Stable isotope analysis of plant water and soil water across two vegetation types in the northern Qinghai-Tibet Plateau

Jing Li[1], Fawei Zhang[2], Yangong Du[3], Yunying WANG[1], Yuting Lan[4], Bo Fan[1], H Zhou[5], Bin wang[6], Guangmin Cao[1], and Xiaowei Guo[2]

[1]Northwest Institute of Plateau Biology Chinese Academy of Sciences
[2]Northwest Institute of Plateau Biology, Chinese Academy of Sciences
[3]Northwest Institute of Plateau Biology
[4]Northwest Institute of Plateau Biology, Chinese Academy of Science
[5]Northwest Plateau Institute of Biology, Chinese Academy of Sciences
[6]NSW Department of Primary Industries, NSW DPI, Wagga Wagga, NSW Australia, 2650

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Abstract

Studying the interrelation of soil water and plant water is essential for an in-depth understanding of eco-hydrological processes. However, water use relationships and comparative studies between shrubs and alpine grassland of the northern Qinghai-Tibet Plateau remain poorly understood. In this study, we compared δ18O and δ2H values of water from soil, plant, precipitation, and groundwater between P. fruticosa shrub and alpine grassland locations at two neighboring sites in order to better understand the interface between plant and surrounding soils of shrubs and grasslands in the northern Qinghai-Tibet Plateau. Our results showed that δ18O and δ2H of soil water, precipitation, and plant water varied significantly over time and water sources in P. fruticosa shrub and alpine grassland sites. Both soil evaporation and plant transpiration at the P. fruticosa shrub site were relatively lower than they were at the alpine grassland site. Alpine grassland plant water had a stronger dynamic fractionation effect in the process of transportation and was more sensitive to environmental conditions. However, plants at the P. fruticosa shrub site displayed more flexible water use patterns, shifted their water sources between shallow soil water and deep soil water. Shrubs from alpine grassland leaded to changes in grassland water use, thereby changing soil water storage. The results of this study will provide theoretical basis for improving the availability and sustainability of soil water, provide guidance for meadow management from ecohydrological processes on the northern Qinghai-Tibet Plateau.

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Jing Li [1,2#], Fawei Zhang [1,3#], Yangong Du [1,3], Yunying Wang [1,2], Yuting Lan[1,3], Mengke Si [1,3], Bo Fan[1,3], Huakun Zhou[1,3], Bin Wang[4], Guangmin Cao [1,3*], Xiaowei Guo[1,3*]

1 Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China

2 College of Resources and Environment, University of the Chinese Academy of Sciences, Beijing, China

3 Qinghai Provincial Key Laboratory of Restoration Ecology for Cold Regions, Xining, Qinghai, China

4 New South Wales Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga 2650, Australia

# These authors contribute equally.

* Correspondence: Xiaowei Guo and Guangmin Cao, Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining,810008, China; Email: *guoxw@nwipb.cas.cn*; Tel & fax: +86 09716123010

Abstract:

Studying the interrelation of soil water and plant water is essential for an in-depth understanding of eco-hydrological processes. However, water use relationships and comparative studies between shrubs and alpine grassland of the northern Qinghai-Tibet Plateau remain poorly understood. In this study, we compared $\delta^{18}O$ and $\delta^{2}H$ values of water from soil, plant, precipitation, and groundwater between *P. fruticosa* shrub and alpine grassland locations at two neighboring sites in order to better understand the interface between plant and surrounding soils of shrubs and grasslands in the northern Qinghai-Tibet Plateau. Our results showed that $\delta^{18}O$ and $\delta^{2}H$ of soil water, precipitation, and plant water varied significantly over time and water sources in *P. fruticosa*shrub and alpine grassland sites. Both soil evaporation and plant transpiration at the *P. fruticosa* shrub site were relatively lower than they were at the alpine grassland site. Alpine grassland plant water had a stronger dynamic fractionation effect in the process of transportation and was more sensitive to environmental conditions. However, plants at the *P. fruticosa*shrub site displayed more flexible water use patterns, shifted their water sources between shallow soil water and deep soil water. Shrubs from alpine grassland leaded to changes in grassland water use, thereby changing soil water storage. The results of this study will provide theoretical basis for improving the availability and sustainability of soil water, provide guidance for meadow management from ecohydrological processes on the northern Qinghai-Tibet Plateau.

Keyword: Ecohydrological processes, Alpine meadow water, Qinghai-Tibet Plateau, Stable water isotopes, *P. fruticosa* shrub water, Soil water

Introduction

Water is one of the most crucial limiting factors determining the dynamic trends of plant in arid and semiarid ecosystems (Li et al., 2013). The availability of the primary water sources (i.e., soil water, groundwater) absorbed by plants have significant changes spatially and temporally, and determine the growth status of plants, and the distribution and growth status of plants affect the ecological structure and functions of the soil/plant system (White&Smith, 2013; Wu et al., 2019). In addition, the interaction of soil and plant water is a vital component of eco-hydrological processes (Dawson&Ehleringer, 1991; Feng et al., 2016; Chang et al., 2019). Therefore, there is an growing interest in researching studies on the interface between plants and surrounding soils (Sprenger et al., 2017; Roebroek et al., 2020).

The Qinghai-Tibet Plateau (QTP) is known as the "Chinese Water Tower" and is an essential component of China's "three screens and two belts" ecological security strategic pattern. QTP is particularly vulnerable to hydrological changes under climate change (He et al., 2020). Data observed from 2001 to 2018 indicated that both soil water storage and number of days of seasonal frozen soil declined over that period. Shrub meadows (*Potentilla fruticosa*) and alpine grasslands are the dominant vegetation types in the QTP. They have important water conservation functions (Dai et al., 2021), and play an important role in maintaining the water-heat balance and ecological barrier function of the QTP (Dai et al., 2019a). Previous studies of soil and plant between grasslands and shrubs only focused on exploiting different hydrological niches of plant and soil (Walker et al., 1981), soil moisture–vegetation feedbacks and their possible effects (D'Odorico et al., 2007), and functional differences in soil and plant (Ryel et al., 2008) by modeling. Additionally, physiological and physical characteristics (Volkmann et al., 2016), such as precipitation patterns (D'Odorico, et al., 2007), soil water availability (Gow et al., 2018) and distribution of fine roots (Lanning et al., 2020; Wang et al., 2021) affect the plant water use patterns, the plants influence soil water availability of different soil layers (Fu et al., 2017), with shallow soil water being affected by precipitation, and deep soil water being affected by groundwater (Feng, et al., 2016). However, on the one hand, water-use patterns of plants on the QTP only conducted on the*Achnatherum splendens* grassland (Jiang et al., 2021) and alpine riparian plants (Huawu et al., 2019), which both have an environment along the river and located in the west of QTP, on the other hand, many mechanisms and significance between shrubs and grasslands are not well understood, and little research on water-use patterns and relationships between shrubs and grasslands has been conducted on the north of QTP.

The isotopic variation of both plant water and soil water can provide valuable information on the interactions between plant water and soil water (Vargas et al., 2017; Che et al., 2019), providing an effective and powerful method for revealing and partitioning the different potential water sources used by plants (Dawson et al., 2002; Rothfuss&Javaux, 2017). During water absorption by roots and transportation along shoots prior to transpiration, no isotopic fractionation of water occurs in terrestrial plants (Ehleringer&Dawson, 1992; Dawson, et al., 2002), except for the halophytes concluding coastal wetland species and woody xerophytes, that H (but not O) fractionate due to symplastic movement of water during uptake (Ellsworth&Williams, 2007) (Brum et al., 2019).

In this study, we compared δ^{18}O and δ^{2}H from different water sources in a pair of neighboring sites, and distinguished water use sources of *P. fruticosa* shrubs and alpine grassland plants on different seasons in order to in-depth understanding of the interaction of soil water and plant water for *P. fruticosa* shrubs and alpine grasslands on the QTP. The results are expected to provide a theoretical basis for the management of the alpine grassland ecosystem, and sustainable use of the soil water in the northern QTP.

2. Materials and methods

2.1. Study area and experimental design

This study was conducted in Ganchaitan, close to Haibei station (101°19'E, 37deg37'N, 3100 m), and included two neighboring sites (i.e., shrubs and grassland) in the northeastern Qinghai-Tibet Plateau, located in Qinghai Province, China, (Figure 1). The region is in a semiarid, cold, high-altitude climate zone(Dai et al., 2019b). The mean annual precipitation is approximately 562 mm. Almost 80% of the annual precipitation falls during the growing season (i.e., from May to September). The mean annual air temperature is approximately -1.7, with the maximum temperature occurring in July (9.8) and the minimum temperature occurring in January (-14.80) (Dai, et al., 2021). Alpine shrubs and meadows are the dominant vegetation types at the study sites. The dominant primary vegetation is the shrub *P. fruticose* ; herbs include *Elymus nutans, Poaprsten, Kobresia humilis, Double-stigma Bulrush,* and *Polygonum vivipsrum* . The characteristics of the community were investigated and shown in Table 1.

Table1 Community characteristics of grassland and shrub sites.

Community characteristics	Experimental sites	Experimental sites
	Shrub	Grassland
Density (plants/5 m × 5 m)	5.23±1.22	29.11±1.89
Height (cm)	97.02±4.62	30.41±3.26
Coverage (%)	10.89±0.98	82.21±1.23
DBH or basal diameter (cm)	0.78±0.12	0.25±0.11

Note: three sub-sampled quadrats (5 m × 5 m) determined plant density, height, coverage, and DHB (basal diameter) at each site.

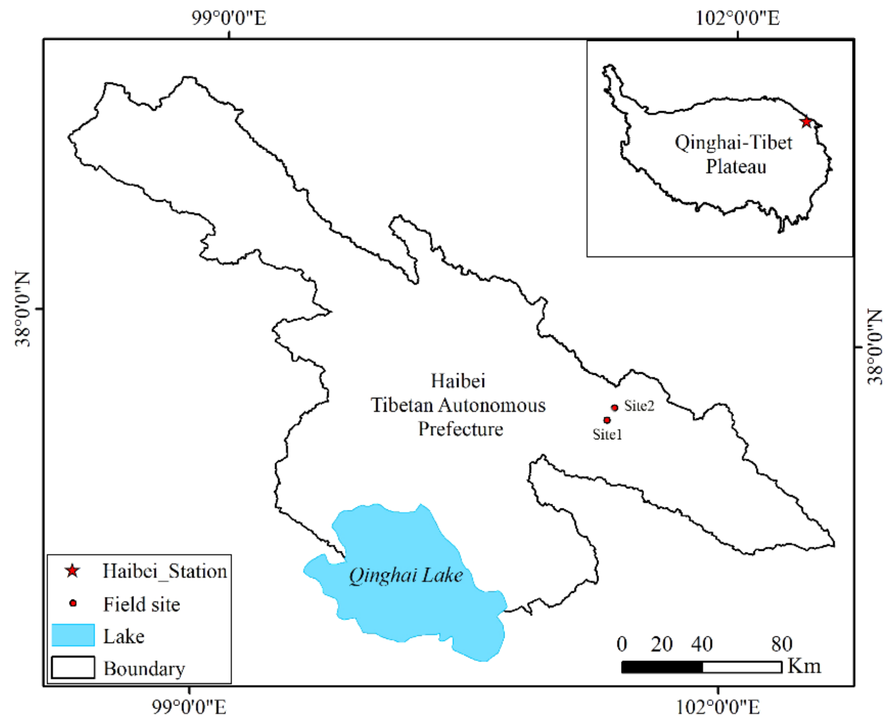


Figure1. Location of sample sites (a), shrub site (b) and grassland site (b).

2.2. Field sampling

We collected the samples, concluding the plant and soil, from each of the two experimental sites during the re-green season, growing season, and withering season in 2021 (20 May, 15 June, 16 July, 16 August, 7 September, and 12 October). Three replicates per plant species were randomly chosen on each sampling date in the two sites, and we collected a total of 72 xylem water samples. The lignified stem sections of*P. squamosa* were sampled at a height of 10 cm above the soil surface. In order to avoid the evaporative gas exchange in the bark tissue leading to isotopically enriched water, we removed the outer bark and phloem on the stem to separate the xylem for isotope analysis (Dawson&Ehleringer, 1993; Martín-Gómez et al., 2017). Three soil cores were extracted for soil samples at 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, 50–60 cm, 60–80 cm, and 80–100 cm within a 2 m radius around the selected plants. A total number of 360 soil samples were collected for isotopic analysis. All plant and soil samples were sealed in 20 ml glass vials with parafilm, and stored in a refrigerator at -10 degC until water extraction was performed using cryogenic vacuum distillation.

Precipitation and groundwater were sampled concurrently with soil water and plant water from May to October. The event-based precipitation samples were collected using a polyethylene bottle at the herder's home, 2 km away from our sites during the study period. Groundwater samples were collected once a week from a well 2 km away from our sites. The well had a depth of 2 m, and was commonly used for groundwater monitoring. A total of 162 and 72 samples from precipitation and groundwater, respectively, were all transferred into clean polyethylene bottles and stored in a refrigerator at -10 degC until water extraction was performed using cryogenic vacuum distillation.

The soil water content (SWC) was determined using an automatic soil moisture monitoring system (CR800; Campbell, USA) with sensors installed at depths of 5 cm, 10 cm, 15 cm, 20 cm, 30 cm, 40 cm, 50 cm, 60 cm, 80 cm, and 100 cm below the soil surface. Precipitation measurements were collected using a precipitation gauge (52,203, RM Young, USA) at a height of 0.5 m. Temperatures were obtained from a meteorological station (Molis 520; Vaisala, Finland). All data were recorded every 30 min.

2.3. Isotopic analysis

Precipitation, groundwater, plant water, and soil water samples were all extracted by the cryogenic vacuum distillation technique (LI-2100pro) in order to avoid the influence of high salinity moisture on the accuracy of the instrument. After all samples were equilibrated to room temperature, extraction was started, setting 3 h for plant water and soil water, 2h for precipitation and groundwater samples to achieve full recovery of sample water according to West et al. (2006). All of the extracted water from samples were transferred into 2 ml vials for analysis, and then analyzed stable isotopes (δ^2H and δ^{18}O) using an isotope ratio spectrometer (LGR-TLWIA-912). The instrument was equipped with an autosampler (PAL-LSI) for sample injection, and post-processing software (LWIA Post Analysis Full Installer v4.4.1) for test diagnosis, checking, and quantifying problems in the analysis (e.g., interference from organic pollutants, injection volume error) through detailed analysis of high-resolution absorption spectra. The organic contamination on plant water need correction procedures to eliminate the influence according to (Schultz et al., 2011). The measurement precision of the liquid water isotope analyzer was 0.3δ^{18}O. The isotopic compositions of δ^{18}O and δ^2H were expressed as an isotope ratio:

$$\delta sam(\%0) = \left(\frac{R\text{sam}}{R\text{std}} - 1\right) \times 1000\%0$$

where δsam was the isotopic difference for the samples relative to the Vienna Standard Mean Ocean Water (VSMOW) standard, and Rsam and Rstd were the molar abundance ratios (^{18}O/^{16}O and^{2}H/^{1}H) in the sample and standard, respectively.

2.4. Data analysis

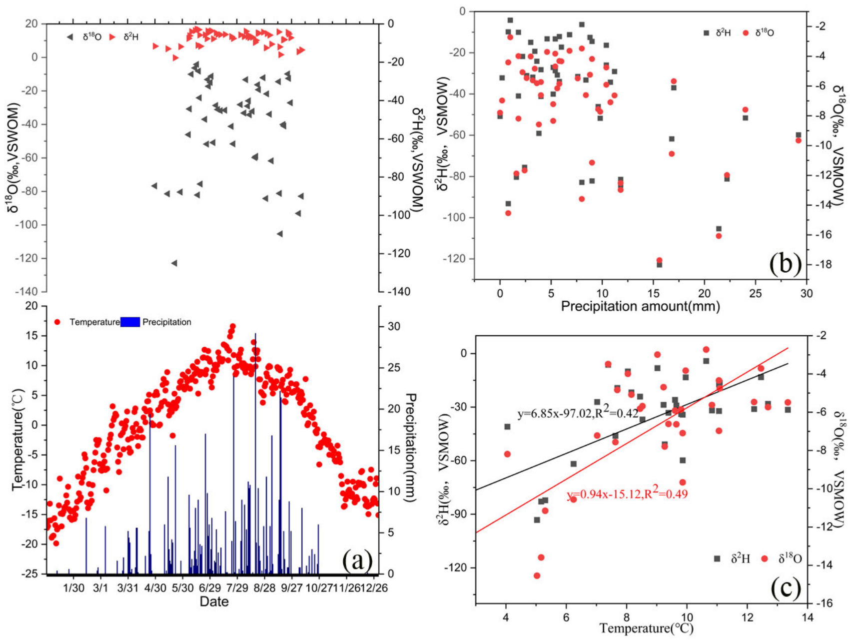
The Bayesian mixing model Mix SIAR (version 3.6.3) was used to identify the source of water absorbed by plant. The Mix SIAR model is a method to identify the proportional contributions of each water source (Stock and Semmens, 2013) according to the mean isotopic values (δ^2H and δ^{18}O), which were considered as the mixture data of the potential water sources (Dawson&Ehleringer, 1993; Beyer et al., 2018). The inputs of original data (for example, the 0-20 cm soil layer data was input as 0-10 cm and 10-20 cm isotope data), the discrimination data (the TDF data in the model), the running time of Markov chain Monte Carlo (MCMC), and the diagnosis method of the model results were according to (Zhou et al., 2021). The average value was output from the model. Three potential soil water sources were identified to facilitate subsequent analysis (i.e., shallow soil water (0–20 cm), middle soil water (20–60 cm), and deep soil water (60-100 cm)), according to the variability in the soil water content and the impacts of precipitation pulse.

One-way analysis of variance (ANOVA) followed by the post hoc Turkey's test at $p = 0.05$ was used to assess hydrometeorological parameters of sampling date and sites. Two-way ANOVA was performed to examine the significant effects of hydrometeorological parameters and their interactions. Pearson's correlations were tested at the $p = 0.05$ level. All statistical analyses were conducted using R software version 4.0.3, and all figures were plotted using Origin 9.1.

3. Results

3.1. Precipitation, temperature, and isotopes

A unimodal distribution was exhibited by temperature and precipitation amount in 2021, respectively (Figure 2). Temperature peaked at the end of July (16.61) and decreased monotonically to the end of October (-6.62) during the sampling period. Precipitation activity was concentrated in the same period, accounting for 86.10% (465 mm) of the total annual precipitation (540 mm). Four heavy precipitation events (> 20 mm) occurred in this period, with daily precipitation amounts of 29 mm on 18 August, 24 mm on 25 July, 22 mm on 14 September, and 21mm on 15 September. The δ^{18}O and δ^2H of precipitation showed large variations, the degree of enrichment and evaporative fractionation in May and October was more than in June to September, indicating that seasonal conditions are important factors that affect the isotope characteristics of precipitation (Figure 2).

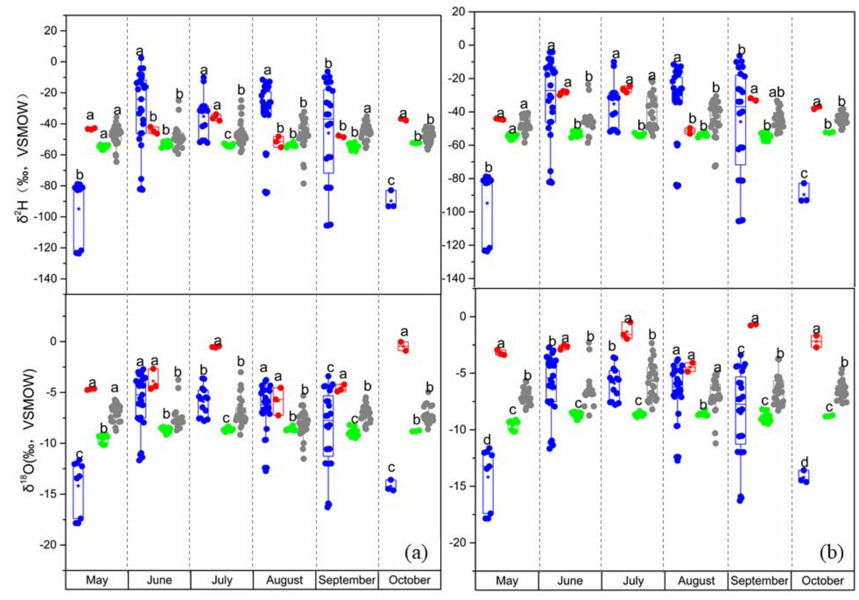


Φιγυρε 2. Πρεςιπιτατιον ανδ τεμπερατυρε ιν 2021 (α), τηε δ^{18}Ο ανδ δ^{2}Η οφ διφφερεντ πρεςιπιτατιον αμουντ (β) ανδ διφφερεντ τεμπερατυρε (ς) δυρινγ τηε σαμπλινγ περιοδ

3.2. Isotopic compositions in precipitation, plant water, soil water, and groundwater

The δ^{18}O and δ^{2}H values in the two sites varied greatly between water sources and months (Figure 3). The average values of δ^{2}H were -53.86and -38.48respectively. The respective average values of δ^{18}O were -8.83water, and plant water, a progressive increase in δ^{18}O or δ^{2}H existed from precipitation to soil water to plant water for each month (Figure 3). Moreover, the δ^{18}O and δ^{2}H in precipitation water increased initially and then decreased significantly from May to October ($p < 0.05$). The δ^{18}O and δ^{2}H of plant water responded well to the trends of precipitation from May to July, but not well from August to October. The δ^{18}O and δ^{2}H of soil water and groundwater showed no significant differences across months for the two sites (p¿0.05). Groundwater isotopes were consistent with the deeper soil, remained relatively stable.

Precipitation Plant water Ground water Soil water

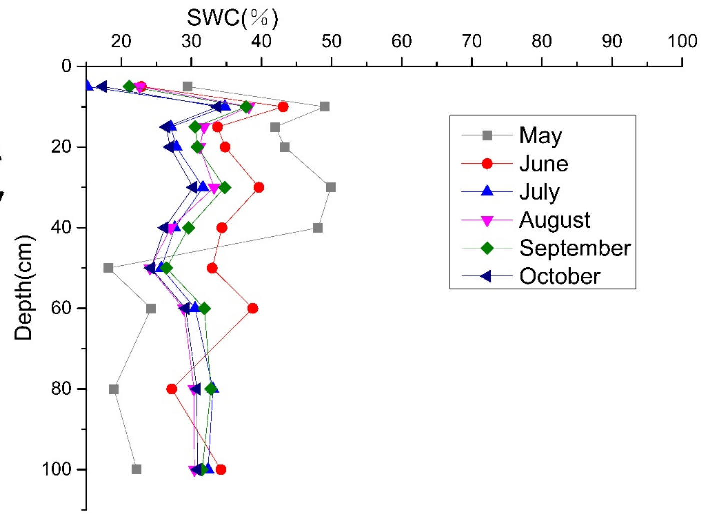


Φιγυρε 3. ἅριατιον οφ δ^{18}Ο ανδ δ^{2}Η οφ πρεςιπιτατιον, σοιλ ωατερ, πλαντ ωατερ, ανδ γρουνδωατερ βετωεεν σηρυβ (α) ανδ γρασσλανδ (β) σιτες

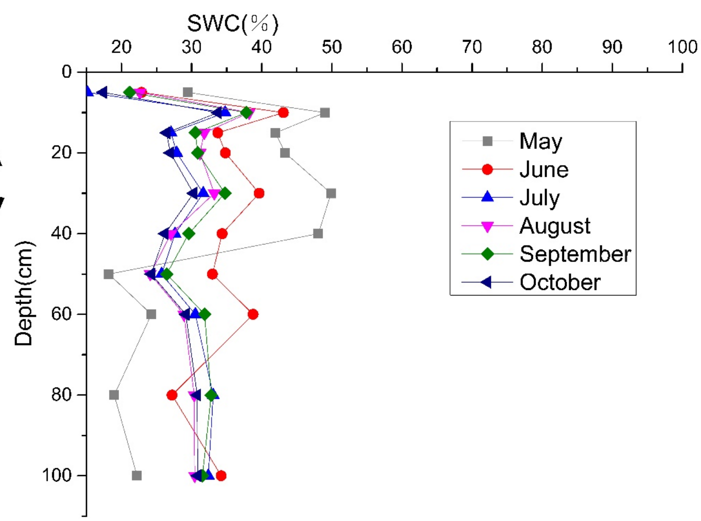
3.3. Comparisons of SWC and isotopes in shrub and grassland sites

There was a significant difference in SWC between shrub and grassland sites ($p < 0.05$) (Figure 4a). However, δ^{2}H in soil water showed no significant difference between shrub and grassland sites ($p = 0.068>0.05$), in contrast to δ^{18}O ($p < 0.05$) (Figure 4b). Likewise, significant differences in plant water between shrub and grassland sites for δ^{18}O were observed ($p < 0.05$), but non-significant differences were observed in plant water ($p = 0.06>0.05$) for δ^{2}H (Figure 3a). Both δ^{18}O and δ^{2}H values in shallow soil water (0–20 cm) were highly variable, and relatively stable in deeper soil layers for both the two sites (Figure 4b). The isotopic values in surface soil water were enriched in June and July, but were depleted in August and September (Figure 4b). The δ^{18}O and δ^{2}H values in the grassland plants were greater than in the shrubs from May to September, but almost the same in October, indicating that grassland plant water has a stronger dynamic fractionation effect in the process of transportation and is more sensitive to environmental conditions comparing with shrub plant water.

C


SWC(%)
20 30 40 50 60 70 80 90 100
0 20 40 60 80 100
Depth(cm)
May
June
July
August
September
October

C


SWC(%)
20 30 40 50 60 70 80 90 100
0 20 40 60 80 100
Depth(cm)
May
June
July
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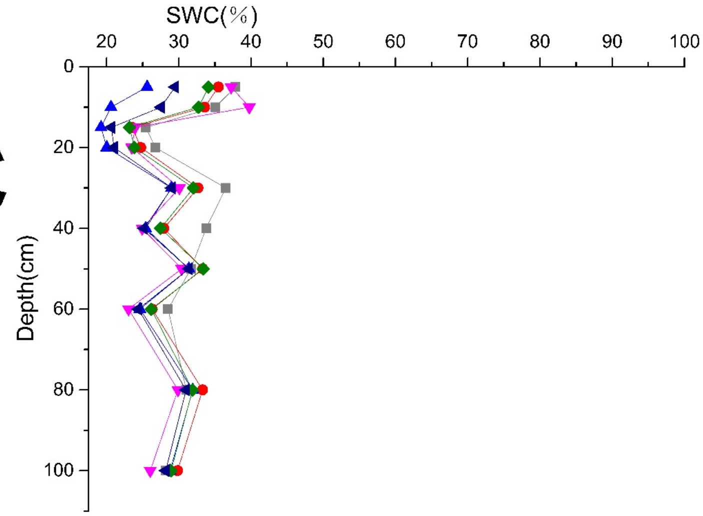
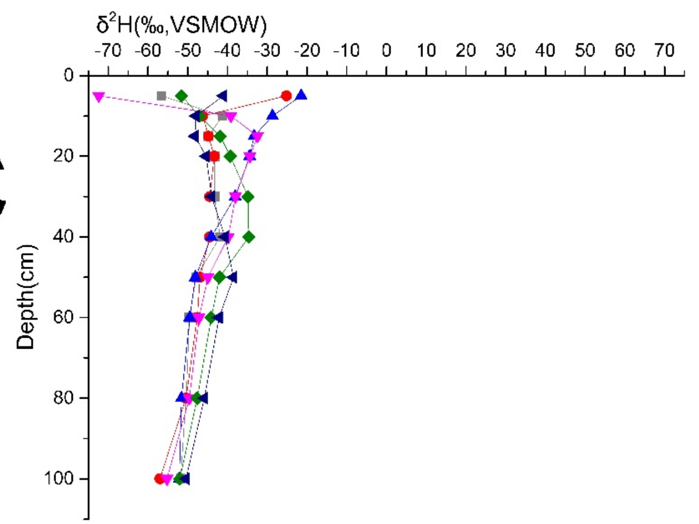
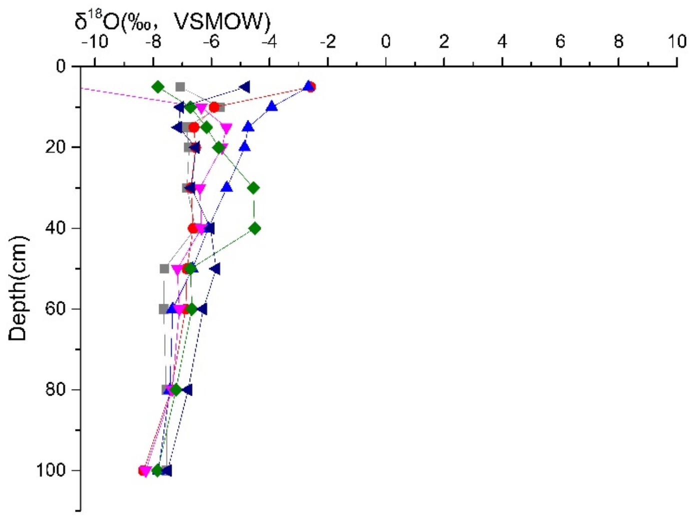
C


SWC(%)
20 30 40 50 60 70 80 90 100
0 20 40 60 80 100
Depth(cm)

C

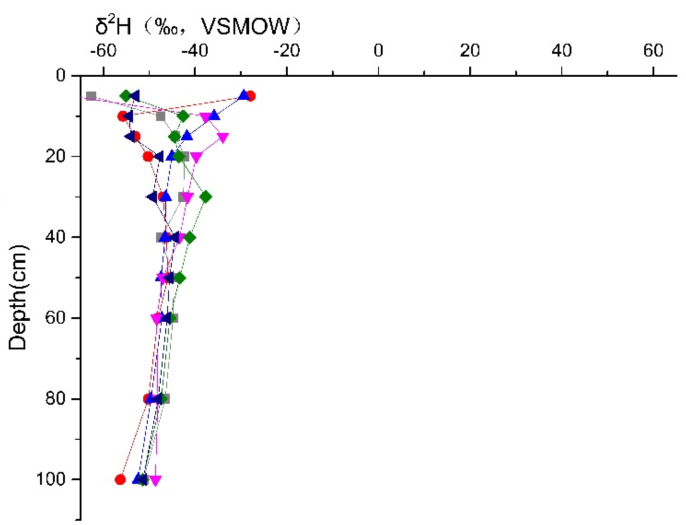

δ²H(‰,VSMOW)
-70 -60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70
0 20 40 60 80 100
Depth(cm)

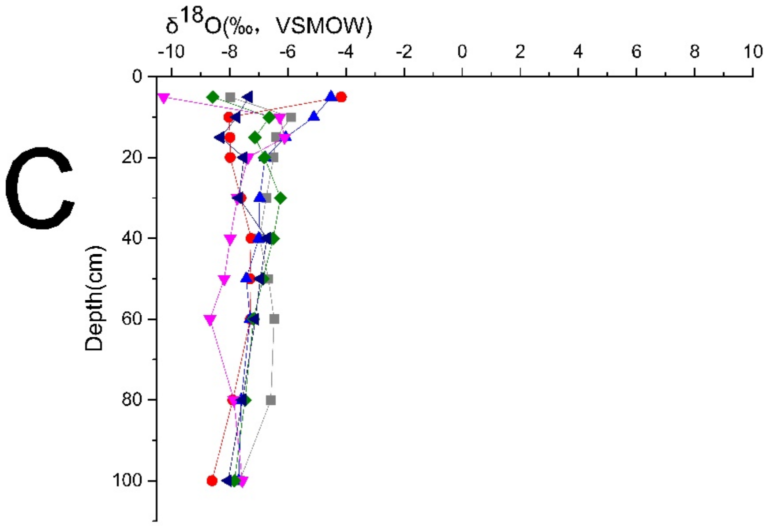
C



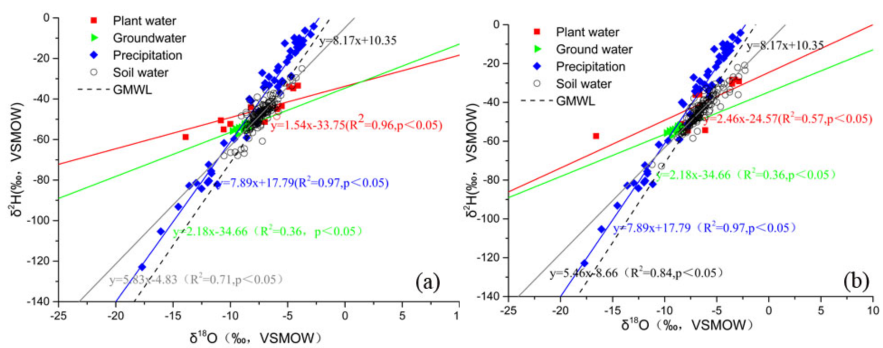
May June July August September October

C





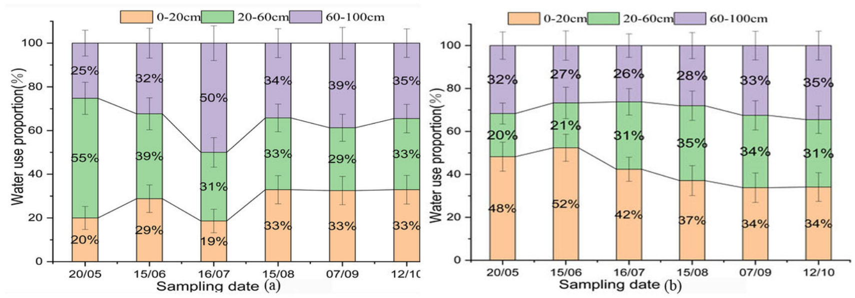
Φιγυρε 4. Σοιλ ωατερ ςοντεντ βετωεεν σηρυβ (α) ανδ γρασσλανδ σιτες (β), αριατιονς οφ δ^{18}Ο ανδ δ^{2}Η αλυες βετωεεν σηρυβ (ς) ανδ γρασσλανδ σιτες (δ)



The δ^{18}O and δ^{2}H showed significant correlations in plant water, precipitation, and soil water during the study period (Figure 5). The equation $\delta^{2}H = 7.89 \times \delta^{18}O + 17.79$ ($R^2 = 0.97$; $p < 0.05$) was used to describe the local meteoric water line (LMWL), the slope was smaller than the global meteoric water line, GMWL: $\delta^{2}H = 8.17 \times \delta^{18}O + 10.35$; (Rozanski et al., 1993), indicating that evaporation is a main reason of arid climate and kinetic fractionation. The slopes and intercepts of the soil water line equations at both sites were smaller than those of the LMWL (Figure 5), indicating that the ongoing evaporation influence soil water (Yang&Fu, 2017). The linear relationships of δ^{18}O and δ^{2}H in soil water between shrub and grassland sites, which were $\delta^{2}H = 5.83 \times \delta^{18}O - 4.83$ ($R^2 = 0.71$) and $\delta^{2}H = 5.46 \times \delta^{18}O - 8.66$ ($R^2 = 0.84$), respectively, were significant, indicating that soil water evaporation was significantly different for plants between shrub and grassland sites. Moreover, the relationships between δ^{18}O and δ^{2}H were $\delta^{2}H = 1.54 \times \delta^{18}O - 33.75$ ($R^2 = 0.96$) for plant water at the shrub site, and $\delta^{2}H = 2.46 \times \delta^{18}O - 24.57$ ($R^2 = 0.57$) at the grassland site.

Φιγυρε 5. Ρελατιονσηιπς οφ δ^{18}Ο ανδ δ Η οφ διφφερεντ ωατερς βετωεεν σηρυβ (α) ανδ γρασσλανδ(β)

3.4 Variation of plant water utilization



Plants in different sites used different fractions of shallow, middle, and deep soil waters with different growth periods (Figure 6). The fractions from the shallow soil layer used by plant varied from 18.6% to 52.4%, and the minimum value at the shrub site was on 16 July. In contrast, the maximum value was observed at the grassland site on 15 June. The proportional contributions of middle soil water and deep soil water acquired by plant were 54.8% and 50%, respectively, at the shrub site. At the onset of the growing season and fast-growing season from May to July, plant at shrub and grassland sites extracted water from the middle soil layer and the shallow soil layer, respectively. Water utilization from shallow, middle, and deep soil water reached equilibrium in August. The water use contributions from the three layers remained unchanged during the late growing season and withering season. The plants at the shrub site had a higher degree of flexible plasticity in water use compared with the plants at the grassland site.

Figure 6. Seasonal variations in water use proportions at the shrub and grassland

4. Discussion

4.1. Variations in the isotopic composition of different water

The $\delta^{18}O$ and $\delta^{2}H$ in precipitation water initially increased and then decreased significantly from May to October. Some scholars have concluded that precipitation $\delta^{18}O$ and $\delta^{2}H$ produce a dominant factor and are consequence of the effects of temperature and precipitation regarding the isotopic inheritance from precipitation to plant water (Liu et al., 2021). There is a clear difference between our results and their conclusion. This difference could be caused by air trajectory that is a very important factor influencing moisture source (Wu et al., 2016). Transition times between a westerly circulation and the Southeast Asia monsoon supplying isotopically regarding moisture sources depleted atmospheric water vapor (Lihui et al., 2015).

Plant water responded well to the trends of $\delta^{18}O$ and $\delta^{2}H$ in precipitation from May to July, but not well from August to October. Several studies have previously documented the consistency between precipitation and plant water (Phillips&Ehleringer, 1995; Meinzer et al., 2006; Sprenger et al., 2016; Plavcová et al., 2018). Our result may be due to drought stress in our study area. On the one hand, soil water is the primary source of plant water, and soil moisture meets the needs of plant growth from May to July. However, during the rapid growth of plants (August and Septermber), fierce water competition stimulates plants to use water in each soil layer in a balanced manner. This is an adaptation mechanism established to promote self-growth until October. On the other hand, soil water isotopic compositions resulting from soil water evaporative differences influenced the plant water uptake pattern of plants (Rothfuss&Javaux, 2017).

The isotopic values and SWC exhibited larger variability in shallow soil layers, compared with deeper soil layers from May to October for both the two sites. Groundwater isotopes were consistent in the deeper soil and remained relatively stable. This result is consistent with results of previous studies in arid and semi-arid regions (Fischer et al., 2017; Wang et al., 2019; Guo et al., 2020). Both $\delta^{18}O$ and $\delta^{2}H$ values were enriched in June and July, depleted in August in the shallow soil layers (Figure 5). The enriched soil water in the shallow soil layers corresponded well to the lower SWC in July, the isotopically depleted soil water matched well with the high SWC, the results probably attributable to less rainfall and intensive evaporation (Gazis&Feng,

2004) in July, and rainfall recharge with negative isotopic values (Wang, et al., 2019) in August. All the results indicate that precipitation recharge, evaporation, and antecedent moisture all influence soil water isotopic compositions (Hsieh et al., 1998; Brooks et al., 2015).

4.2. Comparisons between *P. fruticosa* shrub and alpine grassland

A series of water line equations between $\delta^{18}O$ and $\delta^{2}H$ were established to compare the differences in water interaction from shrub and grassland sites (Figure 5). Soil and plant water samples were well described by linear regressions, resulting of laying at an angle to the LMWL, this is consistent with previous studies (Goldsmith et al., 2012; Evaristo et al., 2015; Che, et al., 2019). The slopes and intercepts of these water lines were determined by the relative evaporation rates of the different water isotopes (Crawford et al., 2014; Benettin et al., 2018; Bowen et al., 2018; Chi et al., 2019), indicating the different magnitudes of evaporative enrichment of isotopes in soil water and plant water. The slope and intercept of soil water at the grassland site (5.46 and -8.66, respectively) were slightly lower than those at the shrub site (5.83 and -4.83, respectively), suggesting soil evaporation was slightly greater at the grassland site than at the shrub site. This was probably because the shrub site had a denser coverage shading the soil surface compared with the grassland site, just as daily evaporation rates were slightly lower for understory vegetation than for grassland during the growing season (Crawford, et al., 2014; Schwärzel et al., 2020). Nevertheless, the slopes and intercepts of $\delta^{18}O$ and $\delta^{2}H$ in plant water at the grassland site (2.46 and -24.57, respectively) were higher than the slope and intercept at the shrub site (1.54 and -33.75, respectively). The differences likely resulted from substantial variabilities for leaf water at shrub and grassland sites, evaporative distinctions of $^{2}H/^{1}H$ and$^{18}O/^{17}O$ on the primary of leaf water(Farquhar et al., 2007), and the transpiration from an area of grass is greater than the transpiration from a similar area of shrubs.

Because the two sampling sites neighbored each other, they had similar topographical and geological conditions, and precipitation $\delta^{18}O$ and $\delta^{2}H$ were also similar. The $\delta^{18}O$ in soil water and plant water showed significant differences between shrub and grassland sites ($p < 0.05$) in contrast to the non-significant differences in $\delta^{2}H$ between the two sites ($p = 0.068$ and 0.06 for soil water and plant water, respectively), resulting in different slopes and intercepts for the water line equations from soil water and plant water between the two sites. Isotope-fractionated differences between $\delta^{18}O$ and $\delta^{2}H$ were probably associated with local microenvironment and heterogeneity differences of surroundings between the grassland and shrub sites despite the same general conditions (Ellsworth&Williams, 2007).

4.3. Implications for meadow eco-hydrological processes

In this study, $\delta^{18}O$ and $\delta^{2}H$ at the grassland site were greater than at the shrub site during the growing season, but almost the same during the withering season, indicating that grassland plant water had a stronger dynamic fractionation effect in the process of transportation and was more sensitive to environmental conditions. Furthermore, plants at the shrub site displayed more flexible water use patterns that shifted shrub water sources between shallow water and deep soil water, and the grassland site was more susceptible to drought stress. From this perspective, alpine shrub sites on the QPT were formed from the long-term encroachment of *P. squamosa* . This result is consistent with the results published previously by many other researchers who found that shrub populations are a result of the proliferation and range expansion of woody plant species in arid and semiarid grassland ecosystems (Van Auken, 2000; Maestre et al., 2009; Archer et al., 2017) , and that areal expansion of shrubs is one of the most threatening forms of grassland degradation in arid and semiarid areas (Eldridge et al., 2011). Shrubs from alpine grassland leaded to changes in grassland water use, thereby changing soil water storage(Li et al., 2022).

Many previous studies have shown that vegetation has profound effects on maintaining local hydrological processes (Feng, et al., 2016; Jia et al., 2017). Plant water use strategies can be used to exploring all available water sources by isotopic compositions of xylem water (Wu, et al., 2019). The contrasting plant water use patterns identified in our study contribute to the differential sensitivity to interannual variations of available moisture input. Hence, appropriate management measures, such as recovering natural environmental characteristics and moisture status, should be implemented to maintain grassland ecosystem sustainability.

5. Conclusions and future directions

The $\delta^{18}O$ and $\delta^{2}H$ values of precipitation, soil water, and plant water varied significantly over months and water sources at the alpine grassland and *P. fruticosa*shrub sites on the QTP. The relationships of $\delta^{18}O$ and $\delta^{2}H$ indicated that both soil evaporation and plant transpiration at the *P. fruticosa* shrub site were relatively lower than they were at the alpine grassland site. The grassland plant water had a stronger dynamic fractionation effect in the process of transportation and was more sensitive to environmental conditions, but the plants at the *P. fruticosa* shrub site displayed more flexible water use patterns that shifted shrub water sources between shallow soil water and deep soil water. Moreover, the relationship in $\delta^{18}O$ and $\delta^{2}H$ between precipitation and plant water, and the factors influencing precipitation maybe resulted from various possibilities. These results promote better understanding of the interface between plant and surrounding soils between *P. fruticosa* shrub and alpine grassland sites, provide guidance for meadow management from the perspective of eco-hydrological processes on the QTP.

Although our study used advanced technology exploring the interrelation of soil water and plant water at the alpine grassland and *P. fruticosa* shrub sites and drawn important conclusions in-depth understanding of eco-hydrological processes, the factors influencing $\delta^{18}O$ and $\delta^{2}H$ values maybe resulted from various possibilities. These possibilities need to be further examined through greater variety of species, more potential water sources, multi-site continuous observation, and longer time scales in the future.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

J Li performed the research, wrote the paper; F Zhang analyzed data, and G Cao, Y Du, Y Wang, Y Lan, M si, B fan, H Zhou and B Wang verified the results; X Guo conceived the study.

DATA AVAILABILITY

The original data supporting the conclusions of this article are available in Dryad, https://doi.org/10.5061/dryad.h44j0zpnb.

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