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Response of plant functional traits to grazing for three dominant species in alpine steppe habitat of the Qinghai–Tibet Plateau, China

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Abstract Above-ground biomass (AGB) is an important indicator of grassland ecosystem performance. Easily measured plant functional traits (PFTs) may provide useful predictors of the response of plants to grazing. Understanding the response of PFTs to grazing and the relationship between PFTs and AGB is very important for effectively predicting the response of ecosystems to grazing and rangeland management. A grazing experiment was conducted in Gangcha County, Qinghai Province, in the northeastern part of the Qinghai-Tibet Plateau in 2012 and 2013. We investigated the response of PFTs in three dominant species (Elymus nutans, Kobresia humilis, and Stipa purpurea) to grazing, using six stocking rates. Plant height (PH), plant weight, leaf area, and leaf dry biomass of these three dominant species had significantly negative relationships with stocking rate. Leaf thickness (LT) of these three species usually showed a unimodal response to grazing. Specific leaf area generally showed a quadratic relationship with grazing intensity. No consistent effects of grazing were observed on nitrogen content per unit mass (N_{mass}) and nitrogen content per unit area (N_{area}). PH, leaf area, and leaf dry mass (LDM) were positively associated with AGB, but LT, N_{mass}, and N_{area} had no statistically significant association with AGB. We thus conclude that PH, leaf area, and LDM best predict the effects of grazing on AGB. Finally, 2.87 sheep/ha is recommended as the

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Qinghai Academy of Animal and Veterinary Sciences, Key Laboratory of Alpine Grassland Ecosystem in the Three River Head Waters Region Jointly Funded by Qinghai Province and Ministry of Education, 810016 Xining, China optimal stocking rate in this region to maintain the health of this grassland ecosystem and to allow for sustainable development.

Keywords Above-ground biomass · Alpine steppe · Dominant species · Plant functional traits · Stocking rate

Introduction

Livestock grazing is the most important human use of the world's grasslands (Milchunas and Lauenroth 1993). Grazing affects grasslands in multiple ways: it affects above-ground biomass (AGB; Gao et al. 2008; Schoenbach et al. 2009), creates changes in community structure and composition (Milchunas et al. 1998; Hendricks et al. 2005; Catorci et al. 2012), and regulates the morphology, physiology, and reproductive allocation of plants (Hickman and Hartnett 2002; Cerato et al. 2010).

As an important indicator of grassland ecosystem performance under grazing management (Seaquist et al. 2003), increased grazing intensity generally negatively affects AGB (Liang et al. 2009; Schoenbach et al. 2011). Grime (1998) proposed a biomass ratio hypothesis, which postulates that ecosystem properties should be related to species traits and the contribution of dominant species to the total biomass of a community. Previous studies tested this hypothesis and verified that some leaf traits exist that could predict ecosystem properties and AGB (Cruz et al. 2010; Garnier et al. 2004; Pontes et al. 2007). Zheng et al. (2010) pointed out that leaf traits of dominant species had positive relationships with AGB. Leaf traits represent plant functional traits (PFTs), which include whole plant and leaf morphology as well as root traits (Díaz et al. 2001; Cingolani et al. 2005; Zheng et al. 2011; Leuschner et al. 2013). However, little is known about the relationships between various PFTs and AGB under grazing in the alpine steppes of the Qinghai-Tibet Plateau; this region includes habitats that are very vulnerable to both ongoing climate change and grazing (Klein et al. 2004). An investigation of the relationship between PFTs and AGB is needed to explore a useful method that can be used to predict AGB.

Plants vary in how their PFTs respond to grazing. Previous studies have shown that changes of PFTs are related to plant phenotypic plasticity under grazing (Díaz et al. 2001; Zhao and wang 2009; Zheng et al. 2010, 2011). In recent years, a number of studies have been conducted to predict the responses of plants to grazing using easily measured PFTs including plant height (PH), leaf area, leaf dry mass (LDM), and specific leaf area (SLA; Díaz et al. 2001; Vesk et al. 2004; Cingolani et al. 2005). Landsberg et al. (1999) and Díaz et al. (2001) reported that PH and leaf size decreased with increasing grazing intensity. Vesk et al. (2004) found that tall plants often form smaller plants when they experience higher grazing pressure. Westoby (1998, 1999) reported that populations of species with a larger SLA declined faster at low grazing pressure and also regenerated faster under heavy grazing than plants with a smaller SLA. Zheng et al. (2011) also found that SLA of Leymus chinensis initially decreased during light to moderate grazing intensity and then increased under heavy grazing during a dry year. Recently, researchers have used leaf thickness (LT) and leaf N content to analyze the responses of various plant species to grazing. LT is probably connected with the tolerance of plants to grazing (Zhao and Wang 2009); the leaf N content of different species also responds in different ways to grazing. Zheng et al. (2011) reported nitrogen content per unit mass (N_{mass}) and per unit area (N_{area}) of L. chinensis increased with increased grazing intensity in a dry year; however, increased grazing intensity resulted in decreased N_{area} and had no effect on N_{mass} in a wet year.

Previous efforts have mostly focused on the relationships between PFTs and grazing intensity, but did not find an appropriate stocking rate that is adapted to local environmental conditions. Moreover, results from studies of particular communities cannot be generalized because of different climatic conditions and grazing histories in different areas (Vesk et al. 2004; Díaz et al. 2007). For example, contradictory views exist related to the prediction of various species' responses using simple traits in sub-humid upland as well as in semi-arid and arid rangelands (Díaz et al. 2001; Vesk et al. 2004). Therefore, additional study is needed to analyze the response of PFTs to grazing so that we can gain a better understanding of the patterns of those responses and to provide better predictive power of the response of plants to grazing.

Grazing management and animal production serve as very essential tools for local social and economic development on the Qinghai–Tibet Plateau (Yu et al. 2012). However, in recent decades, a larger number of areas have been heavily grazed by yak and Tibetan sheep. Grassland degradation has become a severe problem in alpine steppe habitats of the Qinghai–Tibet Plateau (Li et al. 2011; Dong et al. 2012; Yu et al. 2012). Grazing is the main factor that leads to the degeneration of functioning grassland ecosystems, while also resulting in a reduction of productivity and a deterioration in water and soil quality in this region (Dong et al. 2012; Yu et al. 2012; Yan et al. 2013). *Elymus nutans, Kobresia humilis*, and *Stipa purpurea* serve as three dominant, valuable and widely distributed grass species in alpine steppe of the Qinghai–Tibet Plateau. To a large extent, the changes in the PFTs of these three species can reflect the characteristic dynamics of an alpine steppe community under grazing management.

Therefore, *E. nutans*, *K. humilis*, and *S. purpurea* of the alpine steppe in the Qinghai–Tibet Plateau were selected for analysis to answer the following questions in this study: how do PFTs of these three species respond to different stocking rates? What are the relationships between the PFTs and AGB of these three dominant species with a gradient of increased grazing intensity?

Materials and methods

Study area description

The experimental site was selected in Gangcha County in the northeastern districts of Oinghai Province (37°21'N. 100°04'E, 3313 m a.s.l., Fig. 1). Based on long-term meteorological data (1961-2006), the Gangcha County meteorological station reported the annual temperature ranged from -17.5 °C (January) to 11.0 °C (July) and annual precipitation was 370.5 mm with 79-93 % of precipitation falling during the short and cool summer (May-September). The patterns of mean precipitation were similar between 2012 and 2013, but the monthly temperature was little higher in 2013 than in 2012 (Fig. 2). The alpine steppe vegetation type was dominated mainly by E. nutans (Poaceae), K. humilis (Cyperaceae), and S. purpurea (Poaceae). Companion species included Artemisia scoparia (Compositae), Astragalus polycladus (Leguminosae), Heteropappus altaicus (Compositae), Koeleria cristata (Poaceae), Potentilla bifurca (Rosaceae), Potentilla multifida (Rosaceae), and Radix bupleuri (Apiaceae). The mean AGB was about 50–280 g/m². About 13-20 vascular plant species and 103-604 individual plants were recorded per m^2 . The alpine meadow soil type (Xu et al. 2015) had an average pH, soil organic carbon, and total N and P content at depths of 0-10 cm of 7.67, 21.16 g/kg, 5.74 g/kg and 0.73 g/kg, respectively.

Experiment design

The grazing experiment began in June 2010. Grazing lands were subjected to 3 years of grazing from January to December. The experimental treatment consisted of stocking sheep at six stocking rates (0, 1.72, 2.29, 2.87, 4.13, and 5.62 sheep/ha) using six sizes of fenced plots (0.58, 2.91, 2.18, 1.74, 1.45, and 1.25 ha, respectively;



Fig. 1 Location of the grazing experiment; an *inset* map shows the vicinity of the project area within China. A *solid black triangle* indicates each experimental site



Fig. 2 Mean air temperature and the distribution of monthly rainfall in the study area in 2012 and 2013. *Note* meteorological data were collected at an eddy tower approximately 2000 m from the experimental site

Zheng et al. 2012). Three replicates were conducted for each stocking rate. These six stocking rates were based on herbage use ratios of 0, 30, 40, 50, 60, and 70 %, respectively, and were expressed as SR_0 , $SR_{1.72}$, $SR_{2.29}$, $SR_{2.87}$, $SR_{4.13}$, and $SR_{5.62}$, respectively. A total of 28 castrated 16-month-old Tibetan sheep with an average weight of 17 kg were grazed continuously on the plots. Grazing lasted from 8:00 am to 7:00 pm with no supplementary feeding; the sheep were given water to drink twice daily. During the grazing experiment, no sheep died and none were slaughtered.

Trait measurement

During August 5–10 in 2012 and 2013, 30 individuals (ramets) of each of the three species—*E. nutans*, *K. humilis*, and *S. purpurea*—were randomly selected in each

replicate. PH was measured as the distance from the basal stem to the natural crown of each individual plant. After height measurement, each individual was clipped at the base of the stem and the above-ground clippings were placed into individual envelopes for each plant, then returned to the laboratory. These specimens were oven dried at 70 °C for 24 h to a constant mass and weighed to obtain the plant weight (PW). In addition, 30–40 fully expanded, but not senescent, healthy leaves were chosen from 30 to 40 different sexually mature individuals for each species (Zheng et al. 2011). Mean area per leaf (LA, cm^2) was measured based on fresh leaves using a Yaxin-1241 leaf area meter (Beijing Yaxin Science Instrument Technology Co., Ltd., Beijing, China). LT (mm) was measured with an electronic Vernier caliper (Wuxi Xigong Tools & Measuring Instruments Corporation, Wuxi, Jiangsu, China). Next, these leaf samples were taken to the laboratory and the mean dry mass per leaf (leaf mass in g) was estimated by oven drying the samples to a constant weight (65 °C) and then weighing the leaf samples. Mean SLA ($cm^2 g^{-1}$) was calculated using the above index measurements (Cornelissen et al. 2003). Moreover, oven-dried leaf samples from each species were crushed by a FZ102 sample mill plant disintegrator (Taisite Instrument, Ltd., Tianjin, China) and leaf N content was analyzed with Kjeldahl methods (Kjeldahl 2300 Analyzer Unit, Foss, Sweden). Leaf N content was represented as both mass base (N_{mass} , %) and area base (N_{area} , g m⁻²), the latter being calculated as N_{mass} divided by SLA (Cornelissen et al. 2003; Zheng et al. 2011).

AGB measurement

AGB measurement was based on the methods of Zheng et al. (2011). Four 2×2 -m movable iron cages were set in each plot to exclude grazers. The iron cages were randomly moved to a new location each month until the end of September. From the beginning of June, vegetation in two pairs of 1×1 -m quadrats was, respectively, measured inside and outside the iron cages in each plot. Then, samples were collected in each quadrat, returned to the laboratory and oven dried at 75 °C for 24 h to a constant mass and weighed. AGB of the two paired quadrats inside and outside cages was measured in the following months: July, August, and September. The AGB increment equaled the difference of biomass outside and within each iron cage, measured monthly. $B_{\rm u}$ and b_{g} represented total AGB produced monthly within and outside of each iron cage, respectively. The annual biomass accumulation (B_a) that is AGB was evaluated by biomass of June added to the biomass increment of each month and was expressed as the following formula used by Zheng et al. (2011):

$$B_{a} = b_{g1} + (B_{u2} - b_{g1}) + (B_{u3} - b_{g2}) + (B_{u4} - b_{g3})$$
(1)

where B_{ui} and b_{gi} represent the total biomass collected inside and outside of iron cages during the sampling time *i*, respectively, in June, July, August, and September with i = 1, 2, 3, and 4, respectively. Generally, AGB peaks in August; therefore, the AGB of each stocking rate nearly equals B_a . For SR₀, the AGB in August was the same with B_a .

Statistical analysis

Statistical analyses were performed by SPSS 13.0 software (2004, SPSS Inc., Chicago, IL, USA) by applying several methods. A linear regression model was used to analyze the tendency for changes in PH, PW, leaf area, and LDM along the grazing intensity gradient. Quadratic regression analysis was used to assess the changes in LT, SLA, N_{mass}, and N_{area} at different stocking rates. Prior to regression analysis, we calculated the mean value of PFTs from 30 individuals as well as all leaves of each species in each replicate and only used each of the three replicates as the data points in the regression analysis model. Pearson correlation analysis was used to test the relationship between the response of AGB and PFTs to grazing. In addition, we used regression analysis to evaluate the relationship between AGB and PFTs (PH, PW, LA, LDM). Prior to regression analysis, PFT data and AGB were transformed using a natural logarithm to improve the normality of the data.

Results

Response of plant height and plant weight

Elvmus nutans

Grazing significantly affected PH and PW of the three species analyzed here (Fig. 3). For example, PH and PW

of E. nutans were very significantly negatively associated with stocking rate in both 2012 ($R^2 = 0.92$ and 0.84, respectively, P < 0.001, Fig. 3a, d) and 2013 $(R^2 = 0.78 \text{ and } 0.88, \text{ respectively}, P < 0.001, \text{ Fig. 3a},$ d). PW decreased by 88 % in 2012 and 92 % in 2013 at $SR_{5.62}$ when compared with SR_0 (Fig. 3d). PH and PW of S. purpurea showed a significant linear decrease along the grazing gradient in 2012 ($R^2 = 0.87$ and 0.84, respectively, P < 0.001, Fig. 3b, e) and in 2013 $(R^2 = 0.83 \text{ and } 0.64, \text{ respectively}, P < 0.001, \text{ Fig. 3b},$ e). PW declined by 54 % in 2012 and 30 % in 2013 at SR_{5.62} compared with SR₀ (Fig. 3e). For K. humilis, PH had a significantly negative relationship with grazing intensity in 2012 and 2013 ($R^2 = 0.81$ and 0.74, respectively, P < 0.001, Fig. 3c). PW of K. humilis showed a quadratic response to stocking rate, and peaked at $SR_{1,72}$, then decreased at higher stocking rates in 2012 and 2013 (Fig. 3f). At SR_{5.62}, PW decreased by 57 % in 2012 and 30 % in 2013 when compared with SR_0 .

Response of leaf functional traits

Stocking rate had significant effects on LA, LT, LDM, and SLA of the three species analyzed here, although the effect was not significant for SLA of *K. humilis* (Fig. 4). For *E. nutans*, LA and dry mass were significantly negatively associated with stocking rate in both 2012 ($R^2 = 0.85$ and 0.82, respectively, P < 0.001, Fig. 4a, g) and 2013 ($R^2 = 0.78$ and 0.82, respectively, P < 0.001, Fig. 4a, g). LT showed a quadratic response to stocking rate and reached the lowest point at nearly SR_{2.29} in both 2012 ($R^2 = 0.39$, P = 0.026, Fig. 4d). SLA had a different

Kobresia humilis



Stipa purpurea

Fig. 3 Relationships between plant heights and weights of Elymus nutans, Kobresia humilis, and Stipa purpurea and stocking rates



Fig. 4 Relationships between leaf area, leaf thickness, leaf dry mass, and specific leaf area of *Elymus nutans*, *Kobresia humilis*, and *Stipa purpurea* and stocking rates

response to grazing in each of the 2 years. SLA increased significantly in 2012 ($R^2 = 0.46$, P = 0.001, Fig. 4j) and initially decreased and then increased in 2013 ($R^2 = 0.43$, P < 0.014; Fig. 4j). SLA reached a minimum at SR_{2.87}.

For S. purpurea, LA and dry mass had a significant negative relationship with grazing intensity both in 2012 (LA: $R^2 = 0.48$, P = 0.001; LDM: $R^2 = 0.85$, P < 0.001, Fig. 4b, h) and 2013 (LA: $R^2 = 0.37$, P = 0.004; LDM: $R^2 = 0.78$, P < 0.001, Fig. 4b, h). LT and SLA had a unimodal response to stocking rate in both 2012 (LT: $R^2 = 0.48$, P = 0.007; SLA: $R^2 = 0.41$, P = 0.020, Fig. 4e, k) and 2013 (LT: $R^2 = 0.44$, P = 0.013; SLA: $R^2 = 0.47$, P = 0.009, Fig. 4e, k). SLA of S. purpurea reached the lowest value at SR_{2.87}. For K. humilis, LA and LDM showed significantly negative relationships with grazing intensity in

both 2012 ($R^2 = 0.84$ and 0.85, respectively, P < 0.001, Fig. 4c, i) and 2013 ($R^2 = 0.76$ and 0.82, respectively, P < 0.001, Fig. 4c, i). LT was significantly positively correlated with grazing intensity in 2012 ($R^2 = 0.46$, P = 0.010) and had a unimodal response to grazing intensity in 2013 ($R^2 = 0.36$, P = 0.014; Fig. 4f). SLA showed no significant response to grazing and was smallest at SR_{2.87} in both years (Fig. 4l).

 N_{mass} of *E. nutans* increased with an increase in the stocking rate in 2012 ($R^2 = 0.50$, P = 0.006, Fig. 5a), although it initially decreased and then increased in 2013 ($R^2 = 0.49$, P = 0.007; Fig. 5a). N_{area} of *E. nutans* showed a similar trend in that it initially declined and then increased with an increasing stocking rate in both 2012 ($R^2 = 0.54$, P = 0.003, Fig. 5d) and 2013 ($R^2 = 0.55$, P = 0.002, Fig. 5d). For *S. purpurea*, N_{mass} and N_{area} showed a significant quadratic response to an



Fig. 5 Relationship between N_{mass} and N_{area} of *Elymus nutans*, *Kobresia humilis*, and *Stipa purpurea* and stocking rates. N_{mass} nitrogen content per unit mass, N_{area} nitrogen content per unit area

increased stocking rate in that they both initially increased, then peaked at SR_{2.87} and then decreased in both 2012 (N_{mass} : $R^2 = 0.46$, P = 0.010; N_{area} : $R^2 = 0.39$, P = 0.026, Fig. 5b, e) and 2013 (N_{mass} : $R^2 = 0.65$, P < 0.001; N_{area} : $R^2 = 0.45$, P = 0.011, Fig. 5b, e). Grazing had no significant effect on N_{mass} and N_{area} of K. humilis (Fig. 5c, f).

Correlation between PFTs and AGB under grazing

AGB had a significantly negative relationship with stocking rate in both 2012 and 2013 ($R^2 = 0.79$ and 0.80, respectively, P < 0.001) and there was a little reduction for AGB as stocking rates increased from SR_{2.29} to SR_{2.87} (Fig. 6). PH, PW, LA and LDM were significantly positively related with AGB in *E. nutans* and *S. purpurea* (P < 0.01, Table 1). For *K. humilis*, PH, LA, and LDM were positively correlated with AGB but PW had no obvious relationship with AGB (Table 1).

In addition, linear regression analysis indicated that these traits including PH, PW, LA, and LDM in *E. nutans* and *S. purpurea* were significantly correlated with AGB (P < 0.001, Fig. 7). For *K. humilis*, PH, LA, and LDM were very significantly correlated (P < 0.001, Fig. 7a, c, d) with AGB; however, no significant relationship was found between *PW* of *K. humilis* and AGB (P > 0.05, Fig. 7b).

Discussion

Response of plant functional traits of three species to grazing intensity

PFTs provide very important information related to grazing management in grasslands; for example, they

may provide effective predictors of the methods by which plant species respond to grazing (Díaz et al. 2001; Klimesova et al. 2008). In addition, to some degree PFTs affect the functioning of grassland ecosystems (Pontes et al. 2007; Zheng et al. 2010). Scientists have developed six main models for simulating the response of plants to grazing: (1) the generalized model (Milchunas et al. 1988), (2) the rangesuccession model (Dyksterhuis 1949; Arnold 1955), (3) the competitor, stress-tolerator and ruderal model (Grime 1977; Grime et al. 1996), (4) the leaf-heightseed model (Westoby 1998, 1999), (5) resources availability model (Coley et al. 1985), and (6) physiological model (Briske and Richards 1995). In the last few years, several researchers have used PFTs to predict the response of plants to grazing (Díaz et al. 2001; Adler et al. 2005; Cingolani et al. 2005; Zheng et al. 2011). The changes in PFTs of plants reflect phenotypic plasticity to a certain degree. Plant morphological plasticity such as a change in PFTs is a comprehensive manifestation of how a species adapts to dynamic changes of the external environment (Louault et al. 2005). Under a relatively high stocking rate, plants commonly generate one or more avoidance strategies such as the regeneration of tillers, reduction of PH and leaf size, dynamic changes in SLA, and anatomical changes of plant leaves (Díaz et al. 2001; Klimesova et al. 2008; Zhao and wang 2009; Zheng et al. 2011).

This study found a consistent response of PFTs in the three species analyzed here; PH, LA, and LDM all decreased with increased grazing intensity, and plants tended to have a shorter height, smaller leaves, and smaller LDM as the stocking rate increased. Under heavy grazing pressure, these dominant species exhibited miniaturization. These results agree with previous



Fig. 6 Relationship between above-ground biomass (AGB) and stocking rates. *AGB* above-ground biomass

studies in other grassland ecosystems (Landsberg et al. 1999; Díaz et al. 2001; Zheng et al. 2011).

The PW of E. nutans and S. purpurea showed a significant declining trend as the stocking rate increased. This result is consistent with the findings of Zheng et al. (2011). PW of K. humilis initially increased from ungrazed to very light grazing pressure and then decreased with increased grazing intensity. PW of K. humilis at ungrazed sites was lower than at very light grazing intensity, which probably occurred because at ungrazed areas the community coverage and species density were very high; the relatively short plants of K. humilis were shaded by much taller plants such as E. nutans and S. purpurea. Therefore, without grazing the net leaf photosynthetic rate of K. humilis was relatively small because the plants were being shaded leading to a reduction in PW. Bjorkman and Holmgren (1963) and Thomas and Bazzaz (1999) reported that shaded plants tend to have lower light-saturated photosynthetic rates than plants exposed to the sun. The decrease of PW of the three species analyzed here was probably caused by the reduction in both LA and leaf biomass at higher grazing intensities, which further weaken the assimilation capacity and productivity of the plants (Milla and Reich 2007; Zheng et al. 2011).

Leaf thickness was connected with grazing; plants with thicker leaves were more likely to easily adapt to the stress of higher grazing pressure (Cunningham et al. 1999; Zhao and Wang 2009). LT of three species almost significantly decreased as grazing pressure increased from ungrazed to moderate grazing pressure (Fig. 4d–f). This implies that these three species are suitable for light grazing conditions. In contrast, LT increased as grazing pressure increased from moderate to heavy. Species would likely enhance their LT to mediate the effects of high grazing pressure.

SLA, an important trait that reveals the investment of a plant in growth (Westoby 1998), can reflect the expected return based on previously captured resources (Wilson et al. 1999). This study showed SLA of the three species analyzed here had different responses to grazing. SLA is related to grazing response; that is, species with high and low SLA show different responses under grazing (Westoby 1999). In this study, the value of SLA in these three species was similar under grazing (Fig. 5j-1). However, E. nutans had a greater LA when compared with K. humilis and S. purpurea. Vesk et al. (2004) showed grazers preferred larger leaves for a given LA. Moreover, E. nutans had a higher N_{mass} , which provides better forage quality than the other two species; this characteristic might cause E. nutans to be preferred by grazers more than K. humilis and S. purpurea.

Clearly, in this study, the SLA of these three species reached its lowest amount at a moderate stocking rate (Fig. 4j–l). Zheng et al. (2011) found the SLA of L. chinensis initially decreased under light and moderate grazing intensity and then increased during heavy grazing in typical steppe habitat. Our results also displayed an inflection point for SLA of these three species at a moderate stocking rate. Westoby (1999) showed that species with a low SLA predominate at a moderate grazing intensity when compared with species with a high SLA, which implies that species with a relatively low SLA can compete better at a moderate grazing intensity. Moreover, some studies have shown that SLA decreased as the plant life span increased (Reich et al. 1991, 1992). Although species with a high SLA had a short payback time for a unit of dry matter invested in leaf tissue (Poorter and Remkes 1990), leaves with a high SLA are short-lived and relatively vulnerable to herbivores (Coley et al. 1985; Grime et al. 1996). In contrast, leaves having a low SLA had a better capacity to retain captured resources in resource-poor environments

 Table 1
 Pearson correlation coefficients between plant functional traits of the three species analyzed here and above-ground biomass

Species	Plant functional traits							
	PH	PW	LT	LA	LDM	SLA	$N_{ m mass}$	Narea
Elymus nutans Stipa purpurea Kobresia humilis	0.829** 0.843** 0.737**	0.860** 0.776** NS	NS NS NS	0.847** 0.810** 0.889**	0.837** 0.826** 0.861**	NS NS NS	NS NS NS	NS NS NS

Data were log-transformed before analysis to improve normality

LA leaf area, LT leaf thickness, LDM leaf dry mass, N_{area} nitrogen content per unit area, N_{mass} nitrogen content per unit mass, PH plant height, PW plant weight, SLA specific leaf area

Correlation significance (P) levels of the effects are denoted as follows: not significant, ^{NS} (P > 0.05); significant ** (P < 0.01)



Fig. 7 Linear regression analysis between plant height (a), plant weight (b), leaf area (c) leaf dry mass (d), and above-ground biomass (AGB) under grazing. LA leaf area, LDM leaf dry mass, PH plant height, PW plant weight. En, Elymus nutans; Kh, Kobresia humilis, Sp, Stipa purpurea. Data were log-transformed prior to analysis to improve normality of data

(Wilson et al. 1999). In alpine steppe of the Qinghai-Tibet Plateau, the environmental conditions are characterized by relatively strong radiation, larger temperature differences between day and night, and this is a typical cold and windy climate. These types of environmental constraints affect the morphology of plants in alpine steppe. Generally, a large proportion of plants inhabiting alpine steppe have a relatively smaller leaf size and thicker leaves; some species have leaves with specific morphological characteristics such as having a columnar or acicular structure (He et al. 2007). Wilson et al. (1999) reported that LT was strongly negatively related with SLA, which suggests plants with a small leaf size and thicker leaves tend to have a lower SLA in alpine steppe habitat, allowing them to adapt to the unique environments of alpine steppe of the Qinghai-Tibet Plateau.

The leaf N content of the three species analyzed here responded differently to increased stocking rates. Gastal et al. (2010) reported that the response of species to grazing was based on growth form, morphology, and physiology. Different species also had varying strategies used to obtain available soil N (Xu et al. 2011). Selective grazing by herbivores might lead to different responses of leaf N content to grazing in individual species (Singer and Schoenecker 2003). The present findings suggest the N response to grazing for the three species analyzed here was relatively complicated in alpine steppe, which agrees with previous studies (Singer and Schoenecker 2003; Zheng et al. 2011). Therefore, leaf N content was a poor indicator of grazing response. If we want to discover how leaf N content of plant species regularly responds to grazing, a long-term experiment investigating the changes in leaf N content will be needed.

The changes in PFTs observed in this study are most likely caused by phenotypic plasticity, because the duration of the present grazing treatment was extremely short. However, the variability of PFTs within plant populations is likely caused at least in part by genetic variability; thus, long-term grazing could lead to genetic shifts in populations of these species (Silvertown et al. 2006; Strauss et al. 2008).

Correlations between AGB and species functional traits under grazing

As expected, the results demonstrated the response of AGB to increased grazing intensity was positively and significantly related to the response of PH, LA, and LDM in the three species analyzed here. Many studies have shown that PH, leaf size, and LDM were consid-

ered the most important traits that can be used in predicting the responses of plants to grazing (Landsberg et al. 1999; Díaz et al. 2001; Cingolani et al. 2005). This study suggests that as grazing pressure increased the reduction in PH, LA, and LDM was more limited and grazing restrained the growth of dominant species, and further influences AGB. We conclude that the PFTs and AGB of dominant species respond synergistically to grazing. The PW of E. nutans and S. purpurea had a significant positive relationship with AGB, which agrees with the study of Zheng et al. (2011). AGB was not significantly correlated with PW of K. humilis. PW of K. humilis probably had greater variability and initially increased as grazing pressure increased from ungrazed to light grazing intensity, and then decreased until a high stocking rate was reached and then increased under grazing. N_{mass} and N_{area} of the three species did not manifest a strong relationship with AGB under grazing. Most likely this occurred because these traits are more sensitive to environmental changes such as precipitation (Adler et al. 2005; Zheng et al. 2011) and soil nutrients (Osem et al. 2004; Pakeman 2004) than to grazing.

Conclusions

Our findings have important implications for predicting changes in the functioning of grassland ecosystems based on grazing intensity and for formulating practical management measures in alpine steppe of the Qinghai– Tibet Plateau and similar rangelands. This study proposes that simple traits, including PH, LA, and leaf biomass, are the best predictors of the responses of these species and AGB to grazing of the traits analyzed here. Moreover, according to the change in trends observed in SLA and AGB, a stocking rate no higher than 2.87 sheep/ha should be used in alpine steppe of the Qinghai–Tibet Plateau to maintain healthy and sustainable development of this grassland ecosystem.

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