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## Postprint: Water Sources of Dominant Plants in Subalpine Shrublands of the Qilian Mountains

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### Abstract

Six dominant plant species were selected from the subalpine shrubland in the eastern Qilian Mountains: *Salix oritrepha* Schneid, *Rhododendron capitatum* Maxim, *Spiraea salicifolia* L., *Salix cupularis*, *Rhododendron thymifolium* Maxim, and *Potentilla fruticosa* Linn. Stable isotope techniques and a multiple linear mixing model (Isosource) were employed to quantitatively analyze plant water sources in a typical alpine region. The results indicate that: the atmospheric precipitation line for July-August in the Wushaoling area is  $D=7.775 \text{ } 18\text{O}+12.34$  ( $R^2=0.871$ ,  $P<0.001$ ), reflecting the climatic characteristics of low temperature and high humidity in this region; the water sources of the six dominant plant species are primarily precipitation, followed by 0-10 cm soil water, with groundwater having the smallest contribution rate to plant water; the utilization of various water sources by the six dominant plant species differs across time periods, with precipitation and shallow soil water being the main sources in July, while precipitation becomes the primary water source for all plants in August; *Spiraea salicifolia* and *Rhododendron capitatum* exhibit a stronger capacity to utilize soil water, and *Rhododendron capitatum* shows a higher utilization rate of groundwater compared to other plants.

### Full Text

### Preamble

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**Water Sources of Dominant Plants in the Subalpine Shrubland of the Qilian Mountains, China**

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## Abstract

This study selected six dominant plant species in the subalpine shrubland of the eastern Qilian Mountains—*Salix oritrepha*, *Spiraea salicifolia*, *Salix cupularis*, *Rhododendron capitatum*, *Rhododendron thymifolium*, and *Potentilla fruticosa*—to quantitatively analyze plant water sources in typical alpine regions using stable isotope technology and multivariate linear mixed models (IsoSource). The local meteoric water line (LMWL) equation was  $D = 7.775 \delta^{18}O + 12.34$  ( $R^2 = 0.871$ ,  $P < 0.001$ ), reflecting the climatic characteristics of low temperature, high humidity, and strong evaporation in the study area. The results showed that the six dominant plant species primarily utilized precipitation throughout the entire growing season, with soil water from 0–10 cm depth serving as the secondary water source, while groundwater contributed the least. The six species exhibited temporal variation in their utilization proportions of different water sources. In July, all six species mainly used precipitation and shallow soil water. *Rhododendron capitatum* showed higher groundwater utilization than other species. The ability of *Spiraea salicifolia* and *Rhododendron capitatum* to utilize soil moisture was particularly strong.

**Keywords:** stable isotope; water source; subalpine shrub; Qilian Mountains

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## Introduction

Water is a critical factor for plant growth, and its availability directly influences plant development and distribution patterns. Tracking and analyzing plant water sources can predict spatiotemporal vegetation distribution changes under altered water availability, aid in plant selection for degraded ecosystem restoration, and provide scientific basis for developing reasonable climate change adaptation strategies. Traditional plant water research primarily relies on excavation methods for direct root system investigation, combined with comprehensive analysis of multiple factors such as shrub interception and transpiration rates, including plant leaf stomatal conductance and sap flow characteristics. However, these methods have limitations for precise analysis of plant water sources. Root excavation is time-consuming, causes partial damage to plants, and plant water indicators are influenced by various environmental factors and plant water use patterns. Stable isotope tracer technology offers high sensitivity and accuracy and has become an effective tool for tracing plant water sources.

International scholars have long used stable isotope technology to trace plant water sources, conducting in-depth studies across different habitats. Most riparian

plants have mixed water sources, and fog water can also be an important water source in coastal desert areas. Domestic research using stable isotope technology to trace plant water sources started later but has achieved significant results. For example, Chu Jianmin et al. studied several desert plants in Minqin and Ejina Banner, finding that *Nitraria tangutorum* and *Calligonum mongolicum* showed significant differences in water source selection during dry seasons. Other studies have revealed that plants employ more long-term effective water use patterns, such as utilizing deep soil water and groundwater through well-developed root systems. Previous research in China has focused primarily on arid, semi-arid, and humid regions, with relatively few studies in alpine areas. Some studies have investigated water sources of *Myricaria squamosa* in the Qinghai Lake basin, finding that riverside populations depended heavily on groundwater and river water, while populations approximately 150 m from the river utilized more soil water.

Within the same habitat, different plant species exhibit variations in water utilization, and competition forms when water is limited. Therefore, studying water use by different plant species within the same community is necessary to comprehensively understand how water factors affect plant distribution and survival. Subalpine shrubland is an important vegetation type in the Qilian Mountains with high water conservation capacity. However, under climate change impacts and disturbance from agricultural and pastoral activities, shrub degradation and destruction have become serious, affecting hydrological processes in cold regions. This paper quantitatively analyzes water sources of dominant plants in the subalpine shrubland of the eastern Qilian Mountains, examining water source selection by different plants in the same habitat and temporal changes in water source selection by the same plant species. The aim is to comprehensively understand the water cycle and ecohydrological processes of subalpine shrubland in alpine regions, providing basis for ecological restoration and environmental construction in subalpine zones.

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## 1. Study Area Overview

The study area is located in the subalpine shrubland of the eastern Qilian Mountains on the northeastern edge of the Tibetan Plateau, in Zhaxi Xiulong Township, Tianzhu Tibetan Autonomous County, Gansu Province (102°47' 25" E, 37°10' 19" N, 3009.3 m). The terrain is higher in the west and lower in the east, featuring both mountainous and valley landforms. The climate is cold and humid, with an extreme minimum temperature of -34.9°C, extreme maximum temperature of 28°C, and a plant growing season of 120–140 days. The annual sunshine duration is 2600 hours. Annual precipitation is 4160 mm (note: this value appears unusually high and may contain a typographical error in the original text), concentrated in the period with temperatures of -0.1–1.2°C. Soils are primarily subalpine meadow soil and subalpine chernozem. High-cold rhododendron shrubland and high-cold willow shrubland are distributed in belts on

shady slopes above 3050 m, while *Potentilla* shrub grassland is distributed on terraces and valley floors, and sunny slopes are dominated by high-cold meadow steppe.

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## 2. Sampling Methods

Six local dominant species were selected as study subjects: *Salix oritrepha*, *Salix cupularis*, *Rhododendron capitatum*, *Rhododendron thymifolium*, *Spiraea salicifolia*, and *Potentilla fruticosa*. To minimize the influence of external factors such as light on isotope analysis results, sampling was conducted from 9:00–11:00 on July 23, August 2, and August 14, 2015. Healthy individuals of similar height were selected in relatively uniform terrain. Non-lignified branches approximately 0.3 cm in diameter were collected, with bark quickly removed and the samples immediately placed in threaded plastic bottles sealed with Parafilm. In the vicinity of sampled plants, soil samples were collected from layers at 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, and 70–80 cm depths. One portion was immediately placed in threaded plastic bottles sealed with Parafilm for water extraction, while another portion was placed in aluminum boxes for gravimetric soil moisture determination. River water samples were collected from the nearest Jinqiang River tributary, precipitation was collected using a standard rain gauge from the meteorological station, and groundwater samples were collected from the nearest deep well (approximately 150 m from the sample plot). River water and precipitation were placed in threaded plastic bottles and sealed with Parafilm.

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## 3. Water Extraction and Isotope Determination

Water extraction from plant and soil samples, as well as  $D$  and  $^{18}O$  determination of different water bodies, was completed at the Stable Isotope Laboratory of Northwest Normal University. A LI-2000 automatic vacuum condensation extraction system (Beijing Lica United Technology Limited Company) was used for water extraction via vacuum distillation. A DLT-100 liquid water isotope analyzer (Lios Gatos Research, Mountain View, USA) was used for  $D$  and  $^{18}O$  determination, with measurement precision of  $\pm 0.6\text{‰}$  for  $D$  and  $\pm 0.2\text{‰}$  for  $^{18}O$ . The LWI A-Spectral Contamination Identifier v1.0 software was used for spectral contamination correction of measurement data. All values are expressed relative to V-SMOW (Vienna Standard Mean Ocean Water).

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## 4. Data Analysis Methods

The contribution rates of different water sources to plants were calculated using the multisource linear mixing model IsoSource. By setting small increments,

all possible contribution rate combinations were listed, and solutions with differences less than the tolerance were considered feasible. In calculations, the increment was set to 2‰ and tolerance to 0.01. The  $^{18}\text{O}$  value ranges were -9.32‰ to 5.89‰, and D ranges were -57.67‰ to 9.63‰. During sample measurement, hydrogen isotope errors were larger than oxygen isotope errors, and the  $^{18}\text{O}$  variation range was relatively smaller. When using IsoSource with hydrogen isotopes alone, almost no results were obtained, indicating that using oxygen isotopes alone for calculating water source contributions is more accurate. Therefore, only  $^{18}\text{O}$  values were used in calculations. For precipitation  $^{18}\text{O}$  values, a weighted average method was employed, using precipitation before plant sampling as potential water sources with each precipitation amount as weights. Significance testing for differences in hydrogen and oxygen isotope values among different plant species and water sources was performed using SPSS 21.0.

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## Results

### 4.1 Isotopic Characteristics of Different Water Bodies

All samples from the subalpine shrubland plot in the eastern Qilian Mountains showed significant differences in stable hydrogen and oxygen isotope ratios among different water bodies. The average D value was  $(-44.93 \pm 3.43)\text{‰}$ , and the average  $^{18}\text{O}$  value was  $(-6.10 \pm 0.34)\text{‰}$ . Precipitation isotope ratios differed significantly from those of other water bodies. Zhu Jianjia et al. calculated the average D value at the Golmud plot in Qinghai as  $(-68.3 \pm 1.85)\text{‰}$  and  $^{18}\text{O}$  as  $(-10.26 \pm 0.16)\text{‰}$ , while Zhu Xiuqin found the average D value in the karst plot of central Yunnan Province as  $(-72.19 \pm 0.74)\text{‰}$  and  $^{18}\text{O}$  as  $(-7.08 \pm 0.74)\text{‰}$ . These differ from our results, indicating substantial variation in isotope values across different regional water bodies. Plant water and soil water from different periods and soil layers also showed significant differences in hydrogen and oxygen isotope ratios. The D and  $^{18}\text{O}$  values of groundwater and river water were similar, with average values of  $(-43.89 \pm 1.06)\text{‰}$  and  $(-5.34 \pm 0.??)\text{‰}$  respectively. After precipitation reaches the surface, stable isotopes undergo further fractionation during infiltration, plant transpiration, and surface/groundwater runoff formation, leading to significant depletion.

Table 1: Hydrogen and oxygen stable isotope ratios in different water bodies in the subalpine shrub area of the eastern Qilian Mountains (mean  $\pm$  SE) /‰

Table 2: Variation of hydrogen and oxygen stable isotope ratios in soil water along soil profiles (mean  $\pm$  SE) /‰

Table 3: Hydrogen and oxygen stable isotope ratios in different plant water (mean  $\pm$  SE) /‰

## 4.2 Relationships Between Different Water Bodies

Based on precipitation samples, the local meteoric water line for the subalpine zone of the eastern Qilian Mountains was established as:  $D = 7.775 \text{ }^1\text{O} + 12.34$  ( $R^2 = 0.871$ ,  $P < 0.001$ ). The slope is slightly lower than the global average, while the intercept is higher, indicating strong evaporation, low temperature, and high humidity in the region. The high altitude leads to low temperatures, and the inland location causes strong evaporation, resulting in a lower slope. Groundwater and river water plot to the upper left of the meteoric water line but close to it, indicating that precipitation clearly recharges both. Most plant water points plot to the lower right of the meteoric water line, showing significant isotopic enrichment during plant transpiration. Except for a few soil water points far from the meteoric water line, most plot near it, indicating clear precipitation recharge to soil water.

During the sampling period, total precipitation was 4188 mm (note: likely a topographical error), with 223 mm in July (71.6% of annual total) and 543 mm in August (13.7% of annual total). Precipitation showed clear seasonal variation, with summer precipitation accounting for 69.8% of the annual total and winter precipitation less than 5%. Small rainfall events (<5 mm) accounted for 17.6% of annual precipitation. The  $^1\text{O}$  range in river water samples was -9.32% to -8.53%, while in groundwater it was -9.03% to -8.42%, and in precipitation it was -8.26% to -0.76%. The narrow and similar ranges of  $^1\text{O}$  in groundwater and river water indicate that precipitation is their recharge source in the subalpine zone of the Qilian Mountains.

[Figure 1: see original paper] Figure 1: D- $^1\text{O}$  plots of precipitation, soil water, groundwater, and plant water in the subalpine shrub area of the eastern Qilian Mountains

[Figure 2: see original paper] Figure 2: Precipitation from July to August 2015 in the subalpine shrub area of the eastern Qilian Mountains

## 4.3 Water Sources of Different Plant Species

Available water sources for plants include precipitation, groundwater, and river water. The hydrogen and oxygen isotope differences among these sources exceed experimental error, making them relatively independent water sources suitable for quantitative analysis. Calculations showed that river water contributed less than 0.5% to all plants on average, and since the plot location was higher than the river, river water influence was ignored. The six plant species showed different water source utilization patterns, with precipitation as the main source, 0-10 cm soil water as the secondary source, and groundwater contributing the least. Precipitation showed obvious advantages in replenishing plant water sources.

From July 23 to August 14, 2015, five rainfall events were collected, each lasting relatively long, likely related to continuous July rainfall effects. Precipitation contributed 53.6%, 45.03%, 50.23%, 54%, 36.33%, and 29.5% to *Salix*

*oritrepha*, *Spiraea salicifolia*, *Salix cupularis*, *Rhododendron capitatum*, *Rhododendron thymifolium*, and *Potentilla fruticosa* respectively. The main water sources for the first five species were precipitation and surface soil water, with precipitation being more dominant. For *Potentilla fruticosa*, precipitation and surface soil water were also main sources, but their contributions were lower compared to the other four species.

All six species showed consistent soil water utilization patterns: utilization decreased with soil depth from 0–10 cm, reached a secondary peak at 10–20 cm, then gradually decreased with further depth. Although each species utilized soil water from different layers in varying proportions, utilization of 10–80 cm soil water was relatively uniform across layers. On average, *Rhododendron thymifolium* showed relatively high soil water utilization, while *Salix oritrepha* and *Potentilla fruticosa* showed relatively low utilization.

[Figure 3: see original paper] Figure 3: Water source percentage of dominant plant species in the subalpine shrub area of the eastern Qilian Mountains

Plant water absorption is typically related to root distribution, with shallow-rooted plants mainly using surface soil water and deep-rooted plants mainly using deep soil water and groundwater. *Rhododendron capitatum* has fewer lateral roots, with thicker roots twisting through 10–20 cm depth, while fine roots are concentrated in the 0–30 cm surface layer to obtain water and nutrients. This study showed that 0–10 cm soil water contributed 26.8% of total soil water contribution to *Rhododendron capitatum*, and 10–20 cm soil water contributed 36.33%, consistent with findings that 53–77% of total active roots of *Rhododendron thymifolium* are concentrated in 0–20 cm depth. *Potentilla fruticosa* distributes most of its underground root system in the shallow 0–20 cm soil layer, with soil water contributing 69.8% of total contribution to this species, consistent with research showing most active roots of *Potentilla fruticosa* are located in 0–20 cm depth. *Spiraea salicifolia* has relatively thick main roots but well-developed lateral roots, with fine roots accounting for the highest proportion among the six species after *Rhododendron capitatum*, consistent with this study's results. *Salix oritrepha* and *Salix cupularis* have relatively thin main roots but well-developed lateral roots, though *Salix cupularis* has higher utilization of 0–80 cm soil water than *Salix oritrepha*. Subalpine shrubland in the Qilian Mountains mainly utilizes shallow soil water, which aligns with root distribution patterns—roots concentrated in shallow soil facilitate water absorption, especially with lateral root dominance.

#### 4.4 Temporal Changes in Plant Water Sources

Different plants utilize water differently across growth stages. The sampling period (July–August) represented the vigorous growth period for the six dominant species in the eastern Qilian Mountains subalpine shrubland. All species mainly used precipitation and shallow soil water, but showed distinct temporal variation in water source selection. July had frequent rainfall events, and

while the six species utilized rainfall differently, precipitation was the main water source for all, contributing 29.4%, 22.8%, 26.1%, 26.4%, 57.6%, and 32.9% to *Salix oritrepha*, *Spiraea salicifolia*, *Salix cupularis*, *Rhododendron capitatum*, *Rhododendron thymifolium*, and *Potentilla fruticosa* respectively. Compared to July, August showed significantly increased contributions of precipitation to all six species (44.8%, 73.6%, 80.9%, 62.8%, 54.9%, and 61.2% respectively), indicating that while plants utilize stable sources like soil water and groundwater, they also take advantage of precipitation when available.

Soil water is plants' most direct water source, and changes in soil moisture directly affect root water absorption. Comparing soil moisture content across different layers, the 0–30 cm layer had high water content likely because sampling occurred shortly after rainfall events, leaving insufficient time for infiltration to deeper layers. On July 23, soil water utilization by *Potentilla fruticosa* was 28.4% for 0–10 cm depth, decreasing to 10.1% on August 2 and 9.8% on August 14, associated with reduced precipitation frequency and amount. *Salix oritrepha* showed 29.5% utilization of 0–10 cm soil water on August 2, decreasing to 16.03% on August 14. *Rhododendron capitatum* and *Spiraea salicifolia* showed smaller decreases in utilization over time, indicating their stronger ability to utilize shallow soil water.

Utilization of soil water in 10–40 cm layers showed a trend of first decreasing then increasing and decreasing again across time for most species, while *Rhododendron thymifolium* and *Spiraea salicifolia* showed continuous decreases. The 40–80 cm layers had relatively low water content, related to insufficient precipitation recharge depth and shallow groundwater tables. *Potentilla fruticosa* showed peak utilization of deep soil water on August 2, while *Spiraea salicifolia* and *Rhododendron thymifolium* showed different patterns, possibly related to water competition with other plants.

Although the six dominant species utilized little groundwater, temporal variation existed. *Rhododendron capitatum* showed higher average groundwater utilization (9.5%) than other species. Groundwater, as a stable water source, is also important when rainfall is insufficient to recharge soil water. However, declining groundwater levels can directly affect soil moisture and nutrient distribution, triggering plant water competition. In this study area, the low groundwater table during the rainy season at Wushaoling, combined with shallow root distribution of the studied shrubs, resulted in relatively low groundwater contribution rates.

[Figure 4: see original paper] Figure 4: Water source percentage of dominant plant species in the subalpine shrub area of the eastern Qilian Mountains at different times

[Figure 5: see original paper] Figure 5: Variations of soil water content along soil profiles in the subalpine shrub area of the eastern Qilian Mountains

## Conclusions

The local meteoric water line for the Wushaoling region in July-August was  $D = 7.775 \delta^{18}O + 12.34$  ( $R^2 = 0.871$ ,  $P < 0.001$ ), indicating low temperature, high humidity, and strong evaporation. Groundwater and river water plot to the upper left of but close to the meteoric water line, while plant water and soil water show significant isotopic enrichment, plotting mostly to the lower right. Most points cluster near the meteoric water line, indicating clear precipitation recharge to river water, groundwater, and soil water.

All six dominant species showed highest precipitation utilization rates. Soil water utilization showed a trend of first decreasing, then increasing, then decreasing again with depth, but utilization of 0-10 cm soil water was relatively high. Groundwater contributed minimally to all species. The six dominant species in the eastern Qilian Mountains subalpine shrubland primarily used precipitation and 0-10 cm soil water, with temporal variation in water source utilization. Precipitation and shallow soil water contributed similarly to *Spiraea salicifolia*, *Rhododendron capitatum*, and *Rhododendron thymifolium*, while *Salix cupularis* and *Potentilla fruticosa* showed higher precipitation use efficiency than *Salix oritrepha*. The former two also utilized deep soil water more efficiently than the latter.

Different plants have different water use strategies. Plants with similar water uptake layers in the same habitat compete for water sources, with *Spiraea salicifolia* and *Rhododendron capitatum* showing particularly obvious competition. The sampling period coincided with the rainy season, and changes in soil moisture recharge and groundwater table affected plant water use strategies. The water competition relationships among the six dominant species and other plants require further study. Future research should conduct quantitative analysis in other months or years to verify these conclusions, and integrate multiple factors such as shrub interception and transpiration rates to comprehensively understand their effects on plant water sources.

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