Leaf Elemental Stoichiometry of *Stellera Chamaejasme* L. in Response to Environmental Factors in Degraded Grasslands Across Northern China

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Abstract

Plant leaf stoichiometry reflect its adaptations to environments. Leaf stoichiometry variations across different environments have been extensively studied among grassland plants, but little is known about intraspecific leaf stoichiometry, especially for widely distributed species, such as *Stellera chamaejasme* L. In order to evaluate the biogeographical drivers for leaf elemental stoichiometry in *S. chamaejasme*, leaf and soil samples were collected from 29 invaded sites in the two plateaus of distinct environments [the Inner Mongolian Plateau (IM) and Qinghai-Tibet Plateau (QT)] in Northern China. Leaf C, N, P, and K and their stoichiometric ratios, and soil physicochemical properties were determined. Results showed that mean leaf C, N, P, and K concentrations were 498.60, 19.95, 2.15, and 6.57 g kg^{-1}, respectively; the C/N, C/P, and N/P ratios were 25.46, 246.22, and 9.84, respectively. Only leaf K was significantly different between the two environments studied. Soil physicochemical properties of *S. chamaejasme* invaded area varied wildly, suggesting this wide ranging species tend to be insensitive to variation in soil nutrient availability. C and N content of *S. chamaejasme* leaves were unaffected by any environmental factors. However, the stoichiometric homeostasis of P and K was observed. The correlation between leaf P and climate factors was significant only in IM, while leaf K was significantly related to climate factors only in QT. Partial least squares path modeling suggested that soil exerted a significant effect on LP and climate affected leaf P and K both directly and indirectly in QT, while LP appeared to be limited mainly by climatic factors via direct ways and LK was not affected significantly by any environmental factors in IM. This study evaluated the *S. chamaejasme* leaf elemental stoichiometry and their relationships with environmental variables, which can help understand the plant biogeographic patterns and adaption strategy in degraded grasslands in China.

Introduction

Ecological stoichiometry is the study of the balance of energy and multiple chemical elements in ecological interactions, which plays an important role in analyzing the composition, structure, and function of a concerned community and ecological system (Elser et al. 2000; Sterner and Elser 2002). Over the last few decades, a particular focus of ecological stoichiometry has been to document large-scale patterns of, and the driving factors for, plant carbon: nitrogen: phosphorus (C:N:P) stoichiometry (Güsewell 2004; Reich and Oleksyn 2004; He et al. 2006, 2008; Sardans et al. 2012). The relationship between leaf stoichiometry, geographic patterns, and climate factors have been studied in both global and regional scale. Geographical variation in foliar ecological stoichiometry is a challenging issue to plant ecologists (Chown et al. 2004; Reich and Oleksyn, 2004; Yang et al. 2015; Wang et al. 2019). Meanwhile, homeostasis (H) of element composition is one of the central concepts of ecological stoichiometry, and its strength is related to the ecological strategy and adaptability of the species (Sterner and Elser 2002; Persson et al. 2010). Stoichiometric homeostasis can help predict the strategies used by different plant species to cope with limited resources (Sterner and Elser 2002; Hessen et al. 2004). The nutrient conservatism of high H-species could be an important mechanism contributing to their success, particularly in natural (unmodified) terrestrial ecosystems, where nutrient supply is often limiting and highly variable (Elser et al. 2010, Yu et al. 2010). Indeed, the stoichiometric homeostasis of plants varied with species, growth stages, and element types (Peng et al. 2016; Yan et al. 2016; Yu et al. 2011, 2015).

Stellera chamaejasme L., a toxic perennial weed, has been established and is now abundant in the alpine meadow on the eastern Tibetan Plateau and typical steppe on eastern Inner Mongolia Plateau of China (Sun et al. 2009; Guo et al. 2019). It has become one of the most serious weeds threatening a wide range of grasslands (Liu et al. 2004). Much attention has been given to *S. chamaejasme* because of its potential hazards on the grassland ecological safety and its impact on animal husbandry sustainability (Li et al. 2016; Jin et al. 2018; Guo et al. 2019; He et al. 2019; Ma et al. 2020). Despite this, no similar phylogeographical study had ever been conducted on *S. chamaejasme*. Plant nutrient and stoichiometry are key foliar traits with great ecological importance, but existing publications provide limited insight into biogeographic leaf nutrient and stoichiometry patterns for *S. chamaejasme* (Guo et al. 2019; Sun et al. 2009). As habitat heterogeneity tends to increase with geographical distance, wide-ranging species can usually use a wide array of resources and tolerate broad environmental conditions or physiological stresses and flourish over a larger area (Geng et al. 2011; Zhang et al. 2013). Recent studies have assumed that wide-ranging species always have stronger homeostasis or weak relationship in nutrient concentrations than narrow-ranging species in response to environmental factors (e.g. soil fertility) (Yu et al. 2010; Geng et al. 2011). Accordingly, we naturally associate the widespread of *S. chamaejasme* with its stoichiometric homeostasis and understand its distribution and invasiveness considering the stoichiometric homeostasis.

Several studies on a regional and global scale reported that changes of plant leaf N and P element stoichiometry are associated with many biotic and abiotic factors, including climate variables, soil properties, species, and plant functional groups (Güsewell 2004; He et al. 2006, 2008; Ordoñez et al. 2009; Zhang et al. 2019; Sun et al. 2017; Yang et al. 2015). However, species are commonly collected from a few individuals from one or a few populations and averaged at the population or species level, disregarding the intraspecific variability (Albert et al. 2010). Investigating the geographic variation within species can help to uncover the mechanism of relationships between plant tissue nutrients and environments (Hu et al. 2017), which exclude the confounding effects of taxonomic and phylogenetic structure like those found to influence the geographic patterns in leaf nutrients, and their linkages to climate and soil. Since relationships between environment and plant traits along environmental gradients could be presented as evidence of environmental control over species distribution, examining plant-environment (e.g. climate and soil nutrient availability) interactions may provide some insights into the underlying mechanism of *S. chamaejasme* distribution in degraded grasslands. However, no studies have yet incorporated information on geographic patterns in leaf stoichiometry of *S. chamaejasme* in relation to environmental factors.

This study assesses the stoichiometry and patterns of *S. chamaejasme* leaves in degraded grasslands across northern China. The distinct habitats of Qinghai-Tibetan Plateau (QT) and Inner Mongolia Plateau (IM) provide a unique opportunity to test whether there are significant differences in leaf stoichiometry under different environmental conditions and to examine how and to what extent soil and climate modify leaf stoichiometry of *S. chamaejasme* across degraded grasslands. In general, most researchers focused on the roles of C, N, and P stoichiometry in the ecological process from individuals to ecosystems, but potassium (K) is an essential macronutrient that has been partly overshadowed by C, N, and P (Reich and Oleksyn 2004; Han et al. 2011; Sardans et al. 2012; Sardans and Peñuelas 2015). Our study also showed the K concentration of *S. chamaejasme* leaves, which broadens the contents of ecological stoichiometry. We hypothesized that 1) *S. chamaejasme*, a wide-spread weed, would exhibit small variation in leaf stoichiometry in relation to soil factors; in other words, *S. chamaejasme* may have stoichiometric homeostasis, and, 2) due to QT and IM differ in their limiting factors to vegetation, the relationship between *S. chamaejasme* and environmental factors may be related to different factors in the two habitats. To test our hypotheses, we first explored the overall biogeographic patterns of C, N, P, and K stoichiometry of *S. chamaejasme* leaves from 29 sampling sites in two grassland ecosystems in northern China. We then disentangled the effects of climate and soil on the overall plant stoichiometry pattern and compared the difference between the two habitats.

Materials And Methods

Study area

Stellera chamaejasme is a poisonous plant widely distributed in degraded grasslands in China. The typical grassland of the Inner Mongolia Plateau (IM) and the alpine steppe of Qinghai-Tibetan Plateau (QT) are the two main grassland types and present a wide *S. chamaejasme* distribution. Twenty-nine sites were selected (10 sites in IM, 19 sites in QT, Fig. 1), extending from longitude 99.68 to 118.16 °E and latitude 33.35 to 44.77 °N, along with altitudes ranging from 1060 to 3500 m (Supplementary 1). The mean annual temperature (MAT) and mean annual precipitation (MAP) ranged from 1.29 to 8.19 °C and 143.84 to 587.53 mm, respectively.

Plant and soil sampling

Field measurements were conducted in June-July 2019, which was the vigorous growth stage for *S. chamaejasme*. At least 30 *S. chamaejasme* plants were randomly collected in each sampling site, then were subdivided into three subsamples, and the leaves of the subsamples were mixed into a composite sample. The samples were ground into fine powder for testing the content of elements (carbon, nitrogen, phosphorus, potassium). Concentrations of total C (LC) and total N (LN) of the *S. chamaejasme* leaves were determined sequentially by a FLASH 2000 elemental analyzer (Thermo Fisher Scientific, MA, USA). Total leaf P (LP) and K (LK) content were determined by using an AA-6300 Atomic absorption spectrophotometer (Shimadzu, Japan).

Three soil samples (0-15 cm in depth) were collected from each sample site. Each sample was thoroughly mixed and air-dried. Roots in the soil were removed by hand and passed through a 100-mesh sieve. Then, the soil was analysed for soil carbon (SC), nitrogen (SN), phosphorus (SP), potassium (SK), ammonium nitrogen (SAN), nitrate-nitrogen (SNN), available potassium (SAK), available phosphorus (SAP), the potential of hydrogen (pH), electrical conductivity (Ec), and water content (WC). Soil physicochemical properties were measured as by Bao (2000), SC and SN by the FLASH 2000 elemental analyzer, SK by NaOH fusion-flame photometry, SP by NaOH fusion-Mo/Sb colorimetry, SAN and SNN by Auto Discrete analyzer, and SAK were determined by the flame atomic absorption spectrophotometer. To measure SAP, air-dried and pre-weighed soil was extracted using 0.5 mol L^{-1} $NaHCO_3$ and P concentration in the extract was determined by the ammonium molybdate method. Soil pH was measured in a 1:2.5 soil:water suspension, and soil Ec was measured using a conductivity meter. Soil water contents were determined gravimetrically by oven-drying subsamples at 105 ^{o}C for 24 h.

Data Analysis

The means, standard deviations (SD), coefficients of variation (CV), maximum and minimum of leaf element concentrations and their ratios, and soil physicochemical properties were calculated for all sites, regions (Inner Mongolia and Qinghai Tibet). The data exhibited significant heteroscedasticity and non-normal distributions. Thus, these variables were transformed using the natural logarithm before analysis to eliminate outliers or homogeneity of variances. Differences between QT and IM were evaluated by Independent-Samples T Test.

We calculated H according to the model: $y = cx^{1/H}$, where y is the leaf element concentration of *S. chamaejasme*, x is the total nutrient contents or available nutrients in the soil, and c is a constant. The values of H and c were obtained when we analyzed the relationship between y and x using regression analysis. A high value of H indicates strong stoichiometric homeostatic regulation.

In order to determine the influence of climate factors, we obtained raw daily precipitation and temperature data (2010-2019) from the China Meteorological Administration and calculated annual precipitation and temperature using the Kriging interpolation method in ArcGIS (ESRI (Environmental Systems Research Institute), Redlands, CA, USA). Therefore, climate data for MAT, MAP for the sample sites were obtained. Regression analyses were performed to determine the correlation of leaf element contents and climate factors (MAT, MAP). Scatter plots were used to visualize the relationships among leaf element contents and climate factors (MAT, MAP), and appropriate regression equations were developed.

Partial least squares path modeling (PLS-PM) was employed to explore the direct, indirect, and interactive effects between all environmental variables for leaf element contents (The R package pls pm (0.4.9)). The model included the following variables: Leaf elements (LP, LK), climate factors (MAT, MAP), and soil factors (SK, SAK,SNN, and SpH for LP in IM, SP, SAN and SNN for LK in IM, SP, SAP, SNN and SpH for LP in QT, SC, SK, SAP, SWC, and SpH for LP in QT), after testing for collinearity of soil factors with the multivariate analog of Levene's test using the "betadisper" function in the vegan package. Indirect effects are defined as multiplied path coefficients between predictor and response variables, including all possible paths excluding the direct effect. The final model was chosen among all constructed models based on the Goodness of Fit (GOF) statistic according to the model`s overall predictive power.

Results

Pattern of leaf ecological stoichiometry and soil physicochemical properties of *S. chamaejasme*

The mean value (mean), standard deviation (SD), coefficients of variation (CV%), maximum and minimum of leaf ecological stoichiometry of *S. chamaejasme*, and soil chemical and physical properties were listed in Table 1, and a comparison was made between two main habitats of *S. chamaejasme* (Fig. 2). Leaf C, N, P, K and C:N, C:P, N:P of *S. chamaejasme* varied gently across all study sites. The mean leaf C, N, P, and K were 498.60 g kg^{-1}, 19.95 g kg^{-1}, 2.15 g kg^{-1}, and 6.00 g kg^{-1}, respectively, and CV% of leaf P was the largest. Moreover, the mean leaf C:N ratio was 25.46, C:P ratio 246.22, N:P ratio 9.84. Inconsistent with the pattern of leaf results, soil physicochemical properties of *S. chamaejasme* invaded area varied wildly. Soil C, N, P, and K exhibited large variations, primarily ranging c. 5.87-84.74 g kg^{-1} for C; 0.24-7.43 g kg^{-1} for N, 0.20-0.82 g kg^{-1} for P, and 0.95-30.55 g kg^{-1} for K. Variation in soil K content across all study sites was about 32 times (Max/Min), which was the most variable element among the four total elements. Soil mean C:N, N:P, and C:P ratios were 13.99, 77.50, and 6.34, respectively. For available soil nutrients, soil NN variation was considerably larger than that for the AP, AK, and AN content, as evidenced by coefficients of variation (CVs) (Table 1). Similarly, soil WC, pH, and Ec showed a greater variation throughout the sampling areas.

When compared leaf element contents of *S. chamaejasme* in QT and IM, we found that only leaf K concentrations were significantly different between the two habitats, which was greater in QT. Moreover, most soil physicochemical properties were higher in QT than those in IM, except SAN, SNN, and Ec. Specifically, soil P, K, SAP, WC, and pH were significantly higher in QT than IM, but soil Ec was significantly lower in QT.

Table 1 Descriptive statistics for regional *S. chamaejasme* leaf ecological stoichiometry and soil physicochemical properties in Northern China

Overall (n=29)		Mean	SD	CV(%)	Maximum	Minimum
Leaf	C (g kg^{-1})	498.60	22.07	4.43	536.20	451.33
	N (g kg^{-1})	19.95	2.09	10.48	23.48	15.29
	P (g kg^{-1})	2.15	0.52	24.19	3.19	1.40
	K (g kg^{-1})	6.57	1.18	17.96	9.08	4.15
	C:N	25.46	2.51	9.86	33.17	22.49
	C:P	246.22	61.75	25.08	359.69	154.99
	N:P	9.84	2.61	26.52	14.89	5.29
Soil	C (g kg^{-1})	46.11	21.63	46.91	84.74	5.87
	N (g kg^{-1})	3.75	1.70	45.24	7.43	0.24
	P (g kg^{-1})	0.57	0.17	29.82	0.82	0.20
	K (g kg^{-1})	20.80	5.86	28.17	30.55	0.95
	C:N	13.99	8.08	57.70	45.21	7.72
	C:P	77.50	25.49	32.89	130.24	24.87
	N:P	6.34	2.17	34.15	10.50	0.61
	AP (mg kg^{-1})	5.29	1.96	37.00	10.42	2.36
	AK (mg kg^{-1})	175.91	96.39	54.80	407.33	42.33
	AN (mg kg^{-1})	19.17	7.89	41.16	42.43	7.64
	NN (mg kg^{-1})	14.12	14.20	100.57	83.35	3.13
	WC	0.18	0.09	50.00	0.35	0.03
	pH	7.90	0.51	6.46	8.81	6.82
	Ec (μs cm^{-1})	247.21	221.21	89.48	874.50	40.00

Note: SD is standard deviations and CV is coefficient of variation

Ecological stoichiometry homeostasis of *S. chamaejasme* in degraded grassland

The relationships between leaf elements of *S. chamaejasme* and soil were evaluated using the homeostasis index (H), obtained from the homeostasis model. In Fig. 3, H of C and N were 0, indicating that the leaf of *S. chamaejasme* could not retain carbon and nitrogen. The H of AN and NN were 0, showing the stronger effect of both forms of soil inorganic nitrogen on leaf nitrogen of *S. chamaejasme*.

Additionally, H of P and K were higher than 1, and H_K (13.16) was greater than H_P (4.17). Likewise, H_{AP} (4.17) was smaller than H_{AK} (13.21) in *S. chamaejasme* leaves. In summary, the H index of *S. chamaejasme* changed between 4.17 and 13.21.

Spatial variation of leaf elements of *S. chamaejasme* in relation to climatic factors

No significant relationships among leaf C and N content and climatic factors (MAT and MAP) were found using data for all sample sites or regions. For all study sites, only leaf K content was correlated with climate factors ($P<0.001$) with greater K with increasing MAT (Fig.4). For the regions, it should be noted that in IM, the relationship between leaf P and climatic factors was significant, but K was not; on the contrary, the K content of *S. chamaejasme* leaves was related to climatic factors but P was not in QT. To be specific, leaf P concentration increased with increasing MAT and MAP in IM. Moreover, with increasing MAT, leaf K had an increasing trend, but increasing MAP showed an opposite trend in QT.

Relative roles of soil and climatic factors

Both P and K content of *S. chamaejasme* leaves were affected by soil and climatic factors simultaneously. Thus a more in-depth analysis using partial least squares path modeling revealed direct and indirect effects of the environmental drivers on leaf P and K content of *S. chamaejasme* in different regions (Fig. 5). The standardized direct, indirect, and total effects of soil and climate variables on the leaf P and K in QT and IM are presented in Table 2. Firstly, the influence of climatic factors on soil were bigger in IM than that in QT, but the effect of climatic factors on soil was significant on LP in IM. Secondly, we found that soil factors had significant effect only on leaf P in QT, however, the effects of soil factors on LP or LK in IM and LK in QT were insignificant. Thirdly, the direct effect of climate factors on LP or LK in IM and LK in QT were greater than the indirect, while the direct effect on LP in QT was less than the indirect impact. These results suggest that LP or LK were affected by different mechanisms in QT and IM regions. Moreover, the goodness of fit (GOF) was 0.3205 and 0.3556 for LP and LK in QT, respectively, and 0.5490 and 0.4431 in IM. The relatively low predictive power of the model of QT suggested that most variation remained unexplained.

Table 2 Summary of the total effects on the leaf P and K of *S. chamaejasme* in Qinghai Tibet Plateau (QT) and Inner Mongolia Plateau (IM)

Leaf elements	Relationships	QT			IM		
		Direct	Indirect	Total	Direct	Indirect	Total
LP	Climate → Soil	0.369	0	0.369	0.682*	0	0.682
	Climate → LP	-0.456	0.192	-0.264	0.665	0.123	0.788
	Soil → LP	0.519*	0	0.519	0.180	0	0.180
LK	Climate → Soil	0.331	0	0.331	0.590	0	0.590
	Climate → LK	-0.476*	-0.127	-0.604	0.248	0.181	0.428
	Soil → LK	-0.385	0	-0.385	0.306	0	0.306

Discussion

Overall patterns of leaf ecological stoichiometry and soil physicochemical properties of *S. chamaejasme* across northern China

The patterns of N and P status in plant biomass, and especially in leaves, have been intensely studied (Koerselman and Meuleman 1996; Reich and Oleksyn 2004; Han et al. 2005; He et al. 2006, 2008; Song et al. 2014; Fan et al. 2016), but few studies have attempted to document intraspecific leaf stoichiometry, especially for poisonous weeds in grasslands. This study presents, to our knowledge, the first analysis of leaf element concentrations (C, N, P, K) and ratios (C:N, C:P, N:P) of *S. chamaejasme* across degraded grassland in northern China. Our results show that leaf C (498.60 g kg^{-1}), N (19.95 g kg^{-1}), and P (2.15 g kg^{-1}) of *S. chamaejasme* were higher than the mean value of all species in the China Grassland Transect (Fan et al. 2016), and there was no obvious difference between two habitats of *S. chamaejasme.* N and P are the most important limiting nutrients for primary productivity in terrestrial ecosystems (Elser et al. 2007), and a high concentration of N and P in *S. chamaejasme* leaves means high nutrient uptake efficiency of *S. chamaejasme* in degraded grasslands, which could facilitate *S. chamaejasme* outcompete other species in nutrient-poor environments. Moreover, K is one of the essential macronutrients that play critical roles in various metabolic processes, but it has been partly overshadowed in ecological stoichiometry by nitrogen and phosphorus (Shin 2014; Adams and Shin 2014). Although most studies did not involve the K content in plant leaves, it is worth noting that K concentrations of *S. chamaejasme* were significantly greater in QT than that in IM. The reason may be that the content of nutrients in plants can be constrained by nutrient supply in the soil, and the content of soil K is significantly higher in QT, therefore generating this difference. The leaf C:N ratio of *S.*

chamaejasme was 25.46, C:P ratio 246.22, N:P ratio 9.84. Generally, it is not uncommon that using N:P ratios of plant biomass as indicators of N or P limitation in various studies (Koerselman and Meuleman 1996; Tessier and Raynal 2003). The low N/P ratio in *S. chamaejasme* might imply that its growth is restricted by N, which was consistent with the results reported by Guo et al. (2019).

We found that *S. chamaejasme* could survive in a soil environment with considerable variation, which is consistent with the fact that *S. chamaejasme* is wide-ranging species with a wide geographic range in China grassland (Zhang et al. 2015). The soil condition for *S. chamaejasme* growth varies considerably from site to site. Soil physicochemical properties varied with a difference of more than 10 times between the maximum and the minimum included C (14.43 times), N (30.94 times), K (32.27 times), NN (26.66 times), WC (10.60 times), Ec (21.86 times). Moreover, one sampling site (Haiyan in QT) showed the greatest soil physicochemical properties. For example, the content of soil P was lowest (0.20g $kg^{-1)}$, and soil WC was the minimum (0.03), but pH reached a maximum (8.81), indicating *S. chamaejasme* could tolerate an extreme environment. This may provide a competitive advantage for *S. chamaejasme* against other plant species and help explain its rapidly expansion in degraded grasslands. Additionally, we also found that the *CV* of NN was 100.57%, greater than for AN (41.16 %), indicating that *S. chamaejasme* was less sensitive to the NN variation.

Generally, Tibetan alpine grasslands and Inner Mongolian temperate grasslands, which have different limiting factors, are both zonal grassland types in China (Li et al. 2020). Alpine grasslands are mainly limited by low temperatures in the growing season, while temperate grasslands are affected by drought (Fan et al. 2016). Accordingly, our analysis indicated that some soil physicochemical properties of *S. chamaejasme* for the regions were significantly different. Soil WC and pH for Qinghai-Tibet were significantly higher, and Ec lower than those for Inner Mongolia. However, apart from SP, SK and SAP, soil C and N concentrations and other soil available nutrients (AN, NN, AK) for the regions were insignificantly different. These findings suggest that climate imposes important controls on soil nutrients.

Relationships between leaf C,N, P and K and environmental variables

Plant nutrient concentrations and their correlations with soil nutrient conditions are considered effective tools for exploring plant adaptation and resource utilization strategies in a severe environment (Ordoñez et al. 2009; Hong et al. 2015; Geng et al. 2011). Stoichiometric homeostasis (H) is the ability of plants to maintain their element composition relatively stable regardless of changes in nutrient availability via various physiological mechanisms (Sterner & Elser 2002; Persson et al., 2010). The degree of stoichiometric homeostasis can be indicated by the homeostatic coefficient (H), which reflects the ability of plants to maintain a stable nutrient composition regardless of changes in environmental nutrients (Sterner and Elser 2002; Hessen et al. 2004; Giordano 2013; Wang et al. 2019). It is well known that stoichiometric homeostasis had been reported in dominant palatable species (Li et al. 2016; Yu et al. 2015; Peng et al. 2016) in grasslands. However, this has not been established in unpalatable species. Since poisonous plants represent the majority of the plant species detected after grasslands have been degraded, reveal the eco-physiology characteristics of poisonous weeds will help us better understand why poisonous weeds can spread widely on degraded grasslands. We found H_C and H_N of *S. chamaejasme* were 0, indicating that *S. chamaejasme* could not maintain carbon and nitrogen internally. Compared with other grassland species, *S. chamaejasme* had no H_N, which was different from previously reported results for other species (Wu and Wang 2019; Yu et al. 2011). Our data clearly show that the stoichiometric homeostasis coefficient of P (H_P) of *S. chamaejasme* was higher than N (H_N), indicating *S. chamaejasme* was relatively less sensitive responses to soil P than soil N. However, H_N was consistently higher than H_P at the levels of community, family, and species in China reported by previous studies (Peng et al. 2016; Yu et al. 2015). Like many other terrestrial ecosystems, grassland ecosystems face an ongoing increase of atmospheric nitrogen deposition in recent decades (Menge & Field, 2007). The increase of N availability in the soil leads to ecosystems limited by N have gradually transformed to P-limitation or other elements (Galloway et al. 2008; Lebauer and Treseder 2008). Thus *S. chamaejasme* with H_P could survive outstandingly than species without H_P in the future P-limited surroundings. Moreover, although globally N and P are considered of paramount importance to plant function, it is widely known that many other elements are also important in specific contexts or regions (Han et al. 2011). We also found that H_K was greater than H_P, implying K could be used as another important element that indicates the degree to which an organism maintains homeostasis.

Our results indicate that in the macro scale, leaf C and N do not directly correlate with meteorological factors (MAT and MAP), which is in agreement with previous studies conducted in the grassland biomes of China (He et al. 2006). The weak relationships observed between leaf C, N, and climatic variables may result from plant growth, development, metabolism, phenological and life-history traits rather than from the specific geographic environment. On the contrary, there were close relationships between leaf P and K and climatic factors (Fig. 4). The relationship between leaf P and climate factors was significant only in IM, and the K content of *S. chamaejasme* leaves was significantly related to climate factors only in QT. We noticed that the correlation of leaf P and MAP (R^2=0.5523) was greater than the relationship between P and MAT (R^2=0.4886) in IM, and the relationship between K and MAT (R^2=0.3338) was greater than that with MAP (R^2=0.2920) in QT. These again reflect the different limiting factors of plant growth in different regions (Fan et al. 2016). It is a reasonable assumption that precipitation is a more important limiting factor than the temperature for vegetation growth in arid and semi-arid regions like Inner Mongolian Plateau temperate grasslands. In contrast, the temperature is more likely to have a greater effect on leaf element concentrations than precipitation in Qinghai Tibet Plateau alpine grasslands with high-altitude and low temperature. We also found that only LK was negative related with MAP in QT. In fact, K leaches more easily from leaves than N and P (Sardans and Peñuelas 2015); hence it is easy to ascertain the increasement of MAP in the studies leading more leaf leaching of K in *S. chamaejasme.*

To explore complex relationships between soil and climatic factors on leaf P and K contents of *S. chamaejasme*, we conducted a PLS-PM analysis. We found soil exerted a significant effect on leaf P and climate affected leaf P and K both directly and indirectly in QT, while LP appeared to be limited mainly by climatic factors and via direct ways and LK was not affected significantly by any environmental factors in IM. More specifically, climatic factors had an significant influence on soil in IM. Meanwhile, climatic variables had stronger direct effects on LP than the indirect effects in IM. This was contradicting our knowledge that climate factors often affect leaf elements through their influence on soil nutrient status (Luo et al. 2021). However, the arid conditions of the Inner Mongolia Plateau (Arid and Semi-Arid Areas) have no doubt restricted grassland plants growth by insufficient water, and Zhang et al found that drought has become the primary natural disaster threatening to Inner Mongolia grassland ecosystem (Zhang et al. 2009). In addition, climatic factors affected soil state insignificantly and directly or indirectly influenced leaf P or K in QT. Soil factors had significant influence over LP and the influence of climatic variables on LK was significant, indicating the effects of climatic factors and soil factors on LP or LK occurred separately. Our model suggests that underlying mechanisms behind the LP or LK content were different in the two habitats.

In addition, species natural habitats will be subject to more disturbances in the future due to climate change and habitat degradation caused by intensive anthropogenic activities (Wei et al. 2019; Chen et al. 2020). In future works, continuing wide-scale sampling and considering the influence of human activities are required to further develop a deeper understanding of the geographic patterns in *S. chamaejasme*.

Conclusion

Our study is, to our knowledge, the first to comprehensively document the chemistry of multiple mineral elements (C, N, P, K) of *S. chamaejasme* leaves and its surrounding soil physiochemical properties and quantify the potential controls and variability at a large scale. We found that leaf C, N, and P of *S. chamaejasme* were higher than the national average of grassland species, and there was no obvious difference between the two habitats, but K concentrations were significantly greater in QT than that in IM. Inconsistent with the pattern of leaf results, soil physiochemical properties of *S. chamaejasme* invaded area varied wildly, and most of them were greater in QT. *S. chamaejasme* could survive in different soil environments, which supported the fact that wide-ranging species tend to be insensitive to variation in soil nutrient availability.

Based on the homeostasis model, our result clearly show that only the stoichiometric homeostasis of P and K in *S. chamaejasme* across north China was observed, and H_K was higher than H_P, which differs from previously reported results for other species. In addition, leaf P and K were affected by climatic variables. We found the relationship between leaf P and climate factors was significant only in IM, while the K content was related significantly to climate factors only in QT.

In sum, both C and N content of *S. chamaejasme* leaves were unaffected by any environmental factors, but P and K in the two habitats were influenced by different factors. This study demonstrates that soil exerted a significant effect on leaf P and climate affected leaf P and K both directly and indirectly in QT, while LP appeared to be limited mainly by climatic factors and via direct ways and LK was not affected significantly by any environmental factors in IM by conducting a PLS-PM analysis. The study contributes to the understanding of biogeographic patterns and adaption strategy of *S. chamaejasme* in the degraded grasslands in China.

Declarations

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material (data transparency)

All the data were summarized in the manuscript itself. Please contact the corresponding author regarding any additional queries related to the dataset generated and analyzed during the current study. The datasets in this study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

LZ Guo and D Huang conceived the ideas and designed methodology; L Liu, HZ Meng, L Zhang and W He collected the data; LZ Guo and K Wang analysed the data; LZ Guo and D Huang led the writing of the manuscript; W He and VJ Silva reviewed and edited the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Ethics approval

The sampling of plant and soil did not require permission from any local or national authority as sampled. The sampled species are not classifed as endangered and are not under any protection in the sampled area.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

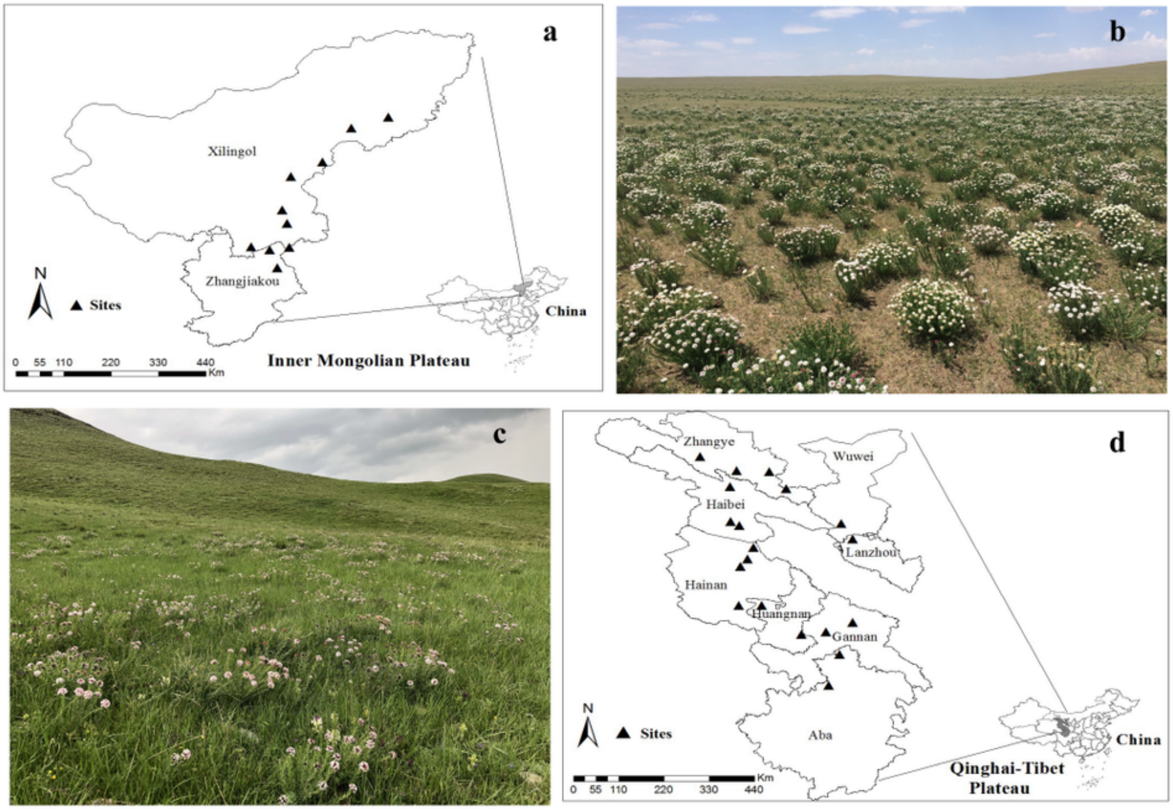

Figure 1

Location of the study and sampling sites. (a) Sampling sites on Inner Mongolia Plateau; (b) S. chamaejasme coverage in Taipusi Banner on Inner Mongolia Plateau; (c) Sampling sites on Qinghai-Tibetan Plateau; and (d) S. chamaejasme coverage in Qilian County on Qinghai-Tibetan Plateau.

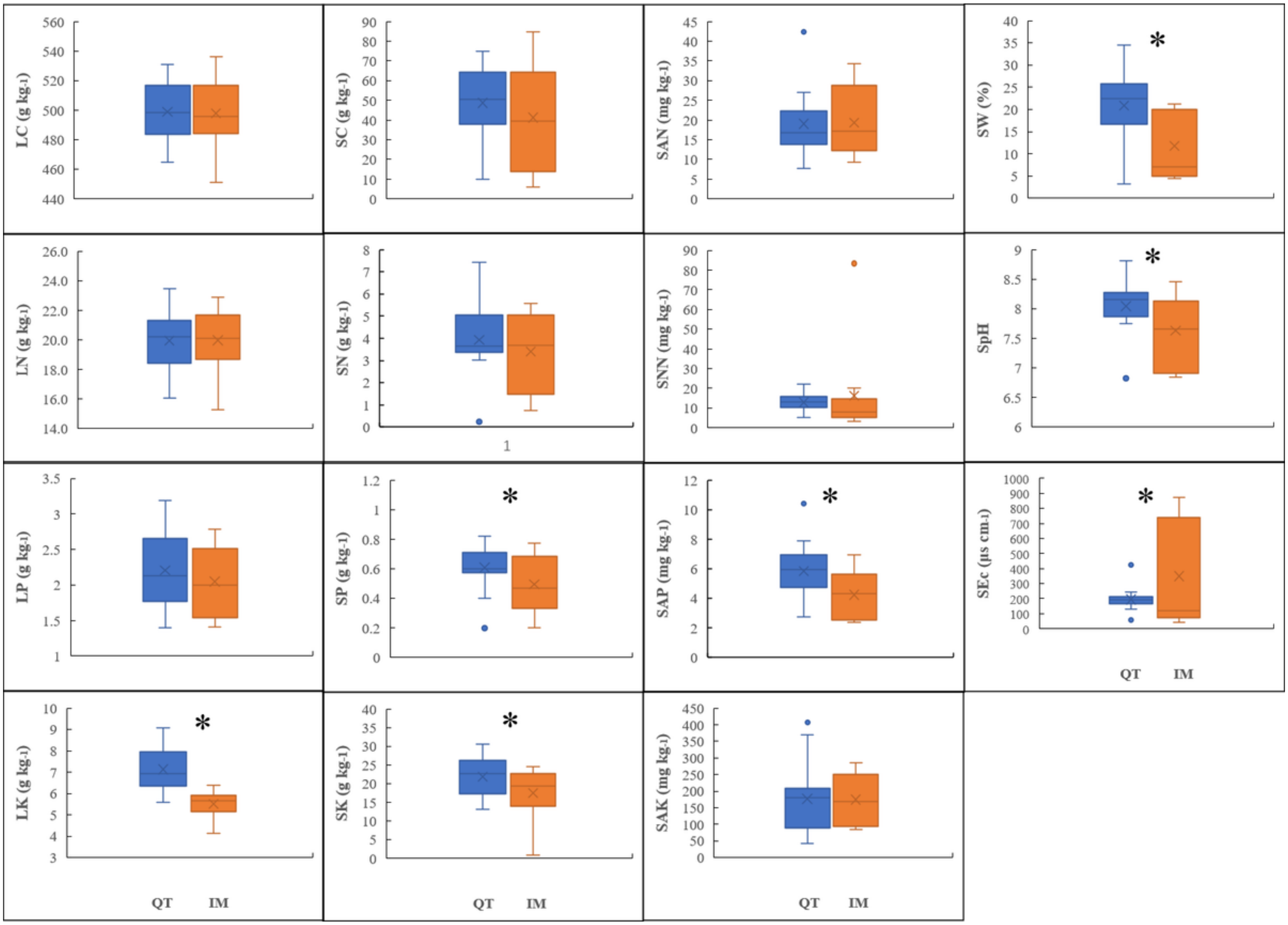

Figure 2

Comparison of leaf element contents and soil physiochemical properties in Qinghai Tibetan Plateau (QT, n=19) and Inner Mongolian Plateau (IM, n=10) Note: Significant differences (P < 0.05 on the basis of t-test) between the QT and IM were indicated by *.

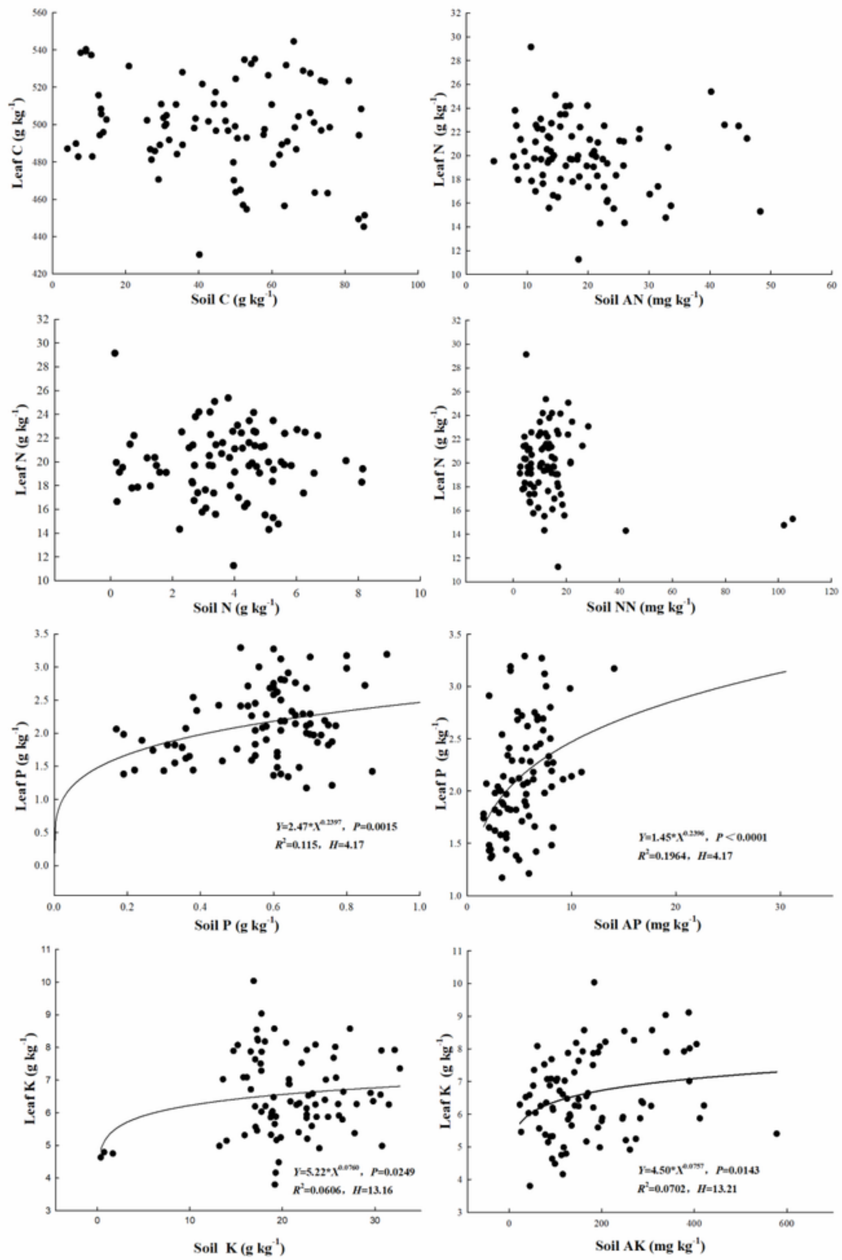

Figure 3

Relationship between the content of C, N, P, K elements in S. chamaejasme leaves and soil

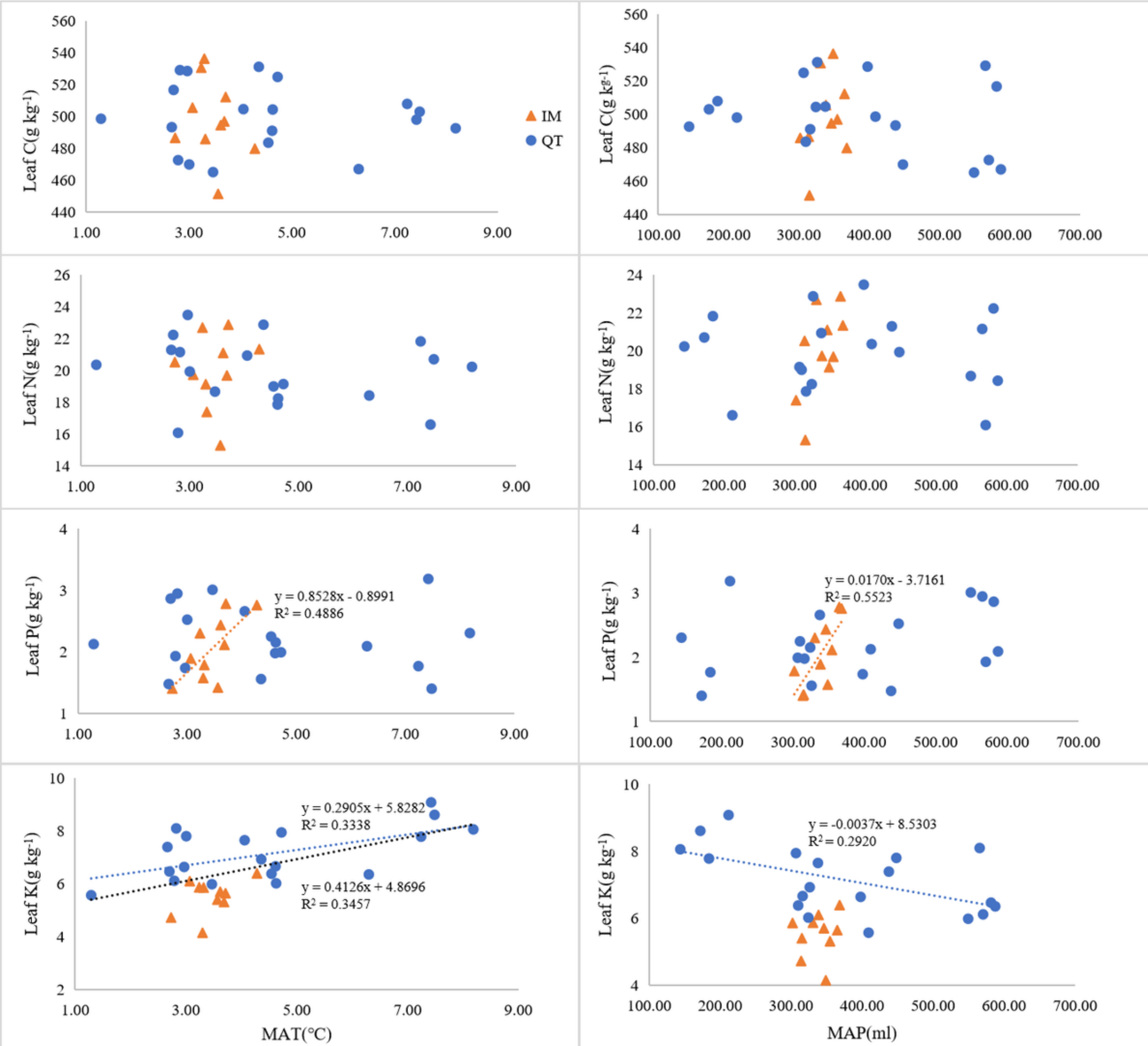

Figure 4

Leaf C, N, P, K content of S. chamaejasme in relation to climate factors (MAT & MAP) in Qinghai Tibet Plateau (blue circles) and Inner Mongolia Plateau (red triangles).

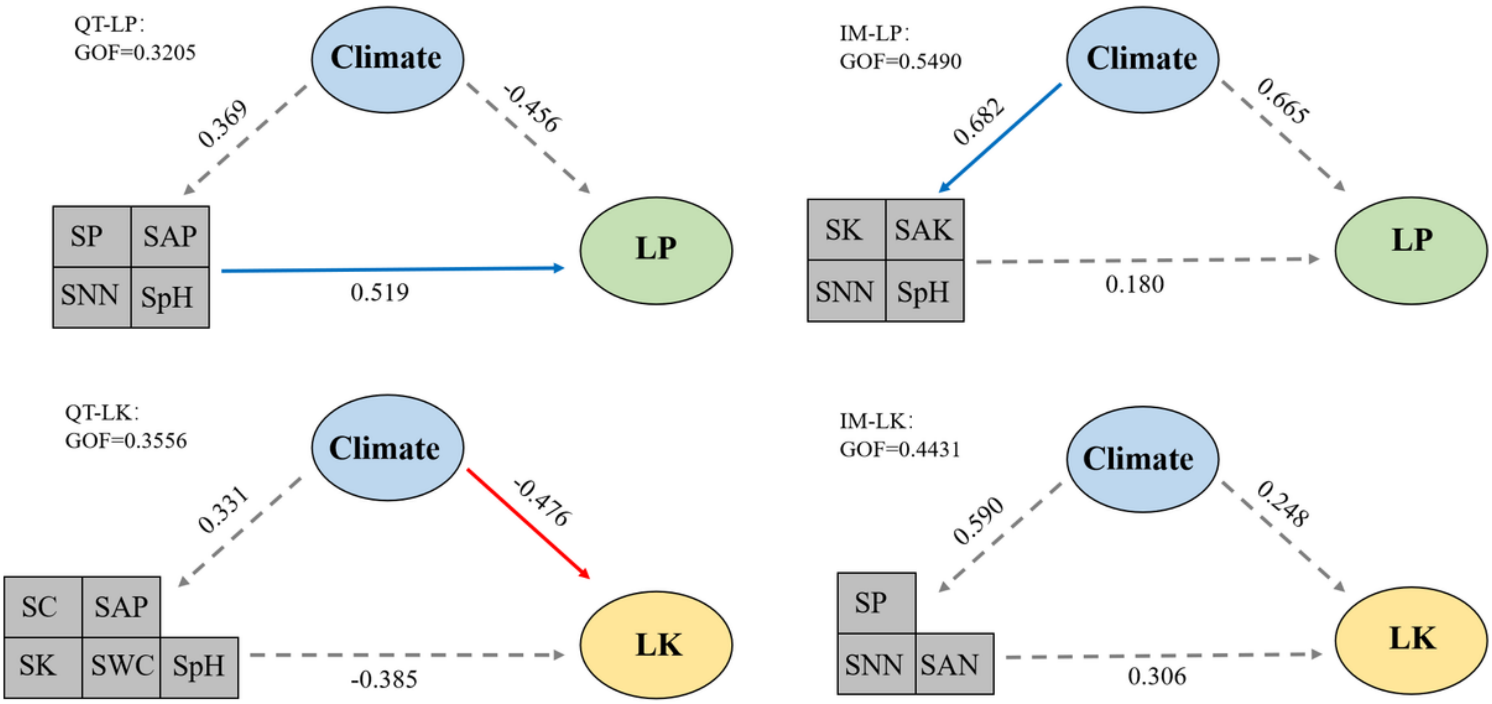

Figure 5

Effects of different variables (soil and climatic variables) on the leaf P and K of S. chamaejasme in Qinghai Tibet Plateau (QT) and Inner Mongolia Plateau (IM) based on partial least squares path modeling. The blue arrows represent positive pathways, and the red arrows indicate negative pathways. The standard path coefficients are showed on the arrow. GOF, goodness of fit of the statistical model.

Supplementary Files

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