Received: 13 June 2022 | Revised: 27 February 2023 | Accepted: 2 March 2023

DOI: 10.1002/eco.2541

RESEARCH ARTICLE

WILEY

Dry season plant water sourcing in contrasting tropical ecosystems of Costa Rica

Ricardo Sánchez-Murillo[1] | **Diego Todini-Zicavo**[2,3] | **María Poca**[4] | **Christian Birkel**[5,6] | **Germain Esquivel-Hernández**[7] | **María Marta Chavarría**[8] | **Giulia Zuecco**[3] | **Daniele Penna**[9]

[1]Department of Earth and Environmental Sciences, University of Texas at Arlington, 500 Yates Street, Arlington, Texas, 76019, USA

[2]University School for Advanced Studies (IUSS), Pavia, Italy

[3]Department of Land, Environment, Agriculture and Forestry, University of Padova, Legnaro, Italy

[4]Grupo de Estudios Ambientales, Instituto de Matemática Aplicada San Luis, Universidad Nacional de San Luis, CONICET, San Luis, Argentina

[5]Water and Global Change Observatory, Department of Geography, University of Costa Rica, San José, Costa Rica

[6]Northern Rivers Institute, University of Aberdeen, Aberdeen, UK

[7]Stable Isotopes Research Group and Water Resources Management Laboratory, Chemistry School, Universidad Nacional, Heredia, Costa Rica

[8]Programa de Investigación, Área de Conservación Guanacaste (ACG), Guanacaste, Costa Rica

[9]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Florence, Italy

Correspondence

Ricardo Sánchez-Murillo, Department of Earth and Environmental Sciences, University of Texas at Arlington, 500 Yates Street, Arlington, Arlington, TX 76019, USA.
Email: ricardo.sanchezmurrillo@uta.edu

Funding information

International Atomic Energy Agency, Grant/Award Numbers: RLA7024, CRP 22904, RC-19747, COS7005; Research Office of the Universidad Nacional, Grant/Award Numbers: SIA-414-17, SIA-411-17, SIA-0236-16, SIA-0101-14, SIA-0482-13; Leverhulme Trust; University of Florence

Abstract

Tracer-aided studies to understand plant water uptake sources and dynamics in tropical ecosystems are limited. Here, we report the analysis of dry season source water uptake patterns of five unique ecosystems of Costa Rica across altitudinal (<150–3,400 m asl) and latitudinal (Caribbean and Pacific slopes) gradients: evergreen and seasonal rainforests, cloud forest, Páramo and dry forest. Soil and plant samples were collected during the dry season in 2021. Plant and soil water extractions were conducted using centrifugation. Stem water extracted volume and stem total water content were calculated via gravimetric analysis. Water source contributions were estimated using a Bayesian mixing model. Isotope ratios in soil and stems exhibited a strong meteoric origin. Enrichment trends were only detected in stems and cactus samples within the dry forest ecosystem. Soil profiles revealed nearly uniform isotopic profiles; however, a depletion trend was observed in the Páramo ecosystem below 25 cm. More enriched compositions were reported in cactus samples for extracted water volumes above ~20% (*adj.* $r^2 = 0.34$, $p < 0.01$). The most prominent dry season water source in the evergreen rainforest (74.0%), seasonal rainforest (86.4%) and cloud forest (66.0%) corresponded to well-mixed soil water. In the Páramo ecosystem, recent rainfall produced by trade wind incursions resulted in the most significant water source (61.9%), whereas in the dry forest, mean annual precipitation (38.6%) and baseflow (33.1%) were the dominant sources. The latter highlights the prevalence of distinct water uptake sources between recent cold front rainfall (near-surface soil storage) to more well-mixed soil moisture during the dry season, revealing ecohydrological processing previously unknown in this tropical region.

KEYWORDS

centrifugation, ecohydrology, plant water sources, stable isotopes, tropics, water extraction, water source uptake

https://doi.org/10.1002/eco.2541

SIGNIFICANCE STATEMENT

Tropical ecosystems are navigating from prolonged droughts to extreme rainfall events. However, the ecohydrological functioning and water vapour feedback across these dynamic and fragile environments are commonly understudied. This study provides new insights into our knowledge of water uptake in unique low and high elevation tropical ecosystems under distinct rainfall regimes. The water source contribution analysis denoted the prevalence of water uptake pools between cold front rainfall to more well-mixed soil moisture during the dry season. In contrast to studies across temperate ecosystems, our results showed nearly uniform soil isotope profiles with depth, with negligible fractionation during the dry season.

1 | INTRODUCTION

Plants are essential regulators of the water cycle, as they control which water pools are being returned to the atmosphere and contribute to building the critical zone (Brantley et al., 2017; Dawson et al., 2020; Fan et al., 2017). Plant rooting depth and environmental conditions determine water sources for vegetation along time and across space. As such, plants may use or switch sources of root water uptake from the unsaturated or saturated zone or capillary fringe (Amin et al., 2020; Barbeta & Peñuelas, 2017; Evaristo & McDonnell, 2017; Jobágy et al., 2021) or even rock moisture (Hahm et al., 2020; Miguez-Macho & Fan, 2021). Thus, source water uptake studies are critical for understanding plant-mediated global changes and their impacts on the hydrological cycle.

Stable isotopes of hydrogen and oxygen have been used for tracing water in ecohydrological and ecophysiological studies in the past decades (Ehleringer & Dawson, 1992; Gat, 1996). The use of stable isotopes for such studies relies on the fact that isotope ratios of $^{18}O/^{16}O$ and $^{2}H/^{1}H$ are sensitive to phase changes, such as evaporation and condensation. Therefore, they provide valuable information on sources or processes to which they have been exposed (Kendall & McDonnell, 2012). In other words, isotope ratios are used as fingerprints of the water molecule to investigate water origin, mixing and age through catchments and the soil–plant–atmosphere continuum (SPAC) (Sprenger et al., 2016, 2019). Different plant functional types (e.g., trees, perennial herbaceous and succulents) and species present different rooting systems for accessing specific water pools (Ehleringer & Dawson, 1992). Transpired water by plants exhibits an isotopic enrichment as isotopic fractionation occurs in the leaves (Dawson & Ehleringer, 1993; Yakir & Sternberg, 2000). Thus, the isotopic composition of water from leaves differs from that absorbed through root water uptake (e.g., Bodé et al., 2020). Therefore, studies focusing on source water uptake are typically based on stem water sampling representing root water sources.

For over a decade, many studies have reported an offset between stem water and potential plant water sources in forest trees (e.g., Brooks et al., 2010; Evaristo et al., 2015; Hervé-Fernández et al., 2016) as well as agriculture trees (Penna et al., 2021). Various authors argued that this is expected considering soil water heterogeneity in space and time, which can influence water isotopic composition found in plant stems in a given moment (Barbeta et al., 2020; Goldsmith et al., 2019; Sprenger & Allen, 2020). However, others showed that isotopic fractionation may occur during root water uptake for xerophytic and halophytic plants (e.g., Ellsworth & Williams, 2007; Lin & da Sternberg, 1993; Zhao et al., 2016), as well as in plants associated with arbuscular mycorrhizas (Poca et al., 2019). Recent studies have directed attention toward stem water heterogeneity (Barbeta et al., 2020, 2022; Knighton et al., 2020). Stems may present a heterogeneous pool of water due to the storage of different spatiotemporal soil water sources (Goldsmith et al., 2019; Knighton et al., 2020) but also due to non-xylem water in the stem co-extracted with water in the xylem lumen (Barbeta et al., 2022; Bowers & Williams, 2022; Zhao et al., 2016). In this sense, methodological issues related to how and which pools of water are extracted from soils and plants are under scrutiny (Allen & Kirchner, 2022; Barbeta et al., 2022; Zuecco et al., 2022). Two fundamental questions are currently under debate: (1) What type of water is being extracted, chemically bound (immobile) or mobile water participating in the evapotranspiration flux? And (2) to what extent the ratio of immobile/mobile water extracted is affecting the relative sample position in the dual-isotope space, and consequently introducing errors in further calculations and modelling of plant water uptake sources and depths?

Most studies on source water uptake have concentrated in temperate regions (e.g., Allen et al., 2019; Barbeta et al., 2019, 2020, 2022; Brooks et al., 2010; Geris et al., 2015; Goldsmith et al., 2019; Hervé-Fernández et al., 2016; Knighton et al., 2020; Zhao et al., 2016), while the tropics have received lesser attention (Goldsmith et al., 2012; Evaristo et al., 2016; de Wispelaere et al., 2017; Jiménez-Rodríguez et al., 2020; Amin et al., 2020; Kühnhammer et al., 2022). From a diversity and species richness perspective, tropical areas pose a challenging scenario (Myers et al., 2000). For example, five centres reach species richness of more than 5,000 species/10,000 km^2 (e.g., Costa Rica-Chocó, Atlantic Brazil, Tropical Eastern Andes, Northern Borneo and New Guinea; Rafiqpoor et al., 2005), which in turn complicates tracking source water due to the lack of few dominant species (as in temperate forests), and possibly increase the spectrum of water uptake strategies in a particular ecosystem. Tropical environments are also challenging for numerous other reasons, such as limited accessibility to field sites due to extreme weather conditions and abrupt land use changes hampering the selection of representative measuring sites (Aparecido et al., 2018; Wright et al., 2018). As a positive remark, tropical environments present strong orographic and rainfall effects on rainfall isotopic composition, moisture recycling and a high evapotranspiration demand, which altogether strengthens isotopic ratio differences among potential plant water sources (Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020).

Costa Rica and, in general, the Central America Isthmus have been navigating from prolonged droughts to above-normal tropical cyclone seasons during the last decade (Sánchez-Murillo, Durán-Quesada, et al., 2019). In this regard, most research efforts have been geared toward understanding rainfall generation and water availability for anthropogenic activities (Caballero et al., 2012; Hidalgo et al., 2013; Hund et al., 2018; Lyon et al., 2022). However, information about how plants and, more generally, how contrasting tropical ecosystems respond or adapt to water shortages or surplus is still lacking. A study in a wet lowland forest during a dry season showed a lack of evaporation signal on soil water, which coincided with the Local Meteoric Water Line (LMWL) (Jiménez-Rodríguez et al., 2020). The authors further evidenced the compartmentalization of plant water use by different plant functional types. A preliminary study in Costa Rica's rainforest region extracted water from soil samples that coincided with rainfall isotope seasonality and groundwater and evidenced minimal enrichment (Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020). In a very recent study, Kühnhammer et al. (2022) applied continuous in situ vapour measurements of stable isotopes of hydrogen and oxygen in soils and plants of a dry forest in northwestern Costa Rica. They compared this method with destructive sampling followed by cryogenic vacuum extractions, the most common extraction method for obtaining water in soils and stems. They found a general agreement between both methods. Still, there is a gap of knowledge in applying tracer-aided approaches to understanding source water uptake across Costa Rica's diverse environments.

A substantial advantage of Costa Rica is the availability of a 7-year continuous integrated isotopic and hydro-meteorological monitoring database across the country (Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020). With this strong background in meteoric waters, we aimed to move forward in describing dry season source water uptake in the five main ecosystems throughout Costa Rica across altitudinal and latitudinal gradients: evergreen and seasonal rainforests, cloud forest, Páramo and dry forest (Figure 1). These ecosystems are representative of three main life zones: dry forest lowlands, middle-elevation forests and the highland forests. The main objective of this study was to explore underlying factors driving the differences and similarities among vegetation water source uptake in contrasting tropical ecosystems. The driest stage toward the end of the dry season was selected as representative of the recurrent critical water availability conditions that the Central American region has been experiencing in the last decade. For this purpose, we

1. applied a centrifuge technique to extract soil and stem mobile water at the end of the dry season;
2. analysed the isotopic composition of the extracted soil and stem water together with precipitation, surface water and groundwater of each ecosystem to explore water uptake throughout the climatic and geomorphic gradients of Costa Rica;
3. quantified water source contributions to plant water uptake through Bayesian mixing model analysis. Due to the large diversity of species, the stem isotopic mixture from the most dominant plants was considered representative of the overall ecosystem functioning.

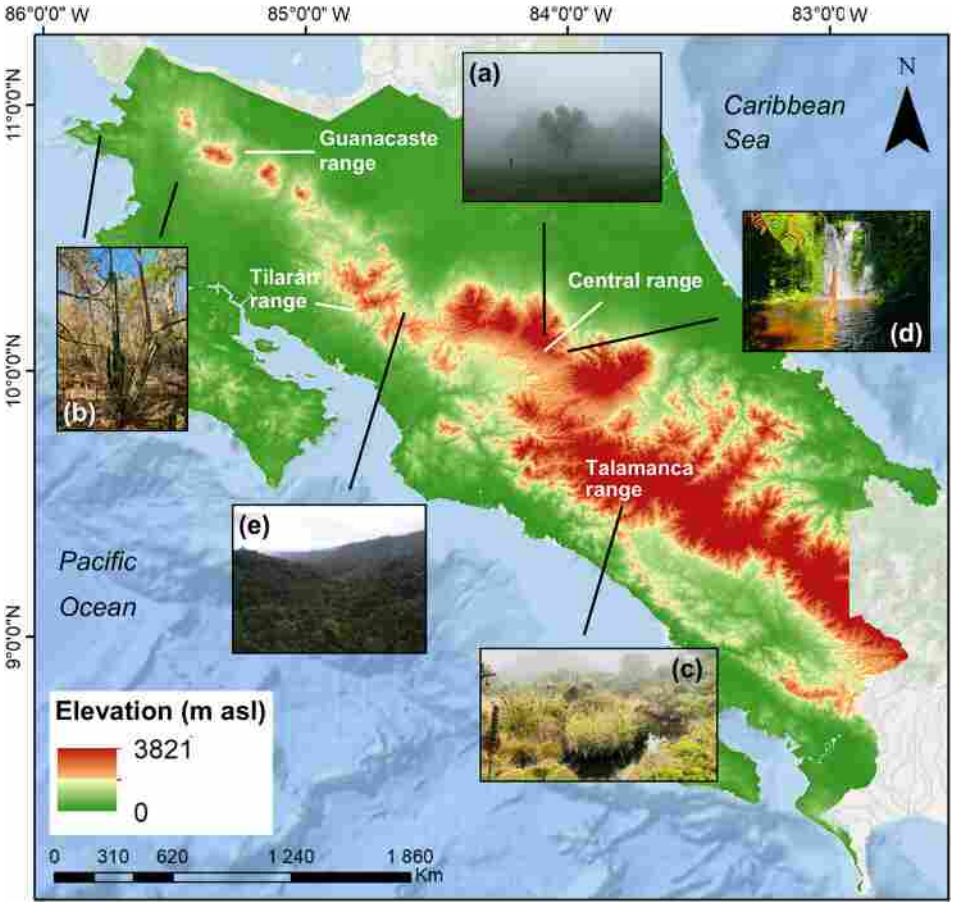

FIGURE 1 Map of the study area, including sampling locations in five unique tropical ecosystems across Costa Rica: (a) cloud forest, (b) dry forest, (c) Páramo, (d) evergreen rainforest and (e) seasonal rainforest. Sampling elevation ranged from sea level (b) to $\sim$3,400 m asl (c). The photographs denote the typical landscape during the dry season.

4. Although cacti are not the most abundant plant in the dry tropical forest of Costa Rica, they constitute an outstanding example of extreme plant adaptation to rainfall deficits in the tropics of Central America. Therefore, we sampled cacti on different substrates to additionally understand their water sources during the dry season.

2 | ECOSYSTEM GENERALITIES

Biomes in Costa Rica are influenced by the precipitation, temperature and evapotranspiration regime and their location on the Caribbean or Pacific slope (Esquivel-Hernández et al., 2017). Dry forests are water-limited ecosystems with an annual potential evapotranspiration (PET) to precipitation (P) ratio >1. This biome is the southernmost sector of Meso-America's dry deciduous lowland forests and is therefore restricted to the northern Pacific lowlands of Costa Rica. The climate is mainly dry to sub-humid, with more than 95% of the annual precipitation (<1,500 mm) falling during the rainy season (mid-May to November; Figure 2a), while the remaining 5% of the rain falls during the dry season (April to mid-May; Figure 2a). The northern lowland forests are dominated by deciduous, semi-deciduous and evergreen trees that annually withstand six dry months (Kappelle, 2016; Stan & Sanchez-Azofeifa, 2019). Numerous cactus species can also be found in this ecosystem (Morales, 2001).

The Páramo of Costa Rica has a mean annual temperature of ~9.5°C and a mean annual precipitation of ~2,000 mm/year. The Páramo is an ecosystem that primarily develops in southern Costa Rica (Cordillera of Talamanca) above the tree line (~3,000–

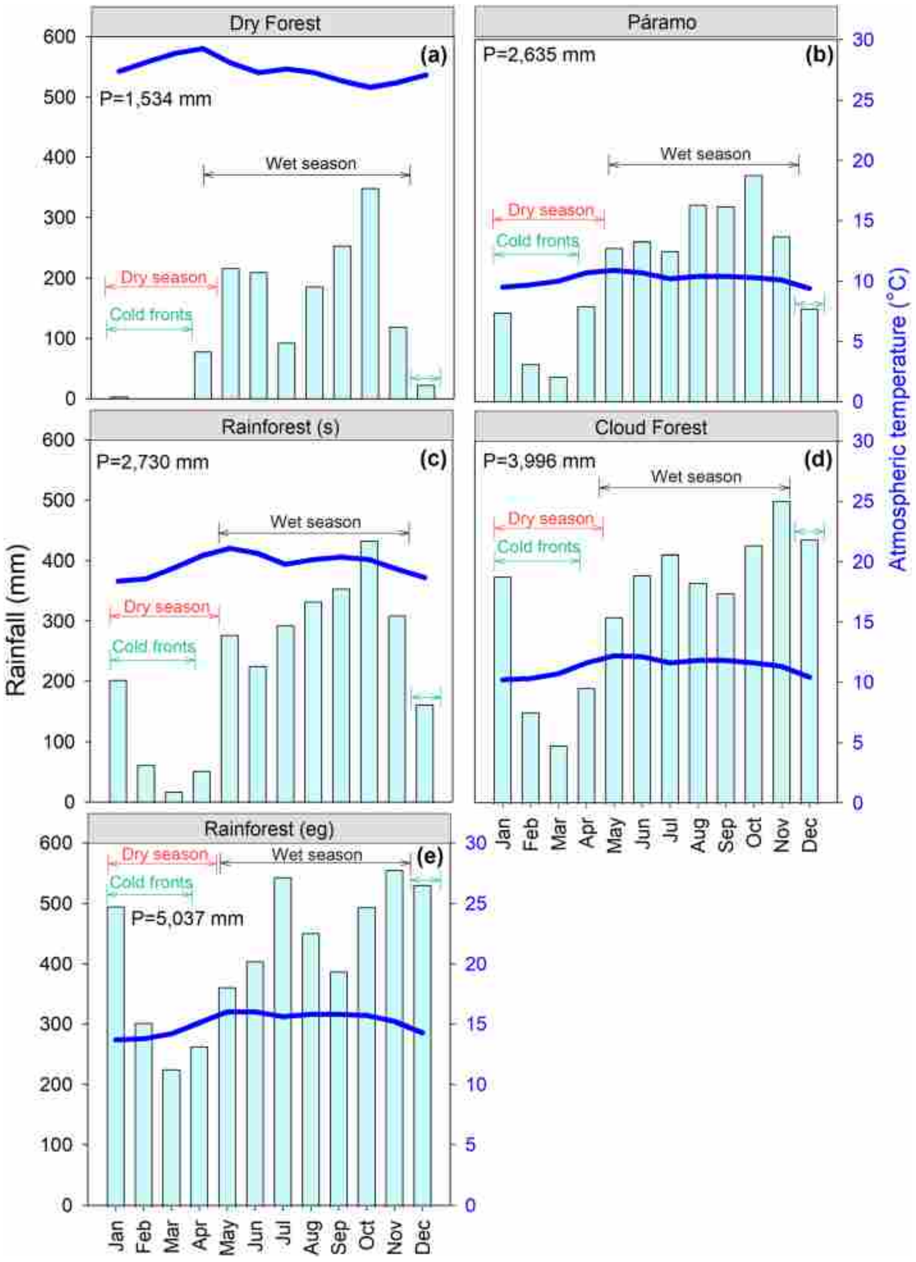

FIGURE 2 Representative climograph for each ecosystem (panels a–e) during the 2021 hydrological year. Bars represent monthly rainfall in mm. Solid blue lines denote the mean atmospheric temperature in °C. The timing of the dry (red), cold fronts (green) and wet (black) seasons are denoted by colour-coded bi-directional arrows. Annual precipitation is also indicated (s, seasonal; eg, evergreen).

3,100 m asl). This high-elevation biome is characterized by high water production and regulation capacities. Vegetation consists of 0.5 to 2.5 m tall bamboo (*Chusquea subtessellata* Hitchc.) and grass (*Festuca dolichophylla* J.Presl) (Esquivel-Hernández et al., 2018, 2019, 2021; Kappelle & Horn, 2016) (Figure 2b). The mountainous rainforests (600–3,000 m a.s.l., Holdridge, 1978) of Costa Rica extend from the northern and central volcanic Cordilleras of Costa Rica into the Cordillera de Talamanca, southeastward down to the border with Panama. The forest structure may differ significantly between the Caribbean and Pacific slopes due to the orientation (windward vs. leeward), rainfall amount, elevation and soil characteristics (Clark et al., 2015). As a result, the seasonal rainforest situated at the continental divide draining into the Caribbean is characterized by a well-defined dry season, unlike the evergreen rainforest located on the Caribbean slope (Figure 2c,e). For instance, mean annual rainfall typically varies between 3,300 and 3,500 mm at Barva volcano in the central volcanic Cordillera (Salas-Navarro et al., 2019). Overall, these mountainous forests exhibit a high degree of spatial heterogeneity and species diversity and are characterized by tall trees (up to 50 m) with abundant woody lianas and herbaceous vines (Kappelle, 2016; Veintimilla et al., 2019).

Cloud forests are located near the continental divide on the Pacific and Caribbean slopes of the volcanic Cordillera (Central and Tilarán) and Cordillera of Talamanca. They are characterized by persistent immersion in clouds, an important source of moisture during the dry season (December–April; Figure 2d). Mean annual rainfall is ~2,700 mm, with strong seasonal variability. Cloud forests typically include lower montane wet forests and lower montane rainforests, where wind-blown mist and cloud cover are integral components of the climate (Karmalkar et al., 2008; Rhodes et al., 2006).

3 | MATERIALS AND METHODS

3.1 | Soil and plant sample collection

Soil, stem and cactus samples were collected only during the dry season of 2021 (February–April) during a 1–2 day campaign at each site. It is important to highlight that our study aims to underpin ecohydrological similarities and differences between water uptake sources from the most predominant plants per ecosystem plot (i.e., as a composite ecosystem mixture); therefore, variations among species, diversity, density and/or plant size metrics were out of the scope during the field campaigns. Table S1 includes the families, genera and species, when identified, after the field campaigns. In addition, Table S1 includes a plant classification based on Raunkiær's life-form categories (Ellenberg & Mueller-Dombois, 1974). As a summary, stem samples included the following main families per ecosystem: (1) dry forest (Fabaceae and Dilleniaceae), (2) Páramo (Asteraceae, Garryaceae, Loganiaceae, Loranthaceae, Poaceae and Winteraceae), (3) evergreen rainforest (Clusiaceae, Melastomataceae and Poaceae), (4) seasonal rainforest (Araceae, Arecaceae, Fabaceae, Euphorbiaceae, Gesneriaceae, Maranthaceae, Melastomataceae, Myrtaceae, Ochnaceae and Pterophyta) and (5) cloud forest (Melastomataceae, Poaceae, Lauraceae, Cornaceae and Meliaceae). Cacti species included different living substrates across the tropical dry forest: *Acanthocereus tetragonus* (L.) Hummelinck (soil substrate), *Peniocereus hirshtianus* (K.Schum.) D.R. Hunt (soil substrate), *Opuntia guatemalensis* Britton & Rose (soil substrate), *Strophocactus testudo* (Karw. ex Zucc.) Bauer (tree substrate), *Stenocereus Aragonii* (F.A.C.Weber) Buxb. (tree substrate), *Melocactus curvispinus* Pfeiff. (coastal rock substrate) and *Hylocereus costaricensis* (F.A.C.Weber) Britton & Rose (tree substrate).

Sampling campaigns included five unique tropical ecosystems of Costa Rica: (1) dry forest (df, Area de Conservación Guanacaste, northwestern Pacific slope, <150 m asl; 10.8394°N, −85.6183°W), (2) cloud forest (cf, Barva volcano, central Pacific slope, 2,400 m asl; 10.1188°N, −84.1080°W), (3) evergreen rainforest (rfeg, Cerro Dantas, central Caribbean slope, 2,000 m asl; 10.0946 N°, −84.0586°W), (4) seasonal rainforest (rfs, Reserva Biológica Alberto Manuel Brenes, northern Caribbean slope, 1,500 m asl; 10.2284°N, −84.6397°W) and (5) Páramo (p, Cerro de la Muerte, southeastern Caribbean slope, 3,400 m asl; 9.5538°N, −83.7556°W) (Figure 1). Two soil profiles were sampled (triplicates were collected) per ecosystem from 0 down to ~75 cm in depth using a soil auger and shovel. A 5 × 5 m quadrangle was used in the field to select the most abundant plants via visual inspection. Stem samples (in triplicates) were collected from non-photosynthetic sections using a garden pruner. Stem external periderm was immediately eliminated to avoid residual bark water or dew water contamination. Cactus samples (triplicates) were collected under the botanical supervision of the Area de Conservación Guanacaste (ACG) personnel from three different substrates (i.e., soil, trees and rocks). One matured cactus was selected, and three different sections were collected for tree and soil-based substrates. Three spherical individuals were collected in the case of rock-based cacti. Soil and stem samples were collected in 50 ml pre-weighted centrifuge tubes, covered with Parafilm, placed in airtight plastic bags and transported and stored in the dark at approximately 5°C until analysis. All samples were transferred to the laboratory on the same day.

3.2 | Soil and plant water extraction

Water from soil and plant samples were obtained through centrifugation. The selection of the centrifugation method was based on the following rationale and assumptions:

1. The large abundance of clay-enriched soils and high organic matter in the study areas may induce a strong isotopic bias during soil cryogenic extractions (Alvarado et al., 2015; Orlowski, Breuer, & Mcdonnell, 2016; Orlowski, Pratt, & McDonnell, 2016; Gaj et al., 2017).
2. In contrast to temperate landscapes, in the tropics of Costa Rica, even in the driest northwestern Pacific region, mean annual precipitation is close to ~1,500 mm; therefore, soils hold relatively large mobile water contents between the field capacity and the wilting point.

3. As the main ecohydrological goal was to separate mobile water effectively, extraction pressures are relevant and should be considered in advance when selecting water extraction methods. Commonly, the applied tension in cryogenic extraction is greater than 100 MPa (Sprenger et al., 2015), while porous cup samplers or lysimeters extract mobile water at tensions lower than 200 kPa (Geris et al., 2015). Based on the centrifugation characteristics described below, we applied an average tension of ~2.4 MPa. Therefore, our experiment was designed to analyse only available mobile water at the end of the dry season.
4. Our study provides the plant-extracted water (in %) to test potential isotopic bias due to the overall extraction volume rather than an extraction or recovery efficiency.

Preliminarily, all soil, stem and cactus samples were weighed (±0.01 g) at room temperature in the laboratory. If needed, soil or stem material was removed to reach an equal mass in the centrifugation tubes. Centrifugation was conducted in 50 ml Falcon tubes (Sorvall, Newton, CT, USA) at 11,000 rpm (equivalent to ~2.4 MPa of tension; based on Bowers et al., 2020) and 5°C for 1.5 h, using an Eppendorf 5804R centrifuge. Extended centrifugation time (2–3 h) did not result in more significant water extraction amounts. For centrifugation, soil sample amounts ranged from 39.6 to 46.3 g, while stem mass sections ranged from 17.2 to 26.8 g. Cactus sample amounts for centrifugation were, on average, 23.6 g. Immediately after centrifugation, the extracted water was transferred with a 1,000 μl micropipettor to a 2 ml glass vial and sealed with a septa cap. When the extracted water volume was below 1.0 ml, a 300 μl glass insert was used to place the sample inside the 2 ml vials to avoid any analytical disruption due to low water volumes in the injection vials. After centrifugation, stem and cactus samples were dried for 48 h at 105°C using a Yamato DKN-602C programmable forced convection oven. Stem water extracted (in %; extracted water/total stem water in the sample) and total stem water (in %; total stem water in the sample/total sample mass) were calculated via gravimetric measurements. High soil clay contents precluded water extraction measurements since particles were firmly stuck to the plastic centrifugation tubes, impeding the dry-oven procedure (see Section 3.2, 1).

3.3 | Isotopic analysis

Isotopic analysis was conducted at the Stable Isotopes Research Group facilities of Universidad Nacional (Heredia, Costa Rica) using an LWIA-45-EP water isotope analyser (Los Gatos, USA). Calibrated secondary standards MTW ($\delta^2H = -130.3$ ‰, $\delta^{18}O = -16.7$‰), USGS45 ($\delta^2H = -10.3$‰, $\delta^{18}O = -2.2$‰) and CAS ($\delta^2H = -64.3$ ‰, $\delta^{18}O = -8.3$‰) were used to normalize the results as well as to assess the quality and drift control procedures. The (Bowers & Williams, 2022) $O/^{16}O$ and $^2H/^1H$ ratios are presented in delta notation δ (‰), relative to the VSMOW-SLAP scale. The long-term analytical uncertainty was ±0.5‰ for δ^2H and ±0.1‰ for $\delta^{18}O$. Deuterium excess (‰) was calculated as *d*-excess = δ^2H-8·$\delta^{18}O$ (Dansgaard, 1964). The line-conditioned excess (‰) was calculated as lc-excess = δ^2H-a·$\delta^{18}O$-b (Landwehr & Coplen, 2006), where a and b correspond to the slope and intercept of the LMWLs for each site, respectively. Isotope results of soil and stem water extracts were routinary checked for spectral interferences using the Spectral Contamination Identifier post-processing software (LWIA-SCI, Los Gatos Research Inc., California, USA). This post-processing tool compares recorded spectra from the soil and stem samples with those from known standards samples to produce a metric of contamination from either narrow-band (methanol) or broad-band (ethanol) substances and indicates the likelihood of spectral interference (Leen et al., 2012; Orlowski et al., 2013; Schultz et al., 2011) via OA-ICOS. This analytical issue is even more problematic during vapour-mode analyses (Nehemy et al., 2019). In our study, no sample was found to be contaminated. Since centrifugation is less invasive in extracting immobile bounded soil or stem water, it favours the extraction of available and mobile water pools (Figueroa-Johnson et al., 2007; Geris et al., 2015; Orlowski, Pratt, & McDonnell, 2016).

3.4 | Complementary rainfall, surface water and groundwater isotope data

The isotopic composition of the extracted soil, stem and cactus water was analysed together with complementary rainfall, surface water and groundwater (spring water) isotope data of each ecosystem collected by the Stable Isotopes Research Group (Universidad Nacional) and the Water and Global Change Observatory (Universidad de Costa Rica). For the dry forest region, surface water from the Tempisque River ($N = 139$, 2018–2021; weekly unpublished data) and rainfall in Liberia ($N = 421$, daily; 2014–2020; Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020) were included. Páramo rainfall ($N = 516$; daily, 2015–2021) and surface water ($N = 85$; biweekly and monthly, 2015–2021) were obtained from a continuous monitoring effort in the Chirripó National Park (Esquivel-Hernández et al., 2021) and Cerro de la Muerte (Sánchez-Murillo et al., 2022). Rainfall ($N = 308$, weekly 2013–2021) and spring water ($N = 661$; daily and weekly 2014–2021) data were obtained from the Sacramento station in the Barva volcano cloud forest (Salas-Navarro et al., 2019; Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020). Rainfall ($N = 1{,}082$; daily 2016–2021) and surface water ($N = 545$; daily and weekly 2016–2021) isotope data from the evergreen rainforest site (Cerro Dantas) were obtained from the continuous monitoring station in the Quebrada Grande watershed (Mayer-Anhalt et al., 2022; Sánchez-Murillo, Romero-Esquivel, et al., 2019). Finally, rainfall ($N = 135$; event-based and daily 2013–2018) and surface water ($N = 285$; event-based and daily 2013–2018) tracer data were obtained from the San Lorencito seasonal rainforest site (Correa et al., 2020; Dehaspe et al., 2018).

3.5 | Bayesian mixing model analysis

The R package MixSIAR (Stock et al., 2018) was used with uninformative priors and 10,000 Markov Chain Monte-Carlo (MCMC) iterations (5,000 burn-in) to evaluate the contribution of water source endmembers to a mixture. All chains always converged, and Gelman's statistics were significant at $p < 0.05$. This preliminary mixing assessment aimed to underpin plant water uptake from the most predominant plants per ecosystem plot during a single sampling event at the end of the dry season of 2021. Our mixing calculations were constrained under the following assumptions and conditions: (1) The extracted plant water was treated as a representative mixture of the natural variability in each ecosystem; (2) all soil profiles were treated as a single endmember per ecosystem since no clear trends with depth were observed, with the exception of the Paramo soil profile and the dry forest that resulted in zero extracted mobile soil water; (3) available long-term isotope data from surface water and groundwater sources were used as valid end-members representing potential reservoirs for water uptake during the dry season; (4) the isotopic differences among the endmembers are sufficiently large (Allen & Kirchner, 2022), and therefore, we expect minimal effects of the extraction method on the plant water source estimations; (5) variations between species were out of the scope of the experimental design; and (6) precipitation was divided into four periods, annual, the second rainfall maxima of the rainy season (September–October 2020), and cold front rainfall between November–December (2020) and January–February (2021). This selection was based on the previous knowledge of isotopic variations in meteoric waters across Costa Rica, where two depleted incursions are observed in May–June and September–October (see Sánchez-Murillo, Durán-Quesada, et al., 2019), and the relative dominance of younger waters contribution to streamflow generation (<95 days; Mayer-Anhalt et al., 2022). Cold front rainfall inputs (Figure 2) are also highly relevant for mountainous ecosystems across the continental divide (i.e., Páramo, seasonal and evergreen rainforests and the cloud forest) (Herrera, 2016). However, these events do not generate rainfall over the Pacific slope (i.e., Foehn effect; Elvidge & Renfrew, 2016), which is the case of the dry tropical forest ecosystem in northwestern Costa Rica.

The mixture consisted of stem (most dominant plants) isotope values, and the source contributions to this mixture were isotope ratios of (1) soils, (2) average surface water (SW; stream and rivers), (3) groundwater (GW; spring), and (4) average precipitation during three distinct rainfall periods (P). The tracers ($\delta^{18}O$ and $\delta^{2}H$) and two second-order variables (*d*-excess and lc-excess) were used for this analysis. Cactus water was not included as these measurements always fell outside (largely enriched) the source isotope mixing diagrams of available sources. Therefore, the assumption of the mixing model that requires a chemically distinct source composition was not valid. We evaluated the latter with dual-isotope diagrams per ecosystem. While in the Paramo soil water, $\delta^{18}O$ values ranged from −5‰ to −10‰ below 25 cm; preliminary dual-isotope plots indicated that plants were not linked to deeper soil water pools.

4 | RESULTS

4.1 | Soil and plant water extraction

The water amount extracted via centrifugation was determined gravimetrically to analyse the effects of the water extracted volume on the stem and cactus isotopic ratios. Figure 3 shows box plots of extracted water (in %) (panel a) and total water in the stem sample (in %) (panel b). Cactus samples exhibited larger extracted water values than the non-photosynthetic tissues from all plant functional types ranging from 1.1% to 35.3%, with a median value of 7.8 ± 10.1% (Figure 3a). Cacti were composed of sample sections with high water content (82.7–96.3%), with a median value of 91.0 ± 3.8% (Figure 3b). Dry forest plant samples presented the lowest water content (38.5–68.1%) and water extraction volume (1.4–5.9%), with median values of 48.0 ± 11.6% and 2.82 ± 1.29%, respectively (Figure 3a,b). The seasonal and evergreen rainforest samples also exhibited large stem water contents, with median values ranging between 80.4 ± 13.8% and 72.4 ± 8.6%, respectively (Figure 3b). More water was extracted from the seasonal rainforest plant samples (4.5 ± 3.6%) than from the evergreen forest ones (3.0 ± 3.5). The cloud forest and Páramo plant samples revealed similar water contents varying between 65.1 ± 10.7 and 62.9 ± 14.9, respectively. However, water extraction was larger in the cloud forest plant samples (6.6 ± 2.2%) than in the Páramo samples (4.8 ± 1.8%).

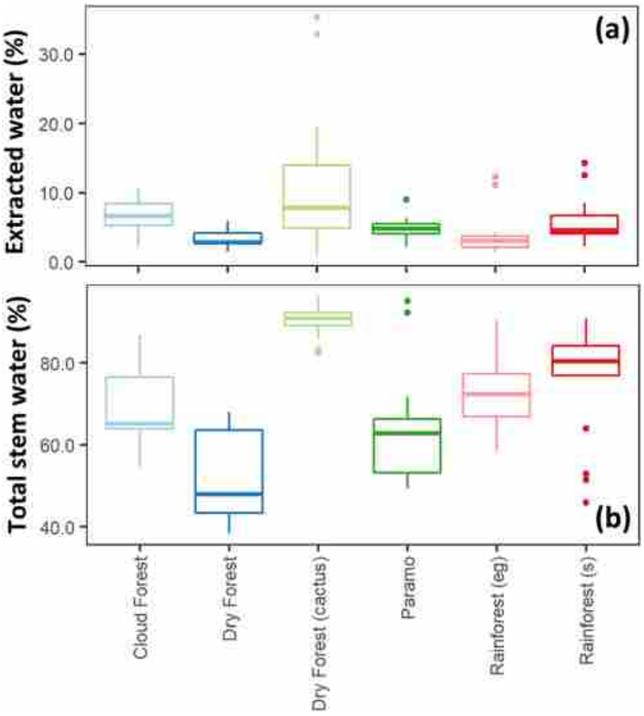

FIGURE 3 Box plots for (a) centrifugated extracted water (%) and (b) total gravimetric stem water (%) for each ecosystem (s, seasonal; eg, evergreen)

4.2 | Extracted water amount and isotope relationships

Figure 4 shows the relationship between the extracted water amount expressed in percentage versus δ^{18}O compositions. A moderate significant linear regression (*adj.* $r^2 = 0.34$, $p < 0.01$) was found in the cactus samples, where those above ~20% of extracted water presented more enriched compositions (Figure 4). Those samples corresponded with cacti growing in soil substrates. The Páramo, cloud forest and seasonal rainforest stem sections exhibited weak and negative trends (Figure 4). The dry forest and evergreen forest samples presented poor relationships. Overall, mean water extracted volumes ranged from 210 to 2,350 μl, and no clear water amount-dependent patterns were detected in the stem samples.

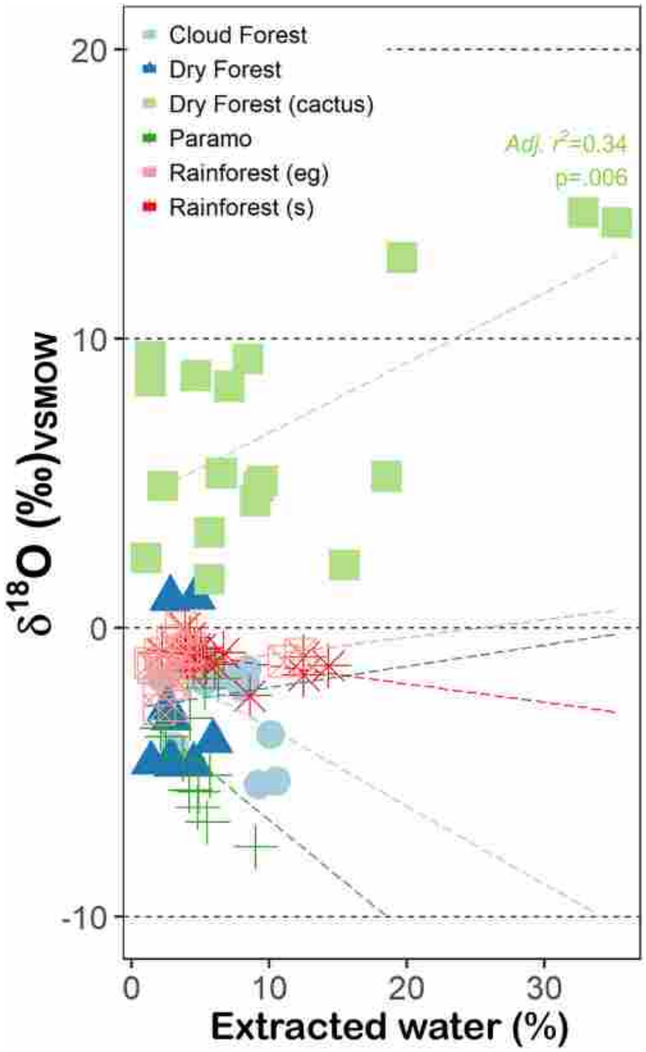

FIGURE 4 Linear regressions (colour-coded dashed lines) between centrifugated extracted water (%) and δ^{18}O (‰) composition for each ecosystem (s, seasonal; eg, evergreen). Only the cactus samples resulted in a significant regression.

4.3 | Variability in the isotopic composition of different water sources

Figure 5 shows dual-isotope diagrams per ecosystem, including rainfall, surface water, groundwater, soils, stem water and cactus water. In general, LMWLs denoted water-energy ecohydrological characteristics across ecosystem types and slopes (the Caribbean versus Pacific slopes) of Costa Rica (Esquivel-Hernández et al., 2017). For example, in the dry forest region, local conditions are influenced by strong subcloud evaporation, low relative humidity, high ambient temperatures and a marked dry season (5–6 months), which in turn results in a lower slope (7.6) and intercept (6.2, Figure 5b) when compared to more humid ecosystems (large moisture recycling with greater slopes and intercepts; Figure 5a,c,d). Similar LMWL trends have been reported in other regions of the Dry Corridor of Central America (Sánchez-Murillo, Esquivel-Hernández, Corrales-Salazar, et al., 2020). The Páramo exhibited LMWLs close to the GMWL (Figure 5c). Altogether, surface water and groundwater revealed a clear meteoric origin, while soil and stem samples exhibited moderate to weak departures from meteoric conditions (Figure 5a–e). Cactus samples showed a marked enrichment independently of their living substrate (soil, trees or rocks) (Figure 5b).

In the dry forest ecosystem, δ^{18}O in rainfall and surface water ranged from 1.6‰ to −15.3‰ with a median value of −5.8 ± 3.2‰ and from −5.3‰ to −10.3‰ with a median value of −6.3 ± 0.8‰ (Figure 6a), respectively. Stem samples ranged from 1.0‰ to −4.8‰ with a median value of −3.5 ± 2.6‰. Cactus samples revealed a more extreme enrichment, with δ^{18}O values ranging from 1.7‰ to 14.4‰ (median = 5.3 ± 4.0‰) (Figure 6a). In the dry forest, second-order variables indicated a strong enrichment process. Median stem *d*-excess and lc-excess values were −9.2‰ and −16.9‰, respectively. Cacti median *d*-excess and lc-excess values were even more extreme, −35.6‰ and −39.7‰, respectively (Figure 6b,c). Surface water in this region also exhibited low lc-excess values ranging from 9.8‰ to −4.2‰ (median = 2.6 ± 3.6‰). These conditions indicated an apparent deviation from mean rainfall *d*-excess (9.0 ± 4.8‰) and suggested strong secondary evaporation processes during the dry season. No soil water was extracted from the dry forest ecosystem via centrifugation (applied tension ~2.4 MPa), due to the very dry conditions.

Surface water, soil and stem water samples denoted a meteoric bias toward more enriched compositions in the evergreen rainforest site. The δ^{18}O in rainfall and surface water ranged from 0.7‰ to −21.1‰ with a median value of −4.5 ± 4.0‰ and from −2.3‰ to −12.0‰ with a median value of −5.7 ± 1.6‰ (Figure 6a), respectively. Soil samples ranged from −1.0‰ to −4.7‰ with a median value of −1.8 ± 0.9‰ (Figure 6a). No large δ^{18}O or lc-excess variations were measured in the soil profile (Figure 7). The δ^{18}O of stem samples ranged from −0.01‰ to −2.9‰ with a median value of −1.2 ± 0.7‰ (Figure 6a). Both rainfall (13.6 ± 4.5‰) and stream water (14.7 ± 3.6‰) exhibited similar *d*-excess values, reflecting the dominance of young water in streamflow (<95 days; Mayer-Anhalt et al., 2022). The *d*-excess and lc-excess ranged from 3.2‰ to 26.2‰ and from −2.4‰ to 12.2‰ for soils and from 12.1‰ to 21.7‰ and −2.3‰ to 7.6‰ for stems, respectively (Figure 6b,c).

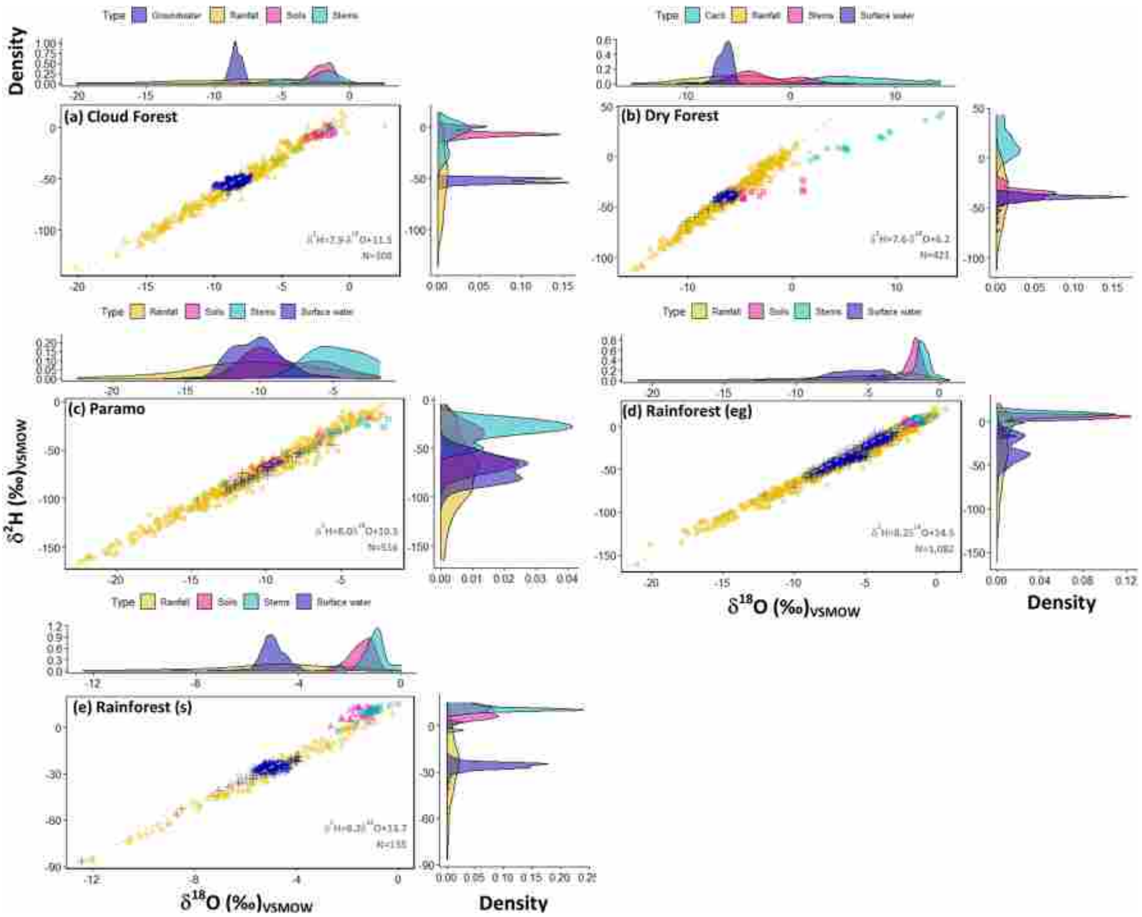

FIGURE 5 Dual-isotope plots for each ecosystem (a–e) (s, seasonal; eg, evergreen). LMWLs (orange dashed line) and the total number of rainfall samples are included for reference. Marginal density plots for $\delta^{18}O$ and $\delta^{2}H$ are included for all endmembers per ecosystem.

The seasonal rainforest site exhibited a smaller rainfall variation with $\delta^{18}O$ values ranging from −0.3‰ to −12.4‰ (median = −4.7 ± 2.5‰). Stream isotopic compositions reflected a strong connection with rainfall inputs, where $\delta^{18}O$ values ranged from −1.7‰ to −12.4‰ (median = −5.0 ± 0.7‰) (Figure 6a). Like the evergreen forest, the soil profile exhibited a nearly uniform $\delta^{18}O$ composition with depth, with no significant lc-excess variation (Figure 7). Soil samples ranged from −1.0‰ up to −2.7‰ with a median value of −1.5 ± 0.4‰, while stem sections varied from −0.01‰ to 2.4‰ with a median value of −1.0 ± 0.5‰ (Figure 6a). Both rainfall (12.6 ± 2.9‰) and stream water (14.0 ± 2.5‰) exhibited similar *d*-excess values. Stream lc-excess varied from −5.7‰ to 6.7‰ (median = 1.4 ± 2.6‰). Soil (median = 7.3 ± 3.8‰) and stem (median = 4.6 ± 1.7‰) samples suggested moderate to low enrichment (Figure 6b,c).

Strong orographic distillation resulted in more depleted median $\delta^{18}O$ rainfall compositions in the cloud rainforest (median = −7.4 ± 3.9‰) and the Páramo sites (median = −10.5 ± 4.2‰) (Figure 6a). The cloud forest soil profile was also uniform in terms of $\delta^{18}O$ compositions with depth in the range of the evergreen and seasonal rainforest isotope ratios. However, lc-excess values in the cloud forest were, in general, the lowest among the sites (Figures 6b,c and 7). In contrast to all the ecosystems, the Páramo revealed a more variable isotope pattern with soil depth, with more depleted values below 25 cm (up to ~10‰), agreeing with the long-term mean annual rainfall $\delta^{18}O$ composition (Figure 7). Spring and stream water in the cloud forest and Páramo sites also depicted a strong meteoric origin with median $\delta^{18}O$ values between −8.4 ± 0.4‰ and −11.6 ± 1.1‰.

The median $\delta^{18}O$ composition of stem sections in the cloud forest (−1.9 ± 1.5‰) (Pacific slope) is in the range of the nearby evergreen ecosystem (Caribbean slope) since rainfall in both sites is affected by similar meteorological features (Figures 1 and 6a). Similarly, soil $\delta^{18}O$ ratios varied from −1.1‰ to −3.2‰ (median = −2.0 ± 0.6‰). Rainfall and spring water presented relatively high *d*-excess values, 11.9 ± 5.0 and 14.4 ± 2.5, respectively (Figure 6b). Spring lc-excess values were consistently lower than rainfall, with a median of 2.0 ± 2.5‰ (Figure 6c). Both soil (10.8 ± 3.7‰) and stem sections (11.7 ± 1.3‰) exhibited *d*-excess values similar to rainfall. Soil (median = −0.8 ± 3.6‰) and stem (median = −0.1 ± 1.4‰) samples also suggested moderate to low evaporation (Figure 6c). A similar isotope trend was measured in the Páramo site. Median $\delta^{18}O$ (*d*-excess) compositions in soils (−9.4‰; 13.4‰) and stem sections (−4.8‰; 8.7‰) reflected mean annual $\delta^{18}O$ and *d*-excess rainfall compositions, respectively. However, median lc-excess values in soils (2.9‰) and stem (−1.8‰) samples were closer to stream water (−0.9‰), reflecting the influence of secondary evaporation.

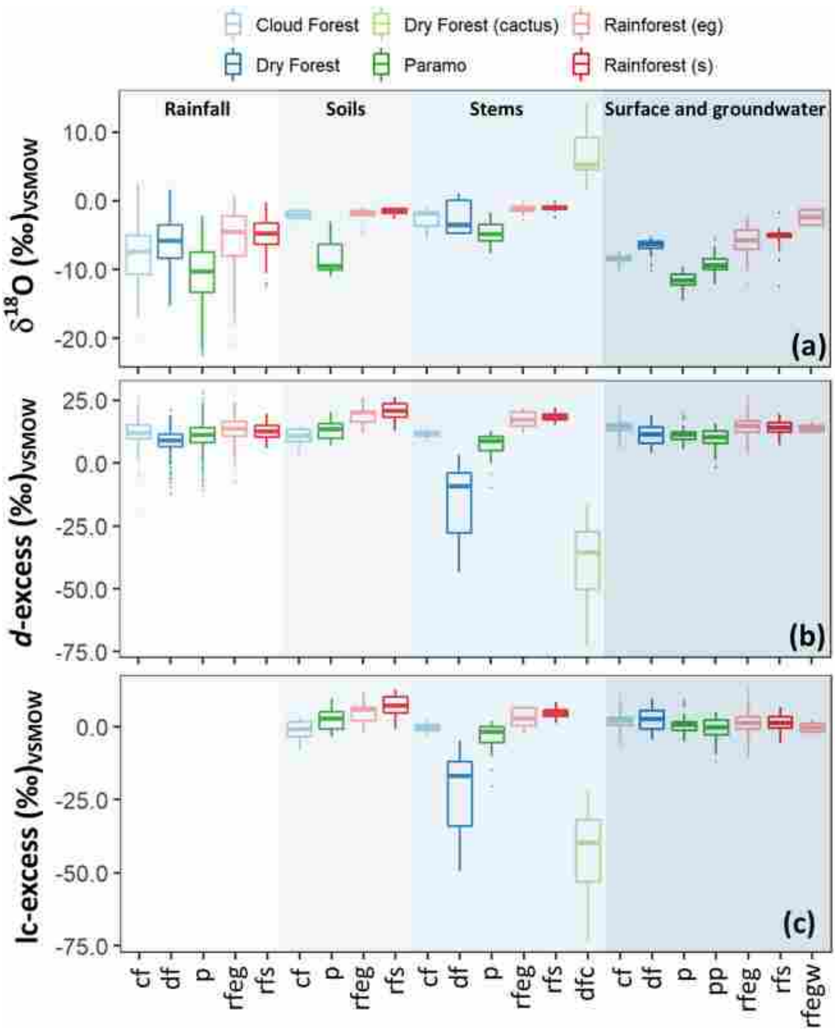

FIGURE 6 Box plots for (a) $\delta^{18}O$ (‰), (b) *d*-excess (‰) and (c) lc-excess (‰), including rainfall, soil water, stem water, cactus water, surface water and groundwater samples for each ecosystem (cf, cloud forest; df, dry forest; p, Páramo; rfeg, rainforest evergreen; rfs, rainforest seasonal; c, cactus; pp, peatbogs; w, swamps)

4.4 | Source water uptake

The Bayesian mixing modelling was designed to understand the relative contributions of precipitation (i.e., three different periods with distinct isotopic ratios), soil water (up to 1 m) and surface and groundwater to the plant water mixture (Table 1). The latter considers the water uptake from the most prominent plants as representative of the ecosystem functioning; however, it is clear that different species will encompass unique water uptake strategies and mechanisms (Dubbert et al., 2023; Tron et al., 2013). Figure 8 shows the dual space between $\delta^{18}O$ and $\delta^{2}H$ with standard deviations from the mean visualized as error bars for all water source endmembers and the ecosystem stem water mixture. Apart from the dry forest, all stem mixtures were influenced mainly by the soil and the most recent cold front rainfall (January–February) isotopes, which were closely represented by average rainfall and surface water isotopes. The dry forest exhibited the only stem water mixture that significantly deviated from the LMWL along which almost all water sources and mixtures plot (Figure 8).

The seasonal rainforest stem mixtures exhibited soil water source contribution of >80%, followed by recent rainfall of around 10% (Table 1). The evergreen rainforest had slightly reduced soil water contributions of 74%, followed by the cloud forest with 66%. The Páramo stem mixture was constituted mainly by recent rainfall with 62% and then by 12% of soil water sources. The dry forest stem mixtures exhibited average precipitation as the most important source contribution (39%), followed by surface water (33%). The uncertainties of the two dominant water source contributions were moderate but increased for less important sources (Table 1).

5 | DISCUSSION

Rainfall isotopic variations have been widely studied in the past decade across Costa Rica (Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020, and references therein). The well-known isotope separation between windward (Caribbean) and leeward (Pacific) slopes (Sánchez-Murillo & Birkel, 2016), coupled with strong orographic distillation, offer unique and traceable isotopic incursions into soil water transport and plant water uptake across contrasting tropical ecosystems of Costa Rica.

Our results provide the first isotope-based water source analysis across contrasting ecosystems in the tropics. Isotope findings are

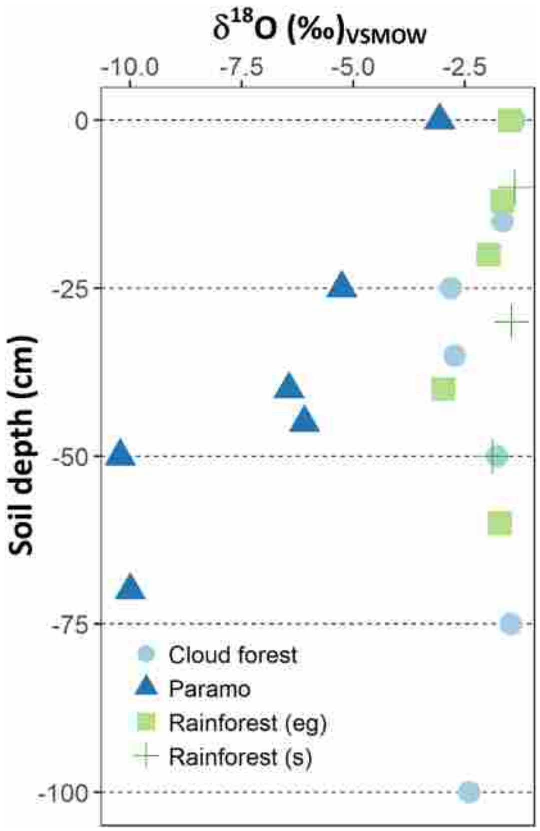

FIGURE 7 Soil depth (cm) versus mean δ^{18}O (‰) compositions for each ecosystem (s, seasonal; eg, evergreen)

presented as a good example of tropical conditions from sea level to high elevation landscapes (∼3,400 m asl) and across a marked precipitation gradient from the evergreen rainforest site (∼4.1–6.1 m/year; Sánchez-Murillo, Romero-Esquivel, et al., 2019; Mayer-Anhalt et al., 2022) to the dry seasonal forest (<1.5 m/year; Baron Ruiz, 2021: Kühnhammer et al., 2022). Furthermore, we showcase the practicality of the centrifugation method for obtaining mobile water from soil and stem samples for determining its isotopic composition and, thus, water source uptake.

5.1 | Mobile water extraction by centrifugation and isotope interpretation

The advent of laser spectroscopy (i.e., lower analytical cost and faster injection and integration times, 2–10 min, when compared to IRMS) has motivated the introduction of new in-house and field extraction techniques, such as vapour equilibration, centrifugation, microwave in-line distillation, Scholander pressure chamber, high-pressure mechanical squeezing and passive lysimeters (Bowers et al., 2020; Bowers & Williams, 2022; Fischer et al., 2019; Millar et al., 2018, 2022; Orlowski et al., 2019; Orlowski, Pratt, & McDonnell, 2016; Sprenger et al., 2015; Zuecco et al., 2022). While these new extraction techniques offer a large potential in decreasing the analytical bias by intricating fractionation processes during low-volume cryogenic extractions (few µl to ∼1 ml; rarely provided as part of the analytical metadata), there is still a lack of standard operating protocols to conduct robust inter-comparisons, as clearly pointed out in a recent review by Millar et al. (2022). The cryogenic vacuum distillation extraction method has been the most widely used analytical technique for tracing plant water sources (e.g., Amin et al., 2020; Barbeta et al., 2020; Brooks et al., 2010; Hervé-Fernández et al., 2016). From a physicochemical perspective, cryogenic vacuum distillation is an invasive water extraction technique that certainly results in high extraction efficiencies (Wen et al., 2022). However, in soil and plant samples, the cryo-extracted water inexorably reflects a combination (of unknown proportions) between chemically bounded water (i.e., immobile mineralogical or biological water) and soil/xylem mobile water (i.e., the analyte of interest involved in the evapotranspiration process) (von Freyberg et al., 2020, and references therein). In this regard, a large conundrum exists to (1) underpin the ratio of mobile and immobile soil/xylem water relative to the total water contained in a particular sample and (2) how this extraction ratio potentially biases (either in a depletion or enrichment direction) the 'true' isotopic value of water flowing through soil and xylem at a given time. The latter may concatenate or propagate errors in mixing calculations or modelling applications. However, the concern has been raised that this technique collects bulk stem water (sap + symplastic + capillary + fibre water), not only sap water (Allen & Kirchner, 2022; Barbeta et al., 2022; Penna et al., 2018; Song et al., 2021; Wen et al., 2022), thus hindering water source uptake through the SPAC. The centrifuge method has been previously used for extracting water for isotopic analysis (Bowers et al., 2020; Li et al., 2007; Millar et al., 2018; Orlowski, Pratt, & McDonnell, 2016; Sprenger et al., 2015; Tsuruta et al., 2019). Only Millar et al. (2018) tried it for plant tissues, but unlike our methodology, they used a pre-treatment of maceration process. Barbeta et al. (2022) have recently compared results by using cryogenic extraction and a cavitron, which follows the same principle of the centrifuge, by using a rotor in which a long stem is attached, and centrifugal force expels water from the centre toward both sides of the stem. They concluded that sap water collected with the cavitron better represents water uptaken by roots than compared to bulk stem water obtained by cryogenic vacuum distillation.

Here, we show that the extraction of mobile water through common centrifugation of stem, cactus and soil samples is possible across a wide range of different ecosystems. Remarkably, this was possible in the dry season, when water is expected to be in more tension in both substrates. Overall, triplicates of 8–10 samples were extracted per day (24–30 individual samples), providing the ability to process many samples in a short timeframe. Water extraction from dry forest soil samples was unsuccessful, revealing an extraction limit related to matric potential (below approximately −2.4 MPa; at 11,000 rpm) in soils with significant clay contents at the end of the dry season. Previous studies have identified extraction thresholds for cup lysimeters (0.05 to 0.10 MPa), mechanical squeezing and centrifugation (∼8,000 rpm) (up to −1 MPa) and cryogenic vacuum distillation (≥100 MPa) (Bowers et al., 2020; Li et al., 2007; Orlowski, Breuer, & Mcdonnell, 2016; Orlowski, Pratt, & McDonnell, 2016; Sprenger

TABLE 1 Bayesian mixing model source contributions (mean, standard deviation SD, quartile and interquartile percentages) to stem water for all ecosystems except the dry forest cacti

Ecosystem	Sources	Mean	SD	2.5%	5%	25%	50%	75%	95%	97.5%
Seasonal rainforest, s	P	0.018	0.018	0.000	0.001	0.005	0.013	0.026	0.054	0.067
	P_Jan/Feb21	*0.071*	*0.054*	*0.003*	*0.005*	*0.028*	*0.060*	*0.102*	*0.176*	*0.199*
	P_Nov/Dec20	0.017	0.017	0.000	0.001	0.005	0.012	0.024	0.049	0.061
	P_Sep/Oct20	0.012	0.012	0.000	0.001	0.004	0.009	0.017	0.036	0.045
	Soil	***0.864***	***0.058***	***0.733***	***0.755***	***0.829***	***0.871***	***0.906***	***0.946***	***0.954***
	SW	0.018	0.018	0.000	0.001	0.005	0.013	0.024	0.054	0.068
Cloud forest, cf	P	0.047	0.042	0.001	0.003	0.016	0.035	0.066	0.132	0.156
	P_Jan/Feb21	*0.174*	*0.143*	*0.007*	*0.013*	*0.064*	*0.139*	*0.25*	*0.457*	*0.540*
	P_Nov/Dec20	0.044	0.04	0.001	0.003	0.014	0.032	0.061	0.125	0.151
	P_Sep/Oct20	0.034	0.031	0.001	0.002	0.011	0.025	0.048	0.099	0.117
	Soil	***0.660***	***0.138***	***0.332***	***0.397***	***0.584***	***0.683***	***0.763***	***0.841***	***0.858***
	GW	0.041	0.037	0.001	0.002	0.014	0.031	0.059	0.116	0.136
Dry forest, df	***P***	***0.386***	***0.235***	***0.017***	***0.035***	***0.187***	***0.372***	***0.573***	***0.790***	***0.832***
	P_Nov/Dec20	0.088	0.080	0.003	0.005	0.027	0.067	0.127	0.251	0.288
	P_Sep/Oct20	0.195	0.165	0.006	0.011	0.067	0.148	0.282	0.528	0.617
	SW	*0.331*	*0.227*	*0.014*	*0.027*	*0.131*	*0.299*	*0.495*	*0.749*	*0.806*
Páramo, p	P	0.070	0.063	0.002	0.004	0.023	0.053	0.100	0.198	0.231
	P_Jan/Feb21	***0.619***	***0.142***	***0.262***	***0.361***	***0.544***	***0.636***	***0.715***	***0.812***	***0.850***
	P_Nov/Dec20	0.062	0.058	0.002	0.003	0.019	0.046	0.087	0.177	0.209
	P_Sep/Oct20	0.049	0.046	0.001	0.002	0.015	0.035	0.069	0.143	0.167
	Soil	*0.123*	*0.122*	*0.003*	*0.006*	*0.037*	*0.085*	*0.174*	*0.363*	*0.442*
	SW	0.077	0.069	0.002	0.004	0.025	0.057	0.110	0.217	0.248
Evergreen rainforest, rfeg	P	0.021	0.020	0.001	0.001	0.007	0.015	0.029	0.059	0.071
	P_Jan/Feb21	*0.186*	*0.081*	*0.038*	*0.057*	*0.129*	*0.183*	*0.239*	*0.324*	*0.356*
	P_Nov/Dec20	0.020	0.020	0.001	0.001	0.007	0.015	0.028	0.059	0.072
	P_Sep/Oct20	0.013	0.013	0.000	0.001	0.004	0.010	0.018	0.038	0.049
	Soil	***0.740***	***0.097***	***0.527***	***0.568***	***0.681***	***0.747***	***0.809***	***0.886***	***0.909***
	SW	0.020	0.019	0.000	0.001	0.006	0.014	0.028	0.057	0.073

Note: The dry forest calculations were not based on rainfall in January and February 2021 (no rain) nor soil isotopes as sources. The seasonal rainforest did use average rainfall sources as no measurements in 2020/2021 were available. We also indicate the two most prominent sources in black and italics and grey (second most important).

et al., 2015). Water amount-dependent trends have challenged the interpretation of plant water isotopes obtained through cryogenic vacuum distillation or centrifugation based on evidence of large (depleted or enriched) hydrogen fractionation (Diao et al., 2022; Orlowski et al., 2019; Tsuruta et al., 2019). We specifically checked for the relationship between the isotopic composition and percentage of extracted water and found no apparent association. The exception was cacti sections, where samples above ~20% of extracted water presented a more enriched composition. Altogether, the centrifuge method seems promising, effective and time-consuming for extracting water for source partition analysis. We encourage further research using centrifugation across a wide diversity of plant species and functional types, and soil type samples, as well as its comparison with other extraction methods and their efficiencies.

5.2 | Water sources across a climatic and geomorphic gradient

The Páramo plants revealed a close relationship to recent rainfall events produced during the strongest trade winds (December–February), where light but constant rain saturates the landscape over several days. In this high-elevation ecosystem of central Costa Rica, rainfall is mainly modulated by enriched rain events during cold fronts (December–March) and depleted incursions during the influence of the ITCZ and the indirect impact of tropical cyclones (Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020). They sustained a carbon-rich ecosystem (Sánchez-Murillo et al., 2022) with a larger soil water retention capacity (Blanco et al., 2018). In this ecosystem, soil isotope compositions were more enriched (−2.5‰ to −5.0‰) between 0 and

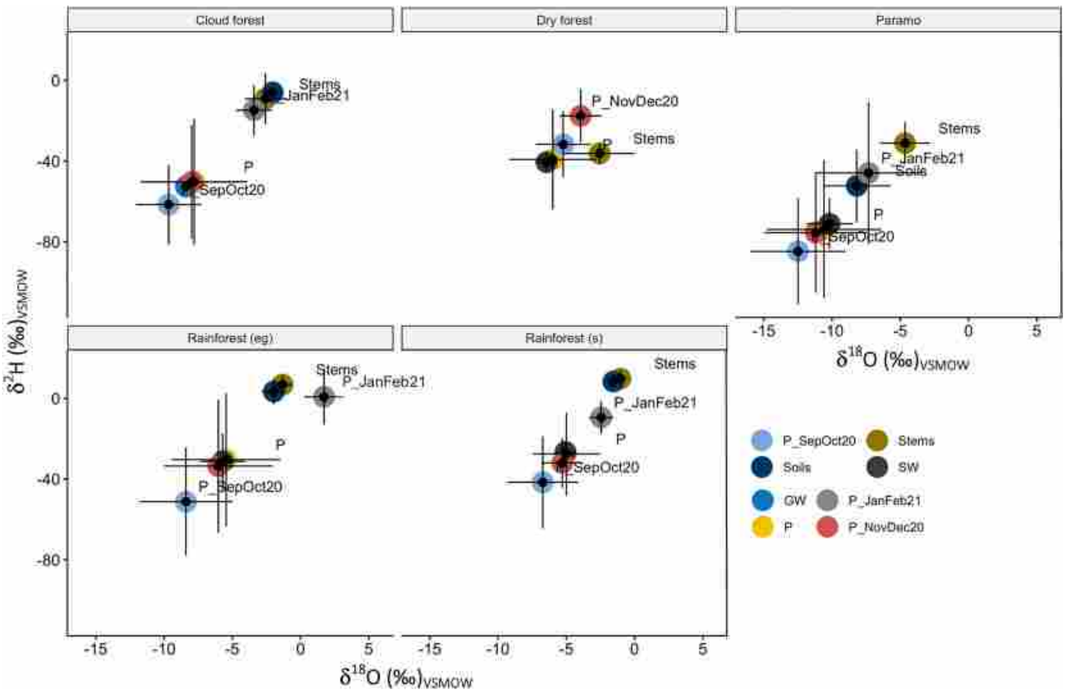

FIGURE 8 Dual isotope plot for all ecosystems, including water sources, with error bars reflecting the standard deviation of measured isotope values contributing to the stem isotope mixtures. Only GW (spring) instead of SW was available at the cloud forest site, and the dry forest did not allow to extract soil water nor did it rain from December 2020 until May 2021 (s, seasonal; eg, evergreen).

25 cm, representing the recent pulse of cold fronts. In contrast, soils below 50 cm described the isotopic composition of mean annual rainfall (Figure 7). During the dry season, the most dominant plants in the Páramo (∼3,400 m asl) used predominantly mobile water from cold fronts (62%) and, to a lesser degree, deeper soil water (12.3%) (Table 1 and Figure 8).

The cloud forest (2,400 m asl) (66%) and evergreen rainforest (2,000 m asl) (74%) plants showed a significant preference for relatively shallow (0–50 cm) well-mixed soil water (Figure 8). However, these plants also consumed recent cold fronts' rainfall, 17.4% and 18.6%, respectively. The seasonal rainforest revealed the strongest preference for well-mixed soil water (86.4%), followed by a lower influence of recent rainfall (7.1%), compared to other humid ecosystems. Using a tracer-aided model, Correa et al. (2020) evaluated how topography and vegetation modulated water flux and age in this seasonal rainforest site. The authors highlighted that stream water was a mixture of younger soil water and slightly older, deeper soil water and shallow groundwater with a maximum age of roughly 2 years during drought conditions. These results are consistent with our stem water isotopic findings biassed toward well-mixed soil moisture.

The ecohydrological prevalence of cold front rainfall uptake is highly relevant since multiple ecosystems across the interior mountain ranges of Costa Rica depend on this type of rainfall input during the dry season. However, recurring ENSO warm events (e.g., 2009–2010, 2014–2015, 2015–2016 and 2018–2019) have resulted in a notable suppression of the trade winds and in net rainfall deficit during the wet season across the Pacific slope (Hidalgo et al., 2015). For instance, Quesada-Román et al. (2020) found negative (positive) growth correlations during cold (warm) phases in the most representative Páramo shrub (i.e., *Hypericum irazuense*). The latter may force plants across high elevation ecosystems to compete for deeper water sources or constrain their growth as warm ENSO events strengthen in future climate scenarios (Aronson et al., 2019; Gushchina et al., 2020).

In contrast, plants in the dry forest relied on a more diverse and resilient mechanism. Water source contribution indicated a moderate uptake from the past hydrological year (38.6%) biassed toward the second rainfall maxima between September and October (19.5%). However, since the dry season in northwestern Costa Rica spans from mid-November to May (∼5–6 months) with a notable decrease in soil moisture, plants also revealed a moderate dependency on surface water (33.1%), which can be interpreted as groundwater contribution (deeper routing and competition) during a common and prolonged baseflow regime under high evaporative conditions (Arciniega-Esparza et al., 2022; Hund et al., 2021). The most prominent rainfall deficits in this region are experienced during the warm phase of ENSO (Babcock et al., 2016). While cold fronts do not contribute to rainfall events across the low elevation dry forest landscapes, ENSO significantly reduces precipitation amounts during the wet season (May to October). Cooley et al. (2019) reported that this region reached an all-time rainfall low in 2015 (the strongest Niño on record; Paek et al., 2017) over a 64-year record, with a wet season Standard Precipitation Index as low as −3.46, resulting in significant declines in green vegetation.

Cacti samples were collected in the dry forest ecosystem lying on three substrates (soil, tree and coastal rock cliff). These plants are characterized by shallow root systems (∼7–20 cm), rapid uptake of recent precipitation and a thick waxy cuticle to prevent water losses (Nobel, 2007: Hultine et al., 2016). Cacti have Crassulacean acid metabolism (CAM), a specialized type of photosynthesis in which stomata are closed during daylight but open in the darkness (fixing CO_2 with phosphoenolpyruvate carboxylase and accumulating organic acids in cell vacuoles), resulting in significant water savings (Gibson & Nobel, 1986; Hultine et al., 2016). A fully hydrated cactus stem has a water content of 90–94% (Gibson, 1998). The cactus stem sections we sampled exhibited similar water contents ranging from 82.7% (*S. testudo*; tree substrate) to 96.3% (*A. tetragonus*; soil substrate). *S. testudo* consistently showed a dehydration pattern in all triplicates (82.7–85.8%), followed by *O. guatemalensis* (89.0–91.0%) (soil-based). The largest amount of water extraction was reported for *A. tetragonus* samples (up to 35.3%). These samples also showed the lowest lc-excess values (−73.0‰), while tree-based (−46.7‰) and coastal rock-based (−33.3‰) resulted in less extreme evaporative losses. Tree-based cacti are shaded, while coastal rock-based cacti receive a constant ocean moistening breeze, resulting in more favourable environmental conditions. In contrast, soil cacti are exposed to a prolonged soil moisture deficit between mid-November and early May, encompassing our sampling period.

Water in cactus stems usually exhibit very high $\delta^{18}O$ values due to evaporative fractionation during storage in or transport through the plant (+4 to +20‰ in $\delta^{18}O$) (English et al., 2007), while $\delta^{18}O$ values in the spine tissue showed even more enriched compositions, +30 to +50‰, due to strong fractionation processes ($\varepsilon = +27$ for cellulose; Roden et al., 2000; Hultine et al., 2019). It is important to highlight that besides root water absorption, cacti can collect water through spines, absorb it through trichomes, and store it in mucilage (Kim et al., 2017); which altogether may affect the isotopic composition of water extracted from stem samples. The cacti $\delta^{18}O$ values (+1.7 to +14.4‰) in our study agree with typical cacti isotope ratios across arid environments (Boutton et al., 1999; English et al., 2007; Hultine et al., 2016). Similarly, large internal isotope gradients (vertical and radial) can emerge in cactus stems from the stem base to the apex. For example, in a controlled irrigation experiment, Hultine et al. (2019) reported a large (20–26%) $\delta^{18}O$ difference in a 90 cm saguaro cactus between the apex and the stem base. In our study, cactus samples were collected in the soil-based species' upper sections (apex) and the middle sections of tree-based samples. Coastal-rock cacti were sampled individually due to their spherical structure. Cacti $\delta^{18}O$ values are related to differences in water residence time, the degree of evaporative enrichment (i.e., stomata conductance and diffusion fractionation) and the water vapour in the surrounding atmosphere (Hultine et al., 2016). Since our cacti samples are circumscribed to the same isotope rainfall and water vapour regime (northwestern Costa Rica), $\delta^{18}O$ may reflect differences in residence time (+14.4‰, *A. tetragonus;* +1.7‰, *S. Aragonii*) and ecophysiological responses.

Water held in cactus has been used as a reliable proxy of climate changes, mainly to underpin ENSO's variability (English et al., 2007, 2010; Moreno-Millán, 2017; Skrzypek et al., 2013). Water residence time in cacti can vary seasonally since stomatal conductance and transpiration loss decreases during dry periods (mid-November to May), leading to higher residence times and progressively higher $\delta^{18}O$ of plant water (Hultine et al., 2019). In the case of the dry forest, the enriched isotope compositions and low *d*-excess and *lc*-excess (Figures 5 and 6) revealed strong fractionation processes potentially linked to water storage during the dry season and effects of the recent ENSO warm events in the region (e.g., 2009–2010, 2014–2015, 2015–2016 and 2018–2019).

To our knowledge, (1) the variety of distinct tropical ecosystems sampled in this study, at the driest edge of the hydrological year in 2021, (2) the relatively low complexity of the analytical procedure and (3) the isotopic results aligned with the well-known meteoric conditions across Costa Rica (Sánchez-Murillo, Esquivel-Hernández, Birkel, et al., 2020) provide evidence of the potential usefulness of the centrifugation method for ecohydrological studies across the wet tropics when continuous (i.e., 1 Hz) vapour measurements are unfeasible in remote and mountainous tropical ecosystems (i.e., non-existent or unreliable electrical sources for long-term monitoring periods). Spiked tests or comparisons with other methods were out of the scope of the present work, but the authors recognize the value of future experiments to better constrain analytical errors.

6 | CONCLUSIONS

This research provides novel isotope-based evidence of water uptake between groundwater, soil water, stream water, precipitation and plant water across contrasting and complex tropical ecosystems, offering new methodological and functional insights. From a methodological point of view, our study contributed to the highly debated issues related to water extraction techniques for isotopic analysis. The centrifugation method we applied extracted mobile water from all plant and cactus samples with no evidence of extraction-induced fractionation, suggesting that this approach can be used as a potential method in the wet tropics.

This study also provides advancements in our knowledge of water uptake and the ecohydrological functioning of tropical ecosystems, which are highly heterogeneous environments and, therefore, particularly complex to investigate. The water source contribution analysis denoted the prevalence of distinct water uptake mechanisms between recent cold front rainfall to more well-mixed soil moisture. However, in all ecosystems, plant water was closely related to relatively recent (most likely <1 year) meteoric, soil and surface waters. In contrast to what studies have reported for temperate ecosystems, our results showed nearly uniform soil isotope profiles with depth, with negligible fractionation during the dry season.

Further studies should be focused on a detailed characterization of species-based water use (i.e., the age distribution of transpiration) (Fresne et al., 2022), in a broader spectrum of soil tensions and during enriched and depleted rainfall isotope incursions (Sánchez-Murillo, Durán-Quesada, et al., 2019) across the main ecosystem gradients of Costa Rica (Beyer & Penna, 2021; Kappelle, 2016). Cactus samples, in particular, show great potential as a paleoenvironmental proxy for the dry forest ecosystem. More systematic studies should include radial and vertical isotopic differences, seasonality and a more robust number of replicates per substrate (soil, tree and coastal rocks).

ACKNOWLEDGEMENTS

This study was partially supported by International Atomic Energy Agency grants COS7005, RC-19747, CRP 22904 and RLA7024. Funding from the Research Office of the Universidad Nacional (Heredia, Costa Rica) through grants SIA-0482-13, SIA-0101-14, SIA-0236-16, SIA-411-17 and SIA-414-17 was crucial for maintaining rainfall, surface water and groundwater monitoring sites. MP is thankful to the Management and Environmental Studies Master Program at Universidad Nacional (Heredia, Costa Rica), which supported her travel expenses to Costa Rica for the purpose of this research. CB would like to thank the Leverhulme Trust (ISOLAND) and the UCR-OACG for partly supporting research time. We thank the access granted by Dr. Oscar Rojas-Carrillo to the POLIUNA laboratory facilities (Universidad Nacional) to conduct the centrifugation experiment. The authors also thank various helping hands and sampling permits from the Área de Conservación Guanacaste, Cerro Dantas Wildlife Refuge, Chirripó National Park, Reserva Biológica Alberto Manuel Brenes and Empresa de Servicios Públicos de Heredia. DTZ acknowledges the support received from the University of Florence (Italy) for international mobility (grants were derived from the decree No. 20440(165), which was part of the Strategic Plan for the period 2019-2021).

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORSHIP CONTRIBUTIONS

RSM, DTZ and MP designed the experiment. RSM and DTZ conducted the sampling campaigns and isotopic analysis. RSM curated the isotopic data. CB performed the model computation. RSM, MP, CB and GEH prepared the original draft. MP, DTZ, GZ and DP edited the original draft. All authors contributed to the final manuscript version.

ORCID

Ricardo Sánchez-Murillo https://orcid.org/0000-0001-8721-8093
María Poca https://orcid.org/0000-0001-9160-1036
Giulia Zuecco https://orcid.org/0000-0002-2125-0717
Daniele Penna https://orcid.org/0000-0001-6915-0697

REFERENCES

Allen, S. T., & Kirchner, J. W. (2022). Potential effects of cryogenic extraction biases on plant water source partitioning inferred from xylem-water isotope ratios. *Hydrological Processes*, *36*(2), e14483. https://doi.org/10.1002/hyp.14483

Allen, S. T., Kirchner, J. W., Braun, S., Siegwolf, R. T. W., & Goldsmith, G. R. (2019). Seasonal origins of soil water used by trees. *Hydrology and Earth System Sciences*, *23*, 1199–1210, https://doi.org/10.5194/hess-23-1199-2019

Alvarado, A., Mata, R., & Kappelle, M. (2015). *Soils of Costa Rica: An agroecological approach* (pp. 64–93). Costa Rican Ecosystems.

Amin, A., Zuecco, G., Geris, J., Schwendenmann, L., McDonnell, J. J., Borga, M., & Penna, D. (2020). Depth distribution of soil water sourced by plants at the global scale: A new direct inference approach. *Ecohydrology*, *13*, e2177. https://doi.org/10.1002/eco.2177

Aparecido, L. M. T., Teodoro, G. S., Mosquera, G., Brum, M., Barros, F. D. V., Pompeu, P. V., Rodas, M., Lazo, P., Müller, C. S., Mulligan, M., & Asbjornsen, H. (2018). Ecohydrological drivers of Neotropical vegetation in montane ecosystems. *Ecohydrology*, *11*(3), e1932. https://doi.org/10.1002/eco.1932

Arciniega-Esparza, S., Birkel, C., Chavarría-Palma, A., Arheimer, B., & Breña-Naranjo, J. A. (2022). Remote sensing-aided rainfall–runoff modeling in the tropics of Costa Rica. *Hydrology and Earth System Sciences*, *26*, 975–999. https://doi.org/10.5194/hess-26-975-2022

Aronson, E. L., Dierick, D., Botthoff, J. K., Oberbauer, S., Zelikova, T. J., Harmon, T. C., Rundel, P., Johnson, R. F., Swanson, A. C., Pinto-Tomás, A. A., Artavia-León, A., Matarrita-Carranza, B., & Allen, M. F. (2019). ENSO-influenced drought drives methane flux dynamics in a tropical wet forest soil. *Journal of Geophysical Research – Biogeosciences*, *124*, 2267–2276. https://doi.org/10.1029/2018JG004832

Babcock, M., Wong-Parodi, G., Small, M. J., & Grossmann, I. (2016). Stakeholder perceptions of water systems and hydro-climate information in Guanacaste, Costa Rica. Earth. *Perspectives*, *3*(3), 1–13. https://doi.org/10.1186/s40322-016-0035-x

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J. C., & Ogée, J. (2022). Evidence for distinct isotopic compositions of sap and tissue water in tree stems: Consequences for plant water source identification. *New Phytologist*, *233*(3), 1121–1132. https://doi.org/10.1111/nph.17857

Barbeta, A., Gimeno, T. E., Clavé, L., Fréjaville, B., Jones, S. P., Delvigne, C., Wingate, L., & Ogée, J. (2020). An explanation for the isotopic offset between soil and stem water in a temperate tree species. *New Phytologist*, *227*(3), 766–779. https://doi.org/10.1111/nph.16564

Barbeta, A., Jones, S. P., Clavé, L., Wingate, L., Gimeno, T. E., Fréjaville, B., Wohl, S., & Ogée, J. (2019). Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences*, *23*(4), 2129–2146. https://doi.org/10.5194/hess-23-2129-2019

Barbeta, A., & Peñuelas, J. (2017). Relative contribution of groundwater to plant transpiration estimated with stable isotopes. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-09643-x

Baron Ruiz, O. J. (2021). *Calibration and validation of a SWAT model for the quantification of water provision ecosystem service for the conservation area of Guanacaste*. M.Sc. Thesis. University of Alberta.

Beyer, M., & Penna, D. (2021). On the spatio-temporal under-representation of isotopic data in ecohydrological studies. *Frontiers in Water*, *3*, 16. https://doi.org/10.3389/frwa.2021.643013

Blanco, C. M. G., Gomez, V. M. B., Crespo, P., & Ließ, M. (2018). Spatial prediction of soil water retention in a Páramo landscape: Methodological insight into machine learning using random forest. *Geoderma*, *316*, 100–114. https://doi.org/10.1016/j.geoderma.2017.12.002

Bodé, S., de Wispelaere, L., Hemp, A., Verschuren, D., & Boeckx, P. (2020). Water-isotope ecohydrology of Mount Kilimanjaro. *Ecohydrology*, *13*(1), e2171. https://doi.org/10.1002/eco.2171

Boutton, T. W., Archer, S. R., & Midwood, A. J. (1999). Stable isotopes in ecosystem science: Structure, function and dynamics of a subtropical savanna. *Rapid Communications in Mass Spectrometry*, *13*(13), 1263–1277. 10.1002/(SICI)1097-0231(19990715)13:13<1263::AID-RCM653>3.0.CO;2-J

Bowers, W. H., Mercer, J. J., Pleasants, M. S., & Williams, D. G. (2020). A combination of soil water extraction methods quantifies the isotopic mixing of waters held at separate tensions in soil. *Hydrology and Earth System Sciences*, *24*(8), 4045–4060. https://doi.org/10.5194/hess-24-4045-2020

Bowers, W. H., & Williams, D. G. (2022). Isotopic heterogeneity of stem water in conifers is correlated to xylem hydraulic traits and supports multiple residence times. *Frontiers in Water*, *4*, 861590. https://doi.org/10.3389/frwa.2022.861590

Brantley, S. L., Eissenstat, D. M., Marshall, J. A., Godsey, S. E., Balogh-Brunstad, Z., Karwan, D. L., Papuga, S. A., Roering, J., Dawson, T. E., Evaristo, J., Chadwick, O., McDonnell, J. J., & Weathers, K. C. (2017). Reviews and syntheses: On the roles trees play in building and plumbing the critical zone. *Biogeosciences*, *14*(22), 5115–5142. https://doi.org/10.5194/bg-14-5115-2017

Brooks, R. J., Barnard, H. R., Coulombe, R., & McDonnell, J. J. (2010). Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience*, *3*(2), 100–104. https://doi.org/10.1038/ngeo722

Caballero, L. A., Rimmer, A., Easton, Z. M., & Steenhuis, T. S. (2012). Rainfall runoff relationships for a cloud forest watershed in Central America: Implications for water resource engineering. *JAWRA Journal of the American Water Resources Association*, *48*, 1022–1031. https://doi.org/10.1111/j.1752-1688.2012.00668.x

Clark, D. B., Hurtado, J., & Saatchi, S. S. (2015). Tropical rain forest structure, tree growth and dynamics along a 2700-m elevational transect in Costa Rica. *PLoS ONE*, *10*(4), e0122905. https://doi.org/10.1371/journal.pone.0122905

Cooley, S. S., Williams, C. A., Fisher, J. B., Halverson, G. H., Perret, J., & Lee, C. M. (2019). Assessing regional drought impacts on vegetation and evapotranspiration: A case study in Guanacaste, Costa Rica. *Ecological Applications*, *29*(2), e01834. https://doi.org/10.1002/eap.1834

Correa, A., Birkel, C., Gutierrez, J., Dehaspe, J., Durán-Quesada, A. M., Soulsby, C., & Sánchez-Murillo, R. (2020). Modelling non-stationary water ages in a tropical rainforest: A preliminary spatially distributed assessment. *Hydrological Processes*, *34*, 4776–4793. https://doi.org/10.1002/hyp.13925

Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus*, *16*(4), 436–468. https://doi.org/10.3402/tellusa.v16i4.8993

Dawson, T. E., & Ehleringer, J. R. (1993). Isotopic enrichment of water in the "woody" tissues of plants: Implications for plant water source, water uptake, and other studies which use stable isotopic composition of cellulose. *Geochimica et Cosmochimica Acta*, *57*, 3487–3492. https://doi.org/10.1016/0016-7037(93)90554-A

Dawson, T. E., Jesse Hahm, W., & Crutchfield-Peters, K. (2020). Digging deeper: What the critical zone perspective adds to the study of plant ecophysiology. *New Phytologist*, *226*(3), 666–671. https://doi.org/10.1111/nph.16410

de Wispelaere, L., Bodé, S., Hervé-Fernández, P., Hemp, A., Verschuren, D., & Boeckx, P. (2017). Plant water resource partitioning and isotopic fractionation during transpiration in a seasonally dry tropical climate. *Biogeosciences*, *14*(1), 73–88. https://doi.org/10.5194/bg-14-73-2017

Dehaspe, J., Birkel, C., Tetzlaff, D., Sánchez-Murillo, R., Durán-Quesada, A. M., & Soulsby, C. (2018). Spatially distributed tracer-aided modelling to explore water and isotope transport, storage and mixing in a pristine, humid tropical catchment. *Hydrological Processes*, *32*, 3206–3224. https://doi.org/10.1002/hyp.13258

Diao, H., Schuler, P., Goldsmith, G. R., Siegwolf, R. T. W., Saurer, M., & Lehmann, M. M. (2022). On uncertainties in plant water isotopic composition following extraction by cryogenic vacuum distillation. *Hydrology and Earth System Sciences*, *26*, 5835–5847. https://doi.org/10.5194/hess-26-5835-2022

Dubbert, M., Couvreur, V., Kübert, A., & Werner, C. (2023). Plant water uptake modelling: Added value of cross-disciplinary approaches. *Plant Biology*. *25*, 32–42. https://doi.org/10.1111/plb.13478

Ehleringer, J. R., & Dawson, T. E. (1992). Water uptake by plants: Perspectives from stable isotope composition. *Plant, Cell & Environment*, *15*(9), 1073–1082. https://doi.org/10.1111/j.1365-3040.1992.tb01657.x

Ellenberg, D., & Mueller-Dombois, D. (1974). *Aims and methods of vegetation ecology* (p. 547). Wiley.

Ellsworth, P. Z., & Williams, D. G. (2007). Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant and Soil*, *291*(1), 93–107. https://doi.org/10.1007/s11104-006-9177-1

Elvidge, A. D., & Renfrew, I. A. (2016). The causes of foehn warming in the lee of mountains. *Bulletin of the American Meteorological Society*, *97*(3), 455–466. https://doi.org/10.1175/BAMS-D-14-00194.1

English, N. B., Dettman, D. L., Sandquist, D. R., & Williams, D. G. (2007). Past climate changes and ecophysiological responses recorded in the isotope ratios of saguaro cactus spines. *Oecologia*, *154*, 247–258. https://doi.org/10.1007/s00442-007-0832-x

English, N. B., Dettman, D. L., & Williams, D. G. (2010). A 26-year stable isotope record of humidity and El Niño-enhanced precipitation in the spines of saguaro cactus, Carnegiea gigantea. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *293*(1–2), 108–119. https://doi.org/10.1016/j.palaeo.2010.05.005

Esquivel-Hernández, G., Mosquera, G. M., Sánchez-Murillo, R., Quesada-Román, A., Birkel, C., Crespo, P., Célleri, R., Windhorst, D., Breuer, L., & Boll, J. (2019). Moisture transport and seasonal variations in the stable isotopic composition of rainfall in Central American and Andean Páramo during El Niño conditions (2015–2016). *Hydrological Processes*, *33*, 1802, hyp.13438–1817. https://doi.org/10.1002/hyp.13438

Esquivel-Hernández, G., Sánchez-Murillo, R., Birkel, C., Good, S. P., & Boll, J. (2017). Hydroclimatic and ecohydrological resistance/resilience conditions across tropical biomes of Costa Rica. *Ecohydrology*, *10*, e1860. https://doi.org/10.1002/eco.1860

Esquivel-Hernández, G., Sánchez-Murillo, R., Quesada-Román, A., Mosquera, G. M., Birkel, C., & Boll, J. (2018). Insight into the stable isotopic composition of glacial lakes in a tropical alpine ecosystem: Chirripó, Costa Rica. *Hydrological Processes*, *2018*, 1–16. https://doi.org/10.1002/hyp.13286

Esquivel-Hernández, G., Sánchez-Murillo, R., & Vargas-Salazar, E. (2021). Chirripó hydrological research site: Advancing stable isotope hydrology in the Central American Páramo. *Hydrological Processes*, *35*, e14181. https://doi.org/10.1002/hyp.14181

Evaristo, J., Jasechko, S., & McDonnell, J. J. (2015). Global separation of plant transpiration from groundwater and streamflow. *Nature*, *525*(7567), 91–94. https://doi.org/10.1038/nature14983

Evaristo, J., & McDonnell, J. J. (2017). Prevalence and magnitude of groundwater use by vegetation: A global stable isotope meta-analysis. *Scientific Reports*, *7*(1), 1, 44110–12. https://doi.org/10.1038/srep44110

Evaristo, J., McDonnell, J. J., Scholl, M. A., Bruijnzeel, L. A., & Chun, K. P. (2016). Insights into plant water uptake from xylem-water isotope measurements in two tropical catchments with contrasting moisture conditions. *Hydrological Processes*, *30*(18), 3210–3227. https://doi.org/10.1002/hyp.10841

Fan, Y., Miguez-Macho, G., Jobbágy, E. G., Jackson, R. B., & Otero-Casal, C. (2017). Hydrologic regulation of plant rooting depth. *Proceedings of the National Academy of Sciences*, *114*(40), 10572–10577. https://doi.org/10.1073/pnas.1712381114

Figueroa-Johnson, M. A., Tindall, J. A., & Friedel, M. A. (2007). Comparison of $\delta^{18}O$ composition of water extracted from suction lysimeters, centrifugation, and azeotropic distillation. *Water, Air, and Soil Pollution*, *184*, 63–75. https://doi.org/10.1007/s11270-007-9399-8

Fischer, B. M. C., Frentress, J., Manzoni, S., Cousins, S. A. O., Hugelius, G., Greger, M., Smittenberg, R. H., & Lyon, S. W. (2019). Mojito, anyone? An exploration of low-tech plant water extraction methods for isotopic analysis using locally-sourced materials. *Frontiers in Earth Science*, *7*, 150. https://doi.org/10.3389/feart.2019.00150

Fresne, M., Chun, K. P., Fabiani, G., Hrachowitz, M., McGuire, K., Moussa, A., Schoppach, R., & Klaus, J. 2022 Integrating transpiration and xylem water stable isotopes in a process-based model to determine transpiration and discharge age distributions, EGU General Assembly 2022, Vienna, Austria, 23–27 May 2022, EGU22-3046, https://doi.org/10.5194/egusphere-egu22-3046

Gaj, M., Kaufhold, S., Koeniger, P., Beyer, M., Weiler, M., & Himmelsbach, T. (2017). Mineral mediated isotope fractionation of soil water. *Rapid Communications in Mass Spectrometry*, *31*, 269–280. https://doi.org/10.1002/rcm.7787

Gat, J. R. (1996). Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences*, *24*(1), 225–262. https://doi.org/10.1146/annurev.earth.24.1.225

Geris, J., Tetzlaff, D., McDonnell, J., Anderson, J., Paton, G., & Soulsby, C. (2015). Ecohydrological separation in wet, low energy northern environments? A preliminary assessment using different soil water extraction techniques. *Hydrological Processes*, *29*, 5139–5152. https://doi.org/10.1002/hyp.10603

Gibson, A. C. (1998). Photosynthetic organs of desert plants. *Bioscience*, *48*(11), 911–920. https://doi.org/10.2307/1313295

Gibson, A. C., & Nobel, P. S. (1986). *The cactus primer*. Harvard University. https://doi.org/10.4159/harvard.9780674281714

Goldsmith, G. R., Allen, S. T., Braun, S., Engbersen, N., González-Quijano, C. R., Kirchner, J. W., & Siegwolf, R. T. (2019). Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest. *Ecohydrology*, *12*(2), e2059. https://doi.org/10.1002/eco.2059

Goldsmith, G. R., Muñoz-Villers, L. E., Holwerda, F., McDonnell, J. J., Asbjornsen, H., & Dawson, T. E. (2012). Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. *Ecohydrology*, *5*(6), 779–790. https://doi.org/10.1002/eco.268

Gushchina, D., Zheleznova, I., Osipov, A., & Olchev, A. (2020). Effect of various types of ENSO events on moisture conditions in the humid and subhumid tropics. *Atmosphere*, *11*(12), 1354. https://doi.org/10.3390/atmos11121354

Hahm, W. J., Rempe, D. M., Dralle, D. N., Dawson, T. E., & Dietrich, W. E. (2020). Oak transpiration drawn from the weathered bedrock vadose zone in the summer dry season. *Water Resources Research*, *56*(11), e2020WR027419. https://doi.org/10.1029/2020WR027419

Herrera, W. (2016). *Climate of Costa Rica. Costa Rican ecosystems* (pp. 19–29). University of Chicago Press.

Hervé-Fernández, P., Oyarzún, C., Brumbt, C., Huygens, D., Bodé, S., Verhoest, N. E. C., & Boeckx, P. J. H. P. (2016). Assessing the 'two water worlds' hypothesis and water sources for native and exotic evergreen species in south-central Chile. *Hydrological Processes*, *30*(23), 4227–4241. https://doi.org/10.1002/hyp.10984

Hidalgo, H., Durán-Quesada, A. M., Amador, J. A., & Alfaro, E. J. (2015). The Caribbean low-level jet, the inter-tropical convergence zone and precipitation patterns in the intra-Americas sea: A proposed dynamical mechanism. *Geografiska Annaler. Series A, Physical Geography*, *97*(1), 41–59. https://doi.org/10.1111/geoa.12085

Hidalgo, H. G., Amador, J. A., Alfaro, E. J., & Quesada, B. (2013). Hydrological climate change projections for Central America. *Journal of Hydrology*, *495*, 94–112. https://doi.org/10.1016/j.jhydrol.2013.05.004

Holdridge, L. R. (1978). *Ecología Basada en Zonas de Vida*. IICA.

Hultine, K. R., Dettman, D. L., English, N. B., Williams, D. G. (2019). Giant cacti: Isotopic recorders of climate variation in warm deserts of the Americas. *Journal of Experimental Botany*, *70*(22), 6509–6519. https://doi.org/10.1093/jxb/erz320

Hultine, K. R., Williams, D. G., Dettman, D. L., Butterfield, B. J., & Puente-Martinez, R. (2016). Stable isotope physiology of stem succulents across a broad range of volume-to-surface area ratio. *Oecologia*, *182*, 679–690. https://doi.org/10.1007/s00442-016-3690-6

Hund, S. V., Allen, D. M., Morillas, L., & Johnson, M. S. (2018). Groundwater recharge indicator as tool for decision makers to increase socio-hydrological resilience to seasonal drought. *Journal of Hydrology*, *563*, 1119–1134. https://doi.org/10.1016/j.jhydrol.2018.05.069

Hund, S. V., Grossmann, I., Steyn, D. G., Allen, D. M., & Johnson, M. S. (2021). Changing water resources under El Niño, climate change, and growing water demands in seasonally dry tropical watersheds. *Water Resources Research*, *57*, e2020WR028535. https://doi.org/10.1029/2020WR028535

Jiménez-Rodríguez, C. D., Coenders-Gerrits, M., Wenninger, J., Gonzalez-Angarita, A., & Savenije, H. (2020). Contribution of understory evaporation in a tropical wet forest during the dry season. *Hydrology and Earth System Sciences*, *24*(4), 2179–2206. https://doi.org/10.5194/hess-24-2179-2020

Jobágy, E. G., Lorenzo, S., Buono, N., Páez, R., Diaz, Y., Marchesini, V., & Nosetto, M. D. (2021). Plants versus streams: Their groundwater-mediated competition at "El Morro," a developing catchment in the dry plains of Argentina. *Hydrological Processes*, *35*, e14188. https://doi.org/10.1002/hyp.14188

Kappelle, M. (Ed.). (2016). *Costa Rican ecosystems*. University of Chicago Press. https://doi.org/10.7208/chicago/9780226121642.001.0001

Kappelle, M., & Horn, S. P. (2016). The Páramo Grasslands of Costa Rica's Highlands. In M. Kappelle (Ed.), *Costa Rican ecosystems* (pp. 492–523). University of Chicago Press. https://doi.org/10.7208/chicago/9780226121642.003.0015

Karmalkar, A. V., Bradley, R. S., & Diaz, H. F. (2008). Climate change scenario for Costa Rican montane forests. *Geophysical Research Letters*, *35*, L11702. https://doi.org/10.1029/2008GL033940

Kendall, C., & McDonnell, J. J. (Eds.). (2012). *Isotope tracers in catchment hydrology*. Elsevier.

Kim, K., Kim, H., Park, S. H., & Joon Lee, S. (2017). Hydraulic strategy of cactus trichome for absorption and storage of water under arid environment. *Frontiers in Plant Science*, *8*, 1777. https://doi.org/10.3389/fpls.2017.01777

Knighton, J., Kuppel, S., Smith, A., Soulsby, C., Sprenger, M., & Tetzlaff, D. (2020). Using isotopes to incorporate tree water storage and mixing dynamics into a distributed ecohydrologic modelling framework. *Ecohydrology*, *13*(3), e2201. https://doi.org/10.1002/eco.2201

Kühnhammer, K., Dahlmann, A., Iraheta, A., Gerchow, M., Birkel, C., Marshall, J. D., & Beyer, M. (2022). Continuous in situ measurements of water stable isotopes in soils, tree trunk and root xylem: Field approval. *Rapid Communications in Mass Spectrometry*, *36*(5), e9232. https://doi.org/10.1002/rcm.9232

Landwehr, J. M., & Coplen, T. B. (2006). Line-conditioned excess: A new method for characterizing stable hydrogen and oxygen isotope ratios in hydrologic systems. In *International conference on isotopes in environmental studies* (pp. 132–135). IAEA Vienna.

Leen, J. B., Berman, E. S. F., Liebson, L., & Gupta, M. (2012). Spectral contaminant identifier for off-axis integrated cavity output spectroscopy measurements of liquid water isotopes. *The Review of Scientific Instruments*, *83*(4), 044305. https://doi.org/10.1063/1.4704843

Li, F., Song, X., Tang, C., Liu, C., Yu, J., & Zhang, W. (2007). Tracing infiltration and recharge using stable isotope in Taihang Mt., North China. *Environmental Geology*, *53*, 687–696. https://doi.org/10.1007/s00254-007-0683-0

Lin, G., & da Sternberg, L. S. L. (1993). Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In *Stable isotopes and plant carbon-water relations* (pp. 497–510). Academic Press. https://doi.org/10.1016/B978-0-08-091801-3.50041-6

Lyon, S. W., Fischer, B. M. C., Morillas, L., Rojas Conejo, J., Sánchez-Murillo, R., Suárez Serrano, A., Frentress, J., Cheng, C.-H., Garcia, M., &

Johnson, M. S. (2022). On the potential of biochar soil amendments as a sustainable water management strategy. *Sustainability*, *14*, 7026. https://doi.org/10.3390/su14127026

Mayer-Anhalt, L., Birkel, C., Sánchez-Murillo, R., & Schulz, S. (2022). Tracer-aided modelling reveals quick runoff generation and young streamflow ages in a tropical rainforest catchment. *Hydrological Processes*, *36*(2), e14508. https://doi.org/10.1002/hyp.14508

Miguez-Macho, G., & Fan, Y. (2021). Spatiotemporal origin of soil water taken up by vegetation. *Nature*, *598*, 624–628. https://doi.org/10.1038/s41586-021-03958-6

Millar, C., Janzen, K., Nehemy, M. F., Koehler, G., Hervé-Fernández, P., Wang, H., Orlowski, N., Barbeta, A., & McDonnell, J. J. (2022). On the urgent need for standardization in isotope-based ecohydrological investigations. *Hydrological Processes*, *36*(10), e14698. https://doi.org/10.1002/hyp.14698

Millar, C., Pratt, D., Schneider, D. J., & McDonnell, J. J. (2018). A comparison of extraction systems for plant water stable isotope analysis. *Rapid Communications in Mass Spectrometry*, *32*(13), 1031–1044. https://doi.org/10.1002/rcm.8136

Morales, J. F. (2001). *Orquídeas, cactus y bromelias del bosque seco* (p. 182). Instituto Nacional de Biodiversidad.

Moreno-Millán, J. (2017). *Isotopic analysis in columnar cactus (Stenocereus griseus) spines in the Desierto de la Tatacoa-climatic and physiological approaches*. Uniandes.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853–858. https://doi.org/10.1038/35002501

Nehemy, M. F., Millar, C., Janzen, K., Gaj, M., Pratt, D. L., Laroque, C. P., & McDonnell, J. J. (2019). ^{17}O-excess as a detector for co-extracted organics in vapor analyses of plant isotope signatures. *Rapid Communications in Mass Spectrometry*, *33*, 1301–1310. https://doi.org/10.1002/rcm.8470

Nobel, P. S. (2007). Cactus roots: Depth and sensitivity to extreme temperatures. In *VI international congress on cactus pear and cochineal* (Vol. 811) (pp. 383–388). ISHS. https://doi.org/10.17660/ActaHortic.2009.811.52

Orlowski, N., Breuer, L., & Mcdonnell, J. J. (2016). Critical issues with cryogenic extraction of soil water for stable isotope analysis. *Ecohydrology*, *9*, 3–10. https://doi.org/10.1002/eco.1722

Orlowski, N., Frede, H. G., Brüggemann, N., & Breuer, L. (2013). Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *Journal of Sensors and Sensor Systems*, *2*(2), 179–193. https://doi.org/10.5194/jsss-2-179-2013

Orlowski, N., Pratt, D. L., & McDonnell, J. J. (2016). Intercomparison of soil pore water extraction methods for stable isotope analysis. *Hydrological Processes*, *30*, 3434–3449. https://doi.org/10.1002/hyp.10870

Orlowski, N., Pratt, D. L., & McDonnell, J. J. (2019). Intercomparison of soil pore water extraction methods for stable isotope analysis and interpretation of hillslope runoff sources. *Hydrological Processes*, *33*, 2939–2954. https://doi.org/10.1002/hyp.13539

Paek, H., Yu, J.-Y., & Qian, C. (2017). Why were the 2015/2016 and 1997/1998 extreme El Niños different? *Geophysical Research Letters*, *44*, 1848–1856. https://doi.org/10.1002/2016GL071515

Penna, D., Hopp, L., Scandellari, F., Allen, S. T., Benettin, P., Beyer, M., Geris, J., Klaus, J., Marshall, J. D., Schwendenmann, L., Volkmann, T. H. M., von Freyberg, J., Amin, A., Ceperley, N., Engel, M., Frentress, J., Giambastiani, Y., McDonnell, J. J., Zuecco, G., … Kirchner, J. W. (2018). Ideas and perspectives: Tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes—Challenges and opportunities from an interdisciplinary perspective. *Biogeosciences*, *15*, 6399–6415. https://doi.org/10.5194/bg-15-6399-2018

Penna, D., Zanotelli, D., Scandellari, F., Aguzzoni, A., Engel, M., Tagliavini, M., & Comiti, F. (2021). Water uptake of apple trees in the Alps: Where does irrigation water go? *Ecohydrology*, *14*(6), e2306. https://doi.org/10.1002/eco.2306

Poca, M., Coomans, O., Urcelay, C., Zeballos, S. R., Bodé, S., & Boeckx, P. (2019). Isotope fractionation during root water uptake by Acacia caven is enhanced by arbuscular mycorrhizas. *Plant and Soil*, *441*(1), 485–497. https://doi.org/10.1007/s11104-019-04139-1

Quesada-Román, A., Ballesteros-Cánovas, J. A., Guillet, S., Madrigal-González, J., & Stoffel, M. (2020). Neotropical Hypericum irazuense shrubs reveal recent ENSO variability in Costa Rican Páramo. *Dendrochronologia*, *61*, 125704. https://doi.org/10.1016/j.dendro.2020.125704

Rafiqpoor, D., Kier, G., & Kreft, H. (2005). Global centers of vascular plant diversity. *Nova Acta Leopoldina NF*, *92*(342), 61–83.

Rhodes, A. L., Guswa, A. J., & Newell, S. E. (2006). Seasonal variation in the stable isotopic composition of precipitation in the tropical montane forests of Monteverde, Costa Rica. *Water Resources Research*, *42*, W11402. https://doi.org/10.1029/2005WR004535

Roden, J. S., Lin, G., & Ehleringer, J. R. (2000). A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochimica et Cosmochimica Acta*, *64*, 21–35. https://doi.org/10.1016/S0016-7037(99)00195-7

Salas-Navarro, J., Sánchez-Murillo, R., Esquivel-Hernández, G., & Corrales-Salazar, J. L. (2019). Hydrogeological responses in tropical mountainous springs. *Isotopes in Environmental and Health Studies*, *55*(1), 25–40. https://doi.org/10.1080/10256016.2018.1546701

Sánchez-Murillo, R., Durán-Quesada, A. M., Esquivel-Hernández, G., Rojas-Cantillano, D., Birkel, C., Welsh, K., Sánchez-Llull, M., Alonso-Hernández, C. M., Tetzlaff, D., Soulsby, C., Boll, J., Kurita, N., & Cobb, K. M. (2019). Deciphering key processes controlling rainfall isotopic variability during extreme tropical cyclones. *Nature Communications*, *10*, 4321. https://doi.org/10.1038/s41467-019-12062-3

Sánchez-Murillo, R., & Birkel, C. (2016). Groundwater recharge mechanisms inferred from isoscapes in a complex tropical mountainous region. *Geophysical Research Letters*, *43*, 5060–5069. https://doi.org/10.1002/2016GL068888

Sánchez-Murillo, R., Esquivel-Hernández, G., Birkel, C., Correa, A., Welsh, K., Durán-Quesada, A. M., Sánchez-Gutiérrez, R., & Poca, M. (2020). Tracing water sources and fluxes in a dynamic tropical environment: From observations to modeling. *Frontiers in Earth Science*, *8*, 571477. https://doi.org/10.3389/feart.2020.571477

Sánchez-Murillo, R., Esquivel-Hernández, G., Corrales-Salazar, J. L., Castro-Chacón, L., Durán-Quesada, A. M., Guerrero-Hernández, M., Delgado, V., Barberena, J., Montenegro-Rayo, K., Calderón, H., Chevez, C., Peña-Paz, T., García-Santos, S., Ortiz-Roque, P., Alvarado-Callejas, Y., Benegas, L., Hernández-Antonio, A., Matamoros-Ortega, M., Ortega, L., & Terzer-Wassmuth, S. (2020). Tracer hydrology of the data-scarce and heterogeneous Central American Isthmus. *Hydrological Processes*, *34*, 2660, hyp.13758–2675. https://doi.org/10.1002/hyp.13758

Sánchez-Murillo, R., Gastezzi, P., Sanchez-Gutierrez, R., Perez-Salazar, R., Esquivel-Hernandez, G., & Poca, M. (2022). Exploring dissolved organic carbon variations in a high elevation tropical peatland ecosystem: Cerro de la Muerte, Costa Rica. *Frontiers in Water*, *3*, 742780. https://doi.org/10.3389/frwa.2021.742780

Sánchez-Murillo, R., Romero-Esquivel, L. G., Jiménez-Antillón, J., Salas-Navarro, J., Corrales-Salazar, L., Álvarez-Carvajal, J., Álvarez-McInerney, S., Bonilla-Barrantes, D., Gutiérrez-Sibaja, N., Martínez-Arroyo, M., Ortiz-Apuy, E., Salgado-Lobo, J., Villalobos-Morales, J., Esquivel-Hernández, G., Rojas-Jiménez, L. D., Gómez-Castro, C., Jiménez-Madrigal, Q., Vargas-Gutiérrez, O., & Birkel, C. (2019). DOC transport and export in a dynamic tropical catchment. *Journal of Geophysical Research – Biogeosciences*, *124*(6), 1665–1679. https://doi.org/10.1029/2018JG004897

Schultz, N. M., Griffis, T. J., Lee, X., & Baker, J. M. (2011). 2011. Identification and correction of spectral contamination in 2H/1H and 18O/16O measured in leaf, stem, and soil water. *Rapid Communications in Mass Spectrometry*, *25*(21), 3360–3368. https://doi.org/10.1002/rcm.5236

Skrzypek, G., Paul, D., & Wojtuń, B. (2013). The altitudinal climatic effect on the stable isotope compositions of Agave and Opuntia in arid environments—A case study at the Big Bend National Park, Texas, USA. *Journal of Arid Environments*, *92*, 102–112. https://doi.org/10.1016/j.jaridenv.2013.02.002

Song, X., Chen, Y., Helliker, B. R., Tang, X., Li, F., & Zhou, Y. (2021). Reply to Evaristo et al.: Strong evidence for the need of correcting extraction bias in an early study of ecohydrological separation. *Proceedings of the National Academy of Sciences*, *118*(17), e2103604118. https://doi.org/10.1073/pnas.2103604118

Sprenger, M., & Allen, S. T. (2020). What ecohydrologic separation is and where we can go with it. *Water Resources Research*, *56*(7), e2020WR027238. https://doi.org/10.1029/2020WR027238

Sprenger, M., Herbstritt, B., & Weiler, M. (2015). Established methods and new opportunities for pore water stable isotope analysis. *Hydrological Processes*, *29*(25), 5174–5192. https://doi.org/10.1002/hyp.10643

Sprenger, M., Leistert, H., Gimbel, K., & Weiler, M. (2016). Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes. *Reviews of Geophysics*, *54*(3), 674–704. https://doi.org/10.1002/2015RG000515

Sprenger, M., Stumpp, C., Weiler, M., Aeschbach, W., Allen, S. T., Benettin, P., Dubbert, M., Hartmann, A., Hrachowitz, M., Kirchner, J. W., McDonnell, J. J., Orlowski, N., Penna, D., Pfahl, S., Rinderer, M., Rodriguez, N., Schmidt, M., & Werner, C. (2019). The demographics of water: A review of water ages in the critical zone. *Reviews of Geophysics*, *57*, 800–834. https://doi.org/10.1029/2018RG000633

Stan, K., & Sanchez-Azofeifa, A. (2019). Tropical dry forest diversity, climatic response, and resilience in a changing climate. *Forests*, *10*, 443. https://doi.org/10.3390/f10050443

Stock, B. C., Jackson, A. L., Ward, E. J., Parnell, A. C., Phillips, D. L., & Semmens, B. X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ*, *6*, e5096. https://doi.org/10.7717/peerj.5096

Tron, S., Laio, F., & Ridolfi, L. (2013). Plant water uptake strategies to cope with stochastic rainfall. *Advances in Water Resources*, *53*, 118–130. https://doi.org/10.1016/j.advwatres.2012.10.007

Tsuruta, K., Yamamoto, H., Katsuyama, M., Kosugi, Y., Okumura, M., & Matsuo, N. (2019). Effects of cryogenic vacuum distillation on the stable isotope ratios of soil water. *Hydrological Research Letters*, *13*(1), 1–6. https://doi.org/10.3178/hrl.13.1

Veintimilla, D., Ngo Bieng, M. A., Delgado, D., Vilchez-Mendoza, S., Zamora, N., & Finegan, B. (2019). Drivers of tropical rainforest composition and alpha diversity patterns over a 2,520 m altitudinal gradient. *Ecology and Evolution*, *9*, 5720–5730. https://doi.org/10.1002/ece3.5155

von Freyberg, J., Allen, S. T., Grossiord, C., & Dawson, T. E. (2020). Plant and root-zone water isotopes are difficult to measure, explain, and predict: Some practical recommendations for determining plant water sources. *Methods in Ecology and Evolution*, *11*, 1352–1367. https://doi.org/10.1111/2041-210X.13461

Wen, M., He, D., Li, M., Ren, R., Jin, J., & Si, B. (2022). Causes and factors of cryogenic extraction biases on isotopes of xylem water. *Water Resources Research*, *58*(8), 2022WR032182. https://doi.org/10.1029/2022WR032182

Wright, C., Kagawa-Viviani, A., Gerlein-Safdi, C., Mosquera, G. M., Poca, M., Tseng, H., & Chun, K. P. (2018). Advancing ecohydrology in the changing tropics: Perspectives from early career scientists. *Ecohydrology*, *11*(3), e1918. https://doi.org/10.1002/eco.1918

Yakir, D., & Sternberg, L. D. S. L. (2000). The use of stable isotopes to study ecosystem gas exchange. *Oecologia*, *123*(3), 297–311. https://doi.org/10.1007/s004420051016

Zhao, L., Wang, L., Cernusak, L. A., Liu, X., Xiao, H., Zhou, M., & Zhang, S. (2016). Significant difference in hydrogen isotope composition between xylem and tissue water in Populus euphratica. *Plant, Cell & Environment*, *39*(8), 1848–1857. https://doi.org/10.1111/pce.12753

Zuecco, G., Amin, A., Frentress, J., Engel, M., Marchina, C., Anfodillo, T., Borga, M., Carraro, V., Scandellari, F., Tagliavini, M., Zanotelli, D., Comiti, F., & Penna, D. (2022). A comparative study of plant water extraction methods for isotopic analyses: Scholander-type pressure chamber vs. cryogenic vacuum distillation. *Hydrology and Earth System Sciences*, *26*, 3673–3689. https://doi.org/10.5194/hess-26-3673-2022

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sánchez-Murillo, R., Todini-Zicavo, D., Poca, M., Birkel, C., Esquivel-Hernández, G., Chavarría, M. M., Zuecco, G., & Penna, D. (2023). Dry season plant water sourcing in contrasting tropical ecosystems of Costa Rica. *Ecohydrology*, e2541. https://doi.org/10.1002/eco.2541