

RESEARCH ARTICLE

Water use efficiency of *Cupressus gigantea* is higher in mountain slope habitats compared to riverside habitats

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Abstract

Cupressus gigantea is an endemic species of the Yarlung Zangbo River in Tibet, China, and is designated as a national key protected wild plant species. It is uniquely distributed in the specific sections from Jiacha County to Danniang village Milin City along Yarlung Zangbo River in various habitats such as the waterline along the Yarlung Zangbo River, terraces, and mountain slopes. In this study, we utilized stable isotope technology (δD , $\delta^{18}O$, and $\delta^{13}C$), combined with the IsoSource model, to quantitatively determined the sources of water absorption and the water use efficiency of *C. gigantea* in various habitats, including riverside area, terraces and mountain slopes. The results showed that the content of soil total nitrogen, soil nitrate nitrogen, soil ammonium nitrogen, and soil organic carbon are significantly higher on mountain slope than in the other two habitats, while the total carbon content, water content, and total nitrogen content of *C. gigantea* leaves are significantly lower on mountain slope than in the other two habitats ($P < 0.05$). The water use efficiency (WUE) of *C. gigantea* in the mountain slope habitats is $107.8 \mu\text{mol mol}^{-1}$, which is significantly higher than that of other habitats ($P < 0.05$), with the WUE of *C. gigantea* in the riverside habitat being the lowest at $35.7 \mu\text{mol mol}^{-1}$. *C. gigantea* distributed on the mountain slope and terrace uses soil water as the main water source. During the normal rainy season, the proportion of soil water utilization is the highest, accounting for more than 60%; in the dry season, the utilization of river water and groundwater increases, while soil water utilization decreases. *C. gigantea* distributed along the riverside mainly absorbs water from the Yarlung Zangbo River, about 63%. In August when the rainfall increases in the rainy season, the use of soil moisture increases, and in the dry season, river water becomes the main water source. This indicates that with the alternation of rainy and dry seasons, the water absorption sources

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and strategies of *C. gigantea* adjusted accordingly. The research results provide an important scientific basis for the protection and management measures of the *C. gigantea* population.

Introduction

The sources of water absorbed by plants generally include soil water, river water, groundwater, atmospheric precipitation, and various other sources. The proportion of hydrogen and oxygen isotope ratios in water varies among these sources due to isotopic fractionation of water [1]. For example, lighter water molecules evaporate more readily, leading to heavier isotopes being enriched in condensed phases like precipitation, river water and groundwater, while lighter isotopes are more prevalent in evaporated water vapor. Therefore, it is possible to analyze which sources of water are used by comparing and analyzing the isotope content of water in different water bodies and plants. This analysis can directly reflect the differences in the source of water absorbed by plants in response to environmental changes, as well as the changes in vegetation water use, adaptability to the natural environment, and ecological stress that plants experience [2,3]. The IsoSource model was used to calculate the relative utilization of multiple water sources by plants, as well as the contribution rate and range of these water sources to plant water [4]. In this method, all possible combinations of each source contribution (0–100%) are examined in small increments (e.g., 1%). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (e.g., $\pm 0.1\%$) are feasible solutions, from which the frequency and range of potential source contributions can be determined. In the mountainous areas of California along the banks of perennial rivers, the early transpiration of trees during the growing season mainly comes from the soil water, while during most of the dry seasons, it comes from groundwater [5]; Studies on the soil-water-atmosphere continuum indicate that the soil depth at which root water uptake occurs largely depends on seasonal climate fluctuations, as observed using stable isotopes tracers of water movement [6]. The water use of *Pinus strobus* shifts between deep and surface soil layers under the influence of precipitation [7]. Research on plants in the Tapares River in the eastern Amazon region of the tropical rainforest found that during the dry season with little precipitation, trees continuously deepened their soil water absorption [8]. A Study on plants in the riparian forest ecosystem of the lower reaches of the Heihe River found that different water sources contribute differently to plants with groundwater being an important contributor to the growth of desert plants such as *Populus euphratica* [9]. In the desertification grassland ecosystem of Ordos, simulated rainfall as a single water source showed that the attenuation period of precipitation in typical desert plant water is 7 days [10].

Plant water use efficiency is a comprehensive physiological and ecological indicator for evaluating the suitability of plant growth. It reflects the relationship between plant water consumption and dry matter production. It is one of the basic and

important characteristics of plants in respond to arid environments, enabling plants to maintain the availability of soil water and thereby improving their resilience to drought conditions [11]. The stable carbon isotope method is based on the stable carbon isotope composition of plants and is currently internationally recognized as an acceptable method for determining long-term plant water use efficiency (WUE). Research has shown a strong correlation between WUE and the stable carbon isotope ratio $\delta^{13}\text{C}$ value. The higher the $\delta^{13}\text{C}$ value of the plant, the higher its WUE, indicating a more economical and conservative water utilization mode [12–14]. Variation in stomatal conductance and water stress-induced changes in the degree of stomatal limitation of net photosynthesis were the major controls on variation in $\delta^{13}\text{C}$ during 1998–2001 in a grassland near Lethbridge, Canada [15]. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values as an indicator of WUE of *Pinus koraiensis* were correlated with DBH, light environment [16].

Cupressus gigantea is an endemic species along the banks of the Yarlung Zangbo River in the Tibet Autonomous Region, and is also a national first-class key protected wild plants. Currently, its natural distribution range is known to be limited to both sides of the Yarlung Zangbo River from Jiacha County to Danniang Township, Nyingchi City, and Jubai Conservation Park on the bank of Niyang River which is one of important tributary of Yarlung Zangbo River. Although the existing distribution range is narrow, the climate zone within its distribution range changes from semi-arid to semi humid area. The largest individual of *C. gigantea* has been recorded being more than 2500 years old, with a height of about 55 m, a diameter at breast height of 2.3 m, and a single plant volume of up to 230 m³ [17,18]. The growth of *C. gigantea* from seedlings to mature trees is very slow. Additionally, due to the drought conditions and shallow soil layers in its habitats, the natural regeneration process is highly challenging. The conversion rate from seed rain to established saplings is only 0.008% [19]. *Cupressus gigantea* is an invaluable ecological asset for the environment and climate of the Qinghai and Tibet Plateau, and is also a unique feature of the Yarlung Zangbo River landscape. Its distribution is scattered or clustered in patches. Without proper protection practice, any damage to the population or blockage of regeneration could result in significant losses.

Water is a critical factor limiting the growth and distribution of plants in arid and semi-arid areas. Plants can adapt to environmental changes by adjusting their sources of water absorption according to different environmental conditions [6,20–22]. In arid environments, plant growth is generally limited by the temporal and spatial availability of water and nutrients [23–26]. The functional traits and population growth of plants in arid and semi-arid ecosystems largely depend on the availability of water in the environment and the plant's ability to utilize that water [27]. Therefore, this study explores the following scientific questions: (1) Are there significant differences in soil characteristics across different habitats? (2) Does *C. gigantea*, which grows in arid environments, exhibit a stronger capability to regulate water use efficiency? (3) Does *C. gigantea* utilizes different sources of water at various stage of growing season?

Materials and methods

Study area

The research site locates in Xizang, China. This area has a typical plateau temperate semi-arid and semi humid monsoon climate, and the vegetation is *C. gigantea* sparse forest. There is a large diurnal temperature variation. The average annual temperature is above 2°C, with the highest temperature in July reaching 17.9 °C and the lowest temperature in January dropping to −12 °C. The annual average sunshine hours are 1600–1900 hours. The average annual precipitation is about 500 mm, concentrated from June to September, accounting for 75% to 88% of the total annual rainfall. The maximum rainfall typically occurs from July to August, while the minimum rainfall occurs from November to February. The annual evaporation is 1201 mm. The soil consists of brown sandy loam with a lot of gravel, characterized by a light texture, low bulk density, weak water storage capacity, and low water content. The climate zone is semi-arid, with a harsh ecological environment and sparse vegetation. *Cupressus gigantea* trees are distributed along the riverside, extending towards the slope direction and up to the upper and middle parts of the mountain. The distribution habitat of *C. gigantea*

Table 1. Description of three habitats in pH value of soil layer (0–40 cm), habitats characteristics, diameter at breast height (DBH), height of and crown width of *C. gigantea* individuals involved in current experiments and the species compound of plant community at three habitats in Tibet, China. Different lowercase of pH value indicates the significant difference among habitats ($P < 0.05$).

Habitats	pH value	Description of habitats traits	DBH(cm)	Height (m)	Crown width(m)	Species in community
Riverside	8.45 ± 0.018 ^a	Vegetation is sparse, with a coverage rate of about 5%; The soil layer is thin with the gravel accumulates due to the influence of water flow. The diameter of gravel is about 10 cm or more. In summer, there are abundant water sources, and the water level and above the river-bank are eroded by water flow.	34.5	7.5	4.1 × 3.2	<i>Rosa sikangensis</i> , <i>Sophora moorcroftiana</i> , <i>Potentilla griffithii</i> , <i>Sanguisorba filiformis</i> , <i>Artemisia wellbyi</i> ,
			18.7	5.9	3.5 × 3.8	
			30.0	7.0	4.0 × 3.8	
			25.6	6.4	3.8 × 3.6	
			43.0	7.4	4.6 × 4.5	
			38.5	7.0	4.3 × 4.2	
Terrace	8.32 ± 0.026 ^b	There are diversity of grasses and shrubs in vegetation, with coverage of about 40%. The surface soil is sandy loam, with the thickness being 30–40 cm and a good soil structure. There is a gravel layer under 40 cm. The terrain is relatively flat.	36.0	5.5	3.5 × 4.3	<i>Ceratostigma willmottianum</i> , <i>Leptodermis pilosa</i> , <i>Spiraea canescens</i> , <i>Cotoneaster microphyllus</i> , <i>Cleistogenes squarrosa</i> , <i>Eleusine indica</i> , <i>Arundinella anomala</i>
			31.0	5.8	3.4 × 4.0	
			32.4	7.3	5.3 × 4.5	
			21.4	6.8	3.9 × 4.2	
			18.3	5.3	3.2 × 3.0	
			45.7	8.3	4.9 × 4.6	
Mountain slope	8.22 ± 0.019 ^c	The vegetation is relatively rich, with a coverage of about 45%. The slope of this habitat is generally between 30–50 degree and the soil layer is thin The upper layer of the soil is dark gray, mostly light brown, and the bottom is mostly gravel.	27.3	6.5	3.2 × 3.5	<i>Artemisia persica</i> , <i>Leptodermis pilosa</i> , <i>Cotoneaster microphyllus</i> , <i>Gentiana crassicaulis</i> , <i>Artemisia annua</i> , <i>Drynaria sinica</i> , <i>Chenopodium foetidum</i>
			30.4	7.2	4.6 × 4.8	
			23.5	7.0	4.0 × 4.2	
			35.3	7.1	5.0 × 4.8	
			37.4	7.5	5.3 × 5.5	
			46.8	8.0	5.7 × 5.9	

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can be roughly divided into riverbank, terrace, mountain slopes and steep cliff. This study selected three typical habitats for investigation: the riverside, terrace, and the mountain slope, each with varying vegetation composition (Table 1).

A sample plot measuring 100 m × 100 m (length × width) was established within each habitat. The tree height, diameter at breast height, and crown width of each *C. gigantea* individual were measured. Concurrently, soil, plant leaf, and stem samples were collected for analysis of carbon isotopes, physiological, and biochemical indicators. The samples were then transported to the lab and stored in a 4°C refrigerator for water extraction to measure hydrogen and oxygen isotope. It is characterized by a distinct monsoon climate with four clearly defined seasons of the distribution area of *C. gigantea*. Therefore, sampling was conducted during three seasons corresponding to the plant's growth period, spring (May), summer (August), and late autumn (Nov.). During experiments, the actual sampling occurred at four events: August 2022 (rainy season, summer), May 2023 (dry season, Spring), August 2023 (rainy season, summer), and November 2023 (before winter, late autumn) (Fig 1).

Sample collection

Plants and soil samples

Three groups of *C. gigantea* individual were selected based on a diameter at breast height: 0–20 cm, 20–40 cm, and greater than 40 cm. Leaves, branches and roots samples were collected from six individuals in each group. High branches with sufficient light in the middle of the crown were chosen for collecting leaf and branches samples for isotope analysis, water content, and other indicators. To prevent isotope fractionation, the phloem of the branches was removed, and the samples were quickly placed into 8 ml glass sample bottles with transparent threaded openings (CNW Technology, Germany). The bottle was labeled and sealed with Parafilm sealing film (American National Can, USA) before being refrigerated and stored at 4°C in the laboratory for subsequent water extraction and analysis of hydrogen and oxygen isotopes.

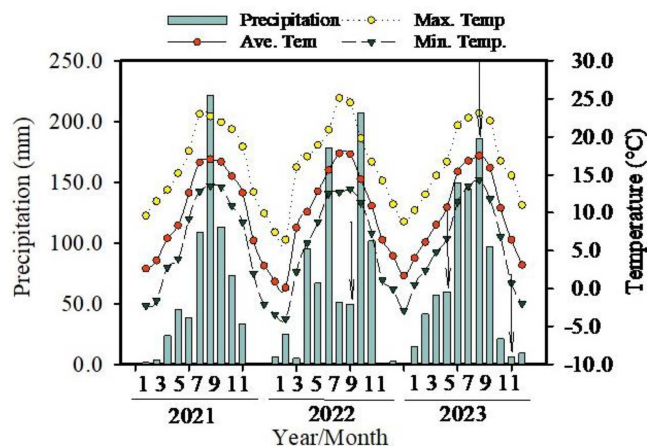


Fig 1. Changes in rainfall (mm), monthly mean atmospheric temperature (°C), monthly maximum and minimum temperatures (°C) during 2021 to 2023. Arrow means the month when the experiments were conducted in Aug. 2022, May 2023, Aug. 2023, Nov. 2023 respectively.

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It was reported that the root system of *C. gigantea* is relatively shallow, with the root occupying 73.06% of the vertical range of 0–40 cm [28]. This may be related to the fact that the average soil layer thickness in the natural distribution area of *C. gigantea* is only 30–40 cm. Therefore, this study collected soil samples from 0–20 cm and 20–40 cm soil layers to examine the absorption and utilization of water sources by *C. gigantea*. Soil samples were collected from 50–100 cm away from the trunk in different directions. The samples were quickly placed into transparent threaded 10 ml glass sample bottles, labeled, sealed with a sealing film, and then refrigerated and stored at 4°C in the laboratory for subsequent water extraction and analysis of hydrogen and oxygen isotopes. Three soil samples were collected for each selected *C. gigantea* tree. Additionally, soil samples were collected from each habitat to measure physiological and biochemical indicators. Three leaf samples from each diameter class of *C. gigantea* were collected, weighed fresh, and then heated at 105°C for 30 minutes. The samples were subsequently dried at 60°C for 48 hours until they reached a constant weight. Weight changes were recorded, and water content of the leaves was calculated. After drying, the samples were ground, crushed, and sieved through an 80-mesh sieve for carbon isotope determination. For the measuring stable carbon isotope, leaf samples were collected only in August 2022 due to the relatively stable $\delta^{13}\text{C}$ values.

Water samples

Water samples from the Yarlung Zangbo River were collected during each sampling period near the research site, placed in glass bottles, seal with sealing film, and refrigerate for storage. Three replicates were collected for each sampling period. Underground water samples were collected from the well at Zhaxitang village, with three replicates for each sampling period. Rainwater samples were collected during the sampling period, with at least three rainfall events collected from August to September 2022, May to June 2023, July to August 2023, and October to November 2023. All samples are sealed in 8 ml glass sample bottles, labeled with the year, month, and day, refrigerated, and transported back to the laboratory for storage at 4°C until measurement.

Samples analysis

Measurement isotope and water source determinaiton

Water was extracted from *C. gigantea* branches and soil samples using a low-temperature vacuum extraction method to prevent isotope fractionation during the extraction process. The hydrogen and oxygen stable isotope analysis of both

extracted water samples and field-collected water samples was performed using a stable isotope ratio mass spectrometer (253 plus) to determine the δD and $\delta^{18}O$ values. The experimental results were corrected using the Vienna Standard Mean Ocean Water (VSMOW), and the measured isotope results were reported as the difference (‰) relative to VSMOW standard value. The stable isotope ratio is expressed as follows:

$$\delta D \text{ (or } \delta^{18}O \text{)} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\%$$

R_{sample} : isotope ratio of samples; R_{standard} : isotope ratio of standard materials.

The multi-source linear mixed model (IsoSource) determines the total contribution rate of all sources by summing to 100%, and then calculates the upper and lower limits of the contribution rate error for each source. The procedure involves inputting potential source waters into the IsoSource software: soil water (0–20 cm), soil water (20–40 cm), groundwater, and river water. Next, isotopic values and markers from the *C. gigantea* plant samples are entered. After setting the increment and allowable deviation, the program is run to perform the calculations and save the results.

IsoSource The multiple linear model is as follows:

$$\delta D_p = \sum f_i \delta D_i \quad (1)$$

$$\delta^{18}O_p = \sum f_i^{18}O_i \quad (2)$$

$$\sum f_i = 1 \quad (3)$$

$\delta D_i(\delta^{18}O_i)$: hydrogen and oxygen isotope values of each water source; $\delta D_p(\delta^{18}O_p)$: abundance of hydrogen and oxygen isotopes in plant water; f_i : contribution rate of water source i to plant water content.

This study includes four potential water sources: soil water (0–20 cm), soil water (20–40 cm), groundwater, and river water. Therefore, the multiple linear model used in this study is:

$$\delta D_p = f_1 \delta D_1 + f_2 \delta D_2 + f_3 \delta D_3 + f_4 \delta D_4 \quad (4)$$

$$\delta^{18}O_p = f_1^{18}O_1 + f_2 \delta O_2 + f_3 \delta O_3 + f_4 \delta O_4 \quad (5)$$

$$f_1 + f_2 + f_3 + f_4 = 1 \quad (6)$$

The contribution rates of four water sources to the water content in the xylem of *C. gigantea* are represented as following: f_1 , soil water (0–20 cm), f_2 , soil water (20–40 cm), f_3 , groundwater, and f_4 , river water.

Carbon isotope determination water use efficiency

Carbon isotopes of leaf samples were analyzed using a stable isotope mass spectrometer (Delta plus xp). Calibrated CO_2 was used as the standard for determining the stable carbon isotope ratio, with international standard V-PDB serving as the reference gas. The sample was compared to the standard gas to calculate the $\delta^{13}C$ value of the *C. gigantea* leaves. This value represents the isotope ratio relative to the international standard. The average water use efficiency (WUE) and leaf isotope resolution Δ can be calculated using a linear model using the following formula:

$$\Delta = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{leaf}}) / (1 + \delta^{13}\text{C}_{\text{leaf}}) \quad (1)$$

$$\delta^{13}\text{C}_a = -6.429 - 0.006\exp[0.0217(t - 1740)] \quad (2)$$

$\delta^{13}\text{C}_{\text{leaf}}$ is the carbon isotope ratio of plant leaves, $\delta^{13}\text{C}_a$ is the carbon isotope ratio of environmental CO_2 , which is -6.7‰ [29]; t is the sampling year.

Therefore, the leaf isotope resolution (Δ) can be calculated using the formula above. For this study, with the sampling year being 2022, the calculation is as follows:

$$\delta^{13}\text{C}_a = -6.429 - 0.006\exp[0.0217(2022 - 1740)] = -9.15655(\%) \quad (3)$$

$$\Delta = (-9.15655 - \delta^{13}\text{C}_{\text{leaf}}) / (1 + \delta^{13}\text{C}_{\text{leaf}}) \quad (4)$$

The C_i/C_a ratio is an important physiological and ecological indicator of plants, reflecting the relative amount of net assimilation rate and stomatal conductance in relation to CO_2 demand and supply. The calculation formula is as follows [30]:

$$C_i/C_a = (\Delta - a) / (b - a) \quad (5)$$

$$C_a = 277.78 + 1.350\exp[0.01572(t - 1740)] \quad (6)$$

C_i is the intercellular CO_2 concentration; C_a is the concentration of environmental CO_2 in the study area ($\mu\text{mol mol}^{-1}$); a is the isotopic fractionation coefficient (4.4%) caused by the diffusion of environmental CO_2 in still air; b is the fractionation coefficient (27%) resulting from CO_2 fixation in chloroplasts and its internal diffusion through 1,5-bisphosphoribulose carboxylase (Rubisco); t is the sampling year.

$$C_a = 277.78 + 1.350\exp[0.01572(2023 - 1740)] = 391.43 \quad (7)$$

The formula for calculating the average water use efficiency (WUE) is as follows:

$$\text{WUE} = (C_a - C_i) / 1.6 = C_a(b - \Delta) / 1.6(b - a) \quad (8)$$

$$\text{WUE} = 391.43 \times (27\% - \Delta) / 1.6(27\% - 4.4\%) \quad (9)$$

Determination of biochemical indicators for soil and leave

Leaf and soil indicators were measured at different habitat sampling sites in August 2022. The physical and chemical properties of soil and *C. gigantea* leaves were determined using 10 indicators: soil pH value, soil total nitrogen, soil ammonium nitrogen, soil nitrate nitrogen, soil available phosphorus, soil organic carbon, leaf moisture content, leaf total nitrogen, and leaf total carbon. The soil pH value was measured using an acidity meter; Leaf moisture content was determined by the drying method; Total nitrogen content in soil and leaves was measured using the Kjeldahl nitrogen determination method; Organic carbon content in soil and total carbon content in leaves were measured using the potassium dichromate external heating method; Soil ammonium nitrogen and soil nitrate nitrogen content were determined using the flow analysis method; Soil available phosphorus content was measured using the NaHCO_3 extraction molybdenum antimony colorimetric method.

Data analysis

Data for soil organic carbon, soil total nitrogen, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ of soil (0–40 cm), total carbon, total nitrogen, water content of leaf and water use efficiency of plant leave were taken from six replicates; data for δD values and $\delta^{18}\text{O}$ values of soil were taken from three replicates, and six replicates for plant. All data of the variables were transformed to their natural logarithms to stabilize heterogeneous variances for statistical analysis. One-way analysis of variance (ANOVA) (SPSS 25.0, Armonk, New York, USA) was used to examine the effects of habitats to variables. Variables were compared by least significance difference to determine whether they were significant at $P < 0.05$. Graphic were created using R4.3.1(R Core Team, R: A Language and Environment for Statistical Computing), Origin 2021(OriginLab Corp., Northampton, Massachusetts, USA), ArcGIS 10.8 (Esri Comp., Redlands, California, USA.), and Adobe Illustrator 2022 (Adobe Inc, San Jose, California, USA).

Results

Trait of plant and soil among habitats

The altitude of the three habitats increases progressively from the riverside to the terrace and then to the mountain slope (Table 1). Soil total nitrogen (TN_{soil}), soil organic carbon content (SOC), soil nitrate nitrogen ($\text{NO}_3\text{-N}$), and ammonium nitrogen ($\text{NH}_4\text{-N}$) were significantly higher at the mountain slope compared to the other two habitats (Fig 2a, b, c, d) ($P < 0.05$). The soil pH value was significantly higher at the riverside habitats than at the other two habitats ($P < 0.05$), with no significant difference in pH values between the terrace and mountain slope habitats (Table 1). The total carbon content in the leaves was significantly higher at the terrace compared to the riverbank and mountain slope ($P < 0.05$) (Fig 3a); The total nitrogen in the leaves was the highest at riverside habitats, the lowest at mountain slope habitats, and differed significantly among three habitats ($P < 0.05$) (Fig 3b). The water content of *C. gigantea* leaves was the lowest at mountain slope habitats compared to the other two habitats ($P < 0.05$), with no significant difference between the riverbanks and terrace habitats (Fig 3c).

The $\delta^{13}\text{C}$ values in *C. gigantea* leaves were significantly higher at the mountain slope compared to the other two habitats ($P < 0.05$) (Table 2). The average $\delta^{13}\text{C}$ value at the mountain slope was -23.4 ± 0.67 , with a range of -24.1%

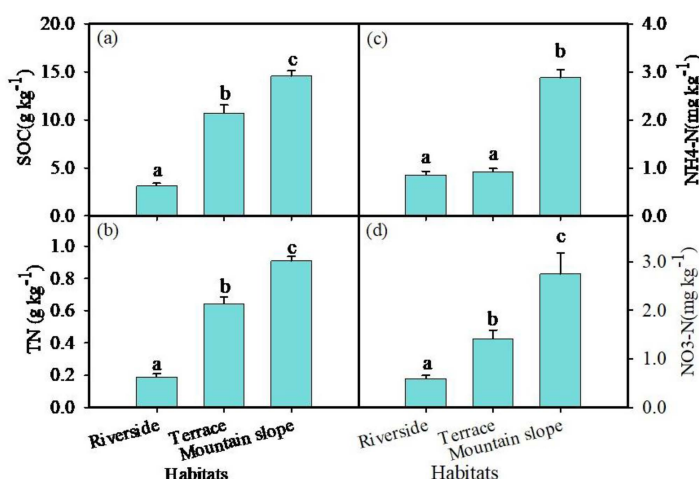


Fig 2. Summary of soil chemical characteristics of soil (0–40 cm) from Riverside, Terrace and Mountain slope carbon in Aug. 2022. (a) organic carbon (SOC). (b) soil total nitrogen (TN). (c) $\text{NH}_4\text{-N}$. (d) $\text{NO}_3\text{-N}$. Values are means of six replicates ($\pm\text{SE}$). Different lowercase letters indicate significant difference among habitats ($P < 0.05$), same letters mean no significant different ($P > 0.05$).

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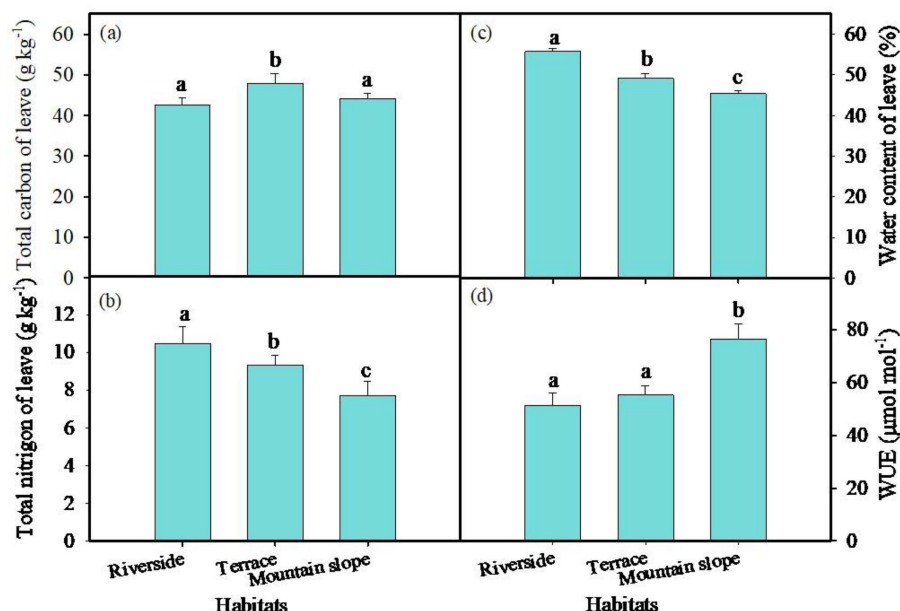


Fig 3. Summary of plant leaf characteristics from Riverside, Terrace and Mountain slope in Aug. 2022. Values are means of six replicates ($\pm$ SE). Different lowercase letters indicate significant difference among habitats ($P < 0.05$), same letters mean no significant different ($P > 0.05$). (a) total carbon (TC_{leaf}). (b) total nitrogen (TN_{leaf}). (c) water content of leaf. (d) water use efficiency (WUE).

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Table 2. Results of linear mixed-effects model analysis (F-values, degree of freedom and significance) testing the effects of growing seasons, habitats, soil layers and their interactions on δD , $\delta^{18}O$ values of soil and plant leaves of *C. gigantea*. Data were performed on $\log_e$ -transformed values. Significant level; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Variables	δD_{soil}	$\delta^{18}O_{soil}$	δD_{plant}	$\delta^{18}O_{plant}$
Seasons	70.73(3, 48)***	23.13(3, 48)***	53.24(3, 60)***	45.15(3, 60)***
Habitats	2.00(2, 48)	0.14(2, 48)	41.78(2, 60)***	38.18(2, 60)***
Soil layers	41.56(1, 48)***	0.33(1, 48)	-----	-----
Season $\times$ habitats	4.18 (6, 48)**	8.68(6, 48)***	11.85(6, 60)***	5.37(6, 60)***
Seasons $\times$ soil layers	23.60(2, 48)***	2.82(3, 48)*	-----	-----
Habitats $\times$ soil layers	7.32(3, 48)**	4.29(2, 48)*	-----	-----
Seasons $\times$ habitats $\times$ soil layers	12.16(6, 48)***	6.84(6, 48)***	-----	-----

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to -22.4‰ . The $\delta^{13}C$ values of the riverside and terrace habitats were $-25.1\text{‰} \pm 0.98$ and $-25.0\text{‰} \pm 1.01$ respectively. Water use efficiency (WUE) was calculated based on the $\delta^{13}C$ value of plant leaves [31,32]. WUE was significantly higher at the mountain slope, with the highest average values of $98.3\text{ }\mu\text{mol mol}^{-1}$ ($P < 0.05$). The WUE of *C. gigantea* was similar at the riverbank and terrace habitats, with an average value of $63.5\text{ }\mu\text{mol mol}^{-1}$ and $64.2\text{ }\mu\text{mol mol}^{-1}$, respectively (Fig 3d). WUE showed a highly positive correlation with soil organic carbon (SOC), soil total nitrogen (TN_{soil}), soil NH_4-N , and soil pH values ($P < 0.01$), and a highly significant negative correlation with leaf water content (LWC) ($P < 0.01$) (Fig 4).

The δD and $\delta^{18}O$ values from different water sources

The δD and $\delta^{18}O$ values of rainfall changed significantly over the experiment period, being most enriched in May 2023, with average value of -46.1‰ and -7.2‰ , respectively. These values were the most impoverished in August, 2022,

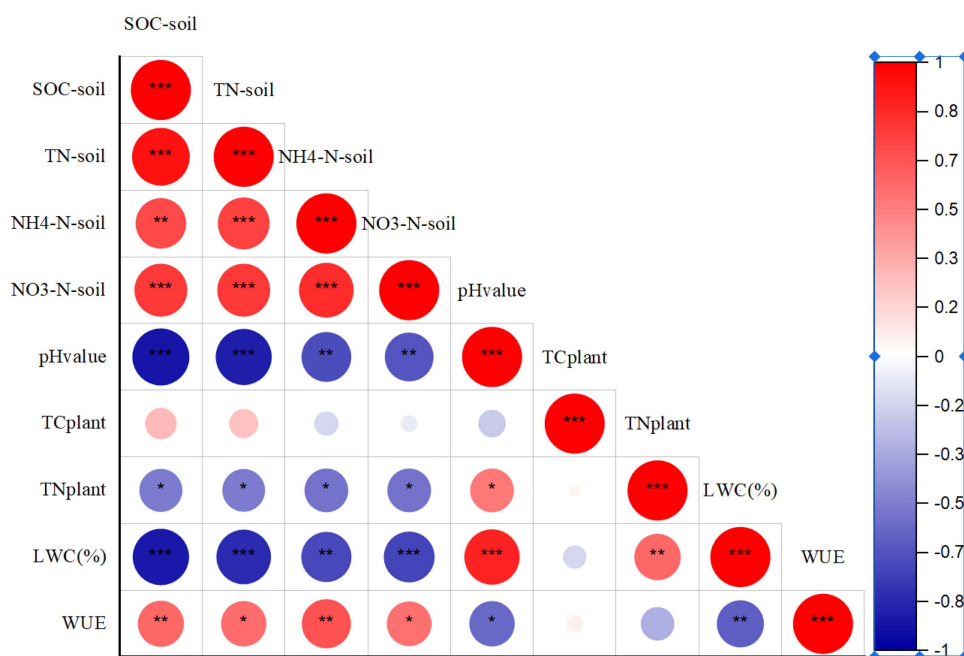


Fig 4. The relationship of water use efficiency of *Cupressus gigantea* in relation to soil organic carbon (SOC), soil total nitrogen (TN_{soil}), NH₄-N, NO₃-N of soil (0–40 cm), total carbon (TC_{plant}), total nitrogen (TN_{plant}), water content of leaf. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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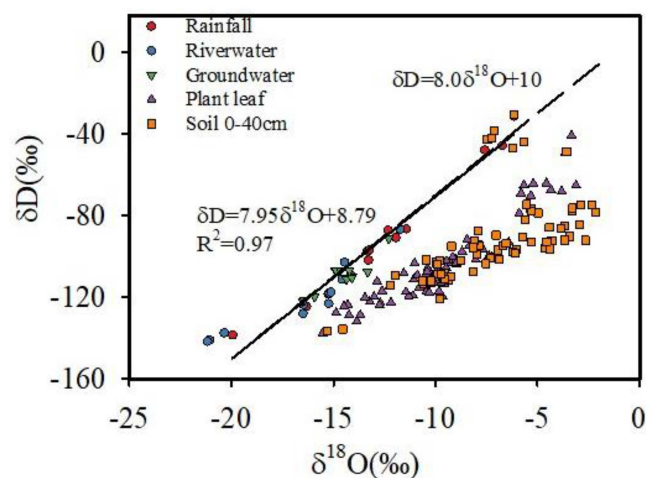


Fig 5. Stable isotopic compositions of rainfall, river water, groundwater, soil water and plant water at the study site. The local meteoric water line is very close to the GMWL whose equation is $\delta D = 8.0 \times \delta^{18}O + 10$.

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with an average value of -103.3‰ and -13.6‰ , respectively (Fig 5). The slope of the Local Meteorological Water Line (LMWL) is slightly lower than that of the Global Atmospheric Waterfall Line (GMWL: $\delta D = 8\delta^{18}O + 10$), which is a more significant trends in arid regions (Fig 5). This is mainly due to the influence of secondary evaporation on the precipitation process, where lighter δD is depleted, and heavier $\delta^{18}O$ is enriched in precipitation. During the study period, the average values of δD and $\delta^{18}O$ in groundwater were -110.1‰ and -14.6‰ respectively, which were distributed slightly lower and

to the right of the atmospheric precipitation line. The average values of δD and $\delta^{18}O$ of the Yarlung Zangbo River water were -121.0‰ and -16.4‰ respectively, which were distributed on both sides of the local atmospheric precipitation line (Fig 5). The average values of soil water δD and $\delta^{18}O$ are -92.5‰ and -7.0‰ , respectively (Fig 5), distributed in the lower right corner of the local atmospheric precipitation line, indicating that precipitation undergoes significant evaporation, enrichment, and fractionation after entering the soil. Due to the influence of evaporation degree and supply source, there are significant differences in the slope and intercept of each water body, but all are smaller than the slope of the regional atmospheric precipitation line, showing varying degrees of deviation. This indicates that each water body is supplied by precipitation (Table 3).

Values OF δD AND $\delta^{18}O$ in xylem water

The δD values of the xylem water of *C. gigantea* at riverside habitats were significantly lower than those of the terrace and mountain slope habitats ($P < 0.05$). However, the values did not change significantly across different seasons, indicating that the water sources of the plants did not switch among seasons in riverside habitats. The average δD and $\delta^{18}O$ values of xylem water were significantly higher in May 2023 than in other seasons, being $-67.3\text{‰} \pm 8.43$ and $-5.9\text{‰} \pm 1.49$ at Terrace habitats, and $65.62\text{‰} \pm 8.50$ and $3.46\text{‰} \pm 1.53$ at Mountain slope habitats (Fig 6a, d). The δD and $\delta^{18}O$ values of the xylem water of the *C. gigantea* at mountain slopes showed significant seasonal variations, with the highest values in May 2023 and the lowest in Aug. 2023 ($P < 0.01$) (Fig 6 a, d). At terrace habitats, the highest value was observed in the early growth season of May 2023, with average δD and $\delta^{18}O$ values of $-67.3\text{‰} \pm 8.43$ and $-5.9\text{‰} \pm 1.49$, respectively. In August 2023, the values were the lowest, being of $-117.7\text{‰} \pm 4.52$ and $-11.19\text{‰} \pm 0.81$ (Fig 6 a, d).

Variation of δD and $\delta^{18}O$ values in different water sources

The average δD and $\delta^{18}O$ values of rainfall in May 2023 were the highest, being -46.1‰ and -7.2‰ , respectively, while they were the lowest in Nov. 2023, being -125.9‰ and 16.7‰ respectively. The change of rainfall among different seasons was quite significantly ($P < 0.001$). The average values of δD and $\delta^{18}O$ in river water in Aug. 2023 were significantly lower than other seasons, being -140.1‰ and -20.9‰ , respectively. However, they were similar between Aug. 2022 and May 2023, Nov. 2023 due to the less rainfall in Aug. 2022. This trend indicates that the changes in the values of δD and $\delta^{18}O$ in the river water displayed a significant trend of impoverishment during the rainy season and gradual enrichment during the non-rainy season. The range of δD and $\delta^{18}O$ value in groundwater are not large, and relatively stable, with average values being -110.1‰ and 14.6‰ , respectively. The average values of δD and $\delta^{18}O$ in groundwater were greater than those of river water, indicating that river water is the main source of groundwater.

The average δD and $\delta^{18}O$ values of soil water at different periods in the study area showed significant differences ($P < 0.05$) (Table 2 and Fig 6b, c, e, f). The δD and $\delta^{18}O$ value of surface soil layer (0–20 cm) at Mountain slope habitats were higher than those at the other two habitats, but no significance differences were observed in the lower soil layer (20–40 cm) (Fig 6b, c, e, f). From a seasonal perspective, the average values of soil water were the lowest in Aug. 2023 and the highest in May 2023.

Table 3. Results of two-way ANOVA analysis (F-values, degree of freedom and significance) of the effects of seasons, habitats and their interactions on leaf water content (LWC), contribution of soil water (0–20 cm) to plant, and (20–40 cm) $\delta^{18}O$ values of soil and plant. Data were performed on $\log_e$ -transformed values. Significant level: * $P < 0.05$; ** $P < 0.01$; * $P < 0.001$.**

Variables	Leave water content	Water contribution of soil layer (0–20 cm)	Water contribution of soil layer (20–40 cm)	Water contribution of ground water	Water contribution of river water
Seasons	14.53(3,60)***	1.99(3,24)	2.23(3,24)	1.50(3,24) ns	2.67(3,24)
Habitats	46.43(2,60)***	27.87(2,24)***	9.49(2,24)**	3.53(2,24)*	32.03(2,24)***
Seasons × habitats	3.44(6,60)**	5.28(6,24)**	0.63(6,24)	1.85(6,24)	3.92(6,24)*

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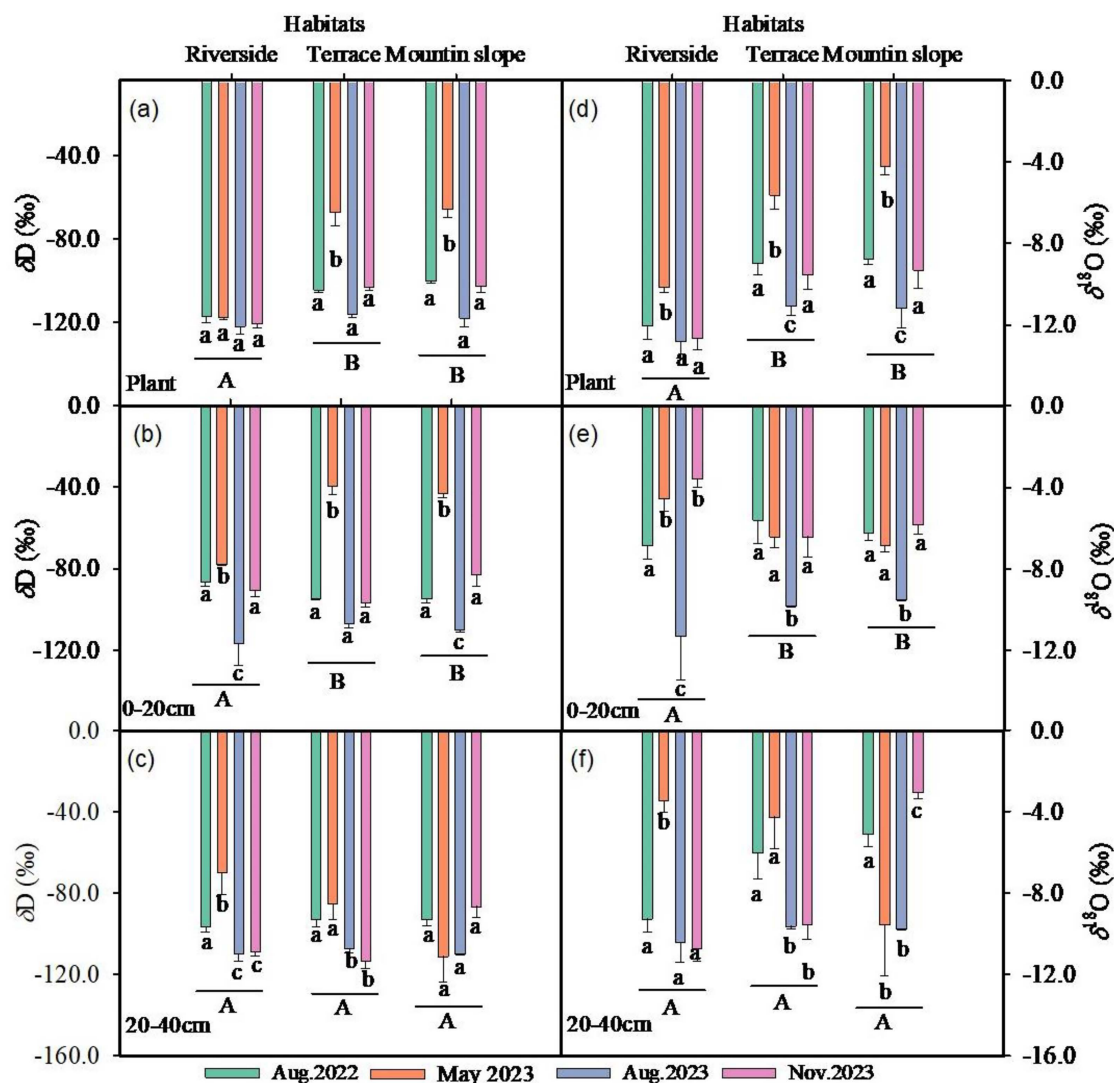


Fig 6. The changes of δD values and $\delta^{18}O$ values of plant (a,d), soil of 0-20 cm layer (b,e), soil of 20-40 cm (c,f) at Riverside, Terrace and Mountain slope habitats in four different sampling period. Values are means of three replicates ($\pm SE$). Different lowercase letters indicate significant difference among habitats ($P < 0.05$), same lowercase letters mean no significant different ($P > 0.05$). Different capital letters mean significant difference among habitats ($P < 0.05$), same capital letters indicate no difference.

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Quantitative analysis of absorption water sources

Using the IsoSource multiple linear mixed model, the contribution rates of potential water sources to *C. gigantea* at different stages in three different habitats of riverside, terrace, and mountain slope were quantified (Fig 7). The results showed significant difference in water sources of *C. gigantea* with the alternation of dry and rainy seasons, and the contribution rates of each potential water source varied significantly among habitats. The utilization ratio of Yarlung Zangbo River water for *C. gigantea* along the riverside was significantly higher than in other habitats except during the rainy season Aug. 2023 (Fig 7a, b, c, d). The utilization ratio of river water for *C. gigantea* at the riverside reached 66.0%, 78.6% and 55.5% in August 2022, May 2023 and November 2023 respectively. In August 2023, the rainfall in the region increased, and the utilization rate of four water sources by *C. gigantea* are similar: 27.7% for upper soil water (0–20 cm), 24.1% for

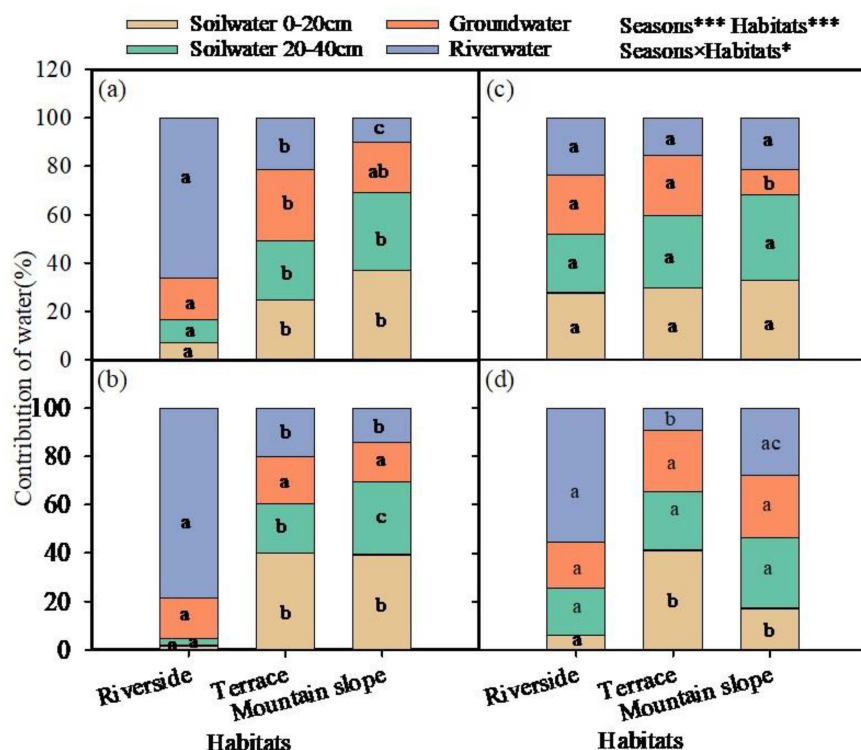


Fig 7. Contribution of four water source, i.e., soil water (0-20 cm), soil water (20-40 cm), river water and ground water to the plant at Riverside, Terrace and Mountain slope habitats of study site in Aug. 2022 (a), May 2023 (b), Aug. 2023 (c) and Nov. 2023 (d). Different lowercase letters indicate significant difference among habitats ($P < 0.05$), same letters mean no difference ($P > 0.05$).

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lower soil water (20–40 cm), 24.7% for groundwater and 23.6% for river water, soil water became the main source of water for *C. gigantea*, with a total contribution of 51.8% (Fig 7). The rainy season in August 2022 was an exception due to extreme drought conditions with low rainfall, and the main source of absorption for the *C. gigantea* was still river water.

The water use of *C. gigantea* in the mountain slope and terrace habitats was relatively stable across different seasons, with soil water as the main source. The utilization rate of river water was significantly lower than that for *C. gigantea* in the riverside areas. The utilization rate of soil water by *C. gigantea* was similar in May, August and November 2023, with utilization ratios exceeding 50%, making it the main source of water in these regions. The lowest utilization ratio of soil water by *C. gigantea* on the mountain slope occurred in November 2023, while the utilization ratio of river water and groundwater increased. In other periods, the utilization ratio of soil water by *C. gigantea* exceeded 60% (Fig 7). The utilization rate of groundwater by *C. gigantea* on the mountain slope was lower in August 2023 than in May, and November 2023 and August 2022 (Fig 7c), likely due to the increased rainfall in August 2023.

Discussion

water use efficiency of *C. gigantea*

Soil, as a carrier for plant survival, exhibits heterogeneous spatial and temporal distribution of its physicochemical properties [33]. As the main distribution center of *C. gigantea*, Lang County has a harsh ecological environment, steep mountains, sandy soil, sparse vegetation. The habitat heterogeneity and fragmentation are severe in the distribution area of *C. gigantea* [34]. One of the fundamental and important characteristics of plants in responding to stressed environments is

their capability to regulate the water use efficiency [35,36]. This enables plants to adapt to changes in soil water availability, thereby enhancing their ability to cope with arid environments [11,37,38]. There are significant differences in the physicochemical properties of soil and *C. gigantea* leaves in different habitats. At mountain slope habitats with high altitude, soil organic carbon, soil nitrate nitrogen, soil ammonium nitrogen, and soil organic carbon were higher than that of terrace and riverside habitats. This may be due to the decrease in atmospheric and soil temperatures with increasing altitude, which makes it difficult for organic matter to decompose, resulting in an increase in surface total nitrogen content [39]; The soil pH value shows a decreasing trend with increasing altitude, and the soil at terrace and mountain slope habitats was closer to neutral than that at the riverbank.

The total carbon content, leaf water content, and total nitrogen content of *C. gigantea* leaves decrease at mountain slope habitats with increasing altitude, and there are significant differences among different habitats. The leaf water content of plants can significantly affect the intensity of photosynthesis and reflect the water and heat conditions of the surrounding habitat. The decrease in water content of *C. gigantea* leaves with increasing altitude suggests that soil moisture conditions in high-altitude areas are slightly lower than those at the riverbank and Terrace. The decrease in total nitrogen content in leaves may be due to the increased total solar radiation and ultraviolet radiation at high altitudes. Plants need to consume their own energy to accumulate resistance to ultraviolet radiation and avoid damage. Additionally, insufficient available soil water affects plant photosynthesis [40–43], while cereal usually can obtain higher biomass and yield in the conditions of high soil nitrogen and water due to intercropping with legumes [44]. The changes in leaf nitrogen content and water content are complementary. Habitat differences such as altitude often cause changes in environmental factors such as light, temperature, and rainfall, which in turn affect plant $\delta^{13}\text{C}$ values and water use efficiency (WUE) [45,46]. The leaves of *C. gigantea* in the mountain slope habitats have the highest $\delta^{13}\text{C}$ value (-22.38%), which is significantly different from those in the other two habitats. The leaves of *C. gigantea* in the riverside habitat have the lowest $\delta^{13}\text{C}$ value (-26.56%). The variation in WUE value of *C. gigantea* among different habitats is consistent with the $\delta^{13}\text{C}$ value. The leaves of *C. gigantea* in the mountain slope habitat have the highest WUE being $107.8 \mu\text{mol mol}^{-1}$, while the leaves in riverside habitats have the lowest WUE being $35.7 \mu\text{mol mol}^{-1}$. This indicates that the water use efficiency of *C. gigantea* leaves has strong resistance in physiology to cope with changes in habitat conditions which quantifies its capacity for water absorption and underlined the understanding of its ecological traits. The difference between the $\delta^{13}\text{C}$ and WUE values of plant leaves is influenced by various factors such as temperature, light intensity, and water content in the environment which reflecting in characteristics of mountain slope, terrace habitats and riverside area. Plants are capable of perceive external environmental changes and regulate their physiological morphology, such as reducing stomatal conductance [47], which affects the $\delta^{13}\text{C}$ value of plant leaves, thereby regulating WUE to adapt to environmental changes [48,49]. Relevant research shows that the leaf $\delta^{13}\text{C}$ value of *Q. aquifolioides* rises with the elevation above 2800 m in the eastern slope of the Himalayas, Tibet, which is similar to the plateau environment of the current study [50]. Additionally, studies on two species, *Hippophae tibetana* and *Populus euphratica* also showed that the $\delta^{13}\text{C}$ value of plants increased with the elevation in Xinjiang [51,52]. The change of $\delta^{13}\text{C}$ and WUE values of *C. gigantea* may be due to the high altitude of the mountain slope habitat, which is far from the Yarlung Zangbo River. The gradient of the land is relatively steep, making it difficult for *C. gigantea* to obtain water, which may lead to an improvement in WUE by adjusting stomatal conductance.

Source of water absorption by *C. gigantea*

The contribution rate of potential water source to *C. gigantea* varies with seasons during the study period. Along the Yarlung Zangbo Riverside, *C. gigantea* primarily absorbed river water during dry seasons with little rain, but switched to soil water when there was enough rainfall in August 2023. However, in August 2022, river water remained the main source of absorption due to less rainfall that year. In the terrace and mountain slope habitats, *C. gigantea* mainly absorbs soil water, accounting for about 60%. There was no obvious pattern in the utilization of water from the upper and lower layers of soil by *C. gigantea* across different seasons. An exception occurred in the terrace habitat, where the proportion of groundwater absorption

increased and the proportion of soil water decreased in August 2022. In the mountain slope habitat, in November 2023, the proportion of *C. gigantea* absorbing soil water decreased, while the proportion of river water and groundwater absorption increased. There may be two possible reasons for these observations: (1) In terrace habitat, the increased absorption rate of groundwater in August 2022 may be due to the drought of that year, leading to low soil moisture content and water redistribution in the roots of *C. gigantea*. The relatively thick soil layer in terrace habitats compared to slopes and riverbanks may allow deeper root system to absorb groundwater, and redistributing water within the plant. Further research is needed to confirm this. 2) In the mountain slope, *C. gigantea* still primarily absorbed soil water, accounting for more than 60%, which may be related to the high WUE of *C. gigantea* in these the habitats. Precipitation is usually the main source of soil moisture replenishment [53,54]. From this point, it can be deduced that the source of water absorption by plants is closely related to their habitat. When the soil moisture content is high, *C. gigantea* will switch to absorbing and utilizing soil water. However, long-term monitoring is needed to determine this relationship definitely, as soil moisture is an important medium connecting vegetation and hydrological processes, and is also a key factor limiting vegetation growth in arid areas [55].

Conclusion

Overall, the values of TN_{soil} , SOC, NO_3-N and NH_4-N of soil were significantly higher in mountain slope habitats compared to riverside and terrace, while the pH value of mountain slope soil was significantly lower than that of other two habitats. The total nitrogen content and water content of leaves in riverside habitats were significantly higher than those in terrace and mountain slope habitats, while the water use efficiency of leaves were the highest in mountain slope habitats. This study indicated that the main water source for *C. gigantea* in the distribution area is soil water in terrace and mountain slope habitats, while *C. gigantea* mainly utilized river water at riverside habitats. This study found that habitat affects water physiology of *C. gigantea*. In future, research about exploring how habitat effecting on the photosynthetic efficiency of *C. gigantea*, as well as its seedling survival and establishment at different habitats. This study provides scientific data to support the conservation of *C. gigantea* by improving soil nutrient and water content.

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Writing – review & editing: Meizhen Liu, Li Luan, Youlu Zuo, Shengyun Liu, Qiaoling He, Jingyu He, Ruru Wu, Gaoming Jiang.

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