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Effects of Enclosure on Plant and Soil Nutrients in Different Types of Alpine Grassland

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Abstract: Enclosure is one of the most widely used management tools for degraded alpine grassland on the northern Tibetan Plateau, but the responses of different types of grassland to enclosure may vary, and research on these responses can provide a scientific basis for improving ecological conservation. This study took one site for each of three grassland types (alpine meadow, alpine steppe and alpine desert) on the northern Tibetan Plateau as examples, and explored the effects of enclosure on plant and soil nutrients by comparing differences in plant community biomass, leaf-soil nutrient content and their stoichiometry between samples from inside and outside the fence. The results showed that enclosure can significantly increase all aboveground biomass in these three grassland types, but it only increased the 10–20 cm underground biomass in the alpine desert. Enclosure also significantly increased the leaf nutrient content of the dominant plants and contents of total nitrogen (N), total potassium (K), and organic carbon (C) in 10–20 cm soil in alpine desert, thus changing the stoichiometry between C, N and P (phosphorus). However, enclosure significantly increased only the N content of dominant plant leaves in alpine steppe, while other nutrients and stoichiometries of both plant leaves and soil did not show significant differences in alpine meadow and alpine steppe. These results suggested that enclosure has differential effects on these three types of alpine grasslands on the northern Tibetan Plateau, and the alpine desert showed the most active ecological conservation in the responses of its soil and plant nutrients.

Key words: enclosure; alpine meadow; alpine steppe; alpine desert steppe; plant nutrient; soil nutrient

1 Introduction

The area of alpine grasslands on the Tibetan Plateau is about 88×10^4 km^2, accounting for 70.1% of its total land area. It is the dominant ecosystem of the ecological security barrier on the Qinghai-Tibetan Plateau. However, due to the environmental conditions of high elevation, cold and drought, the alpine grassland ecosystem is extremely fragile, and much of it has degraded under the combined influences from climate change and increased grazing (Yu et al., 2016). Among the alpine grasslands, the pasture distributed on the northern Tibetan plateau is the largest and most important grassland ecosystem, but nearly half of it has been degraded (Yang et al., 2007). In order to protect the ecological function of the Qinghai-Tibetan Plateau, the Chinese government has comprehensively implemented the "Ecological Security Barrier Protection and Construction Project" on the Tibetan Plateau since 2009. In this project, the enclosures are one of the most important and widely used management

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tools for recovery and conservation of the grassland, and the enclosure area of alpine grasslands on the northern Tibetan Plateau has reached 86000 hm^2, accounting for about 10% of its pasture (Yu et al., 2016).

The effect of enclosure on grassland conservation has been important for the assessment and validation of the project and for making decisions on the management policy. Many studies on enclosures have been conducted on the northern Tibetan Plateau, but most of them have concentrated on the changes of grassland vegetation, such as NDVI (Feng et al., 2019), aboveground productivity（Wu et al., 2009）, species richness and diversity (Wu et al., 2012; Wu et al., 2014; Wu et al., 2015; Yan et al., 2015), and functional traits of plants (Sun et al., 2014; Li et al., 2016), while only limited research has been done on the changes of plant and soil nutrients in response to enclosure (Shen et al., 2016). The ecological conservation of grassland ecosystems includes both grassland plant and soil nutrients, and the stoichiometry between nutrients can reflect energy balance and the interaction of different chemical elements in the ecosystem (Xu et al., 2018). In addition to the specific soil nutrient conditions and assimilation capacity faced by plants (Zhang et al., 2014), the plant and soil nutrients and their stoichiometry are among the important factors for evaluating the effects of fence enclosure on grassland coservation.

On the other hand, the northern Tibetan Plateau spans about 600000 km^2, with an average elevation above 4400 m, and it has a typical continental plateau climate characterized by cold and dry conditions, a short rainy season, large temperature differences between day and night, and the annual temperatures in most areas of this region are below 0 ℃. Moreover, there is an obvious precipitation gradient from east to west on the northern Tibetan Plateau, with annual precipitation decreasing from about 700 mm in the easternmost portion to 50–80 mm in Gar County, Ngari Prefecture (Zhao et al., 2016). Correspondingly, the alpine grassland types transition from alpine meadow to alpine steppe and alpine desert steppe from east to west on the northern Tibetan Plateau (Wang et al., 2002; Li et al., 2011). Studies using NDVI as an indicator have shown that enclosures on the northern Tibetan Plateau have significant spatial heterogeneity in their effectiveness for grassland vegetation conservation (Feng et al., 2019). Therefore, plant and soil nutrients of different types of grassland may have different responses to enclosure on the northern Tibetan Plateau, and studies on this can help to reveal the mechanisms of differential grassland responses to fence engineering and provide a scientific basis for conserving different types of grasslands on the northern Tibetan plateau.

2 Material and method

2.1 Field sites

In this study, three sites representing alpine meadow, alpine steppe and alpine desert were selected on the north Tibetan Plateau as the sampling fields, each located in the typical distribution areas of these three kinds of alpine grassland (Fig. 1).

The alpine meadow field is located in Naqu County (31°38′24″ N, 92°0′36″ E), with an elevation of 4650 m, and mean annual temperature and precipitation from 1981 to 2014 of −0.41 ℃ and 458 mm, respectively. The soil type is alpine meadow soil. The average vegetation coverage is about 80%, and dominant plant species include *Kobresia pygmaea* (with coverage of 60%–70%), *Potentilla nivea*, *Youngia simulatrix*, *Saussurea ceterach*, *P. cuneata*, and others. The enclosed grassland of about 33.33 hm^2 was established in 2010 as a long-term experimental plot of the Lhasa Plateau Ecological Research Station of the Chinese Academy of Sciences.

The alpine steppe field is located in Nyima County (31°48′0″ N, 87°28′48″ E), with an elevation of 4529 m, and mean annual temperature and precipitation from 1981 to 2014 of 0.47 ℃ and 336 mm, respectively. The soil type is alpine steppe soil. The average vegetation coverage is about 30%–40%, and the dominant plant species are *Stipa purpurea*, *Oxytropis chiliophylla*, *Blysmus sinocompressus*, *Heteropappus hispidus*, *P. cuneata*, *P. bifurca*, and others. This field is a national enclosed grassland which was established in 2006 by the local government.

The alpine desert plot is located in Rutog County (33°13′48″ N, 79°27′36″ E), with an elevation of 4297 m, and mean annual temperature and precipitation of 0.06 ℃ and 73.4 mm, respectively. The soil type is alpine desert soil. The average vegetation coverage is about 10%, and the dominant plant species are *S. glareosa*, *Draba torticarpa*, *Ceratoides latens*, and others. This field is a national enclosed grassland which was established in 2007 by the local government.

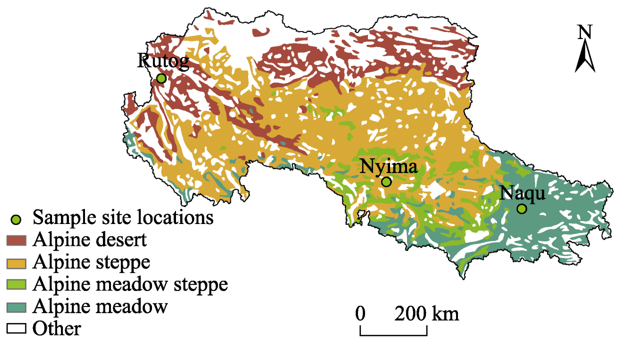

Fig. 1 Spatial pattern and sampling points of the three different types of alpine grassland on the northern Tibetan Plateau

2.2 Samples collection

In each of the three sites of alpine grasslands, paired plots were set about 50 m from the fence, with the grazing-banned grasslands inside the enclosure used as the conservation plots, and the grasslands outside the enclosure used as control plots. Above and underground biomass, plant and soil samples were collected at points both inside and outside the fence at each field site in early August 2018.

Aboveground biomass of plants was obtained by mowing. In each field, five 50 cm×50 cm quadrats were randomly sampled inside and outside fence, and all plant species in each quadrat were recorded. After mowing and classification according to species, plant samples were placed into marked envelopes and brought back to the laboratory, dried in an oven at 65 ℃ for 48 h and weighed. Underground biomass was obtained by soil drilling. After plant samples in the five quadrats were mowed, 3–4 of them were randomly selected, and five soil samples were drilled (soil auger diameter 3.8 cm) in each plot at 0–10 cm and 10–20 cm, and mixed uniformly. The soil samples were taken back to the laboratory, rinsed in running water, drained, dried in an oven at 65 ℃ for 48 h and weighed (Wang et al., 2017).

One dominant plant species in each type of alpine grassland was selected as a representative for the determination of plant leaf nutrient content. The leaves of each dominant plant species in one plot were mixed as a single sample, and five paired samples for each dominant plant species inside and outside the fence were obtained. The dominant plant species selected were *K. pygmaea* for alpine meadow, *S. purpurea* for alpine steppe and *S. glareosa* for alpine desert. Their dried leaves were ground and screened to determine the nutrient contents.

Soil samples were obtained by soil auger from the same quadrats used for the determination of plant biomass. In each quadrat, five cores were taken from the soil at depths of 0–10 cm and 10–20 cm. To determine soil nutrient contents, soil samples from the same depth were mixed and bagged, taken back to the laboratory, and sieved after being naturally dried.

2.3 Plant and soil nutrient content analysis

Plant nutrients analyzed were organic carbon (C), total nitrogen (N), total phosphorus (P) and total potassium (K). The contents of organic C and total N were determined by elemental analyzer (Elementar Analysensystem GmbH, Germany), and the contents of total P and total K were determined by the HNO_3-ICP-OES method.

Soil nutrients analyzed were organic C, total N, total P, and total K. The determination of organic C content used the potassium dichromate volumetric method. Total N content was determined by the Kjeldahl method. Total P content was determined by the sodium carbonate alkali melting-molybdenum antimony colorimetric method. Total K content was determined by flame photometry.

2.4 Data processing

R software was used to process the data, and single factor analysis of variance was used to test for differences in biomass, leaf-soil nutrient contents and their stoichiometries.

3 Results

3.1 Biomass

The results showed that the above and underground biomass of the three types of alpine grassland have different responses to enclosure (Table 1). Aboveground biomass levels inside the fences of alpine desert, alpine steppe, and alpine meadow were 89.7 g m^{-2}, 238.9 g m^{-2} and 1203.6 g m^{-2}, respectively, which represented increases of 170%, 268% and 109% compared to those of samples taken outside the fence, and all of them showed extremely significant differences (P <0.01).

Table 1 Biomass inside and outside the fence in three types of alpine grassland

Sampling sites	Grassland types	Plots	Soil depth (cm)	Aboveground biomass (g m^{-2})	Underground biomass (g m^{-2})
Rutog	Alpine desert	Inside fence	0–10	89.7±3.8A	11.3±10.2Aa
			10–20		11.6±4.6Aa
		Outside fence	0–10	33.2±1.4B*	23.64±6.9Aa
			10–20		31.29±7.5Ba
Nyima	Alpine steppe	Inside fence	0–10	238.9±13.0A	408.2±54.5Aa
			10–20		31.1±19.6Ab
		Outside fence	0–10	64.9±0.9B*	403.1±106.4Aa
			10–20		43.9±9.5Ab
Naqu	Alpine meadow	Inside fence	0–10	1203.6±34.9A	5831.0±2204.6Aa
			10–20		520.2±12.0Aa
		Outside fence	0–10	575.3±26.6B*	5485.6±1613.5Aa
			10–20		651.5±134.7Ab

Note: Different capital letters indicate significant differences between inside and outside fence (P<0.05), different lowercase letters indicate significant difference in the different soil depths under the same community (P<0.05). * indicates a significant difference between inside and outside of the fence at the 0.01 level.

However, enclosure only significantly increased the 10–20 cm underground biomass in alpine desert (P<0.05), and more biomass was concentrated in 10–20 cm soil than in 0–10 cm soil. Contrary to alpine desert, underground biomass in alpine steppe and alpine meadow was mainly concentrated in the 0–10 cm soil layer, and there were no significantly differences between inside and outside the fence samples. Here, the underground biomass for 0–10 cm soil inside the fence was more than that outside the fence, and the biomass in the 10–20 cm soil layer outside the fence was more than the biomass inside the fence.

3.2 Soil nutrients

Responses of soil nutrient contents in the three types of alpine grassland to enclosure were also different (Fig. 2). In the alpine desert, compared to outside the fence, total N content in the 10–20 cm soil inside the fence was significantly increased by enclosure (P<0.01). The enclosure also significantly increased the total K and organic C contents in the 10–20 cm soil (P<0.05), while the total P content showed no significant difference between inside and outside of the fence (P>0.05).

For the alpine steppe and alpine meadow, none the soil nutrient contents showed significant differences between inside and outside the enclosure. Only the total N in 0-10 cm soil inside the fence was significantly lower than that of 10–20 cm in the alpine steppe (P<0.05). The total N and organic C contents inside and outside the fence showed significant decreases with soil depth in the alpine meadow (P<0.05), and the total P content in the 0–10 cm outside the fence sample was significantly lower than that in the 10-20 cm soil in the alpine meadow (P<0.01).

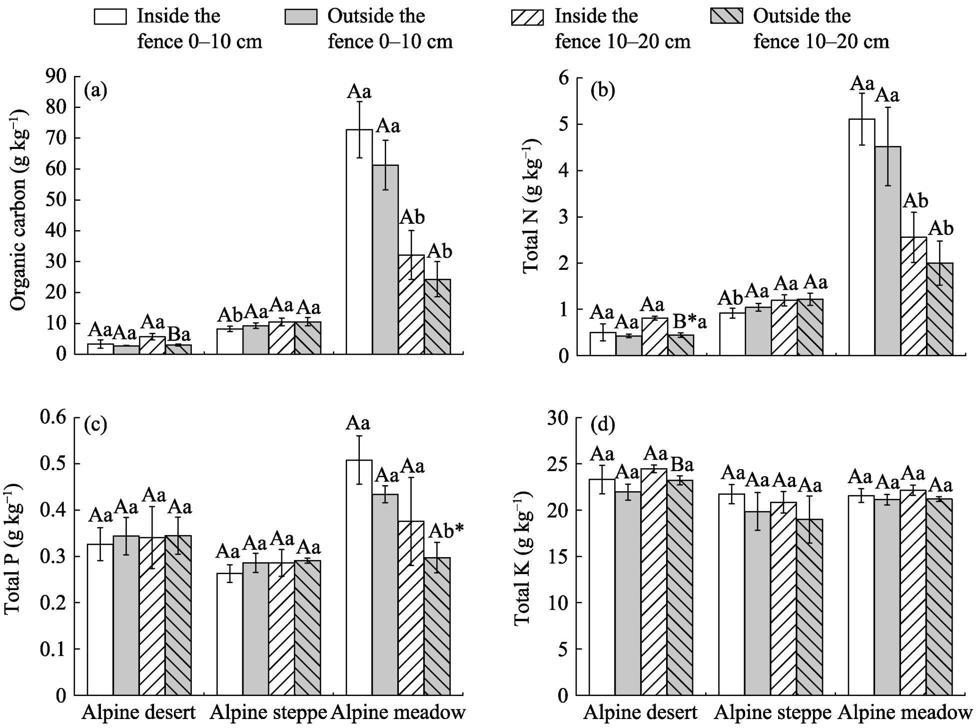

Fig. 2 Soil nutrient contents inside and outside the fence in the three types of alpine grassland

Note: Different capital letters indicate significant differences between inside and outside the fence (P<0.05), different lowercase letters indicate significant differences in the different soil depths under the same community (P<0.05), and the bars represent standard deviations. * indicates a significant difference between inside and outside the fence at the 0.01 level.

3.3 Plant nutrients

Enclosure has different effects on plant leaf nutrient contents in the three types of alpine grasslands (Fig. 3). In the alpine desert, except for C, the leaf N, P and K contents of *S. glareosa* inside the fence were all lower than those outside the fence (P<0.05). In the alpine steppe, leaf C, N, P and K contents of *S. purpurea* inside the fence were also lower than those outside, but only N showed a significant difference (P<0.05), and the other three elements were not significantly different (P>0.05). In the alpine meadow, none of the leaf contents of C, N, P and K in *K. pygmaea* showed a significant difference between inside and outside the fence (P>0.05), although the N and P were lower inside the fence.

3.4 Stoichiometry

Enclosure changed the stoichiometry of nutrients in the plant leaves in the three types of alpine grassland (Table 2). In the alpine desert grassland, C: N, C: P and N: P were all higher inside the fence than outside, of which the C: N and C: P showed significant differences (P<0.05). However, there were no significant differences of C: N, C: P or N: P between inside and outside the fence in either alpine grassland or alpine meadow grassland (P>0.05).

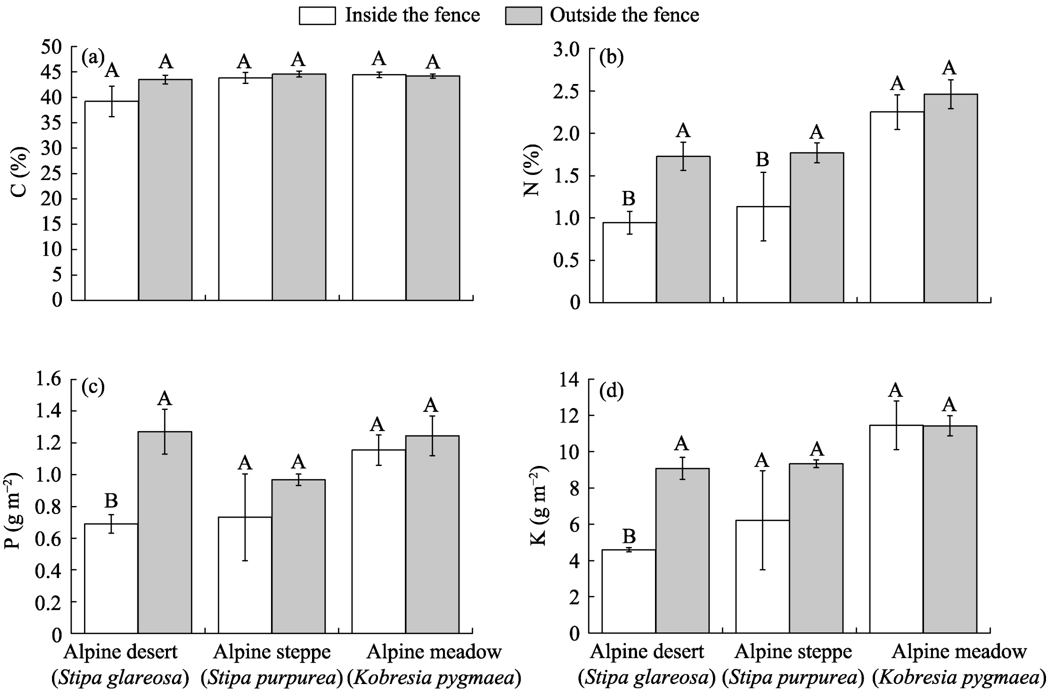

Fig. 3 Plant leaf nutrient contents inside and outside the fence in the three types of alpine grassland

Note: Different capital letters mean significant differences between inside and outside fence (P<0.05), the bars represent standard deviation.

Table 2 Plant leaf nutrient stoichiometry inside and outside the fence in the three types of alpine grassland

Sampling sites	Grassland types	Items	Inside the fence	Outside the fence
		C: N	41.85±3.19A	25.35±2.30B
Rutog	Alpine desert	C: P	56.94±2.87A	34.51±3.55B
		N: P	1.36±0.08A	1.36±0.09A
		C: N	41.39±10.27A	25.27±1.51A
Nyima	Alpine steppe	C: P	65.64±21.28A	46.11±1.63A
		N: P	1.59±0.31A	1.83±0.18A
		C: N	19.85±1.54A	18.01±1.18A
Naqu	Alpine meadow	C: P	38.74±3.71A	35.75±3.28A
		N: P	1.96±0.25A	2.00±0.27A

Note: Different capital letters indicate significant differences between inside and outside the fence (P<0.05).

Soil stoichiometry responses to enclosure also showed differences among the three types of alpine grassland (Table 3). The soil C: N, C: P and N: P inside the fence were all higher than those outside the fence in the alpine desert, and C: P and N: P in the 10–20 cm soil showed significant differences (P<0.05). For the alpine steppe, soil C: N in both 0–10 cm and 10–20 cm, and C: P in 10–20 cm soil were all higher inside the fence than outside, while N: P was lower inside the fence, but none of these showed significant differences (P>0.05). The soil C: N, C: P and N: P of the alpine meadow inside of the fence showed higher values than those outside, but there were also no significant differences (P>0.05).

4 Discussion

Enclosure is a widely used management method for grassland ecological conservation, which can significantly increase the aboveground biomass of grassland vegetation that is reduced due to feeding by livestock (Du et al., 2007). The results of this study showed that the enclosure significantly increased the aboveground biomass in all three types of alpine grasslands, which was consistent with other research in this area (Zhao et al., 2011; Wu et al., 2012). Compared with the increased aboveground biomass, only the underground biomass of 10–20 cm inside the fence in the alpine desert was significantly higher than that outside, while there were no other significant differences. This may

Table 3 Soil stoichiometry inside and outside the fence in the three types of alpine grassland

Sampling sites	Grassland types	Plots	Soil depth (cm)	C: N	C: P	N: P
Rutog	Alpine desert	Inside the fence	0–10	6.52±0.33A	10.01±3.36A	1.53±0.47A
			10–20	7.02±0.89A	16.80±0.30A	2.42±0.33A
		Outside the fence	0–10	6.50±0.39A	8.09±0.72A	1.24±0.04A
			10–20	6.91±0.84A	8.85±0.34B	1.30±0.19B
Nyima	Alpine steppe	Inside the fence	0–10	8.94±0.36A	31.35±4.74A	3.52±0.63A
			10–20	8.81±0.29A	36.72±0.60A	4.17±0.14A
		Outside the fence	0–10	8.86±0.30A	32.47±2.54A	3.67±0.29A
			10–20	8.64±0.21A	36.25±4.70A	4.19±0.46A
Naqu	Alpine meadow	Inside the fence	0–10	14.22±0.34A	142.89±4.78A	10.05±0.36A
			10–20	12.55±0.44A	86.30±9.31A	6.87±0.68A
		Outside the fence	0–10	13.70±1.18A	141.1±15.42A	10.37±1.63A
			10–20	12.16±0.16A	81.42±12.85A	6.70±1.11A

Note: Different capital letters indicate significant differences between inside and outside the fence (P<0.05).

be because most plants of the alpine grasslands on the Tibetan Plateau are perennials, so trampling and grazing by livestock outside the fence also would promote the underground root growth (Yin et al., 2019).

Soil nutrients are important indicators for grassland conservation. The enclosure did not significantly change the soil nutrient status in either alpine meadow or alpine steppe, which was consistent with other research on the alpine grasslands on the Qinghai-Tibetan Plateau (Si et al., 2015; Yin et al., 2019). However, the enclosure did significantly increase the organic C, and total N and K contents of the 10–20 cm soil layer inside the fence of the alpine desert grassland. This may be due to either the hysteresis and dynamic elasticity of grassland soil (Milchunas et al., 1993), the differing types of grasslands and their soil backgrounds, plant growth characteristics or other factors which will affect the changes of grassland soil nutrients (Gao et al., 2017). In this study, soil nutrients in the alpine desert were severely barren, and the average organic C and total N contents of the soil outside the fence were only 2.91 g kg^{-1} and 0.43 g kg^{-1}, respectively, which were far lower than those either in the alpine steppe (9.91 g kg^{-1} and 1.13 g kg^{-1}) or the alpine meadow (42.82 g kg^{-1} and 3.26 g kg^{-1}). However, the input of external substances can easily change the original nutrient composition in the soil significantly. Enclosure removes the effects of grazing and trampling by livestock, increases the input of biomass above and below ground (especially litter), accelerates the release of nutrients and thus increases nutrient content in the soil (An et al., 2015). In addition, due to the gravel and loose soil quality of alpine desert soil, nutrients are easily leached to the lower depths of soil, so that the nutrient content in 10–20 cm soil in the alpine desert after enclosure is significantly increased, and the stoichiometries of C: P and N: P were significantly changed as well. Lower N: P in the soil can indicate that plant growth is restricted by N, whereas a higher N: P can indicate that the plant growth is restricted by P (Huang et al., 2013). In this study, the average soil N: P outside the fence in the alpine desert was 1.24–1.30, while values in the alpine steppe and alpine meadow were 3.67–4.19 and 6.70–10.37, respectively. Soil N: P in alpine desert was far lower than those in alpine steppe and alpine meadow, which suggested that plant growth in the alpine desert was severely restricted by N; and since enclosure increased the N: P in the soil, it was conducive to plant growth and also led to the significant increase of both above and underground biomass inside the fence.

The responses of leaf nutrients in the dominant plants of the three types of alpine grassland to fencing were not completely consistent. Enclosure significantly reduced N, P and K contents of *S. glareosa* leaves in the alpine desert grassland, and N content of *S. purpurea* leaves in the alpine steppe. However, there were no significant differences in C, N, P and K contents of *K. pygmaea* leaves in the alpine meadow inside and outside the fence, although the N and P contents of *K. pygmaea* leaves inside the fence were lower than those outside. Enclosure reduced the N and P contents of plant leaves because of restricted livestock feeding. Most of the plant leaves inside the fence were mature, and with senescence their N and P contents would be decreased. But outside the fence, grazing would stimulate the compensatory growth of the plants and improve nutrient recycling, so that the newly growing leaves would have increased N and P contents (Niu et al., 2016; Cao et al., 2018). The C in plants only plays a supporting role for leaves, and generally does not directly participate in the plant's biomass production (Ding et al., 2012), so enclosure did not change the C content in the plant leaves. This also led to the finding that both C: N and C: P in plant leaves inside the fence were significantly higher than those outside the fence, but there was no difference in leaf N: P in the alpine desert, and so the higher C: N and C: P of plant leaves suggested that plants inside the fence had a higher growth rate (Huang et al., 2013).

5 Conclusions

With respect to the responses of plant and soil nutrients, enclosure had significant conservation effects in alpine desert grasslands, compared with the alpine steppe and alpine meadow. Enclosure not only increased the organic C, N, and K contents in the soil, but also changed the stoichiometric ratios of nutrients in both the soil and plants, thus promoting the growth rate of plants and increasing their above and below ground biomass. However, the controversy surrounding enclosures for grassland management still exists widely. Additional factors such as the duration of enclosure, grassland types, soil properties, changes of the plant growth environment, and different characteristics between plant species will all affect the responses of grassland ecosystems to enclosure. Therefore, research in this area still needs to be further strengthened.

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围栏封育对不同类型高寒草地植物及土壤养分的影响

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摘 要：围栏封育是高寒草地最为常见的保育方式，不同类型草地的封育效应可能会存在差异。开展该方面的研究，可以评估围栏封育对草地恢复的效应，为藏北高原不同类型退化草地的恢复措施提供科学依据。本研究以藏北地区的高寒草甸、高寒草原和高寒荒漠 3 类草地为研究对象，采用单因素方差分析对生物量、叶片–土壤养分含量以及化学计量比进行差异性检验，探讨了围栏封育对这 3 类高寒草地植物和土壤养分的影响。结果表明，围栏封育能显著提高这 3 类草地群落的地上生物量，但仅提高了 10–20 cm 高寒荒漠的地下生物量；围栏封育显著提高了高寒荒漠优势植物叶片的养分含量和 10–20 cm 土壤中全氮、全钾、有机碳的含量，并显著改变了 C、N、P 之间的化学计量比；而在高寒草甸和高寒草原围栏封育仅仅显著增加了高寒草原中优势物种叶片的 N 含量，其他养分指标和化学计量比均没有表现出显著的差异。以上结果表明，从植物和土壤养分来看，围栏封育对高寒荒漠草地的保育作用最为显著。

关键词：围栏封育；高寒草甸；高寒草原；高寒荒漠草原；植物养分；土壤养分