

Effect of grazing exclusion on plant communities varies over time in a Tibetan alpine meadow

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ABSTRACT

Fencing is an important and common grassland management strategy for restoring areas degraded by grazing. However, experimental evidence regarding the differences between the effects of short-term and long-term fencing on grassland structure remains insufficient, and the mechanisms by which fencing influences plant diversity and community structure are not yet fully understood. A long-term fencing experiment was conducted in a Tibetan meadow. We investigated Hill diversity, aboveground net primary productivity (ANPP), plant community height, species richness, and the biomass and height of four plant functional groups (including grasses, forbs, legumes, and sedges) after 1, 2, 3, and 13 years of fencing. This allowed us to compare the differing impacts of fencing on diversity and community structure over time. Additionally, we collected data on environmental factors such as soil compaction, soil moisture, soil nutrients, and plant litter biomass to elucidate the effects of fencing on plant communities. Our results indicated that short-term fencing increased Hill diversity within plant communities. Conversely, long-term fencing significantly decreased Hill diversity in alpine meadows. Furthermore, fencing reduced aboveground net primary productivity across all four assessment periods. Plant diversity exhibited a positive correlation with soil compaction and soil moisture, but a negative correlation with community height, litter biomass, and litter height. Fencing primarily influenced the diversity of litter biomass, community height, and soil nitrate nitrogen content across all biotic and abiotic factors. Our findings demonstrate that the effects of short-term and long-term fencing on plant communities differ markedly. Long-term fencing can adversely affect plant communities through litter accumulation and alterations in soil physical properties; therefore, the duration of fencing must be considered in grassland restoration efforts.

1. Introduction

Grazing is one of the most important management methods for grassland ecosystems (Wu et al., 2019), and can significantly influence both ecosystem structure (Liu et al., 2015) and function (Wang et al., 2019; Zhu et al., 2024). In recent years, rapid population growth and economic development have led to a substantial increase in the number of livestock in many grasslands (Wu et al., 2019). Overgrazing has resulted in decreased biodiversity and productivity, as well as a decline in ecosystem services (Wu et al., 2009; Miao et al., 2022). Extensive grazing bans have been implemented to restore degraded grasslands (Hu et al., 2016), and fencing has emerged as an important and commonly used management technique for this purpose (Miao et al., 2015; Sun

et al., 2020). An increasing number of researchers have focused on the responses of grassland plant communities to fencing (Zhu et al., 2016; Sun et al., 2019; Liu et al., 2020a; Liu et al., 2023).

Most researchers believe that fencing is an effective restoration strategy that can enhance species diversity (Zhu et al., 2016; Liu et al., 2020b) and productivity (Liu et al., 2020b; Liu et al., 2023) by decreasing herbivore consumption (Mofidi et al., 2013; Liu et al., 2014). However, other studies have reported neutral (Yan and Lu, 2015; Li et al., 2018a) or negative effects (Bruckerhoff et al., 2020; Liu et al., 2020c; Li et al., 2022; Suo et al., 2023) of fencing on plant diversity. The impact of fencing on plant diversity appears to vary with grassland type (Huang et al., 2020). Additionally, some studies have indicated neutral (Huang et al., 2020; Sun et al., 2020; Gao et al., 2023) and even negative

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(Suo et al., 2023) effects of fencing on the biomass of plant communities. Beyond the type of grassland, the duration of fencing may also be a significant factor influencing the varying responses of plant diversity and biomass in grasslands (Shang et al., 2008). A meta-analysis has demonstrated that the positive effects of fencing on plant diversity tend to diminish over time (Liu et al., 2023). This suggests that the long-term effects of fencing on plant communities may differ from those observed in short-term studies. However, in contrast to the numerous short-term fencing studies, research on long-term fencing effects is limited. A recent meta-analysis revealed that approximately half of the studies lasted less than five years, and 75 % were conducted over less than ten years (Liu et al., 2023). Comparisons between the long-term and short-term effects of fencing on plant communities at the same site have infrequently been studied, and experimental evidence regarding the variability of fencing effects on plant communities remains scarce. Furthermore, while some studies have explored the impact of fencing on plant communities, most have attributed changes in plant diversity and biomass to factors such as litter accumulation, herbivore exclusion, or competitive pressures among different species (Klein et al., 2004; Wu et al., 2009; Loydi, 2019). Some research has indicated that variations in soil compaction can influence the responses of plant diversity to fencing (Wu et al., 2019). However, it remains unclear which variable serves as the primary regulator of variation in plant diversity among the environmental factors.

Tibetan alpine meadows represent one of the largest highland grazing regions in the world (Wu et al., 2019). However, overgrazing has significantly degraded these meadows (Liu et al., 2017). Fencing has emerged as one of the most popular methods for grassland protection (Liu et al., 2017). Despite its widespread use, the mechanisms by which fencing influences grassland communities in this region remain unclear. To address this gap, a long-term fencing experiment was conducted in a Tibetan meadow, incorporating both fencing and winter grazing treatments. Data on the plant community were collected following short-term (1, 2, and 3 years) and long-term (13 years) fencing treatments to compare their effects on plant communities. We analyzed the relationships among litter, soil physical and chemical properties, plant community diversity, and aboveground net primary productivity to elucidate the effects of fencing on the plant community. We hypothesized that (1) short-term fencing would enhance plant diversity and aboveground biomass by mitigating herbivore disturbance, and (2) long-term fencing would reduce plant diversity due to the shading effects of accumulated litter from taller plants and a decrease in soil water-holding capacity.

2. Materials and methods

2.1. Study sites

The study site is located in the northeast of Hongyuan County, Sichuan Province (102°33' E, 32°48'N, 3500 m above sea level), which is part of the Zoige meadow on the Qinghai-Tibet Plateau. The mean annual temperature at the site is 1.7 °C, and the mean annual precipitation is 756 mm. Alpine meadows are the dominant vegetation type in the area, primarily consisting of *Kobresia littledalei*. Other abundant perennial herbs include *Saussurea nigrescens*, *Galium spurium*, *Anemone rivularis* var. *flore minore*, *Euphrasia spectata*, *Geranium wilfordii*, *Gueldenstaedtia verna*, and *Festuca ovina*. The meadow has been grazed by yaks for an extended period, with a grazing intensity of 2 to 4 yaks per hectare.

2.2. Experimental design

Experiments were conducted using a paired design. Eight blocks within a 1-ha meadow were randomly selected. Each block contained two 1.2 m × 1.2 m plots. One plot was enclosed with a steel frame and stainless-steel screen since late September 2007 to exclude herbivores;

this plot was designated as the fencing treatment. The other plot remained unfenced and was grazed by yaks during the winter months. Grazing commenced in early October each year and ended in mid-May of the following year, with a grazing intensity of 2 adult yaks hm⁻².

2.3. Plant community variables

All community parameters were monitored in early August of 2008, 2009, 2010, and 2020, specifically during the peak plant biomass period in the first three years of the fencing treatment and after 13 years of long-term fencing. A 0.5 m × 0.5 m quadrat was established in each plot (Guo et al., 2021). More than 90 % of the plant species present in the plots were identified within the corresponding quadrats. The number of individuals of each species within the quadrat was counted and converted into the number of plants per square meter, representing the species-level density. The densities of all species in each plot were summed to determine the overall community plant density. Species richness was recorded as the total number of plant species in each plot. The height of each species was randomly measured on three stems within the quadrat, and the average was calculated to represent the height of each species. Community height was computed using the height and relative density of each species, following a weighted average method (Liu et al., 2017), as shown below:

$$H = \sum_{i=1}^S \left(\frac{D_i}{D} \times H_i \right)$$

where S is the species richness (total number of species present) in the plot, D the density of all plant species, and D_i and H_i is the density and the height of the i^{th} species, respectively.

After estimating density, species richness, and height, the above-ground living biomass of plants within the quadrat was clipped by species, packed in paper bags, and then oven-dried at 65 °C for 48 h until a constant weight was achieved. The dry biomass of all plant species in each quadrat was summed and converted to represent the biomass per square meter, which is referred to as aboveground net primary productivity (ANPP). Additionally, the plant litter in each quadrat was collected, oven-dried to a constant weight, and weighed to determine the litter biomass for the year 2020.

Hill's diversity number, often referred to as "effective number of species" or "species equivalents", was chosen to calculate true diversity indices (Hill, 1973). The calculation of Hill's diversity number is outlined as follows (Miao et al., 2018):

$$D_A = \sum_{i=1}^S \left[\frac{N_i}{N} \right]^{1/(1-A)}$$

where S is the species richness (total number of species present) in the plot, N is the total number of species, N_i is the number of the i^{th} species and A is the sequence rank of the diversity measure. This sequence determines the sensitivity of differences in species abundance based on the number of species per square meter. Hill's diversity number fits the two most important diversities well, for $A = 0$, and 1. This is expressed as follows:

$$D_0 = S$$

$$D_1 = \exp(H)$$

where H is the Shannon–Wiener diversity index and the other parameters are the same as those in the previous formula. D_0 represents species richness, D_1 and D_2 can be used to assess species diversity. However, in the present study, D_0 and D_1 were mainly used to analyze species diversity.

All investigated plant species were categorized into four functional groups—grasses, sedges, legumes, and forbs—to further assess the impact of fencing on plant community structure. The biomass of all

species within each functional group was aggregated to calculate the total biomass at the functional group level, allowing for an evaluation of the effects of fencing on different species.

2.4. Determination of soil physical and chemical properties

The soil moisture at a depth of 10 cm was measured using a portable TDR200 soil moisture instrument (Spectrum, USA) in early August 2020. Soil compaction at depths of 1 to 12 ft was assessed using an SC 900 soil compaction meter (Spectrum, USA) during the same period, and the mean compaction value for this depth range was recorded for each plot. Additionally, three soil cores, each 10 cm deep, were collected from each plot using a 5 cm-diameter soil auger following the plant community investigation. The three soil cores were combined, and roots were removed using a 2 mm diameter mesh before dividing the mixture into two samples. One sample was stored at 4 °C to determine soil nitrate nitrogen (N) and ammonium N levels. The other sample was air-dried, passed through a 0.25 mm sieve, and ground to analyze total soil N, available phosphorus (P), and total soil P. Soil nitrate N, ammonium N, available P, and total soil P were measured using a Smartchem 200 Automatic Analyzer (Systea, Italy) based on colorimetric principles (Liu et al., 2011). Total soil N was determined using a Macro Cube element analyzer (Elementar, Germany) following combustion principles (Niu et al., 2016). Soil available N was calculated as the sum of soil nitrate N and soil ammonium N.

2.5. Data analysis

The effects of fencing on the species richness of D_0 and D_1 plant species across different functional groups, aboveground net primary productivity (ANPP), and variations in fencing effects on plant community variables over multiple years were analyzed using repeated measures analyses of variance (RMANOVAs). Paired t -tests were employed to assess differences between the two treatments for each year. The impact of fencing on soil physical variables (including soil moisture and compaction) and soil chemical properties (such as soil nitrate N, ammonium N, available N, total N, total P, and available P) was evaluated through paired t -tests. A piecewise structural equation model (SEM) was conducted to investigate the mechanisms of fencing on D_0 and D_1 , considering variations in community height, litter biomass, soil moisture, soil compaction, and soil nitrate N. Multiple SEM analyses were performed using the path analysis model of AMOS 21.0 (Amos Development Co., Armonk, New York, USA), obtained multiple analysis results (Fig. S1), and based fitting indexes of structural equation model (Table S1), then selected the optimal analysis result (Fig. S1f), while all other analyses were conducted using IBM SPSS version 26 (IBM, Armonk, New York, USA).

3. Results

3.1. Effect of fencing on D_0 , D_1 and plant species richness of different function groups

The effects of fencing on D_0 and D_1 varied significantly over time (Table 1, Fig. 1a, b). Specifically, fencing did not affect D_0 in 2008 and 2010, but it significantly increased D_0 by 16.3 % in 2009 ($P < 0.01$, Fig. 1a) and decreased D_0 by 29.9 % in 2020 ($P < 0.001$, Fig. 1a). Additionally, fencing significantly increased D_1 in 2008 ($P < 0.05$) and 2009 ($P < 0.01$) (Fig. 1b), but it significantly decreased D_1 by 31.7 % in 2020 ($P < 0.01$, Fig. 1b).

The effects of fencing on the species richness of grasses (Table 1, Fig. 1c) and forbs (Table 1, Fig. 1d) exhibited significant temporal variation. Specifically, fencing resulted in a notable increase in species richness of 46.2 % in 2008 ($P < 0.05$, Fig. 1c) and 71.4 % in 2009 ($P < 0.01$, Fig. 1c). However, it led to a significant decrease in grass species richness by 55.6 % in 2020 ($P < 0.001$, Fig. 1c). Fencing did not

Table 1

Results of repeated measurements ANOVAs (F value) for the effects of fencing on D_0 , D_1 , functional groups plant species richness, ANPP.

	Treat	Time	Time * Treat
D_0	4.35 [~]	9.99***	17.24***
D_1	2.26	80.85***	15.13***
Species richness of grasses	0.50	1.92	20.66***
Species richness of forbs	7.85*	2.11	7.34***
Species richness of legumes	1.47	7.93***	2.68 [~]
Species richness of sedges	2.93	21.05***	0.54
Community ANPP	12.64**	6.75***	0.46
Biomass of grasses	62.42***	1.04	0.74
Biomass of forbs	0.38	88.95***	4.10 [~]
Biomass of legumes	6.06*	10.65**	10.68**
Biomass of sedges	0.80	172.96***	8.32***

Significance level: [~], $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

influence the species richness of forbs from 2008 to 2010, but it reduced forb species richness by 30.3 % in 2020 ($P < 0.01$, Fig. 1d). Furthermore, the impact of fencing on legume species richness remained consistent over time (Table 1, Fig. 1e).

3.2. Effect of fencing on Aboveground Net Primary Productivity (ANPP), height, and litter

Fencing significantly reduced the ANPP of plant communities throughout the experimental period; however, the effect of fencing did not vary over time (Table 1, Fig. 2a). Fencing also significantly reduced grass biomass, with no variation in its effect over time (Table 1, Fig. 2b). Additionally, the effects of fencing on the biomasses of forbs, legumes, and sedges varied either marginally or significantly over time (Table 1). Specifically, fencing significantly increased forb biomass by 23.1 % in 2008 ($P < 0.01$, Fig. 2c) and by 29.5 % in 2009 ($P < 0.001$, Fig. 2c). However, long-term fencing slightly decreased forb biomass in 2020 (Fig. 2c). Fencing significantly increased the biomass of legumes by 442.4 % in 2008 ($P < 0.01$, Fig. 2d) but significantly decreased it by 88.6 % in 2020 ($P < 0.01$, Fig. 2d). Furthermore, fencing significantly decreased the biomass of sedges by 15.5 % in 2008 ($P < 0.01$, Fig. 2e) but significantly increased it by 140.5 % in 2020 ($P < 0.01$, Fig. 2e).

Long-term fencing significantly increased the height of the plant community by 69.9 % ($P < 0.001$, Fig. 3a) in 2020. This fencing also significantly elevated the heights of grasses, forbs, and sedges by 42.7 %, 62.5 %, and 150.9 %, respectively ($P < 0.01$; $P < 0.001$; $P < 0.001$, Fig. 3a). Additionally, litter biomass under the fencing treatment in 2020 was 93.8 and 267 times higher than that under the grazing treatment ($P < 0.001$, Fig. 3b; $P < 0.001$, Fig. 3c).

3.3. Effect of fencing on soil physical and chemical properties

Long-term fencing significantly reduced soil compaction and moisture by 46.5 % ($P < 0.01$, Fig. 4a) and 19.7 % (absolute change, $P < 0.001$, Fig. 4b), respectively, in 2020. Additionally, fencing led to a significant decrease in soil nitrate N content by 63.6 % ($P < 0.05$, Fig. 4c) in 2020, while it did not have a significant effect on ammonium N content (Fig. 4d), available N content (Fig. 4e), or total N content (Fig. 4f). Furthermore, fencing significantly increased total soil P and available P by 104.7 % ($P < 0.05$, Fig. 4g) and 28.2 % ($P < 0.05$, Fig. 4h), respectively.

3.4. Relationships between community characteristics and environmental parameters

D_0 and D_1 exhibited a negative correlation with community height, litter biomass, and litter height, while showing a positive correlation with soil compaction and soil moisture (Fig. 5). Additionally, D_1 was negatively correlated with total phosphorus (P) (Fig. 5). Community aboveground net primary productivity (ANPP) and forb biomass were

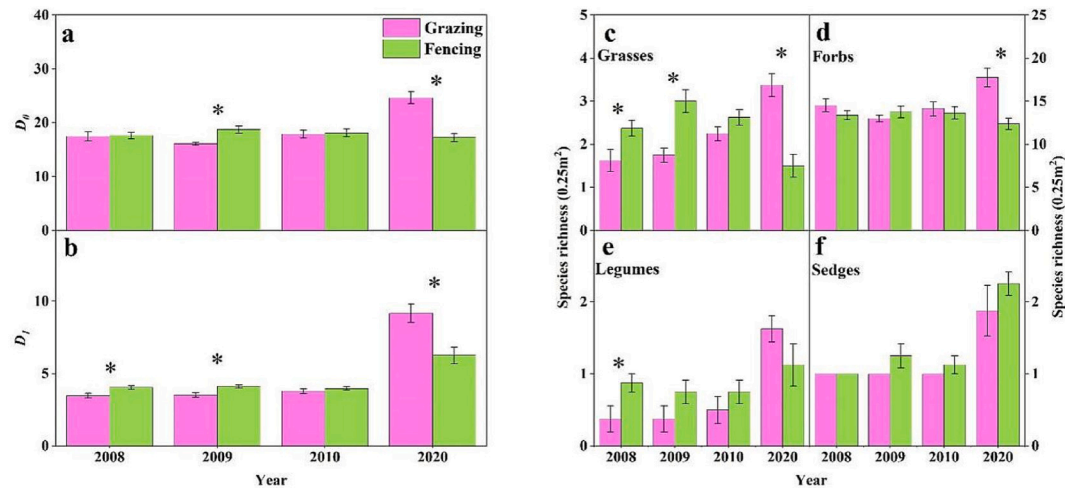


Fig. 1. Mean value (\pm SE) of the D_0 (a), D_1 (b), species richness of grasses (c), forbs (d), legumes (e), and sedges (f) under grazing and fencing treatment in different years. Significance level: *, $P < 0.05$.

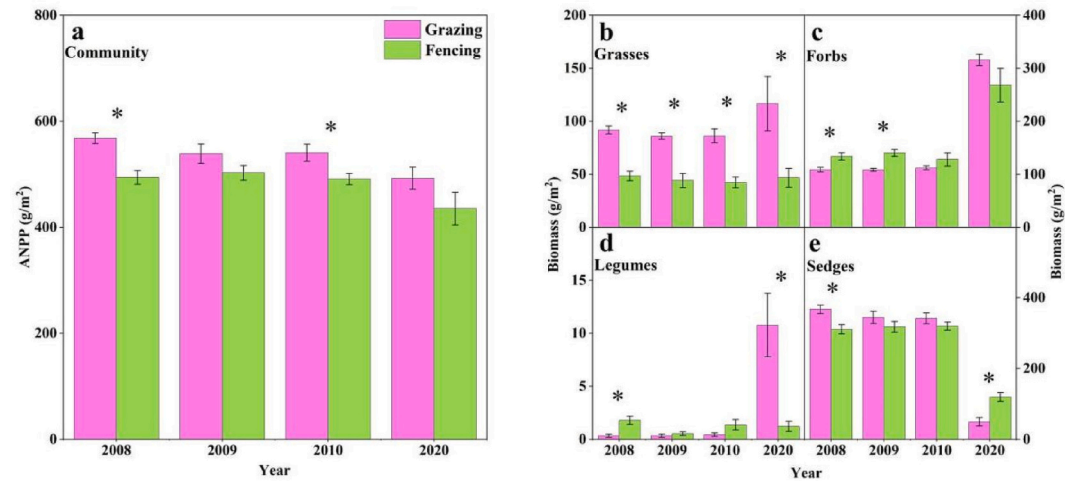


Fig. 2. Mean value (\pm SE) of the ANPP of the plant community (a), biomass of grasses (b), forbs (c), legumes (d), and sedges (e) under grazing and fencing treatment in different years. Significance level: *, $P < 0.05$.

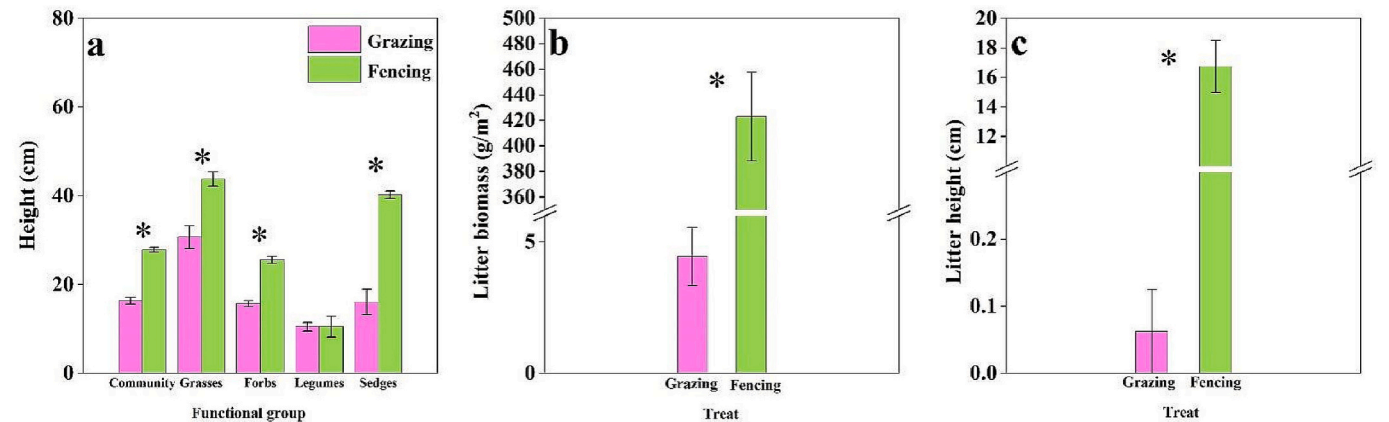


Fig. 3. Mean value (\pm SE) of the height of plant community, and different functional groups(a), litter biomass (b), and litter height (c) under different treatments. Significance level: *, $P < 0.05$.

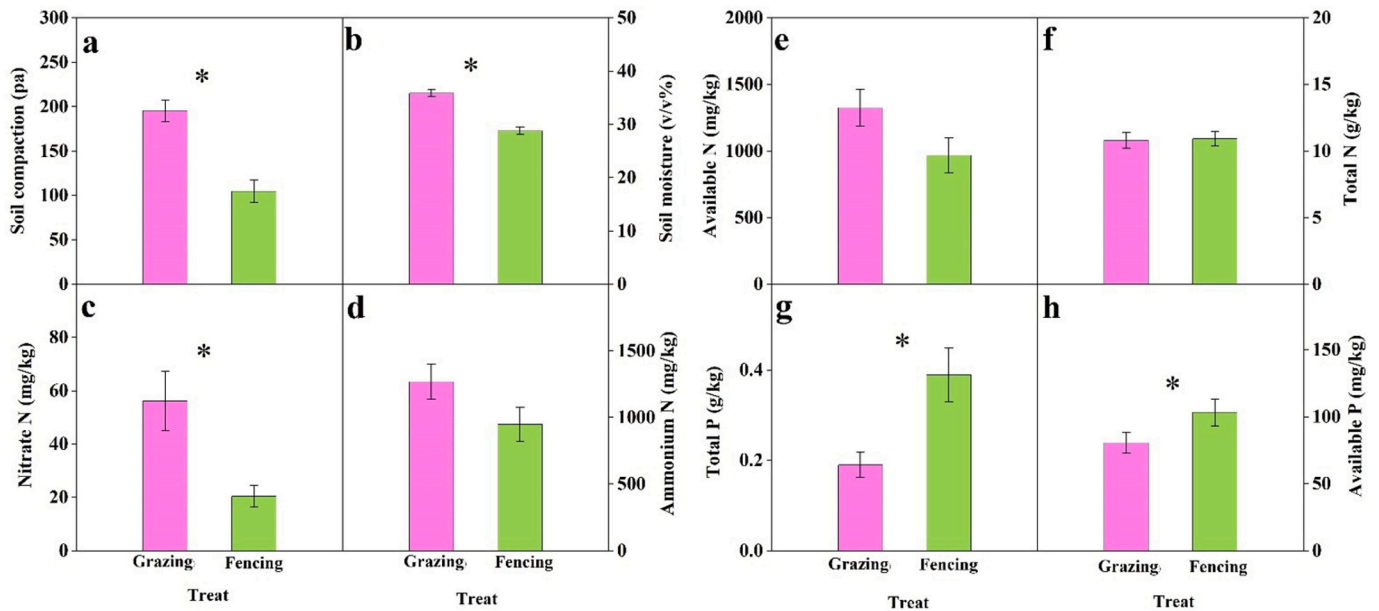


Fig. 4. Mean value (\pm SE) of soil physical variables (soil compaction and soil moisture) and soil chemical properties (soil nitrate N, ammonium N, available N, total N, total P and available P) under grazing and fencing treatment in 2020. Significance level: *, $P < 0.05$.

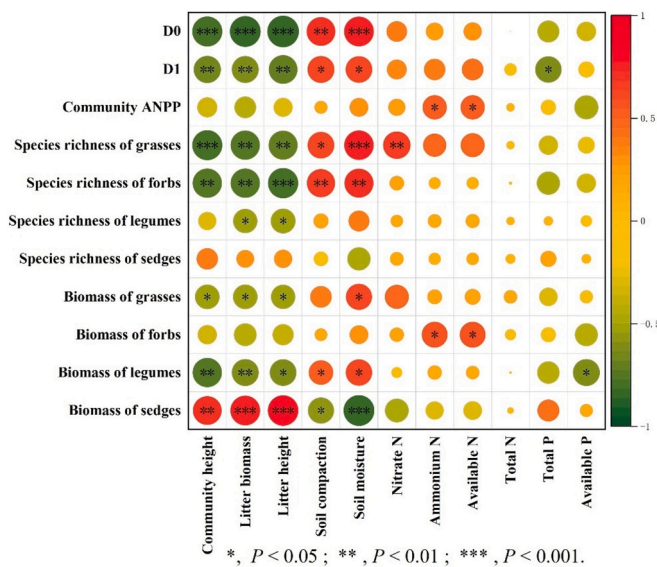


Fig. 5. Correlation heat map among variables of community, litter and soil. Significance level: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

positively correlated with ammonium and available nitrogen (N) (Fig. 5). The species richness of grasses and forbs, along with the biomass of legumes, demonstrated a negative correlation with community height, litter biomass, and litter height, but a positive correlation with soil compaction and soil moisture (Fig. 5). Furthermore, grass species richness was positively correlated with nitrate N (Fig. 5). Legume biomass was negatively correlated with available P (Fig. 5), and legume species richness was negatively correlated with both litter biomass and height (Fig. 5). Grass biomass showed a negative correlation with community height, litter biomass, and litter height (Fig. 5), but a positive correlation with soil moisture (Fig. 5). In contrast, sedge biomass was positively correlated with community height, litter biomass, and litter height (Fig. 5), but negatively correlated with soil compaction and soil moisture (Fig. 5).

SEM results indicated that fencing had a positive effect on

community height, which, in turn, negatively impacted grass species richness. Conversely, grass species richness positively influenced D_0 . Additionally, fencing negatively affected nitrate nitrogen levels, which positively correlated with grass species richness. Fencing also positively influenced litter biomass while negatively affecting the species richness of forbs and legumes. The species richness of legumes positively impacted D_0 , while forb species richness positively affected both D_0 and D_1 (Fig. 6).

4. Discussion

4.1. Effect of short-term fencing on plant community

In the early stages of fencing, we observed an increasing trend in the Hill index diversity of the plant community, which supported our hypothesis. These results contradict some previous studies that reported negative effects (Wu et al., 2009; Shi et al., 2013) and neutral effects (Yan and Lu, 2015) associated with medium- to short-term (6–8 years) fencing of alpine meadows. Wu et al. (2009) and Yan and Lu (2015) concluded that species with lower competitive capacity tend to reduce their density or disappear from plant communities due to competition for light and nutrient availability. However, other studies have demonstrated that short-term (1–5 years) fencing can enhance plant diversity in grasslands (Du et al., 2020; Ren et al., 2024), which aligns with our findings. Short-term fencing restricts animal movement (Sun et al., 2020) and livestock grazing (Huang et al., 2020), both of which are beneficial for plant growth. Consequently, most previous studies have indicated that short- to medium-term fencing creates a favorable environment for plant growth (Al-Rowaily et al., 2012; Liu et al., 2020b). The study site had been subjected to grazing for an extended period before fencing, resulting in the disappearance of many plant species due to animal feeding and the emergence of numerous spatial niches caused by reduced coverage from grazing. Following the fencing treatment, plant reproductive structures, including seeds and buds in the soil, germinated rapidly, which can enhance species richness and diversity. Additionally, fencing can decrease plant species richness through litter accumulation, as litter can impede plant growth and subsequently reduce plant diversity (Klein et al., 2004). However, the meadow is dominated by *Kobresia littledalei* after prolonged grazing. This species is characterized by slender leaves that do not accumulate dense

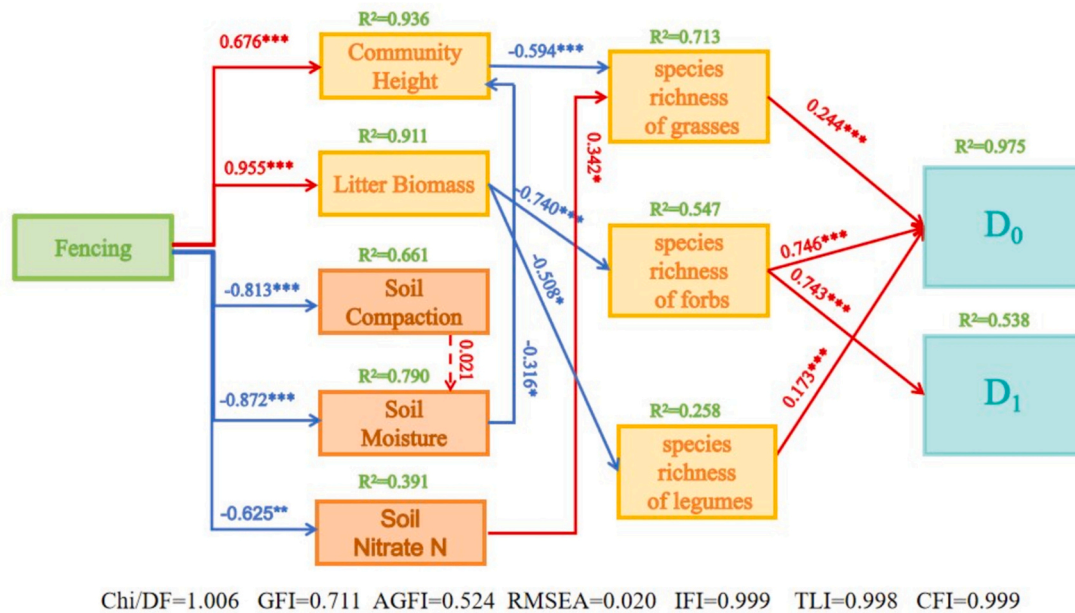


Fig. 6. Structural equation model (SEM) analysis of relationships among D_0 and D_1 , by the variation of community height, litter biomass, soil compaction, soil moisture, and soil nitrate N. Red and blue solid arrows indicate significant positive and negative paths, respectively, and dotted arrows indicate non-significant paths. R^2 , coefficient of determination for all the paths that are directed at the variable above the R^2 . Significance level: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

litter, which would otherwise hinder short-term plant growth. Therefore, the negative effects of fencing due to litter accumulation did not manifest in the short term in this meadow. Furthermore, our results indicated that short-term fencing benefits species richness and legumes in grasslands, consistent with the findings of Wu et al. (2008). Fencing eliminates grazing by livestock, thereby increasing the survival chances of these plants (Al-Rowaily et al., 2012). Since these two types of plants are preferred food sources for yaks, grazing has a significant impact on them.

We found that short-term fencing significantly decreased the ANPP of grassland plant communities in the alpine meadows, which did not support our hypothesis. This result contrasts with previous studies that have demonstrated an increase in biomass under short-term (1–4 years) fencing treatments in alpine meadows (Sun et al., 2020; Liu et al., 2020a; Liu et al., 2020b; Zhan et al., 2022). Fencing typically enhances the aboveground biomass of plant communities by preventing herbivores from feeding, as herbivores can consume substantial amounts of vegetation. However, moderate grazing has been shown to benefit plant growth (Du et al., 2020). In our study, moderate winter grazing was advantageous for plant growth because it mitigated the negative effects of litter accumulation and did not damage the plants during their vigorous growth period. Additionally, a study conducted on the Loess Plateau steppe reported a neutral effect of fencing on ANPP, which is inconsistent with our findings. This discrepancy may be attributed to the differing productivity rates of the two ecosystems. In our research, the productivity of the Loess Plateau steppe was significantly lower than that of the alpine meadow, primarily due to the much lower soil moisture content in the Loess Plateau compared to the Zoige Plateau. Soil moisture content is a crucial factor in regulating various ecological functions within grassland ecosystems (Li et al., 2018b). Given the differences in soil moisture and vegetation growth characteristics between the two regions, the aboveground biomass of alpine meadows may be more sensitive to fencing than that of semi-arid steppes.

Our results indicate that short-term fencing can increase the biomass of forbs and legumes, which aligns with findings from previous studies on short-term (3–5 years) fencing (Wu et al., 2014). This phenomenon may be attributed to the poor palatability of forbs, which leads to reduced grazing pressure and allows them to dominate plant

communities following short-term fencing (Liao et al., 2020). Consequently, forbs may quickly gain a competitive advantage after enclosure by capitalizing on their increased dominance in the absence of grazing (Deng et al., 2017). In contrast, the observed reduction in the biomass of grasses and sedges contradicts previous research that documented benefits for these species from short-term fencing (Wu et al., 2009; Wu et al., 2014; Du et al., 2020). The proliferation of forb and legume plants exacerbates light limitations on the growth of grasses and sedges. While grasses and sedges may grow taller to capture sunlight, this often results in a decrease in their density or the number of tillers. Therefore, although the species richness of grasses increased following short-term fencing, their overall biomass decreased.

4.2. Effect of long-term fencing on plant community

After 13 years of long-term fencing from 2007 to 2020, the decrease in the Hill diversity index under the fencing treatment was contrary to that observed under short-term fencing, thereby supporting our hypothesis. This finding is inconsistent with previous studies that have reported a positive effect of fencing on plant diversity (Zhan et al., 2022; Gao et al., 2023), but aligns with earlier research conducted on alpine meadows (Zou et al., 2016; Wu et al., 2019; Sun et al., 2020). According to prior studies, the negative effects of long-term fencing on plant diversity can be primarily attributed to four factors. First, fencing may influence species diversity by altering litter accumulation. By preventing livestock from grazing, fencing can lead to increased litter accumulation (Cheng et al., 2016). This accumulation may reduce the effective photosynthetic radiation reaching dwarf plants due to self-shading, negatively impacting plant growth (Semmartin and Oesterheld, 1996) and consequently inhibiting the growth and establishment of vegetation, which decreases plant diversity (Klein et al., 2004). Second, fencing can modify community competition, thereby affecting the diversity and density of plant communities. Competition among species intensifies following long-term fencing (Liu et al., 2023). Some less competitive species may experience reduced density or even disappear from plant communities due to competition for light (Grime, 1998) or soil nutrients (Van Der Wal et al., 2004). This results in the community being dominated by a few species with strong colonization abilities, leading to a

decline in species diversity (Borer et al., 2014; Loydi, 2019). Additionally, fencing increased community height in our study, which is consistent with findings from previous research (Zhu et al., 2016; Tang et al., 2016; Gao et al., 2023). According to the overtopping hypothesis, some plants must increase their height to flower above the community canopy to attract pollinators and facilitate seed dispersal (Tang et al., 2016; Liu et al., 2017; Liu et al., 2021). Third, changes in soil compaction are significant mechanisms that regulate plant diversity. Fencing can reduce soil compaction, and increase the water infiltration rate, which may decrease water concentration and soil nitrogen content in the topsoil, potentially negatively impacting plant diversity (Wu et al., 2019). Finally, the impact of fencing on seed dispersal is another possible reason for changes in biodiversity. Previous studies have indicated that fencing can hinder seed transmission and seedling establishment in cattle, which may impede plant community regeneration and subsequently decrease plant diversity (Oesterheld and Sala, 1990). Among the four mechanisms, the SEM results of our study supported the direct regulation of litter, community height, and soil nitrate nitrogen on the diversity index of the plant community. Although the SEM results did not reveal a significant pathway for soil moisture, the significant correlations between plant diversity, soil compaction, and soil moisture suggest that we cannot dismiss the influence of soil physical properties on plant community diversity. The non-significant relationship between plant diversity and soil physical properties may be attributed to heterogeneity among the different plots.

Consistent with the decreasing trend in ANPP in the early stages of fencing, our study also indicated a tendency for long-term fencing to reduce ANPP, although this difference was not statistically significant. These findings contrast with the positive effects of fencing on plant biomass reported in most previous studies (Wu et al., 2019; Yang et al., 2020; Zhan et al., 2022). However, some research has indicated that long-term fencing (greater than 8 years) can diminish or even negate the positive impacts of grazing bans on aboveground plant biomass (Liu et al., 2022). Previous studies have consistently reported that long-term fencing leads to a reduction in aboveground biomass (Jing et al., 2013; Zou et al., 2016). This discrepancy may be attributed to variations in grazing practices. Most prior studies focused on summer or annual grazing as the treatment (Tang et al., 2016; Wu et al., 2019), whereas our study specifically examined winter grazing. First, winter grazing does not disrupt plant growth (Yu et al., 2015), allowing its negative effects on plant development to be considered negligible. Second, winter grazing facilitates the removal of litter through livestock feeding (Gao et al., 2008), which can promote plant growth and advance phenological development. Third, fencing eliminates livestock disturbances and encourages the formation of biological soil crusts on grassland surfaces, which may restrict rainfall and litter input, thereby inhibiting plant growth (Zhao et al., 2006; Zhang et al., 2008). Given these three factors, it is plausible that fencing may lead to a decrease in ANPP compared to the winter grazing treatment. Another aspect contributing to the divergence between our results and those of previous studies is that the variable analyzed in our study, ANPP, does not account for litter biomass. In contrast, other studies often include aboveground biomass, which encompasses litter. Notably, fencing also increased aboveground biomass in our study (Fig. S2). Therefore, our findings suggest that while long-term fencing negatively affects productivity, it does not hinder biomass accumulation.

5. Conclusion

The long-term fencing experiment indicated that short-term fencing positively affected plant communities, while long-term fencing had a negative impact. The negative effects of fencing on diversity were primarily attributed to changes in litter biomass and community height. Future research will focus on determining the specific effects of long-term fencing on plant communities. Our results confirmed the differing impacts of short- and long-term fencing on plant communities

and highlighted the importance of selecting an appropriate restoration period when employing fencing methods for grassland ecosystem restoration; otherwise, it may result in the degradation of the grassland ecosystem.

Data archiving statement

Data available from <https://orcid.org/0009-0009-4224-8403>.

CRediT authorship contribution statement

Taohui Lian: Writing – review & editing, Writing – original draft, Validation. **Yanling Li:** Writing – original draft, Data curation. **Xun Wang:** Software, Data curation. **Shuang Xiang:** Writing – review & editing, Methodology. **Yinzhan Liu:** Writing – review & editing, Supervision, Methodology. **Shucun Sun:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoleng.2024.107420>.

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