylem water in a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains. The

345   **x-axis is the δ¹⁸O of precipitation at a given timestep minus the stream or xylem δ¹⁸O at the previous timestep. The y-axis is the stream or xylem δ¹⁸O at a given timestep minus the stream or xylem δ¹⁸O at the previous timestep. The slope of the regression line through the stream and branch xylem water datasets are equivalent to their respective new water fractions. Blue circles and squares indicate stream and branch xylem water samples collected during the wet season, while red circles and**

350   **squares indicate samples collected during the dry season. ANCOVA indicated no significant differences between the wet and dry seasons.**

Discussion

355   By developing a multi-year time series of precipitation, lysimeter, stream and branch xylem water isotopes, we were able to estimate the seasonal origins of water in key pools and fluxes of the hydrological cycle, as well as generate two different estimates of the proportion of water in these pools and fluxes originating from recent precipitation. Our results demonstrate that lysimeter and stream waters were predominantly comprised of wet season precipitation, even during dry seasons. However, branch xylem

360   waters demonstrated intra-annual variation in their seasonal origins: xylem waters were comprised of wet season precipitation during the wet season, and dry season precipitation during the dry season. In contrast with previous studies focusing on spatial variation in the seasonal origins of water in soil, streams and trees (Allen et al., 2019a; Goldsmith et al., 2022; Martin et al., 2018), our results make use of high frequency sampling to provide insights into temporal variation. Moreover, lysimeter and stream waters displayed low

365   young and new water fractions compared to branch xylem waters, indicating that plants take up water that is younger than is found in other pools and fluxes. Though theoretical predictions lead us to expect water stored within ecosystems to have different ages that water flowing out of ecosystems (e.g., Berghuijs & Allen, 2019), what we observe is the *opposite* of predictions. Here, water taken up by plants is *younger* than water sustaining streamflow, while in the framework of Berghuis & Allen (2019), water taken up by

370   plants is expected to be *older* than water sustaining streamflow. Coupling estimates of the seasonal origin index with the young and new water fractions can contribute to resolving the ages of waters transiting through terrestrial ecosystems.

1. Seasonal origins of water in trees and streams and implications for plant rooting depth

375   The seasonal origin index (SOI) is a measure of the season from which the water in a particular pool or flux originated (Allen et al. 2019). For example, values approaching -1.0 indicate that the sample is mostly comprised of wet-season precipitation. Lysimeter and stream water displayed little intra-annual variability in SOI and the values estimated were nearly always ≤ 0, indicating that these hydrologic compartments were consistently supplied over time by a source of water predominantly comprised of wet-

380   season precipitation.

Soil water lysimeters provide an incomplete picture of soil water isotope dynamics. We expect lysimeter water to be held at a higher (less negative) matric potential and therefore sample a pool of more mobile water than bulk soil water (Goldsmith et al., 2012). Yet, the lack of intra-annual variability in the lysimeter water SOI suggests that the lysimeters either do not sample pools of mobile water or simply do

385   not sample a significant amount of recent precipitation. The lack of recent precipitation signal in lysimeter waters could be due to recent precipitation rapidly mixing with large amounts of existing soil water that

dilutes the signal, significant soil water mixing at depth of sampling (1 m), or the fact that lysimeter waters were collected over a period of two weeks, leading to some temporal averaging in collection alone.

Moreover, there were differences in the SOI (and $\delta^{18}O$) of the two lysimeters based on their geomorphic position: the lysimeter on the hillslope (Fig 3; upwards facing triangles) had more variable SOI than the lysimeter in the riparian zone (Fig 3; sideways facing triangles). In January 2017, we sampled lysimeter and precipitation waters every four-hours and analyzed $\delta^{18}O$ (Fig. S1), which provided insight to differences between the two lysimeters. On short timescales, precipitation $\delta^{18}O$ was variable. The hillslope lysimeter displayed variable $\delta^{18}O$ in response to heterogeneous $\delta^{18}O_{precip}$, while the riparian lysimeter remained relatively constant over several days. This could potentially be due to extensive mixing of waters likely to occur along the flow paths to the riparian zone. Waters along hillslopes, however, are likely not as extensively mixed. Despite the somewhat more variable $\delta^{18}O$ in the hillslope lysimeter, it is worth nothing that on average, the hillslope and riparian lysimeter both still exhibit dampening of the intra-annual precipitation isotope signal.

Branch xylem water, in contrast, displayed marked intra-annual variability in SOI. During the wet seasons, the SOI of branch water was highly variable, but was consistently estimated as < 0. Over the course of the transition to the dry season, SOI became less variable and was consistently estimated as > 0. Thus, the results indicate that trees consistently took up precipitation originating from the same season. The contrasting intra-annual trends in SOI between lysimeter/stream and plant waters emphasize the differences in temporal origins of water that provision streams and trees.

The seasonally changing sources of plant waters observed in this wet environment is in contrast with observations from dry and temperate ecosystems, which is important for understanding the role of stored water in sustaining plants on a global scale. Previous studies of temperate ecosystems, largely carried out at a single time point, have observed that plants do not appear to use summer precipitation during seasonally-dry summers and instead rely on stored winter precipitation (Allen et al., 2019b; Berkelhammer et al., 2020; Martin et al., 2018). Moreover, other studies in dry settings demonstrate that plants use groundwater (Feakins & Sessions, 2010; McCormick et al., 2021; Rempe & Dietrich, 2018), with some positing that water taken up by trees in extremely dry environments may have been in aquifers for decades (Zhang et al., 2017). However, recent modeling results suggest that the use of stored water by plants is overestimated globally due to the overrepresentation of temperate regions in isotope-based ecohydrology studies (Miguez-Macho & Fan, 2021). Moreover, even within a seasonally dry temperate ecosystem, Goldsmith et al. (2022) observed that increasing site mean annual precipitation was significantly related to higher use of summer precipitation during summer (i.e., intra-annual changes in sources of water taken up by plants), a result generally consistent with the pattern we observe in this wet

tropical ecosystem. Taken together, these results emphasize the complex and somewhat non-intuitive influence of hydroclimate on sources of water taken up by plants: in wet environments, plant water sources change intra-annually, while in dry and temperate environments, plants rely on winter or wet season precipitation year-round.

Shallow root systems provide one possible explanation for the changing seasonal origins of plant water in wet environments. In the tropical montane forested watershed studied here, bedrock is well-draining and climate is seasonally wet: two factors that are known to lead to shallow roots (Fan et al., 2017) and facilitate the uptake of recent precipitation by plants. Additionally, given that precipitation outpaces actual evapotranspiration (Clark et al., 2014) even in the driest months (June-September), there is a consistent supply of recent precipitation, lessening the demand for plants to root deeper to access stored water. We infer that plants use a significant portion of water from shallow soils (e.g., < 50 cm), which would be consistent with observations of shallow tree rooting depths from wet tropical forests (Evaristo et al., 2016; Goldsmith et al., 2012; Jackson et al., 1995; Sohel et al., 2021).

2. Water ages in ecosystems

The ages of water taken up by plants are largely unknown (Evaristo et al., 2019; Sprenger et al., 2019), yet crucial for understanding how water moves through ecosystems. Previous studies have focused on determining the depth of water taken up by plants (Evaristo & McDonnell, 2017), potential differences in sources of water used by co-occurring plant species (Guo et al., 2018), or the environmental controls on plant water sources (Allen et al., 2019b). Streamflow in mountainous watersheds is often dominated by old water with damped intra-annual $\delta^{18}O$ cycles (Jasechko et al., 2016; Lutz et al., 2018; Muñoz-Villers & McDonnell, 2012), yet we have very few estimates of how the age of water taken up by plants compares to water in streams (Dai et al., 2022). An emphasis on temporal sampling of branch xylem waters allowed us to estimate that the young water fraction (F_{yw}; water that fell as precipitation within the prior $\sim$ 2 months) and new water fraction (F_{new}; water that fell as precipitation within the prior $\sim$ 2 weeks) of trees (F_{yw} = 37 %; F_{new} = 12 %) was greater than that of streams (F_{yw} = 5 %; F_{new} = 0 %; Fig. 5). This result is consistent with intra-annual patterns in branch xylem water SOI, which indicate that trees took up water that fell as precipitation within the same season (Figs 3 & 4). The low F_{yw} (6 and 15 %) of lysimeter water highlights the significant transit time for water to infiltrate to the 1 m collection depth. The low lysimeter water F_{yw} and relatively higher plant xylem water F_{yw} lends additional support to our theory that plants in this tropical montane cloud forest utilize shallow root networks. Overall, the significantly different F_{yw} in the pools and fluxes of water provide insights towards how water moves through ecosystems.

One possible explanation for the differing F_{yw} and F_{new} between stream and branch xylem water could be differing storage sizes for the reservoirs that sustain each flux. Transit times are equivalent to the

ratio of the storage volume to the flow rate through the storage: given equal flow rates, larger storage

reservoirs should lead to older water ages and vice versa (Botter et al., 2010). Previous work in this tropical

455   montane cloud forest watershed has pointed to permeable bedrock, which may be indicative of a large

groundwater storage reservoir, as one reason for low F_{yw} in stream water (Burt et al., 2022). Other studies

have observed old stream water ages in watersheds with permeable bedrock (Hale & McDonnell, 2016) and

found that large groundwater storage reservoirs provide a mechanistic explanation for the old water in

streams (Hale et al., 2016; Kirchner et al., 2023). Here, water taken up by plants from the shallow soil (as

460   discussed above) is contained within a smaller storage volume than groundwater, leading to a faster transit

time of water through the reservoir and consistent with the high F_{yw} and F_{new} observed in plant waters.

Additionally, we did not find significant differences in F_{new} for stream or branch xylem water

between wet and dry seasons (Fig. 6), suggesting that seasonal change in wetness (and the potential

influence on watershed storage) does not strongly influence the age of water as it does in some other

465   systems (Harman, 2015; Kuppel et al., 2020). The combination of plant and stream water ages has provided

useful information about how water moves through this tropical montane cloud forest ecosystem; future

studies including isotope-based estimates of stream and plant water ages would provide exciting avenues

for further understanding of ecosystem hydrology.

470   **3. The fate of precipitation in ecosystems**

Understanding how precipitation is partitioned between streams and plants over space and time

remains an open question in ecohydrology; however, combining results from different theoretical,

empirical, and modelling approaches can contribute to our more general understanding. More generally,

recent research has demonstrated that precipitation from different seasons is not always evenly partitioned

475   between streamflow and evapotranspiration (Kirchner & Allen, 2020b; Sprenger et al., 2022). Differences

in sources of water that sustain streamflow and evapotranspiration ("ecohydrologic separation") are thought

to be caused – at least in part – by heterogeneity of flow paths in watersheds: some water travels quickly

through the watershed (sustaining streamflow) while other water persists for longer (sustaining

transpiration) (Berghuijs & Allen, 2019). Studies that use empirical observations of water isotopes often

480   emphasize the importance of water originating from winter or wet season precipitation for sustaining plant

transpiration (Miguez-Macho & Fan, 2021). However, the same study by Miguez-Macho & Fan (2021),

when modeling the age of water taken up by plants, found that global annual ecosystems use only 30 %

past precipitation, while isotope-based approaches result in estimates of 50 ± 21 % ecosystem use of past

precipitation. In general, studies that use particle tracking models show that precipitation is partitioned

485   more rapidly to plants than streamflow (Kuppel et al., 2020; Maxwell et al., 2019; Rahimpour Asenjan &

Danesh-Yazdi, 2020; Wilusz et al., 2020). These modeling approaches are likely to be unable to

incorporate the complex reality of preferential flow paths in watersheds, which influence the age of water fluxes through the critical zone (Berghuijs & Allen, 2019; Sprenger & Allen, 2020). Including stable isotope tracer measurements (Sprenger, Llorens, et al., 2022) and accounting for preferential flow paths

490   (Knighton et al., 2019; Sprenger et al., 2018) in hydrologic models improve estimates of plant water ages, suggesting that particle tracking models which assume a well-mixed soil domain do not capture the complexity of hydrologic flow paths and plant-water relations. Though modeling studies can provide information at scales larger than any tracer-based study, they do not easily allow for preferential flow path mechanisms within watersheds. Given that modeling approaches cannot capture complex water flow

495   pathways, more water isotope data are needed to better understand the sources of water taken up by plants and the fate of precipitation in ecosystems.

The provisioning of precipitation to streams and plants is also mediated by hydroclimate. In a Mediterranean catchment, plants use precipitation that is older than streamflow, and the age of water taken up by plants varies intra-annually (Sprenger, Llorens, et al., 2022). Plants in North America take up older

500   water in dry climates and younger water in humid climates (Hahm et al., 2022). Other studies have shown that streamflow (von Freyberg et al., 2018) and plant waters (Allen et al., 2019b; Goldsmith et al., 2022) are comprised of more recent precipitation in wetter climates than drier climates. Our results also show the prevalence of young water in plants and suggests that both wet and dry season precipitation contribute to evapotranspiration, perhaps because precipitation is greater than actual evapotranspiration demands year-

505   round — even in the dry season (Clark et al., 2014). Though in wet environments plants will take up recent precipitation, we still lack a systematic understanding over range of hydroclimates of *how much* wet and dry season precipitation will contribute to each season's evapotranspiration and streamflow.

Conclusions

510   We measured water isotopes of precipitation, lysimeter, stream and branch xylem water in a tropical montane cloud forest to estimate the seasonal origins, as well as young and new water fractions, for the different pools and fluxes of water. Branch xylem demonstrated high intra-annual variability in the seasonal origins of source water for the two-year period during which plant samples were collected and comparatively high F_{yw} (37 %) and F_{new} (12 %). In comparison, stream water demonstrated no intra-annual

515   trend in the seasonal origin index and a low F_{yw} (5 %) and F_{new} (0 %). Taken together, the high F_{yw}, F_{new}, and changing seasonal origin of all three tree species studied here are indicative of transpiration supplied by water within shallow soils that is consistently replenished by recent precipitation, while estimates for streamflow are indicative of water that predominantly fell as wet season precipitation and originated from a large, seasonally buffered water reservoir. Interestingly, soil waters more closely mirrored results from

520 stream waters than plant waters. By simultaneously studying the seasonal origins and ages of water in plants, soils, and streams over time, our results provide an important comparison to past studies in drier climates that show how the amount and seasonality of precipitation interact with subsurface storage and plant rooting patterns to shape the flow of water through ecosystems.

525 Author Contributions

EB, RCdH, AJCQ and AJW designed and carried out the fieldwork. EB and GRG carried out the isotope analyses. EB wrote the code, analyzed the data and made the figures with input from GRG, AJW and RCdH. EB and GRG wrote the manuscript with input from all authors.

530 Competing Interests

The authors declare no competing interests.

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