



Full Length Article

Characteristics and Relationships of Foliar Element Content and Specific Leaf Volume of Alpine Plant Functional Groups

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Abstract

Alpine plants grow in stressful environments with seasonal water shortages, strong ultraviolet and low temperatures. Understanding the characteristics of plant functional traits and their relationship with environmental factors is foundational to recognizing the ecological strategies and adaptive characteristics of plants. In this study, we selected and analyzed 38 alpine species in the Zoige Plateau, a part of the eastern Qinghai-Tibetan Plateau. Next, we simultaneously measured 14 functional traits such as $\delta^{13}\text{C}$ value, foliar C, N, and P element total content, relative water content, specific leaf area (SLA) and specific leaf volume (SLV). A comparison was made using three plant functional groups divided based on water availability, growth form and life form. The results show that the average total contents of foliar C, N, and P were 47.9%, 23.1 mg g⁻¹ and 1.5 mg g⁻¹ for alpine shrubs, and 43.7%, 20.0 mg g⁻¹, 1.3 mg g⁻¹ for herbs, respectively. The SLV of alpine plants had a highly significant positive correlation ($P < 0.01$) to foliar N and P content, total water content, dry mass water content and relative water content. In addition, SLV had a significant positive correlation ($P < 0.05$) to SLA. Among the studied plant leaf functional traits, SLV best characterized the differences in alpine plant groups. © 2018 Friends Science Publishers

Keywords: Alpine meadow; Alpine wetland; Foliar C content; Plant functional groups; Specific leaf volume; Water content

Introduction

Alpine ecosystems are expected to be highly sensitive to global environmental change, and serves as an indicator of climate change (Rammig *et al.*, 2010). Plant functional groups are helpful for understanding the mechanisms which related to the effects of plant species on ecosystem processes and simplify the study of multiple species in an ecosystem (Vitousek and Hooper, 1993). Plant functional groups also serve as the basic unit used to study vegetation change within the environment (Woodward and Cramer, 1996).

The features and strategies of plants to use and adapt to various environments are reflected in some important plant traits. Plant traits could provide important information related to plant growth and adaptation to the environment, and reflect the functional characteristics of plant species in the ecosystem. Therefore, plant traits are also called plant functional traits (Cornelissen *et al.*, 2003). Leaf traits should respond to environmental demands to maintain species fitness and add to adaptive trait (Baruch, 2011). Leaf water content, leaf dry matter content, leaf element content and specific leaf area (SLA) are the important leaf traits (Cornelissen *et al.*, 2003), and are closely related to plant growth strategies and the capacity of plants to use resources.

They also reflect the survival and adaptive strategies of plants used to obtain maximize C gain (Vendramini *et al.*, 2002). The leaf economics spectrum reflects a mixture of direct and indirect causal relationships between traits, and also investment strategies of trade-off between functional traits (Wright *et al.*, 2004).

The characteristics of the elemental content in plants are related to both plant biological traits and habitats. Elemental C, N and P are the most important chemicals used by organisms (Elser *et al.*, 2003). C is the most important element in the dry matter composition of plant, while N and P are generally considered to be the most crucial growth-limiting elements in terrestrial plants. Plant growth processes involve the accumulation of elements and adjustment of their relative proportions (Elser *et al.*, 2010). Water availability also places restrictions on plant morphology. Chen *et al.* (2003) divided typical steppe plants in the Xilin River Basin in Inner Mongolia of China into six plant functional groups based on the availability of water: xerophytes, mesoxerophytes, xeromesophytes, mesophytes, hygromesophytes and hygrophytes.

In alpine and arid environments, plant leaves may become small, irregular or degraded, such as linear, lanceolate or abnormal-rounded leaves of alpine plant

Gentiana farreri, small-orbicular, ovate or ovate-lanceolate leaves of alpine shrub *Potentilla fruticosa*, fleshy-short cylindrical leaves of desert subshrub *Reaumuria soongorica* (Su *et al.*, 2012), degraded leaves of desert shrub *Haloxylon ammodendron* (Su *et al.*, 2007), that make the difficulties to study the leaf functional traits. Such functional or morphological traits are likely the consequence of natural selection, and reflect the co-evolutionary history of plants with climate and soil. Alpine plant functional groups have important indication to environmental change, succession tended to move from alpine meadow to alpine shrub meadow (Su and Shi, 2016). Our aim was to study the plants tolerance to stress from their easy traits. Can a comprehensive index be developed to accurately reflect the leaf functional traits of alpine plants? Herein, we propose specific leaf volume (SLV) as this function index, and analyze the relationship between the value of SLV and other traits, that will help us to understanding the environmental adaptation of alpine plants.

Materials and Methods

Plots and Investigation

The study area is located on the Zoige Plateau, a part of the Eastern Qinghai-Tibetan Plateau. The same latitude and horizontal zone of vegetation on the Zoige Plateau is classified into wetland, swampy meadow, wet meadow, dry meadow, degraded meadow, etc. based on underground and surface water levels and the length of ponding time (Su *et al.*, 2018). The vertical zone is divided into flood plain meadow, lowland meadow/wet meadow, upland meadow/dry meadow, subalpine meadow, subalpine shrub meadow, subalpine shrubbery, subalpine needle forest, alpine meadow, alpine shrub meadow, alpine shrubbery, etc. based on the distance away from rivers and streams, the degree of ponding and terrain height. A comprehensive investigation was conducted on the Zoige Plateau, representative horizontal and vertical belt transects were established. All horizontal and vertical plots are planned on the same level with an area of 20 m×20 m. In each plot, dominant and common species were investigated in 27 different communities from August to September in both 2014 and 2015.

Sampling Analysis

All dominant, subdominant, common species and some rare species of the communities were sampled. Dominant species are the species with largest number of individuals, the size and the projection coverage in the dominant layer of the community. Subdominant species are the species that the number and role are inferior to the dominant species. Common species refers to the common species in the community, and rare species are the species with low frequency in the community.

The late stage of plant growth occurs in late August to early September, plants were collected at this time to achieve

maximum biomass and mature leaves were collected from the sunny side of plants. Collections from ten to fifteen different individuals were made for each species of 50 to 100 clean leaves that were combined to form a mixed sample, which represents a leaf sample of one species. Foliar $\delta^{13}\text{C}$ as well as C, N and P elemental content in leaves from 38 sampled species were determined. Within these species, the leaf water content, SLA and SLV of 18 representative plants were also simultaneously determined.

These plant species from different communities were classified according to groups based on water availability, growth form and life form (Appendix Table 1). The water ecological groups were divided into five groups based on habitat: xeromesophytes, mesophytes, hygromesophytes, hydrophytes and hydrophytes.

The growth form groups were divided into six groups based on physiognomic life form and classification features: tree, shrub, grass, sedge, forb and aquatic. Grass, sedge, and forb include gramineous herbaceous plants, cyperaceous herbaceous plants, and other herbaceous plants, respectively.

Based on Raunkiaer (1934) classification system (Druckenbrod and Dale, 2012), which classifies plant adaptation to harsh and cold environments, life form groups were divided into phanerophytes, chamaephytes, hemicryptophytes, geophytes and therophytes. Trees and shrubs above 25 cm tall belong to phanerophytes, buds or top shoots located on or near the surface as well as short semishrubs less than 25 cm tall and cushion plants were chamaephytes, cluster, semirosette and rosette plants belong to hemicryptophytes, rhizomatous geophytes, swamp plants and aquatic plants were geophytes.

Measurement of Leaf Water Traits, SLA and SLV

One part of the sample was used to determine leaf water content and the other was used to determine SLA and SLV. After the determination, the two samples were merged and crushed for measurement of $\delta^{13}\text{C}$ content and elemental C, N, and P content. The samples were collected, sealed in plastic bags and into storage box and then were sent back to the laboratory. SLV represents the leaf volume per unit of leaf dry-mass. The drainage method was used to determine leaf volume (see Appendix methods and formulas).

Measurement of Foliar $\delta^{13}\text{C}$ Values and C, N, P Content

Stable C isotope ratio was analyzed by a MAT-252 mass spectrometer (Finnigan MAT, Bremen, Germany) in the State Key Gas Geochemical Laboratory of the Lanzhou Institute of Geology, Chinese Academy of Sciences (Su *et al.*, 2012). Foliar element content was measured using a Vario Macro cube Elemental analyzer (Elementar, Hanau, Germany) to measure leaf total C and N content, and the molybdenum antimony resistance colorimetric method measurement of total P content.

Appendix Table 1: Functional group types and foliar $\delta^{13}\text{C}$ values of sampled plant species

Species	Water ecological group	Growth form group	Life form group	Community type and cenotype	$\delta^{13}\text{C}$ (‰)
<i>Potentillafruticosa</i> L.	Xeromesophyte	Shrub	Phanerophyte	Dominant species of subalpine shrubmeadow and subalpine shrubland	-27.22±0.20
<i>Caraganajubata</i> (Pall.) Poir.	Xeromesophyte	Shrub	Phanerophyte	Dominant species of subalpine shrubmeadow, common species of subalpine meadow	-27.45±0.14
<i>Rhododendron thymifolium</i> Maxim.	Xeromesophyte	Shrub	Phanerophyte	Dominant species of alpineshrubland at different elevations	-25.04±0.47
<i>Hippophaerhamnoides</i> ssp. <i>sinensis</i> Rousi	Xeromesophyte	Shrub	Phanerophyte	Common species of subalpineshrubland	-27.50±0.05
<i>Lonicerarapicola</i> var. <i>syringantha</i> Zabel	Xeromesophyte	Shrub	Phanerophyte	Common species of subalpinerocky slopes and subalpineshrub meadow	-26.96±0.09
<i>Daphne giraldii</i> Nitsche	Xeromesophyte	Shrub	Phanerophyte	Common species of subalpineshrubland	-25.17±0.07
<i>Elymusnutans</i> Griseb.	Xeromesophyte	Grass	Hemicryptophyte	Dominant species of uplandmeadow, subdominant species of subalpineshrub meadow, common species of lowlandmeadow	-27.35±0.24
<i>Anaphalisactea</i> Maxim.	Xeromesophyte	Forb	Hemicryptophyte	Common species of lowland meadow	-27.81±0.06
<i>Ajaniatenuifolia</i> (Jacq.) Tzvel.	Xeromesophyte	Forb	Hemicryptophyte	Common species of lowland meadow	-27.72±0.10
<i>Potentillaanserina</i> L.	Xeromesophyte	Forb	Hemicryptophyte	Common species of upland meadow, lowland meadow and wetland	-27.96±0.28
<i>Potentillabifurca</i> L.	Xeromesophyte	Forb	Hemicryptophyte	Common species of floodplain meadow	-27.48±0.14
<i>Leontopodiumhaplophylloides</i> Hand.-Mazz.	Xeromesophyte	Forb	Hemicryptophyte	Common species of upland meadow, swampy meadow and subalpineshrub meadow	-27.93±0.91
<i>Rhodiolakärlowii</i> (Regel) Maxim.	Xeromesophyte	Forb	Hemicryptophyte	Common species of alpinerocky slopes	-26.52±0.08
<i>Piceapurpurea</i> Mast.	Mesophyte	Tree	Phanerophyte	Dominant species of subalpine needle forest	-28.23±0.18
<i>Salix ortrepha</i> Schneid.	Mesophyte	Shrub	Phanerophyte	Dominant species of subalpineshrubland	-27.05±0.71
<i>Spiraeaalpina</i> Pall.	Mesophyte	Shrub	Phanerophyte	Common species of subalpineshrubland	-27.85±0.03
<i>Poapratensis</i> L.	Mesophyte	Grass	Hemicryptophyte	Common species of subalpineshrub meadow	-28.49±0.15
<i>Koeleriacristata</i> (L.) Pers.	Mesophyte	Grass	Hemicryptophyte	Common species of upland meadow	-27.91±0.16
<i>Kobresiapygmaea</i> C.B.Clarke	Mesophyte	Sedge	Hemicryptophyte	Dominant species of subalpine meadow, subdominant species of alpineshrub meadow	-26.46±0.28
<i>Kobresiahumilis</i> (C. A. Mey. ex Trautv.) Serg.	Mesophyte	Sedge	Geophyte	Dominant species of alpine meadow, common species of alpine shrub meadow	-27.14±0.22
<i>Gentianaferrieri</i> Balf. f.	Mesophyte	Forb	Hemicryptophyte	Common species of wetland, swampy meadow and uplandmeadow	-28.07±0.35
<i>Ligulariavirgaurea</i> (Maxim.) Mattf.	Mesophyte	Forb	Hemicryptophyte	Common species of upland meadow, swampy meadow and subalpine shrub meadow	-28.51±0.39
<i>Epilobiumangustifolium</i> L.	Mesophyte	Forb	Hemicryptophyte	Common species of subalpine shrubland	-27.52±0.11
<i>Cremanthodiumbrunneopilosum</i> S. W. Liu.	Mesophyte	Forb	Hemicryptophyte	Common species of subalpine shrub meadow	-27.85±0.14
<i>Descurainiasophia</i> (L.) Webb. ex Prantl	Mesophyte	Forb	Therophyte	Common species of floodplain meadow	-28.28±0.17
<i>Salix taensis</i> Gorz	Hygromesophyte	Shrub	Phanerophyte	Common species of subalpine shrub meadow	-28.32±0.16
<i>Cotoneaster adpressus</i> Bois	Hygromesophyte	Shrub	Chamaephyte	Dominant species of subalpine shrub meadow	-29.80±0.13
<i>Blysmussinocompressus</i> Tang et Wang	Hygromesophyte	Sedge	Geophyte	Common species of swampy meadow	-28.03±0.07
<i>Kobresiasetchwanensis</i> Hand.-Mazz.	Hygromesophyte	Sedge	Geophyte	Dominant species of uplandmeadow, swampy meadow and wetland; Common species of subalpine shrub meadow	-28.28±0.21
<i>Rubusxanthocarpus</i> Bureau et Franch.	Hygromesophyte	Forb	Chamaephyte	Common species of subalpine shrubland	-28.50±0.05
<i>Sanguisorbafiliformis</i> (Hook. f.) Hand.-Mazz.	Hygromesophyte	Forb	Hemicryptophyte	Common species of swampy meadow	-28.12±0.07
<i>Aster alpinus</i> L.	Hygromesophyte	Forb	Hemicryptophyte	Common species of alpine meadow	-30.08±0.12
<i>Carexmuliensis</i> Hand.-Mazz.	Hygrophyte	Sedge	Geophyte	Dominant species of wetland, subdominant species of lowland meadow	-29.36±0.07
<i>Carexmoorcroftii</i> Falc. ex Boott	Hygrophyte	Sedge	Geophyte	Common species of upland meadow	-28.20±0.06
<i>Scirpusdistigmaticus</i> (Kukenth.) Tang et Wang	Hygrophyte	Sedge	Geophyte	Common species of wetland and swampy meadow, subalpine shrubland	-28.88±0.51
<i>Polygonumamphibium</i> L.	Hygrophyte	Forb	Geophyte	Dominant species of floodplain meadow	-28.03±0.08
<i>Hippuris vulgaris</i> L.	Hydrophyte	Aquatic	Geophyte	Common species of floodplain meadow	-28.55±0.03
<i>Batrachiumbungei</i> (Steud.) L. Liou	Hydrophyte	Aquatic	Geophyte	Common species of wetland	-27.33±0.04

Data are mean± SE

Statistical Analysis

For each type trait variable, one-way analysis of variance (ANOVA) was performed to analyze the significant differences with SPSS 16.0 software, and the significant differences were compared between the different classifications in the same types using Duncan's multiple range tests. The correlation between each type was analyzed by linear regression analysis.

Results

Changes of $\delta^{13}\text{C}$ Values in Different Plant Functional Groups

The $\delta^{13}\text{C}$ values of 38 alpine species of typical vegetative communities ranged from -30 to -25‰ with small variation between species on the Zoige Plateau (Appendix Table 1). By comparing different water ecological groups, it

Appendix Methods and Formulas

The leaf relative water content was determined according to the method of Cornelissen *et al.* (2003). Fresh mass (m_1 , g) was determined immediately and the specimens were then kept to deionized water to saturate them for 24 h in the dark, next, specimens were rapidly blotted with absorbent paper to absorbed leaf surface moisture, and weighed to determine saturated fresh mass (m_2 , g) using an electronic balance. Then, leaves were placed into a weighing bottle, fixed at 105°C and dried at 80°C for 24 h to a constant mass, next, leaf dry mass (m_3 , g) was determined. Three replicates were measured for each species. The water traits values were calculated using the following formulas:

Leaf total water content (Tw, %, also called fresh mass water content): $Tw(\%) = \frac{m_1 - m_3}{m_1} \times 100$

Leaf dry mass water content (Dw, %): $Dw(\%) = \frac{m_1 - m_3}{m_3} \times 100$

Leaf relative water content (Rw, %): $Rw(\%) = \frac{m_1 - m_3}{m_2 - m_3} \times 100$

Leaf dry matter content (Dm, %): $Dm(\%) = \frac{m_3}{m_2} \times 100$

Scanner software was used to calculate leaf area. The leaves were flattened and placed on a Canon Scan Lide 110 color scanner (Canon, Tokyo, Japan). Smaller leaves measured in each group were taken as a plurality, and the leaves were not allowed to overlap with each other. The scanner cover plate was closed after checked the leaves to ensure that they were all fully expanded, and then scanned. The leaf area (S , cm²) was accurately calculate by image analysis software (Image J, version 1.47v, National Institutes of Health, Bethesda, Maryland, USA) with three to five replicates (Abràmoff *et al.*, 2004; Juneau and Tarasoff, 2012).

After the leaf area measurement, leaf volume (V , cm³) was determined by measuring a cylinder that was filled with a certain volume of deionized water. Then, the leaf dry weigh (m , g) was obtained according to the method of leaf dry mass measurements. Data for each species were replicated three to five times. The SLA and SLV were based on the following calculation.

Specific leaf area (SLA, cm² g⁻¹): $SLA = \frac{S}{m}$

Specific leaf volume (SLV, cm³ g⁻¹): $SLV = \frac{V}{m}$

$$\delta^{13}C(\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$$

In which R_{sample} and R_{standard} were the C isotope ratios of the sample and standard, respectively, with Pee Dee Belemnite as the standard

wasshowed that the average $\delta^{13}C$ value of xeromesophytes was -27.1‰, the highest of any group, and -28.7‰ for hygromesophytes, the lowest. The average $\delta^{13}C$ for xeromesophytes was significantly different ($P < 0.05$) from that of hygromesophytes and hygrophytes, and no significant differences were observed with mesophytes and hydrophytes (Fig. 1A).

When comparing different growth form groups, the $\delta^{13}C$ value was highest in shrubs (-27.2‰), while there were no significant differences with the grass, sedge, forb and aquatic groups. In addition, the average $\delta^{13}C$ value of herbaceous plants was -28.0‰.

Significant differences in $\delta^{13}C$ value were observed among different life form groups. Phanerophytes had the highest $\delta^{13}C$ value (-27.1‰), similar to that of xeromesophytes. The average $\delta^{13}C$ value of chamaephytes was the lowest (-29.2‰). There was a significant difference ($P < 0.05$) between the $\delta^{13}C$ value of phanerophytes and

chamaephytes, and no significant difference was observed between the other plants (Fig. 1B).

Content and Ratio Changes of Elemental C, N and P in Different Plant Functional Groups

Among the water ecological groups, there were no significant differences of total foliar C content among xeromesophytes, mesophytes, hygromesophytes and hygrophytes, the C content of these four groups averaged 45.0%. The C content of hydrophytes was the lowest (41.4%), and there were significant differences ($P < 0.05$) between hydrophytes and the other four groups (Fig. 2A).

Among the growth form groups, shrubs had the total C content of 47.9%, which was significantly higher ($P < 0.05$) than that of other groups (Fig. 2B). The average C content of the grass, sedge and forb groups was 43.9%. The average C content of *Picea purpurea* was 50.8%, however, as this

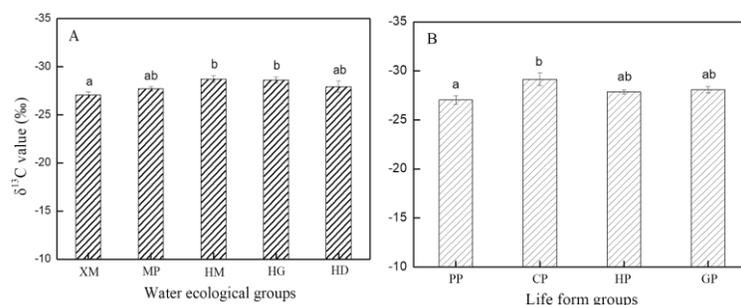


Fig. 1: Comparison of foliar $\delta^{13}\text{C}$ values of plant functional groups in different group types. In panel A and B, bars with different lowercase letters indicate significant differences ($P < 0.05$). XM = xeromesophytes, MP = mesophytes, HM = hygromesophytes, HG = hygrophytes, HD = hydrophytes; PP = phanerophytes, CP = chamaephytes, HP = hemicryptophytes, GP = geophytes

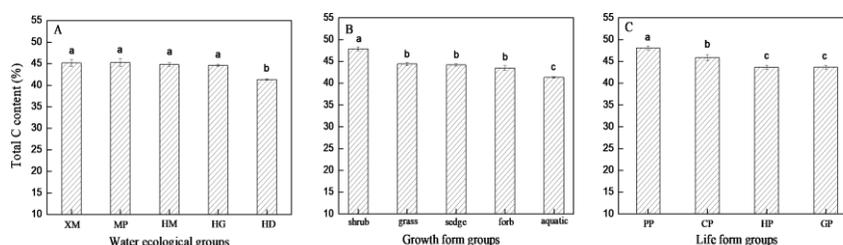


Fig. 2: Comparison of total foliar C content of plant functional groups in different group types. In each panel, bars with different lowercase letters indicate significant differences ($P < 0.05$). XM = xeromesophytes, MP = mesophytes, HM = hygromesophytes, HG = hygrophytes, HD = hydrophytes; PP = phanerophytes, CP = chamaephytes, HP = hemicryptophytes, GP = geophytes

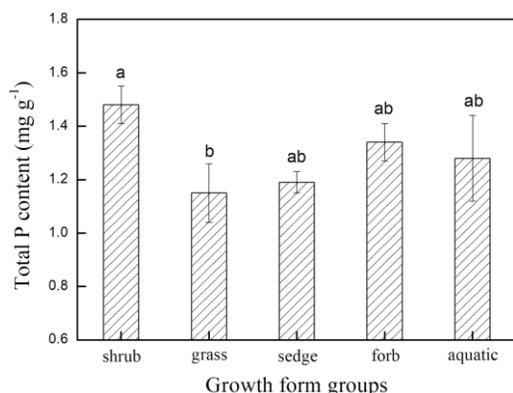


Fig. 3: Comparison of total P contents of growth form groups. In figure, bars with different lowercase letters indicate significant differences ($P < 0.05$)

was the only tree species in the study area, no further statistical analyses were carried out using *P. purpurea*. The foliar C content in sequence was tree > shrub > grass \geq sedge \geq forb > aquatic groups.

Among the life form groups, the C content of phanerophytes and chamaephytes were significantly higher ($P < 0.05$) than that of hemicryptophytes and geophytes (Fig. 2C).

The foliar N content was not significantly different

among water ecological groups. The N content of xeromesophytes, mesophytes and hygromesophytes was similar, with an average of 21.2 mg g^{-1} , while hygrophytes and hydrophytes averaged 19.1 mg g^{-1} . Overall the N content of mesophytes was higher than that of hygrophytes.

Among the different growth form groups, shrubs had the highest N content (23.1 mg g^{-1}), and herbaceous plants species averaged 20.0 mg g^{-1} . The mean value of foliar N content of different alpine species was 20.9 mg g^{-1} .

Among the different life form groups, the foliar N content of phanerophytes was the highest (average of 23.2 mg g^{-1}), while that of geophytes was the lowest (average of 19.8 mg g^{-1}).

No significant difference was observed in the foliar P content between water ecological groups, and the average of the alpine plants was 1.32 mg g^{-1} . But significant differences were observed between growth form groups (Fig. 3). Shrubs had the highest P content (average of 1.5 mg g^{-1}), while grass was the lowest values (average of 1.2 mg g^{-1}), and there was a significant difference between the two ($P < 0.05$). The average P content of herbaceous species was 1.3 mg g^{-1} and that of the tree *Picea purpurea* was 1.2 mg g^{-1} . High-density grass and sedge plants had low foliar P content, and the species of pure *P. purpurea* forest had low P content, which had high-density stands. The P contents of relatively densely growing tree, grass

and sedge plants were lower than those of sparsely growing shrub. There was no significant difference in P content between the different life form groups.

No significant difference was observed between the C:N, C:P and N:P ratios in the leaves of different water ecological groups of alpine plants. The average C:N, C:P and N:P ratios of different groups were 21.9, 351.6, and 16.2, respectively.

No significant difference was observed in the C:N, C:P and N:P ratios between different growth form groups. The C:N, C:P and N:P ratios of the sedge, grass and grass groups were 22.7, 397.3 and 18.4, respectively, these were highest among the various growth form groups. The C:N, C:P and N:P ratios were not significantly different between different life form groups.

Changes of Leaf Water, SLA and SLV of Different Plant Functional Groups

The average total water content of mature leaf of alpine plants was 56.2%, while the water content of dry mass was 136.3%, the relative water content was 64.7%, the dry matter content was 33.2%, the hundred leaves mass was 2.5 g, the SLA was 110.0 cm² g⁻¹, and the SLV was 4.2 cm³ g⁻¹.

Among the water ecological groups, xeromesophyte had the highest SLV (4.8 cm³ g⁻¹, Table 1), this index decreased from xeromesophytes to hygromesophytes. However, the other indices did not exhibit any other clear trends.

Shrubs had the highest SLV (5.2 cm³ g⁻¹) from growth form groups, while the SLV of grass was close to but higher than that of sedge, this tendency was also present in the relative water content (Table 1).

Among the life form groups, SLV showed a decreasing trend in order from phanerophytes > hemicryptophytes > geophytes. In addition, total water content, dry mass water content and relative water content also presented this tendency in order as SLV (Table 1).

From the results of the above three functional group types, SLV best reflected the differences among different groups, the second best indicator was relative water content.

Correlation Analysis of Leaf Functional Traits

To know and screen a typical trait that was used to reflect the functional characteristics of alpine plants, when one trait value was estimated by another trait value, correlation analysis of fourteen functional traits was carried out. The foliar $\delta^{13}\text{C}$ was significantly positively correlated with total C content. The relationship between total N and total P content was close ($P < 0.01$, Table 2). When comparing the foliar N and P content of alpine plants, P content more closely correlated with foliar C content, and the effect was stronger.

The linear correlation between leaf total water content (Tw) and relative water content (Rw) was extremely significantly positive, and between Tw and dry matter content (Dm) was extremely significant negative ($P < 0.01$, Table 2). Therefore, the determination of Tw, Rw and Dm could be calculated by the following formula: $\text{Rw} = 1.56\text{Tw} - 22.86$ ($P < 0.001$), $\text{Dm} = -0.34\text{Tw} + 51.73$ ($P < 0.01$), in which, Tw (%), Rw (%), Dm (%).

SLV had extremely significantly positively correlated with foliar N content, P content, Tw, Dw and Rw ($P < 0.01$). SLA was significantly positively correlated with N content and Tw ($P < 0.05$, Table 2). Compared with SLA, SLV can better reflect the leaf functional traits of alpine plants. For the further analysis, SLV had extremely significantly positive correlations with the five indices, and had the following formulas: $\text{SLV} = 0.31\text{N} - 2.38$ ($P < 0.001$), $\text{SLV} = 4.25\text{P} - 1.40$ ($P < 0.001$), $\text{SLV} = 0.12\text{Tw} - 2.41$ ($P < 0.01$), $\text{SLV} = 0.02\text{Dw} + 1.42$ ($P < 0.01$), $\text{SLV} = 0.06\text{Rw} + 0.50$ ($P < 0.01$), in which, SLV (cm³ g⁻¹), N (mg g⁻¹), P (mg g⁻¹), Tw (%), Dw (%), Rw (%). By comparing these formulas it can be seen the relationship between SLV and foliar N and P was higher than different water contents.

Discussion

The Zoige Plateau is covered by meadow steppe, which is different from the typical and desert steppe. The $\delta^{13}\text{C}$ value is an effective index that can be used to distinguish and reflect the photosynthetic pathway and water use efficiency (WUE) of plants (Lajtha and Michener, 1994), $\delta^{13}\text{C}$ value is positively correlated with the WUE (Farquhar *et al.*, 1989; Marshall and Zhang, 1994). The highest WUE in water ecological groups was xeromesophytes, and in life form groups was Phanerophytes.

C, N and P are the most important chemical elements that affect plant growth and development (Reich *et al.*, 2006). The C:N:P ratios in organisms have been proven to be associated with important ecological processes, such as responses to environmental stress, and are also related to an ecosystem's structure and functions (Sardans *et al.*, 2013). A trade-off exists during the allocation of limited resources between different plant functional traits (Shipley *et al.*, 2006). Global foliar C content of plants was an average of 46.4% (Elser *et al.*, 2000), foliar N content was 20.1 mg g⁻¹, foliar P content was 1.8 mg g⁻¹, and the N:P ratio averaged 13.8 (Reich and Oleksyn, 2004). The foliar N content of Chinese 753 terrestrial plant species was estimated at 20.2 mg g⁻¹, P content was 1.5 mg g⁻¹ (Han *et al.*, 2005). The foliar C content of alpine plants, shrubs and herbaceous plants in the present study averaged 44.8%, 47.9% and 43.7%, respectively (Fig. 2), and for *P. purpurea* this was 50.8%. It can be seen that woody plants in this study have a higher amount than the global average, while herbaceous plants have less than global average. The N and P content

Table 1: Leaf water, SLA and SLV of different plant functional groups

Group types	groups	Tw (%)	Dw (%)	Rw (%)	Dm (%)	HLW (g)	SLA (cm ² g ⁻¹)	SLV (cm ³ g ⁻¹)
Water ecological groups	Xeromesophytes	55.3±2.8	132.5±16.1	66.5±4.7	35.2±1.3	3.2±1.3	119.9±10.4	4.8±0.6
	Mesophytes	60.5±2.4	159.3±15.2	73.5±6.5	32.1±1.1	1.9±0.5	99.7±9.2	3.9±0.3
	Hygromesophytes	50.6±5.8	107.5±24.9	45.1±5.6	29.9±2.4	2.4±0.4	102.8±10.5	3.4±0.3
	Hygrophytes	55.0±4.2	127.1±20.3	58.6±1.4	32.7±3.3	1.8±0.1	105.5±33.5	3.4±0.2
Growth formgroups	Shrub	60.5±1.9	158.8±12.2	78.1±4.3	33.5±0.9	1.6±0.4	106.4±7.8	5.2±0.5
	Grass	49.9±2.6	102.2±11.0	58.0±6.7	35.7±2.1	4.4±2.3	112.6±19.4	3.5±0.7
	Sedge	54.3±2.5	124.4±11.9	55.6±3.1	31.6±1.6	2.1±0.2	105.2±13.4	3.4±0.2
Life formgroups	Phanerophytes	60.5±1.9	158.8±12.2	78.1±4.3	33.5±0.9	1.6±0.4	106.4±7.8	5.2±0.5
	Hemicryptophytes	54.6±3.1	129.0±18.2	61.5±4.4	34.3±1.8	3.7±1.5	117.0±12.8	3.9±0.6
	Geophytes	53.2±3.1	119.3±14.5	53.2±3.8	31.6±2.1	2.0±0.2	104.4±18.7	3.4±0.2

Tw = total water content; Dw = dry mass water content; Rw = relative water content; Dm = leaf dry matter content; HLW = hundred leaves dry mass; SLA = specific leaf area; SLV = specific leaf volume

Table 2: Linear correlation analysis of leaf functional traits in alpine plants

Traits	δ ¹³ C		Total C		Total N		Total P		C:N		C:P		N:P		Tw		Dw		Rw		Dm		HLW		SLA		
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	
Total C	0.54*	(0.03)																									
Total N	0.03	0.19																									
Total P	0.06	0.31	0.81**																								
C:N	0.15	0.11	-0.94**	-0.72**																							
C:P	0.07	-0.11	-0.76**	-0.96**	0.74**																						
N:P	0.01	-0.26	0.00	-0.57*	-0.09	0.60**																					
Tw	0.26	0.32	0.36	0.37	-0.27	-0.31	-0.13																				
Dw	0.26	0.31	0.33	0.36	-0.23	-0.32	-0.17	0.98**																			
Rw	0.38	0.53*	0.32	0.36	-0.12	-0.21	-0.17	0.79**	0.78**																		
Dm	0.01	0.13	-0.22	-0.17	0.31	0.30	0.02	-0.60**	-0.60**	0.01																	
HLW	-0.10	-0.22	-0.24	-0.43	0.27	0.56*	0.50	-0.07	-0.10	0.07	0.19																
SLA	-0.10	-0.27	0.51*	0.41	-0.66**	-0.47	0.06	0.49*	0.44	0.20	-0.56*	0.02															
SLV	0.04	0.24	0.79**	0.81**	-0.70**	-0.71**	-0.25	0.64**	0.61**	-0.26	0.06	0.52*															

r is linearly correlation coefficient, values in bracket indicate ep. *, **, indicate significance at P < 0.05 and 0.01, respectively. Tw = total water content; Dw = dry mass water content; Rw = relative water content; Dm = leaf dry matter content; HLW = hundred leaves dry mass; SLA = specific leaf area; SLV = specific leaf volume

and N:P ratio of alpine plants averaged 20.9 mg g⁻¹, 1.3 mg g⁻¹, and 16.2, respectively. We can see that the N content of the alpine plants in the present study is 4% higher than the global, and 3% higher than Chinese values, approximately consistent. Meanwhile, the P content was significantly lower of 28% than the global average, causing an increase in the N:P ratio.

The foliar N content can represent photosynthetic rate (Grime, 2002). Plants with higher foliar N content and net photosynthetic rates grow faster and are more responsive to climatic warming (Gornish and Prather, 2014). A trend for the N content of shrub or phanerophytes to be higher than that of other groups was obvious. Alpine vegetation has already responded to the changing environmental conditions,

in the Arctic, shrub cover has become denser and higher (Tape *et al.*, 2006; Rammig *et al.*, 2010). With global warming, shrubs will grow faster and may become dominant in alpine vegetation in the future (Gao *et al.*, 2016), which will affect the stability of alpine meadow steppe.

The N:P ratio, to some extent, can reflect the structure, function and nutrient supply of the plant community (Wu *et al.*, 2012), and can be used as an indicator for judging the environment to nutrient supply status of plant growth. A N:P ratio of 14–16 is usually considered in balance, when the ratio is less than 14 plant growth will be restricted by N limitation and if greater than 16 by P limitation (Güsewell, 2004). Compared with our results, the N:P was highest in grass (18.4). Thus, the results of alpine plants restricted by P

were consistent with those of previous analyses, which provide theoretical guidance for improving biomass yield.

The relative water content (Rw) of leaves can reflect the degree of water deficit, and plants with a higher Rw have higher osmotic adjustment function and stronger drought resistance. The Rw of shrub was higher than that of herbaceous plants, and that of grass was higher than that of sedge in herbaceous plants (Table 1). Plant traits are various and the functions are crossing and overlapping with each other (Kattge *et al.*, 2011). The leaf economics spectrum (LES) runs from quick to slow return on investments of nutrients and dry mass in leaves (Wright *et al.*, 2004), and is the functional trait combination in a series of interrelated and coordinated variation (Lavorel, 2013; Poorter *et al.*, 2014; Read *et al.*, 2014). LES does not depend on environmental changes (Cianciaruso *et al.*, 2013), and also difficult to distinguish which species had a higher resistant.

There are three major determinants of vegetation–competition, stress and disturbance—and that each has invoked a distinct strategy on the part of the flowering plant (Grime, 1974). Stress is better restricted to extreme situations (Körner, 2003). High stresses with low disturbance, adopting the stress-tolerant strategy, these plants are leaf form that is often small or leathery, or needle-like (Grime, 1977; 2002). Our results suggested that this kind of leaf form had a higher SLV.

SLA may reflect the ability of plants to obtain resources, is the priority index in the study of ecological comparisons (Garnier *et al.*, 2001), can represent the light use efficiency (Westoby *et al.*, 2002), and may reflect the plants' C balance between captured and used C (Wilson *et al.*, 1999). SLA measures the light-intercepting leaf area per unit of leaf dry-mass (Wright *et al.*, 2004), that's the upper surface area of the leaf, not the entire surface area, and reflect the local part of the photosynthetic organs. Alpine adversity plants have smaller leaf, shape changeable and thickness varies. So it is difficultly to accurately measure the light-receiving area, and the comparability between different species is also poor.

SLV is an important leaf functional trait, which depend on leaf thickness, leaf overall dimension and leaf dry matter content, reflecting the ability of plants to adapt to cold, arid and other extreme environments while resulting from changes in leaf shape, and making leaf area difficult to determine. Leaf volume is a combination of photosynthetic area and thickness, and reflects the whole of photosynthetic organs, with the higher comparability between different plants. From Table 1 that compared the different types of plant functional groups we can see that SLV had a rule change. In the water ecological groups, with the decrease in drought resistance, SLV decreased. In the growth form groups, the SLV of shrubs was greater than that of herbs. In the life form groups, leaves with positions higher of the bud nodes had a larger SLV. SLV is a better indicator trait that can be used to reflect the leaf functional traits of alpine plants.

Plants are generally considered to be poikilotherms that do not thermoregulate. However, empirical data show that plants are actually limited homeotherms that do thermoregulate. Plant thermoregulation and limited homeothermy decouples physiological functioning from climatic variation to promote metabolic homeostasis and maximize carbon assimilation and fitness (Michaletz *et al.*, 2015). Whether the greater SLV of plant has, the stronger with the thermoregulation in leaves, that's needs for further study. Our research suggested that the greater SLV of alpine plant has, the stronger with the resistance to extreme conditions, and this allowed development of the proposed specific leaf volume-stress resistance opinion. Further research is needed on the relationship between leaf anatomical structure and photosynthetic capacity to SLV.

Conclusion

There were close relationships between foliar total N and P content ($r=0.81$, $P<0.01$), in comparison, P content had a closer relationship with C content. Foliar P content of alpine plant is lower than the global and Chinese values, grass is obviously low, which should be taken attention on the management of alpine steppe. The SLV of alpine plants showed a highly significant positive correlation with foliar N content, P content, total water content, dry mass water content and relative water content ($P<0.01$). In addition, SLV was significantly positive correlation with SLA ($P<0.05$). SLV is an important leaf structural trait. Compare with different plant functional groups, alpine shrubs would be affecting the stability of alpine meadow steppe in the future. In practice, using the drainage method to determine leaf volume, put the leaves into distilled water and make sure the leaves were completely submerged and with no bubbles. Reading the values after the water surface stability, avoiding read it for a prolonged soaking, that's could improve the accuracy of the drainage method. We found that SLV best reflected the differences in alpine functional plant groups among the leaf functional traits measured. Further, alpine plants with a greater SLV exhibited stronger resistance to adverse environmental conditions.

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