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Original Research

Adaptation of Leaf Functional Traits of Alpine Meadow Plants to Environmental Factors in the Qilian Mountains

Ling-Ling Song[1]*, Hao Li[2], Bin Xu[2], Zongxing Li[3], Fang Liu[2], Xiaxia Li[2], Zexi Chen[2]**

[1]College of Forestry, Gansu Agricultural University, Lanzhou, Gansu 730070, China
[2]College of Energy and Power Engineering, Lanzhou University of Technology, Lanzhou 730050, China
[3]Key Laboratory of Ecohydrology of Inland River Basin Gansu/Hydrology and Water Resources Engineering Research Center, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, China

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Abstract

This study systematically explored the relationship between leaf functional traits and environmental factors based on 78 quadrats arranged and 35 dominant species of plants investigated in July 2021 in the Qilian Mountains. The results showed significant differences in plant functional traits among different vegetation types. The average values of the nitrogen balance index (NBI), chlorophyll content (Chl), leaf width (LW), flavonol content (Flv), leaf area (LA), and leaf circumference (LC) for shrubs were lower than those of herbs, while the average values of NBI, LA, Chl, LW, Flv, and LC for trees were lower than those of shrubs. The NBI had an obvious positive relationship with Chl, whether for herbs or shrubs and trees, while the NBI had a significant negative relationship with anthocyanin content (Anth) for herbs. The soil temperature decreased from 0-20 cm (20.26ºC) to 80-100 cm (14.9ºC), and the soil temperature of 60-80 cm was equivalent to 80-100 cm. Compared with herbs, shrubs are tall and have higher requirements for the growth environment. The growth and chlorophyll content of shrubs decreased with an increase in altitude. Like herbs and shrubs, trees had significant altitude differences in cold regions. The direct influencing factors of leaf functional traits of plants are climate and soil conditions in cold regions, while the indirect factors are mainly geospatial variations, local environment, and underlying surface conditions.

Keywords: ecological restoration, plant functional traits, influencing mechanism, environmental factors, Qilian mountains region

*e-mail: sll2503@163.com
**e-mail: lizxhhs@163.com

Introduction

A series of natural disasters caused by global climate change and anomalies have caused great damage to ecosystems and natural resources, directly influencing and even threatening human living environments and social security [1]. Therefore, ecological and environmental issues have recently become a global frontier issue that has received much attention, especially in cold regions' ecological security issues [2]. Climate warming will accelerate the global water cycle, leading to changes in precipitation distribution patterns. This change will directly or indirectly impact the balance between plants and the environment, resulting in changes in the competitive relationship between plants and ultimately affecting the stability of biodiversity [3]. Therefore, the study of the functional characteristics of different types of plants in different ecological environments can reflect the adaptive capacity and the long-term response characteristics of plants in the natural environment [4]. The different morphological characteristics of plant functional organs indicate the unique way of balancing and redistributing resources in the inadequacy of water and heat or barren environment and reflect the survival strategies of plants in dealing with different resources [5-6].

The leaf functional traits of plants will also be different at different stages of the growth period due to the changes in hydrothermal conditions. Leaf traits are characteristic parameters at the leaf level that plants exhibit for adapting to the environment and have important indicative significance for environmental changes [7]. They can reflect the adaptation and survival countermeasures of plants to environmental changes and have importance indicating significance for the change of ecological environment [8]. Furthermore, leaf traits have the characteristic of simple measurement and provide access to a large number of complete data. Most researchers also use them as the preferred index to study a plant's ecological adaptability and survival strategy. As the living conditions of plants, soil resources are various manifestations of nutrient cycling, water conservation, and other ecosystem carriers [9]. Plants absorb water and nutrients from the soil, so their physical and chemical properties will affect the diversity of plant communities to a certain extent. The response of plant functional traits studied at the community level to topographic changes may cause uncertainty in the research conclusion of the relationship between functional traits at the community level and soil physical and chemical properties because of the neglect of the influence of different plant phylogenetic backgrounds [10]. The dominant soil factors affecting the functional properties of plant communities are different in different terrains and ecosystems. Therefore, studying the response of plant community functional traits to different terrains and ecosystems can better reveal the adaptive strategies of plants to the environment and the distribution pattern of vegetation in the study area, which is of great significance to the protection and maintenance of the structure and function of the alpine meadow ecosystem in the Qinghai Tibet Plateau [11].

As an important part of the national ecological security strategic pattern, Qilian Mountain plays an important ecological role in water conservation and species diversity [12]. There are rich species and great environmental differences in this area. Meanwhile, the vegetation types in the Qilian Mountains are simple, with sparse growth and low coverage, mainly characterized by alpine grasslands and cushion vegetation. Therefore, it is very urgent to conduct research on plant functional traits in the Qilian Mountains, where vegetation is single and ecologically fragile. This has important support for ecological protection and vegetation restoration in the Qinghai Tibet Plateau, and its theoretical and practical significance is significant [13-15]. Therefore, this study focuses on the natural plant community in the Qilian Mountains and collected plant samples from 78 quadrats arranged, and 35 dominant species of plants were investigated in July 2021. This study aims to: (1) analyze the ecological significance of leaf functional characteristics of trees, shrubs, and herbs in the Qilian Mountains; (2) determine the correlation among leaf functional traits (trees, shrubs, and herbs) of different plant types; and (3) clarify the mechanism of leaf functional traits affecting environmental factors. The results will be helpful in revealing the changes in characteristics and spatial distribution patterns of leaf functional traits in alpine regions and the influential mechanism of leaf functional traits to environmental factors in the alpine region.

Materials and Methods

Study Area

Qilian Mountain is located at the junction of the Qinghai-Tibet Plateau, the Mongolian Plateau, and the Loess Plateau (Fig. 1). It is the water basin of the Heihe River, Shule River, and Shiyang Rivers. As a typical alpine and semi-arid mountain area, the Qilian Mountain area has the characteristics of a large altitudinal range (1700-5800 m), a significant hydrothermal gradient, and a sensitive response to climate change. Qilian Mountain is far from the ocean and has long been controlled by westerly winds. It has the characteristics of continental alpine and semi-humid mountain climates. At the same time, it is affected by warm and humid Pacific air masses, Atlantic water vapor, and Arctic water vapor in different seasons. The Qilian mountain area has an average annual precipitation of about 300-700 mm, mostly in the form of rainfall. The vertical zoning and east-west changes of vegetation in the Qilian mountain area are obvious, and the floristic components also change significantly with altitude, mainly including temperate desert vegetation, alpine shrub and shrub meadow vegetation, alpine

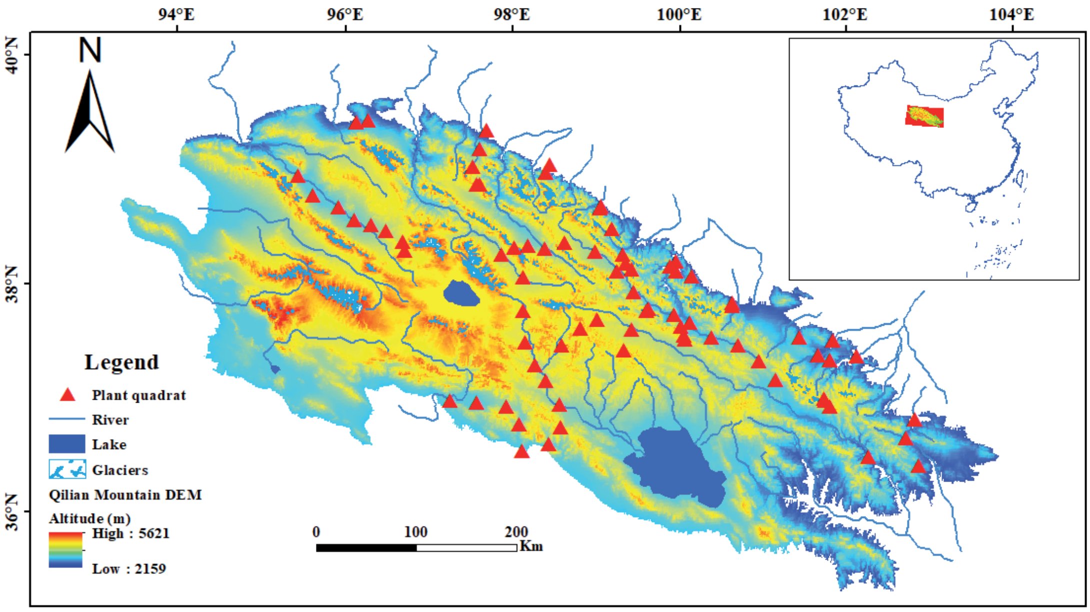

Fig. 1. Study area and the distribution of plant quadrat.

meadow, cushion vegetation and lichen, and alpine sub-ice and snow sparse vegetation. They are often staggered with grasslands, swamps, and water areas in strips or blocks, forming a mountain composite ecosystem. The unique and extreme ecological environment and less human disturbance make this area ideal for studying the functional characteristics of vegetation leaves in Alpine mountainous areas.

Data and Analysis

The sampling work of this study was carried out in July 2021 in the Qilian Mountains. A total of 78 quadrats were arranged (Fig. 1), and a total of 35 dominant plant species were investigated in the Qilian Mountains (Table 1). Because most areas in the Qilian Mountains are uninhabited, sampling in this study was a challenge. In order to ensure smooth and safe on-site work, we conducted on-site investigations along the main transportation routes in the Qilian Mountains. July is the peak period for vegetation growth in cold regions, so we chose to conduct field investigations on plant functional traits in July. The area of the survey quadrat is 20 m × 20 m for trees, and the area of the survey quadrat is 10 m × 10 m for shrubs. 5 small quadrats were then selected from each survey quadrat (the small sample area is 1 m × 1 m) to conduct a vegetation survey for herbs. Four small quadrats and one small quadrat are distributed at the four corners and the middle of the survey quadrat, respectively. To investigate the functional traits of plant leaves, we usually choose three plants of the same species with good growth conditions and similar crown width, height, and diameter. During the process of field investigation and observation sampling, the species, vegetation coverage, abundance, and plant height in each quadrat shall be recorded synchronously, and the basic characteristic values of plants shall be preliminarily determined in this quadrat. At the same time, each sample site's longitude, latitude, and altitude were measured and recorded by GPS. This study conducted a systematic analysis based on 10 functional traits of leaves (Table 2). The leaf area (LA) was measured using an AM-350 portable measurement device. In the process of leaf area measurement, 30 leaves of each plant that was growing well were selected, then the leaf area (LA) was measured one by one, and finally, the average value of 30 leaf areas was calculated. The leaf dry matter content (LDMC) was the ratio of leaf dry weight to leaf fresh weight of each plant species. Nitrogen balance index (NBI), chlorophyll content (Chl), flavonol content (Flv), and anthocyanin content (Anth) were measured by a plant polyphenol chlorophyll meter. The nitrogen balance index (NBI) is the chlorophyll content to flavonol content ratio. The nitrogen balance index (NBI) ranges from 0 to 999. The chlorophyll content (Chl) ($\mu g/cm^2$) ranges from 0 to 150 $\mu g/cm^2$. The flavonol content (Flv) is the phenol index but mainly represents the flavonol content. The range of flavonol content (Flv) is from 0 to 3. Moreover, the anthocyanin content (Anth) is also the phenolic index, which represents the content of anthocyanins. The range of this index is from 0 to 3. The nitrogen balance index is defined as the ratio of chlorophyll to flavonoids, which is an indicator used by the body to reflect the degree of utilization of plant proteins after absorption and digestion [16]. The relative chlorophyll content mainly reflects plants' photosynthetic capacity, while the equivalent water thickness, flavonoid content, and nitrogen balance index, respectively, reflect the water status, antioxidant capacity, nitrogen nutrition status, and growth trend of plants [17].

Table 1. The major plant species investigated in the Qilian Mountains.

Plant type	Major plant species
Herbs	*Artemisia sacrorum, Saussurea amara, Thermopsis przewalskii, Plantago asiatica, Lancea tibetica, Oxytropis myriophylla, Carduus nutans, Saussurea japonica, Aster alpinus, Corethrodendron multijugum, Reaumuria soongarica, Oxytropis ochrocephala, Suaeda glauca, Stellera chamaejasme, Pedicularis sylvatica, Ranunculus japonicus, Saussurea pulchra, Gentiana macrophylla, Zygophyllum xanthoxylon, Potentilla ChInensis, Reaumuria kaschgarica, Comarum salesovianum, Polygonum sibiricum, Ligularia sibirica, Oxygraphis glacialis, Kalidium foliatum, Thermopsis fabacea, Salsola passerina*
Shrubs	Potentilla fruticosa, Potentilla glabra, Caragana sinica, Nitraria tangutorum
Tree	Picea crassifolia, Juniperus przewalskii

Table 2. Main Plant Functional Trait Indicators and Ecological Significance.

Index	Abbreviation	Unit	Definition	Ecological significance
Leaf Length	LL	cm	The length of plant leaves from base to tip	Reflecting the acquisition of resources and the preservation and assimilation of water by plants is the most intuitive manifestation of plant growth and development
Leaf Width	LW	cm	The width of the plant leaves at their widest point	Reflecting the acquisition of resources and the preservation and assimilation of water by plants is the most intuitive manifestation of plant growth and development
Leaf Circumference	LC	cm	The length of a circle around the edge of a plant leaf	Reflecting the acquisition of resources and the preservation and assimilation of water by plants is the most intuitive manifestation of plant growth and development
Leaf Shape Coefficient	SC	/	The ratio of the area of each leaf to the product of its length and width	Known as the shape coefficient or correction factor, this coefficient reflects the shape of plant leaves
Leaf Area	LA	mm^2	The average single-sided projected surface area of a leaf or leaf	Reflecting the ability of plants to capture light
Leaf Dry Matter Content	LDMC	g/kg	The ratio of leaf dry weight to saturated fresh weight	Indicating the amount of resources the leaves acquire and invest in building reflects the plant's ability to resist external factors. The changes are closely related to leaf tissue density, and plants with high dry matter content in their leaves have stronger resistance to physical stress
Chlorophyll Content	Chl	$\mu g/cm^2$	The content of chlorophyll in plant leaves	Directly affecting the potential of plant photosynthesis
Flavonoid Content	Flv	mg/g	The content of flavonoids in plant leaves	Indirect reflection of the response mechanism and adaptation strategy of leaf photosynthetic structure in response to different environments in plants
Nitrogen Balance Index	NBI	/	The ratio of chlorophyll to flavonoids	An important indicator reflecting the growth status of plants, as well as the degree of utilization of food protein by the body after digestion and absorption.
Anthocyanin Content	Anth	mg/g	The content of anthocyanins in plant leaves	Indirect reflection of the response mechanism and adaptation strategy of leaf photosynthetic structure in response to different environments in plants

The most direct factors affecting the functional traits of plant leaves in high-altitude regions are altitude and soil characteristics. Soil moisture content: Using the drying method to determine soil moisture content, fresh soil samples are subjected to fresh weight measurement and then dried in a 105ºC oven to constant weight. The calculation formula for soil moisture content is: soil moisture content (%) = (original soil weight – dried soil weight)/dried soil weight x 100%.

Soil temperature: Soil temperature is measured using an EM50 thermometer. Three random measurements were taken in each soil layer to determine the average value.

Soil bulk density: The soil bulk density was measured using the ring knife method. The ring knife filled with soil samples is placed in a 105ºC oven to dry to a constant weight and weighed. The calculation formula for soil bulk density is: soil bulk density = (ring knife weight after drying + dry soil weight) – ring knife weight)/ring knife volume.

The data used in this study were all processed by EXCEL software. Meanwhile, the drawing was mainly completed using Origin 19.0 software. The Pearson correlation test and one-way ANOVA used in the data analysis of this study were all completed using SPSS 19.0 software. Meanwhile, the arithmetic mean and standard deviation of each personality trait are calculated, and the coefficient of variation (CV) is used to calculate the degree of variation of each functional trait. A single-factor analysis of variance and the least significant difference (LSD) method were used to test the significant differences in leaf traits of plant leaves among different growth types.

Results and Discussions

Variation Pattern

It can be seen from Table 3 that the average value of NBI, Chl, LW, Flv, LA, and LC for shrubs was lower than that of herbs, while the average value of NBI, LC,

Table 3. The general pattern of plant functional traits of plants in the study area (Chl:μg/cm^2; LA:mm^2; LDMC:g/g; LW, LL, LC: mm).

Plant type	Plant Functional Trait	Range	Average Value±SD	CV
Herb plants	NBI	0.1-55.5	20.02±1.19	0.47
	Chl	0.1-70.4	31.05±2.08	0.53
	Flv	0.76-2.04	1.52±0.04	0.21
	Anth	0.01-0.35	0.107±0.01	0.64
	LA	14.9-1975.5	283.35±38.97	1.10
	LW	2.87-172.5	15.87±2.82	0.82
	LL	7.97-97.85	32.61±2.24	0.54
	LC	18.6-295.36	90.13±8.35	0.74
	SC	0.09-1.02	0.49±0.02	0.42
	LDMC	0.09-0.57	0.23±0.01	0.38
Shrub plants	NBI	3.5-47.6	17.91±1.92	0.61
	Chl	3.5-63.7	23.41±2.79	0.68
	Flv	0.87-1.93	1.41±0.05	0.19
	Anth	0.02-0.27	0.12±0.01	0.50
	LA	18.6-274	63.52±9.08	0.82
	LW	3.86-54.24	10.96±1.50	0.79
	LL	5.59-63.8	13.41±1.80	0.77
	LC	18.61-373.1	54.38±11.31	1.19
	SC	0.03-0.83	0.51±0.02	0.29
	LDMC	0.06-0.61	0.37±0.02	0.32
Arbor plants	NBI	1.3-47.8	16.8±7.67	1.01
	Chl	1.0-74.0	23.4±4.69	0.45
	Flv	0.81-1.57	1.26±0.14	0.25
	Anth	0.01-0.29	0.09±0.04	1.11
	LA	15.0-35.75	20.87±3.40	0.36
	LW	1.3-4.71	2.36±0.55	0.52
	LL	11.82-15.75	14.12±0.65	0.10
	LC	20.3-35.09	28.53±2.23	0.17
	SC	0.26-0.47	0.36±0.02	0.11
	LDMC	0.43-0.49	0.45±0.06	0.32

LW, Flv, LA, and Chl for trees was higher than that of shrubs. The adaptability of herbs to alpine environments was stronger than that of shrubs and trees. Thus, herbs were less threatened to suffer nitrogen fertilizer stress than shrubs and trees in high and cold regions. However, the average value of Anth and SC for shrubs was higher than that of herbs, and the Anth and SC for herbs was higher than that of trees. This result also showed that shrubs growing in cold regions have very important ecological significance to the environment of cold regions. In addition, the vulnerability of the alpine environment is a challenge to all plants growing in the cold region [18]. Therefore, all plants growing in the cold region adapt to the growth environment of the alpine region by changing their functional properties, among which the leaf functional traits of herbs and trees have changed more significantly. Furthermore, the average value of LL of trees and shrubs was lower than that of herbs, while the average value of LL of shrubs was lower than that of trees. More importantly, the average value of LDMC of trees was higher than that of shrubs, and the average value of LDMC of shrubs was higher than that of herbs. This result shows that in terms of the ability of various plants to obtain resources in the Qilian Mountains, trees and shrubs have stronger advantages than herbs. The variation in leaf area of herbaceous plants ranged from 14.9 mm^2 to 1975.5 mm^2. More importantly, the range of leaf area change of herbs was larger than that of shrubs and trees. This result indicated that all plants can better adapt to the cold area environment by changing the leaf area, while herbaceous plants can better adapt to the cold area environment by changing the leaf area than shrubs and trees to better carry out photosynthesis and adapt to the rare area environment. Moreover, the coefficient of variation is the main indicator for measuring the degree of dispersion of relevant indicators [13]. Based on their specific value, the relevant indicators can be divided into strong variability indicators ($CV \geq 0.5$), medium variability indicators ($0.2 < CV < 0.5$), and weak variability indicators ($CV \leq 0.2$) [11]. According to this standard, among the leaf functional traits of herbaceous plants in the Qilian Mountains, Flv, LDMC, SC, and NBI are moderately variable leaf functional trait indicators, while Chl, Anth, LA, LW, LL, and LC belong to strongly variable leaf functional trait indicators. For shrub plants, Flv belongs to weakly variable leaf functional trait indicators; LDMC and SC are moderately variable leaf functional trait indicators, while NBI, Chl, Anth, LA, LW, LL, and LC belong to strongly variable leaf functional trait indicators. Therefore, for the leaf functional traits of shrub plants in high cold regions, only Flv is relatively stable, with a weak response to external environmental changes and strong internal stability, and can be used as a relatively stable herbaceous plant leaf functional trait, while other leaf functional traits are more sensitive to environmental changes. For trees, LL, LC, and SC are weakly variable leaf functional trait indicators; LDMC, Chl, Flv, and LA are moderately variable leaf functional trait indicators, while NBI, Anth, and LW are strongly variable leaf functional trait indicators. Therefore, for the leaf functional traits of trees in high-altitude cold regions, only LL, LC, and SC are relatively stable, with a weak response to external environmental changes and strong internal stability, and can be used as relatively stable leaf functional traits of trees, while other leaf functional traits are more sensitive to environmental changes [15].

Correlation Analysis

As can be seen from Table 4, NBI values of herbs, shrubs, and trees showed an obvious positive correlation with Chl values ($P<0.01$). Meanwhile, Chl values of herbs and shrubs showed a significant positive correlation with Flv values, but Chl values of trees showed a positive correlation with Flv, which was not significant. What's more, LW values of herbs were significantly positively correlated with LL, LA, and LC ($P<0.01$). LW values of shrubs were also significantly positively correlated with LC ($P<0.01$), but LW of shrubs was significantly negatively correlated with SC ($P<0.01$). However, LW values of trees were also significantly positively correlated with LA values ($P<0.01$). These results indicated the adaptability of different types of plants to alpine environments. Moreover, LL values of herbs and shrubs and SC showed a significant negative correlation. However, LL values were positively correlated with LC values. However, LL values of herbaceous plants were significantly positively correlated with LA values. This result confirms the above statement. Furthermore, there was a significant positive correlation between Flv and SC in herbs ($P<0.05$). However, Anth was negatively correlated with SC, which once again confirmed that the shape coefficient of herbs was closely related to anthocyanin and flavonol. Meanwhile, the SC of herbs was negatively correlated with LC ($P<0.01$) and LDMC ($P<0.05$), while the SC of shrubs was negatively correlated with LC ($P<0.01$). For plant leaf function traits, the relationship among leaf functional traits of herbs was more complex than that of shrubs, and the relationship among leaf functional traits of trees was the simplest [18].

Soil Properties

The average soil temperature in the 0-20 cm layer was higher than that in the 20-40 cm layer (Fig. 2). Meanwhile, the average soil temperature in the 20-40 cm layer was higher than that in the 40-60 cm layer, which further confirmed the existence of permafrost in cold regions, especially the existence of ground ice in permafrost. More importantly, the soil temperature of 60-80 cm was equal to that of 80-100 cm, which indicated that the soil temperature was basically stable or lower when the soil layer was below 60 cm. The distribution of maximum and minimum values also verified this result. Moreover, the average soil moisture in the 0-20 cm layer

Table 4. The correlation among the leaf functional traits of different plant types.

Herbs	NBI	Chl	LW	LL	Flv	Anth	SC	LDMC	LA	LC
NBI	1									
Chl	.929**	1								
LW	0.045	0.064	1							
LL	0.14	0.183	.466**	1						
Flv	.295*	.517**	0.097	0.101	1					
Anth	-0.058	-0.118	-0.08	-0.098	-0.153	1				
SC	0.084	0.14	-0.065	-.413**	.296*	-.402**	1			
LDMC	0.046	0.029	-0.202	0.009	-0.012	0.172	-.267*	1		
LA	0.032	0.08	.772**	.711**	0.169	-0.142	-0.087	-0.196	1	
LC	0.036	0.027	.501**	.861**	-0.049	0.001	-.521**	0.081	.702**	1
Shrubs	NBI	Chl	LW	LL	Flv	Anth	SC	LDMC	LA	LC
NBI	1									
Chl	.828**	1								
LW	-0.126	-0.117	1							
LL	0.014	-0.027	0.208	1						
Flv	0.16	.341*	0.011	-0.204	1					
Anth	0.124	0.241	-0.192	0.02	-0.231	1				
SC	0.072	0.075	-.513**	-.540**	-0.156	0.263	1			
LDMC	0.272	0.295	0.051	-0.319	-0.017	0.159	0.31	1		
LA	0.011	0.066	0.264	0.172	0.148	0.038	-0.143	0.075	1	
LC	0.005	0.007	.596**	.815**	-0.073	-0.107	-.726**	-0.176	0.056	1
Tree	NBI	Chl	LW	LL	Flv	Anth	SC	LDMC	LA	LC
NBI	1									
Chl	.992**	1								
LW	0.469	0.475	1							
LL	-0.393	-0.467	0.313	1						
Flv	0.502	0.561	0.334	-0.231	1					
Anth	-0.306	-0.409	-0.488	0.383	-0.549	1				
SC	0.068	0.07	0.39	0.366	0.575	0.07	1			
LDMC	0.156	0.036	-0.036	0.338	-0.608	0.763	-0.162	1		
LA	0.16	0.139	.909*	0.676	0.158	-0.244	0.449	0.078	1	
LC	0.261	0.235	0.77	0.44	-0.122	-0.355	-0.172	0.198	0.794	1

**There was a significant correlation at the 0.01 level (bilateral). * There was a significant correlation at the 0.05 level (bilateral).

was higher than that in the 20-40 cm and 40-60 cm layers, while soil moisture in the 80-100 cm layer was higher than that in the 40-60 cm layer. The soil moisture above 60 cm was greatly affected by the changes in the surface environment, especially the infiltration of land precipitation and glacier snow meltwater. However, the supra-permafrost water and the ground ice meltwater mainly recharged soil moisture below 60 cm. On the one hand, most of the plants growing in the alpine region are deep-rooted, cold-tolerant plants because they are affected by the local environment of the alpine region. Therefore, soil moisture in deep soil was very likely to be the main recharge source of plant water in alpine regions. On the other hand, the surface soil has

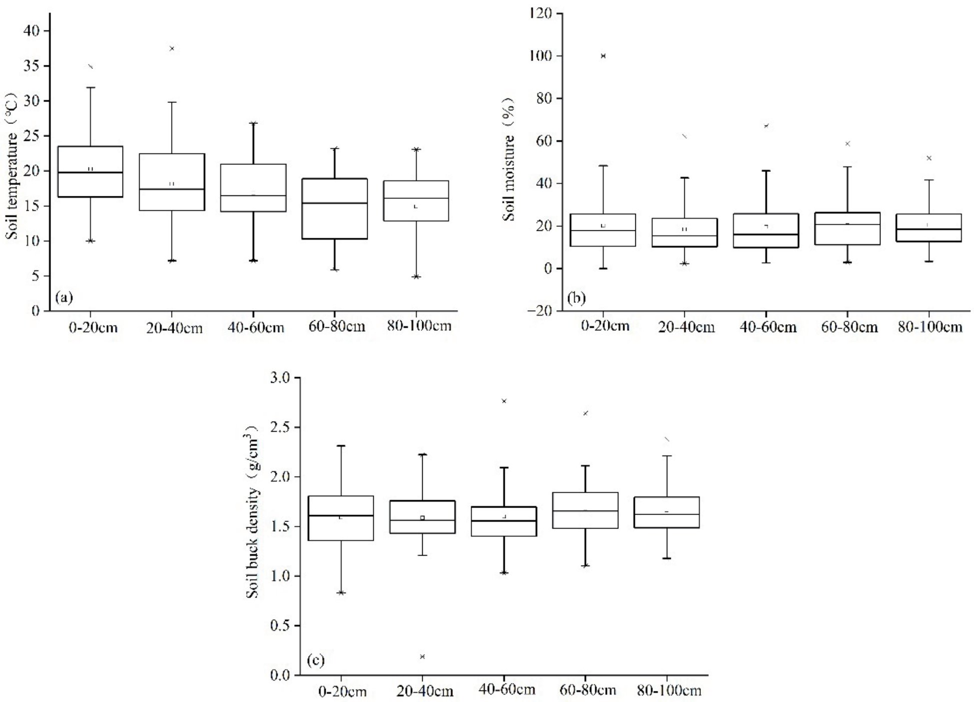

Fig. 2. Box diagram of soil temperature, soil moisture, and soil bulk density (*indicates outliers).

a large water content, which may be mainly caused by the infiltration of precipitation or the infiltration after the melting of glaciers and snow [14]. Furthermore, the soil bulk density fluctuated significantly from 0-20 cm to 80-100 cm in the Qilian Mountains. Therefore, there is no clear change trend in the change characteristics of soil bulk density in different soil layers, which to some extent indicates uncertainty in the soil structure characteristics in different soil layers.

Effects of Soil Physical Properties on Leaf Functional Traits

As the soil temperature increased, the NBI ($P>0.05$) and Chl ($P<0.05$) decreased at first and then increased (Fig. 3), which indicated that the soil temperature had little effect on the growth index of plants in the Qilian Mountains, or NBI and Chl would have a critical value with the increase of soil temperature. That is, when the critical value was greater than the values of NBI and Chl, the soil temperature was negatively correlated with NBI and Chl. On the other hand, when the critical value was less than NBI and Chl, the soil temperature was positively correlated with NBI and Chl. However, soil temperature was negatively correlated with LC, LL, LA, and LW, but not significantly, while it was negatively correlated with Flv ($P<0.05$) and SC ($P<0.01$). This may be mainly because the direct relationship between leaf width, leaf length, leaf area, leaf perimeter, and soil temperature was not particularly close. However, anthocyanin content and LDMC were significantly positively correlated with soil temperature, reflecting that plant leaves' anthocyanin content and the degree of the plant's access to light, water, and nutrients would also increase [19]. Soil temperature is a key factor connecting soil, vegetation, and atmosphere. It is a comprehensive reflection of natural conditions such as climate, vegetation, and terrain, and it is the foundation for vegetation growth and the healthy development of regional ecosystems. In addition, various biochemical processes in soil are influenced by soil temperature. Soil temperature is a very important factor in the nutrient supply of shrub leaves in cold regions, and it is also one of the main factors determining whether shrub plants are limited by nitrogen and phosphorus elements [11].

The same as soil temperature, a significant difference in correlation also appeared between plant leaf functional traits and soil moisture (Fig. 4). Soil moisture was positively correlated with NBI, Chl, Flv, and SC, while it was negatively correlated with Anth and LDMC. This result may be mainly due to the close relationship between the inherent attributes of plants and the adaptation to the cold environment and soil moisture in the Qilian Mountains. Meanwhile, soil moisture and LC, LL, LA, and LW increased first and then decreased. The root system of plants lives directly in the soil, so the amount of soil moisture directly affects the development of plant roots. At the same time, with the increase of soil moisture, the synthesis of nitrogen and protein in plants decreases, and the starch content increases accordingly, making plants grow healthy and robust in alpine regions. Soil moisture is a key factor in plant water absorption and utilization strategies. This affects the internal environment of the community, forcing plants to undergo phenotypic regulation and thereby

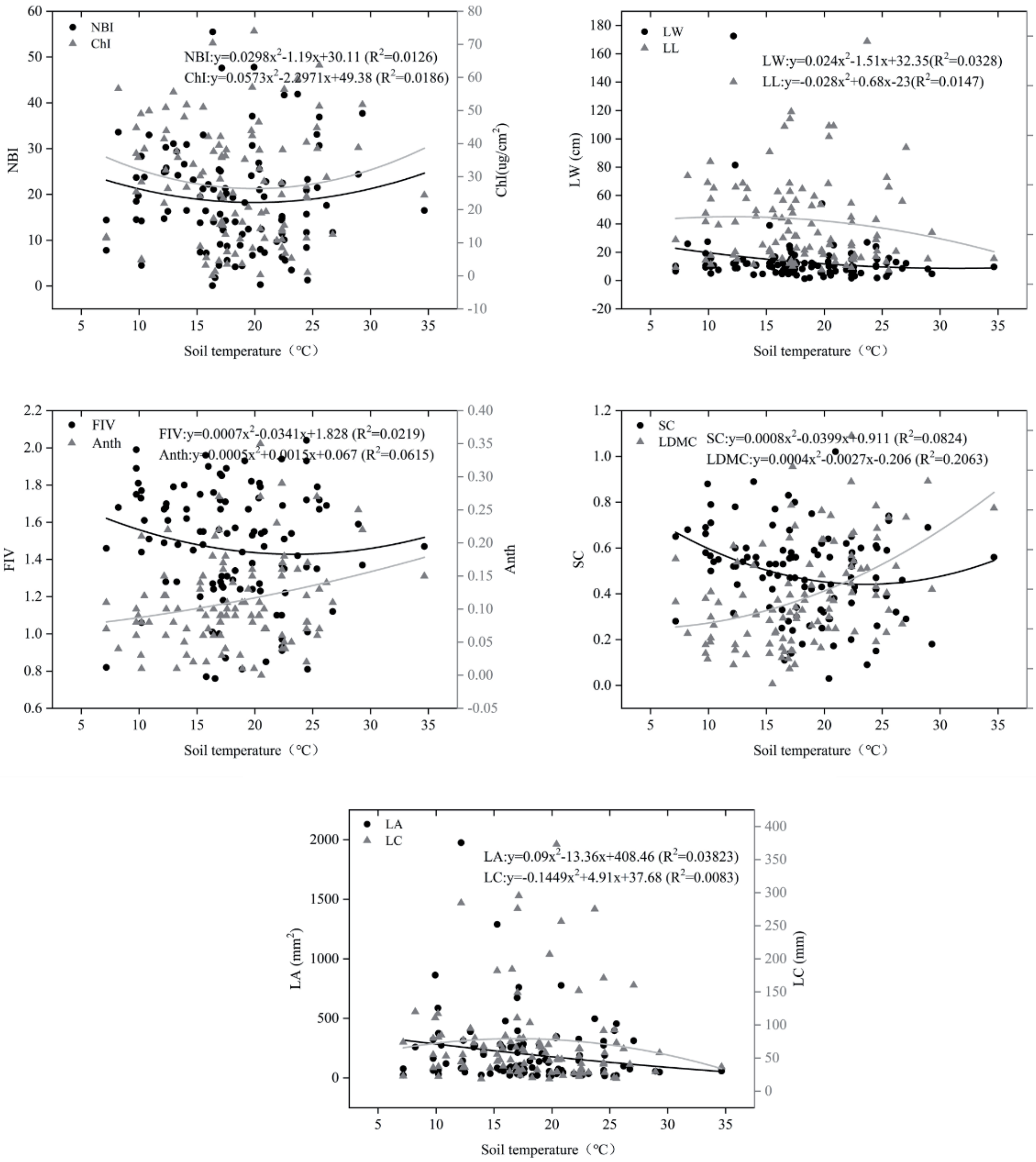

Fig. 3. Relationship between plant leaf functional traits and soil temperature.

improving habitat suitability. Under changing soil moisture conditions, plants must quickly adjust biomass allocation among traits such as specific leaf area and thickness to improve their adaptability to environmental spatial heterogeneity [14].

Soil bulk density decreased first and then increased with NBI, Chl, and Flv, while soil bulk density increased first and then decreased with Anth and LC (Fig. 5). What's more, LDMC had an obvious negative correlation with soil bulk density ($P<0.05$), while soil bulk density was positively correlated with LW ($P>0.05$), LL ($P>0.05$), LA ($P>0.05$), and SC ($P<0.05$). Soil bulk density is one of the important indicators of soil physical and chemical properties, which reflects the advantages and disadvantages of soil properties. Due to the restriction of environmental conditions and the influence of different crop growth characteristics, the soil bulk density of different types of plants has significant differences. More importantly, there are significant differences between soil bulk density and leaf functional traits of different types of plants [20]. Soil bulk density is defined as the dry matter weight of soil per unit volume. It can reflect the physical properties, soil formation processes, and productivity levels of soil. The nutrient content in soil has a significant impact on the growth and nutritional status of plant leaves. Under different nutrient conditions, functional traits such as chlorophyll content and nitrogen-phosphorus ratio in plant leaves will change [11].

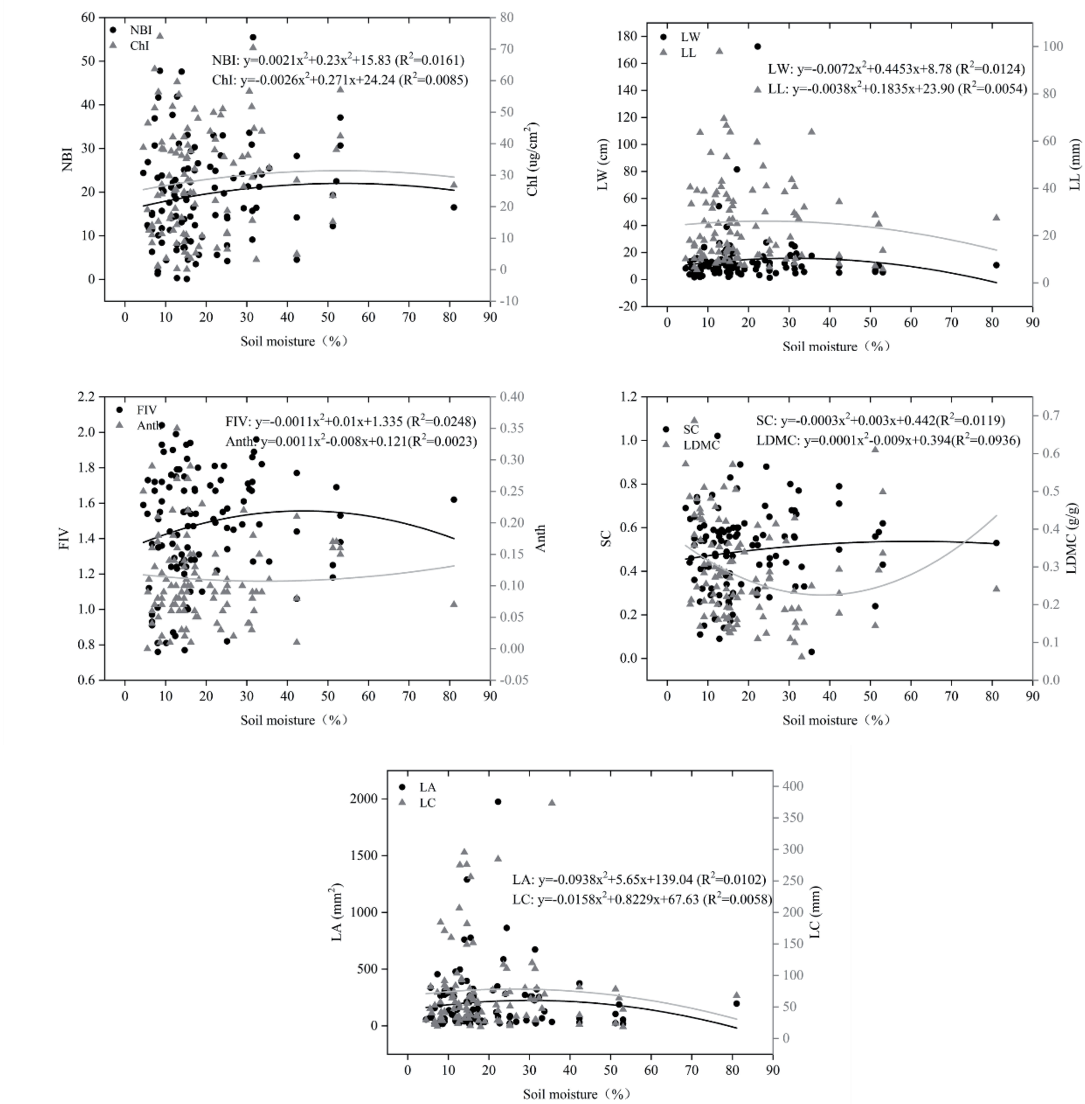

Fig. 4. Relationship between plant leaf functional traits and soil moisture.

Effects of Altitude on Leaf Functional Traits

The change in altitude can affect the growth and development, material metabolism, and functional structure of plants, as well as leaf area, stomatal density, leaf nitrogen content, and other functional characteristics. With the altitude rising, the temperature difference between day and night increases, and the chlorophyll content of plants increases. However, the leaf area of plants also has some changes. In high-altitude areas, plants reduce their leaf area simultaneously to alleviate the water stress caused by strong light and reduce the water deficit caused by the increase in transpiration rate [21]. Meanwhile, with the increase in altitude, the N and P contents, specific root length, maximum potential height, and leaf lignin content of individual leaves and roots of plants have obvious vertical distribution characteristics [22]. It can be seen from Fig. 6 that NBI ($P<0.05$) and Chl ($P<0.05$) of herbs were obviously positively correlated with altitude, which showed that altitude gradient had a greater impact on the growth status of herbs and the chlorophyll content of leaves. The existence of different vegetation types at different altitudes in the Qilian Mountains is sufficient to confirm this result. However, it may take a long time for herbs that grow in cold regions to adapt to the altitude gradient. The altitude was negatively correlated with LC ($P<0.05$), while the altitude was positively correlated with LA ($P<0.05$). However, there was no significant correlation between LL, LW, and altitude. The increase in altitude will lead to a decrease in temperature, while the low temperature will make the growth cycle of leaves relatively short and the cell growth slow. What's more, plants could adapt to the alpine environment better. Only when the leaf area became smaller could plants adapt to the alpine environment better. However, the effect of temperature on leaf functional traits was not determined, but the altitude gradient played a crucial role in affecting the composition of leaf functional traits. Meanwhile, plants may also reduce the temperature by

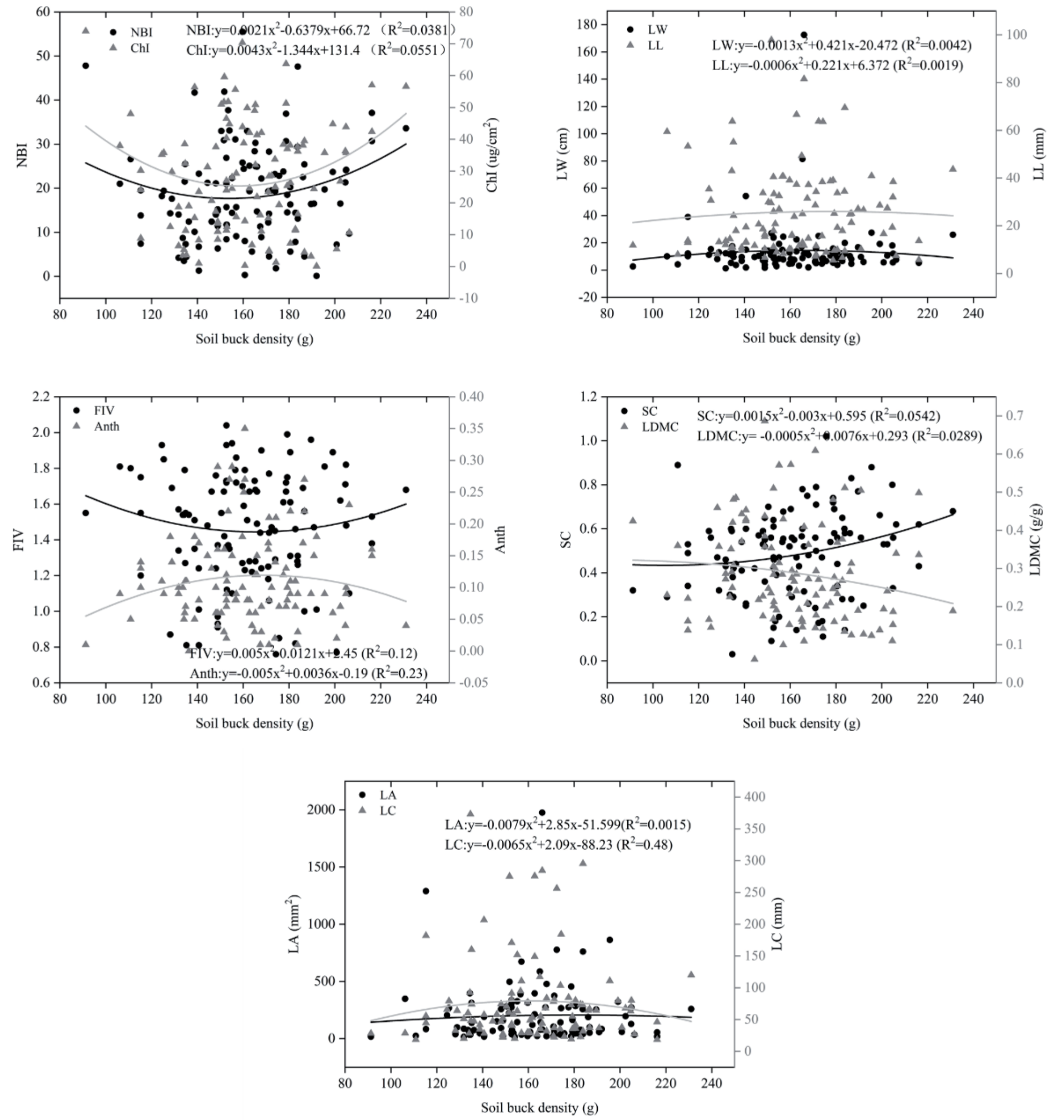

Fig. 5. Relationship between plant leaf functional traits and soil bulk density.

reducing the leaf area, increasing the pores, and finally adapting to the local environment. The lessening of leaf area may, to a certain extent, increase the water-holding capacity of some plants so that the temperature of the leaves rises slowly and ultimately improves the water efficiency of plants [23]. Furthermore, the altitude was negatively correlated with Anth ($P<0.01$), but it was positively correlated with Flv ($P<0.01$). This result indicated that the flavonoid content increased with the increasing altitude, while the anthocyanin content decreased, which may be mainly because the hydrothermal conditions of the local environment of plant growth were closely related to the content of flavonoids and anthocyanins in plants. Meanwhile, SC was positively correlated with altitude ($P<0.01$), while altitude was negatively correlated with LDMC ($P<0.01$). The characteristics of leaf functional traits of herbaceous plants with altitude may mainly be due to the adaptation pattern and degree of plants to the local environment in cold regions.

The changes in characteristics of leaf functional traits of shrubs with altitude gradient were analyzed in Fig. 7. Much the same as herb plants, the altitude was significantly positively correlated with Flv ($P<0.01$), Chl ($P<0.05$), NBI ($P<0.05$), and Anth ($P<0.05$) of shrubs in the Qilian Mountains. This result was mainly due to the gradual change of plant growth environment with increased altitude, which was not conducive to plant growth. In order to adapt to such a harsh environment, plants can change their own functional traits and ultimately maintain normal plant growth. Moreover, altitude was significantly positively correlated with SC and LDMC, which reflected that the shape coefficient and adaptability to the environment of shrubs gradually improved with the increase of altitude in Qilian Mountain. Furthermore, altitude was negatively correlated with LA, LW, LL, and LC. This result was different from that of herbs. This may be mainly due to the different adaptability of different types of plants

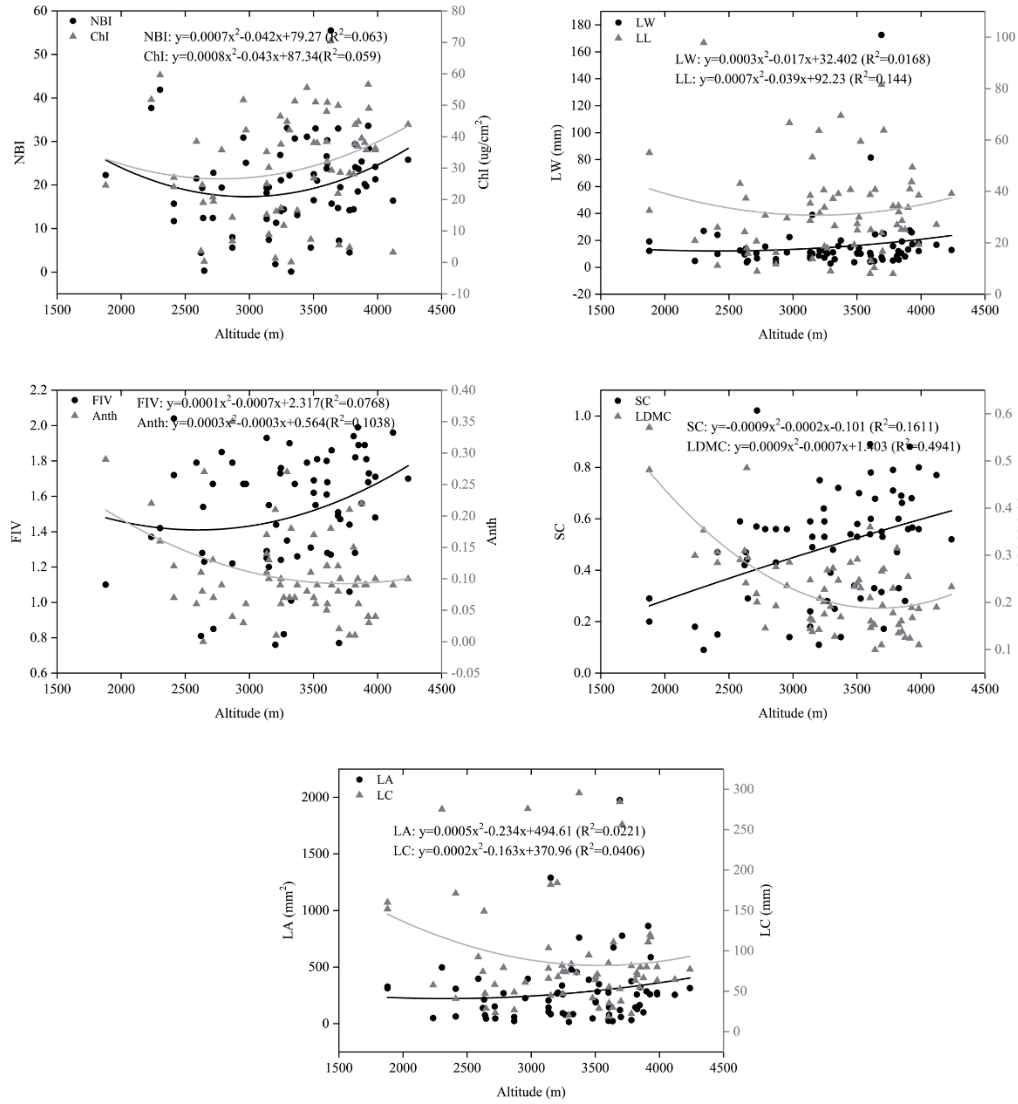

Fig. 6. Relationship between altitude and plant leaf functional traits of herbs.

to the environment and the different demands for the ecological environment.

With the difference in herbs and shrubs, NBI and Chl were significantly negatively correlated with altitude (P<0.01) (Fig. 8), indicating that the growth environment conditions of plants were gradually deteriorating with the increase in altitude and fundamentally affected the changes in leaf functional traits of trees. More importantly, altitude was negatively correlated with Anth (P<0.05) and positively correlated with Flv (P<0.05). The relationship between altitude and Anth for arbors was the same as for the herbs, while it was different for the shrubs. These results may be mainly because arbors were mainly distributed in lower altitude regions in high and cold regions, so the anthocyanin content of arbor leaves decreases with the increase in altitude. Moreover, altitude was negatively correlated with LDMC and SC. However, LA, LL, LW, and LC were positively correlated with altitude. These results may be mainly based on the growth habits and adaptability to the environment of different types of plants. On the other hand, this result may be because of the single species and small number of trees in alpine regions, which makes the few field survey quadrats, which may be another reason for this result. There are few areas suitable for the growth of trees in the high and cold regions; the quadrats investigated are also less. The leaf is the main organ of photosynthesis, which plays an essential component in accumulating nutrients and energy exchange [24]. As the light intensity and ultraviolet radiation increase with the altitude gradient, plants increase the thickness of leaf palisade tissue to reduce the damage of strong light to the leaves and ensure the normal progress of photosynthesis [25]. At the same time, with the altitude gradient, the temperature gradually decreases, and the water supply becomes insufficient. The reduced leaf area helps to reduce the evaporation of leaf water to ensure the normal metabolic function of plant cells. In low-altitude areas, the temperature is mild, the temperature in the growing season is relatively high, and the activities

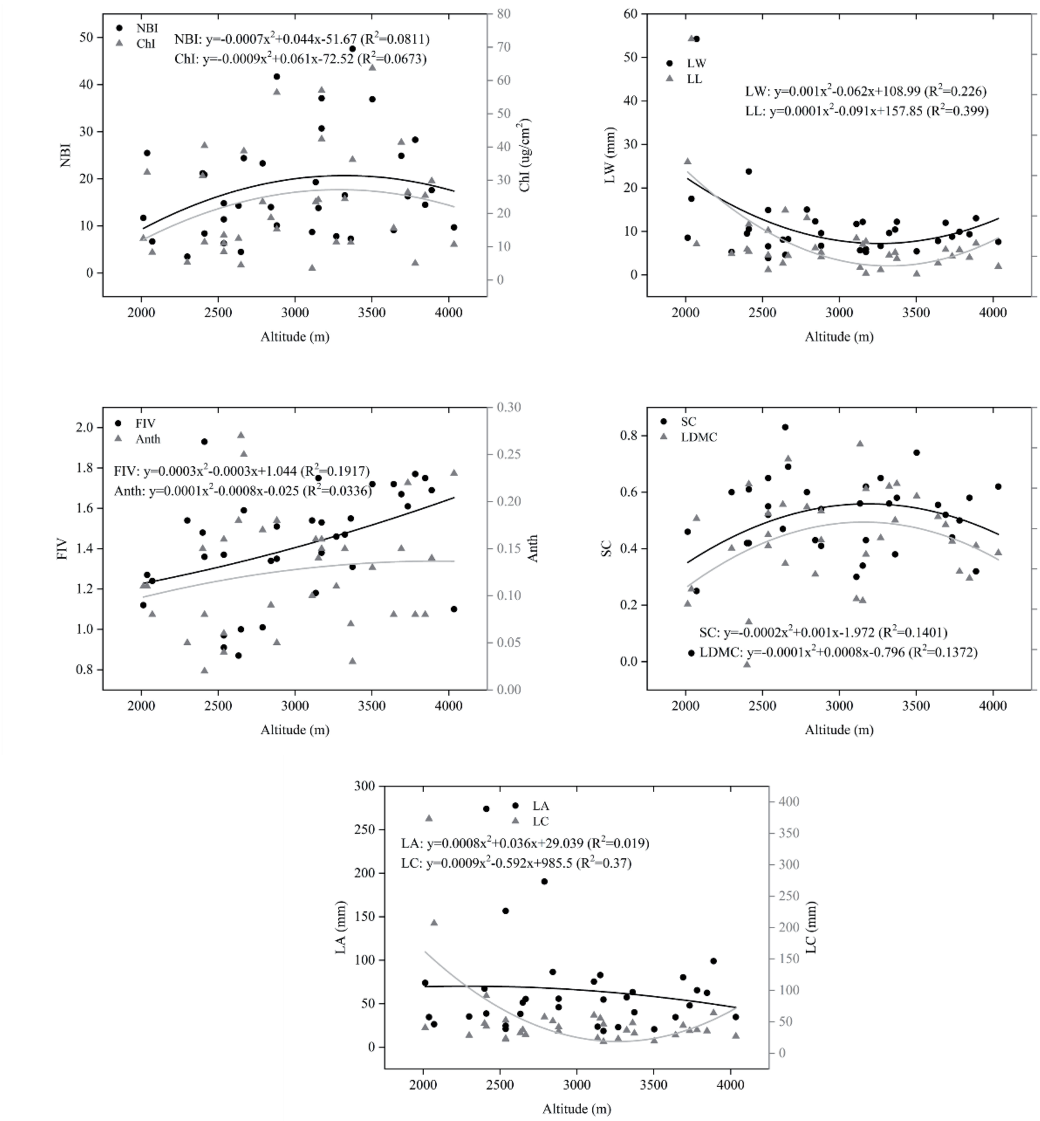

Fig. 7. Relationship between altitude and plant leaf functional traits of shrubs.

of cells and various enzymes in plants are high. At the same time, the solar radiation is weak, and the damage to plant leaves by strong light is lower than that in high-altitude areas. All parts of plants can grow and develop relatively normally, so the plant height, leaf area, leaf perimeter, and specific leaf area are relatively high [22]. Altitude is the most important factor that affects the functional trait of plant leaves because there are obvious gradient differences in precipitation, light intensity, temperature, and other factors at different altitudes and soil development levels; the distribution pattern of water and heat changes, which leads to more complex plant growth conditions [26]. In the process of adapting to complex environmental conditions, plant leaf functional traits will also produce complex adaptive changes.

Influence Mechanism of Leaf Functional Traits

Plant functional traits typically refer to a set of core attributes that plants possess, including aspects related to planting, survival, growth, reproduction, and so on, holding significant ecological significance. Environmental factors such as temperature, precipitation, and pressure induce plants to generate functional traits that adapt to their survival needs, establishing a dynamic balance between them. Temperature directly impacts the exchange of substances between plants and the external environment, affecting plant survival, growth, and metabolism. Water availability plays a crucial role in plant growth, where maintaining a balance between the water lost through leaf transpiration and water absorbed by the roots is essential for normal physiological activity [27]. In arid environments, plants often exhibit smaller leaf areas and thicker and denser leaves, suggesting an evolutionary adaptation to water stress by reducing water consumption. Soil factors, serving as the medium for vegetation growth, contain rich nutrients and substances that create a suitable environment for plant development. Plants directly absorb water, oxygen, carbon, nitrogen, phosphorus, and various minerals

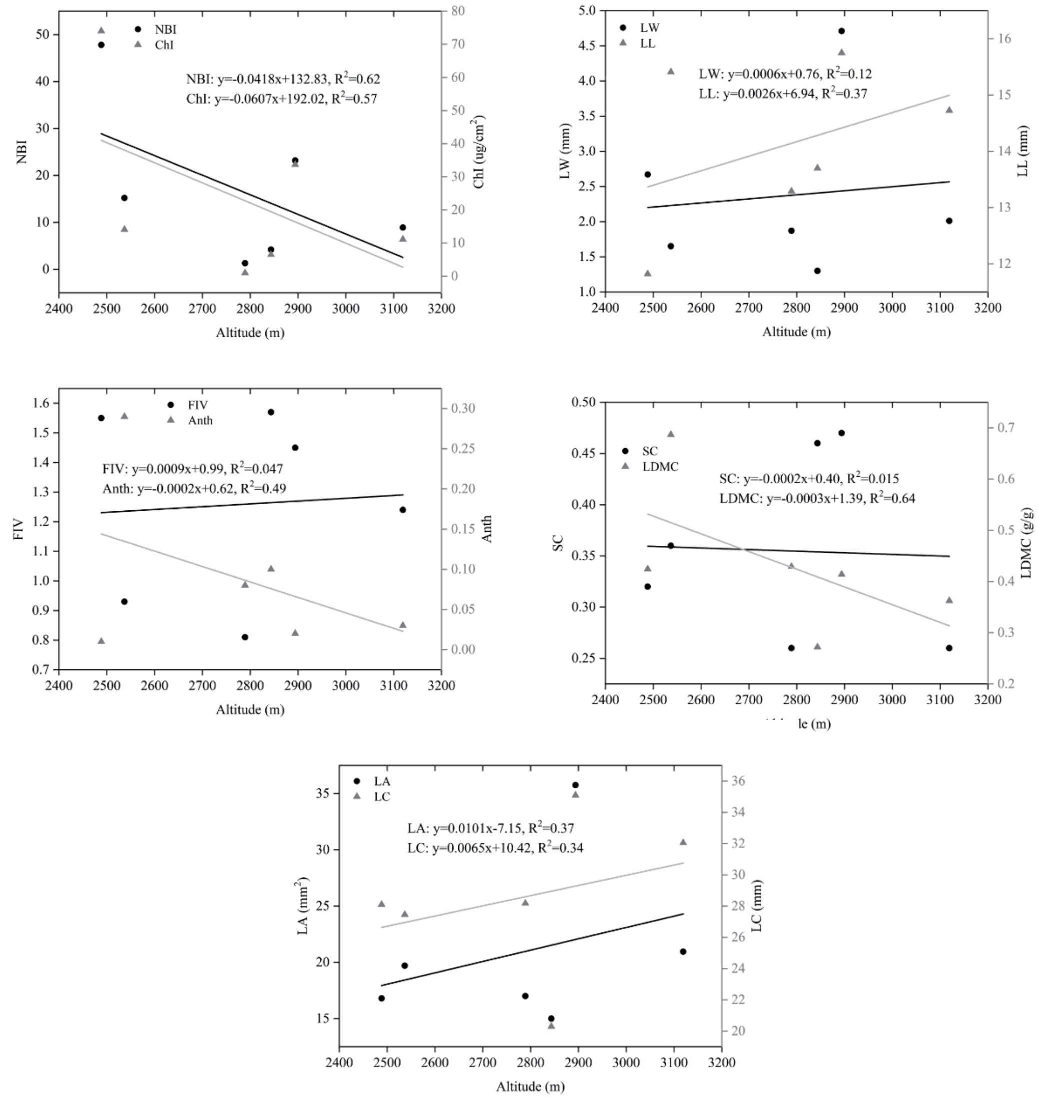

Fig. 8. Relationship between altitude and plant leaf functional traits of trees.

necessary for growth from the soil, with soil influencing plants through nutrients, bulk density, water content, and pH value.

Plant leaves are an important bridge between plants and the external environment, and the changes in their functional properties are affected by the external environment and phylogeny [28]. Plant functional traits are powerful tools for exploring plant adaptation to the environment and studying global change. Leaf functional traits can extend plant characteristics at the leaf level to the entire regional and even global ecosystems, helping us to further reveal the relationship between plants and the environment and ecosystems. Leaf functional traits are mainly affected by light, temperature, and especially water conditions. A large number of studies show that these three environmental factors are the most important factors affecting plant function and growth. The functional traits of plant leaves show different adaptation strategies under different environmental conditions. The relationship between "trait-environment" and "trait-trait" reflects the optimal "adaptation principle" of plant growth and adaptation under natural conditions [11]. Geospatial factors can affect the redistribution of ecological resources such as light, water, and nutrients over short distances, leading to environmental heterogeneity and subsequently impacting plant distribution, community structure, and ecosystem functions. However, plants form different plant functional traits by regulating their physiological and morphological characteristics, thereby affecting various aspects of responding and adapting strategies to changes in geographic space [28].

Among the climatic factors, temperature, precipitation, and illumination are the main factors affecting leaf functional traits. In general, the distribution pattern of continental hydrothermal conditions has an important impact on the horizontal zonal law of leaf functional traits [29]. As an important topographic factor, altitude provides an ideal place to study the response and adaptation of plants to global climate change. Climate,

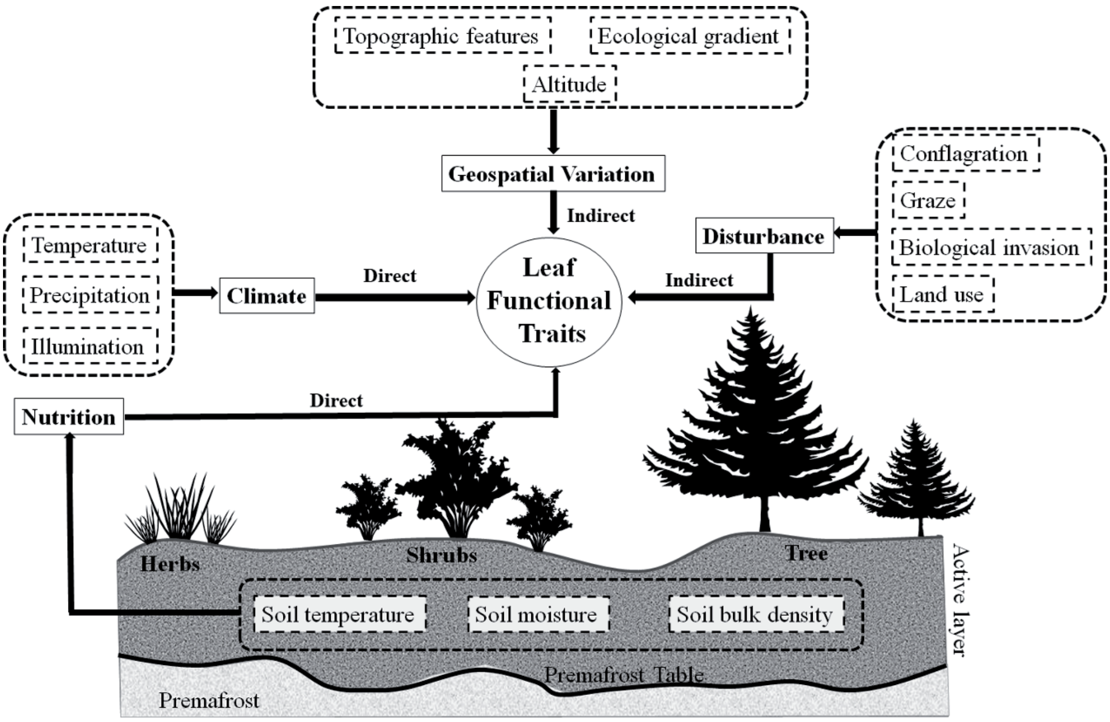

Fig. 9. Conceptual model of changes of plant functional traits with environmental gradients in Qilian Mountains.

soil, and vegetation have not only horizontal zonal characteristics but also vertical zonal characteristics; drastic changes in climate and vegetation can be formed in a small range along the altitude gradient [30]. With the increase in altitude, the temperature gradually decreases, and the precipitation and humidity increase with the increase of altitude below a certain altitude. The vegetation and soil restricted by temperature and moisture conditions also change correspondingly, and the accompanying plant leaf functional traits will also change regularly. Geographical spatial variation includes landform, altitude, slope, and aspect, and its change is closely related to the change of climate and soil [31]. The influence of geographic spatial variation of longitude and latitude topography on plant functional properties mainly comes from the gradient changes of temperature, precipitation, and soil characteristics. Plant leaf functional traits are not only related to climate gradients and adaptation to environmental stress but also closely related to soil. Soil fertility has an impact on plant biomass, leaves, reproduction, physiology, and chemical measurement traits, of which the impact on reproductive traits is the most obvious. The functional types of grasses have the strongest response to fertility [11]. Meanwhile, disturbance includes events that destroy ecosystems, communities, or population structures and change the availability of resources, substrates, or physical environment, such as conflagration, grazing, biological invasion, and land use. Although they usually involve a smaller spatial scale than climate, they occupy a dominant position in many ecosystems [32]. Through the systematic analysis of the relationship between leaf functional traits in the Qilian Mountains and factors such as soil condition and altitude gradient, we found that the direct influencing factors of leaf functional traits of plants in cold regions are climate characteristics and soil condition. The factors that affect plant soil conditions, that is, indirect factors, are mainly local environmental conditions (Fig. 9).

Conclusions

There were significant differences in plant functional traits among herb plants, shrub plants, and arbor plants. The average value of NBI, Chl, LW, Flv, LA, and LC for shrubs was lower than that of herbs, while the average value of NBI, LC, LW, Flv, LA, and Chl for shrubs was higher than that of trees. More importantly, the average value of LDMC of trees was higher than that of shrubs, and the average value of LDMC of shrubs was higher than that of herbs. Herbs can only adapt to better photosynthesis by adjusting leaf area. No matter herbs or shrubs and trees, Chl was obviously positively correlated with NBI ($P<0.01$), while NBI was obviously negatively correlated with Anth of herbs ($P<0.05$). At the same time, Chl was obviously positively correlated with Flv of herbs ($P<0.01$) and shrubs ($P<0.05$), while Chl and Flv of trees were significantly negatively correlated, but the correlation was not significant. The soil temperature decreased from 0-20 cm (20.26ºC) to 80-100 cm (14.9ºC), and the soil temperature of 60-80 cm was equivalent to 80-100 cm. In addition, the soil bulk density fluctuated from 0-20 cm to 80-100 cm. Soil temperature was negatively correlated with Flv and SC. Moreover, soil moisture was positively correlated with NBI, Chl, Flv, and SC, while it was negatively correlated with Anth and LDMC. More importantly, LDMC was negatively correlated with soil bulk density. Meanwhile, soil bulk density was positively correlated with LW, LL, LA, and SC. Furthermore, altitude was positively correlated with NBI, Chl, Flv, SC, and LA of herbs, while it was negatively correlated with LC, Anth, and LDMC. With the different herbs, altitude was positively correlated with NBI, Chl, Flv Anth, SC, and LDMC of shrubs, while it was negatively correlated with LA, LW, LL, and LC. Altitude was negatively correlated with NBI, Chl, Anth, LDMC, and SC, while it was positively correlated with Flv, LA, LL, LW, and LC. The direct influencing factors of leaf functional traits of plants are climate and soil conditions in cold regions, while the indirect factors are mainly local environmental conditions.

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Conflict of Interest

The authors declared that they have no conflicts of interest in this work.

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