



# Article Effects of Precipitation Change and Nitrogen and Phosphorus Additions on Traits and Abundance of Potentilla anserina in an **Alpine Meadow**

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Abstract: Changes in precipitation patterns and eutrophication can cause changes in plant traits and abundance, potentially affecting plant community structure and functions. Here, we studied responses of traits and abundance of Potentilla anserina to precipitation change and nitrogen (N) and phosphorus (P) additions, and the effect of traits on its abundance in an alpine meadow of the Qinghai-Tibet Plateau. We found that precipitation change and N and P additions significantly affected the mean value of traits such as specific leaf area (SLA), leaf dry matter content (LDMC), single leaf area, plant height and individual size, while only P addition significantly affected intraspecific variation of SLA and individual size. Increased precipitation and N and P additions shifted plant traits to more resource acquisitive, and increased plant abundance. Responses of plant traits to P addition were larger than that of N addition. Plant abundance was mainly affected by precipitation, and was limited by N or P dependent on precipitation conditions. In conclusions, our research shows that P. anserina can respond to environmental changes by changing its traits to improve its adaptability, potentially affecting community structure and ecosystem functions.

Keywords: aboveground biomass; eutrophication; functional traits; global change; Qinghai-Tibet plateau

# 1. Introduction

The Qinghai-Tibet Plateau has the highest altitude in the world, known as the "third pole of the world", and is also the most sensitive and ecologically fragile region in response to global climate change [1–4]. Grasslands cover 54–70% of the total area of the Qinghai-Tibet Plateau [5]. Therefore, the Qinghai-Tibet Plateau is widely recognized as an ideal site for studying the response of grassland dynamics to global climate change and human activities [2,6–8].

Potentilla anserina L. belongs to the genus Potentilla in the family Rosaceae and grows on riverbanks, roadsides, hillside grasslands and meadows at altitudes of 500–4100 m [9]. The fleshy, expanded roots of *P. anserina* are rich in nutrients, including protein, fat, sugar, vitamins, organic acids, magnesium, zinc, potassium, and calcium, which are essential for the human body, providing food and important traditional herbal medicines with high economic value [10]. Despite its wide distribution, *P. anserina* growing at altitudes below 2400 m does not form fleshy, expanded roots, and even if it does, the quality is not as good



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as that at higher altitudes [11]. Therefore, the main economic value of *P. anserina* is still concentrated in high altitude areas.

*P. anserina* is also a common companion species in alpine meadows and one of the pioneer and indicator species in degraded grasslands and secondary successional lands [12]. Zhou et al. (2006) suggested that *P. anserina* plays the role of "blocker" in the degradation of alpine meadows, because its strong reproductive ability increases the cover of the community and maintains soil moisture, which prevents the succession of the community to a low cover and soil sanding to a certain extent, and also creates conditions for the restoration of the alpine meadows and the colonization of other plants [12]. Therefore, study of the response of *P. anserina* to global change is not only beneficial to the local exploitation of its economic value, but also to the understanding of its ecological function in alpine meadows under global change.

Plant functional traits are key characteristics that regulate plants by influencing plant growth, survival and reproduction [13,14]. Plant traits largely determine the structure of communities and multiple functions of ecosystems [15,16], so our ability to understand and predict ecosystem function is largely dependent on quantifying the role of functional plant traits and their response to environmental change [17].

The patterns of plant functional traits in response to environmental changes (e.g., eutrophication and precipitation changes) are a hot issue in ecological research [18–20]. Plants can adapt to interspecific competition and environmental filtering through changes in functional traits [19]. Based on the leaf economic spectrum, plant functional traits can characterize plant resource utilization strategies. For example, it was shown that with increasing N content in soil, plants significantly increase height, specific leaf area, leaf N content, increase their ability to capture light resources by adopting an acquisitive resource use approach, and adopt a faster nutrient cycling strategy and thus increase leaf dry matter content, reduce specific leaf area, etc., adopt conservative resource utilization strategies and slow down nutrient cycling to improve plant water retention capacity and reduce water consumption [22]. It has also been shown that in humid areas, plants are mainly limited by soil nutrients [23].

In this study, we investigated the changes in plant traits (such as SLA, LDMC, single leaf area, leaf thickness, plant height and individual size) and abundance of *P. anserina* in alpine meadows with precipitation changes and nitrogen (N) and phosphorus (P) additions. We predicted that the addition of nutrients and increased precipitation could shift plant traits from conservative to greater resource acquisitive (Figure 1). The shift in functional trait strategy facilitates the plants' adaptability to the environment and their ability to access resources, thus enabling the species to rapidly occupy ecological niches in the community and become the dominant species in the community. Thus, we predicted an increase in abundance of *P. anserina* with nutrient addition and precipitation increase.



Conservative strategy: SLA↓, LDMC↑, Leaf size↓, Height↓

> Harsh Habitat e.g. drought, faulty nutrition





**Figure 1.** Plant resource strategy from 'conservative' to 'acquisitive' along the environmental gradient from harsh to superior conditions. SLA: specific leaf area; LDMC: leaf dry matter content.

## 2. Materials and Methods

# 2.1. Site Description

The experimental site is located at the Qinghai Haibei National Field Research Station of Alpine Grassland Ecosystem ( $37^{\circ}37'$  N,  $101^{\circ}12'$  E, c. 3215 m a.s.l.; Figure S1 from the Supplementary Material). The station is located at Haibei, Qinghai, China, in the north-eastern Qinghai-Tibet plateau. This area has a typical plateau continental climate, with long, cold winters and short, cool summers [2,24]. Mean annual precipitation is 488 mm, mainly distributed during the short, cool summer. Mean annual temperature is  $-1.1 \,^{\circ}$ C and ranges from a minimum of  $-15.2 \,^{\circ}$ C in January to a maximum of 9.9  $^{\circ}$ C in July.

## 2.2. Experimental Design

We set up the experiment in June 2018. The experiment used a completely randomized block design with altered precipitation, N addition and P addition as the main treatment factors. Each block contained 12 treatments, crossing three levels of precipitation (no change, drought (50% precipitation reduction) and wet (50% precipitation addition)), two levels of N addition (no addition, 10 g N m<sup>-2</sup> year<sup>-1</sup> (urea)) and two levels of P addition (no addition, 10 g P m<sup>-2</sup> year<sup>-1</sup> (superphosphate)) (Table 1). Each treatment had five replicates, resulting in 60 plots. Each plot was 3 m × 3 m and was surrounded by a 2 m wide buffer zone. Rain shelters were used to control the incoming precipitation amount in the experimental plots. Six 'U'-shaped transparent polycarbonate resin channels were fixed at the 15° angle to intercept rainfall. The collected rainfall from the drought plots was supplied to the wet plots manually after each precipitation event by spray bottle. To account for the effects of shading, we also installed six 'dummy' transparent polycarbonate resin channels in the control plots. Stainless steel sheets were inserted into the soil around the edge of each plot to reduce surface runoff. N and/or P additions were applied in plots in June (early growing season) each year.

	50% Precipitation Reduction (W_)	No Change (W <sub>0</sub> )	50% Precipitation Addition (W <sub>+</sub> )	
No addition $(N_0P_0)$ 10 g N m <sup>-2</sup> year <sup>-1</sup> $(N_+P_0)$	$W_N_0P_0 W_N_P_0$	$W_0 N_0 P_0$ $W_0 N_+ P_0$	$W_+N_0P_0$ $W_+N_+P_0$	
$10 \text{ g P m}^{-2} \text{ year}^{-1} (N_0 P_+)$	$WN_0P_+$	$W_0 N_0 P_+$	$W_+N_0P_+$	
$\frac{10 \text{ g N m}^{-2} \text{ year}^{-1} +}{10 \text{ g P m}^{-2} \text{ year}^{-1} (\text{N}_{+}\text{P}_{+})}$	$WN_+P_+$	$W_0N_+P_+$	$W_+N_+P_+$	

Table 1. Description and abbreviations of the 12 treatments in this study.

## 2.3. Measurement of Plant Traits and Abundance

In August 2020, we randomly sampled the specific leaf area (SLA,  $m^2 \cdot kg^{-1}$ ), leaf dry matter content (LDMC,  $mg \cdot g^{-1}$ ), single leaf area (mm<sup>2</sup>) and leaf thickness (mm) of all healthy leaves and the mature height (cm) and individual size (individual dry weight; g) from five individuals of *P. anserina* in each plot. We harvested all above-ground plant parts of *P. anserina* in a 0.5 m × 0.5 m square of each plot to quantify species abundance in the plant community. All functional variables were measured following the standard protocols developed by [25].

## 2.4. Statistical Analyses

The coefficient of variation (standard deviation/mean) of each trait was calculated as intraspecific trait variation. The correlation between different traits and their intraspecific variation was analyzed by principal component analyses (PCAs). Three-way ANOVAs were used to analyze whether the main effects and their interactions had significant effects on plant traits, intraspecific trait variation and plant abundance. Tukey's Honest Significant Difference (Tukey's HSD) method was used for multiple comparison in different treatments, and for comparisons between two levels the paired *t*-test was used. The relationship

between different functional traits and abundance was analyzed by regression analysis. All data were transformed by  $log_{10}$  to meet the normality hypothesis test. The data were analyzed using R 4.1.3. [26].

# 3. Results

## 3.1. PCA Analysis of Traits and Intraspecific Trait Variation

PCA analysis results show that LDMC, SLA, height, individual size and single leaf area had higher load on the first principal component and leaf thickness had a higher load on the second principal component. The contribution rate of the first principal component was 63.7%, the contribution rate of the second principal component was 18.7%, and the total contribution rate was 82% (Figure 2a). The intraspecific variation of individual size, single leaf area and leaf thickness had a higher load on the first principal component, and the intraspecific variation of SLA, LDMC and height had a higher load on the second principal component. The contribution rate of the first principal component was 40.3%, the contribution rate of the second principal component was 22.2%, and the total contribution rate was 62.5% (Figure 2b).



**Figure 2.** PCA analysis of trait mean (**a**) and variation (**b**) for SLA, LDMC, single leaf area, leaf thickness, height, and individual size.

## 3.2. Response of Traits to Precipitation Change, N Addition, P Addition and Their Interactions

SLA, LDMC, single leaf area, height, and individual size of *P. anserina* had significant responses to precipitation change (W) and P addition (p < 0.01; Table 2). LDMC, single leaf area, height and individual size and had significant responses to N addition (p < 0.01), while SLA had no significant response (Table 2). All interactions had no significant effect on each trait (Table 2). Leaf thickness had no significant response to all treatments.

**Table 2.** Three-way ANOVAs for traits and abundance of *Potentilla anserina* in response to three main factors and their interactions.

Source of Variation	W	Ν	Р	$\mathbf{W}  imes \mathbf{N}$	$\mathbf{W}  imes \mathbf{P}$	$\mathbf{N}  imes \mathbf{P}$	$\mathbf{W}\times\mathbf{N}\times\mathbf{P}$
SLA	9.608 ***	1.782	40.203 ***	0.225	0.944	1.204	0.909
LDMC	12.234 ***	10.093 **	54.556 ***	1.423	2.733	0.130	0.558
Single leaf area	10.501 ***	26.468 ***	34.617 ***	0.575	2.054	0.833	1.146
Leaf thickness	0.909	0.793	0.112	0.867	0.002	3.360	1.561
Height	24.289 ***	41.334 ***	126.907 ***	0.101	2.400	3.540	0.807
Individual size	7.542 **	43.766 ***	26.571 ***	0.837	0.197	0.354	0.177
Abundance	35.918 ***	11.643 **	7.700 *	1.485	3.530 *	0.217	0.361

Note: SLA: Specific leaf area. LDMC: Leaf dry matter content. W: Indicates precipitation treatment. N: Indicates nitrogen addition. P: Indicates phosphorus addition.  $\times$ : Indicates interaction. The values in the table indicate F-values. \* indicates p < 0.05; \*\* indicates p < 0.01; \*\*\* indicates p < 0.001.

Compared to  $W_0$ ,  $W_-$  significantly reduced SLA and single leaf area, and significantly increased LDMC;  $W_+$  had no significant effect on traits (Figure 3). There was significant difference between  $W_+$  and  $W_-$ , for SLA, LDMC, single leaf area, height and individual size, respectively (Figure 3).  $N_+$  significantly reduced LDMC, and significantly increased single leaf area, height and individual size, and had no significant effect on other traits (Figure 3).  $P_+$  significantly increased SLA, single leaf area, height and individual size, and significantly decreased LDMC, and had no significant effect on leaf thickness (Figure 3).



**Figure 3.** Traits under treatments of precipitation change (a,d,g,j,m,p), nitrogen (b,e,h,k,n,q) and phosphorus (c,f,i,l,o,r) additions.  $W_0N_0P_0$ : control group;  $W_+$ : 50% precipitation increase;  $W_-$ : 50% rain reduction;  $N_+$ : nitrogen addition;  $P_+$ : phosphorus addition. Different lowercase letters in the figure indicate the significant difference of the mean value of traits under different treatments, and the Tukey's HSD method or paired *t*-test (for two levels) was used to test the significance.

# 3.3. Response of Intraspecific Variation of Traits and Plant Abundance to Precipitation Change, N Addition, P Addition and Their Interactions

The intraspecific variation of SLA and individual size had a positively significant response to P addition (p < 0.05, Table 3, Figure 4). Other main treatment factors and all interactions had no significant effect on the intraspecific variation of trait.

**Table 3.** Three-way ANOVAs of intraspecific trait variation in *Potentilla anserina* in response to three main factors and their interactions.

Source of Variation	W	Ν	Р	$\mathbf{W}  imes \mathbf{N}$	$\mathbf{W}  imes \mathbf{P}$	$\mathbf{N}  imes \mathbf{P}$	$W \times N \times P$
SLA variation	0.693	0.109	4.916 *	1.177	0.627	0.159	0.043
LDMC variation	0.407	1.302	0.576	1.126	0.394	1.918	0.263
Single leaf area variation	1.496	0.002	0.440	0.412	0.265	0.007	0.601
Leaf thickness variation	0.296	0.126	1.840	0.473	0.435	0.474	1.794
Height variation	2.773	2.427	0.639	0.041	0.117	2.359	0.513
Individual size variation	0.072	1.667	5.561 *	1.661	1.332	1.720	2.781

Note: see note of Table 2. \* indicates p < 0.05.



**Figure 4.** Intraspecific variation of SLA (**a**) and individual size (**b**) of *Potentilla anserina* under phosphorus addition.  $P_0$ : control treatment;  $P_+$ : phosphorus addition. Different lower-case letters in the figure indicate the significant difference of the mean value of traits under different treatments, and the paired *t*-test was used to test the significance.

Three main factors significantly affected plant abundance (Table 2).  $N_+$  significantly increased plant abundance, while drought significantly decreased plant abundance (Figure S2). There was a significantly interaction between precipitation change and P addition (Table 2), while other interactions had no significant effect on plant abundance. Compared to  $W_-P_0$ ,  $W_-P_+$  significantly increased plant abundance (Figure S2).

## 3.4. Relationships of Plant Abundance with Traits and Their Intraspecific Variation

There were significantly positive correlations of abundance with SLA, single leaf area, height and individual size, separately, and a significantly negative correlation of abundance with LDMC (Figure 5). The intraspecific variation of each trait had no relationship with abundance (Table 4).



**Figure 5.** Relationships of abundance with trait mean of SLA (**a**), LDMC (**b**), single leaf area (**c**), leaf thickness (**d**), height (**e**) and individual size (**f**).

Source of Variation	Slope	Intercept	R <sup>2</sup>	p
SLA variation	-0.231	0.853	0.007	0.5247
LDMC variation	-0.231	0.836	0.005	0.5788
Single leaf area variation	0.181	1.224	0.003	0.6584
Leaf thickness variation	0.329	1.475	0.011	0.4339
Height variation	-0.575	0.614	0.051	0.0841
Individual size variation	0.181	1.224	0.003	0.6584

Table 4. Regression analysis of the variation of different traits with plant abundance.

Note: see note of Table 2.

# 4. Discussion

In this study, we explored responses of traits and abundance of *P. anserina* to precipitation changes and N and P addition, and influences of traits on abundance in an alpine meadow on the eastern Qinghai Tibet Plateau. We found that precipitation changes and N and P addition significantly affected traits and abundance of *P. anserina*. We also revealed responses of traits to different treatments and the relationships between traits and abundance. In addition, we found that responses of traits of *P. anserina* to P addition was greater than that to N addition. The mean value of a trait is more important than intraspecific variation in impacts on abundance of *P. anserina* in the community.

## 4.1. Effects of Precipitation Change and N and P Addition on Traits

Plants adopt different strategies of resource utilization to cope with eutrophication and drought, and plant functional traits can reflect plant resource utilization strategies. With the increase of resource availability, plants shifted their strategies from conservative to resource acquisitive [9]. We found that N and P additions significantly increased SLA, single leaf area, height and individual size, and significantly reduced LDMC. These results suggest that under eutrophication, plants can improve their ability to compete for light by increasing SLA, single leaf area, height and individual size, and take a strategy of faster nutrient cycling to adapt to eutrophic environments [27]. Under drought, SLA, single leaf area, height and individual size were significantly reduced while LDMC was significantly increased. These results suggest that in order to adapt to the arid environment, plants adopt conservative strategy to reduce the speed of nutrient cycle.

#### 4.2. Effects of Precipitation Change and N and P Additions on Plant Abundance

In this study, drought reduced abundance of *P. anserine*, suggesting that precipitation was the main factor limiting aboveground biomass, which is consistent with previous studies [28,29]. N addition significantly increased abundance of *P. anserina*, showing that its abundance in this ecosystem was limited by N. Previous studies showed that ecosystems are also limited by N in the temperate steppe [30,31]. Interestingly, there was a significantly interaction between precipitation change and P addition, suggesting that precipitation change can modify the effect of P addition on abundance. Previous research found that P is a limiting factor for biological growth and important ecological processes in many terrestrial ecosystems [32–34]. Our research suggests that water conditions may affect whether P is the limiting factor of the ecosystem. More specifically, we found that P is the limiting factor for plant abundance under drought; or it can be said that P eliminated plant growth restriction caused by drought.

## 4.3. Effects of Mean Value of Traits on Plant Abundance

We found that there was a significant positive correlation of SLA, height, individual size and single leaf area of *P. anserina* with its abundance. This is because the increased height and individual size of the plants establishes them in the upper part of the community, where they can get more sunlight. Plant leaves with high SLA have stronger light acquisition ability [35]. This asymmetry of plant competition for light resources in the community caused by plant height and individual size not only makes the upper layers more competitive but also shades the lower layers, putting the lower layers at risk of species loss [36–38]. Relative to drought, natural precipitation, increased precipitation and nutrient addition all significantly increased SLA, individual size, height, and single leaf area of *P. anserina*. This suggests that the shift in traits towards a resource acquisitive strategy with increased precipitation and nutrient addition lead to a significant increase in species abundance. This corroborates our hypothesis that increased precipitation and nutrients would make *P. anserina* more interspecific competitive, thus enabling it to become the dominant species in the community.

## 4.4. Effects of Intraspecific Trait Variation on Plant Abundance

Albert et al. (2010) proposed that there are variations of traits within plant species, but there is no unified rule for this intraspecific variation and, for the same species, the sources and degrees of intraspecific variation of different traits are different [39]. Genetic diversity and phenotypic plasticity also lead to variation in traits among different individuals of the same species, and this variation allows different individuals of the same species to survive and reproduce in different environments, and is a reflection of the ability of organisms to adapt to their environment [40]. We found that trait mean values generally significantly affected above-ground biomass, while trait variation did not significantly correlate with its abundance either. Thus, the trait mean value of *P. anserina* had more influence on its fitness in the community than trait variation.

# 4.5. Limitation of P on Traits of Potentilla Anserina

N and P, as macroelements required for plant growth [41], can affect the morphological, physiological and chemical properties of plants by altering nutrient cycling in terrestrial ecosystems [42]. Díaz et al. (2016) pointed out that N addition significantly affected plant height and leaf functional traits of plants [13]. In nature, biological N fixation by

N-fixing bacteria is very limited, and the available N in the ecosystem is easily lost from the ecosystem by leaching, volatilization, nitrification and denitrification processes. Therefore, N is the most important nutrient that limits plant growth [43], and many ecosystems exhibit as N-limited. A recent global analysis shows that P restriction is also widely distributed in plant growth and productivity in terrestrial ecosystems [44]. Reed et al. (2015) suggested that there is an increasing need to integrate the P cycle with vegetation dynamics [45].

In our study, although N and P additions both had significant effects on the mean value of plant traits, responses of traits to P addition were more significant than N addition. N addition had no significant effect on SLA, while P addition significantly increased SLA. Moreover, only P addition, not N addition, affected the intraspecific variation of SLA and individual size. This is consistent with previous research, in that Cui et al. (2022) found that the variation of plant traits in a subtropical forest was mainly driven by P [46]. Therefore, our results show that in the alpine meadow ecosystem we studied, the effect of P on plant functional traits is greater than that of N.

## 5. Conclusions

From the perspective of plant functional traits, this study explored the response of mean value and intraspecific variation of traits and abundance of *P. anserina* in an alpine meadow to precipitation change, N and P additions and their interactions, and the relationships of mean value and intraspecific variation of traits with abundance. Our results suggest that drought shifted plant traits from resource acquisitive to more conservative, while nutrients addition shifted plant traits from conservative to more resource acquisitive. The effect of P addition on traits of *P. anserina* was larger than that of N addition. This suggests that we should pay attention to the impact of P on alpine ecosystems and its mechanism in the future research. Additionally, we found abundance of *P. anserina* was related to the mean value of traits, not intraspecific variation of traits. In conclusion, our research results show that species adapt to environmental changes by adjusting their traits, thereby changing the abundance of species in the community, and ultimately potentially affecting community structure and ecosystem functions.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos13111820/s1. Figure S1: Digital Elevation Map (DEM) of the experimental site and China. Figure S2: Abundance of *Potentilla anserina* under treatments of nitrogen addition (a), and combinations of precipitation change and phosphorus addition (b).

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