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REGULAR ARTICLE

Short-term yak-grazing alters plant-soil stoichiometric relations in an alpine meadow on the eastern Tibetan Plateau

Tserang Donko Mipam · Shiyong Chen ·
Jianquan Liu · Georg Miehe · Liming Tian

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Abstract

Background and aims Over-grazing have significantly altered above- and belowground functions in terrestrial ecosystem. However, the influence of grazing intensity on plant-soil stoichiometric relations in alpine ecosystems remains unclear.

Methods We investigated the responses of plant-soil nutrient stoichiometric relationships to four grazing intensities (un-grazing (UG), light grazing (LG), moderate grazing (MG) and heavy grazing (HG)) in an alpine meadow on the eastern Tibetan Plateau. We measured carbon (C), nitrogen (N), phosphorus (P) and potassium (K) concentrations and their ratios in plants and soils in the peak growing season after three years of yak-grazing.

Results Compared to UG and LG plots, heavier grazing intensity at community levels and taxonomic groups decreased plant C and K concentrations by 7.6–10.5% and 17.6–21.3%, respectively, while grazing did not significantly influence plant N and P concentrations. Grazing intensity altered plant C:N and C:P ratios but not C:K and N:P ratios. By contrast, grazing intensity had no significant effects on soil nutrients and stoichiometry. Thus, the differential responses of plant and soil nutrients to grazing intensity caused that plants were more sensitive to grazing than soils.

Conclusions Our results confirm that grazing intensity has differential effects on plant and soil nutrients, implying that plant nutrients do not covary with soil nutrients under changing grazing intensity and challenging the past positive relations between plant and soil nutrients. Further long-term grazing experiments are required to understand the drivers of grazing effect on plant-soil nutrient interactions.

Keywords Ecological stoichiometry · Grazing · Plant nutrients · Soil nutrients · Tibetan Plateau

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T. D. Mipam · J. Liu · L. Tian (✉)
Key Laboratory for Bio-resource and Eco-environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610065, China
e-mail: ttkoko000@gmail.com

T. D. Mipam
Institute of Qinghai-Tibet Plateau, Southwest Minzu University, Chengdu 610041, China

T. D. Mipam · J. Liu
State Key Laboratory of Grassland Agro-Ecosystems, Institute of Innovation Ecology & School of Life Science, Lanzhou University, Lanzhou 730000, China

S. Chen
College of Life Science and Technology, Southwest Minzu University, Chengdu 6110041, China

G. Miehe
Faculty of Geography, Philipps-University of Marburg, Marburg, Germany

Introduction

Above- and belowground components are ecologically linked through nutrient cycling in grassland ecosystems, and their interactions and feedbacks, to a large extent, determine ecosystem processes and functions (Wardle et al. 2004; Bardgett and van der Putten 2014). On the one hand, plants can affect the nutrient supply of soil organisms through providing the quality and quantity of soil substrates (Bardgett and Wardle 2003). On the other hand, changes in microbial community in soils can influence the turnover rates of soil nutrients and the nutrient absorption of roots, which in turn affect plant community and productivity (Hooper et al. 2005). These processes are greatly affected by livestock grazing through defoliation, dung return and trampling in grassland ecosystems (Liu et al. 2015). However, how grazing effects on nutrient interactions between above- and belowground compartments still remains unclear.

Over-grazing has caused serious grassland degradation on the Tibetan Plateau, altering ecosystem functions and resulting in carbon and nutrients losses (Wang and Wesche 2016; Zhou et al. 2017). It has been reported that grazing can affect plant communities, diversity, productivity, nutrients and soil properties (Bai et al. 2012; Wang et al. 2012; McSherry and Ritchie 2013; Zhou et al. 2017). A meta-analysis showed that species richness and diversity decreased with increasing grazing intensities from a moderate level (Herrero-Jáuregui and Oesterheld 2018), while another study indicated that the effect of grazing intensity on richness was dependent on grassland production (Lezama et al. 2014). Compared to non-grazing, grazing decreased plant nutrients in the Eurasian steppe (Bai et al. 2012). In general, grazing-induced variations in soil properties are time-lag effects compared to plant responses. Soil nutrients were directly and indirectly affected by livestock dung and urine and grazing-induced variations in soil heat-water conditions (Wang and Wesche 2016). Previous studies revealed complex patterns of grazing effects on soil carbon (C), nitrogen (N), phosphorus (P) and potassium (K; McSherry and Ritchie 2013; Zhou et al. 2017; Yang et al. 2018). For instance, soil organic carbon (SOC) response to grazing increased by 6–7% for C_4 grasslands and decreased by 18% for C_3 grasslands (McSherry and Ritchie 2013), and soil C and N accumulated under light grazing and lost under moderate and heavy grazing (Zhou et al. 2017). Grazing activities led to K accumulation in alpine meadows (Ma et al. 2016) or deficiency in China's semi-arid grasslands (Cheng et al. 2016). These seemingly inconsistent results suggest that nutrient cycling in response to grazing depends on grassland types and grazing intensity. Furthermore, nutrient availability is closely related to plant diversity and production (Fay et al. 2015). It has been reported that plant diversity declined with nutrient supply, and this effect may be offset by enhanced light availability due to herbivory (Borer et al. 2014). It is therefore of great importance to examine the interactions of grazing-induced plant variables and soil nutrients.

Ecological stoichiometry is a key driver of nutrient cycling, and links different components among plant, soil and microbes in various ecosystems (Zechmeister-Boltenstern et al. 2015). The elements of C, N and P in plants and soils are tightly coupled because of the strict nutrient stoichiometry required by organisms (Tian et al. 2018). Thus, ecological stoichiometry provides a powerful tool for examining the influence of grazing on nutrient interactions between plants and soils (Bardgett and Wardle 2003). There are several processes that grazing affects plant and soil nutrient stoichiometry. First, at the species level, the impacts of grazing intensity on nutrient stoichiometry would be attributed to physiological changes in plant tissues and soil nutrient availability. Second, at the community level, changes in nutrient stoichiometry would be driven by species composition and the species-level responses of nutrition to grazing due to large differences in nutrient concentrations among species (Bardgett and Wardle 2003; Zechmeister-Boltenstern et al. 2015). Third, grazing can enhance root exudates and microbial activity, increasing available nutrients to plants and thus altering plant nutrient concentrations and stoichiometry (Frank 2008). For example, in a transect of China-Mongolia grassland, grazing-altered plant and soil C:N:P ratios are mainly associated with changes in species composition and functional groups (Bai et al. 2012). Nevertheless, few studies have identified the influence of grazing intensity on plant and soil nutrient stoichiometry and their linkages in alpine meadows.

Alpine meadow is a predominant vegetation type and pasture on the Tibetan Plateau, while over-grazing has shifted biogeochemical cycles. Although some studies reported the effect of grazing on plant and soil properties, little attention has been paid to grazing intensity impacts on plant and soil nutrient stoichiometric linkages. In this study, we focused on the changes in plant and soil nutrient stoichiometry and their relations in response to four grazing intensities in an alpine meadow in the eastern Tibetan Plateau. The four grazing intensities include un-grazing, light grazing, moderate grazing and heavy grazing. We hypothesized that (1) plant nutrients did not covary with soil nutrients under changing grazing intensity due to inconsistent soil and plant responses to grazing (Wang and Wesche 2016); and (2) grazing may alter plant-soil nutrient stoichiometric relation because grazing-induced variations in species composition can change nutrient status (Bai et al. 2012).

Materials and methods

Study site and experimental design

The study was conducted at the Qinghai-Tibet Plateau Research Base of Southwest Minzu University, located at a typical alpine pasture, Hongyuan county of the eastern Tibetan Plateau (32°48′ N, 102°33′ E; 3504 m above sea level (asl); Fig. S1). Mean annual temperature and precipitation is approximately 1.5 °C and 747 mm (about 80% during summer seasons) from 1961 to 2013, respectively. The dominant plant species are *Kobresia pygmaea*, *Elymus nutans* and *Kobresia humilis* at the experimental site (Mipam et al. 2019). Based on the differences in species characteristics (e.g. physical and dispersal attributes), we divided those species into five plant taxonomic groups, including *Poaceae*, *Cyperaceae*, *Fabaceae*, *Asteraceae* and *Others*. The soil is classified as Gelic Cambisol (IUSS Working Group WRB 2015).

In 2014, we fenced a 10-ha flat alpine meadow in the study site to exclude grazing for one year in order to ensure the uniformity of initial conditions among grazing treatments. Before setting the experimental layout, we conducted detailed investigations and discussions with local herdsmen and referenced previous studies in this region regarding the yak grazing intensity, and found that the stocking rate of yaks was larger than three yaks per hectare (Mipam et al. 2019). Considering that this region has been experienced overgrazing, we therefore regarded this stocking rate as heavy grazing. Accordingly, moderate grazing and light grazing was set as the stocking rate of 2 yaks ha^{-1} and 1 yak ha^{-1}, respectively. Subsequently, we divided the experimental site into 12 plots under four grazing intensities (un-grazing (UG), no yak; light grazing (LG), 1 yak ha^{-1}; moderate grazing (MG), 2 yaks ha^{-1}; heavy grazing (HG), 3 yaks ha^{-1}). Each grazing intensity has three replicates. Grazing plots aggregate to a total area of 10 ha. The area for each grazing plot is 1 ha and UG plots sum to 1 ha. All plots are randomly distributed in the alpine pasture and are fenced to prevent yaks' movement between plots (Fig. S1). The grazing experiment started in late May in 2015. For each year, yaks are constantly kept for each grazing plot throughout the growing season (late-May to late-September). We selected female yaks with an age of about four years, and the body weights of yaks were approximately 200 kg, ensuring that each yak can feed grasses uniformity to a large extent. We also put one to three water containers (with a volume of about 0.05 m^3) in each grazing plot in order to enable yaks to drink water. The water was artificially transported by employed workers every day during the experimental period. Moreover, although yak is a social animal that may lead to uneven grazing, we observed that yaks usually gather together between adjacent plots when they were at rest based on long-term observations. We also selected six subplots to sample plant and soil samples in each grazing plot in order to eliminate this deviation and spatial heterogeneity. Thus, the sampling protocol and the current grazing intensity have a weak effect on our results. In addition, we have sampled plant parts for determining aboveground biomass (AGB) during the peak season from 2015 to 2017, and the results showed that AGB decreased by 24.7% (6.8–52.6%) under LG plots, 46.3% (33.2–59.7%) under MG plots, and 59.8% (52.6–69.6%) under HG plots compared with UG plots (Fig. S2), suggesting that the stocking rates of yaks of this grazing experiment were appropriately defined.

Plant and soil sampling and measurements

In early September 2017, we randomly selected six subplots in each plot to collect plant and soil samples. We determined species richness by counting the number of plant species in a 50 cm × 50 cm quadrat. All aboveground plant was clipped in each quadrat. These plant samples were classified into taxonomic groups (*Poaceae*, *Cyperaceae*, *Fabaceae*, *Asteraceae* and *Others*). For each taxonomic group, AGB and plant nutrients (e.g. C, N, P and K) were measured. After clipping aboveground plants, soil samples were collected to measure soil nutrients and physical properties for upper 30 cm soils from each subplot based on depth intervals (0–10, 10–20 and 20–30 cm). AGB was determined by oven-drying plant samples at 65 °C for 48 h to a constant weight. Soil moisture and bulk density were measured using metal-ring method, oven-dried at 105 °C for 24 h. Soil and plant C were determined using Walkley-Black dichromate oxidation method. Total nitrogen (TN) was measured with Semi-Micro Kjeldahl method. Total phosphorus (TP) and potassium (TK) were analyzed with Mo-Sb colorimetric method and flame photometric method, respectively. These data were used to calculate the ratios of plant and soil C to N, C to P, C to K, and N to P. Moreover, for each subplot, we determined community-weighted means of C, N, P, K, C to N, C to P, C to K, and N to P ratios by using an equation as follows (Garnier et al. 2007):

$$nurtient_{agg} = \sum_{i=1}^{n} p_i \times nutrient_i$$

where $nutrient_{agg}$ is the community-weighted average of nutrient variables and stoichiometry (C, N, C to N ratio, etc.) for all taxonomic groups collected in a specific subplot, p_i is the proportion of the AGB of group i to the total AGB collected in the specific subplot, and nutrient i is the value of nutrient variables and stoichiometry for group i.

Statistical analyses

We used one-way analysis of variance (ANOVA) and Tukey's HSD post hoc tests to examine the differences in AGB, species richness, soil moisture and bulk density, and the nutrients and stoichiometric characteristics of soils and plants among grazing intensities. We examined the bivariate correlation between soil nutrients and plant nutrients, between soil stoichiometric characteristics and plant stoichiometric characteristics, and between AGB and nutrient stoichiometry of plants and soils under each grazing intensity respectively and together with data from all grazing intensities. Stoichiometric ratios in plants and soils were expressed as mass ratios. Significant difference was considered as $p < 0.05$. The ANOVA was conducted using SPSS 18.0 software, and Pearson correlation was tested by using *psych* package in R software (Revelle 2018).

Results

Responses of plant nutrients and stoichiometry, biomass, and diversity

Community-level plant C concentration showed 0.6%, 7.6%, and 10.5% lower in LG, MG, and HG compared to UG, respectively, with significant higher values in UG and LG than MG and HG (Fig. 1a). Community-level plant N and P concentrations exhibited no significant differences among grazing intensities (Figs. 1b, c). LG had no effect on community-level plant K concentration, while MG and HG significantly decreased it by 17.6% and 21.3%, respectively (Fig. 1d). However, the effects of grazing intensity on plant nutrient concentrations exhibited diverse patterns under different taxonomic groups (Fig. S3a-d). Plant C concentration in UG and LG was higher than that in MG and HG for *Poaceae*, *Cyperaceae*, *Asteraceae*, and *Others*, while no significant difference was found for *Fabaceae* (Fig. S3a). Both plant N and P concentrations had the lowest value of *Poaceae* in LG, whereas they were not affected by grazing for other taxonomic groups (Figs. S3b, c). Plant K concentration gradually decreased with increasing grazing intensity for *Cyperaceae*, *Asteraceae*, and *Others*, but grazing did not affect it for *Poaceae* and *Fabaceae* (Fig. S3d).

At the community level, each grazing treatment did not significantly change plant stoichiometry compared to UG plots, and both plant C:N and C:P ratios in LG were higher than those in MG (Fig. 1e-h). Nevertheless, plant stoichiometry in response to grazing intensity differed among taxonomic groups (Fig. S3e-h). Only LG significantly enhanced plant C:N ratio for *Poaceae* compared to UG, and remarkable higher value observed in LG than that in HG for *Poaceae*, *Cyperaceae*, and *Others*, while no differences were found for *Fabaceae* and *Asteraceae* (Fig. S3e). By contrast, compared to UG, both MG and HG decreased plant C:P ratio for *Poaceae*, and higher value was in LG than that in MG and HG for *Poaceae*, *Fabaceae* and *Others*, whereas no differences were detected for *Cyperaceae* and *Asteraceae* (Fig. S3f). Grazing treatments did not alter plant C:K ratio for *Poaceae*, *Cyperaceae*, *Fabaceae* and *Others*, while plant C:K ratio gradually increased with increasing grazing intensity for *Asteraceae* (Fig. S3 g). Plant N:P ratio was not affected by grazing intensity for *Cyperaceae*, *Fabaceae*, *Asteraceae*, and *Others*, but significantly decreased in MG for *Poaceae* (Fig. S3 h).

For taxonomic group, the lowest biomass was found in HG for each group, and the highest biomass was observed in UG for *Poaceae*, *Cyperaceae*, *Fabaceae and Others* and in LG for *Asteraceae* (Fig. 2). LG did not change community-level AGB, whereas MG and HG significantly decreased it by 46.0% and 57.2% compared to UG, respectively (Fig. S4b). Species

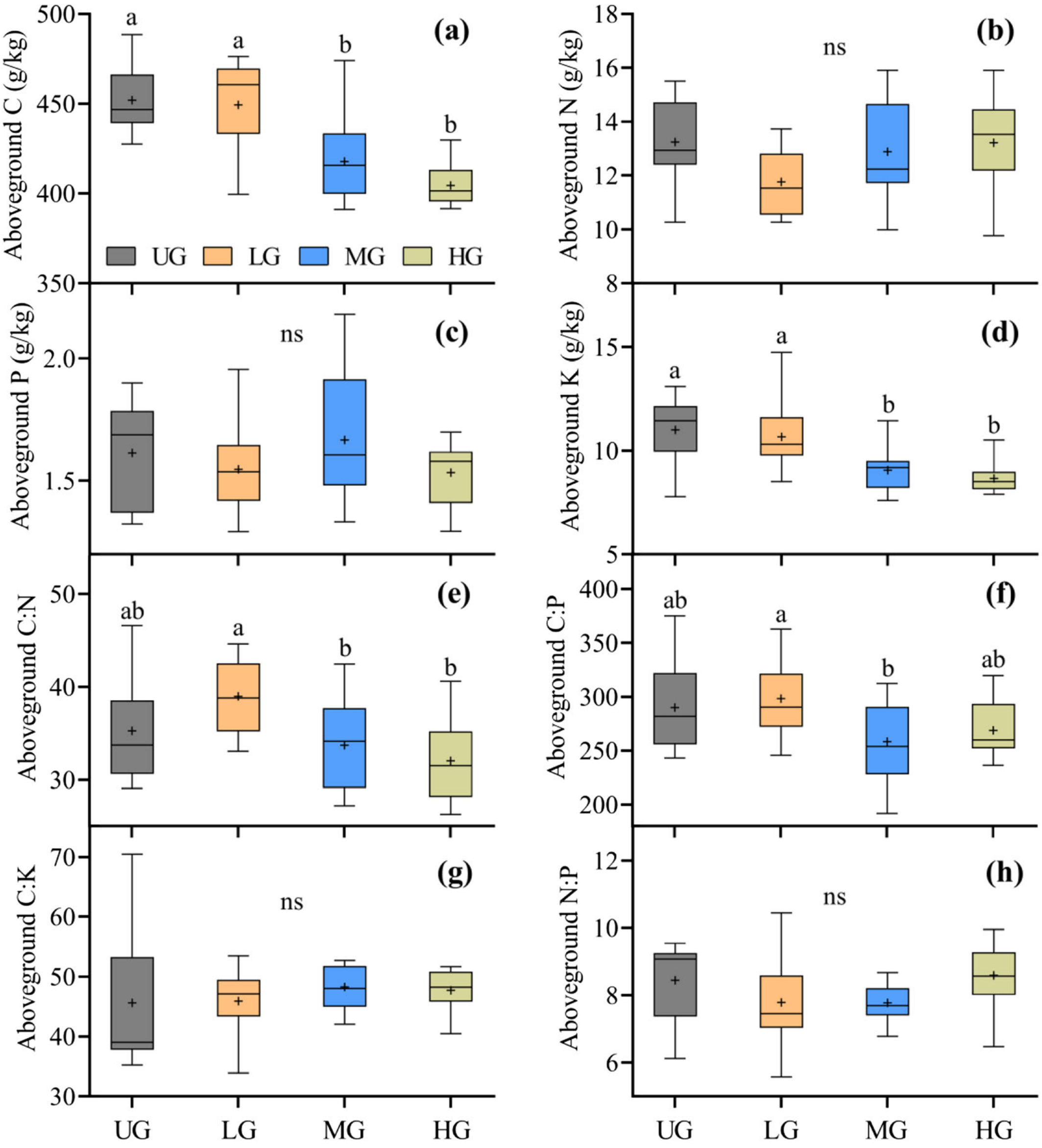


Fig. 1 Community-level nutrient concentrations (**a**-**d**) and stoichiometric ratios (**e**-**h**) of aboveground biomass in response to grazing intensities after 3 years yak grazing experiment. The black and '+', lower and upper edges, and bars in or outside the boxes represent median and mean values, 25th and 75th, and 5th and 95th percentiles of all data, respectively. Both the same letter and ns indicate no significant differences, according to Tukey's HSD post hoc tests at α = 0.05. UG, un-grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing

richness remained stable at about 30 among grazing intensities (Fig. S4d).

Responses of soil environment, nutrients and stoichiometry

For soil environment, although soil moisture and bulk density for the top 30 cm soils showed no significant response to grazing intensity, grazing increased soil moisture by 8.8 to 22.3% and decreased bulk density by 2.1 to 8.0% (Fig. S4a, c). Nearly all soil nutrients and stoichiometry were unaffected by grazing intensity for each depth interval, with an exception of TK, C:P and C:K ratios at the depth of 0–10 cm (Fig. 3). Soil TK concentration was significant higher in MG than that in HG (Fig. 3d), whereas an opposite pattern was detected for soil C:P and C:K ratios (Fig. 3f, g). Also, SOC and TN concentrations seemed to increase in HG at the depth of 0–20 cm (Fig. 3a, b).

Synthesizing the differences in plant and soil nutrients in response to grazing intensity, it can be found that grazing-induced variations in soil nutrients are time-lag compared to plant responses and plant nutrients do not covary with soil nutrients under changing grazing intensity, supporting our first hypothesis.

Relationships between plant and soil nutrient stoichiometry

Overall, C: nutrients stoichiometry showed no significant relations between plants and soils, while plant N and P concentrations weakly increased with soil N and P concentrations (N: $r = 0.11$, $p < 0.05$; P: $r = 0.19$, $p < 0.001$), and plant K concentration was negatively related to soil K concentrations ($r = -0.13$, $p < 0.01$; Fig. 4). These relations differed among grazing intensities. The positive relationship between plant and soil P was only observed in LG and MG, and the negative relation between plant and soil K was found in UG and LG (Figs. S5–8). For nutrient stoichiometry, only plant C:N ratio significantly increased with soil C:N ratio in UG ($r = 0.33$, $p < 0.01$; Fig. S5). The correlation coefficient of the relations between plant and soil K concentration, C:N ratio, and C:K ratio decreased with increasing grazing intensity (Figs. S5–8), which in part support our second hypothesis.

Discussion

Plant nutrients and stoichiometry in response to grazing intensities

Our results showed diverse effects of grazing intensity on plant nutrients. Grazing significantly decreased plant C and K concentrations under heavier intensities (MG and HG), regardless of community levels and taxonomic groups (Figs. 1, S3), consistent with the results from the Eurasian steppe (Bai et al. 2012). The reductions in plant C and K concentrations under heavier grazing plots can be ascribed to three mechanisms. First, the reductions may be due to the decreasing of plant C and K acquisition. Grazing can reduce leaf area through plant removal induced by yaks' feeding and trampling, thus decreasing plant C and K acquisition with increased grazing intensity (Zhang et al. 2015). Second, the reductions would likely be caused by the increased proportion of new-growth tissues due to heavier grazing (Thornton et al. 2000). The new-growth tissues caused by severe grazing led to reductions in C concentration, demonstrated by an isotope experiment that showed 21% of C remobilization to new-growth shoots and tissues after cutting *Festuca pratensis* (Thornton et al. 2000). Third, grazing-induced changes in photosynthetic capacity and resource allocation may be another explanation. Photosynthetic capacity of plants can be largely reduced as a result of tissue removal by heavier grazing, decreasing nutrient absorption and the ability to synthesize nutrients of plants (Briske and Richards 1995). Higher defoliation by heavier grazing can alter resource allocation to roots to maintain minimal root activity (Briske and Richards 1995). However, Yang et al. (2018) observed that grazing enhanced plant C concentration in a Qinghai-Tibet meadow. Such a discrepancy may be partly due to differences in grazing intensities. They set LG, MG and HG as 0.7, 1.2 and 1.6 yaks per hectare, respectively (Yang et al. 2018), which were lower than the corresponding grazing intensities in this study. This suggests that different grazing intensity plays a critical role in affecting the effect of grazing on plant nutrients.

Grazing had no significant effects on plant N and P concentrations (Figs. 1, S3), while previous studies reported that grazing by herbivores could increase plant N and P concentrations due to plant overcompensation (McNaughton 1985; Bardgett et al. 1998; Yang et al. 2018). Such a difference may be associated with different type of livestock that yak and sheep prefer to different plant species. Yaks are preference for Cyperaceae and Poaceae, whereas sheep for Asteraceae. Also, the different responses of grazing activities (trampling, selective grazing, urine and dung) could attribute to such a difference. Urine and dung may increase plant absorbing N and P, whereas selective grazing removed N and P-rich plant leaves and thus produce more poor-N and P litter and excrement, and reduce N and P input to the soil resulting in decreased P uptake from the soil (Olofsson 2009; Liu et al. 2015). It has also been reported that differences in plant N and P concentrations in response to grazing can be caused by the different evolutionary history of grazing (Milchunas et al. 1988).

Plant C:N and C:P ratios were significantly affected by grazing intensity, while grazing intensity did not significantly influence plant C:K and N:P ratios (Figs. 1, S3). The differential responses of plant nutrient stoichiometry to

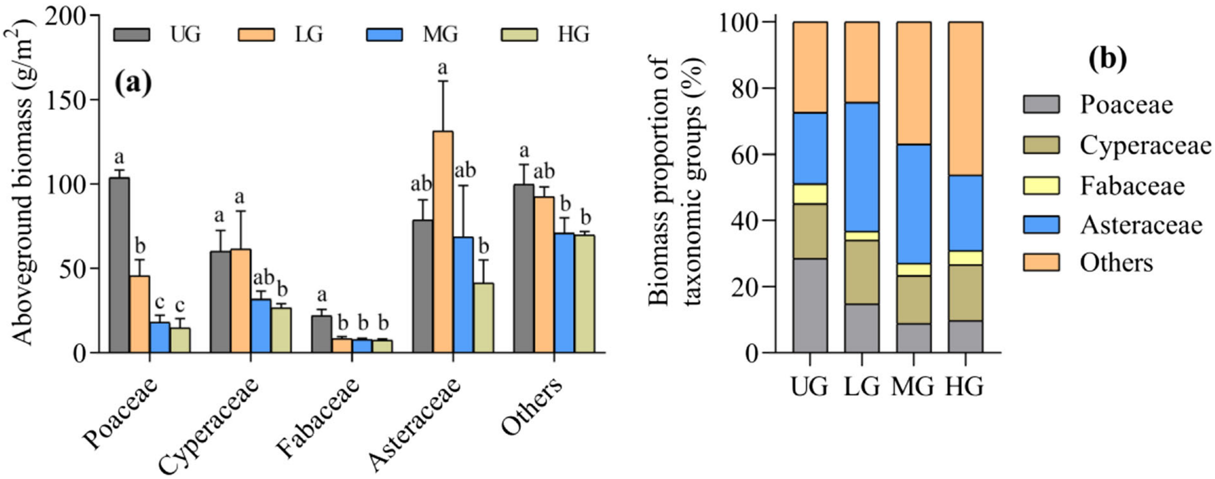


Fig. 2 Aboveground biomass and corresponding proportion of each plant taxonomic group in response to grazing intensities after 3 years yak grazing experiment. Error bars are standard errors. The same letter indicates no significant differences among grazing intensities, according to Tukey's HSD post hoc tests at $\alpha = 0.05$. UG, un-grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing

grazing intensity may be attributed to several processes. First, changes in plant nutrient stoichiometry may be driven by differential variations in plant nutrients in response to grazing intensity. Plant C and K had higher concentrations in UG and LG plots compared to those in MG and HG plots, whereas plant N and P concentrations showed no response to grazing intensity (Fig. 1). Second, soil moisture and nutrient availability may contribute to those different responses. Large differences in soil moisture can lead to different utilization of urine and dung generated by herbivores, resulting in different degrees of mineralization of soil nutrients and thus altering plant nutrient absorption (Shan et al. 2011; Zheng et al. 2012). Third, grazing-shifted in plant composition and dominated species is a major driver to induce changes in plant nutrient stoichiometry induced by grazing (Bai et al. 2012). For instance, the shifts in perennial bunchgrasses, forbs and rhizomatous grasses cause positive, negative or intermediate effects on plant nutrients in response to grazing (Zheng et al. 2012). Grazing-induced changes in species composition may alter plant nutrients and stoichiometry due to different nutrient enrichment abilities of different species. However, soil moisture showed no significant response to grazing intensity (Fig. S4), indicating that grazing-induced changes in

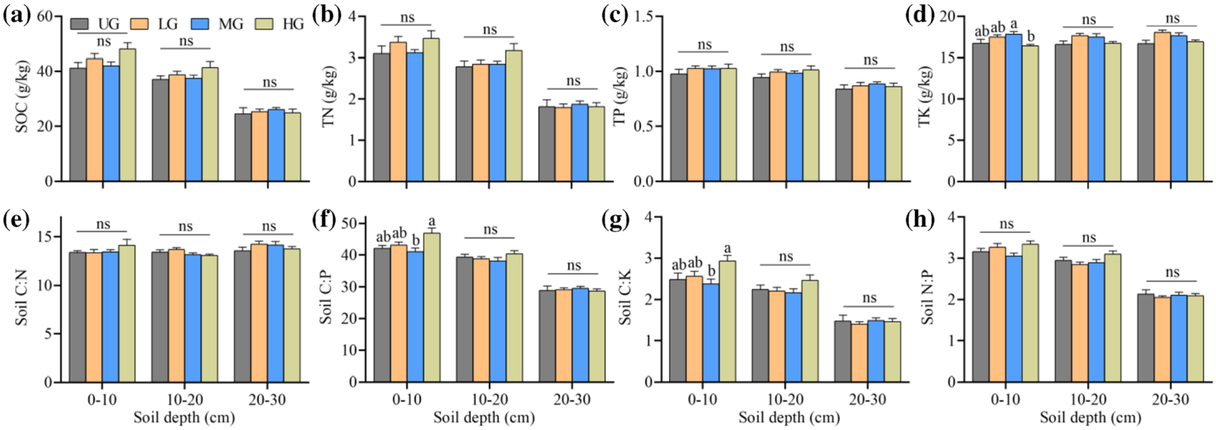
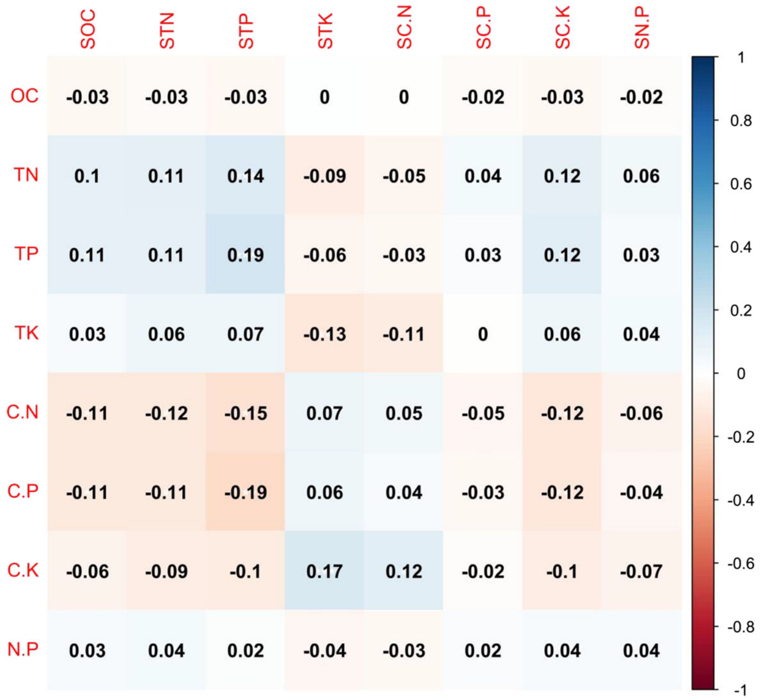


Fig. 3 Nutrient concentrations (**a**-**d**) and stoichiometric ratios (**e**-**h**) for the top 30 cm soils in response to grazing intensities after 3 years yak grazing experiment. Error bars are standard errors. Both the same letter and ns indicate no significant differences, according to Tukey's HSD post hoc tests at $\alpha = 0.05$. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; UG, un-grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing



	SOC	STN	STP	STK	SC.N	SC.P	SC.K	SN.P
OC	-0.03	-0.03	-0.03	0	0	-0.02	-0.03	-0.02
TN	0.1	0.11	0.14	-0.09	-0.05	0.04	0.12	0.06
TP	0.11	0.11	0.19	-0.06	-0.03	0.03	0.12	0.03
TK	0.03	0.06	0.07	-0.13	-0.11	0	0.06	0.04
C.N	-0.11	-0.12	-0.15	0.07	0.05	-0.05	-0.12	-0.06
C.P	-0.11	-0.11	-0.19	0.06	0.04	-0.03	-0.12	-0.04
C.K	-0.06	-0.09	-0.1	0.17	0.12	-0.02	-0.1	-0.07
N.P	0.03	0.04	0.02	-0.04	-0.03	0.02	0.04	0.04

Fig. 4 Heatmap of correlation (Pearson) between nutrients and stoichiometric ratios in plants and soils. The numbers in the figure are R value. The shading from white to blue represents gradation from low to high positive correlation. The shading from white to red represents gradation from low to high negative correlation. The abscissa is nutrients and stoichiometric ratios in soils, including soil organic carbon (SOC), soil total nitrogen (STN), soil total phosphorus (STP), soil total potassium (STK), soil C:N ratio (SC.N), soil C:P ratio (SC.P), soil C:K ratio (SC.K), and soil N:P ratio (SN.P). The ordinate is nutrients and stoichiometric ratios in plants, including organic carbon (OC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), C:N ratio (C.N), C:P ratio (C.P), C:K ratio (C.K), and N:P ratio (N.P). *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

plant nutrients and species composition are the major mechanism underpinning the shifts in plant nutrient stoichiometry. Furthermore, plant C:N, C:P, and C:K ratios were positively related to plant C concentration ($r = 0.35$, 0.48, and 0.21; $p < 0.001$) and negatively associated with plant nutrient concentrations ($r = -0.88$, -0.85, and -0.85; $p < 0.001$; Fig. 5), suggesting that changes in plant N, P, and K concentrations are largely responsible for changing magnitude of plant C:nutrients stoichiometry. Plant N:P ratio was positively related to plant N concentration ($r = 0.76$; $p < 0.001$) and negatively associated with plant P concentration ($r = -0.17$; $p < 0.01$; Fig. 5), indicating that plant N:P stoichiometry is largely determined by variations in plant N concentration.

Soil nutrients and stoichiometry in response to grazing intensities

Grazing intensity exhibited no significant impacts on soil nutrients (Fig. 3). Wang et al. (2017) found that environmental factors, rather than grazing intensity, significantly affected species composition and soil properties over Qinghai-Tibet pastures. Other studies observed positive, negative or non-linear responses of SOC to grazing intensity (i.e. McSherry and Ritchie 2013; Silveira et al. 2014; Eldridge and Delgado-Baquerizo 2017; Zhou et al. 2017). For example, SOC was increased by 6–7% along with increasing grazing intensity on C_3-C_4 mixed and C_4-dominated grasslands, but declined by 18% on C_3-dominated grasslands (McSherry and Ritchie 2013). A recent review showed that SOC and TN accumulated in LG plots but depleted in MG and HG plots (Zhou et al. 2017), depending on climatic zones and grassland types (Abdalla et al. 2018). These differences suggest that soil nutrients in response to grazing depends on specific contexts and grazing intensities (Abdalla et al. 2018).

Compared to UG plots, SOC and TN in surface soils marginally increased in HG plots (Fig. 3). This phenomenon may be related to several factors. First, variations in aboveground net primary production (ANPP) among

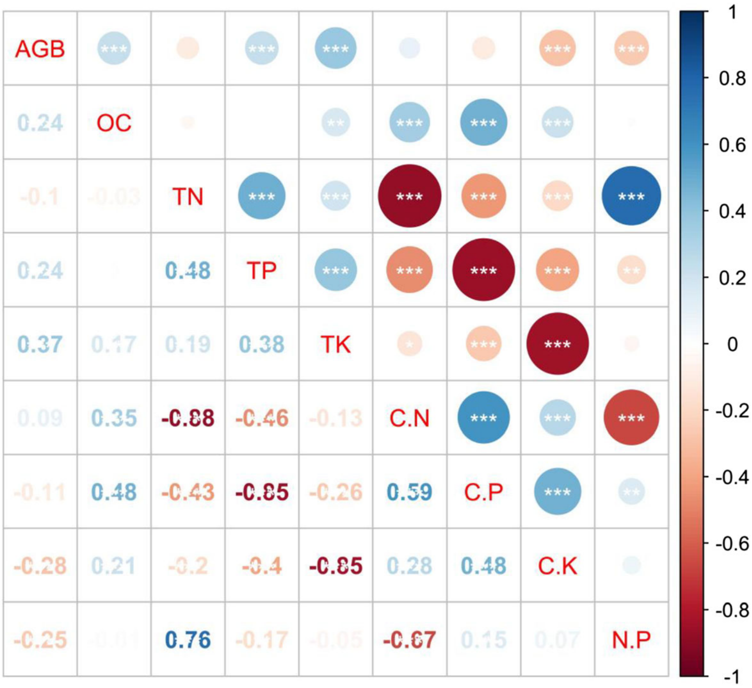


Fig. 5 Heatmap of correlation (Pearson) among aboveground biomass (AGB), nutrients and stoichiometric ratios in plants. The numbers in the figure are R value. The shading from white to blue represents gradation from low to high positive correlation. The shading from white to red represents gradation from low to high negative correlation. OC, organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; C.N, C:N ratio; C.P, C:P ratio; C.K, C:K ratio; N.P, N:P ratio. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

grazing intensities may explain such phenomenon. Our previous results indicated that ANPP under heavier grazing plots was consistently higher than that in UG plots from 2015 to 2017 (Mipam et al. 2019), suggesting that short grazing is conducive to plant growth and the accumulation of SOC. Second, heavier grazing intensities have relative higher urine and dung contents and heavier trampling compared to UG and LG plots, accelerating the decomposition of litter and thus leading to increase SOC and TN (Zheng et al. 2012). It has also been reported that heavier grazing could increase fine and shallow roots, leading to the accumulation of SOC (Li et al. 2011; McSherry and Ritchie 2013). Thus, heavier grazing can enhance the turnover of plant biomass and microbial activity, which in turn releases root exudates and increases SOC and TN (Bai et al. 2012; Yang et al. 2018).

Grazing intensity had no effect on soil nutrient stoichiometry (Fig. 3) due to the insignificant responses of soil nutrients to grazing (Fig. 3), suggesting that short-term yak grazing hardly affect soil conditions. This confirms that grazing-induced changes in soil nutrients and stoichiometry can be time-lag effects compared to plant responses because of the direct effect of herbivores on aboveground plant (Zhou et al. 2011). It has been reported that long-term heavier grazing can lead to large changes in soil nutrients and stoichiometry in Inner Mongolian grassland (Bai et al. 2012). Thus, plant and soil nutrients are expected to be altered if the grazing experiment can be conducted continuously. In addition, positive associations of SOC with TN and TP and of TN with TP were observed, while there were weak or no relations between TK and SOC, TN, and TP (Fig. 6). This suggests that C-N-P in soils is closely coupled in grazing systems. Moreover, soil nutrient stoichiometry was positively related to SOC and TN, with an exception of soil C:N ratio (Fig. 6). Soil C:N and C:K ratios significantly decreased with increasing TN and TK, respectively; indeed, both soil C:P and N:P ratios significantly increased with increasing TP (Fig. 6). These results indicate that changes in soil nutrient stoichiometry in grazing ecosystems are mainly controlled by soil C and N dynamics and slightly by soil P and K changes, whereas soil C:N is weakly controlled by soil C and N changes. In fact, soil C:N in Tibetan grassland is mainly controlled by soil texture and organic matters that consist of conserved proportions of SOC and TN due to the effects of mineralization and immobilization of organisms (Tian et al. 2018). By contrast, soil C:P, C:K, and N:P ratios are affected by complicated factors across Tibetan grassland (e.g. climate, topography, vegetation and soil properties; Tian et al. 2018).

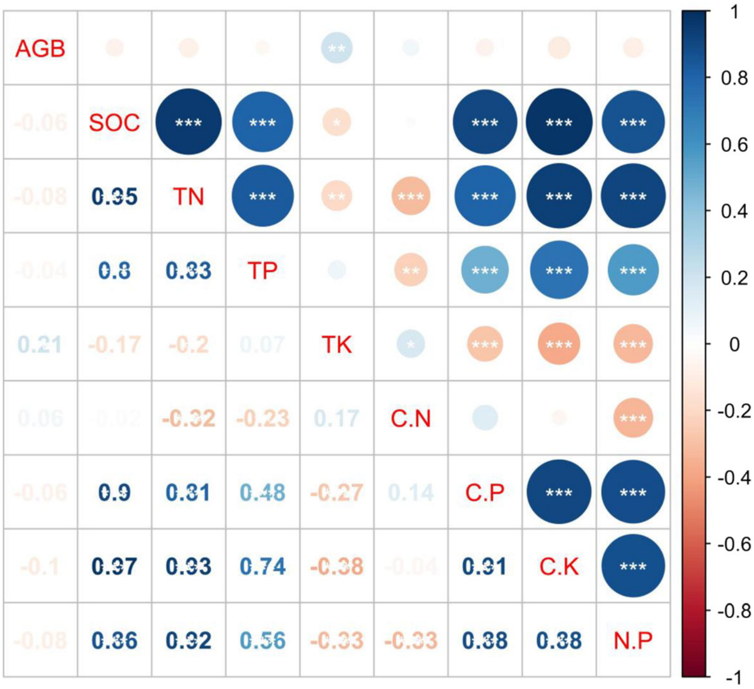


Fig. 6 Heatmap of correlation (Pearson) among aboveground biomass (AGB), nutrients and stoichiometric ratios in soils. The numbers in the figure are R value. The shading from white to blue represents gradation from low to high positive correlation. The shading from white to red represents gradation from low to high negative correlation. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; C.N, C:N ratio; C.P, C:P ratio; C.K, C:K ratio; N.P, N:P ratio. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

Linkages between plant and soil stoichiometry

Plant nutrient concentrations are primarily derived from soil nutrients (Geng et al. 2012, 2017). Nevertheless, inconsistent with this general pattern, our results showed that there were weak relations between plant and soil nutrients and stoichiometry (Figs. 4, S5–8). Several studies also found that plant nutrients did not covary with soil nutrients (Luo et al. 2015; Ye et al. 2015). It has also been reported that plant removal did not significantly affect soil nutrients in a subalpine tundra after eleven years, suggesting that a decoupling between plant and soil nutrients along with species composition succession and environmental changes (Wardle et al. 2013). These weak relations can be attributed to the "dilution effect" of plant size and the demands for plant growth. For instance, plant C uptake and growth rate can be increased under optimal soil conditions (i.e. higher soil fertility), diluting plant nutrient concentrations (Luo et al. 2015). Plants may only absorb nutrients for the demands for their growth, rather than for matching nutrient supply (Luo et al. 2015). Nevertheless, some studies observed tight relationships between plant and soil C concentration in grazing regimes (such as Yang et al. 2018), indicating complicated stoichiometric linkages between above- and belowground. Such a discrepancy can be explained by the following factors. First, soil nutrients are slowly released in alpine meadows due to the lower microbial mineralization and decomposition caused by low temperatures in cold regions (Davidson and Janssens 2006), leading to lower nutrient availability. Second, grazing is considered to enhance soil nutrients via stimulating root exudates and microbial activity (Bardgett et al. 1998), which cannot balance the stoichiometric relationships between plant and soil nutrients (Yang et al. 2018). Third, plant nutrients are largely controlled by nutrient availability in soils because plant absorbs inorganic forms and compounds of nutrients (Zechmeister-Boltenstern et al. 2015).

Previous studies indicated that grazing could increase soil nutrient effects in alpine grassland, showing that soil nutrients and quality, rather than climate factors, influence the trade-off between above- and belowground biomass in grazing grasslands more remarkably compared to that in grazing enclosures (Sun et al. 2018). Veen et al. (2014) found that changes in plant community composition in grazing plots were more stable than that in ungrazed plots, and grazing-induced soil legacy effects impacted plant biomass allocation patterns in grazed grasslands. Moreover, the intermediate grazing hypothesis shows that moderate grazing can enhance primary production via plant compensatory growth and recycling of limited nutrients, promoting the tissue loss of grazing tolerant and nutrient-rich species or inhibiting the growth of palatable species, thus altering the plant-soil nutrient stoichiometric relations in grazing regimes (Bai et al. 2012). The above- and belowground interactions in grazing systems may also be affected by grazing-induced changes in soil microbial biomass and enzymes activities (Yin et al. 2019). However, the underlying mechanisms of plant-soil feedbacks still remain unclear in grazing regimes. Thus, further studies are required to focus on the interactions of long duration of grazing treatments and belowground parts.

In summary, this study showed divergent responses of plant nutrients and stoichiometry to grazing intensity. Plant C concentration, K concentration, C:N and C:P ratios decreased under higher grazing intensities, whereas plant N concentration, P concentration, C:K and N:P ratios exerted no responses to grazing in the short-term yak grazing experiment. Conversely, grazing intensity did not significantly alter soil nutrients and stoichiometry. Moreover, there were insignificant associations of nutrients and elemental ratios between plants and soils in grazing regimes. Further studies are required to deeply explore plant-soil interactions in response to grazing intensity and environmental changes.

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