Sept. 6th, 2023

Dear Dr. Coenders-Gerrits,

Thank you for your time and attention during the peer-review of our manuscript, "The seasonal origins and ages of water provisioning streams and trees in a tropical montane cloud forest." We have replied to reviewer #1's comments directly on the annotated PDF they submitted. Below are detailed replies to reviewer #2, with reviewer #2's comments in *italics* and our replies in **bold**. We thank both reviewers for their thoughtful and constructive suggestions.

During the revision process, we made several improvements to the manuscript and we believe that it is ready for publication. In particular, we 1) distinguished between the soil lysimeter waters collected in riparian versus hillslope zones (in figures and text) and 2) added the new water fraction calculations for soil lysimeter waters. We have improved the figures and revised the text.

We hope that the paper will make an important contribution to the field of ecohydrology by improving our understanding of how water moves through landscapes by exploring the application of multiple metrics in hydrology (young and new water fractions) to tree branch xylem waters.

Thank you again for your support.

Sincerely,

Emily I. Burt
Postdoctoral Fellow
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Orange, CA

The paper "The seasonal origins and ages of water provisioning streams and trees in a tropical montane cloud forest" by Burt et al. presents an assessment of indices of young and new water fractions of tree, soil and stream water as well as indices of the seasonal origin of these water pools in a tropical forest catchment in the Andes of Peru. Even though I find the paper generally well written, I consider that some parts of the methods and discussion sections could be further developed to support the findings of the study. Given the relevance of the paper for the isotope ecohydrology of the understudied Andean region, I consider it is a manuscript suitable for publication in HESS after some points described below are implemented in the manuscript.

General comments

Soil water isotopic data: In most figures the authors show soil water isotopic data without accounting for differences due to landscape position. However, the signals are different between those positions (valley vs hillslope). I suggest to consistently report the soil water isotopic composition results separately, as they show differences useful for interpretation. Please also note throughout the manuscript that soil water for isotopic analysis was collected at 1 m depth, as the variability in the depth and position across the landscape where soil water was collected can cause important differences in the observed isotopic signals. I also encourage the authors to check out papers in which this has been observed in other tropical montane catchments in the northern Andes (e.g., Mosquera et al., 2016; Mosquera et al; 2020; Lahuatte et al., 2022) to put their work in context and support some of their inferences yielded from the presented data.

Description of study site: The interpretation in the discussion section would benefit if the paper would include a more complete description of the study site (e.g., land use, the areal distribution, depth and properties of soils, and the vegetation characteristics such as root depth and leaf area index). Please expand the section with as much information as available, and use for interpretation.

We thank the reviewer for their comments. We are encouraged that they find the paper to be generally well-written and relevant for the field of isotope ecohydrology. We appreciate the reviewer's comments about the lysimeter locations and the importance of distinguishing between the hillslope and riparian lysimeters. Indeed, many of the comments from the other reviewer were also related to the lysimeter data. We revised all the figures in the paper to clearly illustrate the differences between the hillslope and riparian lysimeter. Additionally, we clarified the text relating to the lysimeters, to indicate whether the lysimeter data is from the hillslope or riparian zone.

Regarding the description of the study site, we agree with the reviewer's suggestion to include information about land use, areal distribution, depth and properties of soil, root depth and leaf area index. We added soil organic layer depth (Girardin et al., 2010; Wu et al., 2019), dominant plant genera (Rapp et al.,

2012), leaf area index (Girardin et al., 2014) and available information on plant root depth (Girardin et al., 2013).

Additionally, we added two of the references suggested by the reviewers from studies in tropical montane watershed in Ecuador to help contextualize the data from our study.

Specific comments:

L40-41: replace "twice monthly" in line 40 by "every two weeks" at the end of the sentence in line 41

We prefer the phrase "twice monthly" preceding "collections of precipitation, lysimeter and tree branch xylem waters," rather than describing sampling frequency at the end of the sentence. We believe the sampling frequency will be overlooked at the end of the sentence with the proposed change.

L44: It is important to highlight throughout the manuscript the depth at which soil water was collected (i.e., 1 m; L156), as the isotopic signal would likely be different if soil water would have been collected at different depths. Perhaps it would be even worth noting that soil water was collected below the root zone.

We agree that noting the soil water collection depth is important. We added clarification about the depth of soil water collection to lines 45 and 114. Though we do not have access to detailed measurements of root depth, Girardin et al. (2013) suggests that most roots are in the organic horizon, which is approximately 20–26 cm deep (Girardin et al., 2010; Wu et al., 2019).

We added the following sentence to the discussion to clarify on the tree root zone in this study:

"Though we do not have detailed measurements of rooting depth, Girardin et al. (2013) found that most roots were contained in the soil organic layer, which is approximately 20–26 cm deep (Girardin et al., 2010; Wu et al., 2019)."

L49: please add "in this study" after "2 years old", as that time step is specific for the presented monitoring setup and not general for the method applied.

We agree. This is updated.

L55: I think that the importance of the study lies on the fact that it was carried out in a humid environment, as opposed to drier environments where this type of research has been carried out before. Consider specifying this "HUMID tropical montane cloud forest".

Updated.

L122: is the reported elevation the mean catchment elevation? Please specify.

The reported elevation was the elevation of the sampling location. We updated the text to the mean elevation of the watershed.

L145: please discuss whether the spatial variability in the selection of trees at different distance from the streams could have influenced (or not) in the interpretation of the presented tree water isotopic data.

Undoubtedly there is spatial variation in the tree water isotope composition within the watershed. However, we do not believe there is sufficient evidence within the literature to hypothesize on systematic trends in plant water isotope composition with distance from the stream and this is not central to the questions that we are addressing.

L164-167: the reader would really appreciate a plot of the rainfall amount and stream water level data measured at the study site to put the finding in context in relation to the site hydrometeorological conditions. I strongly suggest the authors to include a plot of these data in the manuscript for facilitate interpretation.

We added rainfall amount and stream discharge to Figure 1.

L164 and L166: report the brand, model and accuracy of the tipping bucket used to measure rainfall amount, and the accuracy of the type and accuracy of the logger used to measure water level.

For the rain gauge, we added the following to line 129: (Tipping bucket raingauge model TB3; Hydrological Services PTY LTD; Liverpool, NSW, Australia; accuracy ± 2 %),

And for the water level logger, we added the following to line 171: accuracy ± 0.2 %

L184-187: Since the samples were analyzed in different laboratories, were some replicate samples analyzed using both instruments to correct for potential analytical discrepancies? If yes, please explain; if not, please discuss the implication in the manuscript.

This concern was raised by Reviewer #2, and we replied as follows:

All three labs use internationally accepted standards that are governed by IAEA (V-SMOW). We carried out post-processing of samples using appropriate calibration, drift and memory correction procedures.

While it would have been ideal to process all samples on the same instrumentation, this was not practical especially given the multi-year duration of

this research project (for example, during that time some of the instruments were decommissioned). Many current studies mix precipitation samples processed by CRDS with xylem water samples processed by IRMS. Moreover, there have been dozens of meta-analyses in isotope ecohydrology that have taken no precautions to account for inter-lab variability. Given the congruence of the data, we have no evidence for there to be systematic errors.

Additionally, each lab utilized rigorous quality control measures:

Chapman Picarro – 4 standards and 5 measurements of an independent QC sample

LBL LGR – 6 measurements of an independent QC sample

Caltech LGR – used sample-standard bracketing and re-ran 3 certified standards every 5 samples.

L188-190: was potential contamination in water samples assessed (e.g., using the ChemCorrect software)? If yes, please explain how this was accounted for and report the number of reanalyzed or discarded samples; if not please discuss the potential implication of contamination if the reported isotopic signals, particularly for tree water data.

Tree water samples were analyzed via IRMS, which precludes the organic compound interference that can occur when analyzing branch xylem waters via isotope ratio infrared spectroscopy.

Stream and soil lysimeter waters were filtered to 0.2μm when collected per standard practice in the literature.

L200: I think the correction was only carried out for xylem water samples? Please update if needed.

We updated line 204 to provide clarity that only the xylem water isotope values were corrected for the SOI and young water fraction calculations.

L210-211 values >+1 and <-1 are reported in Fig. 4. Please describe what they mean.

We added the following text to explain the SOI values outside of the +1 to -1 range:

"Given the high variation in precipitation isotopes, individual xylem water samples responding to a given precipitation event may fall outside of the average seasonal precipitation endmembers, resulting in SOI greater than +1.0 or less than -1.0."

L237: I believe it should be Eqts. 4-6 instead of 4-7. Please update if needed.

Updated.

L264: not sure what "cloud waters" means (e.g., fog, drizzle, or the combination of both). Please specify. Also, isn't the isotopic composition of cloud water actually enriched as it plots above the LMWL? Clarify if needed.

"Cloud waters" refers to cloud water vapor, sampled via a line pumped through a cold trap (from Clark et al., 2014 HESS).

We revised the text to state that cloud water is enriched in deuterium relative to the LMWL.

L270: I suppose the corrected data is shown in the paper figures. If so, please specify in the caption and/or legend of the figures. I would also be useful for the reader to see how the correction changed the measured isotopic composition in supplementary material for reference.

The data in Figure 2 are the uncorrected tree branch xylem water isotope ratios. The rest of the tree branch xylem water isotope displayed in Figure 3 onwards are corrected for evaporation. We added supplementary figure S2, which shows how the data is influenced by the evaporation correction.

L306: there is no fig S2 in the manuscript of supplementary material. Please revise and correct as needed.

We revised the supplement: there are now Figures S1–S5, all properly referenced in the text.

L317: specify the geomorphic position where samples were collected.

Throughout the manuscript, we now carefully distinguish between riparian and hillslope lysimeters.

L387-388: I am not convinced that sampling frequency could cause the observed attenuation in soil water isotopic data. I think this relates to the soil water being sampled at 1 m depth only. I think that the papers suggested in the general comment on this subject above could help to better interpret and support the presented findings.

We added a reference to Lahuatte et al. (2022) in this sentence of the manuscript to highlight the similar attention of soil water isotope signals at 1 m depth. However, as also pointed out by Lahuatte et al., there can be a rather diverse range of hydrologic behaviors that occur because of changes in soil and bedrock. For this reason, we have concerns about drawing closer comparisons between the observations from the study site in this paper and the observations from the

study sites in the four papers recommended by the reviewer. Our study site is heavily forested and underlain by a weathered shale bedrock with poorly classified soils. The study sites in the papers recommended by the reviewer have volcanic bedrock, some contain wetlands/paramo, and some are not forested — complicating comparison to data from our study site.

L396-397: I am not entirely convinced of this potential explanation of the differences in the isotopic composition of soil water. I think this could be related to differences in the water storage capacity of soils at different geomorphic positions across the landscape as previously observed in other montane Andean catchments (e.g., Lazo et al., 2019). This is why reporting the properties, areal extent and depth of soils across the catchment is important for interpretation.

We added a citation to Lazo et al. (2019) in this section of the paper.

L425: what about ecophysiological processes (e.g., the possibility of foliar water uptake due to the influence of fog/cloud water at the study area)? Please expand the discussion considering this point.

The primary effect of clouds and leaf wetting is the suppression of transpiration, which would slow plant water turnover time and thus the uptake of water with a distinct isotopic signal (Gotsch et al., 2014). Foliar water uptake may refill leaves and very small branches at times when there is a significant plant water deficit, but is unlikely to consistently affect water isotope ratios (Goldsmith et al. 2019). While there is evidence that common tree species at this site have the capacity for foliar water uptake (Goldsmith et al., 2017), there is no strong evidence that would suggest sufficient and regular foliar water uptake; precipitation exceeds AET, which means leaf water deficits are minimal.

L512: replace "high" by "strong"

Done.

L519: this is likely because the lysimeters were placed below the root zone. This is why I strongly suggest that the sampling depth for soil water must be reported consistently throughout the manuscript.

We have revised the text to now report the sampling depth consistently throughout the manuscript.

Fig 2: Classify soil water isotopic composition into different locations across the landscape. E.g., the text in the caption of Fig 4 clearly indicates this, and I think it would be valuable to specify this in all figures where this is not clear.

We thank the reviewer for this suggestion. Figures and text distinguish between hillslope and riparian lysimeters.

Fig 3. Same as in Fig. 3

In Figure 3, we now distinguish the data from the hillslope and riparian lysimeters.

SUGGESTED REFERENCES TO CONSIDER

Lahuatte, B., Mosquera, G.M., Páez-Bimos, S., Calispa, M., Vanacker, V., Zapata-Ríos, X., Muñoz, T., Crespo, P., 2022. Delineation of water flow paths in a tropical Andean headwater catchment with deep soils and permeable bedrock. Hydrol. Process. 36, e14725. https://doi.org/10.1002/HYP.14725.

Lazo, P.X., Mosquera, G.M., McDonnell, J.J., Crespo, P., 2019. The role of vegetation, soils, and precipitation on water storage and hydrological services in Andean Páramo catchments. J. Hydrol. 572, 805–819. https://doi.org/10.1016/J.JHYDROL.2019.03.050.

Mosquera, G., Crespo, P., Breuer, L., Feyen, J., Windhorst, D., 2020a. Water transport and tracer mixing in volcanic ash soils at a tropical hillslope: a wet layered sloping sponge. Hydrol. Process. 34, 2032–2047. https://doi.org/10.1002/hyp.13733.

Mosquera, G.M., Célleri, R., Lazo, P.X., Vaché, K.B., Perakis, S.S., Crespo, P., 2016b. Combined use of isotopic and hydrometric data to conceptualize ecohydrological processes in a high-elevation tropical ecosystem. Hydrol. Process. https://doi.org/10.1002/hyp.10927.

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 ($\delta^{18}O$ 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

70   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;

75   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

80   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^{2}H) 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

85   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

90   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

95   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 $\sim 11 \pm 1$ °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.
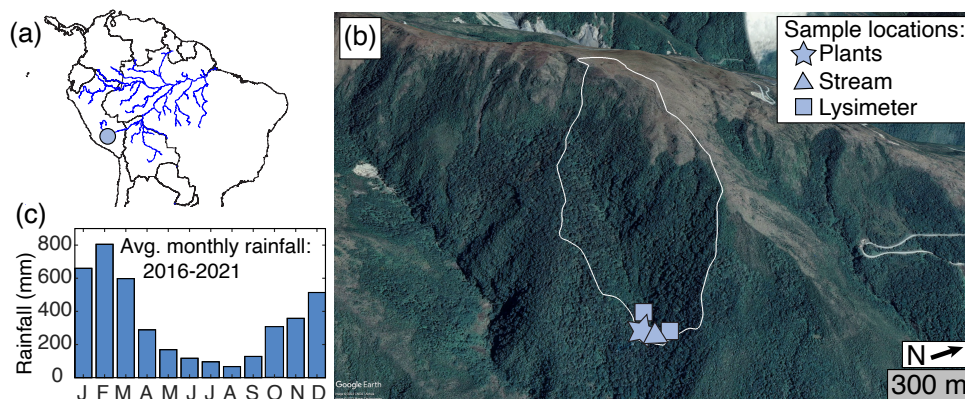
Figure 1. (a) Map of South America; the blue circle represents the approximate location of the tropical montane cloud forested watershed studied in this paper. (b) Google Earth imagery of the study watershed, outlined in white. Plant sampling location is represented by a blue star, stream sampling location 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

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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 $\delta^{18}O$ 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 $\delta^{18}O$ and 1.1 ‰ for δ^2H.

Lysimeter, stream and precipitation waters were analyzed for $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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.

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 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):

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

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

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

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

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 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

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were then used to calculate the $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ dataset with 142 observations, we sampled 142 times, each time from the full dataset of tree water $\delta^{18}O$ values). We repeated this 10,000 times each for the precipitation, stream, and tree water $\delta^{18}O$ 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 $\delta^{18}O$ 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:

$$\delta^{18}O_{p,j} - \delta^{18}O_{x,j-1} \tag{7}$$

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:

$$\delta^{18}O_{x,j} - \delta^{18}O_{x,j-1} \tag{8}$$

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.

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).

## 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. 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 -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.

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 $\delta^{18}O$ 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.
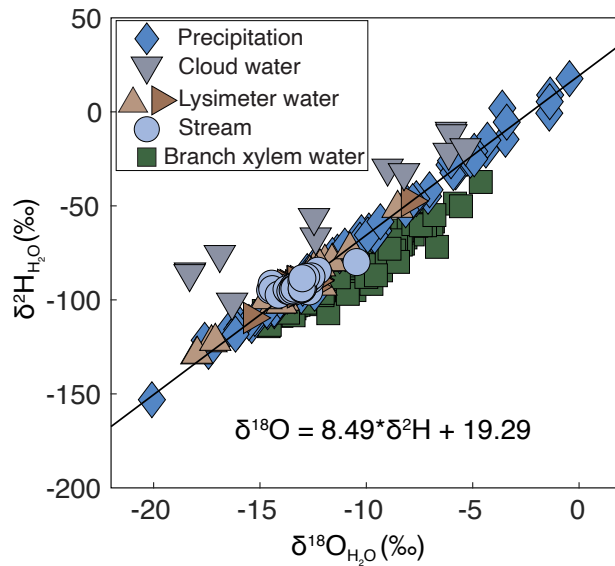
Figure 2. Precipitation, stream, soil, and plant water isotope ratios collected between 2016 and 2020 from a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains. Regression line and accompanying equation represents the local meteoric water line. Cloud water isotope ratios are from Clark et al. (2014).
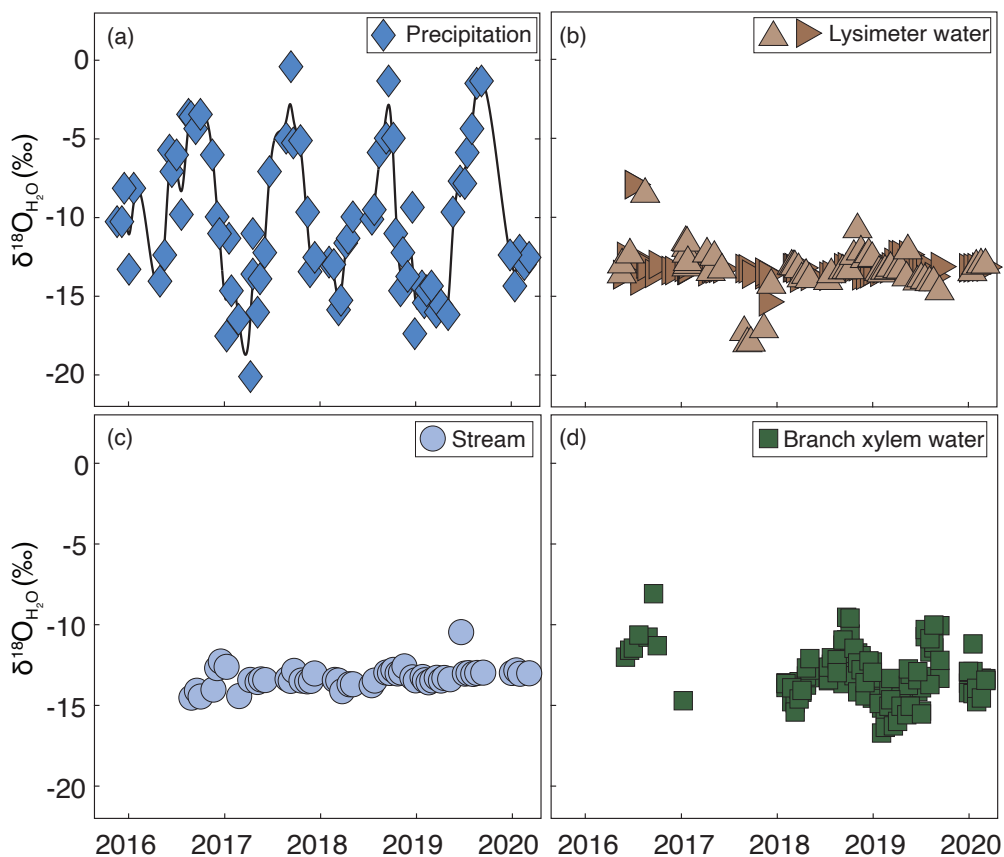
Figure 3. (a) δ¹⁸O of precipitation (solid line shows a smooth spline fit), (b) lysimeter waters, (c) stream water and (d) branch xylem water from a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains.
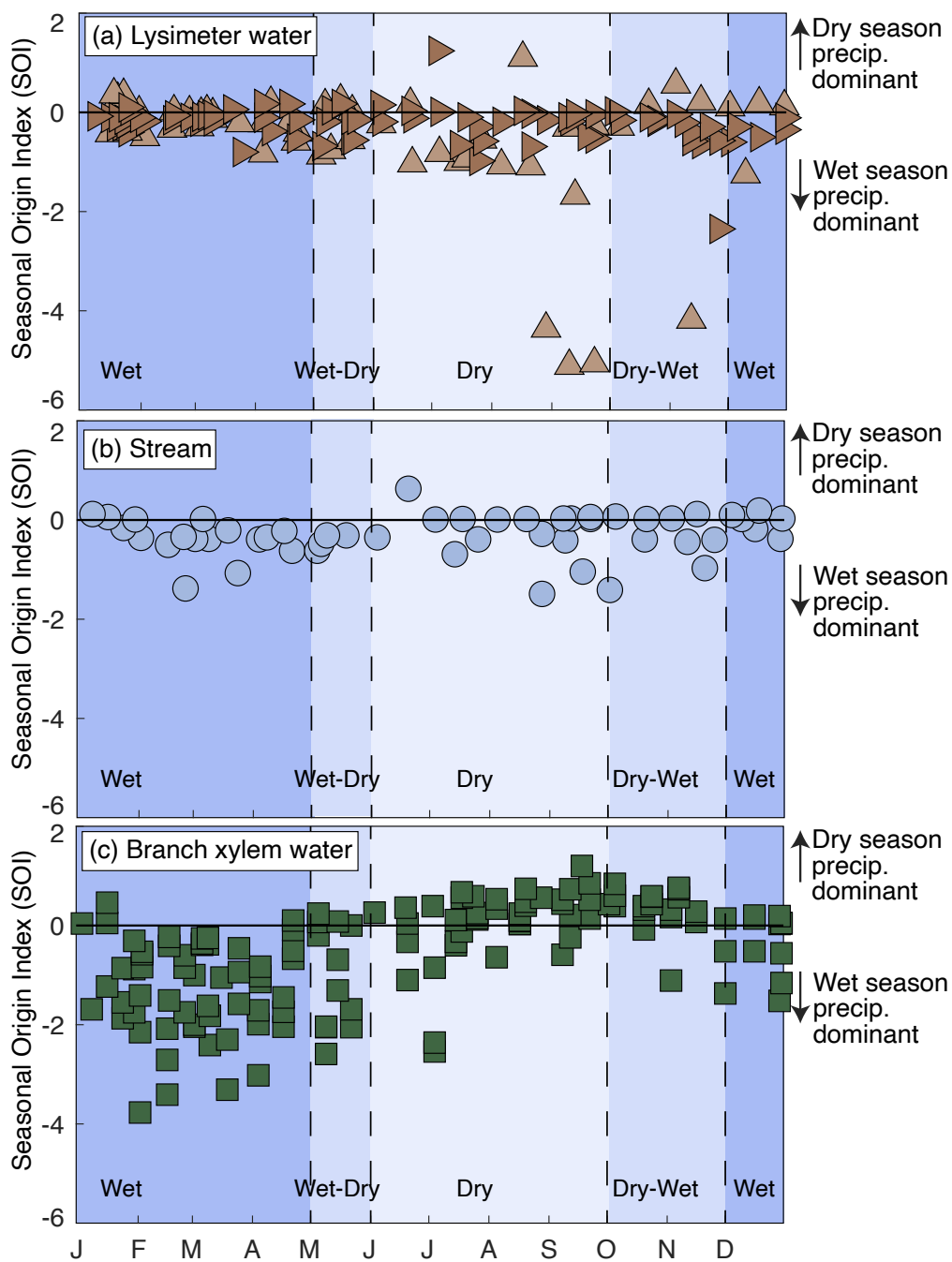
Seasonal origin indices

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).

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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

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 δ^{18}O. The branch xylem water F_{yw} was 37 ± 15 %. The non-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.
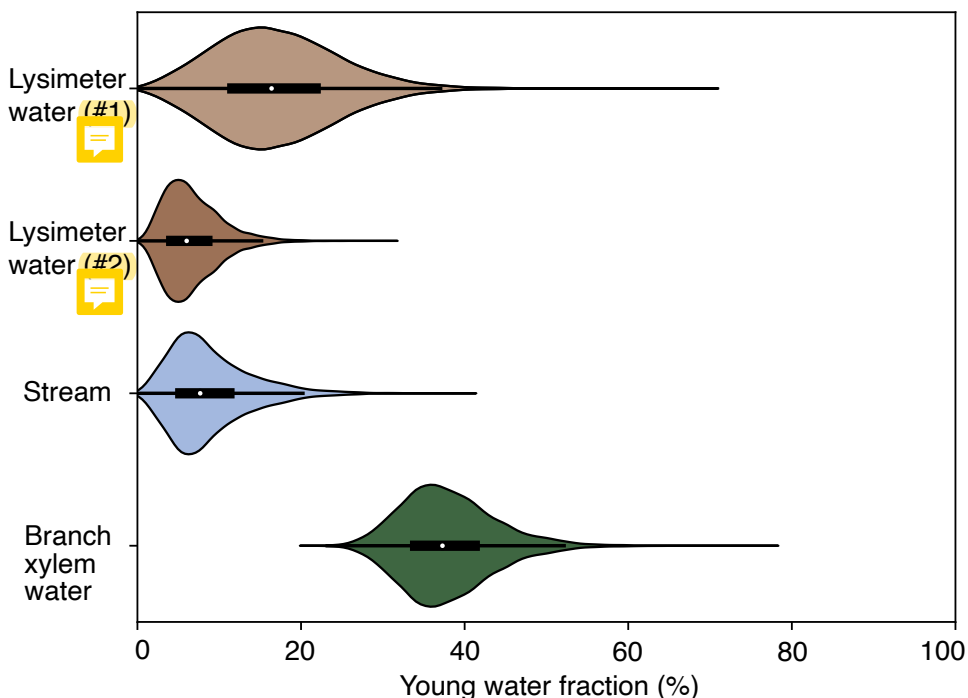

Figure 5. Violin and inset boxplots of the young water fractions (F_{yw}) for lysimeter, stream and branch xylem water, as determined from a bootstrap resampling routine. F_{yw} were calculated using water isotope data from years 2016–2020 in a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains.

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.
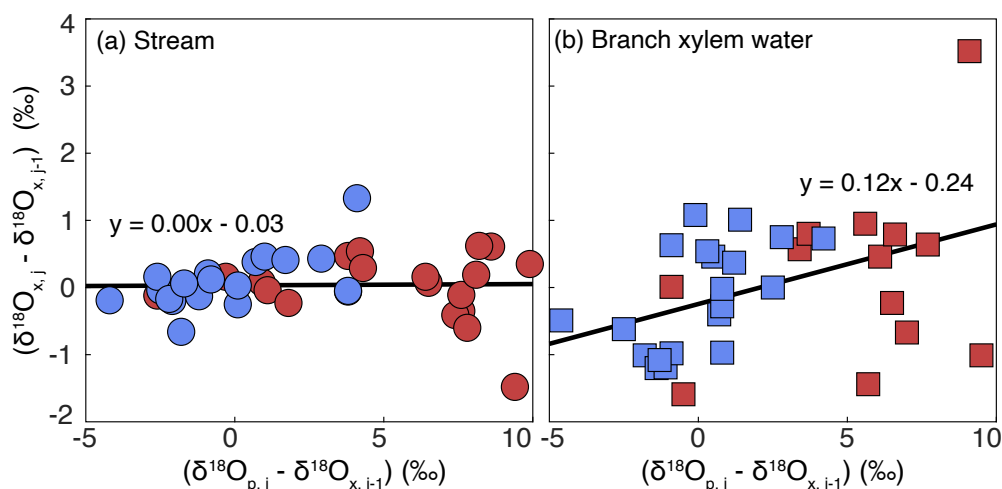
Figure 6. Ensemble hydrograph separation performed on (a) stream and (b) tree branch xylem water in a tropical montane cloud forested watershed in the southeastern Peruvian Andes mountains. The

345   **x-axis is the $\delta^{18}O$ of precipitation at a given timestep minus the stream or xylem $\delta^{18}O$ at the previous timestep. The y-axis is the stream or xylem $\delta^{18}O$ at a given timestep minus the stream or xylem $\delta^{18}O$ 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 +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

...lutes 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

390   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

395   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.

400   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

405   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

410   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

415   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

420   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

425   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

430   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.,

435   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 &

440   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 ~ 2 months) and new water fraction (F_{new}; water that fell as precipitation within the prior ~ 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

445   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

450   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

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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.

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.

Competing Interests

The authors declare no competing interests.

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