

Partitioning evapotranspiration flux components in a subalpine shrubland based on stable isotopic measurements

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ABSTRACT. Soil, vegetation and atmospheric water vapor (0.1~3 m above ground) were sampled in a subalpine shrubland covered with *Quercus aquifolioides* during three days in the early monsoon period in Wolong Nature Reserve, China. In June 2006, the average LAI of *Q. aquifolioides* was 2.05 m² m⁻² and the average community coverage was more than 90%. Isotope turbulent mixing relationships, isotopic values of transpired water from plants and that of evaporating water vapor from soil surface were used to estimate fractions of transpiration and evaporation contributing to the total evapotranspiration (ET). The method worked well for δD , but it was imprecise for $\delta^{18}O$ because the minute isotopic differences between transpired water and soil water. The results from δD showed that fractional contributions of plant transpiration to ET were 74.5±9.9%, 65.6±8.3% and 96.9±2.0% on 21st, 24th and 25th June, 2006, respectively, implying that ET is mostly generated by plant transpiration. Notably, the transpiration from herbage layer for ET was likewise important as that from shrub layer. Our approach is useful for partitioning ET in semiarid subalpine shrubland at an ecosystem scale on short time steps. This approach improves the understanding of water exchange in semiarid ecosystems, and offers an opportunity to measure and validate component fluxes with accurate spatial representation at a common scale.

Keywords: Evapotranspiration; Flux partitioning; *Quercus aquifolioides*; Semiarid shrubland; Stable isotopes.

INTRODUCTION

Evapotranspiration (ET) is one of the important climatic factors controlling energy and mass exchange between terrestrial ecosystems and the atmosphere (Chen et al., 2006) and plays a specially important role in semiarid landscapes (Huxman et al., 2005). During the growing season in many arid and semiarid environments, energy and mass fluxes are temporally and spatially heterogeneous due to monsoonal precipitation which is episodic and localized (Yepez et al., 2003; Williams et al., 2004). Hereinto, ET usually accounts for 90% of precipitation inputs in these ecosystems (Wilcox et al., 2003), and shifts precipitation inputs rapidly in mass and energy cycles between ecosystem and ambient components (Yepez et al., 2003). The change of ET, respectively its two components during the dynamic wetting and drying cycles (Ehleringer et al., 1991, 1999; Jackson et al., 1998; Yepez et al., 2003),

provide a detailed insight how biotic and abiotic factors change vegetation and eco-hydrological processes, such as ecosystem productivity (Huxman et al., 2005; Yepez et al., 2005) and vegetation influences on water and energy exchange (Moreira et al., 1997; Wang and Yakir, 2000; Yakir and Sternberg, 2000).

Effective methods, such as lysimetric method (Wangati and Blackie, 1971), sap flow measurement techniques (Jackson et al., 2000), models and remote sensing (Kairu, 1991; Nagler et al., 2007), and micrometeorological techniques (Lenschow, 1995; Moncrieff et al., 2000), are used to measure or estimate ET. But, there are several limitations in using these methods: lysimetric data are point data and cannot be used for verifying regional ET estimates (Kairu, 1991); the application of sap flow is limited to individual plants, particularly large trees (Kairu, 1991; Ehleringer and Field, 1993); models and remote sensing approaches need a lot of soil, plant and atmospheric input data and field validation to refine at appropriate scales (Kairu, 1991); and micrometeorological methods are unable to distinguish different components of ET (Wang and Yakir, 2000). With these limitations, spatial

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representation is especially difficult to overcome (Wilson and Meyers, 2001).

Stable isotopic tracer methods offer a new opportunity to study the components of ET at the field-scale, from the leaf level to ecosystem (Kao, 1997; Kao and Chang, 1998; Wang and Yakir, 1995; Harwood et al., 1998; Kao et al., 2000; Wang and Yeh, 2003; Kao et al., 2002), and can partition the ET from different compartments of the ecosystem incorporating measurement of water vapor (Gat, 1996; Yakir and Wang, 1996; Brunel et al., 1997; Moreira et al., 1997; Wang and Yakir, 2000; Yepez et al., 2003, 2005; Williams et al., 2004). The basis for using stable isotopes to study ET is the differences between soil evaporation and leaf transpiration; and ET, the sum of evaporation and transpiration, also has a different isotopic characteristic than with the background moisture in most cases (Gat and Matsui, 1991; Wang and Yakir, 2000). These isotopic distinctions and the concentration of moisture around vegetation can be used to estimating ET by generating Keeling plots, the isotope turbulent mixing relationships (Williams et al., 2004). Then, the contributing fractions of evaporation and transpiration to total ET, net ecosystem discrimination and soil disequilibrium effects, can be identified (Yakir and Sternberg, 2000; Yepez et al., 2003, 2005). Combinations of stable isotope analyses and other measurements, such as sap flow (Cramer et al., 1999; Williams et al., 2004) and micrometeorological methods (Yepez et al., 2003; Griffis et al., 2004), also offer unique information about ecosystem functioning and dynamics (Cable and Huxman, 2004). Using stable isotopes to estimate ET flux components is now a widely used approach in terrestrial ecosystem studies (Helliker et al., 2002). However, this method requires high precision, in isotopic sampling and analysis which is currently at the limit of detection (Yakir and Sternberg, 2000).

In this study, we partition ET from a semiarid subalpine shrubland in Wolong Nature Reserve, Western China, with the use of stable isotopes. We generated Keeling plots with data from five layers inside the vegetation to assess ecosystem isotopic flux. We attempt to determine the relative contributions of soil evaporation, shrub and herbage transpiration to ecosystem-scale ET in the semiarid subalpine shrubland.

MATERIALS AND METHODS

Study site

The study site was located on Balang Mountain in Wolong Nature Reserve, China (30°51.437' N, 102°58.308' E, altitude 2,743 m). Around the study site, there is a relatively flat terrain (slope < 5°, extent about 200 m × 800 m). Vegetation at the site was a subalpine shrubland composed of alpine oak (*Quercus aquifolioides* Rehd. Et Wils.), which dominates the slope facing south of Balang Mountain between 2,700 m and 3,600 m altitude. The vegetation composed of alpine oak has obviously xeric characteristics (WNRAB, 1987). Under the canopy, the

dominant herbage species is *Cystopteris montana* (Lam.) Bernh. ex Desv., which belongs to geophytes. Diameter at base height (DbH) and height of *Q. aquifolioides* varied from 2 cm to 5 cm and from 1.1 m to 2.5 m, respectively; the height of herbage varied from 0.01 m to 0.5 m. In June 2006, the average LAI of *Q. aquifolioides* was 2.05 m² m⁻² (LAI-2000, Li-Cor Inc., Lincoln, NE, USA). The average community coverage was more than 90% in the June. The soil type is similar to Cambisols and the soil depth is generally about 50 cm (WNRAB, 1987; Liu et al., 2006).

East Asian Monsoon and Indian Monsoon can influence Wolong from April to October. But on Balang Mountain, the influence of plateau climate is stronger. Hence, this region is dry and cold, and the diurnal range in temperature is large (WNRAB, 1987). Mean annual precipitation amount is 710 mm, and about 62±7% of precipitation occurs from July to September; mean annual evaporation amount, mean annual temperature and mean annual relative humidity are about 800 mm, 3°C and 79%, respectively (Zheng et al., 2006). Precipitation in this region has a high spatial and temporal variability except for July, August and September.

There is a meteorological observation field of Wolong Ecological Station in the east of study site, about 150 m away. In this field, precipitation amount was recorded by CR2-06 pluviometer (Songtao digital technology, Chengdu, China) with an automatic recorder. Evaporation outside vegetation was measured every day by evaporation pan. Wind speeds at 10 m were measured every hour by a ZL wind velocity indicator (Shanghai Meteorological Instrument Factory Co., Ltd., Shanghai, China).

At the study site, actual evaporation from vegetation was estimated every day by another evaporation pan inside vegetation. Wind speeds at 3 m were measured by DEM6 cup anemometer with wind vane (Tianjin Meteorological Instrument Factory, Tianjin, China). Photon flux density (PFD), transpiration rate of alpine oak leaves, and atmospheric pressure were recorded every 15 minutes using a LI-190 Quantum Sensor in combination with a standard chamber of a LI 6400 gas analyzer (Li-Cor Inc., Lincoln, NE, USA). Soil temperature (5 cm depth) was measured by angle pipe geothermometers at three randomly chosen locations. Air temperature and relative humidity were measured every 5 minutes by PT1000 and HIH3610 probes of HT501-II (Hartech Co., Ltd., Hangzhou, China), respectively. Vapor pressure deficit (VPD) and water vapor concentration were calculated from data recorded by HT501-II.

Theory of flux partitioning

Naturally, there are several kinds of stable isotopes in water molecules: ²H (D), ¹H, ¹⁸O, ¹⁷O and ¹⁶O. Thereof, D, ¹H, ¹⁸O and ¹⁶O were measured in this study. Concentrations of these isotopes are expressed as deviation from an international standard (V-SMOW) and using δ notation in per mil (‰):

$$\delta (\text{‰}) = [(R_s/R_{st}) - 1] \times 1000 \quad (1)$$

where R_s and R_{st} are the molar ratio of the heavy to light isotopes in the sample and the standard, respectively.

Soil water and leaf water are the sources of evapotranspiration. In the processes of transfer, isotopic composition of water is modified due to the fractionations of equilibrium isotope effects, kinetic and vital effects, or transport effects (Gat, 1996). Craig and Gordon (1965) described a model to calculate the isotopic ratios of evaporating water vapor from soil surface (δ_E) as:

$$\delta_E = \frac{[\alpha^* \delta_L - h \delta_{ab} - \varepsilon^* - (1-h) \varepsilon_k] / [(1-h) + (1-h) \varepsilon_k / 1000]}{\varepsilon_k / 1000} \quad (2)$$

where δ_L is the isotopic composition of liquid water at the evaporating surface; δ_{ab} is the isotopic composition of the background atmospheric water vapor; α^* is the temperature-dependent equilibrium fractionation factor for ^{18}O or D; $\varepsilon^* = (1 - \alpha^*) \times 1000\text{‰}$; ε_k is the kinetic fractionation factor, about 18.9‰ for oxygen and 17.0‰ for hydrogen in a turbulent boundary layer (Wang and Yakir, 2000); h is the relative humidity (range 0 to 1) normalized to the temperature of the soil surface.

In this paper, $\alpha^* < 1$ and $\alpha^* = 1/\alpha^+$ (Gat, 1996); and α^+ can be calculated by the equation provided by Majoube (1971):

$$^{18}\text{O}\alpha^+ = \frac{[1.137(10^6/T^2) - 0.4156(10^3/T) - 2.0667]/1000}{+1} \quad (3)$$

$$\text{D}\alpha^+ = \frac{[24.844(10^6/T^2) - 76.248(10^3/T) + 52.612]/1000}{+1} \quad (4)$$

where T is soil temperature recorded at 5 cm depth in degrees Kelvin.

From Eq. 2, we see that the evaporating water vapor from soil surface is more depleted in both ^{18}O and D with respect to liquid water (i.e. $\delta_E < \delta_L$). It also shows that this depletion is a function of isotopic compositions of liquid water at the evaporating surface and the atmospheric vapor, the relative humidity, and fractionations associated with the diffusivity of water molecules across the boundary layer (Yakir and Sternberg, 2000). Many experimental results for soil water undergoing evaporation have shown that the predictions, which were generated by Craig-Gordon model, are reliable (Walker and Brunel, 1990; Mathieu and Bariac, 1996a, 1996b).

If isotopic steady state (ISS) of plants lasts sufficiently long and the isotopic enrichment by leaves can be ignored, the transpiration water from leaves will have the same isotopic signature as the source water used by plants (Dawson, 1993; Moreira et al., 1997), which means we can use the isotopic compositions of water from stem or xylem or sap to replace that of transpiration water from leaves (Yakir and Sternberg, 2000). Flanagan et al. (1991), Wang and Yakir (1995) reported that broadleaved species would gradual approach to ISS within 1~3 h after drastic changes in ambient conditions in the laboratory. Notably, because the time required to approach ISS is dependent on the humidity surrounding the leaf and the turnover time of leaf water (Wang and Yakir, 1995), isotopic compositions of transpiration vapor and stem water will not always be

the same during observation periods. This limitation could be overcome by averaging all the values measured over the long-term (Flanagan et al., 1991; Wang and Yakir, 1995; Harwood et al., 1999). In this paper, we assumed the transpiring vegetation to be under isotopic steady state, and then isotopic values of transpiration vapor (δ_T) were determined by analyzing isotopic values of stem water (Moreira et al., 1997; Wang and Yakir, 2000).

To partition the total evapotranspiration flux, we also need to know the isotopic compositions of ET (δ_{ET}). In the ecosystem, the combination of some background amount of a substance and some amount of the substance from sources is usually considered as the atmospheric concentration of this substance (Yakir and Sternberg, 2000). Hence, based on mass balance, δ_{ET} can be estimated by the Keeling plots (isotope turbulent mixing relationships) described by Keeling (1961):

$$\delta_{\text{ebl}} = C_a(\delta_a - \delta_{\text{ET}}) / C_{\text{ebl}} + \delta_{\text{ET}} \quad (5)$$

where δ_{ebl} is the isotopic signature of the substance in the ecosystem which can be collected from the ecosystem boundary layer; C_a , C_{ebl} are the concentrations of the substance in the atmosphere and the ecosystem boundary layer, respectively; δ_a is the isotopic signature of the substance in the atmosphere.

This is a linear relationship, and when used with water vapor the y-intercept reflects the isotopic values of ET (Moreira et al., 1997; Yopez et al., 2003). When using this model to estimate δ_{ET} , Yakir and Sternberg (2000) suggested two assumptions: (1) there is no loss of water vapor from the ecosystem excluding turbulent mixing with the atmosphere; (2) sources of the combined atmospheric water vapor come from evaporated and transpired water vapor and the background atmospheric vapor. Though these assumptions would not be met perfectly in field works, Gat (1996) and Wang and Yakir (2000) considered that isotopic variations caused by these imperfect conditions would not generate significant influence on partitioning ET.

The fractional contributions by transpiration and evaporation to ET are calculated by software Isoerror (version 1.04; Phillips and Gregg, 2001) and IsoSource (version 1.3.1; Phillips and Gregg, 2003) provided by Health and Environmental Effects Research Laboratory of US Environmental Protection Agency (<http://www.epa.gov/wed/pages/models.htm>). When there are only two sources (e.g. combined shrub and herbage transpiration as one source), Isoerror calculates the mean and the standard error of the fractional contributions based on the uncertainty generated by the variability of both sources and the intercept in a linear regression on Keeling plots (Phillips and Gregg, 2001). When there is three or more sources (e.g. if shrub and herbage transpiration are independent processes), IsoSource examines all possible combinations of each source contribution (0~100%) in small increments, then all feasible solutions are statistic to generate the mean and the standard error of the fractional contributions (Phillips and Gregg, 2003).

The sampling heights of water vapor have significant influence on the spatial resolution of Keeling plots (Flanagan and Ehleringer, 1998; Dawson et al., 2002; Yepez et al., 2003). Therefore, Wang and Yakir (2000), Yepez et al. (2003) suggested collecting samples in collection profiles from lower to upper heights to improve spatial representation.

Soil water, plant water and vapor collection

In the growing season, samples were collected before the period of precipitation sets in frequent are heavy (21st, 24th, and 25th June, 2006). Using a hand-auger, soil was sampled from the surface to 10 cm. Sampled branches of alpine oak were 0.5~1.0 cm in diameter, 2~3 cm in length and from each of them the bark was removed. Stems of *C. montana* were collected from the basal portions. Every plant sample was composed of 2~3 stems from different individuals. The sampling period on each day was from 10:00 to 12:00 h and from 13:00 to 15:00 h; and in every hour three soil samples, three branch samples of alpine oak and three stem samples of *C. montana* were collected. Soil and plant samples were placed into screw-cap glass vials (5 ml) and sealed with Parafilm, then stored at about 2°C. Soil and plant water was extracted by cryogenic vacuum distillation (Ehleringer et al., 2000).

The average isotopic signature of water from soil cores (δ_s) was used for δ_L in Eq. 2. Soil temperature (T , 0.05 m depth) was recorded at the same time soil cores were collected. Because δ_T was the isotopic combination of shrub transpiration (δ_{Ts}) and herbage transpiration (δ_{Th}), it could be determined by the weighted average of the isotopic value of bulk vegetation (Yepez et al., 2003). Based on the average canopy cover of the alpine oak of about 60% in the June, the weighted value was calculated as $\delta_T = 0.6 \delta_{Ts} + 0.4 \delta_{Th}$, assuming equal transpiration rates per unit leaf area of both functional groups.

Water vapor was collected from 5 heights at a time (0.1 m, 0.5 m, 1.5 m, 2.0 m and 3.0 m). During the collection period mentioned above, sampling was started at 10:00, 11:00, 13:00 and 14:00 h. For each group vapor was collected during 30 min with a flow rate of 250 ml min^{-1} using a PM850.5 vacuum pump (voltage 6 V, Ruiyi Mechanical Design Center, Chengdu, China). The air was circulated through a set of 45 cm long glass traps (modified from Helliker et al., 2002) which were immersed in a mixture of ethanol and liquid nitrogen (about -80°C). Traps were made of 9 mm (o.d.) Pyrex glass attached to 6~9 mm (o.d.) Cajon Ultra-Torr adapters which framed in 9 mm (o.d.) Swagelok Union Tee. After sampling the traps were sealed with Parafilm and stored at about 2°C. Samples were transferred to 9 mm (o.d.) Pyrex tubes by cryogenic vacuum distillation (Ehleringer et al., 2000).

Near the vapor sampling inlets, probes of HT501-II recorded air temperature (T_a , in Kelvin) and relative humidity (h , as a fraction between 0 and 1) every 5 min. Using T_a , h and atmospheric pressure (P_a , in hPa), water vapor concentration was calculated by (McRae, 1980):

$$[\text{H}_2\text{O}] \text{ (mmol mol}^{-1}\text{)} = 10h[P_A \exp(13.3185t - 1.9760t^2 - 0.6445t^3 - 0.1299t^4)]/P_a \quad (6)$$

where P_A is standard atmosphere pressure (about 1013.25 hPa) and $t = 1 - (373.15/T_a)$.

Using the inverse of average vapor concentration ($1/[\text{H}_2\text{O}]$) during sampling period of each height as independent variables, and isotopic values of water vapor ($\delta^{18}\text{O}$ or δD) collected at the corresponding height as dependent variables, Keeling plots were generated. The isotopic ratio of ET (δ_{ET}) was obtained from the intercept of the Keeling plots (described in Eq. 6). Using δ_T (including δ_{Ts} and δ_{Th}) and δ_s as sources, the fractional contributions of transpiration and evaporation to ET (δ_{ET}) were calculated by Isoerror and IsoSource.

Stable isotope and data analysis

The stable isotope ratio analysis for ^{18}O was processed as follows: at a constant temperature of 25°C, water samples are mixed with 0.1% CO_2 for isotopic equilibrium over 18 h, and then the gas mixture was measured by mass spectrometry. This automated continuous flow analysis was performed by Gas Bench II and MAT-253 (Finnigan MAT, Bremen, Germany).

To measure isotopic concentrations of D, water samples were dropped into the reaction furnace with carbon column (> 1400°C), and then the produced gas was separated and H_2 was transferred to mass spectrometer using helium as carrier gas. This automated continuous flow analysis was performed by TC/EA (Finnigan MAT, Bremen, Germany) and MAT-253.

The water samples were isotopically analyzed at Key Laboratory of Nuclear Resources and Environment, Ministry of Education, East China Institute of Technology. The standard deviation for repeated analysis of laboratory standards with above methods was $\leq 0.2\text{‰}$ for ^{18}O and $\leq 2\text{‰}$ for D. In this study, the sample analyses for ^{18}O and D were repeated five times independently.

In this paper, the parameters were calculated by SPSS (Statistical Package for the Social Sciences, Version 13.0) using standard type I linear regressions. Keeling plots were pictured by Excel 2007.

RESULTS

Environmental conditions during sampling days

The last precipitation event occurred six days before the first sampling day (21st June). And after two precipitation events of 19.2 mm and 5.3 mm, the second and third sampling days (24th and 25th June) were chosen (Figure 1). Amount of outside and inside vegetation evaporation pan were more than 2.1 mm d^{-1} and 0.9 mm d^{-1} , respectively (Figure 1).

Influenced by cloud on 21st June, the PFD was no more than 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 24th and 25th June were sunny and in most of sampling time the PFD was close to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2). The average VPD from 9:00 to

16:00 hours in these three days were 1.40 ± 0.69 kPa, 1.23 ± 0.52 kPa and 1.21 ± 0.28 kPa, respectively, and the peak of VPD on each day was occurring between 9:00 and 10:00 h (Figure 2). From 9:00 to 16:00 h, wind speeds at 10 m ranged from 0.2 to 10.0 m s^{-1} and the daily peak wind speed was occurred around 14:00 h (Figure 2); wind speeds at 3 m respectively were $0.97 \pm 0.51 \text{ m s}^{-1}$, $0.41 \pm 0.18 \text{ m s}^{-1}$ and $0.83 \pm 0.20 \text{ m s}^{-1}$ in the three days (Figure 2). The transpiration rates of alpine oak respectively were $0.50 \pm 0.17 \text{ mmol m}^{-2} \text{ s}^{-1}$, $0.93 \pm 0.50 \text{ mmol m}^{-2} \text{ s}^{-1}$ and $1.61 \pm 0.53 \text{ mmol m}^{-2} \text{ s}^{-1}$ on the three days (Figure 2).

Isotopic ratios of evaporation and transpiration water

Isotopic compositions of soil water (δ_s) ranged from -8.5% to -4.3% for $\delta^{18}\text{O}$, and from -71.9% to -20.1% for δD , respectively. Isotopic ratios of stem water from *Q. aquifolioides* (δ_{Ts}) ranged from -9.3% to -4.7% for $\delta^{18}\text{O}$, and from -66.7% to -22.0% for δD , respectively. Isotopic ratios of stem water from *C. montana* (δ_{Th}) ranged from

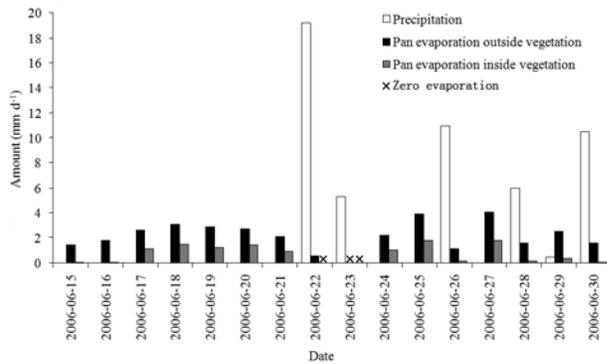


Figure 1. Daily precipitation, pan evaporation outside and inside vegetation from 15th to 30th June, 2006.

Table 1. Parameters used to estimate the isotopic values of evaporating water vapor from soil surface (δ_E) with Craig-Gordon model (Craig and Gordon, 1965). Soil temperature (T) was the average of three randomly chosen locations. Relative humidity (h) was the average at 0.1 m height. The average isotopic values of water at the soil surface (δ_s , 0~10 cm) was used for the isotopic value of liquid water at the evaporating surface (δ_L). δ_{ab} was the average value for vapor collected at 0.1m above the ground. α^* is the temperature-dependent equilibrium fractionation factor; $\epsilon^* = (1 - \alpha^*) \times 1000\%$; ϵ_k is the kinetic fractionation factor for molecular diffusion.

Date	T (\pm SD, K)	h (\pm SD, %)	δ_s (\pm SD, ‰)	δ_{ab} (\pm SD, ‰)	α^*	ϵ^* (‰)	ϵ_k (‰)	δ_E (\pm SD, ‰)	
21st June Morning	289.60 \pm 0.07	40.8 \pm 4.2	$\delta^{18}\text{O}$	-7.0 \pm 1.0	-11.4 \pm 0.6	0.990045	10.0	18.9	-38.9 \pm 1.0
			δD	-33.2 \pm 8.0	-71.7 \pm 2.8	0.921191	78.8	17.0	-150.0 \pm 6.0
Afternoon	289.30 \pm 0.07	53.7 \pm 2.9	$\delta^{18}\text{O}$	-7.3 \pm 0.7	-11.5 \pm 1.8	0.990019	10.0	18.9	-41.9 \pm 2.6
			δD	-33.5 \pm 12.9	-77.5 \pm 0.4	0.920901	79.1	17.0	-161.9 \pm 4.9
24th June Morning	287.32 \pm 0.96	46.4 \pm 25.2	$\delta^{18}\text{O}$	-7.1 \pm 0.5	-14.3 \pm 1.1	0.989844	10.2	18.9	-39.8 \pm 6.0
			δD	-25.5 \pm 2.7	-89.9 \pm 6.7	0.918960	81.0	17.0	-140.8 \pm 23.2
Afternoon	292.87 \pm 0.18	35.3 \pm 3.4	$\delta^{18}\text{O}$	-7.1 \pm 0.6	-10.6 \pm 0.8	0.990324	9.7	18.9	-38.1 \pm 0.8
			δD	-29.8 \pm 6.1	-75.2 \pm 1.7	0.924289	75.7	17.0	-133.1 \pm 5.1
25th June Morning	286.03 \pm 0.69	37.6 \pm 8.9	$\delta^{18}\text{O}$	-6.4 \pm 1.2	-12.5 \pm 1.0	0.989729	10.3	18.9	-37.3 \pm 1.2
			δD	-45.7 \pm 8.9	-77.1 \pm 2.6	0.917679	82.3	17.0	-167.1 \pm 2.7
Afternoon	293.07 \pm 0.66	26.9 \pm 3.0	$\delta^{18}\text{O}$	-7.0 \pm 0.9	-10.2 \pm 2.4	0.990341	9.7	18.9	-37.1 \pm 1.1
			δD	-60.3 \pm 11.0	-59.8 \pm 6.2	0.924474	75.5	17.0	-169.8 \pm 10.2

-8.0% to -4.4% for $\delta^{18}\text{O}$, and from -57.5% to -11.6% for δD , respectively. Isotopic compositions of vapor (δ_a) ranged from -15.9% to -6.4% for $\delta^{18}\text{O}$, and from -102.4% to -55.4% for δD , respectively. These results indicated that isotopic values of evaporating water vapor from soil surface (δ_E , soil evaporation, Table 1) were more isotopically depleted relative to vapor generated by plant transpiration (δ_T , Table 2) during the three sampling days.

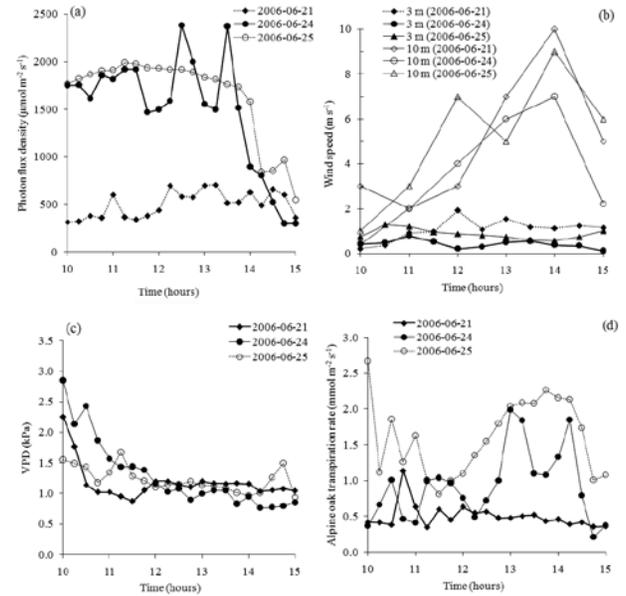


Figure 2. The environmental conditions of sampling days (21st, 24th and 25th June, 2006). (a) Photon flux density ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) from 9:00 to 16:00 h; (b) wind speeds (m s^{-1}) recorded at 3 m and 10 m from 9:00 to 16:00 h; (c) vapor pressure deficit (VPD, kPa) from 9:00 to 16:00 h; (d) transpiration rate of alpine oak ($\text{mmol m}^{-2} \text{ s}^{-1}$) from 10:00 to 15:00 h.

Keeling plot analyses and fractional contributions of sources

Significant regression lines were found in Keeling plots pictured by δD , but those plotted by $\delta^{18}O$ were not significant (significance level 0.05, Table 3). Table 3 also shows the slope and intercept of Keeling plots. Only significant regression lines are shown in Figure 3. All intercepts of Keeling plots were close to symbols which reflected isotopic values of plant transpiration relative to that reflected isotopic compositions of soil evaporation. This meant plant transpiration contributes more to ET than soil evaporation.

Considering shrub and herbage transpiration as one source and soil evaporation as another one, the fractional contributions of plant transpiration to total ET (T/ET) were $96.9 \pm 1.6\%$, $97.7 \pm 2.0\%$ and $95.2 \pm 1.3\%$ for $\delta^{18}O$, $74.5 \pm 9.9\%$, $65.6 \pm 8.3\%$ and $96.9 \pm 2.0\%$ for δD on 21st, 24th and 25th June, respectively. The estimated contributions of soil evaporation given by $\delta^{18}O$ were obviously less than that obtained by δD on 21st and 24th June (Figure 4). Because Keeling plots for $\delta^{18}O$ were not significant, δ_{ET} for ^{18}O might be imprecise. Consequently, we partitioned ET flux into different ecosystem components using δD results. Fractional contributions of transpiration from *Q. aquifolioides* and *C. montana* were also calculated independently (Figure 4). Results showed that the fractions of shrub and herbage transpiration were similar, and it

Table 2. Average isotopic values of stem water from *Quercus aquifolioides* (δ_{TS}) and from *Cystopteris montana* (δ_{TH}), and the weighted average isotopic values for plant transpiration ($\delta_T = 0.6 \delta_{TS} + 0.4 \delta_{TH}$).

Date		δ_{TS} (\pm SD, ‰)	δ_{TH} (\pm SD, ‰)	δ_T (\pm SD, ‰)
21st June	$\delta^{18}O$	-7.0 ± 0.9	-6.1 ± 0.9	-6.7 ± 0.7
	δD	-28.8 ± 5.1	-17.6 ± 4.1	-24.8 ± 3.3
24th June	$\delta^{18}O$	-8.0 ± 1.1	-7.2 ± 0.4	-7.6 ± 0.7
	δD	-30.9 ± 5.5	-21.3 ± 8.7	-26.8 ± 6.0
25th June	$\delta^{18}O$	-7.9 ± 0.5	-6.2 ± 0.6	-7.2 ± 0.3
	δD	-56.2 ± 7.4	-43.2 ± 10.2	-49.4 ± 6.3

Table 3. Slope and intercept of the regression lines between $\delta^{18}O$ or δD values of water vapor collected at different heights (0.1–3 m above ground) and the inverse of the corresponding vapor concentration. The intercept indicates the isotopic values of evapotranspiration (δ_{ET}). C.I. = confidence interval.

Data	Keeling plots					C.I. (95%) for intercept		
		Slope (\pm SD)	Intercept (\pm SD)	R ²	P	n	Lower	Upper
21st June	$\delta^{18}O$	-50.68 ± 33.50	-7.71 ± 1.96	0.139	0.159	16	-11.95	-3.56
	δD	-336.70 ± 56.47	-58.20 ± 3.30	0.718	0.00004*	16	-65.29	-51.14
24th June	$\delta^{18}O$	-71.01 ± 34.33	-8.37 ± 2.40	0.211	0.055	18	-13.46	-3.28
	δD	-322.50 ± 128.80	-64.72 ± 9.01	0.281	0.023*	18	-83.83	-45.63
25th June	$\delta^{18}O$	-40.66 ± 26.37	-8.64 ± 1.58	0.130	0.146	18	-12.01	-5.32
	δD	-309.60 ± 101.78	-53.06 ± 6.08	0.366	0.008*	18	-65.98	-40.18

*The significance level is 0.05.

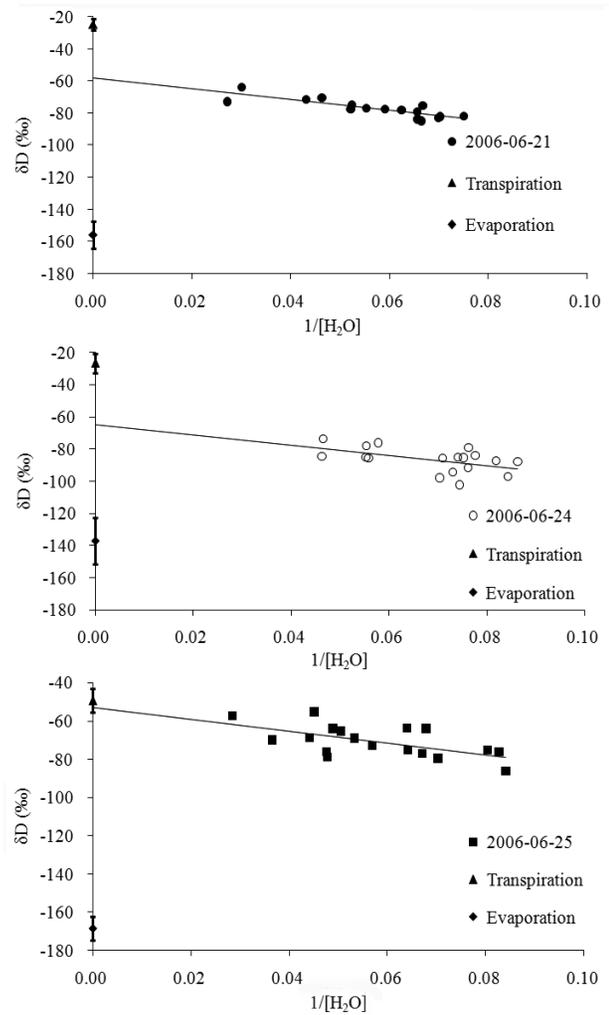


Figure 3. Keeling plots for δD of water vapor collected at different heights (0.1–3 m above ground) on 21st, 24th and 25th June, 2006, respectively. Keeling plots for $\delta^{18}O$ of water vapor in these days are not present because the significances of these regression lines were $p > 0.05$. The samples of water vapor were started to collect at 10:00, 11:00, 13:00 and 14:00 h, and vapor was collected for 30 min with a flow rate of 250 ml min^{-1} for each group. The slope and intercept of the regression lines are shown in Table 3.

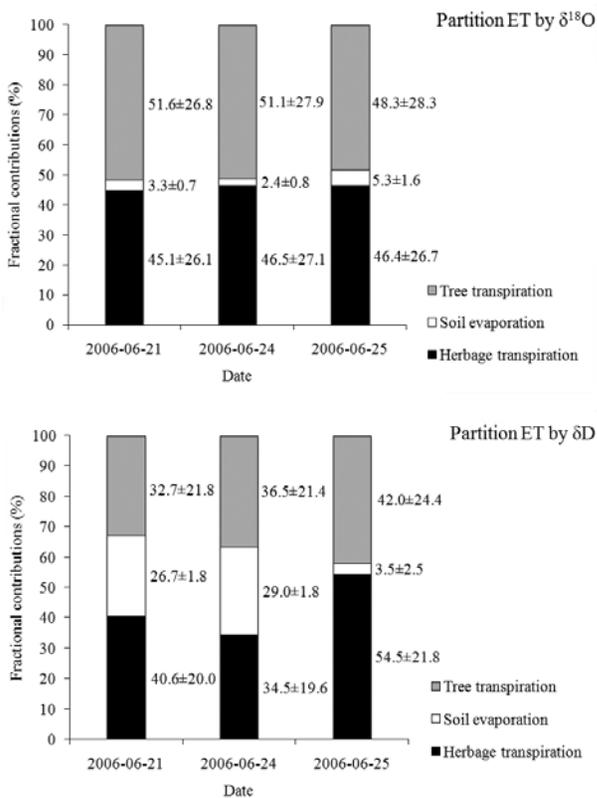


Figure 4. Total ET partitioned into shrub transpiration, herbage transpiration and soil evaporation in the ecosystem dominated by *Quercus aquifolioides* in shrub layer and *Cystopteris montana* in herbage layer. Numbers indicate the estimated fraction of each ecosystem component (\pm SD, %) using IsoSource (Phillips and Gregg, 2003) and Isoerror (Phillips and Gregg, 2001).

implied that herbage layer also have important function in local water exchange.

DISCUSSION

The dynamics of woody plants in semiarid and arid landscapes have important implications for hydrology, ecology, and society due to woody plants potential of changing water cycles in these regions (Huxman et al., 2005). In these arid and semiarid ecosystems, ET is the most important component of the local water cycle (Wilcox et al., 2003). Thus, describing the relations of ET flux and different ecosystem components will tell us more information about ecosystem function in these regions, such as presenting the factors that controlling ecosystem production (Jackson et al., 1998; Huxman et al., 2005) or underlying ecosystem-level water-use efficiency (Yepez et al., 2005). Stable isotopes and Keeling plots provided unique information about the ecosystem ET flux in a subalpine shrubland dominated by *Q. aquifolioides*. Our results demonstrate that distinguishing different components of ET by stable isotopes is feasible in a semiarid subalpine shrubland.

In this study, we assumed that the plants were approach

to isotopic steady state during the sampling periods on each day. Laboratory experiments told us ISS of broadleaved species would gradual approach in 1~3 h after drastic changes in ambient conditions (Flanagan et al., 1991; Wang and Yakir, 1995). Flanagan et al. (1991) and Yepez et al. (2003) suggested that small leaves, relatively constant radiation and VPD, and high transpiration rates would help plants promote a rapid progression to ISS due to a fast turnover time of leaf water. At the study site, alpine oak has evergreen leaves which were 2.5~5 cm in length and 1.5~2.5 cm in width in June, 2006. We collected samples from 10:00 to 12:00 h and from 13:00 to 15:00 h, and the start time was 2.5~3 h after sunrise. From 10:00 to 16:00 h, radiation (represented by photon flux density) and VPD was relative stable (Figure 2) on each day. In these sampling periods, pan evaporation rates were high especially on the 24th and 25th June (Figure 2); evaporation rates on 21st June were lower than on other days but also were close to those reported by Yepez et al. (2003). Therefore, we believe that we collected samples under the ambient conditions that likely promoted a rapid approach to ISS.

Many works indicated that the potential deviations from ISS of transpired vapor (e.g. 1~3‰ for $\delta^{18}\text{O}$) should have minimal influence on partitioning the ET into its components (Flanagan et al., 1991; Wang and Yakir, 1995; Harwood et al., 1998; Yepez et al., 2003, 2005; Williams et al., 2004) because the magnitude of isotopic variation of transpired vapor is too small compared to the highly isotopically depleted soil evaporation (Table 1, Table 2; Gat, 1996; Wang and Yakir, 2000; Yepez et al., 2003). In this work, the maximal variations of total transpired vapor from ISS were 0.7‰ for $\delta^{18}\text{O}$ and 6.3‰ for δD , respectively (Table 2). It would represent 1.8% and 3.7% changes in the ratio of plant transpiration to soil evaporation for $\delta^{18}\text{O}$ and δD , respectively, and these variations fell in current 95% confidence intervals.

δ_{Ts} differed from δ_{Th} (Table 2). We considered that it may be caused by shrub using soil water correspondingly deeper than herbs. The source water in the soil became surprisingly negative on 25th June compared to 24th June. Using δD as a sample, (1) δ_{Ts} on 24th June was $-30.9 \pm 5.5\text{‰}$ and detailed instances were $-26.2 \pm 2.9\text{‰}$ on 10:00 h, $-28.9 \pm 1.8\text{‰}$ on 11:00 h, $-31.5 \pm 6.2\text{‰}$ on 13:00 h and $-36.8 \pm 0.8\text{‰}$ on 14:00 h; (2) δ_{Ts} on 25th June was $-56.2 \pm 7.4\text{‰}$ and detailed instances were $-56.6 \pm 8.7\text{‰}$ on 10:00 and 11:00 h, and $-55.9 \pm 7.2\text{‰}$ on 13:00 and 14:00 h. Forasmuch, we suggested that: following the upper soil horizons went short of soil water, shrubs and herbs became to use deeper soil water which was recharged by precipitation on 22nd and 23rd June that might be rather negative (Xu et al., 2008).

The regression lines using $\delta^{18}\text{O}$ of water vapor at different heights above ground and the inverse of corresponding water concentration were not significant in this study. Two conditions may account for these results: (1) our method depends on the isotopic differences

between δ_T (δ_{Ts} or δ_{Th} or both) and δ_S values (Wang and Yakir, 2000). In this work, $\delta^{18}O$ values of *Q. aquifolioides* and *C. montana* were similar to that of shallow soil water (Table 1 and Table 2). Wang and Yakir (2000) pointed out that the difference between δ_T and δ_S values could diminish if the soil surface is drying up. Besides this aspect, alpine oak preferring to utilize shallow soil water (Xu et al., unpublished) also might diminish the difference between δ_T and δ_S values. The equilibrium enrichment factor (ϵ^*) of δD is much greater than that of $\delta^{18}O$ (Table 1), therefore in this study the isotopic differences between plant and soil water for δD might be less influenced by the water uptake mode and dry shallow soil horizons; (2) the small number of observations (Table 3) leaves us with a relatively high degree of statistical uncertainty. Though Yepez et al. (2003) only used 11 observations to generate a significant regression line of Keeling plots for partitioning understory ET, more observations of different heights and long-playing collection may help to improve the spatial resolution (Flanagan and Ehleringer, 1998; Dawson et al., 2002), the precision and the reliability (Harwood et al., 1999) of Keeling plot analyses.

Results of T/ET showed that plant transpiration was the dominant source for total ET in June, 2006, the early monsoon period. These results also reflected the vegetation which is dominated by alpine oak that used the available moisture efficiently (Yepez et al., 2003). These results consisted with other studies conducted in tropical rain forest ecosystems (Shukla et al., 1990; Nobre et al., 1991; Moreira et al., 1997), arid and semiarid ecosystems (Wang and Yakir, 2000; Yepez et al., 2003, 2005; Williams et al., 2004), but disagreed with a study in fallow bush land of woody shrubs (Brunel et al., 1997). Brunel et al. (1997) reported that plant transpiration only contributed 20% of the total ET. This disagreement may be caused by low vegetation coverage (only 20%) in the study of Brunel et al. (1997). We observed the fractions of soil evaporation for δD decreased obviously on 25th June relative to 24th June (Figure 4). There occurred 19.2 mm and 5.3 mm precipitation on 22nd and 23rd June (Figure 1), respectively, but 24th and 25th June were sunny and pan evaporation rates were high (Figure 2). Therefore, we conclude that soil horizons close to ground surface were drying up along with the plant water uptake, and water for soil surface evaporation in these horizons was absent. This trend of fractions of soil evaporation in ET is in agreement with the findings by Wang and Yakir (2000): the contribution of soil evaporation to total ET is small or negligible in arid and semiarid environments except for short periods after precipitation events. Another comparison in this study, the fraction of soil evaporation to ET based on δD was similar on 21st June to that of 24th June. We mentioned that the transpiration rate was not high due to low PFD on 21st June (Figure 2). Thus, the surface soil horizons did not dry up too much by plant water uptake and evaporation. In contrast with soil evaporation, T/ET as determined via δD was increased from 24th to 25th June. This is consisted with Yepez et al.

(2005) who found T/ET increased over several days after irrigation in semiarid grassland.

CONCLUSIONS

The analyses of stable isotopes and Keeling plots allowed us to partition ET into different flux components in a subalpine shrubland covered with *Q. aquifolioides* in the Wolong Nature Reserve. Our findings indicate that ET is mostly generated by plant transpiration, more than 65% on three sampling days during the early monsoon period. Transpiration from the herbage layer appears to be as important as that from the shrub layer. In this work, using $\delta^{18}O$ to partition ET is unreliable mainly because the isotopic differences between transpired water and soil water were minute. Our findings help to improve our understanding of the water fluxes and functioning of the alpine oak shrubland ecosystem. With further improvements of the method it could possibly be used to study regional energy and water exchange.

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使用穩定同位素方法劃分亞高山灌叢的蒸散組分

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在中國臥龍自然保護區季風期早期的三天裏，採集了川滇高山櫟亞高山灌叢中的土壤、植被和大氣水蒸氣（地面之上 0.1~3 m）樣品進行穩定同位素分析。2006 年 6 月研究區域內川滇高山櫟平均葉面積指數為 $2.05 \text{ m}^2 \text{ m}^{-2}$ ，川滇高山櫟群落蓋度大於 90%。本研究通過分析同位素湍流混合關係、植物蒸騰水和土壤表層蒸發水分的穩定同位素值，估算了蒸騰和蒸發對蒸散總量的貢獻比例。在本研究中運用 δD 得到了較精確的結果；而蒸騰水與土壤表層水之間較小的同位素差異可能導致在運用 $\delta^{18}\text{O}$ 分析時得到的結果不精確。在 2006 年 6 月 21 日、24 日和 25 日， δD 方面的結果顯示植物蒸騰對蒸散的貢獻分別為 $74.5 \pm 9.9\%$ 、 $65.6 \pm 8.3\%$ 和 $96.9 \pm 2.0\%$ ，這表明川滇高山櫟灌叢中的蒸散主要來自於植物蒸騰。值得注意的是，草本層的蒸騰和灌木層的蒸騰對蒸散的貢獻同等重要。從短期和生態系統尺度上看，我們的方法在半乾早亞高山灌叢中能有效劃分蒸散組分。本研究有助於加深對半乾旱生態系統中水分交換的認識，也驗證了一個在普通尺度上有較高空間代表性的測量蒸散組分通量的方法。

關鍵詞：蒸散；劃分通量；川滇高山櫟；半乾旱灌叢；穩定同位素。

