

Temporal partitioning of water between plants and hillslope flow in a subtropical climate

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ABSTRACT

Recent work has suggested that ecohydrological separation may exist between the water sources for recharge and plant water uptake. However, the temporal partitioning of plant transpiration and hillslope flow is still poorly understood. In a growing season, the stable isotopes of precipitation, soil water, groundwater, plant water and hillslope flow in a subtropical climate in Southwest China were determined to assess the compartmentalization of vegetation water use and flow generation. The results suggest that the hillslope flow and plant water have different isotopic characteristics in most cases. The $\delta^{13}C$ -excess values of plants significantly differed from those of the hillslope flow. These different isotopic signatures for plants and the hillslope flow were associated with the different proportions of various water sources in each water pool. Precipitation, the hillslope flow and soil water plot approximately along the local meteoric water line (LMWL), and the studied plant xylem waters plot partly below the LMWL, supporting ecohydrological separation. In this subtropical climate with seasonal droughts, the hydrological separation is temporal and does not occur during the wet season due to the increase in hydrological connectivity. On dry days, the various water sources poorly mix in the subsurface. Thus, the ecohydrological separation between the plant water and hillslope flow water sources varies depending on the rooting depth of plant species and moisture conditions. The implications underlying these findings will be helpful for constructing a process-based ecohydrological model and for understanding the mechanisms underlying the hydrologic interactions between plants and subsurface water flow.

1. Introduction

Most watershed hydrology models and coupled ecology-biogeochemical-hydrology models assume complete mixing for subsurface water, and this assumption is called the black box approach (Hewlett and Hibbert, 1967; McDonnell et al., 2007; Alila et al., 2009). This single ecohydrological reservoir paradigm leads to the concept that roots uptake water from the same pool that is moving to streams (Brooks et al., 2010; Evaristo et al., 2015). However, other studies have demonstrated that the mixing process involving precipitation and soil water is not complete in the vadose zone due to preferential flow pathways and the different mobilities of pre-event water, including the mobile and tightly bound fractions of resident soil water (Brooks et al., 2010; Zhao et al., 2013; McDonnell, 2014; Zhao et al., 2016). Good et al. (2015) estimated that hydrological connectivity ranges from 14 to 59% at the global scale, which suggests a pervasive disconnect between

water bound in soils and water entering streams, although not a complete separation. Dawson and Ehleringer (1991), Ehleringer and Dawson (1992), Brooks et al. (2010) and Penna et al. (2013) found that the isotopic characteristics of water bodies differed in various climates. In addition, it is important to understand the connections between plant water use and hydrologic flow paths, as well as the associated impact on the streamflow regime, to fully comprehend underground hydrological processes (Asbjornsen et al., 2011; Evaristo et al., 2015). These studies highlight the need for an in-depth deeper mechanistic understanding of the link between plant water uptake and streamflow patterns in different ecosystems and climatic regions. An improved understanding of hydrological connectivity is essential at variety of temporal and spatial scales (Good et al., 2015).

Brooks et al. (2010), Goldsmith et al. (2012) and Evaristo et al. (2015) demonstrated a complete separation between vegetation water, which was strongly enriched by evaporation, and stream-recharged

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water, which was less enriched in Mediterranean and tropical climates due to hydrological separation. However, Geris et al. (2015), Hervé-Fernández et al. (2016) and McCutcheon et al. (2017) found that soil waters with different mobilities could be isotopically similar. They suggested that the isotopic distinction between root-absorbed and draining waters may not be an appropriate indicator of the ecohydrological separation of soil waters. Hervé-Fernández et al. (2016) and McCutcheon et al. (2017) concluded that in a rainy temperate climate, the “two water worlds” hypothesis (an ecohydrological separation of water flowing to streams or recharging groundwater and water used by trees) is temporally variable. In addition, water that contributes to groundwater recharge is not always isolated from water used in plant transpiration (Jasechko et al., 2014; Jasechko and Taylor, 2015). Moreover, most of these studies were based on sampling campaigns of 1 to 2 days (i.e., 10 to 15 xylem samples), so their conclusions may not be representative of all situations. Sprenger et al. (2017) noted that a high sampling frequency over time and at various depths is critical when using stable isotopes as tracers to assess plant water uptake patterns within critical zones. However, few experiments have accounted for the temporal variability of the ecohydrological separation (Hervé-Fernández et al., 2016).

Stream water typically features multiple water sources, including precipitation, soil water, irrigation water, domestic wastewater, and groundwater (Dahl et al., 2007; Tang et al., 2008; Tan et al., 2017). Determining why the source of plant water is different from that of stream water may be hindered by the complexity of the water sources of stream water. Answering the question at the hillslope scale is feasible because this scale represents and determines the streamflow signature, which is ultimately generated with little disturbance (Klaus et al., 2013). The ecohydrological connectivity of plant water use and flow generation is more direct at the hillslope scale than at the catchment scale. However, the literature includes a distinct lack of field work and direct evidence relevant to the complex interactions between plant water uptake and flow generation on hillslopes (Butt et al., 2010).

Simple linear mixing (SLM) models have been widely used in partitioning source contributions to plant water based on stable isotope methods (Liu et al., 2015; Zhao et al., 2016; Rothfuss and Javaux, 2017). Traditional SLM models have been used to estimate two or three water sources (e.g., Thorburn and Walker, 1993; Brunel et al., 1995), and relatively recent SLM models can address multiple sources via an iterative mass balance approach (e.g., IsoSource by Phillips and Gregg, 2003). However, such methods do not consider the effects of standard deviations. When employed in a Bayesian inverse modeling framework based on a Markov Chain Monte Carlo (MCMC) calculation, SLM models have been used to correct the isotopic enrichment coefficient (e.g., MixSIR by Moore and Semmens, 2008; SIAR by Parnell et al., 2010). However, few plant source water partitioning studies have used SLM models in Bayesian frameworks (e.g., Leng et al., 2013; Barbata et al., 2015). Parnell et al. (2010) and Evaristo et al. (2016) proved that this approach is useful for partitioning the contributions of various sources of plant water. Rothfuss and Javaux (2017) performed a comparison of the “direct inference” method, the two-end member mixing model and multisource mixing models. The inter-comparison underlined the satisfactory performance of the Bayesian approach of Parnell et al. (2010), which uses a rigorous statistical framework.

Determining the various sources of vegetation water and hillslope flow is helpful for vegetation restoration, river discharge prediction, and ecosystem protection in ecologically fragile regions. Clarifying the water sources of vegetation water use and hillslope flow generation is also important for understanding the underground ecohydrological processes in various hydroclimate environments. Consequently, the objectives of this study were as follows: 1) to explore the water sources, as well as the temporal variation in these sources, of hillslope flow and plant water, including water uptake by cypress, vitex and maize, for a

shallow soil hillslope in a subtropical climate region and 2) to determine whether hydrological separation exists between the water sources of plant transpiration and hillslope flow. The findings of this study improve the general understanding of soil-vegetation interactions and plant water uptake patterns.

2. Material and methods

2.1. Study site description

This research was conducted in a hilly headwater catchment (0.35 km²) in Yanting County, SW China (31°16'N, 105°28'E) (Fig. 1a). Sloping farmland accounted for 50% of this catchment, and the other portions of the catchment consisted of forestland and residential areas. The dominant landscape unit was sloping farmland. The stream was a transient tributary of the Mi River. This study area was characterized by a moderate subtropical monsoon climate with an annual mean temperature of 17.3 °C and a mean annual precipitation of 826 mm/year from 1981 to 2006. The annual precipitation was mainly concentrated in the summer and autumn, with 85.2% of precipitation occurring during the above period.

The studied Regosol according to World Reference Base (IUSS Working Group WRB, 2006) was a loamy soil composed of 27.1% sand, 51.6% silt and 22.3% clay according to the USDA classification of soil texture. The soil had a pH of approximately 8.3, an average bulk density of 1.33 g·cm⁻³, an average organic matter content of 8.75 g·kg⁻¹, and a saturated hydraulic conductivity of 10⁻² to 10⁻¹ mm·min⁻¹ (Zhao et al., 2013).

The studied hillslope was used for farming and had a slope of approximately 6°. There were no observable differences in soil type across the studied hillslope. The soil depth on the hillslope averaged 0.5 m. A 30-m long trench was located 50 m upslope from a pond. The main type of tree, shrub and crop on the study slope was cypress (*Platycladus orientalis* (L.) Franco), vitex (*Vitex negundo* L.) and Maize (*Zea mays* L.), respectively. The upslope and downslope were both covered by *Platycladus orientalis* (L.) Francoes and *Vitex negundo* L. The maize was planted on the middle of the slope.

2.2. Climate and hydrological measurements

The climatic data were collected at a standard meteorological station near the study hillslope. Rainfall quantities were measured using an automatic tipping bucket with an error of 0.1 mm (HOBO event data logger, USA). Three collection grooves were constructed on the surface of the topsoil, the surface of the mother rock and the top of the sandrock (Fig. 1c). The depth of mudstone was 2.1 m. The depths of the troughs for surface flow, interflow and underflow were 2.6 m, 1.8 m and 1.5 m, respectively. The depth of the groundwater table was approximately 2.7 m. The flow amounts were measured using a tipping bucket with a HOBO event recorder located in the troughs. The overland flow and interflow were transient, but the underflow was continuous in the study period.

2.3. Sampling of different compartments

Samples of plant water, soil water (in this case, soil water is bulk soil water), hillslope flow and groundwater were collected from the hillslope either on a daily basis during a rainfall event or otherwise on a monthly basis for isotopic analysis. Precipitation was sampled with a 20-cm-diameter glass funnel connected to a 1-L high-density polyethylene bottle. A table tennis ball was placed in the funnel to reduce evaporation. Overland flow, interflow and underflow (groundwater flow in the deep zone) were collected from a trench. Water samples were taken at 8:00 each day during rainfall events from a collective

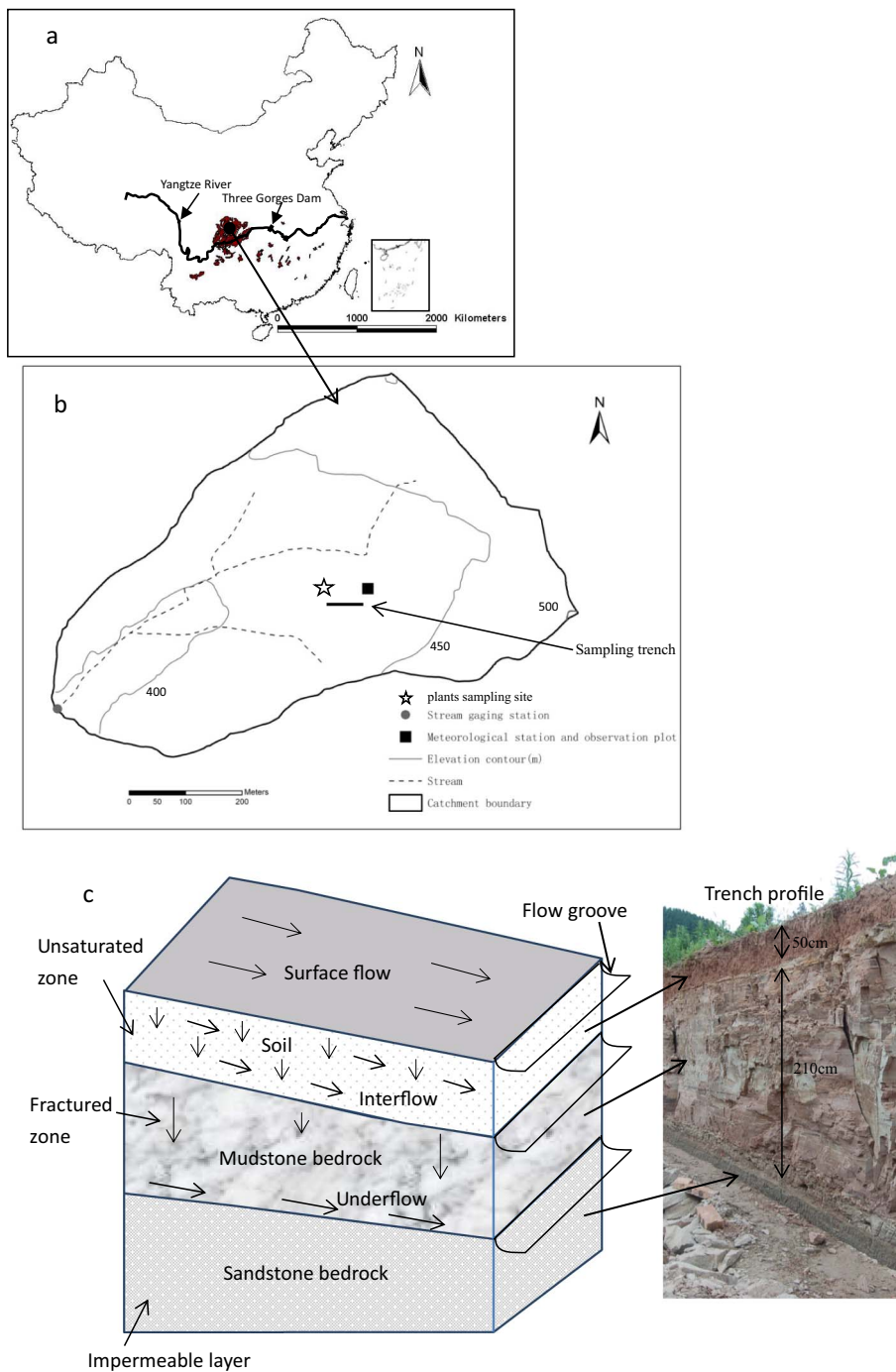


Fig. 1. The distribution of purple soil (Entisol) in China and the study site at the hilly area of purple soil (Entisol), SW China (a), the study catchment and the locations of hillslope flow (horizontal line) (b), and the sketch map of flow measurement (c). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trench built on the bottom of the trench at a downslope location from May to November. In this period, 5 storms (> 50 mm) occurred.

On the slope, suberized twig samples of three individuals of cypress and vitex were collected at an interval of approximately one month. However, three campaigns occurred in June. Maize was sampled from June to August in its growing season. At the same time, stem samples just above the ground were collected from nine maize individuals during the growth period from the seedling to harvest stages. Additionally, 10-cm soil sample columns were collected using a hand auger at depths of 0–10, 10–20, 20–30, 30–40 and 40–50 cm from three slope positions. Samples used for cryogenic vacuum distillation were sampled from the entire 10-cm column. The soil samples were placed

into a 10-ml glass bottle and stored at 4 °C for soil water distillation and isotopic analysis. Plant and soil samples were placed in glass vials with a screw tops and sealed with parafilm to avoid water loss.

Water was extracted from the plant and soil samples via cryogenic vacuum distillation (West et al., 2006). The soil and plant samples were extracted for 3.0 h and 4.0 h at 95 °C, respectively. All samples were weighted pre- and post-water extraction, as well as after an additional sequence of oven drying (48 h at 105 °C). The resulting weights were compared to determine the water extraction efficiency. Only samples that reached a water recovery value higher than 98% were used for further isotope analysis (Araguás-Araguás et al., 1995). Water samples were analyzed for δD and $\delta^{18}O$ based on isotope ratio infrared

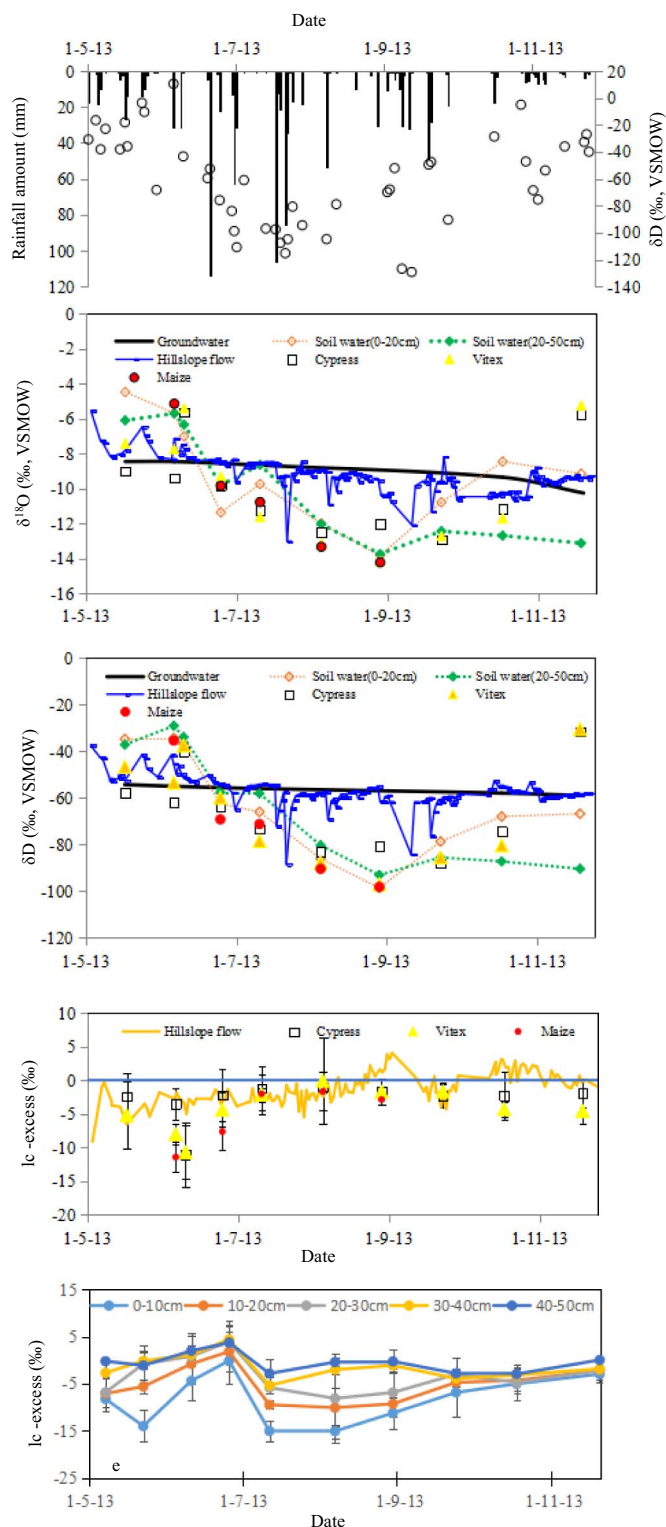


Fig. 2. The rain amount and δD values of precipitation (a), $\delta^{18}O$ of soil water, groundwater, plant xylem water and hillslope flow (b) and δD of soil water, groundwater, plant stem water and hillslope flow (c), and lc -excess of plants xylem water and hillslope flow (d), lc -excess of soil water isotopes (e) at the hillslope during the study period. (PR, Precipitation; GW, Groundwater; SS, Shallow soil water; DS, Deep soil water; HF, Hillslope flow; CY, cypress; VI, Vitex; Ma, Maize).

spectroscopy (IRIS) using a Picarro CRDS connected to a micro combustion module of A0214 (L-2120i, Picarro, USA). The δD and $\delta^{18}O$ measurements for xylem water were corrected following the methods of Schultz et al. (2011). No spectral interference was observed for the IRIS

technique. To address any concerns about potential errors associated with using the IRIS technique compared to the traditional IRMS technique (West et al., 2010), we randomly selected samples to compare the two methods. The randomly selected samples were compared to a CO_2 equilibration method based on the IRMS. The comparison results showed that the residuals generated from both techniques were not significantly different (values ranged from 0.02 to 0.72‰ for $\delta^{18}O$ and 0.03 to 2.28‰ for δD) for both plant xylem water and soil water samples, with an inter-technique correspondence close to unity. Additionally, Evaristo et al. (2015) previously found that there was little difference between the two methods. All δD and $\delta^{18}O$ values were expressed relative to Vienna Standard Mean Ocean Water (VSMOW) in ‰ as follows:

$$\delta_{\text{sample}} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\text{‰} \quad (1)$$

where R is the ratio of deuterium to hydrogen atoms or ^{18}O to ^{16}O atoms of the sample and VSMOW. The measurement precision for δD was 0.5 and 0.1‰, respectively. In addition, comparing LMWL to xylem water lc -excess values is helpful for assessing the compartmentalization of plant water and flowing water. The lc -excess was calculated as previously described (Landwehr and Coplen, 2004):

$$lc - excess = \delta^2H_s - a \times \delta^{18}O_s - b \quad (2)$$

where the subscript ‘s’ represents the sample and a and b are the slope and intercept of the LMWL, respectively.

2.4. Calculations and statistical analyses

An SLM Bayesian model method was employed to determine the relative contributions of different water sources to the composition of plants on the hillslope. The SIAR Bayesian mixing model statistical package for R (Parnell et al., 2010) was used to explore the structure and plausible meaning of the data in the probability space. SIAR solves mixing models for stable isotopic data based on a Gaussian likelihood with a Dirichlet prior mixture for the mean using MCMC methods. The model uses multiple isotope values of ‘consumers’ (in our case, individual values of $\delta^{18}O$ and δD for each plant) and sources (mean plus the standard deviation) and a correction matrix for potential fractionation (set to 0 for both isotope pairs) as inputs. The calculated percentage contributions of various water sources to hillslope flow corresponded to the day of plant sampling. Four potential sources of plant water and hillslope flow were given in the Bayesian model: (1) shallow soil water from 0 to 20 cm, (2) deep soil water from 20 to 50 cm, (3) precipitation, and (4) groundwater. The trophic enrichment factor and concentration dependence of the original model were set to 0. The model was run with 500,000 iterations, and each source’s most likely contribution to plant water was obtained for all plants at the site.

To identify temporal differences between hillslope flow and plant water, their isotopic signatures were compared for the wet season and dry season with respect to LMWL. The comparison of the slope of regression lines of δD versus $\delta^{18}O$ for various water sources used standardised major axis (SMA) estimation method (Warton and Weber, 2002). All statistical analyses were performed in R 3.3.3 (R Core Team, 2017).

3. Results

3.1. Precipitation water

The temporal trends of isotopic ratios in the precipitation water, soil water, groundwater, hillslope flow and plant water are shown in Fig. 2. Table 1 shows the statistical results for the $\delta^{18}O$ and δD data of precipitation, soil water, groundwater, hillslope flow, cypress xylem water, vitex xylem water and maize xylem water in the study period. The isotopic ratios in the precipitation water exhibited the largest fluctuations among those of different waters. Notably, the $\delta^{18}O$ and δD of

Table 1
Statistical analysis of measured deuterium and oxygen-18 values in various water pools during the study period.

| | Number | δD | | | $\delta^{18}O$ | | |
|---------------------|--------|--------------------|--------|-------|------------------|-------|------|
| | | Mean \pm SD | Min | Max | Mean \pm SD | Min | Max |
| Rainwater | 65 | -42.5 \pm 46.1c | -129.2 | 41.4 | -7.1 \pm 4.4c | -17.9 | 4.1 |
| Soil water 0–10 cm | 30 | -61.2 \pm 23.6ab | -107.1 | -26.5 | -8.8 \pm 3.2c | -14.5 | -2.0 |
| Soil water 10–20 cm | 30 | -62.5 \pm 24.2a | -107.9 | -27.6 | -9.3 \pm 2.8b | -14.5 | -4.6 |
| Soil water 20–30 cm | 30 | -65.1 \pm 23.7a | -105.2 | -25.7 | -9.8 \pm 2.9b | -15.1 | -5.0 |
| Soil water 30–40 cm | 30 | -71.3 \pm 21.1a | -103.3 | -26.8 | -10.8 \pm 2.7a | -15.2 | -5.3 |
| Soil water 40–50 cm | 26 | -70.7 \pm 28.1a | -99.4 | -28.1 | -10.3 \pm 3.0a | -14.9 | -4.2 |
| Groundwater | 9 | -56.5 \pm 2.0bc | -59.1 | -54.3 | -8.4 \pm 1.1c | -8.7 | -8.2 |
| Hillslope flow | 159 | -57.6 \pm 8.1b | -88.5 | -37.6 | -9.2 \pm 1.6c | -15.0 | -5.6 |
| Cypress xylem water | 27 | -69.6 \pm 17.3a | -90.4 | -31.3 | -11.0 \pm 2.6a | -14.5 | -5.2 |
| Vitex xylem water | 27 | -73.8 \pm 26.0a | -111.6 | -29.8 | -11.1 \pm 3.9a | -16.7 | -2.1 |
| Maize xylem water | 45 | -78.6 \pm 21.1a | -104.7 | -34.4 | -11.6 \pm 2.8a | -15.2 | -4.7 |

Different letters indicate significant difference at 0.05 level.

precipitation water displayed wide ranges of -17.9‰ to 4.1‰ and -129.2‰ to 41.4‰, respectively. The $\delta^{18}O$ and δD values of rainwater exhibited an obvious seasonal pattern and a variable temporal trend, with depleted values in the summer and autumn and enriched values in the winter.

3.2. Soil water, groundwater and hillslope flow

$\delta^{18}O$ and δD in soil water exhibited less variability, ranging from -15.2 to 2.0‰ and -107.9‰ to -25.7‰, respectively (Table 1). In

deep soil water (20–50 cm), $\delta^{18}O$ and δD were different from the values in shallow soil water (0–20 cm) ($P < 0.05$ using unpaired *t*-test, Fig. 3). The average values of $\delta^{18}O$ and δD in groundwater were -8.4 \pm 1.1‰ and -56.5 \pm 2.0‰, respectively. The isotopic ratios in groundwater had the smallest standard deviation (SD). $\delta^{18}O$ and δD in the hillslope flow ranged from -15.0 to -5.6‰ and -88.5 to -37.6‰, respectively (Table 1). The isotopic values in the hillslope flow water did not significantly differ from those of groundwater ($P > 0.05$ using unpaired *t*-test, Fig. 3).

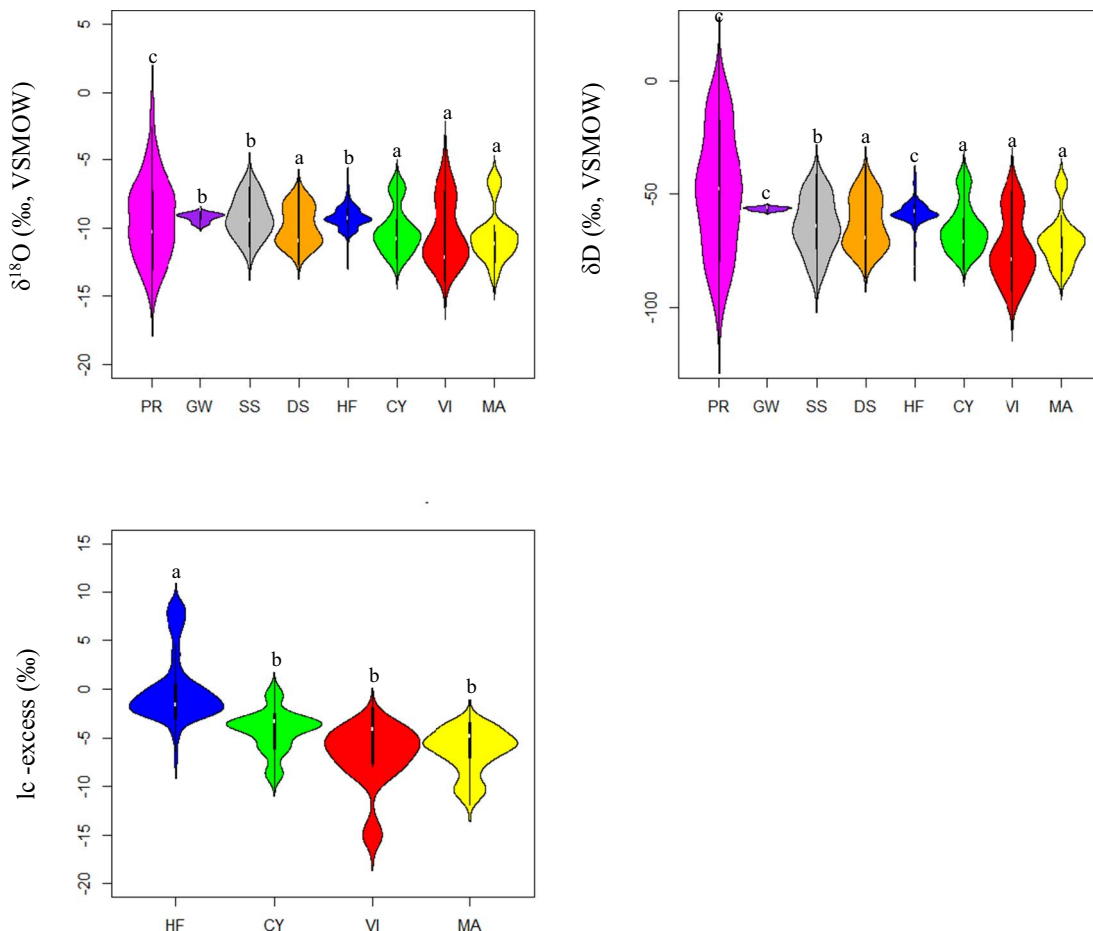


Fig. 3. Violin plots showing the distribution of δD , $\delta^{18}O$ and Ic -excess of various water pools over the sampling campaigns (White point represents the median and the range from the 25th and 75th percentile are shown with a black line within the violin plots). (PR, Precipitation; GW, Groundwater; SS, Shallow soil water; DS, Deep soil water; HF, Hillslope flow; CY, cypress; VI, Vitex; Ma, Maize).

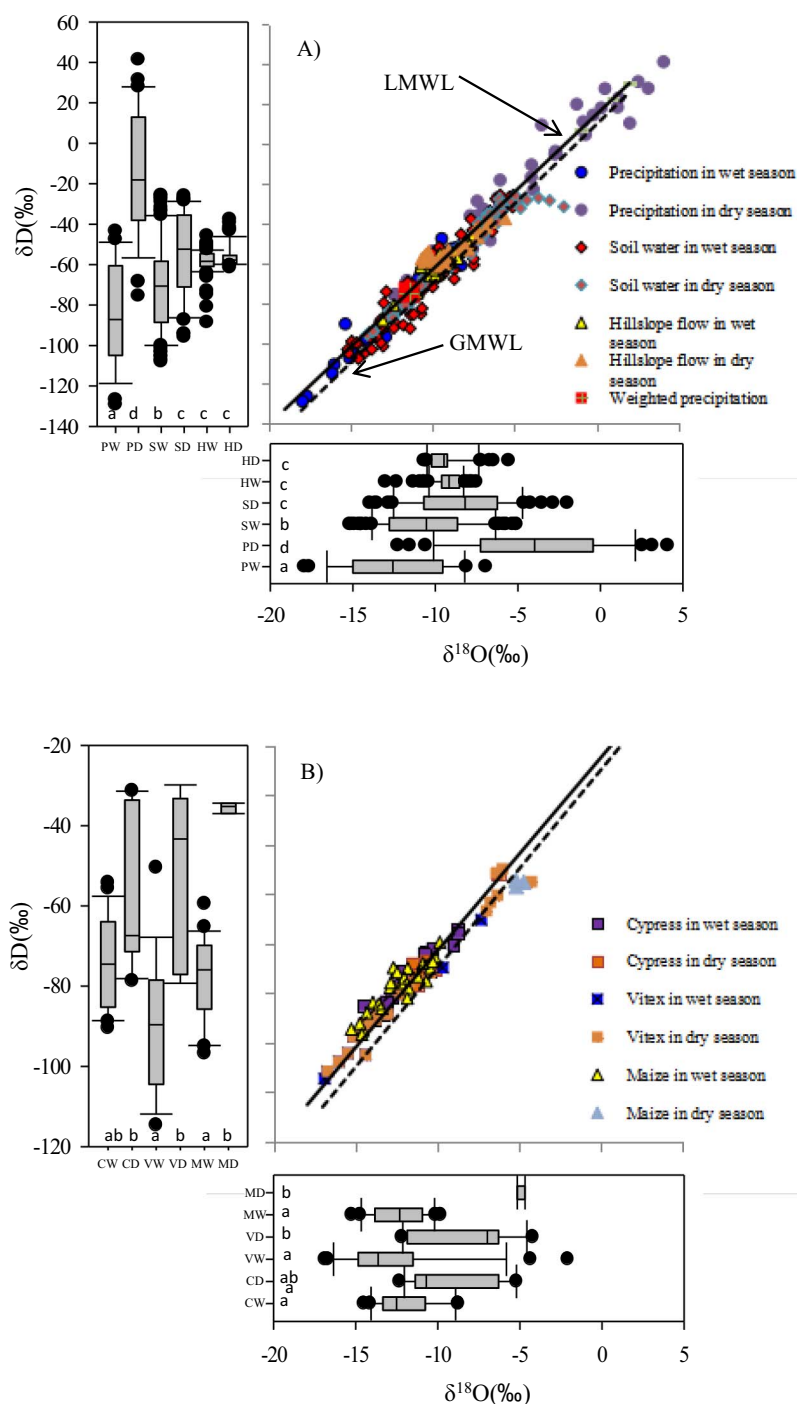


Fig. 4. The linear regressions of $\delta^{18}O$ vs δD of rainwater, soil water and hillslope flow (A) and xylem water of cypress, vitex and maize (B); LMWL ($\delta D = 7.81(\pm 0.17)\delta^{18}O + 15.0(\pm 1.61)$) and GMWL are shown in solid and dotted black lines, respectively. δD and $\delta^{18}O$ of various water pools are summarized on boxplots. Boxplots show 25th, 50th and 75th percentiles, while data extremes are shown by black points. Statistical grouping ($\alpha = 0.05$) is indicated by different letters (PW, precipitation in wet season, PD precipitation in dry season, SW, soil water in wet season, SD, soil water in dry season, HW, hillslope flow in wet season, HD, hillslope flow in dry season, CW, cypress in wet season, CD, cypress in dry season, VW, vitex in wet season, VD, vitex in dry season, MW, maize in wet season, MD, maize in dry season).

3.3. Xylem water and *lc-excess*

The mean $\delta^{18}O$ and δD values of the cypress xylem water were -10.6 ± 2.6 (varying between -14.5 and -5.2 ‰) and -69.6 ± 17.3 ‰ (varying between -90.4 and -31.3 ‰), respectively, which were not significantly different from those of the deep soil water, maize and vitex xylem water ($P > 0.05$ using unpaired *t*-tests) but the isotopes were significantly different from those of the shallow soil (0–20 cm), groundwater and hillslope flow ($P < 0.05$ using unpaired *t*-tests, Fig. 3). The cypress xylem water exhibited the lowest SD of isotopic data compared with other plants. The average δD values of the vitex and maize stem water were -73.8 ± 26.0 ‰ (varying between -111.6 and -29.8 ‰) and -78.6 ± 21.1 ‰ (varying between

-104.7 and -34.4 ‰), respectively. The average δD values of the vitex and maize water did not statistically differ from that of the deep soil water ($P > 0.05$ using unpaired *t*-tests, Fig. 3) but were significantly different than those of the shallow soil (0–20 cm), groundwater, hillslope flow and precipitation water ($P < 0.05$ using unpaired *t*-tests, Fig. 3).

The temporal dynamics of $\delta^{18}O$ and δD in soil water and plant water mimicked the precipitation trend (Fig. 2). Typically, for the cypress and vitex, the water sources switch from a deep water source (e.g., groundwater) in the dry season to shallow soil horizons in the wet season. In addition, the isotopic signature in plant xylem water displayed a lagged phenomenon. The $\delta^{18}O$ and δD in hillslope flow responded to the precipitation input signals but rapidly decreased to the

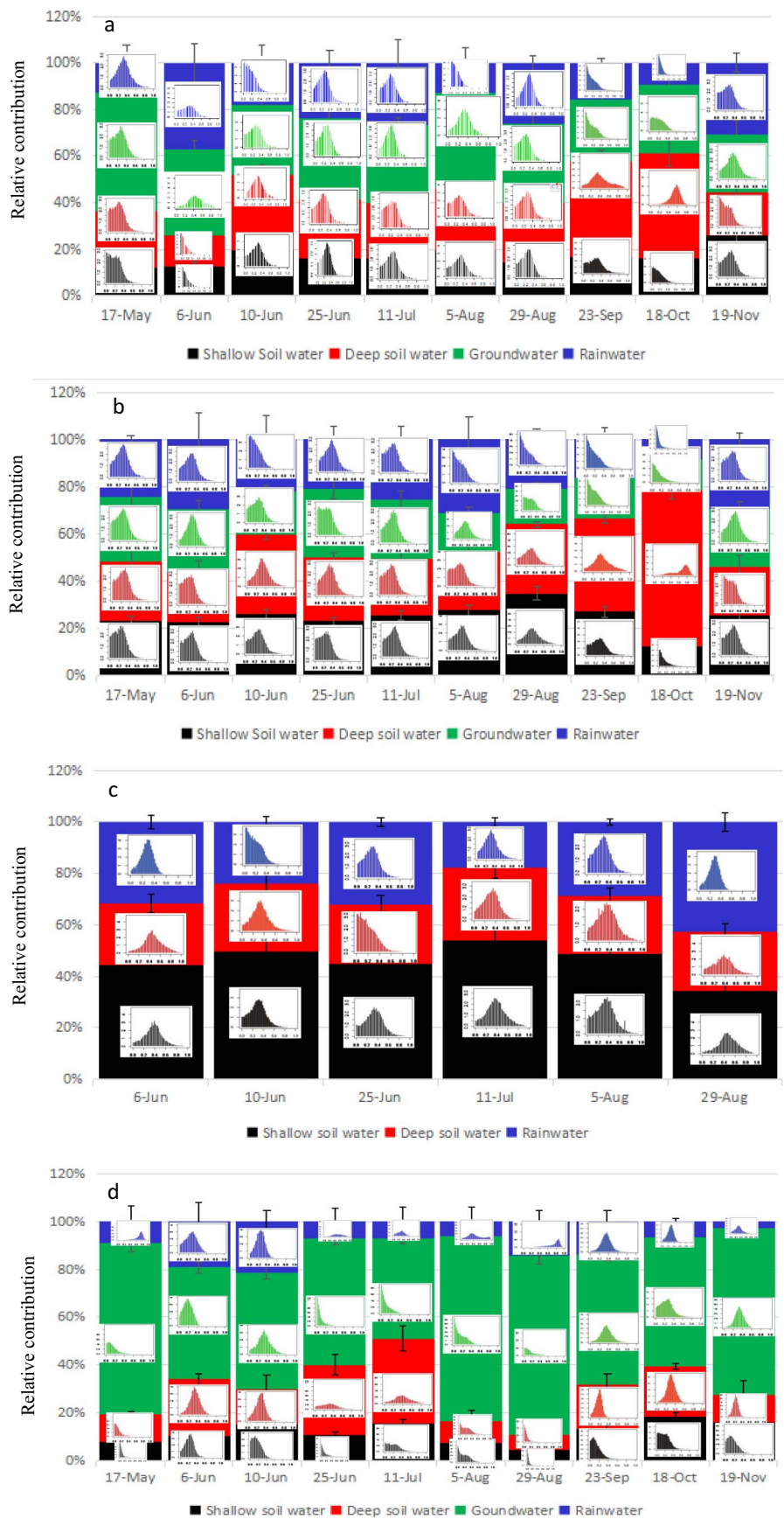


Fig. 5. Source water partitioning using Bayesian mixing model. The respective probability density plots of each putative source water (shallow soil water (0–20 cm), deep soil water (> 20 cm), rainwater, groundwater) superimposed on plots of relative contribution to cypress xylem water (a), vitex xylem water (b), maize stem water (c) and hillslope flow (d). Error bars represent 1 SD. (The y-axis and x-axis of the histograms were density and proportion, respectively).

levels in groundwater after 1–2 days. $\delta^{18}\text{O}$ and δD values in groundwater were relatively stable compared with those in other water pools.

The lc-excess from cypress vitex and maize exhibited differences when compared with the lc-excess of hillslope flow ($P < 0.05$ using unpaired *t*-tests, Fig. 3c). The plants showed no differences in lc-excess when compared with each other ($P > 0.05$ using unpaired *t*-tests, Fig. 3c). In May, June, October and November, the plant lc-excess values were negative, supporting the ecohydrological separation. However, the lc-excess of hillslope flow was less variable and departed from the plant lc-excess in those months. In July, August and September, the hillslope flow and plant xylem water exhibited lc-excess values close to zero (i.e., the lc-excess from xylem water was close to zero and the LMWL, Fig. 2d). These lc-excess values suggest a single water world or ecohydrological connection and not two.

The lc-excess of the hillslope soil water averaged over the upper 20 cm was obviously lower than that of the deeper soil between May and September. Additionally, the lc-excess values of the upper 10 cm were always significantly more negative than those of other layers (Fig. 2e). The lc-excess depth profiles exhibited a persistent pattern of steadily decreasing values with depth, approaching a value of 0‰ for soil water at depths of 40–50 cm. The variability in the lc-excess values of soil water generally decreased with depth (Fig. 2e).

3.4. $\delta^{18}\text{O}$ vs δD plots

Fig. 4 shows linear regressions for $\delta^{18}\text{O}$ vs δD in precipitation water, soil water, plant water, and hillslope flow. The LMWL was $\delta\text{D} = 7.81(\pm 0.17)\delta^{18}\text{O} + 15.0(\pm 1.61)$, with a similar slope but a higher intercept compared with the Global Meteoric Water Line (GMWL), which is $\delta\text{D} = 8.0\delta^{18}\text{O} + 10.0$. The isotopic compositions of plant water and hillslope flow were concentrated near the lower section of the LMWL. Both the water used by plants and the water that forms the hillslope runoff were mainly derived from rainfall enriched in heavy isotopes. The linear regression of δD vs $\delta^{18}\text{O}$ in soil water was $\delta\text{D} = 7.05(\pm 0.17)\delta^{18}\text{O} + 6.41(\pm 1.61)$. The slope and intercept were both lower than the LMWL. This finding reflects the effect of evaporation on soil water. The linear regression of δD vs $\delta^{18}\text{O}$ for hillslope flow was $\delta\text{D} = 7.56(\pm 0.10)\delta^{18}\text{O} + 8.65(\pm 0.83)$. The slope and intercept were both lower than the LMWL. This observation indicates the contribution of an evaporation-affected water source in flow generation.

The linear regression of δD vs $\delta^{18}\text{O}$ in cypress water was $\delta\text{D} = 6.42(\pm 0.29)\delta^{18}\text{O} + 0.66(\pm 3.23)$. Notably, the slope and intercept differed significantly from those of precipitation, soil water and hillslope flow ($P < 0.05$ using SMA estimation). Similarly, the δD vs $\delta^{18}\text{O}$ regression lines for vitex ($\delta\text{D} = 6.82(\pm 0.23)\delta^{18}\text{O} + 2.68(\pm 2.86)$) xylem water also differed from those of most other water sources (precipitation and hillslope flow, $P < 0.05$) but not soil water ($P > 0.05$ using SMA estimation). The parameters of the maize stem water equation ($\delta\text{D} = 5.74(\pm 0.29)\delta^{18}\text{O} - 6.88(\pm 3.61)$) significantly differed from those of the other possible water sources ($P < 0.05$ using SMA estimation).

Isotopes in the hillslope flow and soil water did not display significant differences between wet and dry days ($P > 0.05$ using unpaired *t*-test, Fig. 4a). However, these isotopes differed from those of rainwater and soil water on wet days ($P < 0.05$ using unpaired *t*-test, Fig. 4a). Except for cypress, vitex and maize exhibited different isotopic characteristics on wet and dry days ($P < 0.05$ using unpaired *t*-test, Fig. 4b).

3.5. Water sources for plants and hillslope flow

The potential sources of xylem water and hillslope flow were determined using a Bayesian mixing model. Fig. 5 shows the percentage contributions of precipitation, shallow soil water, deep soil water and groundwater to the hillslope flow and plant water. The respective probability density plots of each end-member were superimposed on the plots of the relative contributions to xylem water and hillslope flow.

Table 2

The residuals of $\delta^{18}\text{O}$ and δD for SAIR model in hillslope flow and plant water source calculations.

| Date | Hillslope flow | | Cypress | | Vitex | | Maize | |
|--------------|-----------------------|------------------|-----------------------|------------------|-----------------------|------------------|-----------------------|------------------|
| | $\delta^{18}\text{O}$ | δD | $\delta^{18}\text{O}$ | δD | $\delta^{18}\text{O}$ | δD | $\delta^{18}\text{O}$ | δD |
| 15 May | 0.67 | 5.65 | 1.41 | 11.46 | 9.9 | 65.27 | / | / |
| 6 June | 1.15 | 11.31 | 2.26 | 16.43 | 3.22 | 24.79 | 0.65 | 5.35 |
| 10 June | 0.47 | 3.27 | 1.90 | 14.67 | 5.65 | 8.47 | / | / |
| 25 June | 0.68 | 4.37 | 3.51 | 17.77 | 7.40 | 18.28 | 4.42 | 21.46 |
| 11 July | 0.44 | 12.31 | 4.27 | 4.05 | 3.74 | 4.94 | 1.43 | 7.74 |
| 5 August | 0.49 | 2.45 | 2.96 | 15.51 | 7.82 | 24.75 | 0.86 | 12.43 |
| 29 August | 1.26 | 21.36 | 2.16 | 13.75 | 2.95 | 14.31 | 0.95 | 3.62 |
| 23 September | 1.07 | 2.66 | 0.71 | 5.65 | 1.44 | 9.29 | / | / |
| 18 October | 1.29 | 6.66 | 1.00 | 5.19 | 0.12 | 0.65 | / | / |
| 19 November | 0.47 | 3.40 | 3.62 | 35.46 | 4.04 | 28.80 | / | / |

Table 2 and Table 3 show the residual and matrix tables of the correlation coefficients for the Bayesian mixing model results. The parameters suggested that the modeling results were relatively accurate. During the study period, the hillslope flow was mainly composed of groundwater, with a contribution of 41.6–77.1% (mean $58.1 \pm 12.6\%$). Therefore, the measured contributions of soil water and rainwater were relatively low for the hillslope flow at the time of sampling. Rainwater contributed to 2.8–21.4% of the hillslope flow, deep soil water ($19.1 \pm 9.9\%$) was more prevalent than shallow soil water ($12.1 \pm 4.4\%$) in the hillslope flow composition.

For plant water use, groundwater was a more important water source for cypress (26.5–51.0%), with a mean of $34.1 \pm 7.7\%$, than for vitex (13.5–28.9%), with a mean of $21.2 \pm 5.7\%$. Cypress and vitex both exhibited the ability to adapt to water availability, as indicated by the balanced contributions of multiple water sources to xylem water (the contribution of shallow soil water was $24.9 \pm 5.5\%$ for vitex and $16.9 \pm 3.9\%$ for cypress; the contribution of deep soil water was $31.0 \pm 13.4\%$ for vitex and $27.6 \pm 9.7\%$ for cypress, and the contribution of precipitation was $22.9 \pm 7.0\%$ for vitex and $21.4 \pm 9.0\%$ for cypress). Maize features a short root system; thus, it can only use soil water and precipitation. Shallow soil water was the main water source (contributing to 34.4–53.8% of stem water) for maize during the growing period. In general, the different isotopic signatures of the plant water and hillslope flow represented the differences in the relative contributions of potential water sources, which varied over time.

4. Discussion

4.1. Precipitation, soil water and hillslope flow

The isotopes in precipitation exhibited depleted values in summer and enriched values in winter. The isotopic ratios of precipitation were negatively correlated with the rainfall amount, but the correlation was not significant. This phenomenon was consistent with the results shown at Wake Island of Dansgaard (1964) and in other cases, such as Hughes and Crawford (2013). However, it contradicts the results of Gat (1996). These differences may depend on the complex effects of location, temperature and rainfall amount. In the precipitation process, the heavy isotopic components (e.g., H_2^{218}O) fall first. Because the volatility of H_2O^{16} is higher than the volatility of heavier isotopes, lighter isotopes fall later during a rainfall episode. The study region has a subtropical humid monsoon climate with rainfall concentrated in the summer. Consequently, precipitation exhibits depleted isotopic characteristics in the summer. The greater isotopic fractionation of ^{18}O compared with that of D due to evaporation during rainfall or mixing between rainfall and evaporation water results in the disproportional enrichment of ^{18}O relative to D and a moderately lower slope for the LMWL.

Table 3

Matrix of correlation coefficients of group proportions. The left columns represent correlation coefficients of source 1 vs sources 2, 3, 4; the second columns represent correlation coefficients of source 2 vs sources 3, 4; the third columns represent correlation coefficients of source 3 vs source 4. Source 1 means shallow soil water, source 2 means deep soil water, source 3 means groundwater, source 4 means recent precipitation.

| Date | Cypress | | | Vitex | | | Hillslope flow | | | Maize | | |
|--------------|---------|-------|-------|-------|-------|-------|----------------|-------|-------|-------|-------|-------|
| 17 May | 0.06 | / | / | -0.35 | / | / | 0.35 | / | / | / | / | / |
| | -0.37 | -0.75 | / | -0.32 | -0.39 | / | -0.44 | -0.89 | / | / | / | / |
| | -0.17 | 0.05 | -0.42 | -0.26 | -0.36 | -0.31 | -0.65 | 0.19 | -0.33 | / | / | / |
| 6 June | -0.20 | / | / | -0.42 | / | / | -0.50 | / | / | -0.59 | / | / |
| | -0.10 | -0.42 | / | -0.32 | -0.33 | / | -0.34 | -0.34 | / | -0.26 | -0.44 | / |
| | -0.19 | -0.41 | -0.52 | 0.04 | -0.40 | -0.46 | 0.28 | -0.43 | -0.56 | 0.18 | -0.32 | -0.43 |
| 10 June | -0.42 | / | / | -0.36 | / | / | -0.28 | / | / | / | / | / |
| | -0.33 | -0.22 | / | -0.35 | -0.17 | / | -0.31 | 0.15 | / | / | / | / |
| | -0.36 | -0.37 | -0.29 | -0.40 | -0.35 | -0.35 | -0.60 | -0.24 | -0.48 | / | / | / |
| 25 June | 0.03 | / | / | -0.02 | / | / | -0.42 | / | / | -0.62 | / | / |
| | 0.04 | -0.25 | / | 0.06 | -0.27 | / | -0.55 | -0.44 | / | -0.60 | 0.01 | / |
| | -0.39 | -0.50 | -0.54 | -0.42 | -0.49 | -0.51 | 0.67 | -0.19 | -0.73 | -0.50 | 0.01 | 0.03 |
| 11 July | -0.53 | / | / | -0.57 | / | / | 0.02 | / | / | -0.46 | / | / |
| | 0.01 | -0.30 | / | -0.17 | -0.24 | / | -0.23 | -0.39 | / | 0.03 | -0.50 | / |
| | -0.23 | -0.33 | -0.63 | -0.33 | -0.27 | -0.40 | 0.02 | -0.24 | -0.78 | -0.23 | -0.33 | -0.43 |
| 5 August | -0.40 | / | / | -0.48 | / | / | -0.16 | / | / | -0.34 | / | / |
| | -0.29 | -0.20 | / | -0.03 | -0.40 | / | -0.76 | -0.23 | / | -0.73 | 0.24 | / |
| | -0.32 | -0.54 | -0.19 | -0.29 | -0.50 | -0.20 | -0.20 | -0.24 | -0.27 | -0.30 | -0.60 | -0.17 |
| 29 August | -0.61 | / | / | -0.69 | / | / | -0.15 | / | / | -0.83 | / | / |
| | 0.07 | -0.05 | / | -0.04 | -0.26 | / | -0.29 | -0.14 | / | 0.07 | 0.03 | / |
| | -0.27 | -0.33 | -0.61 | -0.22 | -0.42 | -0.11 | -0.24 | -0.33 | -0.70 | -0.18 | -0.16 | -0.22 |
| 23 September | -0.61 | / | / | -0.59 | / | / | -0.67 | / | / | / | / | / |
| | -0.41 | 0.03 | / | -0.33 | -0.22 | / | -0.47 | 0.02 | / | / | / | / |
| | -0.45 | 0.04 | -0.32 | -0.36 | -0.17 | -0.28 | -0.15 | 0.38 | -0.78 | / | / | / |
| 18 October | -0.24 | / | / | -0.30 | / | / | -0.30 | / | / | / | / | / |
| | -0.60 | -0.42 | / | -0.47 | -0.45 | / | -0.23 | -0.57 | / | / | / | / |
| | -0.37 | 0.30 | -0.40 | -0.37 | 0.07 | -0.38 | -0.56 | 0.42 | -0.55 | / | / | / |
| 19 November | -0.29 | / | / | -0.28 | / | / | 0.32 | / | / | / | / | / |
| | -0.44 | 0.03 | / | -0.44 | 0.04 | / | -0.48 | 0.67 | / | / | / | / |
| | -0.55 | -0.21 | -0.37 | -0.52 | -0.22 | -0.39 | -0.51 | 0.25 | -0.47 | / | / | / |

Soil water has depleted isotopic levels compared with rainwater. Due to the shallow soil depth and high water infiltration rate of the studied Entisol (Wang et al., 2015), the isotopic distribution along the soil profiles was approximately similar. However, deep soil water at 20–50 cm had a different isotopic signature compared with that of shallow soil water. The difference occurred because the shallow soil was affected by evaporation. The soil water from 0 to 20 cm was more enriched in heavy isotopes than those in deeper soil water. In addition, the lc-excess of shallow soil water was more negative in the shallow soil layers than in the deeper soil water. The evaporation of meteoric water from the soil under nonequilibrium conditions can result in negative lc-excess values (Hervé-Fernández et al., 2016). Thus, the evaporation of shallow soil water was more prevalent than that of deep soil water. The differences between shallow soil and deep soil characteristics have been noted in various environments (Rothfuss et al., 2015; Sprenger et al., 2016).

The isotopic ratios in groundwater did not substantially vary over the study period due to the vast water storage of groundwater reservoir. The lack of a significant difference between the isotopic compositions of groundwater and hillslope flow during most of the time reflects the important role of groundwater in generating hillslope flow. The isotopic values of hillslope flow exhibited lower fluctuations over time than did those of precipitation. Although rainwater caused the isotopic values of hillslope flow to fluctuate, the isotopic ratio of hillslope flow rapidly returned to a relatively stable value, similar to that of groundwater in the days after a rain event. This finding suggests that rainwater contributes to hillslope flow during rain events and groundwater dominates the hillslope flow when the rain stops and the flow discharge is low.

One limitation of this research was that the spatial resolution of soil water sampling was low (e.g. 10-cm increments). A higher resolution could allow researchers to test the efficiencies of soil physical models in simulating the hydrological flow and transport in the critical zone (Oerter and Bowen, 2017; Sprenger et al., 2017). In addition, the soil

samples were only sampled once at each depth and location on the hillslope. However, the spatial variability in the subsurface isotopic composition was generally variable and the variability cannot be fully covered. The uncertainty of soil water isotopic composition in the subsurface increases the difficulty to accurately account for the water source determination for the plant water uptake.

4.2. Xylem water

The δD and $\delta^{18}O$ values of plant xylem water significantly differed from those of the hillslope flow. A number of studies at other sites have also suggested that the isotopic composition of water in the tree xylem and streams considerably differed (Brooks et al., 2010; Mantese et al., 2012; Penna et al., 2013).

The root depth determined the potential and available water sources for plant water use. The cypress and vitex have deep root systems that can utilize both shallow and deep water sources. The cypress and vitex water was associated with all of the potential water sources. The balanced water source composition of plant xylem water implied that cypress and vitex employ a flexible strategy for water use. Additionally, groundwater plays a more important role in cypress than in the vitex, especially on rainless days. Less rainfall and decreasing soil water availability often result in a shift in plant water sources to more stable, deep water sources (Romero-Saltos et al., 2005; Li et al., 2013). David et al. (2007) found that the water that transpired from cypress was mainly associated with uptake groundwater sources. In the shallow Regosol, nutrient contents were relatively limited which may affect the root water uptake (Heinrich, 2006). This would be the ecophysiological rational for the plants to preferably access groundwater, while soil water would as well be available. Maize has a relatively shallow root system that allows it to use only soil water and rainwater. Consequently, the different relative contributions of rainwater and/or resident soil water to plant water and hillslope flow were responsible for

the observed differences in the isotopic signatures of the plant water and hillslope flow.

Apparently, soil water and plant water were affected by evaporation, and the water used by the plants was more isotopically enriched than the hillslope flow water based on $\delta^{18}\text{O}$ vs δD plots. This phenomenon suggests that evaporated water was more important in plant water use than in hillslope flow generation. This finding suggests that an ecohydrological separation occurs, whereby mobile water drains into streams and tightly bound fractions of resident soil water is used by plants (Zhao et al., 2013; Zhao et al., 2016). This result was consistent with the findings of Brooks et al. (2010), who stated that water retained in small soil pores affected by evaporation was mainly taken up by plant uptake.

Another limitation of this research included the known issues of cryogenic extraction (Orlowski et al., 2016; Orlowski et al., 2016; Gaj et al., 2017; Gaj et al., 2017; Newberry et al., 2017). Gaj et al. (2017) stated that the interlayer and absorbed water could not be effectively extract from soils with high clay minerals contents based on the standard temperature for cryogenic soil water extraction (e.g. 95 °C in this study). They suggested that temperatures between 200 °C and 300 °C should be used for soil water extraction. Although the recovery efficiency in this study was 98% and the clay mineral content was not high, a potential source of uncertainty was associated with the cryogenic vacuum extraction method. This uncertainty affected the determination of the proportion of soil water used in the plants.

4.3. *lc-Excess and water sources*

Ecohydrological separation was supported by negative *lc*-excess values for xylem water. The *lc*-excess values of xylem water close to 0, indicate no ecohydrological separation.

Overall, the xylem water of maize exhibited lower but not significantly different *lc*-excess values (i.e., plotted farther from the LMWL) than cypress and vitex, suggesting that plants with short roots withdraw even more evaporated water than plants with deep roots. Additionally, our findings suggest that the water used by plants (i.e., xylem water) had undergone evaporation under nonequilibrium conditions (Hervé-Fernández et al., 2016). While *lc*-excess provides evidence to support the ecohydrological separation hypothesis during dry periods (Brooks et al., 2010; Goldsmith et al., 2012; Penna et al., 2013; McDonnell, 2014; Evaristo et al., 2015), it does not support the same conclusion for wet periods. In wet periods, the *lc*-excess values of hillslope flow and xylem water were similar and close to 0‰ (i.e., the LMWL). Large amounts of rainfall may increase the hydrological connectivity between hillslope flow and plants, resulting in the isotopic homogenization of the vadose zone. Therefore, during these periods, no ecohydrological separation was observed.

Other studies have described either ecohydrological separation (Brooks et al., 2010; Goldsmith et al., 2012; Evaristo et al., 2015) or a full connection (Geris et al., 2015). However, their conclusions may not be representative of all situations because most of these studies only sampled for 1 to 2 days or 10 to 15 xylem samples. Our study accounted for temporal variability and shows that the ecohydrological separation does not occur in all circumstances. The results of this study provide some evidence of ecohydrological connectivity at the study site in periods characterized by wet conditions.

4.4. *Mechanisms of the ecohydrological separation*

Mechanism 1: The results of this research show that groundwater was involved in flow generation and plant transpiration. The different contributions of multiple potential water sources (e.g., groundwater) to plant water and flow could be the reason for the observed isotopic differences. For example, groundwater was always unavailable for short-root maize, which could be an important reason for the observed isotopic composition difference between maize stem water and hillslope

flow. Consequently, the isotopic differences observed between plant water and hillslope flow could be due to the differences in plant species (e.g., root depth) and available water conditions.

Mechanism 2: Water in plants or hillslope flow from different sources (including precipitation, which exhibited large temporal variations in δD herein) did not mix completely in the large subsurface reservoir of the hillslope (Muñoz-Villers et al., 2012). Rain altered the relative contributions of water sources to xylem water and hillslope flow, and the degree of change and duration varied. Notably, the *lc*-excess of plant xylem water and hillslope flow was closer to 0‰ on wet days than dry days, which indicated that there was no ecohydrological separation present under wet conditions. Larger water inputs increase the hydrological connectivity or mixability of the subsurface reservoir. Additionally, Bond et al. (2002) found that the time lag between maximum transpiration and minimum hillslope discharge decreased on wet days, which enhanced the soil hydraulic connectivity of the soil and strengthened the interactions between tree water use and hillslope flow generation. Consequently, the hydrological interactions between plant water uptake and hillslope flow intensified as the water storage of the hillslope increased. The strong hydrological connection between plant water uptake and hillslope flow generation under wet conditions was likely an integrated result of plants using multiple water sources in both shallow and deep soil horizons, as well as the multiple water sources that participated in hillslope flow generation. Namely, the plants exerted a stronger effect on the hydrologic flux in the soil-atmosphere system under wet conditions than under dry conditions.

The well-known water balance equation directly links vegetation water use to hydrologic flow paths. For example, plant transpiration determines the water loss from the soil to the atmosphere (Asbjornsen et al., 2011). The present study showed that the hydrological links between vegetation water use and the flow regime were indirect, especially on dry days. The water loss to transpiration may influence the hydrologic flow paths due to uptake of multiple potential water sources, including rainwater, resident soil water and groundwater, in temporally varying combinations that likely change the streamflow response. These results will be valuable for constructing a process-based ecohydrological model to predict stream discharge and for informing irrigation decisions for farmland during drought periods.

Further research is needed to identify the differences in the stomatal control of transpiration between different plant species and to analyze the relationship between plant water data and the available water content in the soil. The information could be used to support the development and implementation of best management practices (e.g., optimizing the spatial distribution of land use and land management as well as improving irrigation and drainage systems) aimed at the efficient use and maintenance of sustainable water resources at the catchment scale.

5. Conclusions

Two stable isotopes (δD and $\delta^{18}\text{O}$) in hillslope flow and cypress, vitex, and maize stem water were analyzed to identify the ecohydrological differences between plant water use and flow on a hillslope with a shallow Entisol in Southwest China. The isotopic values of plant water were generally different from those of hillslope flow due to the different combinations and relative contributions of multiple water sources (i.e., rainwater, shallow soil water, deep soil water and groundwater). This study provided evidence that supports the ecohydrological separation hypothesis during dry periods, based on observed differences in plots of $\delta^{18}\text{O}/\delta\text{D}$, as well as differences in *lc*-excess values from hillslope flow and xylem water samples. However, the ecohydrological separation hypothesis does not hold in periods characterized by wet conditions. During dry periods, the soil water content was low in the soil, and plants relied on both soil water and groundwater to meet evapotranspiration requirements. However, the hillslope flow, which was characterized by a small flow discharge, was dominated by

groundwater, with little soil water involved because dry soil has little mobile soil water. The isotopes in soil water become enriched under nonequilibrium conditions due to evaporation in the soil, causing xylem samples to deviate from the LMWL. The ecohydrological separation between plant water and hillslope flow varied based on the plant species and moisture conditions, which indicates that water from different sources did not completely mix in the subsurface. These results contrast into question the common assumption in modeling that plants and flows rely on the same water pools. Although the hydrological links between vegetation water use and the flow regime appeared indirect, water loss via plant transpiration may both influence and be influenced by subsurface hydrologic flow paths and subsequently affect the streamflow response at the catchment scale. These findings are useful for developing an accurate process-based ecohydrological model and obtaining an in-depth understanding of the subsurface mixing processes for water from different sources. In the future, high spatiotemporal resolution observations of the various water sources of plant use and hillslope flow generation, as well as plant physiological parameters, are required to better understand the eco-hydrological mechanisms underlying the complex interactions and feedback between plants and subsurface flow on hillslopes.

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