

Study on the Relationship Between Leaf Functional Traits and Soil Factors of *Rhododendron aganniphum* var. *Schizopeplum* at Different Altitude Gradients in Sejila Mountain

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Abstract:

The study of plant leaf functional traits and environmental factors is of great significance for understanding the formation of plant communities under different altitude gradients and their adaptation mechanisms to the environment. This study takes *Rhododendron aganniphum* var. *Schizopeplum* in Sejila Mountain, Tibet as the research object, analyzes the different leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* under different altitude gradients, and investigates the main soil factors that affect the changes in leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*, attempting to reveal the adaptation strategies of *Rhododendron aganniphum* var. *Schizopeplum* in soil environment of high altitude areas. The results showed that: (1) With the increase of the altitude gradient, the leaf area (LA), dry weight (DW), dry matter content (LDMC), leaf length (LL) and specific leaf area (SLA) of *Rhododendron aganniphum* var. *Schizopeplum* decreased, while leaf N content (N), N:P, leaf thickness (LT), leaf P content (P) and leaf tissue density (LD) presented an upward trend with the increase of elevation gradient. The variation coefficient of leaves ranges between 4.60%~57.78%, variation coefficient of P is the lowest (4.60%), and variation coefficient of SLA is the highest (57.78%). Where, variation of P, N, N:P, LT, and LL is weak, that of LDMC, LA and DW is moderate, and that of LD and SLA is strong. (2) There is certain correlation between leaf functional traits, and it adapts to the harsh external environment changes in the high-altitude areas of southeastern Tibet through its own relevant adjustments. Leaf DW, LDMC, and LA can be used as the main indicators for changes in leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*. (3) The leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* are affected by various soil factors. Among the soil factors, TP, AK, and AP are the main soil factors that affect leaf functional traits. The above research results deepen our understanding of the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* at different altitude gradients, and provide scientific basis for the scientific protection and utilization of the germplasm resources and biodiversity of *Rhododendron aganniphum* var. *Schizopeplum* in this area.

Keywords: *Rhododendron aganniphum* var. *Schizopeplum*, leaf functional trait, soil factor, southeastern Tibet.

I. INTRODUCTION

Plant functional traits refer to measurable biological characteristics displayed by plants in response to external environment changes, which directly or indirectly reflect the adaptation strategies of plants to the external environment, play an important role in regulating the resource allocation of plants, and serve as an important link for us to explore plants and external environment[1,2]. Leaf is the organ having the largest contact area with and the most sensitive response to the external environment, which is the main place for photosynthesis of the plant. Its functional traits directly reflect the survival strategy formed by the plant in adaptation to the external environment[3]. At the same time, leaf functional traits feature sensitivity to the external environment, strong ecological plasticity, and easy data measurement, which can effectively reflect the adaptability and ecological trade-offs of plants in different environments[4]. With the advancement of the leaf economic spectrum theory[5], study on relationship between leaf functional traits and the environment has increasingly become a hot issue in ecological research. As a key topographic factor, altitude induces different distribution of water and heat in a small space, which is a natural laboratory for studying the adaptation of plant leaf traits to the external environment[6]. Scholars at home and abroad have conducted a large number of experiments on the changes of leaf functional traits along altitude gradients[7-10]. The research results reveal varying relationship strength and variation scope of the intraspecific traits of different species along altitude gradient. Whether it is consistent with global conclusions on the community scale requires thorough discussion[11].

There are close material connections and interactions between soil and plants[12], and the relationship between plant functional traits and soil is extremely complex. Related research results show that soil nutrients mainly determine the plant leaf traits, while climatic conditions determine the life forms of plants[13]. Zhang Huiwen et al. [14]found that soil water content and total nitrogen content are the main factors affecting the leaf functional traits of spruce in Mount Tianshan. Liu Minxia et al.[15]found that the functional traits of plants on different slopes in alpine meadows are jointly affected by soil moisture and pH. At the same time, studies have found that soil nutrients have a certain impact on the concerted reaction of plant functional traits[16]. Related research results show that the ratio of carbon to nitrogen (C:N) in soil nutrients is negatively correlated with leaf thickness and leaf dry matter content, and positively correlated with specific leaf area, leaf nitrogen content, and leaf phosphorus content[17]. Leaf water content of *Haloxylon ammodendron* is significantly correlated with soil water content, and total leaf phosphorus content is significantly correlated with soil pH[18]. Leaf length, leaf width, leaf area, leaf dry weight of reeds in inland river wetlands are significantly positively correlated with soil surface moisture, and significantly negatively correlated with soil surface salinity[19]. It can be seen that soil nutrients affect the ecosystem function by regulating the functional traits of plants[20].

Rhododendron aganniphum var. *Schizopeplum* belongs to *Rhododendron* of Ericaceae, also known as *Rhododendron fissotectum*, which is native to Changdu Mangkang, Nyingchi Chayu and Milin. The fruit is stubby and erect or slightly curved, and the corolla is pink or white. Continuously distributed in a large area in southeastern Tibet, it is the indicator and dominant species of the Sejila forest line[21,22]. With the continuous in-depth research on plant leaf traits, people gradually realize that studying a single species can let us more accurately reflect the relationship between plant leaf functional traits and the external

environment. Therefore, on the basis of a comprehensive survey, this study carried out a sampling survey of *Rhododendron aganniphum* var. *Schizopeplum* in Sejila Mountain. Through the determination of its leaf functional traits, the change law of the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* with altitude gradient is analyzed. The main soil factors affecting the changes in leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* are investigated. The research results can not only be compared with the research results at the global and community scales, but also can be used to predict the variation characteristics of plant leaf traits under the background of future climate change, enrich the plant trait database, and also provide a scientific basis for the scientific protection and utilization of the germplasm resources and biological diversity of *Rhododendron aganniphum* var. *Schizopeplum*.

II. MATERIALS AND METHODS

2.1 Overview of The Study Area

Located in Nyingchi City, Gongbu Nature Reserve (29°10'-30°15'N, 93°12'—95°35'E), Sejila Mountain belongs to the southward extension of Nyainqentanglha Mountain. This area is in sub-alpine temperate semi-humid climate zone, with warm winter and cool summer, and distinct dry and wet seasons. The average annual temperature is -0.73°C, the average temperature of the hottest month (July) is 9.23°C, the average temperature of the coldest month (January) is -13.98°C, the extreme minimum temperature is -31.6°C, the extreme maximum temperature is 24.0°C, the average annual precipitation is 1134.1 mm. The evaporation is 544.0 mm, accounting for 48.0% of the average annual precipitation. The rainy season is June-September each year. The vegetation in this area mainly includes *Picea likiangensis* var. *Linziensis*, *Rhododendron nivale*, *Quercus semicarpifolia* Smith, *Abies georgei* Orr var. *Smithii*, *Sabina saltuaria*. As a typical mountain system in southeastern Tibet, Sejila Mountain has obvious distribution patterns in vegetation and soil types with the elevation of altitude, which is a natural laboratory for studying the relationship between soil and vegetation in alpine regions[21,23].

2.2 Plot Setting and Sample Collection

At the end of July 2021, with 4003m~4385m above sea level as the study area and a vertical elevation interval of 100m, we selected representative *Rhododendron aganniphum* var. *Schizopeplum* communities, set a 130m long fixed line transect for each, a total of 5 fixed line transects. In each line transect, set up a fixed quadrat of 10m×10m with an interval of 20m on each line transect, a total of 5 quadrats. In each quadrat, randomly select 3 good-growing and disease-free *Rhododendron aganniphum* var. *Schizopeplum*. With the help of long reach chain saw and tree climbing, collect one unobstructed branch with good growth in the four directions of the east, west, south, and north of the middle layer of the canopy, and select 10 disease-free and complete leaves on each branch. There are a total of 120 leaves for each quadrat, with a total of 600 leaves for each line transect. Put it in the middle of the pre-soaked filter paper, place it into a ziplock bag and seal it, then bring it back to the laboratory to measure various traits and indicators. Meanwhile, record the information of each line transect (TABLE I).

TABLE I. Environmental data of the line transect

| NUMBER | ALTITUDE | LONGITUDE | LATITUDE | MAIN VEGETATION |
|--------|----------|-------------------|-------------------|----------------------------------------------------------------------------|
| 1 | 4385m | E94°42'26.0 6" | N29°39'25. 31" | <i>Sabina saltuaria</i> |
| 2 | 4293m | E94°42'31.3 9" | N29°39'19. 93" | <i>Sabina saltuaria, Rhododendron nivale, Potentilla fruticosa, et al.</i> |
| 3 | 4182m | E94°42'37.0 1" | N29°39'15. 55" | <i>Abies georgei Orr var. Smithii, Sabina saltuaria, et al.</i> |
| 4 | 4097m | E94°42'40.6 1" | N29°39'12. 64" | <i>Abies georgei Orr var. Smithii, Potentilla fruticosa, et al.</i> |
| 5 | 4003m | E94°42'45.7 3" | N29°39'07. 62" | <i>Abies georgei Orr var. Smithii, Sabina saltuaria, Rosa, et al.</i> |

The "S" sampling method was selected for sampling using soil auger. Five soil samples of 0~20cm cm soil layer were collected for each quadrat. A total of 125 soil samples were collected, which were put into marked ziplock bags and brought back to the laboratory. The plant residues and gravels in the soil were all picked out and the soil was dried in a natural state, then ground and screened through a 0.25 mm soil sieve to determine the soil nutrient content.

2.3 Determination of Leaf Functional Traits

Bring the collected leaves back to the Tibet Ecological Safety Laboratory, immediately wipe off the moisture of the leaf epidermis with filter paper, put it in a leaf area meter (AM300) to measure the leaf area (LA) and leaf length (LL), and then use an electronic vernier caliper with an accuracy of 0.01 mm. Avoid the main leaf vein, evenly select 3 points along the leaf to measure the thickness, and take the average value as the leaf thickness (LT). Then, put the leaves in a ziplock bag filled with distilled water, and place it in dark environment at 5°C for 8 hours. Immediately after taking it out, use filter paper to absorb the moisture on the leaf surface, and weigh it using an electronic balance with an accuracy of 0.0001g to obtain saturated fresh mass (FW). Then, put the leaves in a cowhide envelope, put them in an oven at 105°C for 20 min deactivation of enzymes, then adjust to 80°C and dry it to a constant dry weight (DW). Finally, the leaves were crushed and sieved to determine the N and P contents of the leaves. Calculate LDMC, SLA, leaf tissue density (LD) with calculation formula as follows:

$$\text{LDMC} = \text{DW} / \text{FW} \quad (1)$$

$$\text{SLA} = \text{LA} / \text{DW} \quad (2)$$

$$\text{LD} = \text{DW} / (\text{LA} \times \text{LT}) \quad (3)$$

Soil pH was measured with a pH meter, soil moisture (SW) was measured with dry weighing method, soil organic matter (SOC) was measured with potassium dichromate external heating method, soil total nitrogen (TN) and leaf N content were all determined by automatic azotometer, soil total phosphorus (TP) and leaf P content were determined by acid-soluble-molybdenum-antimony colorimetric method, soil total

potassium (TK) was determined by acid-soluble-flame photometry; rapidly available phosphorus (AP) was determined by ultraviolet and visible spectrophotometer, rapidly available potassium (AK) was determined by ammonium acetate extraction-flame photometer method²⁴.

2.4 Data Analysis and Processing

Calculate the arithmetic mean values of the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* in all altitude gradients, use single factor analysis and Duncan for multiple comparisons, use Pearson to analyze the correlation between the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*, and use the principal component analysis method to screen out the main indicators affecting change in leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*. Analyze the relationship between the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* and soil factors by the canonical correspondence analysis method. The calculation process and the statistical process were completed by Excel 2010 and SPSS 19.0, and the graphs were plotted by Excel 2010 and Canoco for Windows 4.5. Normally, the variation coefficient (CV) is the ratio of the standard deviation to the mean. $CV \leq 20\%$ suggests weak variation, $20\% < CV \leq 50\%$ indicates moderate variation, and $CV > 50\%$ implies strong variation[25].

III. RESULTS AND ANALYSIS

3.1 Leaf Functional Traits of *Rhododendron aganniphum* var. *Schizopeplum* Under Altitude Gradient

There are significant differences in the 9 leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* in Sejila Mountain (TableII). With the increase of the altitude gradient, the leaf LA, DW, LDMC, LL and SLA of *Rhododendron aganniphum* var. *Schizopeplum* present a downward trend; while N, N:P, LT, P and LD all present an upward trend with the increase of the altitude gradient. The variation coefficient of leaf traits of *Rhododendron aganniphum* var. *Schizopeplum* is ranked in ascending order as follows: $P < N < N:P < LT < LL < LDMC < LA < DW < LD < SLA$. Where, the variation of P, N, N:P, LT, and LL is weak, while the variation of LDMC, LA and DW is medium, and the variation of LD and SLA is strong.

TableII. Leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* under different altitude gradients

| FUNCTIONAL TRAITS | ALTITUDE GRADIENT | | | | | Mean ± SE | CV |
|-----------------------|-------------------|------------------|------------------|-----------------|-----------------|----------------|------------|
| | 4003m | 4097m | 4182m | 4293m | 4385m | | |
| LA (cm ²) | 29.37±8.3 4a | 28.38±8.4 4a | 29.27±6.2 0a | 24.24±4.9 9a | 24.59±6.5 1a | 28.52±9.9 6 | 34.64 % |
| LL (cm) | 11.48±1.8 7 | 10.18±1.6 8bc | 11.07±1.6 5ab | 9.02±0.99c d | 8.99±1.23 d | 10.15±1.8 0 | 17.58 % |
| LT (mm) | 0.48±0.06 b | 0.51±0.08 b | 0.56±0.08a | 0.50±0.08 b | 0.57±0.05 a | 0.54±0.09 | 16.64 % |
| DW (g) | 0.38±0.10a | 0.46±0.06a | 0.42±0.08a | 0.35±0.10 | 0.37±0.09 | 0.41±0.16 | 38.56 |

| | | | | | | | |
|--------------------------|------------------|------------------|------------------|------------------|-----------------|-----------------|------------|
| | b | | | b | ab | | % |
| LDMC (g/g) | 0.30±0.03 b | 0.37±0.05a | 0.28±0.03 b | 0.30±0.04 b | 0.24±0.01 b | 0.30±0.08 | 28.34 % |
| SLA (cm ² /g) | 78.98±18. 46a | 70.76±22. 86a | 69.30±16. 06a | 68.15±11. 35a | 59.93±9.5 8a | 82.26±47. 90 | 57.78 % |
| LD (g/cm ³) | 0.24±0.08a | 0.32±0.04a | 0.22±0.04a | 0.30±0.05a | 0.28±0.01 a | 0.30±0.17 | 56.12 % |
| N (g/kg) | 15.79±17. 72a | 15.01±3.6 0a | 15.01±3.8 2a | 18.54±0.6 9a | 17.51±0.2 0a | 16.37±2.5 7 | 15.14 % |
| P (g/kg) | 0.98±0.09a | 0.98±0.02a | 1.02±0.03a | 1.02±0.05a | 0.98±0.03 a | 0.99±0.05 | 4.60 % |
| N:P | 16.15±2.0 4ab | 15.27±3.1 9b | 14.68±3.2 6b | 18.26±1.0 2a | 17.88±0.4 8a | 16.45±2.6 1 | 15.68 % |

3.2 Correlation and Principal Component Analysis of Leaf Functional Traits of *Rhododendron Aganniphum* var. *Schizopeplum*

3.2.1 Correlation Analysis on Leaf Functional Traits of *Rhododendron Aganniphum* var. *Schizopeplum*

The Pearson correlation test was carried out on the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* under different altitude gradients (Table III). It can be seen that leaf LA and N, N:P of *Rhododendron aganniphum* var. *Schizopeplum* are significantly negatively correlated ($P<0.05$), LA and LL are significantly positively correlated ($P<0.01$); leaf DW and N are significantly negatively correlated ($P<0.05$); leaf N and N:P are significantly positively correlated ($P<0.01$). There is insignificant correlation between the remaining leaf functional traits ($P>0.05$).

Table III. Correlation coefficients of leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*

| | LA | LT | DW | LD MC | SLA | LD | N | P | N:P |
|----------|-------------|------------|-------------|------------|------------|------------|-------------|------------|------------|
| LT | -0.20 3 | 1 | | | | | | | |
| DW | 0.700 | 0.091 | 1 | | | | | | |
| LDM C | 0.381 | -0.60 5 | 0.630 | 1 | | | | | |
| SLA | 0.730 | -0.78 6 | 0.226 | 0.50 0 | 1 | | | | |
| LD | -0.49 6 | -0.24 8 | 0.098 | 0.56 1 | -0.28 9 | 1 | | | |
| N | -0.93 3* | -0.02 3 | -0.89 2* | -0.42 9 | -0.48 1 | 0.316 | 1 | | |
| P | -0.02 3 | 0.182 | -0.11 4 | -0.08 5 | -0.02 8 | -0.30 1 | 0.107 | 1 | |
| N:P | -0.92 1* | -0.06 2 | -0.87 7 | -0.42 7 | -0.46 7 | 0.359 | 0.975 ** | 0.115 | 1 |
| LL | 0.961 ** | -0.27 3 | 0.474 | 0.21 8 | 0.806 | -0.65 9 | -0.80 4 | -0.03 3 | -0.79 8 |

Note: ** indicates a significant correlation at the 0.01 level, * indicates a significant correlation at the 0.05 level.

3.2.2 Principal Component Analysis of Functional Traits of *Rhododendron Aganniphum* var. *Schizopeplum*

Based on the principle that the eigenvalue is greater than 1, three principal components were extracted, and their eigenvalues were 5.227, 2.143, and 1.705 respectively. The contribution rates of these three principal components are 52.268%, 21.429%, and 17.046%, respectively, and the cumulative contribution rate is 90.743 %, indicating that these three main components are the main factors affecting the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*. According to the comprehensive score ranking regarding the principal components of the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*, the leaf DW, LDMC and LA can be used as the main indicators of changes in leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*.

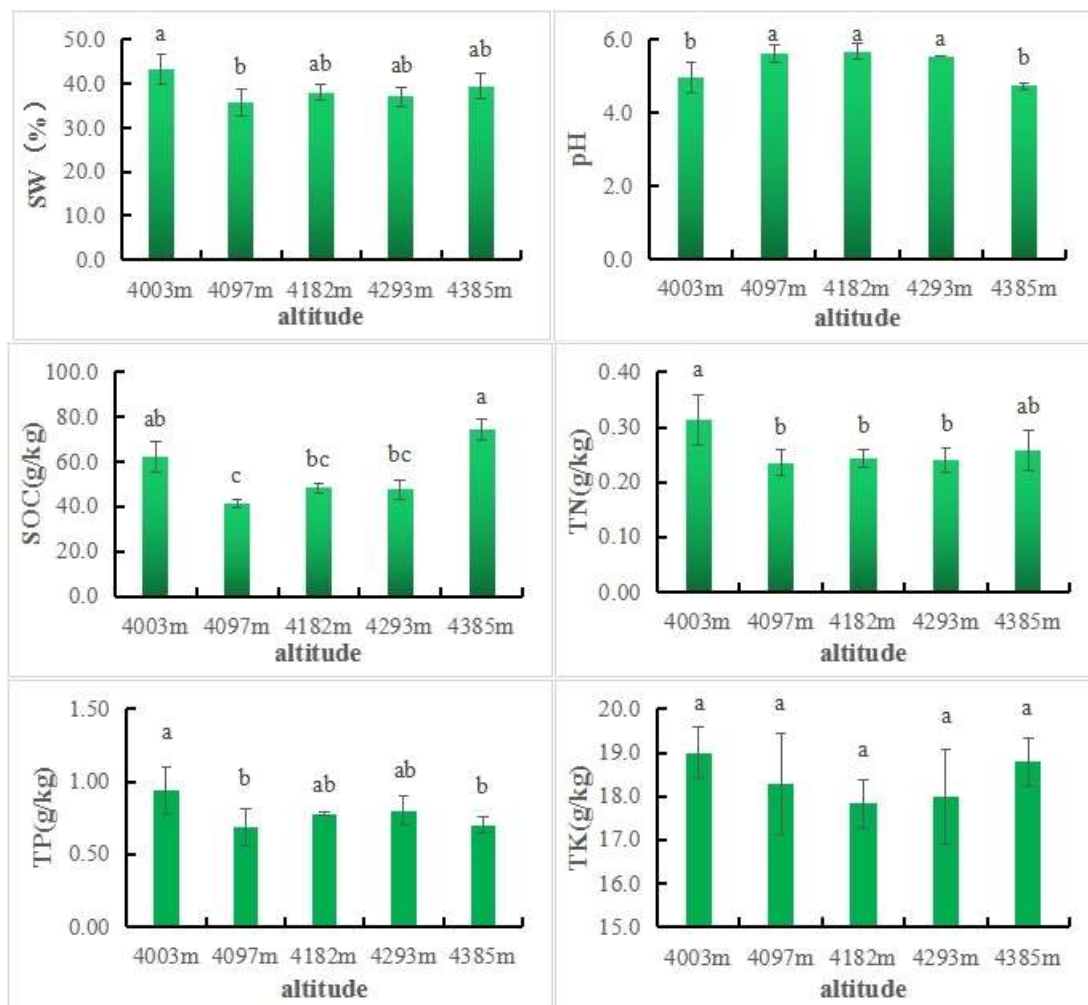
Table IV. Initial factor loading matrix and principal component contribution rate

| FUNCTIONAL TRAITS | PRINCIPAL COMPONENTS 1 | PRINCIPAL COMPONENTS 2 | PRINCIPAL COMPONENTS 3 | COMPREHENSIVE SCORE | COMPREHENSIVE RANKING |
|--------------------------------|------------------------|------------------------|------------------------|---------------------|-----------------------|
| LA | 0.985 | -0.152 | -0.057 | 0.521 | 3 |
| LT | -0.291 | -0.695 | 0.657 | -0.208 | 7 |
| DW | 0.738 | 0.172 | 0.641 | 0.586 | 1 |
| LDMC | 0.561 | 0.763 | 0.143 | 0.530 | 2 |
| SLA | 0.745 | 0.255 | -0.617 | 0.373 | 5 |
| LD | -0.383 | 0.838 | 0.381 | 0.049 | 6 |
| N | -0.942 | 0.129 | -0.304 | -0.569 | 10 |
| P | -0.170 | -0.371 | -0.227 | -0.228 | 8 |
| N:P | -0.928 | 0.203 | -0.273 | -0.538 | 9 |
| LL | 0.913 | -0.251 | -0.307 | 0.409 | 4 |
| eigenvalue | 5.227 | 2.143 | 1.705 | | |
| contribution rates/% | 52.268 | 21.429 | 17.046 | | |
| cumulative contribution rate/% | 52.268 | 73.697 | 90.743 | | |

3.3 Changes of Surface Soil Factors of *Rhododendron Aganniphum* var. *Schizopeplum* Along the Altitude Gradient

Fig 1. shows that the surface soil nutrient content of *Rhododendron aganniphum* var. *Schizopeplum* forest is quite different at different altitudes. The surface soil of *Rhododendron aganniphum* var. *Schizopeplum* forest is acidic. The soil pH at 4003 m and 4385 m is similar, 4.97 and 4.74, respectively.

The difference between the two is insignificant ($P>0.05$), but both are significantly lower than the soil pH at 4293 m~4097 m ($P<0.05$). The SOC content of *Rhododendron aganniphum* var. *Schizopeplum* forest in different altitudes is 41.25~74.44 g/kg, the SOC content is the highest at 4385m, and the SOC content is similar at 4293m and 4182 m. The soil TN content of *Rhododendron aganniphum* var. *Schizopeplum* forest is the lowest at 4097 m(0.23 g/kg) and the highest at 4003 m (0.31 g/kg), which is significantly higher than the soil TN content at other altitude gradients ($P<0.05$). TP content of *Rhododendron aganniphum* var. *Schizopeplum* forest is similar at 4385 m~4097 m, 0.70 g/kg, 0.80 g/kg, 0.78 g/kg, and 0.69 g/kg respectively, reaching the maximum value of 0.94 g/kg at 4003 m, showing significant difference from TP content at the other 4 altitude gradients ($P<0.05$). TK content is between 17.83~19.00 g/kg, and there is no significant difference between different altitude gradients ($P>0.05$). The soil AP content of *Rhododendron aganniphum* var. *Schizopeplum* is the highest at 4003 m, reaching 5.85 mg/kg, and the lowest at 4097 m, which is 2.47 mg/kg. The highest content is significantly different from that of other altitude gradients ($P<0.05$). Soil AK content of *Rhododendron aganniphum* var. *Schizopeplum* forest is the highest at 4003m (94.33 mg/kg) and the lowest at 4293m (37.33mg/kg), showing a significant difference between the two ($P<0.05$).



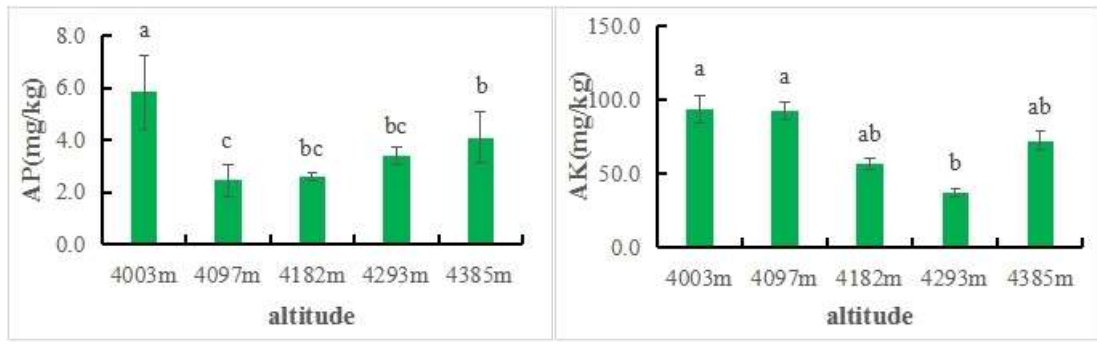


Fig 1: Characteristics of soil factors at different altitudes in the study area

3.4 The Relationship Between Leaf Functional Traits and Soil Factors of *Rhododendron Aganniphum* var. *Schizopeplum*

In order to further analyze the relationship between leaf functional traits and soil factors of *Rhododendron aganniphum* var. *Schizopeplum*, CCA analysis was performed on leaf functional traits and soil factors, with results shown in Fig 2. It can be seen from Fig 2. that soil factors TP, AK, and AP have the greatest impact on leaf functional traits, while TK has the least impact. It can be seen from the figure that soil AK, pH, SW, TP, and TN are positively correlated with leaf LDMC and SLA of *Rhododendron aganniphum* var. *Schizopeplum*, and negatively correlated with LD, N, N:P; soil SOC is positively correlated with leaf P, LD, N, N:P, LT; soil AK, pH are positively correlated with leaf LL, LA, DW.

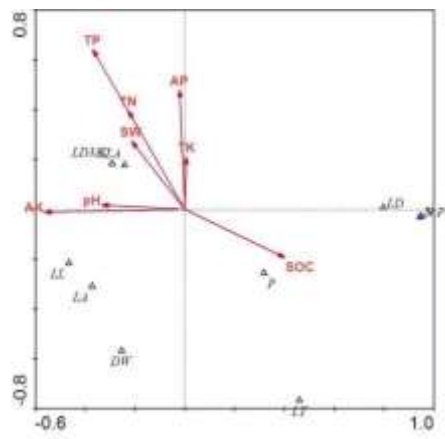


Fig 2: CCA ranking analysis diagram on plant functional traits and soil factors

IV. DISCUSSION

4.1 Changes in Leaf Functional Traits of *Rhododendron Aganniphum* var. *Schizopeplum* with Difference Altitude Gradients

In the study of plant functional traits, leaf traits such as LL, SLA, LDMC can usually reflect the plant ability to acquire and utilize external resources, which are closely related to plant growth[26]. In this study, with the increase of the altitude gradient, the leaf traits of *Rhododendron aganniphum* var. *Schizopeplum* changed from a pioneering strategy characterized by thin and long leaves with big leaf area, big SLA and

LDMC to conservative strategy characterized by thicker and shorter leaves with smaller leaf area, and smaller SLA and LDMC. It indicates that *Rhododendron aganniphum* var. *Schizopeplum* has a higher growth rate and resource acquisition ability in low altitude areas, but uses a conservative strategy in high altitude areas to increase its own nutrient storage and gain a competitive advantage[27]. The N, N:P, and P contents of *Rhododendron aganniphum* var. *Schizopeplum* present an upward trend with the rise of elevation gradient. On the one hand, it may be related to the soil nutrient content, and on the other hand, it may be related to the plant's self-regulation mechanism[28].

Many studies have shown that altitude gradient changes play a crucial role in shaping the leaf functional traits of plants[29,30]. This experiment analyzed the differences of 10 leaf functional traits under 5 altitude gradients, and found that the variation coefficient can reach 4.60%~57.78%, with average variation coefficient at 28.51%, indicating that altitude significantly affects plant functional traits. Among the 10 leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*, the variation coefficient of P is the lowest (4.60%), while the variation coefficient of SLA is the highest (57.78%). This is in line with findings of He Guiping et al.[31] on the leaf traits of woody plants in the forest on northern slope of Motianling, indicating that SLA is more sensitive to changes in altitude gradient, and P is highly conservative.

4.2 Correlation And Principal Component Analysis Of Leaf Functional Traits Of *Rhododendron Aganniphum* Var. *Schizopeplum*

Leaf functional traits do not play a role alone, but there is certain correlation between the traits, and plant ability to adapt to the external environment can be improved through the adjustment and allocation of acquired resources[32]. Therefore, studying the interrelationships between plant functional traits carries very important significance for understanding the adaptation strategies of plants in different habitats. In this study, LA and LL are significantly positively correlated ($P < 0.01$), possibly because longer plant leaf can effectively expand the leaf area. This research result is consistent with findings of Jiao Liang et al.[19], Ma Jiangming et al.[33]. This study found that LA was significantly negatively correlated with N and N:P ($P < 0.05$). On the one hand, N content in plant leaf is closely related to plant photosynthesis. The increase in LL leads to the increase in LA, which results in less N content distributed to unit mass of photosynthetic organs. On the other hand, it may be related to insufficient N in the soil.

In principal component analysis of leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*, the main indicators with higher comprehensive scores should be considered. In this experiment, DW, LDMC, and LA can be used as the main indicators of changes in leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum*. DW, LDMC, and LA comprehensively reflect the plant ability to acquire resources, and the common manifestation is that *Rhododendron aganniphum* var. *Schizopeplum* adapts to the harsh external environment changes in the high-altitude areas of southeastern Tibet through its own related adjustments.

4.3 The Relationship Between Leaf Functional Traits and Soil Factors of *Rhododendron Aganniphum* var. *Schizopeplum*

Plant growth and development depends not only on the physiological characteristics of the plants themselves, but also on external environmental factors, such as soil factors. There is a close interaction between plants and soil factors[34]. On the one hand, the soil provides nutrients and support for the plant growth and development, and affects the changes in plant functional traits. On the other hand, litter residues of plants release nutrients under decomposition of microorganisms and return it to the soil[35]. The results of this experiment show that the main soil factors affecting leaf functional traits include TP, AK and AP, and TK has the least effect on them, indicating that the leaf functional traits of *Rhododendron aganniphum* var. *Schizopeplum* can respond significantly to changes in soil factors to a certain extent. In this study, soil TP and AP were positively correlated with leaf LDMC and SLA, and negatively correlated with leaf P, N, N:P, LD, and DW, indicating that soil P content is the main factor affecting these traits. P not only directly participates in the plant growth and development, but also affects absorption of soil N by plants. This is consistent with the findings of Zhang Kai et al.[36] that soil P content also has a certain effect on the leaf functional traits of the Chinese pine, such as leaf LT, LDMC, TN, etc. Soil SOC is mainly derived from animal, plant and microbial residues. Plant litter enters the soil after decomposition by soil microorganisms, causing different soil SOC contents, thereby affecting plant functions. Studies have shown that SOC is the main soil factor that affects the variation of plant functional traits[37]. Although this study found that soil SOC is positively correlated with leaf P, LD, N, N:P, and LT, it found that soil SOC content is not the main factor affecting plant traits of *Rhododendron aganniphum* var. *Schizopeplum*. This phenomenon needs further exploration and research.

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REFERENCES

- [1] Y. H. Wei, W. Z. Liang, L Han (2021) Leaf functional traits of *Populus euphratica* and its response to groundwater depths in Tarim extremely arid area. *Acta Ecologica Sinica*, 41:5368-5376.
- [2] K. Zhang, J. H. Hou, N. P. He (2017) Leaf functional trait distribution and controlling factors of *Pinus tabuliformis*. *Acta Ecologica Sinica*, 37:736-749.
- [3] B. He, Q. Li, T. Feng (2020) Variation in leaf functional traits of different-aged *Pinus massoniana* communities and relationships with soil nutrients. *Journal of Nanjing Forestry University (Natural Sciences Edition)*, 44:181-190.
- [4] J. L. Funk, W. K. Cornwell (2013) Leaf traits within communities: context may affect the mapping of traits to function. *Ecology*, 94:1893-1897.
- [5] I. J. Wright, P. B. Reich, M (2004) Westoby: The worldwide leaf economics spectrum. *Nature*, 428: 821-827.

- [6] B. J. Graae, P. D. Frenne, A Kolb (2012) On the use of weather data in ecological studies along altitudinal and latitudinal gradients. *Oikos*, 121: 3–19.
- [7] S. W. Liu, Y. B. Ai, Y. H. Liu (2021) Variations in leaf functional traits along the altitude gradient of *Pinus tabuliformis* and its environmental explanations in Beijing Songshan Mountain. *Journal of Beijing Forestry University*, 43:47–55.
- [8] G. Midolo, P. Frenne, N. Hölzel, et al (2019) Global patterns of intraspecific leaf trait responses to elevation. *Global Change Biology*, 25: 2485–2498.
- [9] M. N. Umaña, N. G. Swenson (2019) Intraspecific variation in traits and tree growth along an elevational gradient in a subtropical forest. *Oecologia*, 191: 153-164.
- [10] C. Wang, J. Lu, C. N. Zhou, H. F. Yao, L. Z. JiaYang, B. La (2021) Altitude distribution of leaf functional traits of *Quercus aquifolioides* in southeastern Tibet. *Journal of Forest and Environment*, 41: 366-372.
- [11] Z. J. Li, Q. Tian, L. L. Song (2018) Variation and correlation of leaf traits in woody plants in the north-facing slope of Motianling, Gansu, China. *Journal of Desert Research*, 38: 149-156.
- [12] B. Zhang, X. Z. Lu, J. Jiang, L. Donald, Z. Y. Fu, J. C. Zhang (2017) Similarity of plant functional traits and aggregation in a subtropical forest. *Ecology and Evolution*, 7: 4086-4098.
- [13] J. C. Ordóñez, P. M. Van Bodegom, J. P. M. Witte (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Glob Ecol Biogeogr*, 18:137-149.
- [14] H. W. Zhang, J. Y. Ma, W. Sun, F. H. Chen (2010) Altitudinal variation in functional traits of *Picea schrenkiana* var. *Tianschanica* and their relationship to soil factors in Tianshan Mountains, Northwest China. *Acta Ecologica Sinica*, 30: 5747-5758.
- [15] M. X. Liu, J. Z. Ma (2012) Responses of plant functional traits and soil factors to slope aspect in alpine meadow of South Gansu, Northwest China. *Chinese Journal of Applied Ecology*, 23: 3295-3300.
- [16] D. Li, S. R. L. Kang, M. Y. Zhao (2016) Relationships between soil nutrients and plant functional traits in different degradation stages of *Leymus chinensis* steppe in NeiMongol, China. *Chinese Journal of Plant Ecology*, 40:991-1002.
- [17] M. M. Jager, S. J. Richardson, P. J. Bellingham (2015) Soil fertility induces coordinated responses of multiple independent functional traits. *Journal of Ecology*, 103: 374-385.
- [18] F. Wang, S. J. Guo, B. L. Fan, W. X. Zhang, F. L. Wang (2020) Variation in leaf functional traits of different-aged *Haloxylon ammodendron* communities, and the relationship with soil factors. *Pratacultural Science*, 37: 2486-2496.
- [19] L. Jiao, X. Guan, X. R. Liu, X. G. Dong, F. Li (2020) Functional Traits of *Phragmites australis* Leaves and Response to Soil Environmental Factors in Inland River Wetland. *Arid Zone Research*, 37:202-211.
- [20] Schellberg J, Pontes L D S (2012) Plant functional traits and nutrient gradients on grassland. *Grass and Forage Science*, 67: 305-319.
- [21] W. W. Guo, M. C. Zhuo, J. P. Fang (2020) Anatomical Characteristics and Environmental Adaptability of *Rhododendron aganniphum* var. *Schizopeplum* Leaf in Sejila Mountain, Southeastern Tibet. *Acta Botanica Boreali-Occidentalia Sinica*, 40:0811-0818.
- [22] Z. L. Yang, W. Y. Yu, W. L. Zheng (2021) Variation of fruiting and germination characteristics of *Rhododendron aganniphum* var. *Schiaopeplum* with environmental factors. *Acta Ecologica Sinica*, 41: 101-109.

- [23] Y. Z. Lian, L. H. Cao, H. M. Liu (2021) Characteristics of soil stoichiometric and nutrient contents in high altitude area on the west slope of Sejila mountains. *Journal of Central South University of Forestry & Technology*, 41:140-150.
- [24] T. Gao, H. Quan, J. Lu (2021) Characteristics of topsoil nutrients and their stoichiometry in pinus densata forest in southeast Tibet. *Journal of Northwest A&F University (Natural Science Edition)*, 49:62-70+80.
- [25] J. Qin, H. Y. Kong, H. Liu (2016) Stoichiometric characteristics of soil C,N,P and K in different Pinus massoniana forests. *Journal of Northwest A & F University (Natural Science Edition)*, 44:68-76.
- [26] Q. L. Zhong, L. B. Liu, X. Xu (2018) Variations of plant functional traits and adaptive strategy of woody species in a Karst forest of central Guizhou Province, southwestern China. *Acta Phytocologica Sinica*, 42: 562-572.
- [27] E. Garnier, J. Cortez, G. Billès (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, 85: 2630-2637.
- [28] F. S. Chapin, A. J. Bloom, C. B. Field (1987) Plant responses to multiple environmental factors: Physiological ecology provides tools for studying how interacting environmental resources control plant growth. *Bioscience*, 37: 49-57.
- [29] R. Yang, B. R. Zhang, L. L. Wang (2015) The Response of Plant Functional Traits Group to Gradients of Altitude in Dry-hot Valley of Yuan-Mou. *Ecology and Environmental Sciences*, 24:49-56.
- [30] Z. J. Li, Q. Tian, L. L. Song:Variation and association of woody leaves on the northern slope of Mortianling, Gansu Province. *Journal of Desert Research*,(1),38(2018).
- [31] G. P. He, Q. Tian, Z. J. Li (2018) Change in Leaf Functional Traits of Woody Plants Along Altitudinal Gradients at Species and Community Levels on the Motianling Northern Slope. *Acta Botanica Boreali-Occidentalia Sinica*, 38: 553-563.
- [32] Cornwell W K, Schwilk D W, Ackerly D D (2006) A trait-based test for habitat filtering: Convex hull volume. *Ecology*, 87: 1465-1471.
- [33] J. M. Ma, X. Z. Zhang, S.C. Liang (2012) Leaf traits of common plants in Yaoshan Mountain of Guilin, China. *Journal of Guangxi Normal University (Natural Science Edition)*, 30:77-82.
- [34] J. Ding, Q. Wu, H. Yan (2011) Effects of topographic variations and soil characteristics on plant functional traits in a subtropical evergreen broad-leaved forest. *Biodiversity Science*, 19:158-167.
- [35] P. Wang, L. X. Sheng, H. Yan (2010) Plant functional traits influence soil carbon sequestration in wetland ecosystem. *Acta Ecologica Sinica*,30: 6990-7000.
- [36] K. Zhang, J. H. Hou, N. P. He (2017) Leaf functional trait distribution and controlling factors of Pinustabuliformis. *Acta Ecologica Sinica*, 37: 736-749.
- [37] Y. Y. Duan, L. J. Song, S. Q. Niu (2017) Variation in leaf functional traits of different-aged Robinia pseudoacacia communities and relationships with soil nutrients. *Chinese Journal of Applied Ecology*, 28:28-36.