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Analyzing relationships among water uptake patterns, rootlet biomass distribution and soil water content profile in a subalpine shrubland using water isotopes

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ABSTRACT

Stable isotopic characteristics of plant water represent an integrated response of root systems to water sources with different isotopic signatures. Analysis of these signatures can help to identify many ecological processes involved in the uptake, transport and utilization of different water sources. In August 2003, we collected soil water samples throughout the soil profile from a subalpine shrub ecosystem in Wolong Nature Reserve, West China, along with stem water samples from the two dominant shrub species, *Quercus aquifolioides* and *Salix luctuosa*. Stable isotope contents of the different water samples were determined in conjunction with rootlet biomass distribution of each species and soil water content throughout the soil profile. Results indicated that these subalpine non-phreatophytic shrubs utilized soil water primarily from the top 30 cm of the soil profile. Water uptake patterns were significantly positively correlated with rootlet biomass distribution as well as the soil water content profile. Hence, the two shrubs could play an important role in keeping rainwater from entering river channels quickly, thereby reducing risk of flooding.

1. Introduction

Through its utilization of soil water, vegetation has an important impact on the shallow soil water system [23], and the soil–root–stem water pathway is a major component of subsurface hydrological systems [28]. Moreover, the depth and distribution of plant roots define the area from which plants can potentially absorb soil water [42].

Since root presence may not be a reliable indicator of water uptake dynamics in either time or space [14], traditional methods for measuring or estimating water uptake have many philosophical and practical difficulties [32]. However, analysis of the stable isotope composition of plant water has proven to be an effective tool for understanding spatial utilization of water sources taken up by root systems [3,11,14,34]. Stable isotope analysis can also resolve more qualitative and quantitative problems about many hydrological processes than other techniques that focus on, for example, sap-flux, eddy-flux [23], soil solution chemistry [1], heat pulse or water potential [8].

Waters from different parts of the soil profile have different isotopic signatures as a result of precipitation and groundwater recharge [7,15], hydraulic lift [11,13] and near surface evaporation [9]. In general, precipitation may recharge soil water within about the top 20 cm of soil and sometimes deeper, and groundwater with a relatively shallow water table can recharge soil water from below through capillary rise [7]. Hydraulic lift also can move deeper soil water or groundwater to shallower soil horizons through superficial lateral roots of some plants [11]. Evaporation of water from the soil usually takes place prior to the uptake of water by vegetation [9,36]. Meanwhile, water uptake and transport through the root system are passive processes [28], and if osmotic effects are neglected, plants do not fractionate water during the uptake and transport process except near the leaf [3,4]. Hence, the stable isotopic composition of plant water (e.g. stem water, xylem water or sap) represents the

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integrated response of root systems to water sources with different isotopic signatures [11,14,24] and can help determine the soil depth from which plants obtain water [11,12,13,18].

Although many investigations have focused on the spatial distribution of plant water uptake within the soil profile and its relationship with water sources and root distributions, contradictory findings have resulted in continued interest in the subject. A large number of studies in systems where hydrological inputs to the upper horizons are unreliable and unpredictable confirm that plants obtain most of their water from deep water sources, even at times when water is available near the surface (e.g. [29,37]). In addition, water uptake patterns do not necessarily match the root distribution and the soil water content profile [37]. However, Green and Clothier [19] and Green et al. [20] found a significant correspondence between the pattern of water uptake of some plants and their root distribution before irrigation. They also observed a strong preferential utilization of near surface water after localized irrigation. Similarly, Tang and Feng [36] found that plants primarily utilized near surface soil water during the wet season. Nnyamah and Black [31] proposed that the amount of water extracted from any soil layer depended on both rooting density and soil water content. Thus, Knight [24] supposed that in a system with sufficient water available from rainfall in the shallow soil horizons to satisfy vegetation requirements, water uptake patterns might match the root distribution and the soil water content profile.

In the Wolong Valley, located in the upper watershed of the Yangtze River, alpine shrub is a typical vegetation type and plays an important role in controlling flooding. However, few studies have investigated the role of alpine shrubs in reducing risk of flooding by examining the relationships among their water uptake patterns and rootlet biomass distributions and the soil water content profile. In this study, relationships among stable hydrogen and oxygen isotope values of alpine shrubs, soil water and precipitation, as well as rootlet biomass, were determined. Our objective was to determine whether Knight's [24] hypothesis could be applied in our study area to explain relationships among water uptake patterns of alpine shrubs, root distribution and the soil water content profile, and, further, to discuss the influence of water uptake patterns of shrubs on reducing risk of flooding.

2. Materials and methods

2.1. Study area

Our study site was located on Balang Mountain in the Wolong Nature Reserve in the sub-humid temperate zone of China's Sichuan Province. This area is dominated by a monsoon climate subject to East Asian monsoons (April–August) and Indian monsoons (September–October). Mean annual precipitation, mean annual relative humidity and mean annual temperature are about 711 mm, 80% and 8.9 °C, respectively [40].

The vegetation community on the south slope of Balang Mountain consists of shrubs and herbs from altitudes of 2800 m–3100 m a.s.l. *Quercus aquifolioides* Rehd. Et Wils. dominates the shrub cover (60%–80% cover), whereas *Salix luctuosa* Levl. is the most common shrub species. *Q. aquifolioides* and *S. luctuosa* are non-phreatophytic on Balang Mountain. Herbaceous vegetation is dominated by eight species with coverages less than 15%, but the most common species are *Deyeuxia levipes* Keng. and *Anaphalis contorta* (D. Don) Hook. f. During our study, basal diameters of *Q. aquifolioides* and *S. luctuosa* varied from 2 cm to 5 cm. The heights of *Q. aquifolioides* averaged from 2 to 5 m with a mean height of 3.4 m, and heights of *S. luctuosa* averaged from 1 to 5 m with a mean height of 2.1 m. The height of herbaceous vegetation varied from 0.1 m to 0.3 m. Humus thickness is typically less than 5 cm and litter thickness varies between 2 and 5 cm [40].

2.2. Field sampling

Three sampling plots (10 × 10 m) were established on the southeastern slope of Balang Mountain. Plots A, B and C were established at 3070 m (30°51.810′ N, 102°58.147′ E), 2930 m (30°51.845′ N, 102°58.286′ E) and 2830 m (30°51.782′ N, 102°58.364′ E) a.s.l., respectively.

In all three plots, soil samples were taken of the humus and soil profile using six depth ranges on Aug. 13, Aug. 16 and Aug. 20, 2003: Soil-1 (0–5 cm depth), Soil-2 (5–10 cm depth), Soil-3 (10–20 cm depth), Soil-4 (20–30 cm depth), Soil-5 (30–40 cm depth) and Soil-6 (40–50 cm depth). A total of 63 soil/humus samples were collected from the three plots (54 soil samples and 9 humus samples) for isotopic analysis. In order to calculate soil water contents, additional soil samples were collected on the sampling dates, dried at 105 °C for 48 h and then weighed. On the same dates soil samples were collected, samples of stems (~3 cm in length and 0.5–1.5 cm in diameter) and bark were also taken from three randomly chosen individuals of *Q. aquifolioides* and *S. luctuosa*. To reduce influences of evaporation, all samples were collected before 10:00 am, placed into 10 ml Pyrex tubes that were sealed with a plastic septum and Parafilm, and then stored at −15 °C for later analysis.

The biomass of rootlets of *Q. aquifolioides* and *S. luctuosa* was also determined in the sampling plots. After randomly selecting three plants of each shrub species, we collected 10 × 10 cm clods with the full depth of the soil layer from the humus and each soil layer (see previous paragraph) of a profile located outside 10 cm of the bases of the shrubs [2]. Rootlets (diameter < 5 mm) were extracted by flotation in saturated NaCl solution with a density of 1.2 g cm^{-3}, then weighed to obtain biomass.

Precipitation fell on most days during the sampling period. According to precipitation records, the majority of these precipitation events were less than 5 mm d^{-1}. Monthly mean precipitation and air temperature on Balang Mountain from 2001 to 2003 are shown in Fig. 1.

2.3. Stable isotopic analysis

Water samples were extracted from soil and stem samples using a cryogenic vacuum distillation extraction system [16] and then measured by a continuous flow isotope ratio mass spectrometer coupled with a TC/EA (MAT-253, Thermo-Finnigan Instrument Inc., Germany). With respect to the Vienna Standard Mean Ocean Water (VSMOW), the final results were expressed as:

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

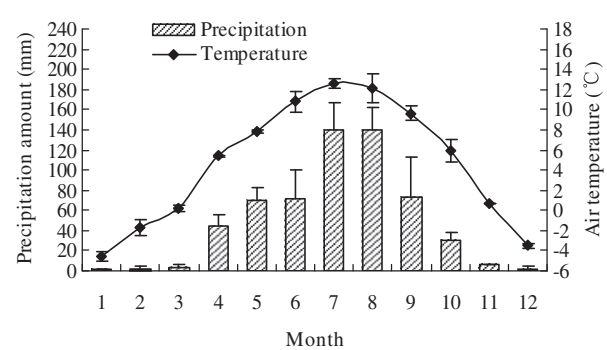

Fig. 1. Mean monthly precipitation and mean monthly air temperature on Balang Mountain using data from 2001 to 2003.

where δ_{sample} is the isotope ratio of the sample relative to VSMOW, R_{sample} is the ratio of $^2H/^1H$ or $^{18}O/^{16}O$ in samples and $R_{standard}$ is the ratio of $^2H/^1H$ or $^{18}O/^{16}O$ in the VSMOW. The analytical precisions were ≤2.0‰ for δD and ≤0.1‰ for $\delta^{18}O$.

2.4. Data analysis

In order to make stable isotope analysis reasonable, two assumptions were made in this study: (1) the isotopic composition of the soil water was laterally homogeneous within the rooting area [3,4,24]; and (2) the timing of samplings of the humus, soil and stems, and time delays associated with transport of isotopes up the plant, were not important [3,4,36].

The local meteoric water line (LMWL) on Balang Mountain during July and August of 2003 [10] was used to analyze the water source characteristics of shrub stems as described below:

$$\delta D = 8.28\delta^{18}O + 8.93. \quad (2)$$

The IsoSource software [33] was used to quantify bounds of the contributions of multiple water sources when the numbers of potential sources were more than the numbers of different isotopic tracers. In this study, the water sources were defined as the soil waters in each layer (from humus to Soil-6) of the three plots, and the mean isotopic values of each layer were used to represent the potential water sources. The mixtures of this model were determined by analyzing the mean isotopic values of the stem waters of every species in the three plots on each sampling day. Within the IsoSource program, the increment of each source was set at 1% and the tolerances of the sum at 0.01‰.

One-way ANOVA was used to evaluate the differences among soil water contents, rootlet biomass and the contributions of various soil waters. Partial correlation analysis was applied to determine whether water uptake patterns of *Q. aquifolioides* and *S. luctuosa* were consistent with their rootlet biomass distribution. The water content profile was considered to be a covariate in partial correlation analysis. All statistical analyses were conducted in the Statistical Package for the Social Sciences (SPSS Version 12.0 for Windows).

3. Results

3.1. Isotopic characteristics of soil water and stem water of shrubs

The δD and $\delta^{18}O$ values of soil water ranged from −87.5‰ to −70.1‰ and from −11.5‰ to −8.0‰, respectively. According to Fig. 2, the range in δD and $\delta^{18}O$ values of *Q. aquifolioides* was similar to that of *S. luctuosa* (Fig. 2b,c).

Comparing the best-fit regression lines for stem water with that for soil water revealed strong similarities, indicating that the stem water originated from soil water at a depth between 0 and 50 cm (Fig. 2). The slopes of the regression lines for soil and stem waters were less than that of LWML ($\delta D = 8.28\delta^{18}O + 8.93$), which indicated that evaporative fractionation of water in the soil had occurred prior to uptake of water by the shrubs.

3.2. Soil water content and rootlet biomass of Q. aquifolioides and S. luctuosa

The mean soil water content of the three sampling plots decreased asymptotically for all three sampling dates with increasing depth of the soil profile (i.e., from humus to soil-6) (Fig. 3). The water contents in the upper layers (humus, 0–5 and 5–10 cm depths) on Aug. 16 exceeded those on Aug. 13 and Aug. 20, while the water contents in the deeper layers (deeper than 10 cm depth of soil) were lower and more stable among the three sampling dates.

The rootlet biomass of *Q. aquifolioides* increased from Soil-6 to the humus layer, *S luctuosa* rootlet biomass is lower at 20–30 and 30–40 cm depths than at any other depths (including 40–50 cm) and the rootlet biomass of *Q. aquifolioides* was similar to that of *S. luctuosa* in the upper soil layers (humus to 20 cm depth) (Fig. 4). In the upper 30 cm of the soil profile (including humus), mean rootlet biomass of *Q. aquifolioides* accounted for 80.5 ± 4.5% of the total root biomass and that of *S. luctuosa* accounted for 76.7 ± 7.6%.

3.3. Water uptake patterns of Q. aquifolioides and S. luctuosa

The variation of δD was similar to that of $\delta^{18}O$ with soil profile depth (left figures in Fig. 5); hence, it was feasible to use coupled water isotopes in the IsoSource model. The water uptake patterns of *Q. aquifolioides* and *S. luctuosa* on the three sampling days were described by distribution patterns of contributions of the soil water as calculated by the IsoSource model. The largest mean contribution of soil water to stem water occurred in the 0–5 cm soil layer (right figures in Fig. 5). On Aug. 13 and 16, the contributions of soil water to stem water were minimal between 5 and 30 cm but then increased somewhat in the deepest layers. Contributions of soil water to stem water on Aug. 20 were slightly different through the soil profile, with the highest contributions occurring from 0 to 10 cm depth and relatively low and constant contributions of all other layers. Thus, both of these subalpine shrubs, which have shallow rooting systems, tended to utilize soil water primarily from the upper soil layers but could also use water from all the layers (humus to Soil-6).

When considering rootlet biomass distribution and water content profile separately, water uptake patterns of *Q. aquifolioides* and *S. luctuosa* were correlated with their rootlet biomass distribution ($r = 0.390$, $p < 0.05$, and $r = 0.502$, $p < 0.05$, $n = 21$, respectively) and water content profile of the soil ($r = 0.703$, $p < 0.001$, and $r = 0.685$, $p < 0.001$, $n = 21$, respectively). However, using the water content profile as a covariate, water uptake patterns of *Q. aquifolioides* were not consistent with their rootlet biomass distribution ($r = 0.151$, $p > 0.25$, $n = 21$). In contrast, water uptake patterns of *S. luctuosa* were consistent with their rootlet biomass distribution ($r = 0.457$, $p < 0.05$, $n = 21$). Therefore, water content variation in the soil profile affected relationships between water uptake patterns of these plants and their rootlet biomass distributions.

4. Discussion

4.1. Advantages of coupled water isotope techniques in studying water pathways among soils, roots and stems

Yi et al. [41] developed a new method for describing characteristics of input water to lakes using coupled isotope tracers (δD and $\delta^{18}O$). We applied this method to characterizing water dynamics among soils and plant roots and stems. Generally, the stable isotope ratios of hydrogen and oxygen in water samples can identify water sources and water dynamics in watersheds [30,43] Previous studies using δD tracers have shown that sugar maple trees can absorb water from groundwater through the root system [44] and that the shrub *Artemisia ordosia* takes advantage of deeper soil water recharged by large rain events in arid regions [6]. In our study, comparison of the similarity in the relationship between δD vs. $\delta^{18}O$ of soil and stem water indicated that stem water of our subalpine shrubs originated primarily from soil water with a depth of 0–50 cm. Moreover, by comparing the regression line for soil water with the local meteoric water line (LMWL), it was clear that

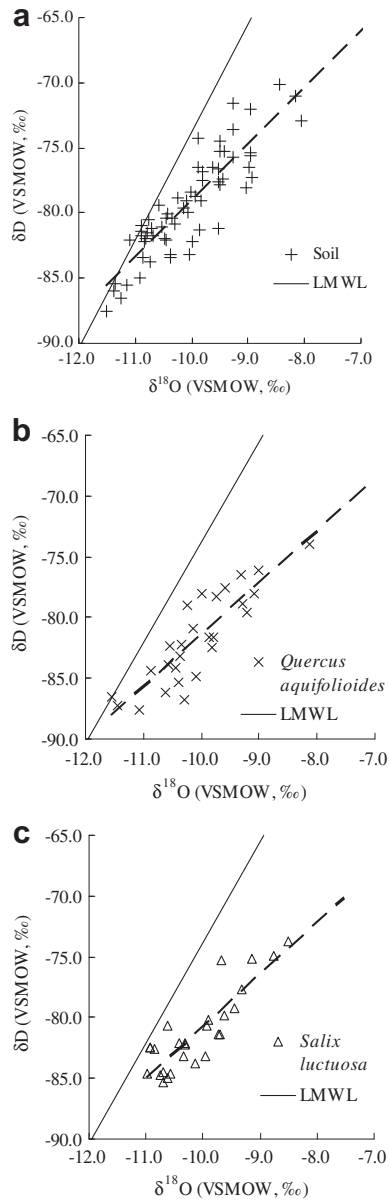

Fig. 2. The relationships between δD and $\delta^{18}O$ for the local meteoric water line (LMWL) and soil and shrub water on the south-facing slope of Balang Mountain. Dashed lines represent the best-fit regression lines for (a) Soil water: $\delta D = 4.34\delta^{18}O - 35.73$ ($n = 63$, $R^2 = 0.78$, $p = 0.000$); (b) Stem water of *Quercus aquifolioides*: $\delta D_Q = 4.29\delta^{18}O_Q - 38.63$ ($n = 27$, $R^2 = 0.75$, $p = 0.000$); and (c) Stem water of *Salix luctuosa*: $\delta D_S = 4.26\delta^{18}O_S - 38.20$ ($n = 27$, $R^2 = 0.75$, $p = 0.000$).

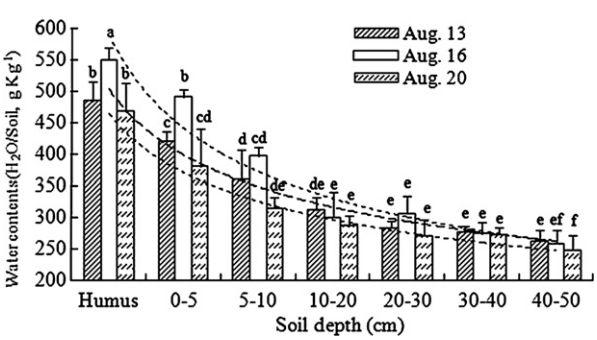

Fig. 3. Mean soil water contents of the three sample plots on the south slope of Balang Mountain on Aug. 13, Aug. 16 and Aug. 20, 2003. Different letters indicate that soil water contents are significantly different ($p < 0.05$; Tukey HSD test).

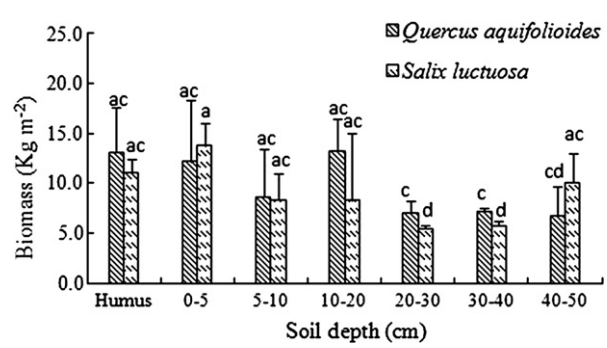

Fig. 4. Rootlet biomass distribution of *Quercus aquifolioides* and *Salix luctuosa* in the soil column from humus to Soil-6 (40–50 cm depth) layers on the south-facing slope of Balang Mountain in August 2003. Different letters indicate significant differences ($p < 0.05$; Tukey HSD test) in rootlet biomass.

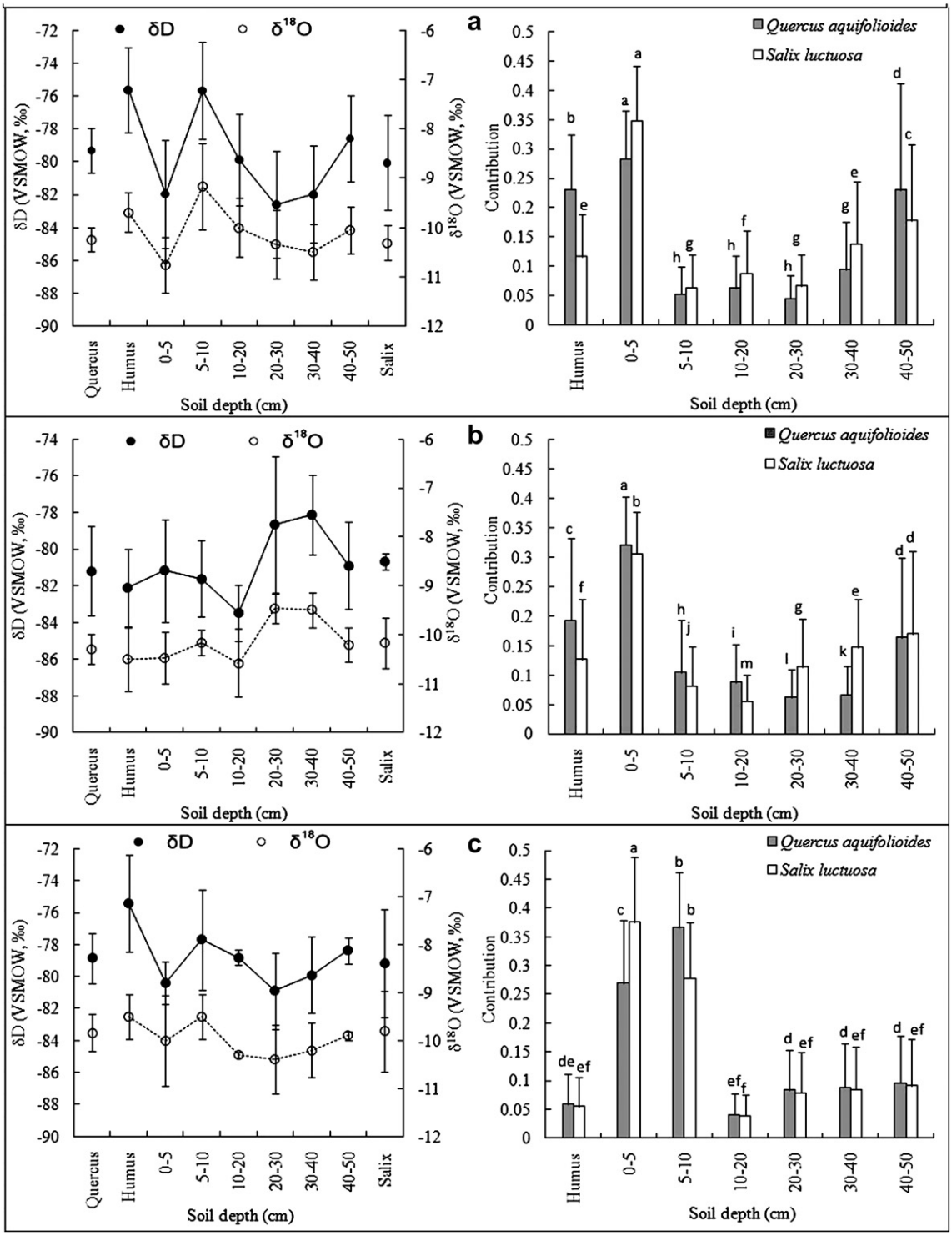

Fig. 5. Isotopic composition of soil water throughout the soil profile and stem water of *Quercus aquifolioides* and *Salix luctuosa* (left side), and water uptake patterns of these shrubs (right side) on the three sampling days on the south-facing slope of Balang Mountain. (a) Aug. 13, 2003; (b) Aug. 16, 2003; and (c) Aug. 20, 2003. On the abscissa axis, Quercus refers to *Q. aquifolioides* and Salix refers to *S. luctuosa*. Different letters indicate significant differences ($p < 0.05$; Tukey HSD test) in soil layer water contributions.

evaporation affected soil waters before they were taken up by the plants. Therefore, the water isotope technique we used was able to provide new insights into explaining plant water sources and water dynamics, whereas traditional methods such as analysis of the chemistry of soil solutions [1] are not able to distinguish these effects.

Quantification of the contributions of water sources to a mixture is another important water isotope application. Traditionally, root water uptake has been described indirectly by excavating the plant's root system [17,22] and observing the variability of sap flow within stems [20]. Wang et al. [38]. applied multiple-source mass balance assessment to estimate the depth of root water uptake of

crops and contributions of soil water to plant water in different growing seasons. In this study, we also quantified contributions to stem water of soil water from different layers of the soil profile with an isotope mixing model (IsoSource) and established relationships between water uptake patterns and root distribution using partial correlation analysis. These studies denote a new way of thinking about simulation of root water uptake that utilizes coupling of stable isotopes of hydrogen and oxygen, and thus, they represent a significant contribution to the study of the water cycle within the soil–root–plant continuum.

4.2. Effects of water uptake patterns of shrubs on reducing risk of flooding

For water uptake patterns to match the root distribution and the soil water content profile, there must be sufficient water available from rainfall in the shallow soil horizons to satisfy the vegetation requirements [24], especially in semiarid and arid areas [29,37]. In our study area in August 2003, the total amount of evaporation outside and inside the vegetated areas was 77.4 mm and 13.5 mm, respectively, while precipitation contributed 114.8 mm according to meteorological records. This meant that much more of total precipitation entered the soil horizons of shrub. Lei et al. [25,26] and Wei et al. [39] reported similar results, indicating that 38% of total precipitation entered the alpine oak shrubland in Miyaluo near our sampling site. At a similar site, He et al. [21] found that a small amount of precipitation (<10 mm d^{-1}) could recharge soil water at 50 cm depth. Moreover, the East Asian and Indian monsoons brought large amounts of precipitation to Wolong Nature Reserve, especially in July to August (Fig. 1), and precipitation fell on 83% of days from July to September during 2001–2003 [27]. Therefore, although sufficient water was available in the shallow soil horizons during the rainy season to satisfy this presupposition of Knight's hypothesis, the hydrological inputs brought by recent precipitation events could increase risk of flooding.

In our study plots, bedrock below the soil layer at about 50 cm deep obstructed root growth of *Q. aquifolioides* and *S. luctuosa* [40]; thus, most of the rootlet biomass of these species was distributed in the upper 30 cm of the soil profile (Fig. 4). This was consistent with other studies that found that most of the roots of woody shrubs were located in the shallow soil horizons [5,22]. Moreover, water uptake patterns of *Q. aquifolioides* and *S. luctuosa* showed that the mean uptake fractions in the top 30 cm of the soil profile during sampling days were 75.4% and 73.1%, respectively (Fig. 5). Hence, the two shrubs in our study had shallow rooting systems and utilized soil water mostly from the top 30 cm. Other studies have also found that vegetation exhibited a strong tendency to absorb soil water from the upper 30 cm of the soil profile at sites with relatively high rainfall [4,7].

In summary, alpine shrubs in our study primarily utilized the shallow soil water resulting from rainwater. Furthermore, significant evaporation existed on southern slopes, and the water holding capacity of the alpine shrub canopy was high [35]. Therefore, the alpine shrub community could play an important role in keeping rainwater from entering river channels quickly, thereby reducing risk of flooding.

5. Conclusions

We analyzed stable isotope contents of soil water and stem water of *Q. aquifolioides* and *S. luctuosa* in conjunction with rootlet biomass and soil water content. We found: (1) Stem water of *Q. aquifolioides* and *S. luctuosa* came directly from the soil profile, with 75.4% and 73.1% of stem water originating from the top 30 cm of the soil profile in August 2003; (2) Water uptake patterns of the two subalpine shrub species matched rootlet biomass distributions or soil water contents throughout the soil profile, but only that of *S. luctuosa* was consistent with both rootlet biomass distribution and soil water content throughout the soil profile; (3) Alpine shrub communities could play an important role in keeping rainwater from entering river channels quickly, thereby reducing risk of flooding.

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