Response of leaf traits of common plants in alpine meadow to plateau pika disturbance

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Abstract. Leaf traits have been proven to reflect the adaptation of individual plants to disturbance environments in a grassland ecosystem. A field survey was conducted to investigate the effects of the disturbance intensity of plateau pika on the leaf traits of a dominant (\textit{Kobresia pygmaea}) and two common plants (\textit{Elymus nutans} and \textit{Anemone rivularis} var. \textit{flore-minore}) in an alpine meadow. This study indicated that the plateau pika disturbance enables the individuals of three plants to exhibit respective plasticity because the three plants had different leaf indices (LI) as the disturbance intensity increased. \textit{K. pygmaea}, \textit{E. nutans} and \textit{A. rivularis} var. \textit{flore-minore} had high specific leaf area (SLA), leaf dry mass content (LDMC), and leaf nitrogen content (LNC) at relatively low, moderate, and high disturbance intensities of plateau pika, respectively. \textit{K. pygmaea}, \textit{E. nutans} and \textit{A. rivularis} var. \textit{flore-minore} suffered low nutrient stress at low, moderate and high disturbance intensities due to high N:P at corresponding disturbance intensities. These results indicated that \textit{K. pygmaea}, \textit{E. nutans} and \textit{A. rivularis} var. \textit{flore-minore} grew well at relatively low, moderate, and high disturbance intensity conditions, respectively, which contributed to the improvement of alpine meadows with a higher proportion of \textit{E. nutans} at a moderate disturbance intensity or the deterioration of alpine meadows with a higher proportion of \textit{A. rivularis} var. \textit{flore-minore} at a high disturbance intensity. Our findings suggest that leaf traits are effective tools to explain how small burrowing herbivore disturbances often lead to the improvement or deterioration of alpine meadows under different disturbance intensities.

Additional keywords: alpine meadow, disturbance intensity of plateau pika, dominant and common plants, improvement or deterioration, leaf traits.

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Introduction

Alpine meadows on the Qinghai-Tibetan Plateau play major roles in ecological barrier and livestock production (Zhang \textit{et al.} 2016). However, they are also sensitive and vulnerable to herbivory disturbances (Sun \textit{et al.} 2009; Wu \textit{et al.} 2009; Sun \textit{et al.} 2015a, 2015b; Niu \textit{et al.} 2016; Zhang \textit{et al.} 2016). The plateau pika (\textit{Ochotona curzoniae}) is an endemic and small herbivore on the Qinghai-Tibetan Plateau, and usually creates extensive disturbances on alpine meadows via burrowing and foraging behaviours (Smith and Foggin 1999; Sun \textit{et al.} 2009; Guo \textit{et al.} 2012a, 2012b; Liu \textit{et al.} 2013; Sun \textit{et al.} 2015a, 2015b; Choying 2016). The impact of this extensive disturbance on alpine meadows often improves or deteriorates alpine meadows, and this may be ascribed to the disturbance intensities of plateau pika. The literature shows that low disturbance intensities result in the improvement of alpine meadows; in contrast, high disturbance intensities encourage alpine meadows to deteriorate (Guo \textit{et al.} 2012a; Jia \textit{et al.} 2014; Pang \textit{et al.} 2015).

The improvement or deterioration of alpine meadows is mainly caused by the replacement of the main plants in the plant community (Li \textit{et al.} 2014; Pang \textit{et al.} 2015). \textit{Kobresia pygmaea} is a dominant plant and \textit{Elymus nutans} and \textit{Anemone rivularis} var. \textit{flore-minore} are common and frequent plants in alpine meadows where plateau pikas are present (Jia \textit{et al.} 2014; Wang \textit{et al.} 2016b; Pang and Guo 2017). To a large extent, the responses of changes in the dominance degree of these three populations to the disturbance intensities of plateau pika can reflect the improvement or deterioration of alpine meadows because \textit{K. pygmaea} and \textit{E. nutans} are of good quality and \textit{A. rivularis} var. \textit{flore-minore} is a major weed (Chen and Jia 2002). The performance of the three plant populations under different disturbance intensities of plateau pika is often achieved through individual plant adaptation to disturbance environments.

Leaf traits are considered to be plastic morphologic traits and are also considered to be good tools to reflect individual plant adaptations to disturbance environments (Díaz \textit{et al.} 2007;
Cruz et al. 2010; Firn et al. 2017) because leaves with diverse shapes and sizes have different carbon assimilations, resource use strategies and energy balances (Rusch et al. 2009; Cruz et al. 2010; Zheng et al. 2010; Firn et al. 2017). Specific leaf area (SLA), leaf dry mass content (LDMC), leaf nitrogen concentration (LNC), leaf phosphorus concentration (LPC), and the leaf index (LI) (ratio of length to width) are generally regarded as important and common leaf traits (Tsukaya 2002; Laliberté et al. 2012; Meng et al. 2015), each of which play different roles in plant growth (Cornelissen et al. 2003). Several studies have verified that SLA, LDMC, LNC, LPC and LI can be used to successfully explain individual plant adaptations to large herbivore disturbance (such as livestock grazing) and predict the growth potential of different plant populations under different grazing intensities (Cruz et al. 2010; Zheng et al. 2010; Laliberté et al. 2012; Wang et al. 2016a). However, the effects of small herbivore disturbance on leaf traits received little attention, and more studies are needed to determine whether leaf traits can be applied to explain the adaptation of individual plants to different disturbance environments of small herbivores. Therefore, this study attempts to investigate the effects of different disturbance intensities of plateau pika on the leaf traits of K. pygmaea, E. nutans, and A. rivularis var. flore-minore, which may explain how different disturbance intensities of plateau pika often improve or deteriorate alpine meadows in the Qinghai-Tibetan Plateau. In this study, we hypothesised that (1) plateau pika, as well as big herbivores, disturbance affects leaf traits; (2) the plant traits of three common plants respond to the disturbance intensities of plateau pika differently.

Materials and methods

Study area description

This study was carried out at Maqu County (100°45′45″–102°29′00″E, 33°06′30″–34°30′15″N) in Gansu Province, China. This county is located in the eastern region of the Qinghai-Tibetan Plateau. The climate in this county is cold and humid, consisting of a short warm season (May–September) and a long cool season (October–April). The mean annual temperature is 1.2°C (ranging from −10°C in January to 11.7°C in July) and the mean annual precipitation is 564 mm, 80% of which occurs from May to September. The annual potential evaporation ranges from 1000 to 1500 mm. According to the Chinese soil classification system (Gong 2001), the soil type is subalpine meadow soil (Yu et al. 2017a). The vegetation type is alpine meadow, the dominant species of which is K. pygmaea, and the subdominant species are E. nutans and A. rivularis var. flore-minore, whereas the other associated species are Polygonum viviparum, Potentilla fruticosa, Oryzopsis munroii, Roegneria kamohi, Festuca rubra and Agrostis matsumurae (Li et al. 2014). Alpine meadow accounts for 89.5% of the total land area in Maqu County, and is used for grazing traditional livestock. Plateau pika is the main species of small mammal herbivore that lives in the alpine meadow and is spread over all fitness habitats. The disturbance intensity is different across all present habitats due to pika biology.

Experimental design and sampling

It is difficult to use the actual density of plateau pika to determine their disturbance intensities in the field because plateau pikas are social mammals and live in family groups consisting of two to five adults and their young (up to eight), which do not disperse in their year of birth (Dobson et al. 1998; Qu et al. 2012, 2013). Therefore, the density of active burrow entrances is always used as an index to estimate the disturbance intensity of plateau pika on the Qinghai-Tibetan Plateau, China (Sun et al. 2009; Guo et al. 2012a, 2012b; Jia et al. 2014; Sun et al. 2015a, 2015b; Pang and Guo 2017; Yu et al. 2017a). This method was also used for the disturbance intensity of black-tailed prairie dogs in north central Colorado, USA (Johnson and Collinge 2004). The population density of plateau pika can grow rapidly within a relatively short period, with a peak in population during early August (Liu et al. 1982; Pang and Guo 2017; Yu et al. 2017b).

Field surveys were conducted in a winter-grazed alpine meadow (no grazing during the survey period) during early August 2015. We randomly chose 12 disturbance sites where plateau pikas were present and burrow entrances were observed in the field and these sites shared a common alpine meadow type and topographic and geomorphic conditions. At each disturbance site, 1 plot of 25 m × 25 m was arranged, and the distance between plots approximately ranged from 0.5 km to 4.5 km. In each plot, the burrow entrances were plugged for three days and the number of plugs cleared by plateau pikas to allow access to the meadow surface was recorded (Guo et al. 2012a, 2012b; Sun et al. 2015a). The number of burrow entrances whose plugs were cleared by plateau pikas in three days per plot was pooled, and its average value was regarded as the active burrow entrances of that plot.

In each plot, five subplots (1 m × 1 m) were designed in a "W" distribution pattern to collect plant leaves. In each subplot, all individuals of K. pygmaea, E. nutans and A. rivularis var. flore-minore with health leaves were harvested by clipping at ground level from 10:00 am to 4:00 pm (Cornelissen et al. 2003). All harvested individuals of each species were wrapped in moist paper and immediately placed in a valve bag, so that they remained water-saturated. These samples were stored in a cool box until further processing in the laboratory.

Leaf trait measurement

All healthy leaves from harvested individuals for each species in each subplot were used to measure leaf traits, from which 30 completely expanded leaves from 15 randomly selected individuals (two leaves per individual) were used to measure leaf length, leaf width, leaf area, leaf dry weight and leaf saturated weight. The average value of 30 selected leaves was regarded as the index value of that subplot. Other leaves were used to measure the P and N concentrations.

Leaf length and width were measured with Vernier callipers, and leaf area was measured with a CI-203 leaf area meter (QC CID, WA, USA). The leaf area of K. pygmaea and E. nutans was difficult to directly measure because their leaves are very narrow. Therefore, we cut green paper of a known (total) area to the desired dimensions and treated several leaves as if they were one. After measuring leaf area, the leaves were placed in water at 5°C in the dark overnight to produce a standard degree of turgor for measuring saturated weight. The lamina samples were blotted, dried with tissue paper to remove surface water, immediately
weighed to measure saturated weight, placed in a 105°C hot oven for 15 min and then stoved to balance weight in a 65°C hot oven for determining the dry weight. SLA was calculated as \( SLA = \frac{\text{leaf area}}{\text{leaf dry weight}} \), and LDMC was calculated as \( LDMC = \frac{\text{leaf dry weight}}{\text{leaf saturated weight}} \). LI was calculated as \( LI = \frac{\text{leaf length}}{\text{leaf width}} \). LNC and LPC were determined by the Kjeldahl method (Foss Kjeltec 8400, FOSS, Hillerød, Denmark) and Mo-Sb colourimetry (UV-2102C, UNICO, Shanghai, China), respectively.

Statistical analysis
The data from five subplots within each plot were pooled to test the relationships between leaf traits and the disturbance intensity of plateau pika. The responses of leaf traits to the disturbance intensity of plateau pika were determined and the disturbance intensity (active burrow entrance densities) was considered to be the fixed factor, which was used to construct a regression analysis. The regression curves of leaf traits/disturbance intensity were obtained by ordinary linear regression, nonlinear regression or quadric regression models; in this study, regression models were ultimately chosen on the basis of their significance. Pearson correlation analysis was used to analyse the relationships among leaf traits. All of the statistical analyses were performed with R Version 3.4.1 (R Core Team 2013).

Results

Relationships between leaf traits of K. pygmaea and the disturbance intensity of plateau pika
With the exception of LPC, the other leaf traits were significantly related to the disturbance intensity of plateau pika for K. pygmaea (Fig. 1) in which LI and LNC showed decreasing trends \( (F = 11.680, P = 0.007, R^2 = 0.493; F = 42.800, P < 0.001, R^2 = 0.792) \), SLA showed a parabolically decreasing trend \( (F = 8.470, P = 0.009, R^2 = 0.576) \), and LDMC first increased and then decreased and peaked at relatively low disturbance intensity.

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**Fig. 1.** (a) LI, (b) SLA, (c) LDMC, (d) LNC, (e) LPC and (f) N : P of Kobresia pygmaea based on a linear model. For a detailed visualisation of the relationship between the disturbance intensity and LI, SLA, LDMC, LNC, LPC and N : P an adjusted local smoothed regression line (black) with its 95% confident interval (grey) was used. LI, leaf index; SLA, specific leaf area; LDMC, leaf dry mass content; LNC, leaf nitrogen concentration; LPC, leaf phosphorus concentration; N : P, the ratio of leaf nitrogen to leaf phosphorus; and Disturbance intensity, active burrow entrance density.
intensities ($F = 38.850, P < 0.001, R^2 = 0.873$) as the disturbance intensity of plateau pika increased.

*Relationships between leaf traits of *E. nutans* and the disturbance intensity of plateau pika*

The disturbance intensity of plateau pika had a significant effect on LI ($F = 12.720, P = 0.002, R^2 = 0.681$), SLA ($F = 10.670, P = 0.004, R^2 = 0.638$), LDMC ($F = 4.950, P = 0.035, R^2 = 0.418$), and LNC ($F = 7.545, P = 0.012, R^2 = 0.543$) of *E. nutans* (Fig. 2); these leaf traits showed downward parabolas, and they all peaked at moderate disturbance intensities, whereas the disturbance intensity of plateau pika had no significant effect on LPC.

*Relationships between leaf traits of *A. rivularis* var. flore-minore and the disturbance intensity of plateau pika*

Leaf traits of *A. rivularis* var. *flore-minore* showed different relationships with the disturbance intensity of plateau pika (Fig. 3). With the increase in disturbance intensity, LI and LNC tended to increase ($F = 8.121, P = 0.017, R^2 = 0.393$; $F = 23.960, P = 0.001, R^2 = 0.676$), SLA presented a parabolically increasing trend ($F = 4.608, P = 0.042, R^2 = 0.400$), LDMC showed an upward parabolic trend ($F = 41.250, P < 0.001, R^2 = 0.880$), and LPC increased first and then decreased ($F = 5.675, P = 0.025, R^2 = 0.459$).

*Relationship among leaf traits of the three dominant plants*

Pearson analysis results showed that the relationship among different leaf traits varied with plant species (Tables 1–3). With the exception of LPC, the relationships among leaf traits for *K. pygmaea* showed significantly positive correlation (Table 1). LDMC had no relationship with LNC for *Elymus nutans*, whereas the relationships among other leaf traits for *Elymus nutans* were similar to those for *K. pygmaea* (Table 2). LNC of *Anemone rivularis* var. *flore-minore* was positively correlated with LI and SLA, whereas LDMC was negatively correlated with LPC (Table 3).

![Fig. 2](image-url)
Discussion

Leaf traits can be used to predict the relative growth potential of dominant plants (Tsukaya 2002; Hikosaka and Osone 2009; Meng et al. 2015; Laughlin et al. 2015; Wang et al. 2016a) whose replacement might reflect the potential change direction of grassland (Guo et al. 2012a; Jia et al. 2014; Pang and Guo 2017). To explain how plateau pika disturbances improve or deteriorate alpine meadows on the Qinghai-Tibetan Plateau,
Our study applies the leaf traits of diverse leaf shapes to investigate the adaptation of a dominant plant and two common plant individuals to different disturbance intensities of plateau pika. Our findings show that the disturbance intensities of plateau pika affect the leaf traits of three plants, similar to the effects of the grazing intensities of large herbivores on leaf traits (Cruz et al. 2010; Zheng et al. 2010; Laliberté et al. 2012; Wang et al. 2016a). Li shows the different trends among *K. pygmaea*, *E. nutans* and *A. rivularis* var. *flore-minore* with the increase in disturbance intensity, indicating that the extended leaf length or leaf width of the three plants is influenced by the disturbance intensities of plateau pika. These results imply that the different disturbance intensities of plateau pika enable different individual plants to exhibit respective plasticity, and their populations will develop respective plasticity for adapting to different disturbance intensities of plateau pika.

The SLA is the light-catching area deployed per unit of previously photosynthesised dry mass allocated to that purpose (Reich et al. 1998) and often reflects the potential relative growth rate or mass-based maximum photosynthetic rate of plants (Cornelissen et al. 2003; Firn et al. 2017). Meanwhile LNC is positively related to plant metabolic activity and can also reflect plant growth because LNC has a positive relationship with photosynthetic capacity (Gulmon and Chu 1981; Hedin 2004; Hikosaka and Osone 2009). In our findings, the SLA and LNC of *K. pygmaea*, the dominant plant, are negatively related to the disturbance intensity, which infers that *K. pygmaea* has a higher potential relative growth rate at a relatively low disturbance intensity and grows better than under moderate and high disturbance intensities. Higher disturbance intensities decrease the soil nutrients (such as soil total N, total P, and organic carbon) and soil moisture (Pang and Guo 2017; Yu et al. 2017a), which leads to a smaller SLA for *K. pygmaea*. This is because the plant develops a smaller SLA in resource-stressed environment than that in resource-rich environments (Cornelissen et al. 2003; Zheng et al. 2010), resulting in a relatively low potential relative growth rate. LDMC was higher at relatively low disturbance intensities than moderate and high disturbance intensities, which implies that the *K. pygmaea* adapts better at relatively low disturbance intensities because leaves with high LDMC show more resistance to physical disturbances by herbivores than leaves with low LDMC (Cornelissen et al. 2003; Sasaki et al. 2013), and plants with higher LDMC tend to adapt well (Cruz et al. 2010; Wang et al. 2016a).

*E. nutans* is one of the common and frequent plants of alpine meadows in our study, and it occupies an essential role in the plant community because of its good quality for livestock. Thus, its high proportion in the plant community indicates the improvement of the alpine meadow. Our results showed that the SLA and LNC of *E. nutans* present downward parabola trends, peaking at moderate disturbance intensities because low or high disturbance intensities of plateau pika cause soil nutrient stress with low soil total N, total P, and organic carbon (Pang and Guo 2017; Yu et al. 2017a). Higher SLA values tend to correspond to a relatively high investment in plant growth rather than leaf defence (Cornelissen et al. 2003; Cingolani et al. 2005), and a higher LNC indicates a higher rate of physiological metabolic activity, which implies that *E. nutans* performs better at moderate disturbance intensities of plateau pika than low or high disturbance intensities. The LDMC of *E. nutans* also first increases and then decreases as the disturbance intensities increase, inferring that *E. nutans* shows better adaptation at moderate disturbance intensity conditions.

*A. rivularis* var. *flore-minore* is another common and frequent plant of alpine meadows, and it is usually considered to be a common weed. Its higher proportion in the plant community deteriorates the quality of alpine meadows. We found that the SLA and LNC of *A. rivularis* var. *flore-minore* present increasing trends with increasing disturbance intensity, which indicates that *A. rivularis* var. *flore-minore* grows well at higher disturbance intensities. Its LDMC shows an upward parabolic trend in which the minimum value is observed at relatively lower disturbance intensities; this shows that *A. rivularis* var. *flore-minore* has a better adaptation at high disturbance intensity than low and moderate disturbance intensities.

The ratio of leaf N to P (N : P) of a given plant tends to be very significant for N and P availability (Cornelissen et al. 2003; Luo et al. 2014), and it is a proven useful diagnostic indicator of N or P limitation to plant growth (Güsewell 2005; Luo et al. 2014). Increasingly, studies agree that lower N : P ratios indicate that plant growth suffers from N deficiency, and vice versa (McGroddy et al. 2004; Zhang et al. 2011; Luo et al. 2014). Our findings show that the leaf N : P ratios of the three plants are below 14 regardless of the disturbance intensities of plateau pika (Figs 1–3), indicating that the growth of the three plants generally suffers from N-limited conditions. However, the disturbance intensities of plateau pika alter the N-limitation status of the three plants. The N-limitation status was first exacerbated and then alleviated for *K. pygmaea* (F = 6.707, P = 0.016, R² = 0.509), whereas it shows an opposite process for *E. nutans* (F = 5.533, P = 0.027, R² = 0.452) when compared with *K. pygmaea*, and the N-limitation status was gradually alleviated for *A. rivularis* var. *flore-minore* (F = 25.760, P < 0.001, R² = 0.692). These findings imply that *E. nutans* suffers less nutrient stress at moderate disturbance intensities than other disturbed intensities and that *K. pygmaea* and *A. rivularis* var. *flore-minore* do the same at relatively low disturbance intensities and at relatively high disturbance intensities, respectively.

Plateau pika disturbance alters the composition of plant communities by selecting plants with leaf traits that confer optimum fitness for those disturbance conditions (Li et al. 2014). Our results indicate that three plants might develop distinctive strategies with different plasticity, relative growth potentials and N or P limited statuses at different disturbance intensity conditions and show different adaptations to varying disturbance intensities caused by plateau pika, the same as marmot disturbances in Hustai National Park of Mongolia (Sasaki et al. 2013). Comprehensively allowing for the performances of SLA, LDMC, LI, LNC and LPC, the three plants present different adaptations to increasing disturbance intensity of plateau pika; *K. pygmaea* adapts well at a relatively low disturbance intensity, *A. rivularis* var. *flore-minore* grows well at a relatively high disturbance intensity, and *E. nutans* performs well at a moderate disturbance intensity, which may contribute to the replacement of the main plants for a given plant community in the long-term. This is one of the most likely
reasons that plateau pika disturbance leads to an improved alpine meadow with a high proportion of *E. nutans* or deteriorated alpine meadows with a high proportion of *A. rivularis* var. *flor-e-minore*. Therefore, our results verify that leaf traits can be used to explain that the disturbance intensities of plateau pika cause alpine meadows to generate different outcomes to some extent.

**Conclusion**

The leaf traits of plants have been shown to reflect the adaptation of individual plants to disturbance environments, and our study investigates the effects of the disturbance intensities of plateau pika on the SLA, LDMC, LI, LNC and LPC of a dominant plant and two common plants and attempts to clarify how the disturbance intensities of plateau pika improve or deteriorate alpine meadows. Our findings show that *K. pygmaea*, *E. nutans* and *A. rivularis* var. *flor-e-minore* have different SLA, LDMC, LI, LNC and LPC as the disturbance intensity of plateau pika increases, and these results imply that different soil nutrient stresses caused by the disturbance intensities of plateau pika encourage the three plants to develop distinctive plasticity, differently relative growth potentials and relative adaptive abilities, and different N stress statuses, resulting in their distinctive adaptation strategies. The three plants might encourage the changes in proportion of the three plants of a given plant community in the long-term and consequently improve alpine meadows with higher proportions of *E. nutans* and *A. rivularis* var. *flor-e-minore* at moderate disturbance intensity conditions due to high soil N content, or conversely deteriorate alpine meadows with higher proportions of *A. rivularis* var. *flor-e-minore* at high disturbance intensity conditions. Therefore, our study presents a possible hypothesis for how small burrowing herbivore disturbances often lead alpine meadows to develop alternative outcomes by analysing the leaf traits.

**Conflicts of interest**

The authors declare no conflicts of interest.

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