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Water and nutrient use efficiencies of *Stipa purpurea* Griseb. along a precipitation gradient of the Tibetan Plateau

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Abstract: Water and nutrient use efficiencies are important adaptive features of plants in arid and semi-arid environments. In this study, water, nitrogen (N), and phosphorus (P) use efficiencies of *Stipa purpurea* Griesb., an endemic and dominant grass species, were investigated in the alpine steppe along precipitation gradients on the Tibetan Plateau. The leaf N content of *S. purpurea* increased along the precipitation gradient, but leaf P decreased, whereas carbon (C) remained unchanged. Leaf $\delta^{13}C$ (water use efficiency, WUE) and C:N ratio (N use efficiency, NUE) decreased with increasing precipitation. But leaf C:P (P use efficiency, PUE) and N:P ratios increased with increasing precipitation. A trade-off was found between WUE and PUE but not between WUE and NUE. The changes in leaf traits were associated with changes in soil water, organic C, total N and P. These findings offer insights into understanding alpine plant water and nutrient use strategies along a precipitation gradient, as well as facilitate the prediction of alpine ecosystem responses to precipitation changes.

Keywords: grassland; water availability; nutrient limitation; correlation; soil characteristic

Water and nutrients, such as nitrogen (N) and phosphorus (P), are the limiting factors for a plant's optimum growth, functioning, and reproduction. In literature, water use efficiency (WUE) is a measure of a plant's capacity to convert water into biomass and has been consistently viewed as a key link to carbon (C) capture by ecosystems (Gong et al. 2011, Diao et al. 2021). On the other hand, N and P use efficiency (NUE and PUE, respectively) by plants show the ability to take and utilise both of them for maximum biomass production, which in turn indicates the potential for nutrient losses to the environment from ecosystems (Dijkstra et al. 2016). Global climate change effects water and nutrient use efficiency strongly through altered precipitation (Lü et al. 2012, Dijkstra et al. 2016), and the latter controls soil water, nitrogen, and phosphorus availability to plants. As a result, plant growth is affected by influencing physiological processes related to water and nutrient availability and absorption (Liao et al. 2021). By analysing WUE, NUE, and PUE, potentially divergent responses of plants to precipitation change would be helpful in understanding the water and nutrient conservation strategies. Thus, contributing further to the management of plant water and nutrients, especially in arid and semi-arid environments.

Previous studies have suggested that plants generally have lower WUE in humid and sub-humid areas due to larger stomatal conductance and transpiration rates caused by higher air humidity and soil moisture content (Gong et al. 2011, Dijkstra et al. 2016). In arid and semi-arid areas, plants may maintain high WUE to reduce the impact of water deficit and enhance their ability to compete for water under drought conditions (Gong et al. 2011). But in some studies, precipitation or soil moisture showed an increased role or no impact

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on plant WUE (Dijkstra et al. 2016). The divergent response of WUE to precipitation often occurred in different plant species (Chen et al. 2005).

By comparing the WUE, the effect of precipitation on plant nutrient use efficiency is mainly uncertain. Precipitation can impact NUE by modifying plant and soil moisture conditions, but the species-specific NUE of plants varies greatly in the natural environment (Liao et al. 2021). Both precipitation-induced increases decrease, and no significant changes have been observed in plant NUE (Dhillon et al. 2017, Liao et al. 2021, Liu et al. 2021). These conflicting results may be due to variations in soil properties, climate conditions, and plant species (Dijkstra et al. 2016). Although the responses of plant NUE to participation have been widely investigated (Dijkstra et al. 2016), the response of PUE ratio to precipitation has received less attention. Very few studies reported the effects of precipitation or soil moisture on plant PUE, and there seems to be no consistent conclusion (Dhillon et al. 2017). It also needs to be pointed out that almost all of these studies were conducted in crop plants, and few studies were carried out on the relationship between plant PUE and precipitation or soil water in grassland (Dijkstra et al. 2016).

The Alpine steppe is the most widely distributed ecosystem in the arid and semi-arid areas on the Tibetan Plateau (Hong et al. 2016). *Stipa purpurea* Griseb. (Family: Poaceae), an endemic and dominant grass species is widely distributed in the harsh environments of the alpine steppe (Wei et al. 2019). Previous studies have revealed the study results on plant biomass, morphological characteristics, and anatomical structure of *S. purpurea* (Wei et al. 2019, Wu and Wang 2021). However, little is known about its water and nutrient use efficiencies and thus has been considered as a knowledge gap to cover the underlying mechanisms of physiological processes related to the water and nutrient uses by alpine plants. Therefore, the present study aimed to examine the leaf $\delta^{13}C$, C:N, and C:P ratios (as indices of leaf WUE, NUE, and PUE, respectively) of *S. purpurea* in response to the precipitation gradient in the Tibetan Plateau. The main objectives were to: (1) determine the changes in the WUE, NUE, and PUE pattern of *S. purpurea* along the precipitation gradient and (2) explore the relationship of water and nutrient use efficiency with precipitation variables in the Tibetan Plateau.

MATERIAL AND METHODS

Study sites. The study area was the alpine steppe of the Tibetan Plateau, located between 31.23–32.31°N and 80.12–91.35°E (Figure 1). It is dominated by the grass *S. purpurea*. All the study sites were distributed in seven counties, namely, Nakchu, Amdo, Palgon, Nima, Gerts, Gakyi, and Gar, from east to west along a precipitation gradient in the Tibet Plateau. The climate of the area is arid to semiarid. From west to east, along the transect, mean annual temperature

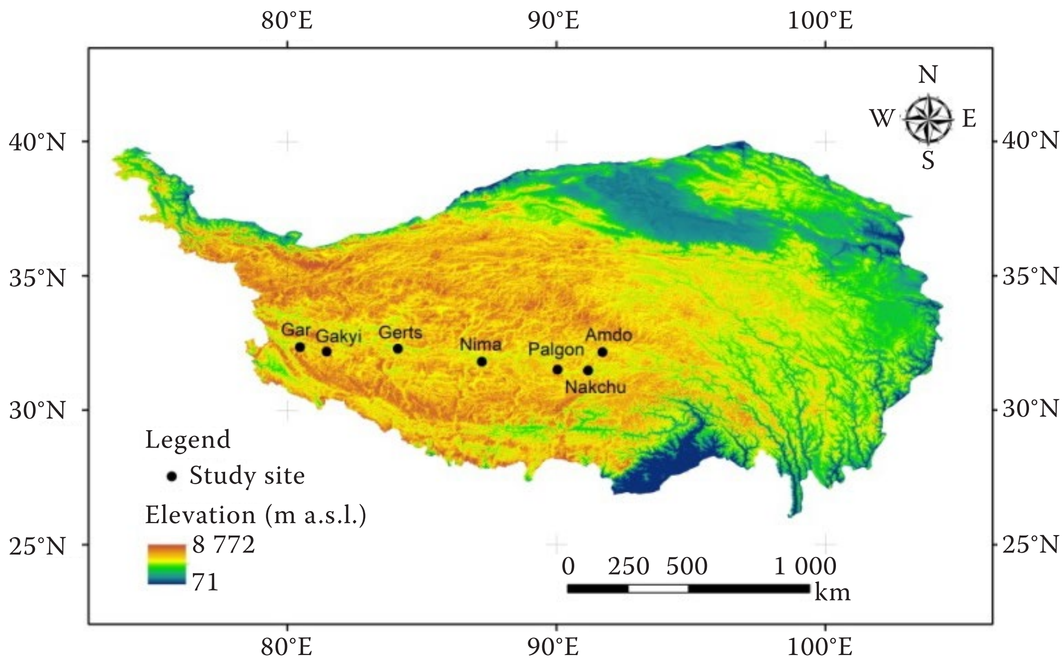

Figure 1. Sampling site locations in *Stipa purpurea* alpine steppe in Tibetan Plateau

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(MAT) decreased from +0.5 to –1.9 °C, and mean annual precipitation (MAP) increased from 100–450 mm. The soil types are all classic calcic, which are defined as cambisols according to the FAO-UNESCO Soil Map of the World soil taxonomy system (Du and Gao 2020). The experimental grass species *S. purpurea* was the widely distributed and dominant species of the steppe with the largest biomass in the study area community.

Sampling and measurements. In total, 7 sites were selected along a precipitation transect on the Tibetan Plateau in August 2019 (Figure 1, Table 1). Three random 10 × 10 m plots were established at each site to collect the plant and soil samples. The plots were set on flat terrain and were located far from human habitats to minimise the effects of microtopography and grazing disturbances. In each plot, newly mature leaves of more than 10 plants of *S. purpurea* were collected. Five soil cores with a 4.0 cm diameter from the surface layer (0–15 cm) at each plot were collected, mixed and taken to the laboratory for water content and nutrient analysis.

All leaf samples were oven-dried at 65 °C for 48 h to constant weight and ground using a mill before passing through a 60-mesh screen. Gravimetric soil water content (%) was measured after drying the soil sample at 105 °C for 48 h. The soil samples were air-dried and ground with the help of a grinder and sieved using a 2 mm mesh width sieve for analysing soil nutrients. The elemental analyser (Elementar Vario Max, Langenselbold, Germany) was used to test soil organic carbon (SOC), soil total N (STN), and leaf N concentration. The sulfuric acid-perchlorate acid heating digestion method was used to measure leaf P concentration and soil total P (STP). Leaf $\delta^{13}C$ (isotope ratio of $^{13}C/^{12}C$) was analysed using an isotope ratio mass spectrometer (Delta Plus, Thermo Finnigan, Berlin, Germany) interfaced with an elemental analyser (Euro EA 3000, EuroVector, Milan, Italy).

Plant WUE was defined as leaf $\delta^{13}C$ values according to the leaf $\delta^{13}C$ values reflected by the Ci:Ca ratio, an important physiological index concerning stomatal behaviour and WUE (Peri et al. 2012). The ratio of the leaf C calculated plant NUE and PUE:N and C:P, which indicates the plant carbon gain per unit N and P (Dijkstra et al. 2016, Zhou et al. 2016).

Statistical analysis. Before carrying out the parametric tests, all data were checked for normality and homogeneity of the variances. Regression analysis was used to test the patterns in leaf $\delta^{13}C$ and C, N and P and their ratios, and SOC, STN, STP, and SWC along the precipitation gradient. Redundancy analysis (RDA) was performed to determine whether leaf traits correlated with soil variables. Statistical procedures were conducted using SPSS version 24.0 (IBM, Armonk, USA) and Canoco version 5.0 (Microcomputer Power, Ithaca, USA).

RESULTS

Leaf C, N, and P contents. Across all sites, the mean values of leaf C, N, and P contents of *S. purpurea* were 432.1, 21.5, and 1.1 g/kg, respectively (Figure 2). The data range for the parameters mentioned before were 401.4–459.5, 16.5–26.0, and 0.8–1.6 g/kg, respectively (Figure 2). The MAP significantly affected leaf N and P contents (Figure 2B, C). Leaf C stayed unchanged along the precipitation gradient (Figure 2A), while N increased (Figure 2B), but leaf P decreased (Figure 2C).

Leaf $\delta^{13}C$, C:N, C:P and N:P ratios. The mean values of leaf $\delta^{13}C$, C:N, C:P, and N:P ratio of *S. purpurea* were –25.8, 20.5, 400.3. and 20.3, respectively. The data range for the parameters mentioned before was observed as 24.0–26.9, 18.4–24.3, 258.3–542.8 and 10.6–29.9, respectively, across all the study sites (Figure 3). The MAP significantly influenced leaf

Table 1. Description of the study site

Site	Latitude (º)	Longitude (º)	Altitude (m a.s.l.)	MAT (ºC)	MAP (mm)	Dominant species
Amdo	32.16	91.71	4 564	–1.9	435.7	*Stipa purpurea*, *Kobre siahumilis*
Nakcu	31.48	91.17	4 589	–1.5	380	*S. purpurea*, *Carex moorcroftii*
Palgon	31.52	90.05	4 576	–0.8	321.7	*S. purpurea*, *C. moorcroftii*
Nima	31.81	87.22	4 540	–0.4	200	*S. purpurea*, *C. moorcroftii*
Gerts	32.29	84.11	4 550	–0.2	170.1	*S. purpurea*
Gakyi	32.18	81.46	4 510	–0.2	120	*S. purpurea*
Gar	32.35	80.47	4 408	0.5	72.1	*S. purpurea*

MAT – mean annual temperature; MAP – mean annual precipitation

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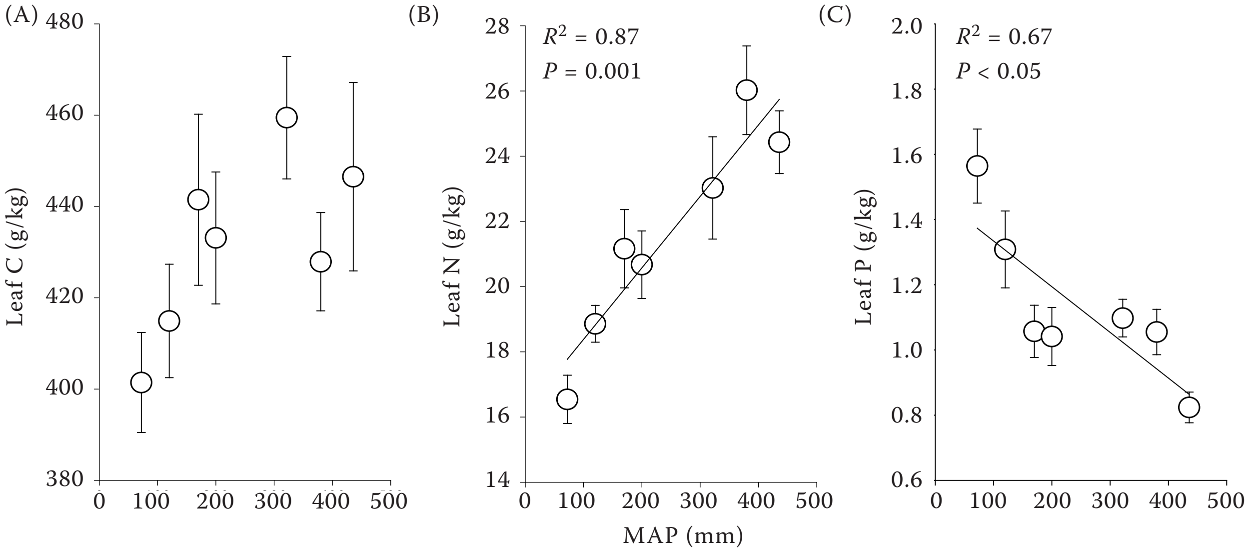

Figure 2. Variation of leaf carbon (C), nitrogen (N), and phosporus (P) contents along the precipitation gradient. MAP – mean annual precipitation

$\delta^{13}C$, C:N, C:P, and N:P ratios. Leaf $\delta^{13}C$ and C:N ratio decreased significantly with increasing MAP (Figure 3A, B). But a significant increase in the leaf C:P and N:P ratios was observed with increasing MAP (Figure 3C, D). Leaf $\delta^{13}C$ was correlated positively with the leaf C:N ratio (Figure 4A) but negatively with the leaf C:P ratio (Figure 4B).

Soil C, N, P and water contents. Soil C, N, and P and water contents exhibited variations across all sites in the alpine steppe, ranging from 5.38–16.44, 1.50–3.72, 0.22–0.44, and 3.71–12.28, respectively (Figure 5), but their average values were 10.26, 2.46, and 0.32 g/kg and 7.73%, respectively (Figure 5A, B, C). Soil C, N, and water contents increased along the

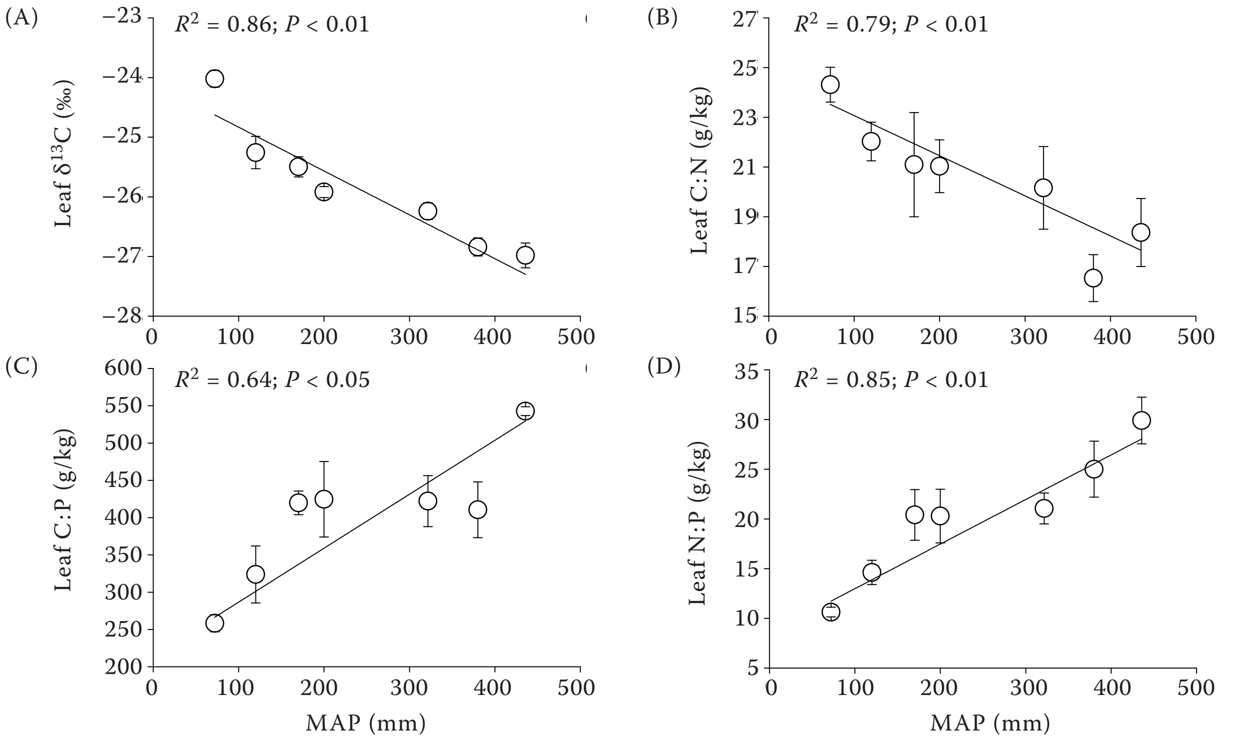

Figure 3. Variation of leaf $\delta^{13}C$, leaf C:N and C:P ratios along the precipitation gradient. MAP – mean annual precipitation

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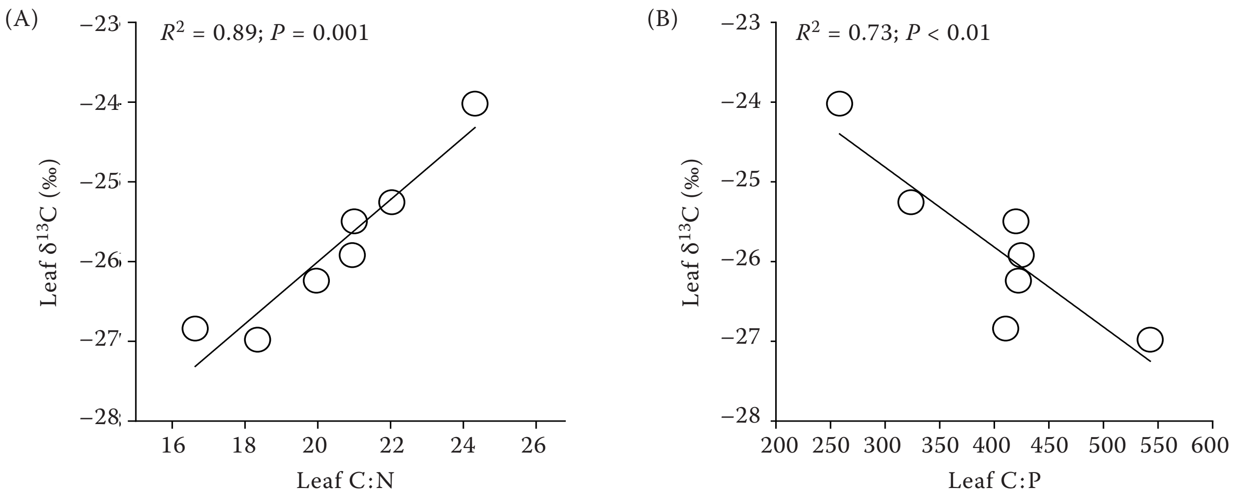

Figure 4. Relationships between leaf $\delta^{13}C$ and leaf C:N and C:P ratios

precipitation gradient (Figures 5A, B, D), while P declined (Figure 5C).

Linking leaf traits with soil variables. The RDA showed that the first and two canonical axes explained 90.3% and 1.4% of the total variation in the leaf traits (Figure 6). Leaf N, C:P, and N:P ratios showed a strong positive correlation with SOC, STN, and SWC but a negative with STP. Leaf P, $\delta^{13}C$, and C:N ratio strongly correlated positively with STP but negatively with SOC, STN, and SWC. There were no strong relationships between leaf C, SOC, STN and STP.

DISCUSSION

Leaf C, N, and P contents of *S. purpurea* responded differently to the MAP. Leaf C content was almost unaffected by MAP, in line with previous research (He et al. 2006), mainly due to the stable plant C composition and structural basis (Ågren 2008). In the present

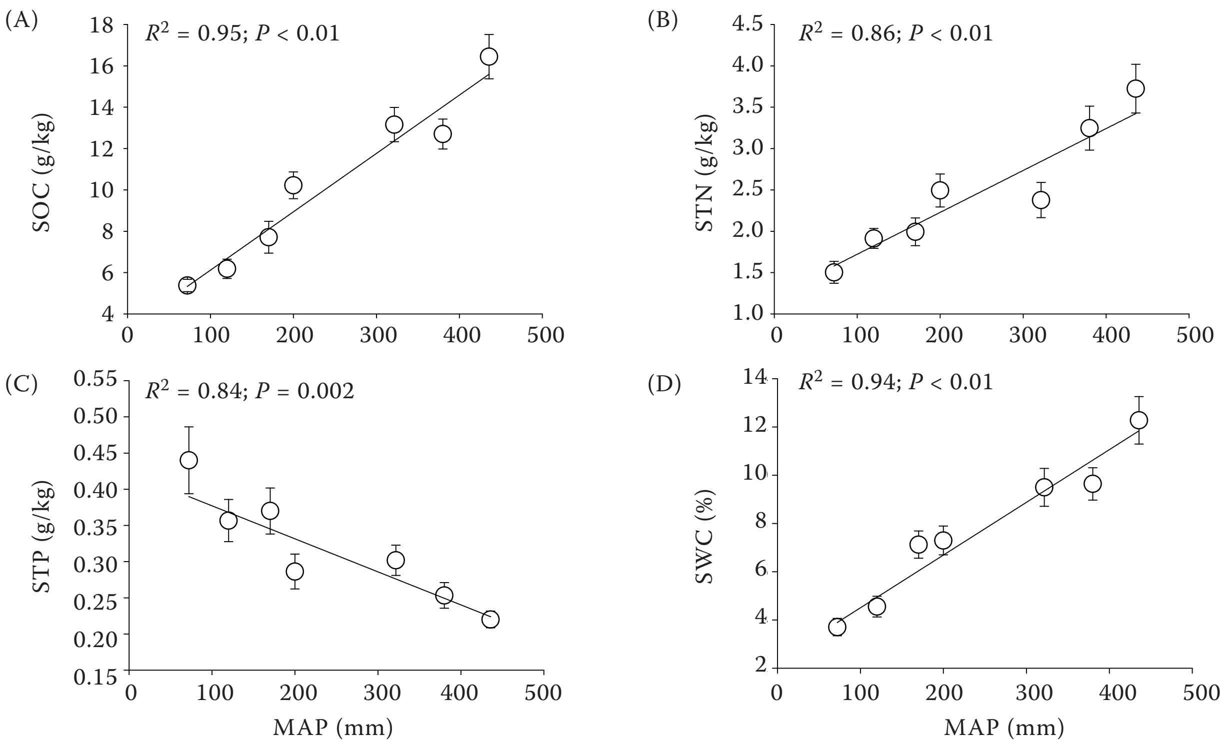

Figure 5. Variation of soil organic carbon (SOC), soil total nitrogen (STN), soil total phosphorus (STP), and soil water content (SWC) along the precipitation gradient

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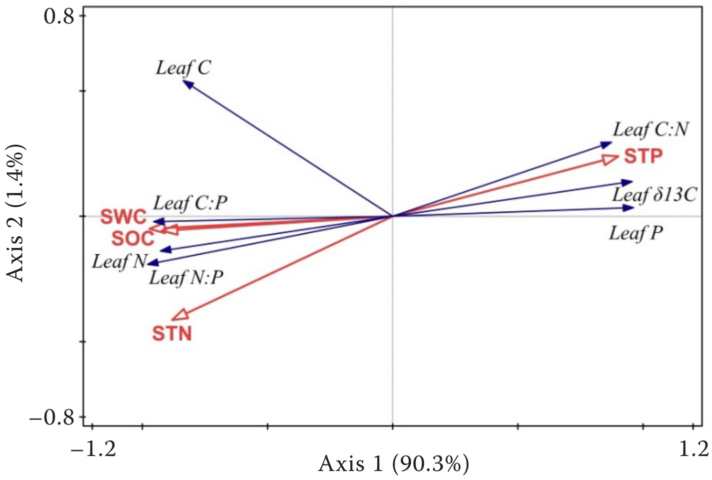

Figure 6. Redundancy analysis of the relationship between leaf traits and soil variables. SOC – soil organic carbon; STN – soil total nitrogen; STP – soil total phosphorus; SWC – soil water content

investigation, the leaf N content was strongly affected by MAP, and the result was not in agreement with He et al. (2008) and Luo et al. (2017). They reported that plant leaf N increased with the pattern of precipitation in arid and semi-arid grassland. This was explained by the strong covariations of soil N along precipitation gradients (Figure 3). Soil N content was positively correlated with precipitation, indicating that soil N availability was improved with increasing precipitation. Soil N is primarily provided by the decomposition of organic matter. This is a consequence of high plant biomass formation due to increased water availability (Dijkstra et al. 2012). Meanwhile, increasing water availability can accelerate soil mineralisation and promote plant N absorption (Zhang et al. 2018).

Unlike leaf N, P was negatively correlated with the MAP in this study. This does not agree with the previous study in this area, which showed that the precipitation did not have a great effect on plant P in alpine grassland ecosystems (Hong et al. 2016, Zhou et al. 2020). This diversified results in leaf P may attribute to the observation scale, i.e., species level in the present study and community level in the previous studies. This study's lower leaf P content probably also couples with lower soil P under higher precipitation (Figure 3). It results mainly because P in soil is mainly derived from rock weathering and its relatively low dispersal ability in soil solution. On the other hand, the higher precipitation could result in more P leaching from the soil and then lead to a decreased supply of P to plants (Xu et al. 2015, Hong et al. 2016). The different behaviours among leaf C, N, and P concentrations along the precipitation gradient suggested that *S. purpurea* has high flexibility of element composition and migration as a result of the trade-off between elements uptake and storage efficiency in the alpine steppe.

Leaf $\delta^{13}C$, an integrative index of leaf WUE (Dijkstra et al. 2016), is an important trait for plants adapted to arid and semi-arid environments (Gong et al. 2011, Diao et al. 2021). In this study, leaf $\delta^{13}C$ decreased along the precipitation gradient, which is in accordance with a common phenomenon that reduced leaf WUE induced by greater water availability (Gong et al. 2011, Dijkstra et al. 2016). This is due to a greater increase in photosynthetic rate than transpiration rate under increasing MAP, which led to a lower water cost accompanied by higher carbon sequestration through a higher photosynthetic rate, thus presenting a higher WUE (Gong et al. 2011). This indicated that alpine plants face drought stress and strengthen the protection of internal water contents.

Leaf C:N and C:P ratios as indices of leaf WUE and NUE, respectively, are also important adaptation strategies for plants in arid and semi-arid environments, where high C:N and C:P ratios reflect high NUE and PUE, respectively (Dijkstra et al. 2016, Zhou et al. 2016, Su and Shangguan 2020). In the present study, the leaf C:N ratio significantly decreased with increasing MAP, which was similar to the study in a semi-arid grassland (Lü et al. 2012, Castellanos et al. 2018, Su and Shangguan 2020) but was inconsistent with the results in the temperate desert steppe in Inner Mongolia (Liao et al. 2021). This could be due to the species-specific N-use strategies of plants (Diao et al. 2021). NUE decreased with increasing MAP can attribute to higher N uptake in higher soil N status following increased precipitation (Lü et al. 2012). Along with the higher N uptake, more leaf N allocation in the fraction of structural proteins (e.g., cell-wall binding proteins) reduces photosynthetic capacity and NUE (Li et al. 2012). In addition, an increase in the content of Rubisco in high leaf N

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status also led to a lower NUE (Feng et al. 2008). The results highlighted that *S. purpurea* could alter the absorption of N source to adapt to different soil N statuses caused by precipitation change, and leaf N allocation may regulate the acclimation of this alpine plant to water deficit stress.

Leaf C:P ratio increased with increased precipitation in this study, which is similar to the study in a semi-arid grassland (Lü et al. 2012). The higher leaf C:P is associated with, the lower plant available P in soil (Figure 4). Plants growing unusually under drought tended to have a high C:P ratio in leaves, which could be achieved with a constant P uptake rate or increased P uptake in response to decreasing P (Sardans and Peñuelas 2012). Thus, the results indicated that *S. purpurea* also altered the use-strategy of P source to adapt to habitats having differences in precipitation.

It should be pointed out that plants often decrease WUE with increased NUE and *vice versa*, suggesting a trade-off between WUE and NUE (Dijkstra et al. 2016). This trade-off can be explained by physiological constraints in the leaf when plants increase leaf intercellular carbon oxide concentration *via* opening their stomata; this increases photosynthesis per unit of leaf N but also increases transpiration, thereby reducing WUE. However, no trade-off, but the synergy between leaf NUE and WUE of *S. purpurea* was found in the present study. Different species have different WUE and NUE. It was reported that a positive correlation between WUE and NUE on grasses in semi-arid grassland (Chen et al. 2005, Zhou et al. 2016) indicated a high WUE which was not in discordance with a high NUE. There was a synergy between the WUE and NUE of *S. purpurea*, suggesting that *S. purpurea* could regulate its WUE and NUE to adapt to changes in precipitation and its productivity could be co-limited by water and N, and could be maintained by increased WUE and NUE. The plants on the Tibetan Plateau not only need to adapt to drought but also need to adapt to low-temperature stress, while at high altitude environment, more N may be used for protection against extreme temperature to increase NUE to minimise N loss, thus, they could simultaneously maintain relatively higher WUE and NUE (Zhou et al. 2016).

In contrast, no clear evidence for a trade-off between leaf WUE and PUE was found (Dijkstra et al. 2016). A trade-off between the WUE and PUE of *S. purpurea* indicates that the phenomenon is a common adaptive strategy in alpine steppe because a high WUE is possible only if the photosynthetic machinery and energy transfer capacity is high, which requires high P concentrations or low PUE (Dijkstra et al. 2016). In addition, inefficient WUE can be associated with higher transpiration fluxes, favouring P uptake and enhancing PUE (Zhou et al. 2016). A negative correlation between WUE and PUE indicates that plant consumes more phosphorus in carbon assimilation due to a low PUE under relatively low water conditions. Therefore, despite a decreasing WUE, *S. purpurea* in relatively humid conditions might fully utilise P resource to maximise its assimilation through gaining per unit of leaf P. Conversely, the plant growing in dry conditions might well underutilise P source to maximise its WUE. This trade-off might be a result of long-term natural selection.

Leaf N:P ratio is widely used to describe the relative limitation of N and P on plant growth (Liu et al. 2021). Usually, the leaf N:P ratio > 16 indicated P limitation, whereas leaf N:P ratio < 14 was indicative of N limitation, and when it was between 14 and 16, plant growth was co-limited by N and P together (Koerselman and Meuleman 1996). In this study, the leaf N:P ratio increased with the increasing MAP, and the average leaf N:P ratio across all sites was 20.3, which was far more than 14 and similar to the reports of Liu et al. (2021), who stated that increases in N:P ratio from increased MAP in other alpine plants. All these indicated that plant *S. purpurea* in most of the study sites suffered P limitation, and increasing precipitation will exacerbate the P limitation of alpine plants in the Tibetan Plateau. The relatively lower soil P content can explain the P limitation in the studied region because plant nutrients are generally coupled with soil nutrients at ecosystem scales (Zhang et al. 2018).

In conclusion, this study revealed that leaf $\delta^{13}C$ and C:N ratio of *S. purpurea* was negatively correlated with precipitation, while C:P and N:P ratios correlated with precipitation in the alpine steppe in the Tibetan Plateau. And there was a tradeoff between WUE and PUE, rather than NUE existed in *S. purpurea*. Also, increased precipitation could further exacerbate the increased P constraints on this alpine plant growth. These findings will improve our understanding of responses of alpine plant water and nutrient use strategies to changes in precipitation in arid and semi-arid areas. Moreover, the dynamics of P relative to N will be critical in predicting the responses of the alpine steppe to precipitation change. Further studies in this research domain should be devoted to measuring growth-related traits such as growth rate, the photosynthetic capacity of plants

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related to plant WUE and nutrient use efficiency to understand the life strategies better.

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