The mechanism underlying grazing shaping stoichiometry of plant community on a grassland of Guizhou subtropical plateau

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Abstract. Livestock grazing is a traditional and major use of grassland in China subtropics. However, plant stoichiometry and how it was affected by grazing in these ecosystems have been poorly explored. Here, the responses in plant nutrition and stoichiometry as well as soil nutrition and stoichiometry under representative grazing intensities (i.e., moderate grazing and heavy grazing) compared with no-grazing were characterized, the drivers of plant stoichiometry were explored in a subtropical artificial grassland, SW China. Wilcoxon test showed that grazing intensity significantly changed the response of soil OC, TN, AN, AP, AK, pH, OC: TN, AN: AP, AN: AK, AP: AK, and AP: AK. Grazing intensity changed the response in N, ASH, EE, ADF, P, N: P, N: Ca and P: Ca of plant. Path analysis showed the effect of grazing intensity on the stoichiometric ratio of nitrogen and phosphorus in plant community was achieved through the removal of herbivores. Our research provides some mechanistic understanding of grazing management of subtropical grassland, and also provides new insights and useful reference for the protection and utilization of grassland in Guizhou Plateau.

Keywords: grazing, vegetation, stoichiometry, driving forces, mechanism, artificial grassland, subtropical region

1. Introduction

Amounting 50 million km² (Du et al 2020), grasslands represent nearly half of the earth's land surface (Ding et al 2020a) and cover ca. three-quarter of the agricultural area (Abdalla et al 2018), and provide numerous essential ecosystem services, including carbon (C) sequestration, climate regulation, support human populations and livestock (Ding et al 2020a), and economic benefits (He et al 2019). Although grazing by livestock is the principal and traditional grassland management technique worldwide (Chang et al 2020, Du et al 2020), greatly altering ecosystem nutrient cycling (Cao et al 2019) as well as many case studies have been conducted (Liu et al 2021), the effects of grazing intensity on the nutrient dynamics of plant–soil systems in grassland is still poorly understood (Hou et al 2020), especially for the effect of grazing on the humid and warm subtropical grassland.
Ecological stoichiometry is key to ecosystem functioning (Fan et al 2015), reflects carbon and nutrient cycling (Ding et al 2019, Ding et al 2020b, Yang et al 2018). The stoichiometric ratio of C, N, P in plant represents the balance strategy of plant nutrient utilization efficiency, and reflects the dynamic balance between soil nutrient supply and plant nutrient demand (He et al 2020), has important implications for nutrient limitation (He et al 2020). However, the above- and below-ground ecological stoichiometry and their relationship remain poorly understood (Fan et al 2015), especially in the China subtropical grassland under different grazing intensities. Based on stoichiometric homeostasis, stoichiometry in previous studies was regarded as a feature of plant that controlled by genes or adapting to the environment (Gong et al 2020). The focus of previous studies is to reveal the plasticity and adaptability of stoichiometry in the species turnover of community level (Gong et al 2020). This change of stoichiometry involves a long period of time that enough to change the composition of plants, but the driving force of community level stoichiometry is still unknown when species composition remains unchanged in the short term.

Therefore, the objective of this study was to address: a) short term grazing can change the nutrient content and stoichiometry of soil and plants; b) the effects of grazing intensity on soil nutrients content and stoichiometry may vary with soil layers; c) grazing intensity may change plant stoichiometry through grazing removal rather than through trampling and manure that affecting soil properties.

2. Material and method

2.1. Study site and experimental design
The study was conducted in Dushan experimental base of Guizhou Grassland Research Institute in the Guizhou Plateau, Southwestern China (25°34′N, 107°37′E, 950-1017 m above sea level). The climate is characterized by a subtropical plateau monsoon humid climate with annual rainfall of 1100-1300 mm and average temperature of 15.0 °C, air humidity of 80%, accumulated temperature above 0°C of 5302°C, accumulated temperature above 10°C of 4538°C, frost-free period of 272 days, and annual sunshine of 1337 hours. The artificial grassland is establishment by mixed sowing (perennial ryegrass: tall fescue: orchardgrass: white clover: alfalfa=3.5:2:2:1:1.5). In this experiment, the rotational grazing system were adopted. There were three grazing intensities, i.e., no grazing, moderate grazing and heavy grazing. The artificial grassland with the same growth of forage grass was selected before grazing for 50 days. The semi-fine wool sheep with the same weight were selected for fixed-point grazing experiment. For moderate grazing, the length of the grazing rope is six m, the grazing time is three days, there are five rotational grazing sites, and the rotational grazing period is 15 d. During the 60 d experiment, four times rotation was performed. For heavy grazing, the length of the grazing rope is two m.

2.2. Vegetation assay
Three 0.5 m × 0.5 m quadrats were set up in each grazing intensity. The aboveground parts of the plants in each quadrat was collected by mowing. Plant N, P and EE were determined using Kjeldahl method, Molybdenum antimony colorimetric method, Soxhlet ether extraction method, respectively. ADF and ASH were determined using the method of zhang (2016). The ratio of N and P was expressed as N: P, and the response (i.e., change rate) of plant nutrients, and stoichiometry of N and P were calculated. Because there is no correspondence between the samples, we calculate the response of each correspondence.

2.3. Soil physicochemical assay
Three sites were selected in each grazing intensity. Nine soil cores in each layer (0-10 and 10-20 cm) were collected using the cutting ring method, and were mixed into one composition sample. Soil pH was determined using a pH meter, OC was determined by the potassium dichromate oxidation-external heating method, TN was determined by national standard determination protocol of soil total nitrogen, AN was determined by Alkali hydrolysis diffusion method, AP was determined by NaHCO₃
extraction-molybdenum antimony resistance colorimetry, and AK extracted with ammonium acetate. The ratio of OC and TN was expressed as OC:TN (Ding et al 2019), the ratio of soil AN and AP was expressed as AN:AP, the ratio of soil AN and AK was expressed as AN:AK, the ratio of soil AP and AK was expressed as AP:AK (Ding et al 2020b). The response of soil nutrients and stoichiometry was calculated. Because there is no correspondence between the samples, we calculate the response of each correspondence.

2.4. Statistical analyses
Wilcoxon test was used to determine the significant differences among groups in R (R Core Team, 2019). Path analysis was implemented to examine the potential pathways that can account for how grazing intensity and soil layer alter the response in plant stoichiometry using “lavaan” package (Yves, 2012). A model was acceptable at Bollen-Stine Bootstrap p > 0.05.

3. Results and discussions
Soil physicochemical properties and stoichiometry may change due to the trampling, excretion of livestock and redistribution of plant elements under different grazing intensities. Grazing has positive or negative effects on soil physicochemical properties (Hou et al 2020). In this study, Wilcoxon test showed that grazing intensity significantly changed the response of soil OC, TN, AN, AP, AK, pH, OC:TN, AN:AP, AN:AK, and AP:AK (Figure 1). Ji et al (2020) showed that soil OC declined with increasing grazing intensity. Hou et al (2020) found that soil total N, available N, and available P concentrations significantly increased with grazing intensity. Jiang et al (2020) indicated that the effects of grazing on soil C content became less negative or even positive with increasing soil depths. In this study, however, heavy grazing increased soil OC, TN, AN, pH, AN: AP, AN:AK, but decreased topsoil AP, soil AK, topsoil AP:AK; moderate grazing increased subsoil TN, topsoil AN, soil AP, pH, topsoil OC:TN, topsoil AN:AK, soil AP:AK, but decreased subsoil OC, topsoil TN, AK, subsoil OC:TN. These results therefore, showed that the effects of grazing on soil nutrients and stoichiometry depended on grazing intensity and soil depth. Soil element stoichiometry played a substantial role in carbon and nutrient cycling in terrestrial ecosystem (Ding et al 2019, Ding et al 2020b) and plant.

![Figure 1. Response of physicochemical properties and elemental stoichiometry of topsoil and subsoil to grazing gradient. ns, not significant; ***, P < 0.001; ****, P < 0.0001](Image)
Grazing can remove the aboveground biomass of grassland plant through grazing behavior, directly changing the distribution pattern and element stoichiometry of plant elements, change soil physical and chemical properties and elemental stoichiometry through trampling behavior and excretion behavior, in turn indirectly change the distribution pattern and elemental ratio of plant elements. The heavy grazing intensity increased plant N, ASH, EE, N:P, but decreased plant ADF, P, N:Ca and P:Ca; moderate grazing intensity plant N, EE, P, but decreased plant ADF, ASH, N:Ca and P:Ca. Furthermore, Wilcoxon test showed that the response in N, ash, EE, ADF, P, N: P, N:P, N:Ca and P:Ca of plant were changed by grazing intensity (Figure 2). It is particularly noteworthy that compared with moderate grazing intensity, heavy grazing intensity significantly increased the response of N, ash, EE, N:P of plant, but significantly decreased the response of P, N:Ca and P:Ca of plant. Nitrogen and phosphorus played vital roles in plant functioning, and are among the most important limiting nutrients in terrestrial ecosystems (Han et al 2005). Nitrogen (N) and phosphorus (P) are two vital elements for plant vegetative growth and reproduction and plant–soil trophic feedbacks (Gong et al 2020). Furthermore, the differences of N and P responses in grassland plant indicated that heavy grazing intensity might aggravate the deficiency of plants P.

![Figure 2](image_url)

**Figure 2.** Response of nutrient and element stoichiometric ratio of grassland vegetation to grazing gradient. MG - moderate grazing; HG - heavy grazing; GI - grazing intensity. ns, not significant; ****, P < 0.0001

Short-term heavy grazing accelerates nutrient cycling of the soil–plant system in grassland ecosystems (Hou et al 2020), thus disentangling the driving forces that shape the stoichiometric characteristics of C, N and P in the plant is important to better understand the multiple nutrient dynamics of soil–plant system with grazing intensity. The stoichiometry of plants could be constrained by a wide array of factors (Borer et al 2015). The potential mechanisms control over the plant stoichiometry were grouped into five pathways that may operate simultaneously:

(a) Previous efforts suggested that local plant diversity and species composition might change the plant nutrient composition at community level (Borer et al 2015). Bai et al (2012) speculated that the alterations in species composition and functional groups as well as foliar N and P contents for the same species can account for the changes in C:N:P stoichiometry. Therefore, we inferred that because of the differences in the stoichiometry of different species, if plant species change over a long period of time, it may lead to changes in the stoichiometry at the community level.

(b) Grazing impacted nutrient resorption from senescing leaves (Li et al 2020), and grazing effects on plant nutrient resorption efficiency differed among species (Zhang et al 2020). If grazing induced changes in plant species, the stoichiometry of plants might change due to the change of element utilization efficiency. Furthermore, grazing increased plant N and P resorption at community level (Zhang et al 2020). If the effects of plant N and P resorption were allometry, the stoichiometry at the community level would occur due to grazing.
(c) The stoichiometry of different compartments of plants is different, and animals selectively eat some plants species and some compartments. As a result, the stoichiometric ratio of different organs was changed by grazing (Ma et al 2019). In this study, grazing intensity positively and directly drove the changes in plant N: P, and plant N: P was not affected by the alterations in soil AN, AK, AP, OC: TN, AN: AP, and AN: AK (Figure 3), indicated that short term grazing changed plant stoichiometry through direct removal.

(d) Soil directly supply for plant with nutrients. Previous studies of plant stoichiometry and stoichiometric homeostasis have focused primarily on the role of nutrient supply as a constraint on plant tissue chemistry (Borer et al 2015). Grassland soil was affected (Abdalla et al 2018) by trampling, ingestion and excretion by herbivores (Chai et al 2019). Some studies found that grazing increases soil C (Abdalla et al 2018), N (Alves et al 2020) and promotes plant N uptake (Shen et al 2019). Grazing decreased soil AN, but increased soil AP (Ma et al 2019), thus changed soil AN:AP. Nutrients released through herbivore feces had the potential to influence plant-available nutrients (le Roux et al 2020) and affected stoichiometry. However, others studies indicated that grazing significantly decreased soil TN, TP (Ma et al 2019), OC, AP, and C:N (Liu et al 2021). Although many uncertainties exist, grazing changed soil nutrients and stoichiometry. Our study also found that grazing intensity changed the response of soil nutrients and stoichiometry (Figure 3). These changes might drive changes in plant stoichiometry. Cao and Chen (2017) found that plant N:P was strongly correlated to soil N:P. These might verify the above viewpoint. However, in this present study, plant N: P was not affected by the alterations in soil AN, AK, AP, OC: TN, AN:AP, and AN:AK. This suggested that although short-term grazing may affect the soil through trampling and excretion, this effect had not been transferred to the plant stoichiometry, which further indicated that short-term grazing reshaped the plant stoichiometry through direct removal.

(e) As we all know, grazing animals directly remove plants by feeding, while trampling and excretion directly modify the properties of soil. These two direct effects may exist simultaneously in grazing. They influence and interact with each other, and jointly change the plant stoichiometry. He et al (2019) found that grazing intensity modulates the C, N and P cycles in grassland ecosystems by affecting nutrient use efficiency of plant and physicochemical processes of soil. This finding also supported our view.

Collectively, our research provides some mechanistic understanding of grazing management of subtropical artificial grassland in Guizhou plateau, and also provides new insights and useful reference for the protection and utilization of grassland in Guizhou Plateau.

Figure 3. Path analysis showing how grazing intensity and soil layers affect soil nutrient and stoichiometry, as well as plant stoichiometry. Solid lines are significant and dashed lines are insignificant. Bollen-Stine bootstrap P-value > 0.05.
4. Conclusions
For short-term grazing, the effect of grazing intensity on the stoichiometric ratio of nitrogen and phosphorus in plant community is achieved through the removal of herbivores. It is not changed by the effect of trampling and excretion of herbivores on the nutrient and stoichiometry of soil.

Acknowledgments
This study was supported by the Youth fund of Guizhou Academy of Agricultural Sciences (qiannongkeyuanqingnianjijin [2018] 12), Key projects of Natural Science Foundation of Guizhou Province (qiankehejichu [2018] 1419) and Guizhou science and technology plan (qiankehezhicheng [2018] 2371). Puchang Wang and Wen Zhang contributed equally to this study.

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