Interrelation of soil water and plant water revealed by hydrogen-oxygen isotopes across alpine shrub and grassland in northern Qinghai-Tibet Plateau

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Abstract

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The alpine grassland shrubbization of the northern Qinghai-Tibet Plateau on the background of global change and overgrazing, is a prominent and serious problem. However, the water competition ability of shrubs and alpine grasslands is rarely reported. Here, we tracked the $\delta^{18}O$ and $\delta^{2}H$ of soil water, plant water, precipitation, and groundwater, analysed sources of water use in shrub and grassland by Mix SIAR model. Our results showed that both $\delta^{18}O$ and $\delta^{2}H$ in soil, precipitation, and plant varied significantly over time, groundwater remained relatively stable in*P. fruticosa* shrub and alpine grassland sites during observation. Considering groundwater, precipitation, soil water, and plant water, a progressive enrichment in $\delta^{18}O$ or $\delta^{2}H$ existed from groundwater and precipitation to soil water to plant water for each month. Alpine grassland was more susceptible to drought stress, had a stronger partitioning effect in dynamic transport than shrub. The *P. fruticosa* shrub displayed more flexible water utilisations, and was more competitive for water than grasslands. Furthermore, the plants in alpine shrub and grassland reached water use balance in August. Shrubs degraded from alpine grassland changed water use pattern of grassland, thereby changing soil water storage. These results contribute to in-depth understanding the alpine grassland shrubbization from water use patterns of grassland and shrub plants on the northern Qinghai-Tibet Plateau.

Keyword: Stable isotopes, Alpine meadow water, *P. fruticosa* shrub water, Soil water, Mix SIAR model, Northern Qinghai-Tibet Plateau

Introduction

Water is one of the most crucial limiting factors determining the community dynamic trends and productivity of plant in arid and semiarid ecosystems (Li et al., 2013). The primary water sources, such as soil water and groundwater, absorbed by plants determine the growth status of plants, 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). Additionally, the interaction of soil and plant water is a vital component of eco-hydrological processes (Dawson&Ehleringer, 1991; Chang et al., 2019).

Previous studies about interrelation of soil and plant mostly focused on exploiting different hydrological niches (Walker et al., 1981), soil moisture–vegetation feedbacks and their possible effects (D'Odorico et al., 2007), and functional differences (Ryel et al., 2008) by modeling. Precipitation patterns (D'Odorico, et al., 2007), soil water utilization (Gow et al., 2018; Lanning et al., 2020) and roots distribution (Wang et al., 2021a) affect the plant water use patterns. They were all based on traditional situ observations. Subsequently, some scholars found that isotopic variation of both plant water and soil water provide an effective and powerful way to reveal and partition the different potential water sources used by plants (Vargas et al., 2017; Che et al., 2019; Rothfuss&Javaux, 2017).

The Qinghai-Tibet Plateau (QTP) is known as "Chinese Water Tower" and is an essential component of "three screens and two belts" ecological security strategic pattern (Li et al., 2022), that both show the important role of QTP in the construction of ecological civilization. However, QTP is particularly vulnerable under overgrazing and climate change (He et al., 2020). Data observed from 2001 to 2018 indicated that both soil water storage declined over that period. Shrub (*Potentilla fruticosa*) meadows 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., 2019). However, on the background of global change and overgrazing, the problems of grassland shrubs and the decline of water conservation functions caused by grassland degradation, are threatening the national strategic position of water resources in the Qinghai-Tibet Plateau (Guo et al., 2020). Grassland degradation changes carbon accumulation rate (Sun et al., 2020), soil nutrients (Wu et al., 2020), evapotranspiration (Ji et al., 2022), and plant water use efficiency (Wang et al., 2021b), but the interrelation of water competition from shrubs and alpine grasslands are not well understood, and little research on water-use patterns and relationships between *P. fruticosa* shrubs and alpine grasslands has been conducted on the northern QTP.

To fill the gaps, we compared $\delta^{18}O$ and $\delta^{2}H$ from different water sources in a pair of neighboring sites, combined with field observations, and distinguished water use sources of *P. fruticosa* shrubs and alpine grassland plants on different seasons through Mix SIAR model to in-depth understand the interaction of soil water and plant water for *P. fruticosa* shrubs and alpine grasslands. Our results provided a theoretical basis for the alpine grassland shrubbization, and made contribution to restoration of the alpine grassland in the northern QTP under global climate change.

2. Materials and methods

2.1. Site description

This study was conducted in Ganchaitan, close to Haibei station (101°19'E, 37deg37'N, 3335 m), and included two neighboring sites (i.e., shrubs site and grassland site, the distance between them was 460 m) 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). The soil type in the study area was classified as Mollic Gryic Cambisols based on the classification of soil systems in China (Liu et al., 2018). Vegetation characteristics of the sampling sites were investigated and shown in Table 1.

Table1 Vegetation characteristics of the sampling 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
Root biomass (g/m^3)	2447±349	1656±640
Dominant species composition and plant community description	*Potentilla fruticosa L. Kobresia humilis,Double-stigma Bulrush, Polygonum viviparum L.,* and *Elymus nutans Griseb.*	*Elymus nutans Griseb., Polygonum viviparum L., Double-stigma Bulrush, Poa prsten, Kobresia humilis*

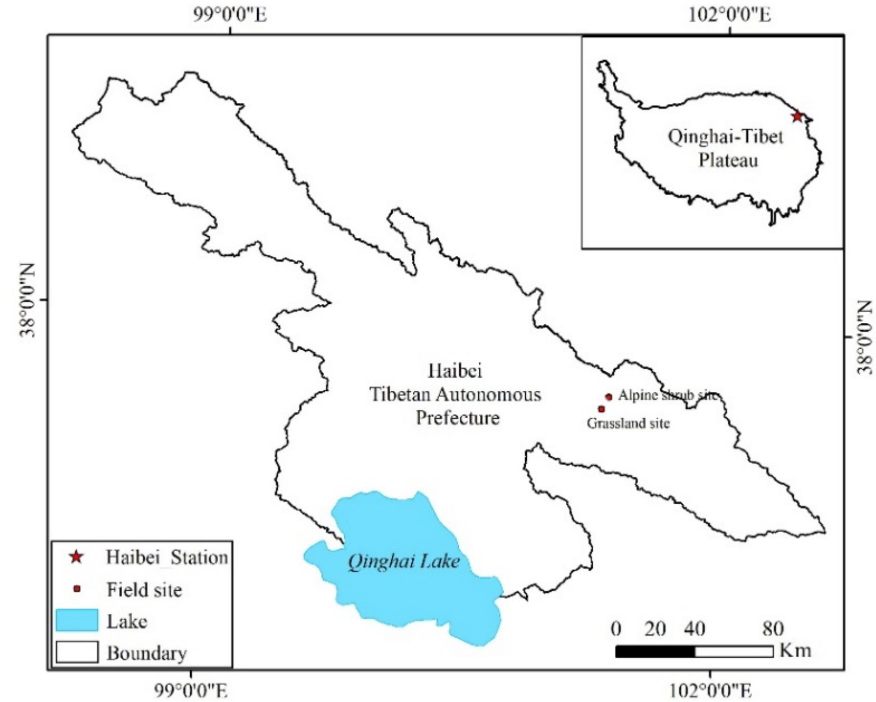


Figure 1. Location of sample sites (a), shrub site (b) and grassland site (c).

2.2. Sample collections and isotope analysis

2.2.1 Sample collection

We collected the samples, including the plant, soil, precipitation and groundwater. All the samples were frozen at -10°C prior to vacuum distillation, for consistent handling while avoiding the effects of fractionation. In addition, our preliminary experimental results showed that the isotope ratio spectrometer had more stable operating state after extraction of precipitation and groundwater.

The plant and soil samples were collected from the shrub and grassland 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). Plant species were randomly chosen on the sampling date in the two sites. The whole plants of herbs were used for experiments after removing leaves. Whereas, the stem sections were removing the outer bark from *P. fruticosa* were used for isotope analysis (Martín-Gómez et al., 2017). The soil samples were collected around the selected plants within 2 m by soil cores, the soil cores were divided into 10 layers (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), each soil samples were analyzed as separate samples. Three replicates for each of the soil layers and plants samples were collected in the two experimental sites. A total number of 72 and 360 samples of plants and soils, respectively, were sealed for isotopic analysis.

Precipitation and groundwater were sampled concurrently from May to October in 2021. The event-based precipitations were sampled using bottles at herder's home, 2 km away from our sampling sites during the sampling period, the number of replicates (one, two or three) from every event-based precipitation were determined according to the amount of precipitation. Groundwater were sampled once a week from a well 2 km away from our sites, three replicates were collected. 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.

The soil water content (SWC) was determined by 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.

2.2.2. Isotopic analysis

Precipitation, groundwater, plant water, and soil water samples were all extracted by the cryogenic vacuum distillation technique (LI-2100pro, Lica United Technology Limited, Beijing, China) 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, 2 h for precipitation and groundwater samples according to West et al. (2006). All of the extracted water was transferred into 2 ml vials, then analyzed stable isotopes (δ^2H and $\delta^{18}O$) using an isotope ratio spectrometer (LGR-TLWIA-912, Los Gatos Research, San Jose, CA, USA). 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 measurement precision was $0.3\delta^{18}O$. The organic contamination on plant water need correction procedures to eliminate the influence (Schultz et al., 2011). The isotopic compositions of $\delta^{18}O$ 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, Rsam was the abundance ratios ($^{18}O/^{16}O$ and$^2H/^1H$) of samples, Rstd was the abundance ratios of standard.

2.2.3. Statistical analysis

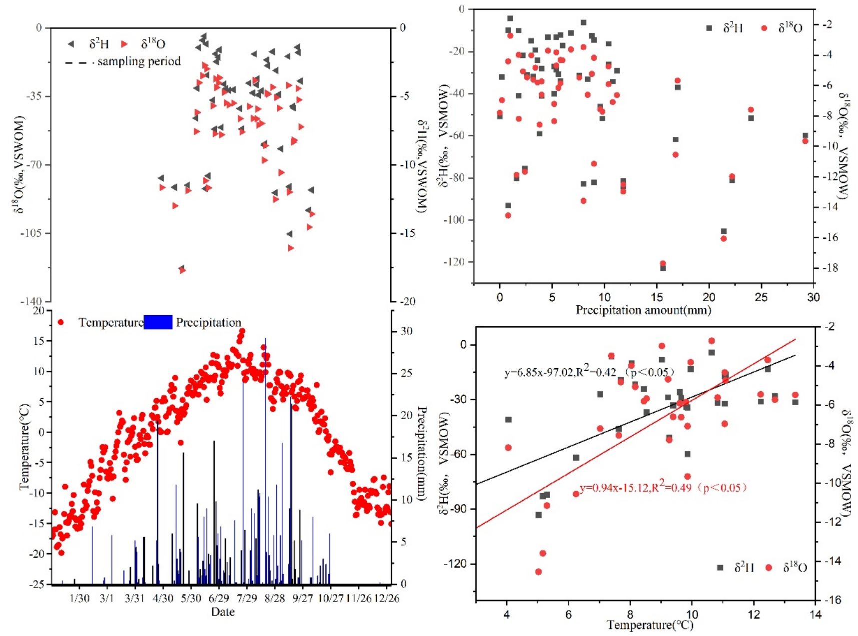
All statistical analyses were conducted using R (software version 4.0.3, https://www.r-project.org/), and all figures were plotted using Origin 9.1(https://www.originlab.com/). One-way analysis of variance (ANOVA) followed by the post hoc Turkey's test at p = 0.05 was used to assess hydrometeorological 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.

The Bayesian mixing model Mix SIAR (http://conserver.iugo-cafe.org/user/ brice.semmens/Mix SIAR) was used for identifying the proportions of contributions from each water source according to the δ^2H and $\delta^{18}O$, 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.

3. Results

3.1. Temperature, precipitation and its isotopes

A unimodal distribution was exhibited by temperature and precipitation amount in 2021, respectively (Figure 2a). Temperature peaked at the end of July (16.61) and decreased monotonically to the end of October (-6.62) during the observation. Precipitation activities were concentrated in the same time, accounting for 86.10% (465 mm) to the annual precipitation (540 mm). The $\delta^{18}O$ 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, seasonal conditions are important factors that affect the isotope characteristics of precipitation, however, precipitation amount had little influence to δ^{18}O and δ^{2}H in precipitation (Figure 2b), temperature explained 49% to δ^{18}O, whereas, 42% to δ^{2}H (Figure 2c).

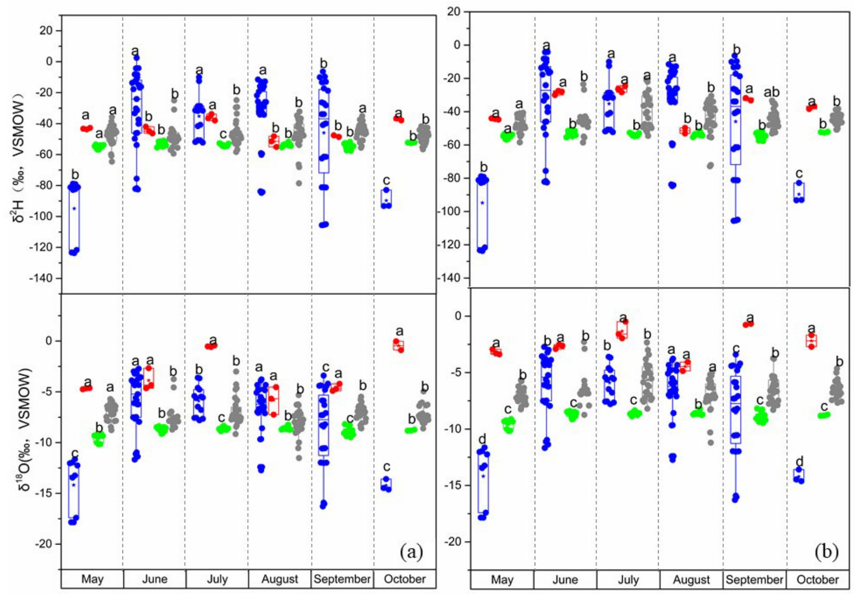


Note: the δ^{18}O and δ^{2}H in precipitation, precipitation amount and temperature in the figure were all daily means.

Φιγυρε 2. Τηε δ^{18}Ο ανδ δ^{2}Η ιν πρεςιπιτατιον, πρεςιπιτατιον αμουντ 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). Generally, the average values of δ^{2}H were -53.86-52.25water, and plant water, respectively. The respective average values of δ^{18}O were -8.83Considering groundwater, precipitation, soil water, and plant water, a progressive enrichment in δ^{18}O or δ^{2}H existed from groundwater and precipitation to soil water to plant water for each month. The δ^{18}O and δ^{2}H in precipitation water enriched initially and then depleted significantly from May to October ($p < 0.05$). Moreover, the trends of δ^{18}O and δ^{2}H in plant water responded well to the trends of precipitation from May to July, but not well from August to October. Likewise, significant differences were observed for δ^{18}O in plant water between shrub and grassland sites ($p < 0.05$), but non-significant differences were observed for δ^{2}H ($p = 0.06>0.05$). There were no significant differences in the δ^{18}O and δ^{2}H of soil water and across months for the two sites (p¿0.05). Groundwater isotopes remained relatively stable during the sampling period.

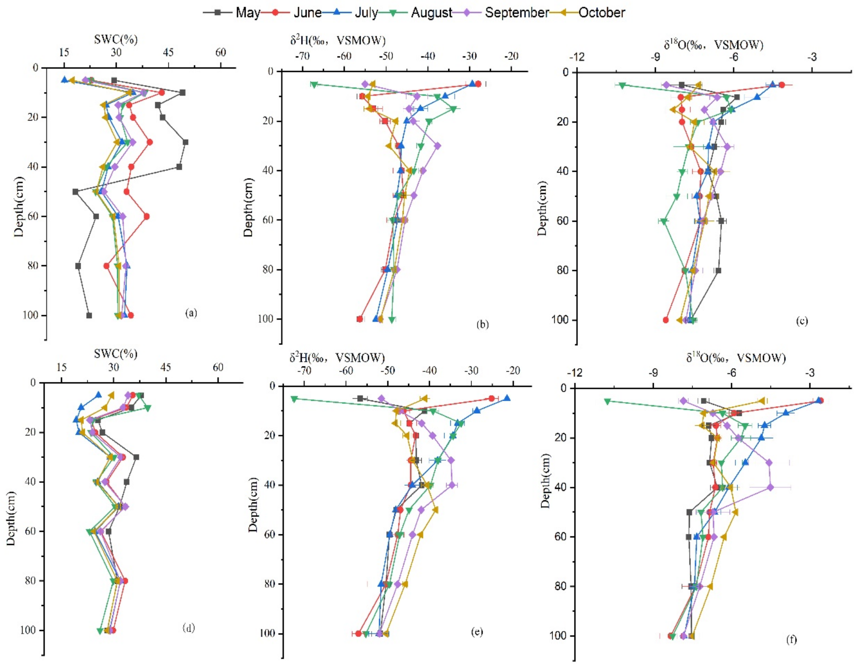


Precipitation Plant water Ground water Soil water

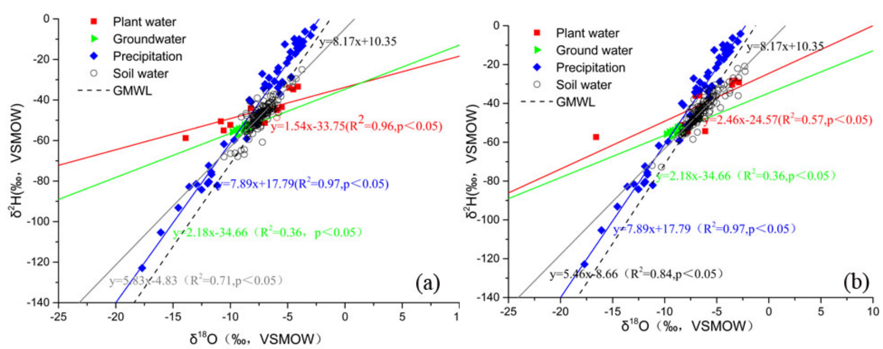
Φιγυρε 3. ἄριατιον οφ δ^{18}Ο ανδ δ^{2}Η οφ πρεςιπιτατιον, σοιλ ωατερ, πλαντ ωατερ, ανδ γρουνδωατερ βετωεεν σηρυβ (α) ανδ γρασσλανδ (β) σιτες

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

Soil water content, variations of δ^{2}H and δ^{18}O of shrub and grassland sites were shown in Figure 4. There was a significant difference in SWC of the same soil layers between shrub and grassland sites ($p < 0.05$), the SWC of shrub site ranged from 15.13 ± 0.06% to 49.87 ± 0.29%, whereas ranged from 19.25 ± 0.02% to 39.76 ± 0.24% in grassland site during the sampling period (Figure 4a and 4d). However, δ^{2}H in soil water showed no significant difference between the two sites ($p > 0.05$) (Figure 4b and 4e), in contrast to δ^{18}O ($p < 0.05$) (Figure 4c and 4f). Both δ^{18}O and δ^{2}H in shallow soil water (0–20 cm) were more highly variable than deeper soil layers. The isotopic values in surface soil water were enriched in June and July, depleted in August and September. The δ^{18}O and δ^{2}H values in the grassland soil were enriched than in the shrubs from May to September, but almost the same in October, indicating that grassland is more sensitive to environmental changes, plant water has a stronger partitioning effect in dynamic transport comparing with shrub plant water.



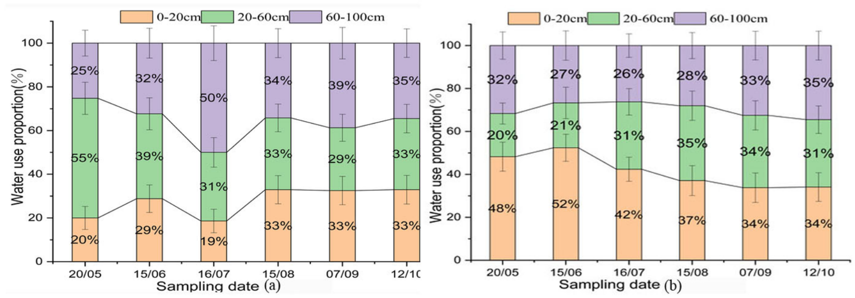
Φιγυρε 4. Σοιλ ωατερ ςοντεντ (α), αριατιονς οφ δ^{2}Η (β) ανδ δ^{18}Ο (ς) ιν σηρυβ, σοιλ ωατερ ςοντεντ (δ), αριατιονς οφ δ^{2}Η (ε) ανδ δ^{18}Ο (φ) ιν γρασσλανδ σιτες



The δ^{18}O and δ^2H showed significant correlations in plant water, precipitation, and soil water during the study period (Figure 5). The local meteoric water line (LMWL) was described by equation, δ^2H $=7.89 \times \delta^{18}$O $+17.79$ ($R^2 = 0.97$; $p < 0.05$) , its slope was smaller than the global meteoric water line (GMWL), δ^2H $= 8.17 \times \delta^{18}$O $+ 10.35$; (Rozanski et al., 1993), indicating that evaporation is a main reason of arid climate and kinetic fractionation in the research sites. The linear relationships of δ^{18}O and δ^2H in soil water were significantly indicated that the different evaporation effect between shrub and grassland sites. Additionally, their slopes and intercepts at the two sites were smaller than those of the LMWL, for the ongoing evaporation influence soil water (Yang&Fu, 2017). Moreover, Soil water, plant water and LMWL laid at an angle on the both sites, indicating that these water types contained water that had been recycled through terrestrial evaporation and precipitation.

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 during different growth periods (Figure 6). The plant water utilization of shallow soil water ranged from 19% to 52%, 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 55% and 50%, respectively, at the shrub site. At the onset of the growing season and fast-growing season from May to July, plants 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 contributions of the three layers remained unchanged during the late growing season and withering season. The plants at the shrub site had 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 shrub site (a) and grassland site (b)

4. Discussion

4.1. Variations of the isotopic composition in different water

The $\delta^{18}O$ and $\delta^{2}H$ in precipitation initially enriched and then depleted significantly from May to October. Some scholars have concluded that $\delta^{18}O$ and $\delta^{2}H$ in precipitation are consequence of the effects of temperature and precipitation amount. The consequence produce a dominant factor for plant and soil waters, regarding the isotopic inheritance from precipitation to plant water, and their studies conducted in Ordos Plateau, where located in monsoon regions(Liu et al., 2021). There is a clear difference between their conclusions and our results. Precipitation amount had little influence to $\delta^{18}O$ and $\delta^{2}H$ in precipitation, temperature explained 49% to $\delta^{18}O$, whereas, 42% to $\delta^{2}H$. It may be caused by air trajectory, which influence $\delta^{18}O$ and $\delta^{2}H$ in precipitation (Wu et al., 2016) by influencing moisture source. Our research sites located in south of Qilian Mountains, which situated the intersection of the alpine regions, the northwest regions, and the monsoon regions (Yang et al., 2019). They had more frequency of transition times than Ordos Plateau, where influenced by internal circulation and monsoon circulation (Gou et al., 2011), influencing the moisture sources depleted atmospheric water vapor.

Plant water trends 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 of precipitation and plant water (Phillips&Ehleringer, 1995; Meinzer et al., 2006; Sprenger et al., 2016; Plavcová et al., 2018). Our different results may be due to that precipitation intensity exerts little effect on the soil water as almost all rainfall returns to the atmosphere via evapotranspiration (Dai et al., 2019a). Soil water is the primary source of plant water, 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 growth until October. Furthermore, soil water isotopic compositions resulting from soil water evaporative differences influenced the plant water uptake pattern of plants (Rothfuss&Javaux, 2017).

The $\delta^{18}O$, $\delta^{2}H$ and SWC displayed larger variability in shallow soil depths, compared with deeper soil depths from May to October for the both sites. Groundwater isotopes were consistent in the deeper soil and remained relatively stable. This result is consistent with previous studies of arid and semi-arid regions (Fischer et al., 2017; Wang et al., 2019; Guo, et al., 2020). Both $\delta^{18}O$ and δ^2H values were enriched in June and July, depleted in August in the shallow soil layers. The isotopically enriched soil water of shallow depths matched well with the lower SWC, in contrast to the depleted to the higher, the results probably attributable to less precipitation and intensive evaporation (Gazis&Feng, 2004) in July, and rainfall recharge with negative isotopic values (Wang, et al., 2019) in August. The results indicate that precipitation recharge, evaporation, and antecedent moisture all influence soil water isotopic compositions (Brooks et al., 2015).

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

The $\delta^{18}O$ in soil water and plant water showed significant differences between shrub and grassland sites ($p < 0.05$) in contrast to non-significant differences in δ^2H, resulting in different slopes and intercepts for the water line equations of soil water and plant water. Isotope-fractionated differences 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).

The water line equations between $\delta^{18}O$ and δ^2H were established. 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; Che, et al., 2019). Their slopes and intercepts from soil water at the grassland site were slightly than those at the shrub site, 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, as daily evaporation rates were slightly lower than grassland (Crawford et al., 2014; Schwärzel et al., 2020). Nevertheless, the slopes and intercepts in plant water lines at the grassland site were higher than the slope and intercept at the shrub site. The differences resulted from substantial variabilities for plant water at shrub and grassland sites, evaporative distinctions of$^2H/^1H$ and$^{18}O/^{17}O$ on the primary of leaf water (Farquhar et al., 2007) , and the transpiration from an area of grassland is greater than the transpiration from a similar area of shrubs.

4.3. Implications for grassland shrubbization of alpine meadow

The plants at alpine *P. fruticosa* shrub extracted water from the middle soil layer to deep soil water preferentially from May to June, and extracted water from shallow, middle and deep soil in a balanced manner from July to October. The results were different from the results carried out on north of Qilian Mountains, where has a drier climate. Their results had shown that *P. fruticosa* used the shallow soil water except for August, when used the middle soil water (Zhang et al., 2022). This difference may be due to the different ages of plant (Wang, et al., 2021a), the different geographical locations (Li et al., 2022), or the different symbiotic species (Wu, et al., 2019). Additionally, the different sources of the soil water from the eco-hydrological processes maybe another reason.

Both $\delta^{18}O$ and δ^2H at the grassland site were enricher than at the shrub site during the growing season, but almost the same during the withering season. Grassland was more susceptible to drought stress, plants at grassland site had a stronger partitioning effect in dynamic transport, comparing with plants at the shrub site displayed more flexible water utilisations. From the perspective of plant water use patterns and evolution, alpine shrub on the northern QPT were formed from the long-term encroachment of *P. fruticosa* . This result is consistent with the results published previously by Klein et al. (2007) , who found that shrub populations are a result of proliferation and expansion of woody plant, and that areal expansion of shrubs is one of the most threatening forms of grassland degradation (Eldridge et al., 2011) . The contrasting plant water use patterns identified in our study provided theoretical basis for the alpine grassland shrubbization, contributed to predict vegetation functional dynamics and the ecohydrological process under current and future climatic conditions should take species-specific plasticity and responses to short-term water supply fluctuations into account.

5. Conclusions and future directions

The $\delta^{18}O$ and δ^2H values of precipitation, soil water, and plant water varied significantly over months at the alpine grassland and *P. fruticosa* shrub sites on the northern QTP, those of groundwater remained relatively stable during the sampling period. Considering groundwater, precipitation, soil water, and plant water, a progressive enrichment in $\delta^{18}O$ or $\delta^{2}H$ existed from groundwater and precipitation to soil water to plant water for each month. Alpine grassland was more susceptible to drought stress, had a stronger partitioning effect in dynamic transport than shrub. The *P. fruticosa* shrub displayed more flexible water utilisations, and was more competitive for water than grasslands. Furthermore, the plants in alpine shrub and grassland reached water use balance in August to October. Shrubs degraded from alpine grassland changed water use pattern of grassland, thereby changing soil water storage. These results contribute to understand the alpine grassland shrubbization from water use patterns of grassland and shrub plants on the northern Qinghai-Tibet Plateau, promote better understanding of the interface between plant and surrounding soils from the perspective of eco-hydrological processes on the QTP.

Our study explored the interaction of soil water and plant water at the alpine grassland and *P. fruticosa* shrub sites using stable isotopes, drawn important conclusions in the process of alpine grassland shrubbization. However, greater variety of species, more potential water sources (dew, creek and deeper groundwater), multi-site continuous observation, and longer time scales need to be took into consideration to further examine in the future.

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DECLARATIONS

Ethical Approval: The paper is not currently being considered for publication elsewhere. All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

Consent to Participate: Informed consent was obtained from all individual participants included in the study.

Consent to Publish: The participant has consented to the submission of the case report to the journal.

Conflict of Interest: None.

AUTHOR CONTRIBUTION

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

DATA AVAILABILITY

The data that support the findings of this study are openly available in Dryad, https://doi.org/10.5061/dryad.h44j0zpnb.

REFERENCES

Beyer M, Hamutoko J, Wanke H, Gaj M, Koeniger P. 2018. Examination of deep root water uptake using anomalies of soil water stable isotopes, depth-controlled isotopic labeling and mixing models. *Journal of Hydrology* 566**:** 122-136.

Brooks P D, Chorover J, Fan Y, Godsey S E, Maxwell R M, McNamara J P, Tague C. 2015. Hydrological partitioning in the critical zone: Recent advances and opportunities for developing transferable understanding of water cycle dynamics. *Water Resources Research* 51**:**6973-6987.

Chang E, Li P, Li Z, Xiao L, Zhao B, Su Y, Feng Z. 2019. Using water isotopes to analyze water uptake during vegetation succession on abandoned cropland on the Loess Plateau, China. *Catena*181**:** 104095.

Che C, Zhang M, Argiriou A A, Wang S, Du Q, Zhao P, Ma Z. 2019. The Stable Isotopic Composition of Different Water Bodies at the Soil–Plant–Atmosphere Continuum (SPAC) of the Western Loess Plateau, China. *Water* 11**:** 1742.

Crawford J, Hughes C E, Lykoudis S. 2014. Alternative least squares methods for determining the meteoric water line, demonstrated using GNIP data. *Journal of Hydrology* 519**:** 2331-2340.

D'Odorico P, Caylor K, Okin G S, Scanlon T M. 2007. On soil moisture–vegetation feedbacks and their possible effects on the dynamics of dryland ecosystems. *Journal of Geophysical Research: Biogeosciences* 112.

Dai L, Guo X, Ke X, Du Y, Zhang F, Cao G. 2021. The variation in soil water retention of alpine shrub meadow under different degrees of degradation on northeastern Qinghai-Tibetan plateau. *Plant and Soil* 458**:** 231-244.

Dai L, Guo X, Zhang F, Du Y, Ke X, Li Y, Cao G, Li Q, Lin L, Shu K. 2019a. Seasonal dynamics and controls of deep soil water infiltration in the seasonally-frozen region of the Qinghai-Tibet plateau. *Journal of Hydrology* 571**:** 740-748.

Dai L, Ke X, Guo X, Du Y, Zhang F, Li Y, Li Q, Lin L, Peng C, Shu K. 2019b. Responses of biomass allocation across two vegetation types to climate fluctuations in the northern Qinghai–Tibet Plateau.*Ecology and evolution* 9**:** 6105-6115.

Dai L; Guo X; Ke X; Du Y; Zhang F; Li Y; Li Q; Lin L; Cao G; Peng C. 2019c. Responses of plant communities to degenerate succession in a shrub-meadow on the Qinghai-Tibet Plateau. *Journal of Ecological Environment* 28**:** 732.

Dawson T E, Ehleringer J R. 1991. Streamside trees that do not use stream water. *Nature* 350**:** 335-337.

Eldridge D J, Bowker M A, Maestre F T, Roger E, Reynolds J F, Whitford W G. 2011. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecology letters*14**:** 709-722.

Farquhar G D, Cernusak L A, Barnes B. 2007. Heavy water fractionation during transpiration. *Plant physiology* 143**:** 11-18.

Fischer B M, van Meerveld H I, Seibert J. 2017. Spatial variability in the isotopic composition of rainfall in a small headwater catchment and its effect on hydrograph separation. *Journal of Hydrology*547**:** 755-769.

Gazis C, Feng X. 2004. A stable isotope study of soil water: evidence for mixing and preferential flow paths. *Geoderma* 119**:**97-111.

Goldsmith G R, Muñoz-Villers L E, Holwerda F, McDonnell J J, Asbjornsen H, Dawson T E. 2012. Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. *Ecohydrology* 5**:** 779-790.

Gou X, Hu Z, Cui G, He H, Sun J, Hao L, Gu L. 2011. Monsoon changes and their correlation with summer precipitation on the Ordos Plateau.*Arid Zone Resources and Environment* 25**:** 79-83.

Gow L, Barrett D, O'grady A, Renzullo L, Phinn S. 2018. Subsurface water-use strategies and physiological responses of subtropical eucalypt woodland vegetation under changing water-availability conditions.*Agricultural and Forest Meteorology* 248**:** 348-360.

Guo X, Dai L, Li Q, Qian D, Cao G, Zhou H, Du Y. 2020. Light grazing significantly reduces soil water storage in alpine grasslands on the Qinghai-Tibet plateau. *Sustainability* 12**:** 2523.

He J; Liu Z.; Yao T; Sun S; Lv Z; Hu X; Cao G; Wu X; Li L; Pu H. 2020. Restriction factors and restoration techniques of degraded grassland restoration on the Qinghai-Tibet Plateau. *Science and Technology Herald* 38**:** 66-80.

Ji Z, Pei T, Chen Y, Wu H, Hou Q, Shi F, Xie B, Zhang J. 2022. The driving factors of grassland water use efficiency along degradation gradients on the Qinghai-Tibet Plateau, China. *Global Ecology and Conservation* **:** e02090.

Klein J A, Harte J, Zhao X-Q. 2007. Experimental warming, not grazing, decreases rangeland quality on the Tibetan Plateau. *Ecological Applications* 17**:** 541-557.

Lanning M, Wang L, Benson M, Zhang Q, Novick K A. 2020. Canopy isotopic investigation reveals different water uptake dynamics of maples and oaks. *Phytochemistry* 175**:** 112389.

Li H, Wang Z, Zhang F, Yi L, Guo X, Li Y, Lin L, Cao G, Li Y, Zhou H.2022. Characteristics of soil water-holding functions in alpine grassland-dwarf tarragon meadows at the southern foot of the Qilian Mountains. *Soil and water conservation research* 29**:**135-141+146.

Li J, Zhang F, Si M, Lan Y, Li B, Lin L, Du Y, Cao G, Guo X. 2022. Response of Soil Water Storage to Meteorological Factors in Alpine Shrub Meadow on Northeastern Qinghai–Tibetan Plateau. *Diversity*14**:** 185.

Li X-Y, Zhang S-Y, Peng H-Y, Hu X, Ma Y-J. 2013. Soil water and temperature dynamics in shrub-encroached grasslands and climatic implications: results from Inner Mongolia steppe ecosystem of north China. *Agricultural and forest meteorology* 171**:** 20-30.

Liu H, Mi Z, Lin L, Wang Y, Zhang Z, Zhang F, Wang H, Liu L, Zhu B, Cao G. 2018. Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proceedings of the National Academy of Sciences* 115**:** 4051-4056.

Liu J, Wu H, Cheng Y, Jin Z, Hu J. 2021. Stable isotope analysis of soil and plant water in a pair of natural grassland and understory of planted forestland on the Chinese Loess Plateau. *Agricultural Water Management* 249**:** 106800.

Li Z, Duan R, Ke H, Liu X, Xie G, Gao W, Song L, Zhang B, Gui J, Xue J. 2022. Research progress of ecohydrology in the source region of the Yangtze River based on hydrochemical characteristics. *Glacier permafrost* 44: 288-298.

Martin-Gomez P, Serrano L, Ferrio J P. 2017. Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology. *Tree physiology* 37**:** 511-522.

Meinzer F C, Brooks J, Domec J C, Gartner B L, Warren J, Woodruff D, Bible K, Shaw D. 2006. Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques.*Plant, cell & environment* 29**:** 105-114.

Phillips S L, Ehleringer J R. 1995. Limited uptake of summer precipitation by bigtooth maple (Acer grandidentatum Nutt) and Gambel's oak (Quereus gambelii Nutt). *Trees* 9**:** 214-219.

Plavcova L, Hronkova M, Šimková M, Květoň J, Vráblová M, Kubásek J, Šantrůček J. 2018. Seasonal variation of δ18O and δ2H in leaf water of Fagus sylvatica L. and related water compartments. *Journal of Plant Physiology* 227**:** 56-65.

Rothfuss Y, Javaux M. 2017. Reviews and syntheses: Isotopic approaches to quantify root water uptake: a review and comparison of methods.*Biogeosciences* 14**:** 2199-2224.

Rozanski K, Araguás-Araguás L, Gonfiantini R. 1993. Isotopic patterns in modern global precipitation. *Geophysical Monograph-American Geophysical Union* 78**:** 1-1.

Ryel R J, Ivans C Y, Peek M S, Leffler A J. 2008. Functional differences in soil water pools: a new perspective on plant water use in water-limited ecosystems. *In* Progress in botany. Springer. 397-422.

Schultz N M, Griffis T J, Lee X, Baker J M. 2011. Identification and correction of spectral contamination in 2H/1H and 18O/16O measured in leaf, stem, and soil water. *Rapid Communications in Mass Spectrometry* 25**:** 3360-3368.

Schwärzel K, Zhang L, Montanarella L, Wang Y, Sun G. 2020. How afforestation affects the water cycle in drylands: A process-based comparative analysis. *Global change biology* 26**:** 944-959.

Sprenger M, Leistert H, Gimbel K, Weiler M. 2016. Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes. *Reviews of Geophysics* 54**:**674-704.

Sun J, Zhou T C, Liu M, Chen Y C, Liu G H, Xu M, Shi P L, Peng F, Tsunekawa A, Liu Y. 2020. Water and heat availability are drivers of the aboveground plant carbon accumulation rate in alpine grasslands on the Tibetan Plateau. *Global ecology and biogeography* 29**:**50-64.

Vargas A I, Schaffer B, Yuhong L, Sternberg L d S L. 2017. Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. *New Phytologist* 215**:** 582-594.

Walker B H, Ludwig D, Holling C S, Peterman R M. 1981. Stability of semi-arid savanna grazing systems. *The Journal of Ecology* **:** 473-498.

Wang J, Fu B, Jiao L, Lu N, Li J, Chen W, Wang L. 2021a. Age-related water use characteristics of Robinia pseudoacacia on the Loess Plateau.*Agricultural and Forest Meteorology* 301**:** 108344.

Wang J, Fu B, Lu N, Wang S, Zhang L. 2019. Water use characteristics of native and exotic shrub species in the semi-arid Loess Plateau using an isotope technique. *Agriculture, Ecosystems & Environment*276**:** 55-63.

Wang Y, Pei W, Cao G, Guo X, Zhou H, Du Y. 2021b. Moderate Grazing Increases Water Use Efficiency for Environmental Health in Alpine Meadows of the Tibetan Plateau. *Frontiers in Ecology and Evolution* 9.

White J C, Smith W K. 2013. Water sources in riparian trees of the southern Appalachian foothills, USA: A preliminary study with stable isotope analysis. *Riparian Ecology and Conservation* 1**:**46-52.

Wu H, Li X-Y, Jiang Z, Chen H, Zhang C, Xiao X. 2016. Contrasting water use pattern of introduced and native plants in an alpine desert ecosystem, Northeast Qinghai–Tibet Plateau, China. *Science of the Total Environment* 542**:** 182-191.

Wu H, Zhao G, Li X-Y, Wang Y, He B, Jiang Z, Zhang S, Sun W. 2019. Identifying water sources used by alpine riparian plants in a restoration zone on the Qinghai-Tibet Plateau: Evidence from stable isotopes. *Science of the Total Environment* 697**:** 134092.

Wu J, Wang H, Li G, Ma W, Wu J, Gong Y, Xu G. 2020. Vegetation degradation impacts soil nutrients and enzyme activities in wet meadow on the Qinghai-Tibet Plateau. *Scientific reports* 10**:**1-17.

Yang Y, Fu B. 2017. Soil water migration in the unsaturated zone of semiarid region in China from isotope evidence. *Hydrology and Earth System Sciences* 21**:** 1757-1767.

Zhang F, Jia W, Zhu G, Zhang Z, Shi Y, Yang L, Xiong H, Zhang M. 2022. Using stable isotopes to investigate differences of plant water sources in subalpine habitats. *Hydrological Processes* 36**:**e14518.

Zhou Y, Gao X, Wang J, Zhao X. 2021. Study on the source of water uptake by roots of Lycium barbarum in irrigated area of Qaidam Basin.*Chinese Journal of Ecological Agriculture* 29**:** 400-409.