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Stable carbon isotope composition and intrinsic water use efficiency in plants along an altitudinal gradient on the eastern slope of Yulong Snow Mountain, China

Feng Jiang[1], Tao Pu[2], Zi-Jing Xue[3], Yan-Wei Ma[1], Xiao-Yi Shi[1,2*] and Fu-Xi Shi[3*]

Abstract

Background Stable carbon isotope composition ($\delta^{13}C_p$) can be used to estimate the changes in intrinsic water use efficiency (iWUE) in plants, which helps us to better understand plants' response strategies to climate change. This study focused on the variations in $\delta^{13}C_p$ and iWUE for the different life-form plants (i.e., herbs, shrubs, and trees) along an altitudinal gradient (3300, 3600, 3900, 4100, 4300, and 4500 m) on the eastern slope of Yulong Snow Mountain, southeastern margin of the Qinghai-Tibet Plateau. The response mechanisms of $\delta^{13}C_p$ and iWUE for different life-form plants to altitude were thoroughly analyzed in this mountain ecosystem.

Results The $\delta^{13}C_p$ values of plants on the eastern slopes of Yulong Snow Mountain ranged from $-30.4‰$ to $-26.55‰$, with a mean of $-28.02‰$, indicating a dominance of C_3 plants. The $\delta^{13}C_p$ and iWUE values varied among different life-form plants in the order of herbs > shrubs > trees, particularly in 3600, 3900, and 4300 m. The $\delta^{13}C_p$ and iWUE values for herbs and shrubs increased with altitude and were mainly controlled by air temperature. The two parameters for trees exhibited a trend of initial decrease followed by an increase with altitude. Below 3900 m, the $\delta^{13}C_p$ and iWUE values decreased with altitude, influenced by soil moisture. However, above 3900 m, the two parameters increased with altitude, mainly regulated by air temperature. In addition, iWUE was positively correlated with leaf P content but negatively correlated with leaf N:P ratio, especially for herbs and trees, suggesting that P plays a key role in modulating iWUE in this region.

Conclusions The differentiated responses of water availability for different life-form plants to a higher altitudinal gradient are regulated by air temperature, soil moisture, and leaf P content in the Yulong Snow Mountain. These results provide valuable insights into understanding the water–carbon relationships in high-altitude ecosystems.

Keywords Stable carbon isotope, Water use efficiency, Altitude, Life form, Yulong Snow Mountain

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Background

With global climate warming, there have been significant changes in factors controlling plant growth, such as rising atmospheric CO_2 concentrations (Rahman et al. 2019), snow-ice meltwater (Geyman et al. 2022; Cai et al. 2021), and changes in global rainfall patterns (Chen et al. 2021). The changes have had a profound impact on plant growth and physiological behavior (Rahman et al. 2019). Water use efficiency (WUE) is a key physiological parameter that can be used to assess plant photosynthetic capacity and the trade-off between CO_2 fixation and water loss (Ma et al. 2021; Lavergne et al. 2019). Leaf-scale intrinsic WUE (iWUE) is defined as the net photosynthetic rate (A) divided by the stomatal conductance to water vapor (g_s) (Driscoll et al. 2020). Consequently, plants can adjust stomatal conductance and CO_2 assimilation rate to alter their iWUE in response to environmental factors such as water availability and temperature (Lin et al. 2023; Lawson and Vialet-Chabrand 2019; Yi et al. 2019). For example, Farquhar et al. (1982) proposed that the stable carbon isotope composition of C_3 plants could provide information on plant iWUE, where a more positive carbon isotope composition corresponds to a higher iWUE (Gong et al. 2022; Vantyghem et al. 2022; Wu et al. 2022; Hussain et al. 2019; Yin et al. 2008). Leaf $\delta^{13}C_p$ is primarily related to the ratio of intercellular (C_i) to atmospheric (C_a) partial pressure of CO_2 (C_i/C_a; Farquhar et al. 1989). When the C_i/C_a ratio remains relatively constant, iWUE increases proportionally with a rise in C_a (Belmecheri et al. 2021; Ma et al. 2021). Therefore, a decrease in stomatal conductance usually leads to an increase in leaf $\delta^{13}C_p$ and iWUE because stomatal conductance limits CO_2 assimilation (Ponton et al. 2006; Farquhar and Sharkey 1982).

Variations in leaf-scale $\delta^{13}C_p$ and iWUE can be used to study plant responses to environmental change (Hussain et al. 2019). Generally, the two parameters can vary widely with altitude and other environmental factors (Kong et al. 2017). Altitudinal changes can substantially alter climatic conditions such as temperature, atmospheric pressure, and precipitation (Zhu et al. 2023; Hultine and Marshall 2000), which can affect plant $\delta^{13}C_p$ and iWUE (Rumman et al. 2018; Wu et al. 2015; Jung et al. 2014). For instance, Zhou et al. (2013) reported the leaf $\delta^{13}C_p$ was positively corrected with altitude, while it was negatively corrected with atmospheric pressure in the Qinghai-Tibet Plateau. This may be due to the decrease in partial pressure of CO_2 as the air pressure decreases with increasing altitude, resulting in a lower concentration of CO_2 in the atmosphere. In addition, altitudinal changes affect the redistribution of temperature and moisture conditions (Du et al. 2022), making the effects of differences in hydrothermal conditions on plant $\delta^{13}C_p$ and iWUE more complex. For example, Pu et al. (2021) found that the higher the elevation, the higher the iWUE. This is because stomatal closure decreases C_i/C_a in cold regions, leading to an increase in leaf $\delta^{13}C_p$ (Lin et al. 2023; Camarero et al. 2021; Ponton et al. 2006). Conversely, McDowell et al. (2009) observed a decrease in $\delta^{13}C_p$ with an increase in altitude in semi-arid regions. This was attributed to the increase in precipitation at high altitudes, which improved the discrimination between ^{13}C and ^{12}C in plant leaves, leading to a decrease in $\delta^{13}C_p$ values. Moreover, nutrient status, such as nitrogen (N) and phosphorus (P) in leaves can affect $\delta^{13}C_p$ and iWUE by influencing leaf photosynthesis (Huang et al. 2023; Rumman et al. 2018). Nonetheless, the variations in leaf-scale $\delta^{13}C_p$ and iWUE at different altitudes remain uncertain. Investigating whether differences in water and heat conditions as well as leaf N and P contents affect variations in $\delta^{13}C_p$ and iWUE significantly for plants with different life forms along altitudes is crucial to deepen the understanding of carbon–water coupling processes in mountain ecosystems.

Yulong Snow Mountain is located on the southeastern margin of the Qinghai-Tibet Plateau and belongs to a typical monsoon temperate glacier region, exhibiting high sensitivity to climate change. Seven main vertical zonal vegetation types make the area an ideal place to study the water–carbon coupling process (Chang 2014). The main vertical zonal vegetation types could be categorized into coniferous forests, evergreen broad-leaved forests, thickets, meadows, and fluvial rocky beach vegetation (Yang et al. 2008). The rapid retreat of temperate glaciers directly affects soil hydrothermal properties and various environmental factors. It is a representative site for the response mechanism of $\delta^{13}C_p$ and iWUE to altitudinal gradients for different life-form plants. In the present study, the effects of different habitat conditions induced by altitude on leaf-scale iWUE values were analyzed based on the stable carbon isotope technique. Our objectives were (1) to determine the distribution pattern of $\delta^{13}C_p$ on the eastern slope of Yulong Snow Mountain; (2) to analyze the difference in $\delta^{13}C_p$ and iWUE among different life-form plants; (3) to reveal spatial variations of $\delta^{13}C_p$ and iWUE with altitudes; and (4) to explore the factors controlling iWUE for different life-form plants across the altitude gradient. The findings of the present study could enhance our understanding of plant metabolism and the responses of the water–carbon coupled process of a typical temperate glacial area.

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Methods

Study area

Yulong Snow Mountain (26°59′–27°17′N, 100°4′–100°15′E) is located on the southeastern edge of the Qinghai-Tibet Plateau (Fig. 1), the southern edge of the Hengduan Mountain, is China's most southerly modern glacier distribution area (He et al. 2016). Yulong Snow Mountain falls within the south temperate zone of the plateau monsoon climate, characterized by a unique mountainous monsoon climate. Due to significant altitude differences from the valley to the mountain summit, there are distinct vertical climate variations, encompassing cold, temperate, and warm zones with high humidity, abundant sunshine, and ample ultraviolet radiation (Yang et al. 2008). The area features diverse vegetation across different elevations, with elevations ranging from 2000 to 2900 m characterized by Yunnan pine forests and slope cultivation zones. Elevations between 2900 and 3100 m feature high-mountain pine and oak needle-broadleaf mixed forests. The zone between 3100 and 3800 m constitutes a subalpine coniferous forest region, consisting of fir, redwood, and spruce forests (either pure or mixed). The altitude range of 3800 to 4500 m delineates the alpine scrub meadow belt. Below 4100 m, lies the alpine scrub meadow, predominantly characterized by diverse species of cushion or creeping rhododendron. Between altitudes of 4100 and 4350 m, only sporadic occurrences of alpine flowstone beach plants are observed (Guo et al. 2009).

Sampling design and measurements

In late July 2022, three survey plots of 20 m×20 m were set up along the east slope of Yulong Snow Mountain at altitudes of 3300, 3600, 3900, 4100, 4300, and 4500 m (i.e., a total of 18 plots), and different dominant plants were sampled in each plot, which could be classified into three different life-forms, namely, trees, shrubs, and herbs (Additional file 1: Table S1). For different life-form plants, plants of similar height or diameter at breast height to the respective plants with good growth were taken as each target plant; sunny, healthy, and mature leaves for the year were collected from each plant, and the same number of leaves were collected from each plant for each species depending on the number of leaves to be mixed as a sample of that plant species. Upon returning to the laboratory, collected leaf samples were placed in an oven and subjected to dehydration at 105 °C for 15 min, followed by drying at 60 °C to a constant weight. Each dried plant sample was then pulverized using an onyx mortar and fully automated ball mill and passed through a 100-mesh sieve as the product to be tested. At the same time, soil samples with a diameter of 5 cm from 0 to 30 cm were collected using a soil drill, and the soil moisture contents were measured using the oven drying method, which were calculated using the following formula:

$$\text{Soil moisture content (\%)} = (w_1 - w_2)/w_2 \times 100\% \tag{1}$$

where w_1 is soil wet weight; w_2 is soil dry weight.
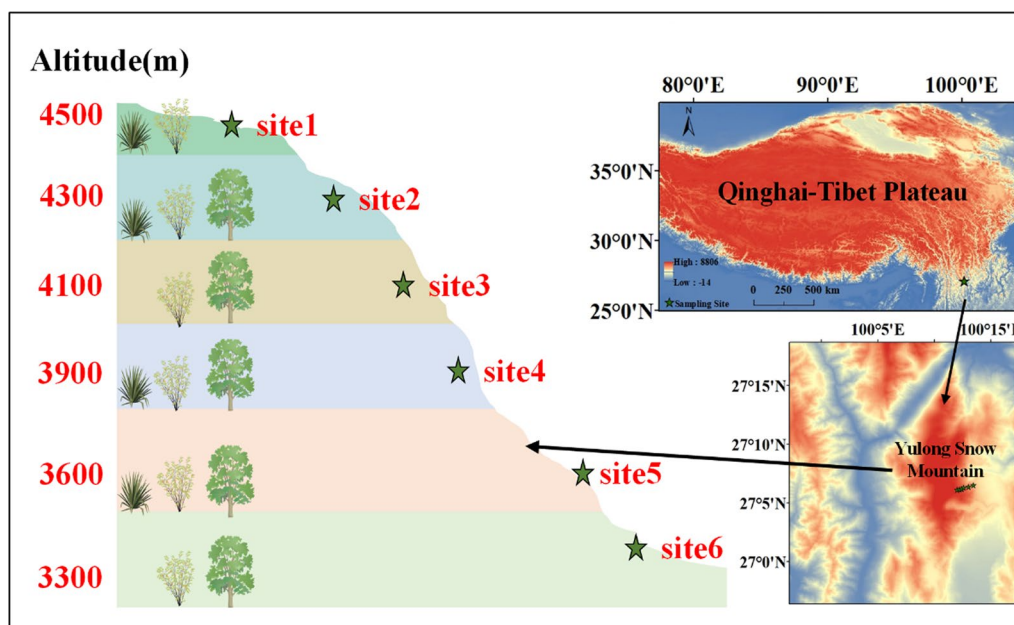
Fig. 1 Sampling sites on the eastern slope of Yulong Snow Mountain

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The temperature data for Lijiang City at 2400 m were obtained from the National Meteorological Data Centre (http://data.cma.cn/). According to Xin's (2016) findings, the direct decrease rate of temperature on the eastern slope of Yulong Snow Mountain at different altitudes in July varies as follows: the direct decrease rate of temperature between 2400 and 3046 m is 0.67 ℃/100 m; The direct decrease rate of temperature between an altitude of 3046–4300 m is 0.53 ℃/100 m; The direct decrease rate of temperature between 4300 and 4800 m above sea level is 0.48 ℃/100 m. Based on the average values of temperatures from 1979 to 2023 at 2400 m, the July temperatures at different altitudes of Yulong Snow Mountain can be obtained by using the interpolation method (Additional file 1: Table S2).

Plant powder sample of 0.06–0.09 mg was weighed and wrapped in a pre-ignited tin cup, and an isotope ratio mass spectrometer (IRMS) (Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany) coupled with an elemental analyzer (Flash EA 2000 HT, Thermo Fisher Scientific, Bremen, Germany) was used to determine plant leaf stable carbon isotope abundance $\delta^{13}C_p$ values, which were calculated using the following formula:

$$\delta^{13}C_p(‰) = \left(R_{sample} - R_{standard}\right) / R_{standard} \times 1000‰ \tag{2}$$

where R_{sample} is the relative abundance of the sample; $R_{standard}$ is the relative abundance of $^{13}C/^{12}C$ in the international common standard substance VPDB (Vienna Pee Dee Belemnite). The international standard substances cellulose (IAEA-CH3; −24.724‰), L-glutamic acid (USGS40; −26.39‰), and the laboratory secondary standard substance urea ($CO(NH_2)_2$; −41.30‰) were also used to calibrate the samples and instrumental operating conditions, and the precision of the measurements was < 0.03‰.

Calculation of intrinsic water use efficiency (iWUE)

Plant intrinsic water use efficiency (iWUE, $\mu mol·mol^{-1}$) can be estimated from the carbon isotope discrimination value $\Delta^{13}C$ and the atmospheric CO_2 concentration C_a, and can be expressed as (Farquhar and Richards 1984):

$$iWUE = \left[C_a(b - \Delta^{13}C)\right] / 1.6(b - a) \tag{3}$$

where $a = 4.4‰$, representing the diffusive fractionation coefficient of $^{13}CO_2$ as it passes through the stomata; $b = 27‰$, the fractionation coefficient during carboxylation of $^{13}CO_2$ by Rubisco enzyme; $\Delta^{13}C$ is the stable carbon isotope discrimination of the plant, as calculated in Eq. (4); and C_a is the atmospheric CO_2 concentration ($\mu mol·mol^{-1}$), as calculated in Eq. (5); 1.6 is the diffusion ratio of water vapor and CO_2 in air.

Stable carbon isotope discrimination ($\Delta^{13}C$) was calculated as (Farquhar et al. 1982):

$$\Delta^{13}C = (\delta^{13}C_a - {}^{\delta 13}C_p) / \left[\left(1 + \delta^{13}C_p\right)/1000\right] \tag{4}$$

where $\delta^{13}C_p$ and $\delta^{13}C_a$ are the carbon isotope ratios of plant leaves and atmospheric CO_2, respectively.

Atmospheric CO_2 concentration (C_a) and carbon isotope ratios ($\delta^{13}C_a$) were calculated according to the following equations, respectively (Driscoll et al. 2020; Feng 1998):

$$C_a = 277.78 + 1.350 \exp[0.01572(t - 1740)] \tag{5}$$

$$\delta^{13}C_a = -6.429 - 0.006 \exp[0.0217(t - 1740)] \tag{6}$$

where t is the sampling year, in this study $t = 2022$, substituting into Eq. (5) and (6), C_a was calculated to be 391.43 $\mu mol·mol^{-1}$ and $\delta^{13}C_a$ was −9.16‰.

Additionally, C_i/C_a is calculated according to the following formula (Farquhar et al. 1982):

$$C_i/C_a = (\Delta^{13}C - a)/(b - a) \tag{7}$$

### Statistical analyses

Univariate analysis of variance (ANOVA) was employed to assess differences in $\delta^{13}C_p$ values, C_i/C_a, and iWUE of plants with various life forms. The effects of elevation, life forms, and their interactions on $\delta^{13}C_p$ values and iWUE were investigated using two-way ANOVA. Regression analysis was conducted to correlate $\delta^{13}C_p$ values and iWUE of different life forms with altitude gradients. Pearson's correlation coefficient was calculated to assess the relationships between the variables, and the level of significance was set at 0.05. All statistical analyses of the experimental data were carried out using IBM SPSS Statistics 26 and Excel 2013, while figures were generated using Origin 2023.

Results

Distribution pattern of $\delta^{13}C_p$ in plant leaves

In this study, a total of 128 samples from different life forms were collected at six altitudes on the eastern slope of Yulong Snow Mountain. The samples comprised 36 herb samples, 48 shrub samples, and 44 tree samples. The distribution of leaf $\delta^{13}C_p$ values is shown in Fig. 2. The results showed that the leaf $\delta^{13}C_p$ values fluctuated between −30.4‰ and −26.55‰, with a mean value of −28.02‰. Most leaf $\delta^{13}C_p$ points were

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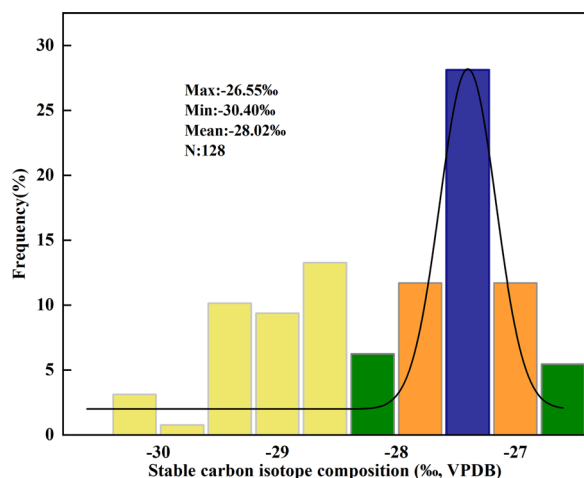
Fig. 2 Distribution of the stable carbon isotope composition in the Yulong Snow Mountain

located in the range of -29.5‰ to -27‰, accounting for 90.63% of the samples. The maximum $\delta^{13}C_p$ value across all samples was -26.55‰, which was observed at 4500 m (herbs). The minimum $\delta^{13}C_p$ value was -30.40‰, occurring at 3900 m (trees).

Differences in leaf-scale $\delta^{13}C_p$, C_i/C_a, and iWUE among different life forms

Significant differences in the leaf-scale $\delta^{13}C_p$, C_i/C_a, and iWUE values were observed among different life forms in the Yulong Snow Mountain region (Fig. 3, $P < 0.05$). The mean $\delta^{13}C_p$ value for herbs was -27.27 ± 0.45‰, with a C_i/C_a mean of 0.63 ± 0.02 and an iWUE mean of 90.69 ± 5.11 μmol·mol^{-1}. For shrubs, the mean $\delta^{13}C_p$ value was -28.20 ± 0.91‰, the C_i/C_a mean was 0.67 ± 0.04, and the iWUE mean was 80.13 ± 10.36 μmol·mol^{-1}. Trees had a mean $\delta^{13}C_p$ value of -28.43 ± 0.87‰, a C_i/C_a mean of 0.68 ± 0.04, and an iWUE mean of 77.58 ± 9.91 μmol·mol^{-1}. According to the multiple comparison analysis, significant differences were found in the $\delta^{13}C_p$ and iWUE among herbs, shrubs, and trees (Fig. 3a, b and c, $P < 0.05$). Both $\delta^{13}C_p$ values and iWUE values followed the order of herbs > shrubs > trees.

To further validate these findings, the present study selected three altitudinal gradients, that is, 3600 m, 3900 m, and 4300 m, where three life forms coexist simultaneously. As shown in Fig. 3d, e, and f, the mean $\delta^{13}C_p$ values for herbs, shrubs, and trees at 3600 m were -28.18 ± 0.09‰, -29.07 ± 0.14‰, and -29.01 ± 0.34‰, respectively. The C_i/C_a values were 0.67 ± 0.01, 0.71 ± 0.01, and 0.71 ± 0.02. The iWUE values were 80.34 ± 0.98 μmol·mol^{-1}, 70.23 ± 1.64 μmol·mol^{-1}, and 70.89 ± 3.92 μmol·mol^{-1}, respectively. Significant differences were observed in $\delta^{13}C_p$ values, C_i/C_a, and iWUE

between shrubs and trees ($P < 0.05$). At an altitude of 3900 m (Fig. 3g, h and i), the mean $\delta^{13}C_p$ values for herbs, shrubs, and trees were -27.06 ± 0.09‰, -29.22 ± 0.11‰, and -29.35 ± 0.95‰, respectively. The C_i/C_a values were 0.62 ± 0.01, 0.72 ± 0.01, and 0.73 ± 0.04. The iWUE values were 93.10 ± 1.00 μmol·mol^{-1}, 68.59 ± 1.21 μmol·mol^{-1}, and 67.05 ± 10.76 μmol·mol^{-1}, respectively. No significant differences were observed in the $\delta^{13}C_p$ values, C_i/C_a, and iWUE values for herbs, shrubs, and trees ($P > 0.05$). At an altitude of 4300 m (Fig. 3j, k and l), the mean $\delta^{13}C_p$ values for herbs, shrubs, and trees were -27.17 ± 0.29‰, -27.45 ± 0.59‰, and -27.92 ± 0.62‰, respectively. The C_i/C_a values were 0.62 ± 0.01, 0.64 ± 0.03, and 0.66 ± 0.03. The iWUE values were 91.82 ± 3.44 μmol·mol^{-1}, 88.66 ± 7.08 μmol·mol^{-1}, and 83.31 ± 7.48 μmol·mol^{-1}, respectively. There were significant differences in the $\delta^{13}C_p$ values, C_i/C_a, and iWUE for herbs, shrubs, and trees ($P < 0.01$). The results indicate that different life forms at the same altitudinal gradient follow a general trend, with $\delta^{13}C_p$ and iWUE values showing the pattern: herbs > shrubs > trees.

Changes in leaf-scale $\delta^{13}C_p$, C_i/C_a, and iWUE across the altitude gradient

For the herbs, there was a significant positive correlation between leaf-scale $\delta^{13}C_p$ and iWUE with altitude ($P < 0.01$). Meanwhile, C_i/C_a showed a negative correlation with altitude ($P < 0.01$). With increasing altitude, $\delta^{13}C_p$ and iWUE exhibited an increasing trend, with an average increase of 0.11‰ in $\delta^{13}C_p$ and 1.28 μmol·mol^{-1} in iWUE for each 100 m rise (Fig. 4a, d and g). Similarly, there was a positive correlation for shrubs between leaf $\delta^{13}C_p$ and iWUE with altitude ($P < 0.01$). Meanwhile, C_i/C_a showed a negative correlation with altitude ($P < 0.01$). As altitude increased, $\delta^{13}C_p$ and iWUE showed an increasing trend, with an average increase of 0.03‰ in $\delta^{13}C_p$ and 0.34 μmol·mol^{-1} in iWUE for every 100 m rise (Fig. 4b, e and h). For trees, the leaf $\delta^{13}C_p$ and iWUE values exhibited a parabolic trend with altitude, initially decreasing and then increasing. At an altitude of 3900 m, $\delta^{13}C_p$ and iWUE values began to decrease until they reached the lowest values at 3900 m. Subsequently, both $\delta^{13}C_p$ and iWUE values gradually increased, reaching their maximum at 4300 m (Fig. 4c, f and i).

Factors controlling variation in leaf-scale iWUE

As altitude increases, the soil moisture content increases, but air temperature decreases (Additional file 1: Table S2 and Fig. 5a). Previous studies showed approximately 90% of the rainfall occurs during the monsoon season, and a large amount of moisture is transported by the prevailing south-west and south-east monsoon from the Indian and

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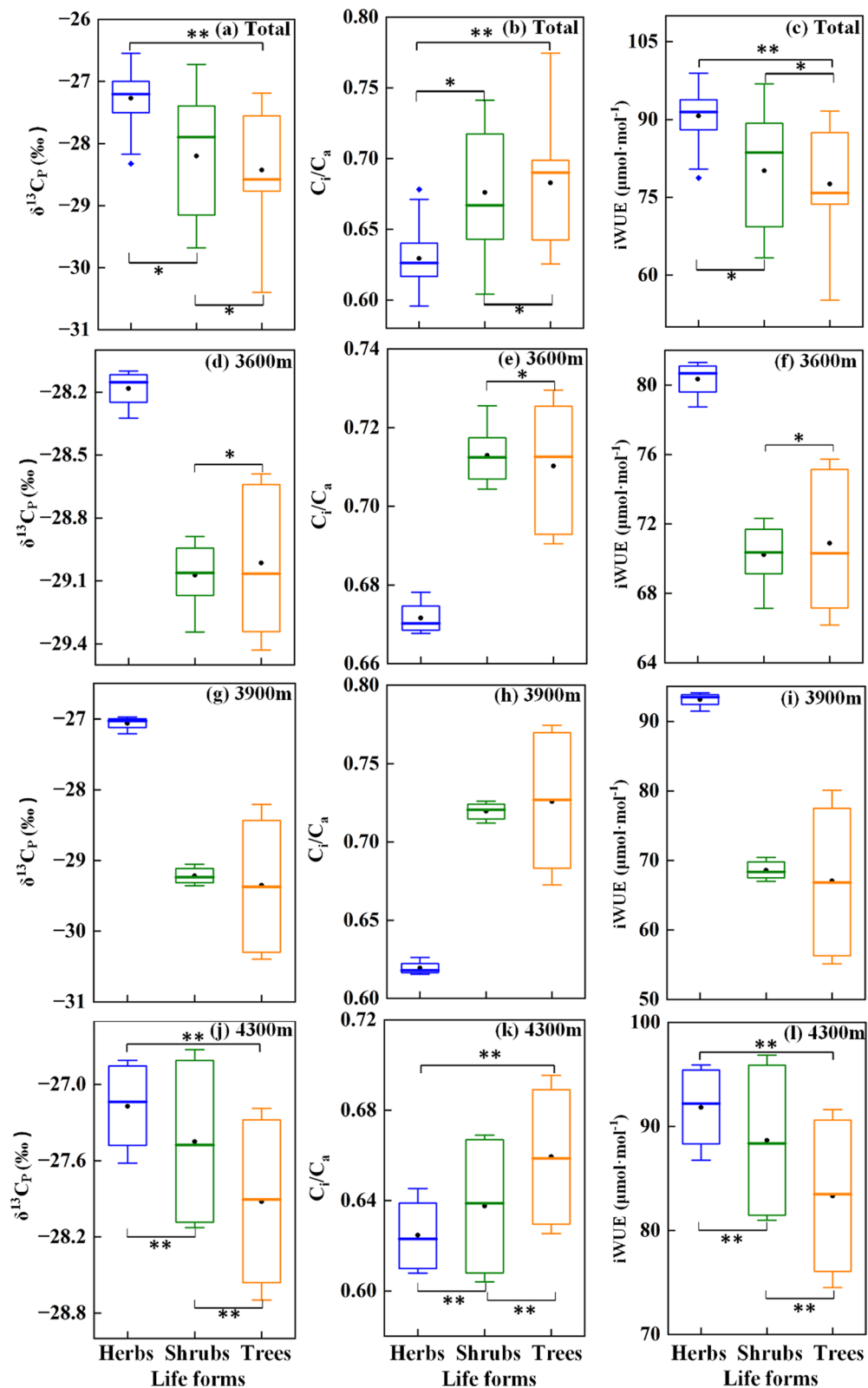
Fig. 3 Differences in leaf-scale $\delta^{13}C_p$, C_i/C_a, and iWUE values for different life forms in the Yulong Snow Mountain. Different lowercase letters indicate significant differences, * indicates $P < 0.05$, and ** indicates $P < 0.01$
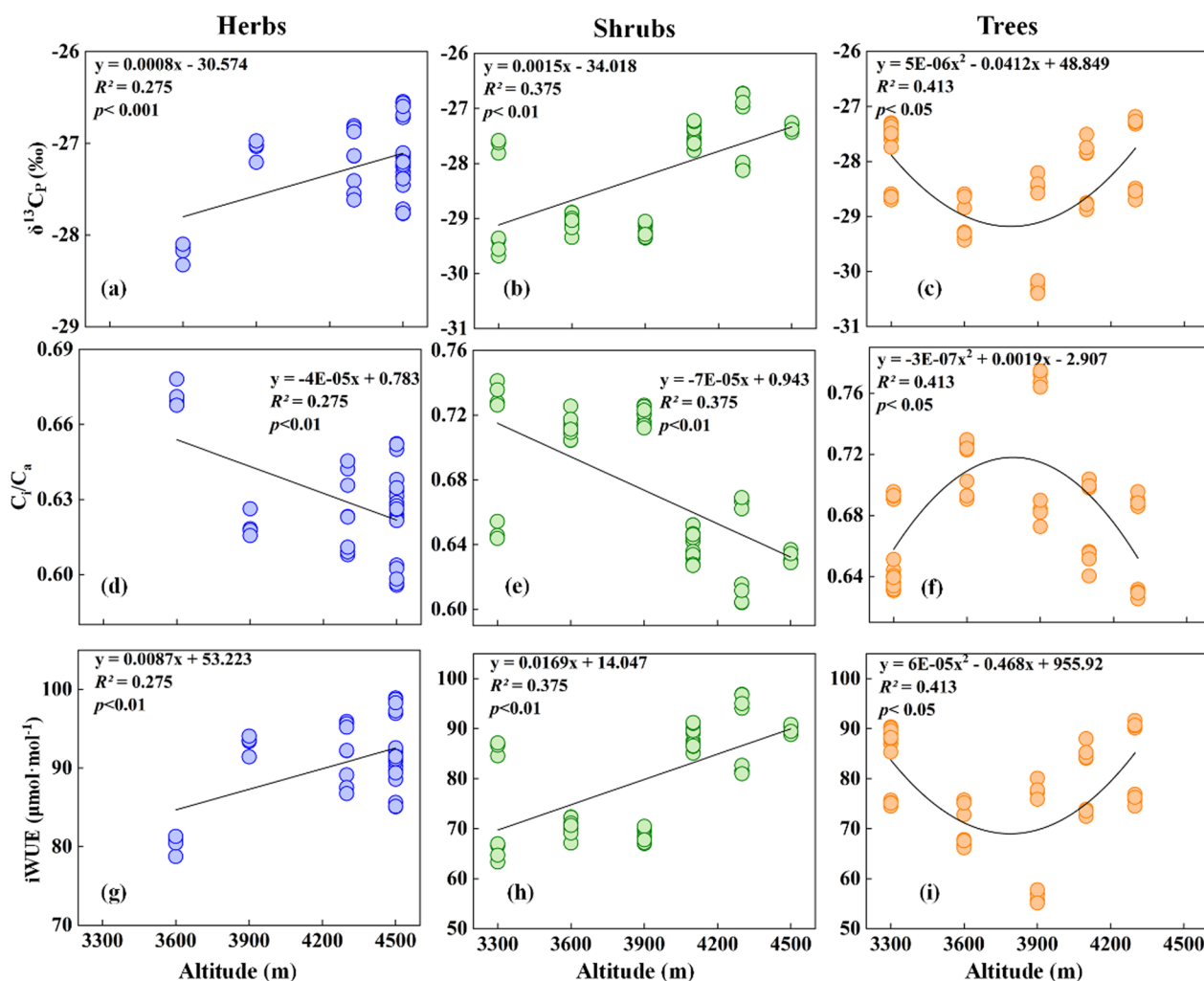
Fig. 4 Variations in leaf-scale $\delta^{13}C_p$, C_i/C_a, and iWUE values along an altitude gradient in the Yulong Snow Mountain

Pacific Oceans (Niu et al. 2019). Thus, the soil moisture content was the highest (203%) at 4100 m, and the lowest (115%) at 3300 m due to the heavy rainfall in July (sampling period). It could be found that iWUE significantly negatively correlated with air temperature for herbs and shrubs ($P<0.01$). With decreasing air temperature, iWUE gradually increased for the two life forms (Fig. 5b and c). However, the iWUE for trees at <3900 m decreased with increasing soil moisture content (Fig. 5d; $P<0.01$), but increased with decreasing air temperature at >3900 m (Fig. 5e, $P<0.01$).

Leaf-scale iWUE in relation to leaf nutrients

There was no significant correlation between iWUE and leaf N content for herbs, shrubs, and trees (Fig 6a, d, and g, $P > 0.05$). A significant positive correlation was observed between iWUE and leaf P content for herbs

and trees ($P < 0.01$; Fig 6b and h), while the correlation between iWUE and leaf P content was not significant for shrubs (Fig 6e, $P > 0.05$), Additionally, a significant negative correlation was observed between iWUE and leaf N:P ratio for herbs and trees ($P < 0.01$; Fig 6c and i), while the correlation between iWUE and leaf N:P ratio was not significant for shrubs (Fig 6f, $P > 0.05$).

Discussion

Distribution pattern and influence of life forms on $\delta^{13}C_p$ and iWUE values

Terrestrial plants can be categorized into C_3, C_4, and CAM plants according to photosynthetic pathways, and there are obvious differences in leaf-scale $\delta^{13}C_p$ values for plants with different photosynthetic pathways. Due to the differences in photosynthetic carboxylase and carboxylation spatiotemporally, the recognition and rejection of ^{13}C vary, resulting in different fractionations.

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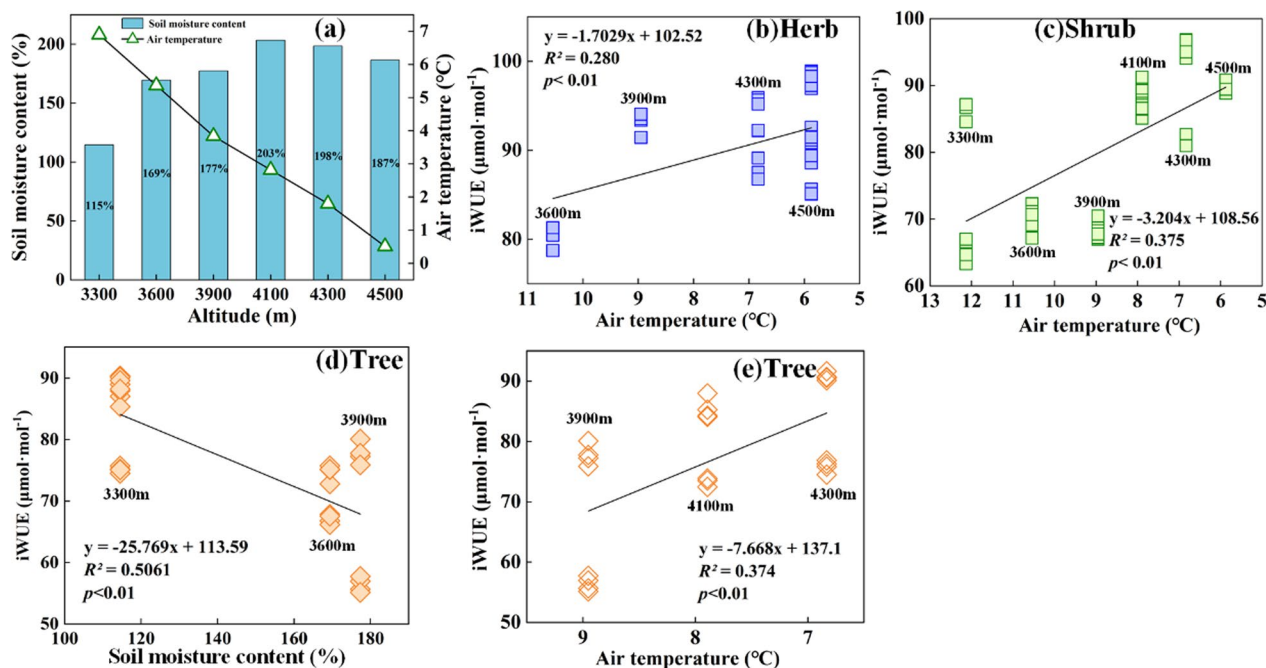
Fig. 5 The relationships of the iWUE with soil moisture content and air temperature for different life forms in the Yulong Snow Mountain

Therefore, the $\delta^{13}C_p$ values of plants with different photosynthetic pathways are significantly different (Farquhar et al. 1989). In the present study, the leaf-scale $\delta^{13}C_p$ from 128 plant samples was $-30.4‰$ to $-26.55‰$. Therefore, the plants are typical C_3 plants, with an average $\delta^{13}C_p$ value of $-28.02‰$. The value is lower than the average leaf $\delta^{13}C_p$ value of terrestrial plants in China ($-27.15‰$) reported by Li et al. (2017), as well as the average values for arbuscular mycorrhizal plants ($-27.01‰$) and ecto-mycorrhizal plants ($-27.12‰$) studied by Zhang et al. (2022), and it is also lower than the global average for C_3 species ($-27.0‰$) reported by Kohn (2010). The driver of these differences is the water availability, where $\delta^{13}C_p$ tends to be lower in humid regions and higher in arid areas. In humid regions, plant carbon fixation is used primarily for vegetative growth and higher productivity related to producing large leaf areas, wide vessels, and other functional traits reflecting hydrophytic strategies. This is manifested by lower water storage ability compared to species adapted to arid environments (Li et al. 2022). In a dry environment, plants will reduce stomatal conductance to decrease water loss; however, this also decreases carbon fixation efficiency (Santos et al. 2017). During the wet season, plants tended to open stomata to absorb more CO_2, increasing the ratio of intercellular to ambient CO_2 concentrations (C_i/C_a). Therefore, under favorable moisture conditions, the $\delta^{13}C_p$ values of plants on the eastern slope of Yulong Snow Mountain

were significantly lower compared to those in other arid environments.

Different genetic and environmental factors contribute to the differences in the morphology of herbs, shrubs, and trees, resulting in different water use efficiency (Ponton et al. 2006). The results of the study have shown a pattern of leaf-scale $\delta^{13}C_p$ and iWUE values among different life forms, in the order of herbs > shrubs > trees. Song et al. (2008) observed that the order of leaf $\delta^{13}C_p$ values of dominant species on the Qinghai-Tibet Plateau was xerophilous plants > sedges > shrubs > broad-leaved evergreens > evergreen conifers, which is consistent with the results of this study. Root system length varies among life forms, with trees and shrubs generally having deeper root systems and more extensive lateral roots than herbs (Schenk and Jackson 2002). This enables trees and shrubs to access relatively stable water sources (Duan et al. 2008). Species with deeper roots and more reliable water sources tend to have more negative leaf-scale $\delta^{13}C_p$ values, indicating lower iWUE values (Song et al. 2008). Thus, herbaceous plants on the Yulong Snow Mountain showed higher $\delta^{13}C_p$ and iWUE values compared to shrubs and trees.

Factors controlling $\delta^{13}C_p$ and iWUE for different life forms across the altitude gradient

Changes in water and thermal conditions from altitudinal changes can affect plant $\delta^{13}C_p$ and iWUE values

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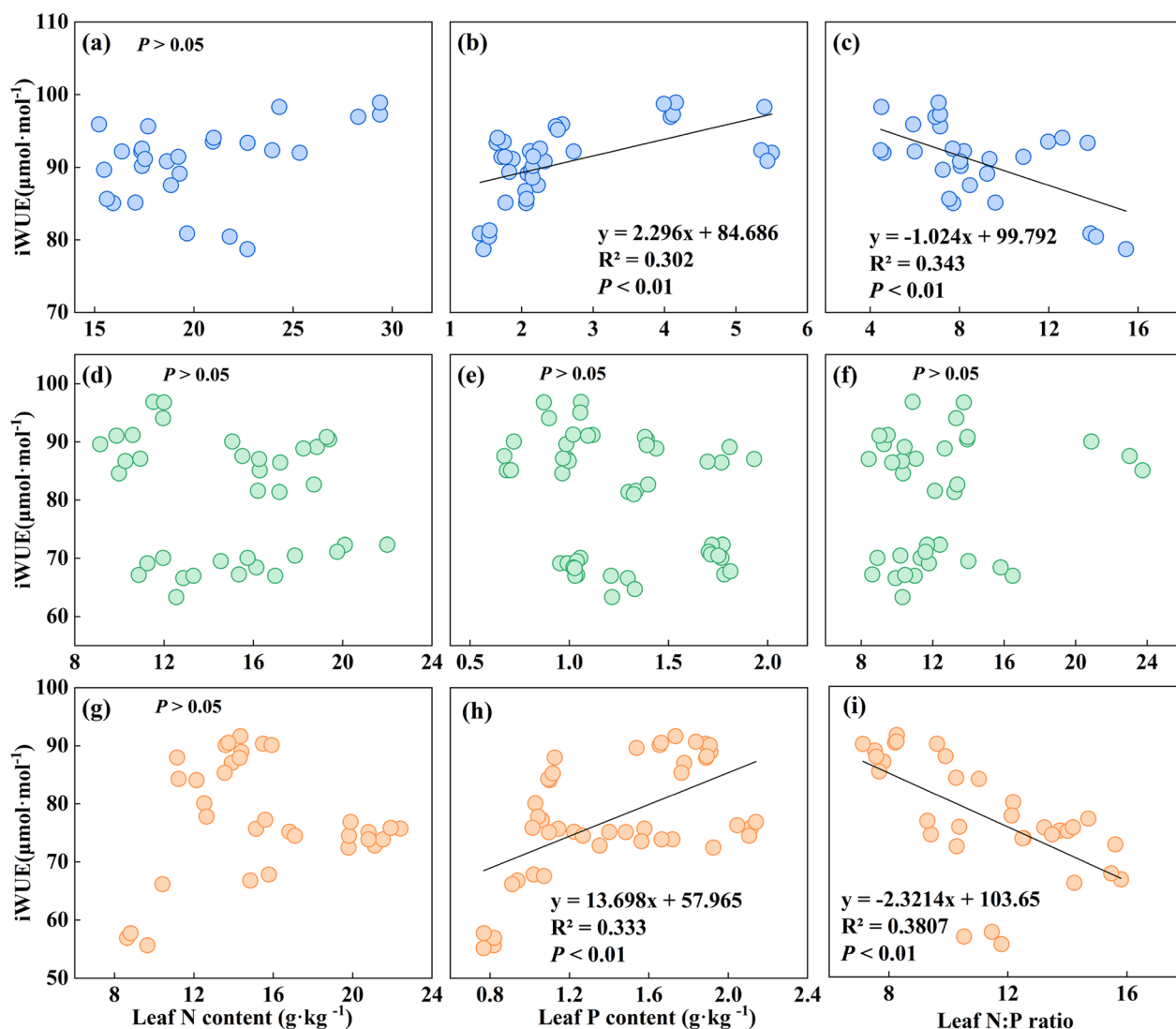
Fig. 6 Relationships of iWUE with leaf N content, leaf P content, and leaf N:P ratio for different life-form plants from Yulong Snow Mountain. Note: Blue circles represent herbs (**a–c**), green circles represent shrubs (**d–f**), and orange circles represent trees (**g–i**). The linear fits were used to test the coupling between $\delta^{13}C_p$ and leaf nutrients with the significance level set at 0.05

(Midolo et al. 2019). Firstly, leaf-scale $\delta^{13}C_p$ values and iWUE values are proxies for plant response to drought stress (Vantyghem et al. 2022). The effects of drought on $\delta^{13}C_p$ and iWUE vary across different regions (Liu et al. 2015). For example, Song et al. (2017) found that in southern China, moderate and extreme droughts led to a decrease in iWUE, whereas severe droughts usually led to a slight increase in iWUE. However, Guo and Xie (2006) observed an opposite relationship between $\delta^{13}C_p$ and precipitation in different regions of the Qinghai-Tibet Plateau, with a negative correlation in the southern region and a positive correlation in the northern region. Moreover, leaf-scale iWUE values vary across

different seasons. Song et al. (2017) found that iWUE was higher during the wet season and lower during the dry season. Consequently, soil water content is identified as a key variable affecting leaf-scale iWUE under different altitude conditions (Li et al. 2022). As soil water content decreased, plant stomatal conductance diminished, resulting in a decrease in the ratio of C_i to C_a, thereby enhancing plant iWUE (Berriel et al. 2019; Liu et al. 2013; Miller et al. 2001). In this study, herbs and shrubs exhibited a positive correlation between $\delta^{13}C_p$ and altitude (Fig. 4). In contrast, trees showed a parabolic trend in $\delta^{13}C_p$ for altitude, initially decreasing and then increasing (Fig. 4). The results indicated that the soil water content

(0–30 cm) collected at different altitudes from the Yulong Snow Mountain did not exhibit a clear correlation with iWUE. This is because soil water content exceeded 115% at all altitudes, suggesting saturated soil conditions that sufficiently meet the water requirements for plant growth (Fig. 5a). Herbs and shrubs with shorter root systems absorb water from shallower soil layers. However, being larger with deeper and more extensive root systems, trees access water from deeper soil layers (Dawson and Pate 1996; Ehleringer et al. 1991). Our results clearly showed that the leaf-scale iWUE of the trees decreased with increasing soil water content below 3900 m (Fig. 5d). The leaf-scale $\delta^{13}C_p$ and iWUE values were negatively correlated with elevation below 3900 m, and positively correlated with elevation above 3900 m, with 3900 m being a clear inflection point (Fig. 4c and i). Below 3900 m, soil water content may play a key role in plant growth. Both precipitation and soil water content increase with altitude, leading to increased leaf stomatal conductance and enhanced transpiration, resulting in lower $\delta^{13}C_p$ and iWUE values in plants (Ren and Yu 2011; Wang et al. 2011; Brendel et al. 2003; Van De Water et al. 2002).

In addition to water conditions, temperature differences from elevation changes are also important factors affecting plant $\delta^{13}C_p$ and iWUE. Temperature generally decreases with increasing altitude, and lower temperatures lead to higher $\delta^{13}C_p$ values and iWUE (Wang et al. 2023; Körner and Paulsen 2004). In this study, herbs and shrubs showed a negative correlation with increasing air temperature (Fig. 5b and c). Similarly, the $\delta^{13}C_p$ and iWUE values of trees were progressively positive with increasing altitude at 3900 m and above, with air temperatures mainly below 9 °C (Fig. 5e). In high-altitude regions, low temperatures subject plants to cold stress, resulting in reduced stomatal conductance, and subsequently lowering plant transpiration rates and photosynthetic rates. However, transpiration rates decrease more rapidly than photosynthetic rates (Cregg and Zhang 2001), which results in an increase in iWUE in colder high-altitude areas. Meanwhile, in such cold regions, the closure of stomata in response to plant stress also reduces C_i/C_a (Körner et al. 1991). Simultaneously, low temperatures reduce photosynthetic enzyme activity, weakening $^{12}CO_2$ assimilation and increasing plant leaf $\delta^{13}C$, contributing to the increase in iWUE (Wang et al. 2023). In high-altitude regions on the Yulong Snow Mountain, as air temperatures gradually decrease, and with limited available water, the control exerted by temperature on carbon isotope fractionation strengthens. It then becomes a significant factor influencing the variation in plant carbon isotope fractionation with altitude. Therefore, the leaf-scale $\delta^{13}C_p$ and iWUE of herbs and shrubs

are primarily influenced by air temperature (Fig. 5b and c). However, the abrupt change in iWUE with altitude in trees suggests a shift in influencing factors. At altitudes below 3900 m, trees are relatively tall, with deep and well-developed roots that absorb deep soil water and are affected by soil water availability (Fig. 5d). Above 3900 m, trees are smaller with shallower roots, absorbing water from shallow soil layers. Here, the soil water content is already saturated, resulting in a minor impact. Therefore, the control effect of temperature on stable carbon isotope fractionation of trees is reinforced at the above 3900 m (Fig. 5e), which becomes the main control factor of iWUE with altitude.

Relationships between iWUE and leaf nutrients

Leaf N and P contents are two key elements regulating the physiological and ecological function of plants (Chen et al. 2011; Elser et al. 2010). Many previous studies have shown that there are remarkably close coupling relationships between iWUE and leaf nutrients (e.g., N and P) in terrestrial ecosystems (Huang et al. 2023; Rumman et al. 2018; Cornwell et al. 2018; Gong et al. 2011; Hultine and Marshall 2000). In theory, the N and P nutrients in leaves primarily affect iWUE through their impact on photosynthesis (Hultine and Marshall 2000). On one hand, N is a vital component of chlorophyll and enzymes; an increase in N content can further affect photosynthesis capacity and the carbon assimilation efficiency by influencing plant chlorophyll and photosynthetic carboxylation enzyme (Cornwell et al. 2018; Hultine and Marshall 2000). However, during the biochemical C assimilation processes, $^{12}CO_2$ is more reactive than $^{13}CO_2$, making it easier to diffuse and assimilate into photosynthetic products (Liu et al. 2010). Therefore, an increase in N content leads to higher iWUE in plants (Gong et al. 2011). On the other hand, as leaf N content increases, the leaf mass per unit area increases, leading to increased internal diffusion limitations, increasing leaf-scale $\delta^{13}C_p$ values (Williams and Ehleringer 2000). In the present study, there was no significant correlation between iWUE and leaf N content for herbs, shrubs, and trees (Fig. 6a, d, and g). This is mainly possible because P, as an important element for energy storage and cell structure, is a limited element in this region compared to N (Chen et al. 2011). Moreover, in terms of limiting plant development and growth, leaf P content may be more important than leaf N content (Ostertag 2010; Reich et al. 2009). Zheng et al. (2020) also found that leaf P content generally responds stronger to nutrient addition than leaf N content, regardless of fertilizer or species type. A previous study has shown that increasing leaf P can reduce soil daily transpiration, thereby increasing iWUE due to the decrease

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in net photosynthetic assimilation rate being less than the decrease in transpiration (Singh et al. 2008). Leaf N:P ratio was mainly influenced by P content because the variable of leaf P content is larger than leaf N content, and hence leaf N:P ratio is mainly determined by leaf P content (Chen et al. 2011; Ordoñez et al. 2009). Therefore, iWUE is positively correlated with leaf P content but negatively correlated with leaf N:P ratio for herbs and trees. However, it is worth noting that the correlation of iWUE with leaf N, P, and N:P ratio for shrubs was not significant (Fig. 6d–f), suggesting that the relationship between iWUE and leaf nutrients for shrubs remains to be further investigated in the future.

Conclusion

On the eastern slope of Yulong Snow Mountain, the $\delta^{13}C_p$ values of plant leaves fluctuated between $-30.4‰$ and $-26.55‰$ from 3300 to 4500 m. Significant differences were observed in leaf-scale $\delta^{13}C_p$ and iWUE among herbs, shrubs, and trees, with the order of $\delta^{13}C_p$ and iWUE values: herbs > shrubs > trees. The $\delta^{13}C_p$ and iWUE values for herbs and shrubs increased gradually with the altitudinal gradient, which was controlled by air temperature. However, the $\delta^{13}C_p$ and iWUE values for trees exhibited a quadratic pattern characterized by a decrease followed by an increase. Below 3900 m, the $\delta^{13}C_p$ and iWUE values decreased with increasing altitude, primarily influenced by soil water availability. Above 3900 m, the $\delta^{13}C_p$ and iWUE values increased with increasing altitude, mainly influenced by air temperature. Moreover, leaf-scale iWUE was positively correlated with leaf P content but negatively correlated with leaf N:P ratio, especially for herbs and trees. Therefore, the combined impact of changes in water and thermal conditions as well as leaf N and P nutrients from altitudinal variation strongly influenced the variations in leaf-scale $\delta^{13}C_p$ and iWUE values in the Yulong Snow Mountain. These findings shed light on how plants adapt to high-altitude environments and the water–carbon relationships in mountain ecosystems.

Abbreviations
$\delta^{13}C_p$	Stable carbon isotope composition
iWUE	Intrinsic water use efficiency
N	Nitrogen
P	Phosphorus

Supplementary Information

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Additional file 1. Supplementary tables.

Author contributions
Feng Jiang: Methodology, Supervision, Software, Visualization, Writing – original draft, Data curation. Tao Pu: Conceptualization, Methodology, and Reviewing. Zi-Jing Xue: Investigation and Data curation. Yan-Wei Ma: Investigation and Data Curation. Xiao-Yi Shi: Conceptualization, Funding, Writing – review and editing. Fu-Xi Shi: Conceptualization, Writing, and Reviewing.

Availability of data and materials
The data generated and analyzed during the present study are available from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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