Asian Journal of Agriculture and Biology
https://doi.org/10.35495/ajab.2024.234

Original Article

Influence of grazing intensity and regime on soil nitrogen fixation dynamics in alpine grasslands

Ali Nawaz[1,2], Muhammad Khalid Afzal[3,4]*

[1]School of Grassland Science, Beijing Forestry University, Beijing-100083, China
[2]Department of Agronomy, College of Agriculture, University of Sargodha-40100, Punjab, Pakistan
[3]College of Forestry, Beijing Forestry University, Beijing-100083, China
[4]Institute of Forest Sciences, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur-63100, Pakistan

Corresponding author's email: mkhalidafzal007@gmail.com
Received:15 November 2024 / Accepted: 27 January 2025 / Published Online: 22 February 2025

Abstract

The present study was conducted to investigate the influence of grazing intensity and management regimes on soil nitrogen cycling and related ecosystem processes in alpine grasslands. Rotational grazing (RG), characterized by alternating short grazing periods at high stocking densities with rest intervals, has been proven to be a practice that enhances herbage production and improves grassland resilience compared to continuous grazing (CG). Our results showed that Proteobacteria are the main ANF phylum under all grazing levels. Grassland sowing is a primary restoration means for heavily degraded alpine meadows on the Tibetan Plateau, but much less information is known about soil nutrient dynamics under such grazing regimes. This study evaluated topsoil nutrient levels (phosphorus, potassium, and nitrogen) and physical properties under three grazing intensities: light, moderate, and heavy. It was concluded that increasing grazing intensity elevated soil organic matter, temperature, and nutrient concentrations (nitrogen, potassium, and phosphorus) while reducing soil pH and water content. These findings demonstrate that intensity and regime of grazing significantly influence dynamics in soil nitrogen fixation processes.

Keywords: Grazing intensity, Rotational grazing, Grassland restoration, Alpine grasslands, Soil nitrogen fixation, Tibetan Plateau

How to cite this article:

Nawaz A and Afzal MK. Influence of Grazing Intensity and Regime on Soil Nitrogen Fixation Dynamics in Alpine Grasslands. Asian J. Agric. Biol. 2025: 2024234. DOI: https://doi.org/10.35495/ajab.2024.234

Introduction

One of the primary functions of grasslands is grazing, which has the potential to greatly increase the ecosystem's susceptibility to human interference and global climate change due to its effects on the ecosystem's capacity to cycle nutrients and deliver services. Moreover, it may hasten the decomposition of deposited soil particles (Abdalla et al., 2018). One of the biggest sustainability issues is the damage that grazing does to the ecosystem of alpine grasslands. Numerous studies have examined how grazing affects the plant and soil characteristics of the alpine ecosystem. Grazing has been shown to increase the diversity of vegetation communities, improve root-to-shoot ratios, and significantly reduce nitrogen (N) levels (Lu et al., 2017). On the other hand, the impact of grazing on the fundamental soil elements may be significantly more complex than previously thought, given the inconsistent results of numerous research (Hao and He, 2019). For instance, whereas research has shown that grazing lowers biomass and soil nutrient concentrations, other studies have shown that a more practical grazing strategy for sustainable grassland management may involve more intense grazing (Dong et al., 2020). It has been frequently confirmed that soil nitrogen upsurges throughout grazing prohibiting, despite some studies concluding no substantial alterations in soil nutrients between grazing exclusion and different grazing intensities (Yang et al., 2016). Thus, more investigation is needed to completely understand the impact of grazing on soils. The results of this study may be very important for developing strategies for sustainable management and eco-compensation. Nitrogen is known to be the primary nutrient that limits plant growth in high- and mid-latitude regions and is essential for the synthesis of nucleic acids and proteins. A fundamental biological process called nitrogen cycling is rapidly changing because to human perturbations including reclamation, nitrogen fertilizer, and grazing. This is due to the fact that many people are concerned about the consequences of excess nitrogen and N_2O emissions (Canfield et al., 2010; Jain et al., 2013; Zhong et al., 2014). The availability of N reserves is the primary factor proposed to explain the manufacturing process in terrestrial ecosystems. The primary natural nitrogen input type for ecosystems is biological nitrogen fixation, a process in which some prokaryotic microbes use the catalytic activity of nitrogenase to convert atmospheric nitrogen molecules to ammonia (Barron et al., 2009; Reed et al., 2011; Zheng et al., 2018). Every year, inert N_2 is converted into around 120 million tons of active nitrogen through biological nitrogen fixation. Soil fertility is increased and the soil nitrogen cycle is strengthened by microbial nitrogen fixation (Gupta et al., 2014). It also replenishes the nitrogen that is lost to the atmosphere from the soil.

Approximately 41.4% of China's geographical surface is made up of its various grasslands, of which 84.3% is utilized for human purposes (Li et al., 2020). Grasslands cover about three times the area of agricultural land. Grasses ecosystems make up a sizable amount of China's natural resources. They provide the foundation for animal husbandry and livestock grazing in the large pastoral regions that are home to over 20 million people, which makes them crucial to the country's food security. China is home to three main categories of natural grasslands: temperate, alpine, and subtropical shrubby tussock rangelands. The Qinghai-Tibetan Plateau is home to alpine grasslands, which are composed of hygrophilous forbs such as *Carex*, *kobresia*, and *Cyperaceae* that are resistant to low temperatures. It has long been recognized that the biogeochemical cycling of soil N and its stoichiometric fluctuation in terrestrial ecosystems are significant aspects related to nutrient restriction and ecological stability, aside from soil element concentrations. The vegetation-soil-microbial feeding cycle controls the stoichiometry and buildup of soil components and is impacted by management techniques and environmental factors (Jiang et al., 2018). Grasslands are subject to a variety of human management approaches and make about 25% of the planet's geographical area. Grazing is a common land-use type in grasslands that aids in managing the fluxes and cycles of nutrients. To understand grassland ecosystems and determine the extent to which grasslands contribute to global nitrogen fluxes, it is imperative to investigate the role that herbivores play in controlling soil nitrogen levels. Alpine meadows cover somewhat more than 35 percent of the plateau. These are the main grazing areas in eastern Tibet. However, because the amount of N held in the soil is not well-documented, it is challenging to evaluate the effects of different management practices on the biogeochemical processes that control the exchange of nitrogen between the soil and the atmosphere. This study sought to determine how yak grazing intensities affected the seasonal dynamics of soil N content and denitrification in alpine environments. Livestock grazing modifies the nitrogen cycles and may affect the rate at which soil accumulates. Regarding how grazing affects the amount of N in the soil, there is contradicting information. Research has demonstrated that grazed pastures have substantially higher soil N levels than non-grazed enclosures (Eldridge et al., 2016). Objectives of this research is to clarifying the effects of grazing regime and intensity on soil nitrogen fixation, total and accessible nitrogen, soil nitrification, denitrification, total nitrogen content, total phosphorus content, and total potassium content.

Material and Methods

Study area

The investigation was conducted at the Tiebujia Grassland Restoration Station, which is located in Gonghe County, Hainan Tibetan Autonomous Prefecture, Qinghai Province, China (99°35′N37°02′E). The study site is located at an elevation of 3210 meters above sea level. The predominant plateau continental climate brings long days and bitterly cold winters. It was a typical plateau semiarid steppe with an average annual temperature of -0.7°C and 377.10 mm of precipitation. At the experimental sites, there is a yearly evaporation of 1484 mm. The experiment site's soil type is categorized as dark chestnut soil with a clay texture under Chinese soil taxonomy. *Poa pratensis, Elymus breviaristatus*, and *Artemisia scoparia* are the three dominant species in the alpine steppe vegetation (Qin et al., 2022). The map study site shown in Figure 1 is where various soil samples were taken to determine the intensities of grazing. The mean annual air temperature and mean annual precipitation anomalies throughout 2016–2100 are shown in Figure 2. The various grazing intensities no grazing (NG), light continuous grazing (LCG), medium continuous grazing (MCG), and heavy continuous grazing (HCG) were depicted in Figure 3.

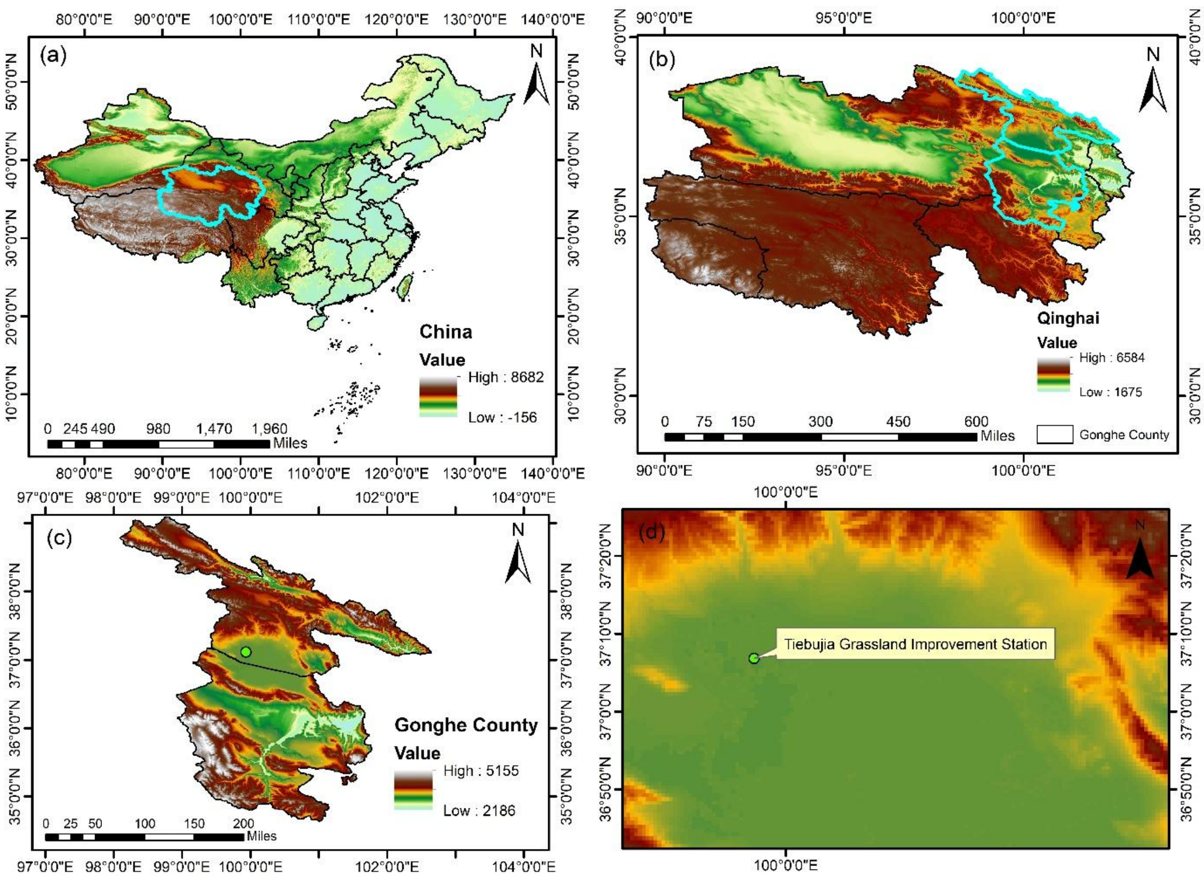


Figure-1. Map of the Study area

https://doi.org/10.35495/ajab.2024.234

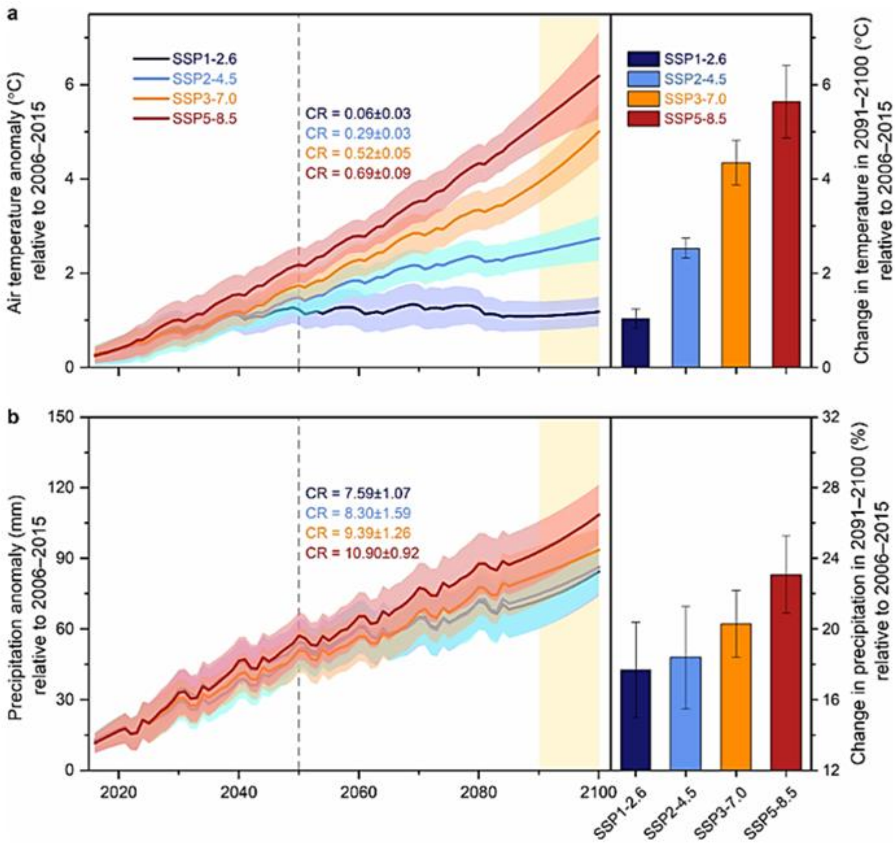


Figure-2. (a) Mean annual air temperature and (b) mean annual precipitation anomalies during (2016–2100) (Zhang et al., 2022)

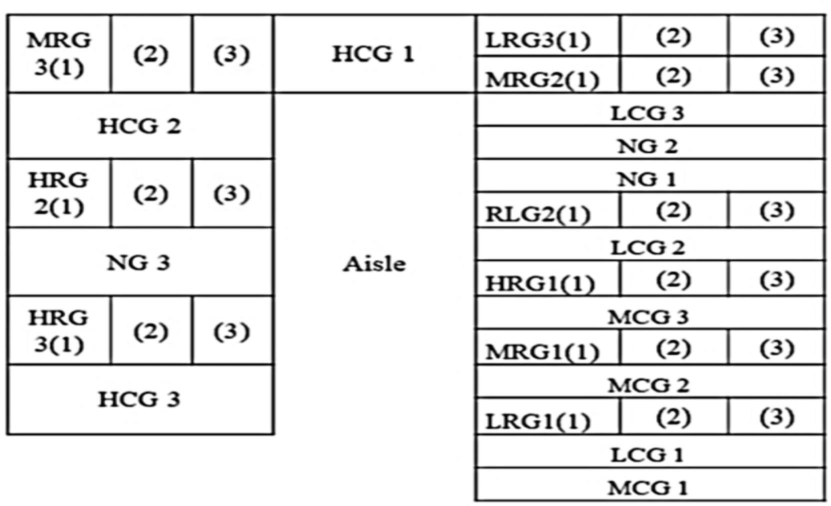


Figure-3. Experimental plots NG, LCG, MCG, and HCG for no, light, medium, and heavy continuous grazing, respectively.

https://doi.org/10.35495/ajab.2024.234

Soil Sampling

The research area was used as a free grazing pasture with a reasonable stocking rate before animal exclusion in order to encourage vegetation regeneration following grazing. Within a 21-ha alpine steppe paddock, the grazing experiment was carried out in twenty-one 100 m × 100 m plots. One of the treatments included continuous grazing (CG), which was divided into three groups: no grazing (NG), heavy continuous grazing (HCG), and medium continuous grazing (MCG). Every type of grazing treatment, including medium continuous grazing (MCG), light continuous grazing (LCG), heavy continuous grazing (HCG), and no grazing (NG), was randomly assigned to an experimental plot. The grazing experiment ran for roughly 60 days per year. To stop livestock from grazing, the same plots were roped off from each other. From the field, new soil samples (0–20 cm) were collected. After being gathered, samples were passed through a 2 mm sieve. After screening, weigh around 100g of soil to determine the amount of nutrients present.

Soil Physical Properties

Soil temperature (ST), soil organic matter (SOM), and soil water content (SWC) were assessed with a soil moisture thermometer. Samples were taken and measurements of SOM, SWC, and ST were made at the sites where soil cores were close by.

Soil Physicochemical analysis

Using a stainless-steel cutting ring with dimensions of 5.0 cm in diameter, 5.0 cm in height, and 100 cm^3 in volume, the pH, total phosphorus (TP) (g/kg), total potassium (TK) (g/kg), and total nitrogen (TN) (g/kg) were computed. The materials will be physically mixed together and any notable living plant material or gravel removed before the start of the inquiry. A 2 mm mesh filter will be utilized following the crushing and air drying of the samples. To find the concentrations of TN, TP, and TK, 50 grams of the subsamples were crushed in a mortar and sieved through a 0.25 mm screen. To ascertain the TN content, the Kjeldahl method was applied. Once the samples have been treated with sulfuric acid, perchloric acid, and molybdenum, the total porosity content is determined using the antimony, anti-colorimetric method. Orthophosphate salt is created at high temperatures when sulfuric and perchloric acids are mixed with phosphorus-containing minerals and organic molecules. Zhou et al. (2023) employed anti-colorimetry to ascertain the concentration of TP in the solution subsequent to the dissolution of the orthophosphate salt.

Non-symbiotic N_2 fixation

N_2 was calculated using a stable isotope approach for ^{15}N. A 4 g dry-mass soil sample was incubated in an artificial environment that was enriched in ^{15}N and included 99.8 atoms of $^{15}N_2$. A 12 cc exetainer was filled with fresh soil. Following a gas-tight closure, argon (Ar) flush, cautious evacuation, and filling with 7.2 ml of $^{15}N_2$ and 0.8 ml of O_2, each exetainer was sealed. Pressure variations were observed before and after introducing $^{15}N_2$ and O_2 to the artificially created ^{15}N-enriched atmosphere. The average atmospheric composition, expressed as volume per volume percent, was 72.5% $^{15}N_2$, 8.2% O_2, and 19.2% Ar. After being incubated for 72 hours at 15 °C in the dark in this setting, fresh soil samples were dried at 50 °C. Following that, the 15N contents of soil that had been exposed to $^{15}N_2$ (^{15}N labeled) and soil that had not (natural abundance) were ground and analyzed. The ^{15}N atom% was calculated using the isotope ratio of each sample (R sample = $^{15}N/^{14}N$). The $^{15}N_2$ fixation rate (in ng N g $soil^{-1}h^{-1}$) was computed using an isotope dilution model (Schleuss et al., 2021). Grazing can have an impact on the make-up and activity of soil microbial populations, particularly bacteria that fix nitrogen. Microbiological environments can be modified by grazing-related changes in soil moisture, organic matter concentration, and compaction.

$$\mathbf{^{15}N_2 \text{ fixation rate } (mg\ N\ kg^{-1}d^{-1}) = TN\ (mg\ N\ kg^{-1}) \times \frac{(\text{atom\% } ^{15}N_{labeled} - \text{atom\% } ^{15}N_{NA}) \times 10^6}{100 \times t\ (h)}}$$

https://doi.org/10.35495/ajab.2024.234

Where:

- The overall amount of N in the soil (measured in mg/kg) is called TN.
- The amount of N in the labeled sample is called atom% $15N_{labeled}$.
- The amount of N in the control samples is called atom% $15N_{NA}$.
- The incubation time is t (in hours).
- The conversion factor is 10^6 (mg N $kg^{-1}d^{-1}$)

Statistical Analysis

Melting sodium carbonate (Na_2CO_3), an alkali solvent, with soil samples at 920 ◦C resulted in the dissolution of potassium in the solution. Knudsen and Johansen (1989) employed this technique to ascertain the TK content. The physicochemical properties of soil were examined in a lab setting. All data was subjected to two analyses of variance (ANOVA): by sample date and by grazing intensity. To compare grazing treatments, the least significant difference (LSD) test was employed for statistically significant values ($p<0.05$) from ANOVA. To maintain consistency, the study conducts experiments in triplicate and complies with strict quality assurance procedures. Instruments and equipment are calibrated on a regular basis, and analytical-grade chemicals are purchased from approved vendors. User manuals and guidelines are closely followed when collecting data and preparing samples. Outliers are dealt with and cleaned up, and the results are compared to reference standards.

Results

Soil Physical & Physiochemical Properties

Soil Organic Matter Content

Grazing improves the amount of organic matter in the soil. It rises as the intensity of the grazing increases. Soil organic matter (SOM) content is the proportion of soil made up of organic substances. SOM increases as the intensity of grazing increases by time which was significantly highest ($p<0.005$) as compared to other sites. Soil OM levels in the HG site were consistently greater than those in the LG and MG sites for the course of the investigation. LG, MG, and HG had shown (59.71±12.1, 79.9±4, and 98.23±31.4, respectively).

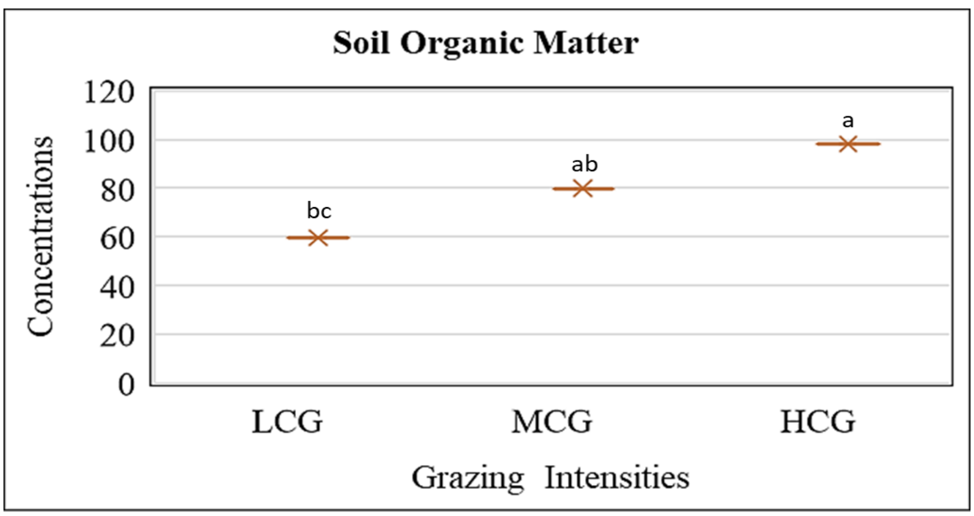


Figure-4. Soil organic matter content

Soil pH

The cycling of nutrients, particularly nitrogen, which affects soil pH, can be impacted by grazing. There were substantial differences in soil pH at different grazing intensities. Light grazing was depicted as the highest soil pH while high grazing demonstrated as lowest pH for soil, as LG, MG and HG (7.1, 6.3 and 5.9, respectively).

https://doi.org/10.35495/ajab.2024.234

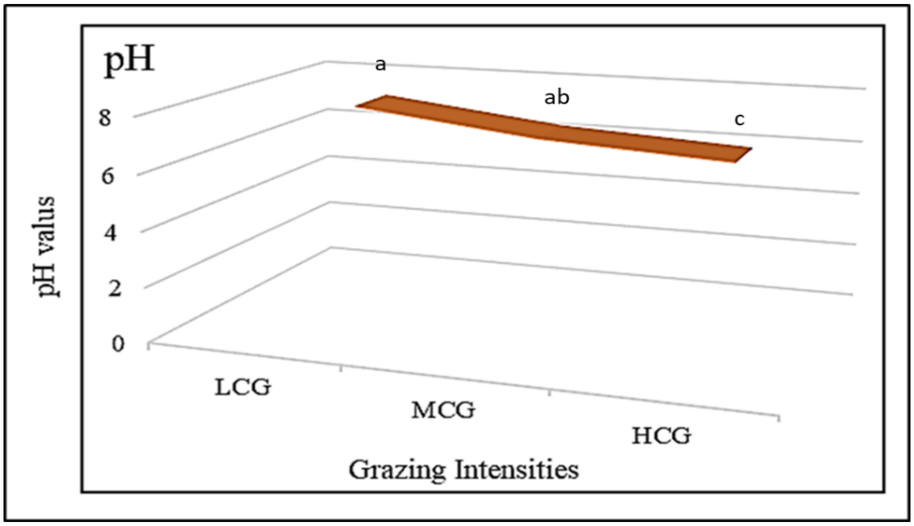


Figure-5. Soil pH (p<0.05)

Table-1. Effects of different grazing intensity on Soil Physical & Physiochemical Properties

Grazing Intensity	pH	SOM	SWC	Soil T	TNC	TPC	TKC
LCG	7.1±0.6[a]	59.71±12.1[bc]	25%	13.2°C	5.92±0.3[bc]	0.38±0.01[c]	12.32±0.3[c]
MCG	6.3±0.8[ab]	79.91±4.16[ab]	20%	18.6°C	9.23±1.09[a]	0.721±0.07[ab]	31.19±0.38[ab]
HCG	5.9±0.4[c]	98.23±31.4[a]	15%	26.8°C	11.78±2.8[a]	0.921±0.12[a]	49.13±1.23[a]

Temperature of the soil and water content

There is a strong correlation between these two factors and the intensity of grazing. Temperature and moisture content of the soil rose quickly. The HG location had much greater soil temperature than the LG and MG sites, which did not significantly differ from one another. During the day, lower vegetation cover can cause greater soil temperatures because more solar radiation reaches the soil surface. LG, MG and HG had exhibited as the lowest to highest temperature according to the day and night time duration. At night, soils with less vegetation cover may cool down faster, resulting in lower nighttime temperatures. LG, MG and HG had different temperatures as 13.2°C, 18.6°C and 26.8°C, respectively. Throughout the experiment, there was a continuous difference in the amount of soil moisture between the HG site and the LG and MG sites ($p < 0.01$). Increasing the intensity of grazing causes a decrease in soil water content (SWC). In terms of water content, the percentages for light, medium, and high grazing intensities were 25%, 20%, and 15%, respectively. Temperature or soil moisture did not significantly correlate with the amount of grazing.

Soil Total Nitrogen Content (STNC)

Soil N varied substantially between different grazing intensities, with a significant difference ($p < 0.001$). The sampling data indicated that the mean concentration of soil N was 5.92, 9.23, and 11.78 g kg^{-1} for the LG site, MG site, and HG site, in that order. Significantly, there were no primary interactions between soil N and grazing intensities. Meanwhile, soil total nitrogen (TNC) varies with an increase in grazing intensities.

https://doi.org/10.35495/ajab.2024.234

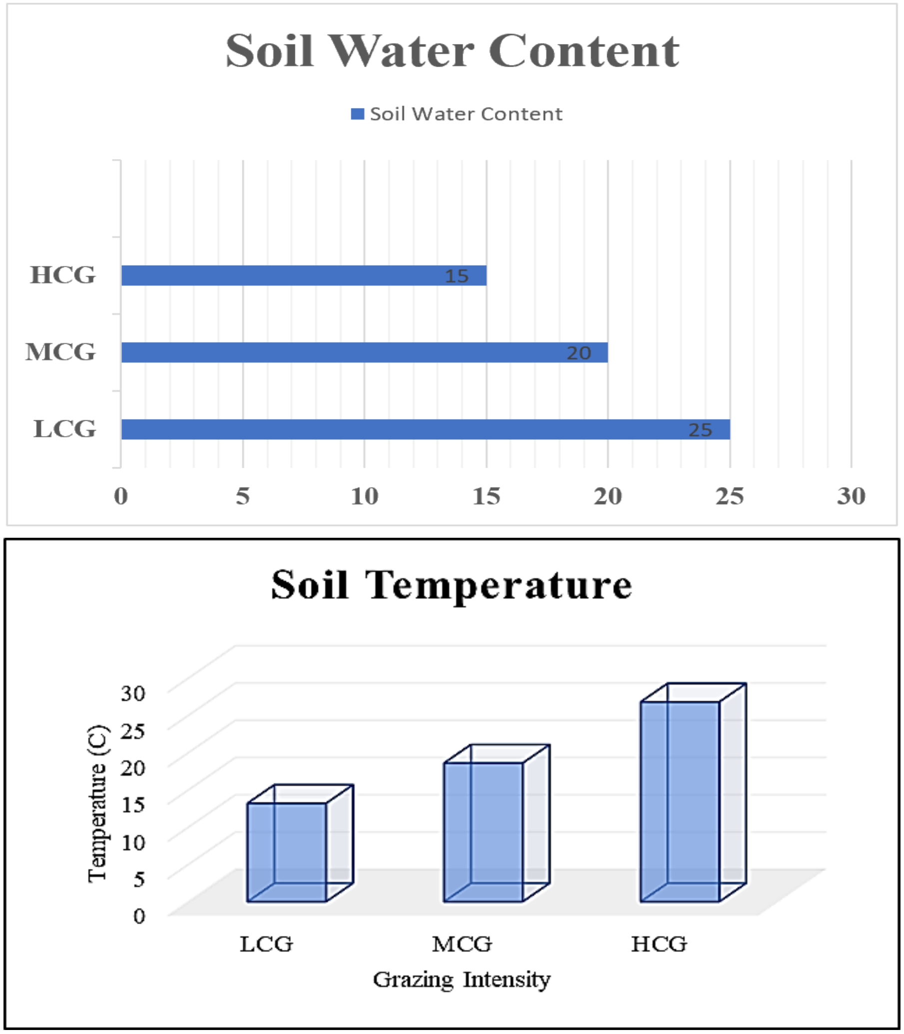


Figure-6. Soil water content and soil temperature

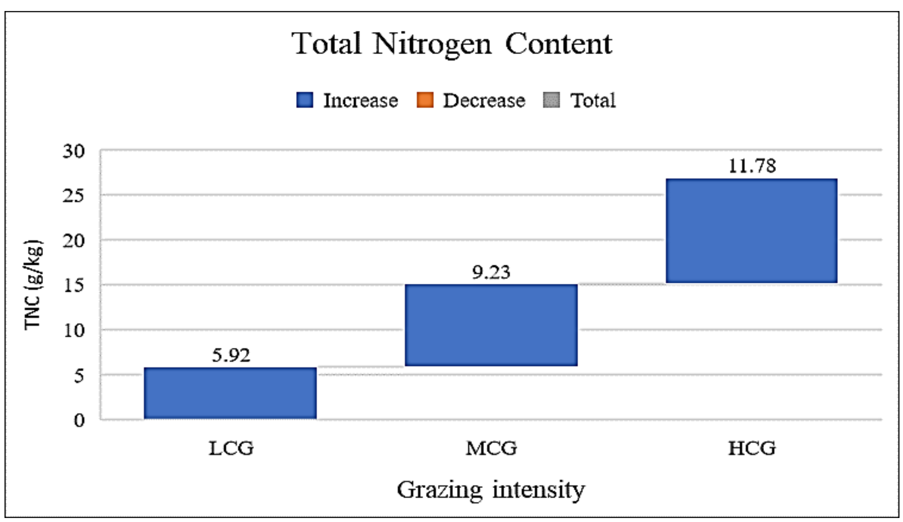


Figure-7. Total nitrogen content of the soil

Soil Total Phosphorus Content (STPC)

Grazing lowers plant biomass, which may result in a reduction in the quantity of phosphorus that plants absorb. Elevated soil phosphorus concentrations may result from less plant uptake. All things considered, the impact of grazing can differ based on variables like grazing intensity and duration, vegetation type, soil properties, and management techniques. The sampling data indicated that the mean concentration of soil P was 0.38±0.01, 0.721±0.07, and 0.921±0.12 g kg-1 for the LG site, MG site, and HG site, respectively, which was significantly greater than others.

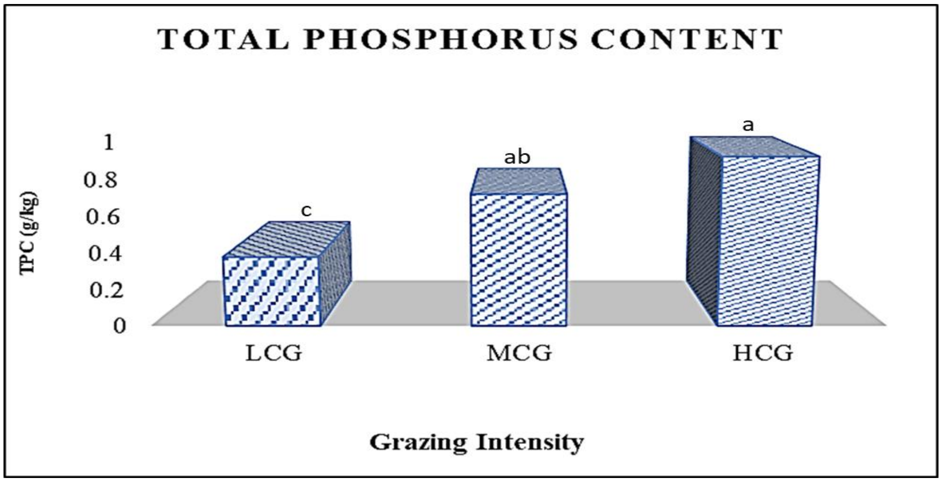


Figure-8. Total phosphorus content of the soil.

Soil Total Potassium Content (STKC)

Soil potassium content varies by varying the intensities as LG, MG, HG has depicted variations by changing the grazing intensities. LG demonstrated as the lowest K level for grazing intensity as compared to the MG which has medium grazing with moderate value. Contrariwise, HG had maximum grazing intensity for increasing the level of K. LG, MG, and HG had the mean concentration for soil K was (12.32±0.3, 31.19±0.38 and 49.13±1.23 g/kg), respectively. Grazing reduces plant biomass, affecting potassium cycling in the environment. When plants are grazed, they absorb less potassium and may leave more in the soil. All things considered, the relationship between soil potassium levels and grazing intensity is complex and varies based on site-specific factors such as vegetation, soil type, climate, and grazing management techniques. To properly comprehend and control the effects of grazing on soil potassium levels, regular soil testing and monitoring are necessary. According to this study, soil potassium content has not explored in the alpine regions yet.

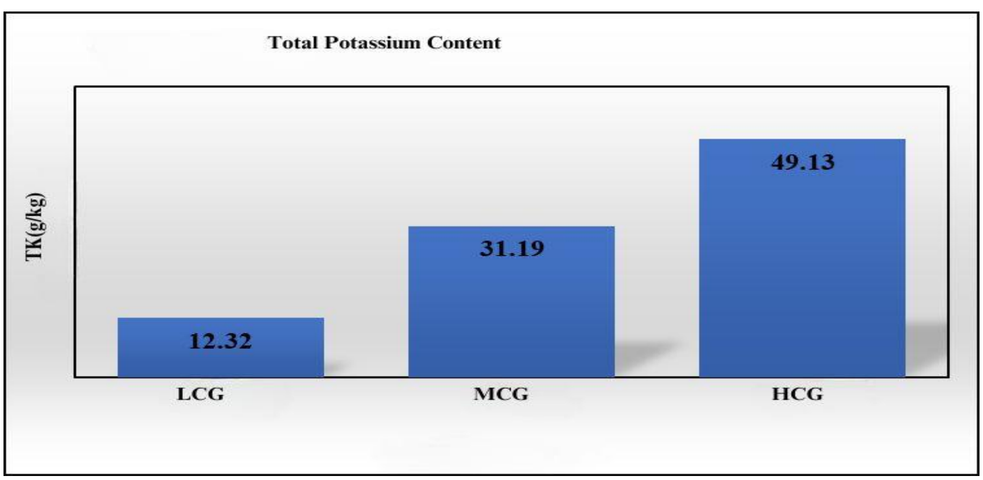
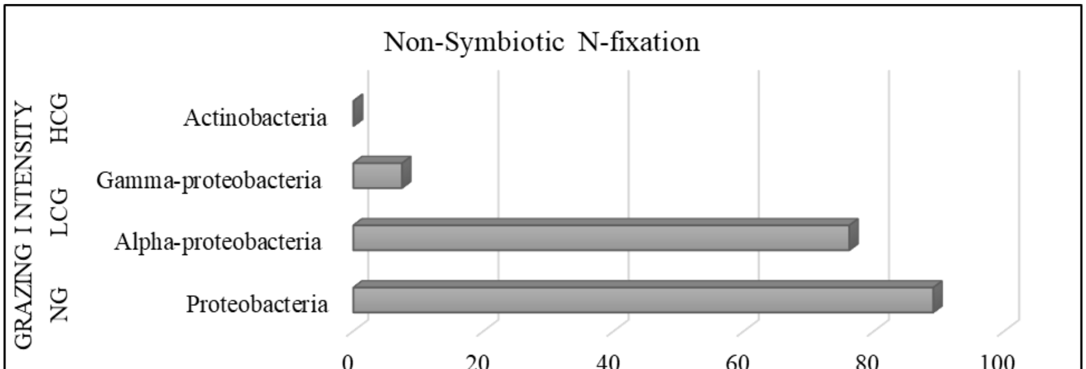


Figure-9. Total potassium content of the soil.

https://doi.org/10.35495/ajab.2024.234

Denitrification and Nitrification

There were notable differences between the two processes at different grazing intensity ($p < 0.001$). Both the denitrification and nitrification gross rates grew quickly. The average gross rates of nitrification and denitrification for the LG, MG, and HG sites were, respectively, 9.76, 10.02, and 11.23 mg N $kg^{-1}d^{-1}$ and 0.23, 0.42, and 0.65 mg N $kg^{-1}d^{-1}$ during the course of the sample dates. On every sampling date, the HG site showed noticeably higher gross nitrification and denitrification than the LG site ($p < 0.001$). Proteobacteria was the predominant phylum after screening, with an abundance of 89.1% under both light and no grazing conditions. With abundances of 76.2% and 7.48%, respectively, at medium grazing intensity, Alpha-proteobacteria and Gamma-proteobacteria dominated the category, whereas Actinobacteria's prevalence was merely 0.08% with heavy grazing intensity. Of all the grassland types, NG and HCG had the highest ANF bacterial species abundance. Configuration of asymbiotic nitrogen-fixing microbial communities demonstrated the lowest percentages for microbial growth during grazing intensities compared to previous studies in China, 2021.



	NG	LCG		HCG
	Proteobacteria	Alpha-proteobacteria	Gamma-proteobacteria	Actinobacteria
Series1	89.1	76.2	7.48	0.08

Figure-10. Configuration of asymbiotic nitrogen-fixing microbial societies under different grazing intensities

Discussion

Incessant grazing as Continuous (CG) & Rotating grazing (RG)

Under a continuous grazing system, animals have unfettered access to the pasture throughout the grazing season. Removing livestock from the pasture at any time during the season is known as pseudo-continuous grazing. Plant species are more tolerant to continuous grazing should make up an efficient continuous grazing system. Over time, the overall nutritional value of the pasture falls because fodder loses some of its nutrients as the plant ages (Bott et al., 2013). When pastures are continuously grazed, certain regions may experience more intense grazing, which will lower plant health and vigor and necessitate longer recovery times to preserve forage cover. Furthermore, compared to rotational grazing systems, continuous grazing systems exhibit a less uniform distribution of animal feces. Rotational grazing (RG), when compared to continuous grazing (CG), promotes vegetation production and thus grassland resilience to intensive grazing as mentioned in figures. Both grazing strategies have their uses in livestock management, but rotational grazing is often better for soil health, pasture quality, and overall ecosystem sustainability. Continuous grazing may be appropriate for some low-intensity grazing systems, but it frequently necessitates careful management to prevent long-term damage (Hao et al., 2013). According to our research studies, rotational grazing enhances the nitrogen dynamics in soil.

https://doi.org/10.35495/ajab.2024.234

Vegetation-soil microbe interactions play an important role in grassland ecosystem health across various grazing systems. Rotational grazing enhances root growth and exudate release into the soil, which provide important sources of energy for microbial populations in the soil. In turn, these microbial interactions stimulate microbial activity, such as nitrogen-fixing bacteria, thereby improving nutrient supply and uptake for the vegetation (Kleppel and Frank, 2022). In addition, the enhanced microbial diversity and activity under RG contribute to the decomposition of organic matter, enriching soil organic carbon and enhancing its structure and fertility. Continuous grazing (CG) often breaks this balance through overgrazing, which reduces root biomass and diminishes microbial populations that are crucial for nutrient cycling. Such disturbances result in soil degradation, plant susceptibility, and lower forage productivity with time. These findings emphasize the relevance of rotational grazing in creating powerful vegetation-microbe interaction that supports sustainable grassland management (Chen et al., 2024).

Soil Physical & Physiochemical Properties

In the current study, soil OM levels in the HG site were consistently greater than those in the LG and MG sites for the course of the investigation. LG, MG, and HG had shown (59.71±12.1, 79.9±4, and 98.23±31.4, respectively) as demonstrated in (figure 4). It refers to all organic components in soil, including a wide spectrum of materials originating from once-living organisms. For the long-term management of the soil and agricultural productivity, maintaining or raising the soil's organic matter content is essential. Reduced tillage, crop rotation, organic additives, and cover crops can all raise SOM levels. Because it affects soil properties, soil organic content has a major effect on both soil fertility and the ecological environment. Structure (Bronick and Lal, 2005), water retention (Rawls et al., 2003), microbial activity (Degens et al., 2000), and nutrient availability. Oelofse et al. (2015) claim that SOC is a complex mixture of organic compounds that vary in terms of turnover rate, recalcitrance, and degree of breakdown.

There were substantial differences in soil pH at different grazing intensities. Light grazing was depicted as the highest soil pH while high grazing demonstrated as lowest pH for soil, as LG, MG, and HG (7.1, 6.3, and 5.9, respectively) as exhibited in (figure 5). For instance, over time, higher rates of nitrification and nitrogen mineralization may cause soil acidification. The particulars of the grazed environment and the balancing elements will determine the overall impact of grazing on soil pH. Monitoring soil pH over time in grazed areas is important for understanding these dynamics and managing soil health (Cheng-Jim et al., 2014).

There is a strong correlation between these two factors and the intensity of grazing. Temperature and moisture content of the soil rose quickly. The HG location had much greater soil temperature than the LG and MG sites, which did not significantly differ from one another. Soil temperature increases with time and increasing grazing intensity (Chang et al., 2012). In this study temperature or soil moisture did not significantly correlate with the amount of grazing.

Soil N varied substantially between different grazing intensities, with a significant difference. This study suggests that grazers may improve soil nitrogen levels. This may be a transient phenomenon, with high levels expected to return. Grazing causes non-preferred plant species to dominate, leading to decreased production and soil nitrogen sequestration. Forage grasses under light to moderate grazing intensities dominate plant communities with significant aboveground biomass productivity. Determining the appropriate grazing intensities for the alpine system is necessary. In certain regions, integrating various grazing strategies could sustainably support rangeland management (Gao et al., 2007).

Grazing lowers plant biomass, which may result in a reduction in the quantity of phosphorus that plants absorb. Elevated soil phosphorus concentrations may result from less plant uptake. Researchers found no appreciable difference in soil total particle levels between grazed and ungrazed areas, suggesting that a meadow steppe is apparently stable. As per the findings of Niu et al. (2016), one possible explanation for the stability of soil phosphorus could be its primary source, weathering. Phosphorus is derived from rocks rather than organic matter degradation, therefore its solubility in soil is low. Vegetation-derived soil organic matter, including litter, root exudates, and dead roots, has a lower impact on soil compared to soil nitrogen. Reduced fungal activity during response grazing may contribute to the apparent stability of phosphorus levels (Zhang et al., 2018).

The various grazing intensities had no statistically significant interaction with the gross rates of nitrification and denitrification. This study shows that compared to light grazing, nitrification, and denitrification rates are higher during intensive grazing as mentioned in (figure 6, 7, 8, 9 and 10). These results showed that increased grazing intensities accelerate the rates at which soil N is transformed. This can be explained in two ways (Olofsson et al., 2001). Grazer excrements provide readily available nitrogen to plants and soil microorganisms. Grazers can alter soil microclimate, including temperature and water content, which influence biological activities in terrestrial ecosystems (Kooch et al., 2020). Soil temperature was found to have a positive correlation with both nitrification and denitrification. According to LeRoux et al. (2003), nitrification in soils rises with water content and falls when anaerobic zones form.

Composition of asymbiotic nitrogen-fixing microbial communities under different grazing intensities

Grazing intensity influences soil nitrogen fixing dynamics in alpine grasslands. Moderate grazing intensity helps to sustain or even improve nitrogen fixation by increasing plant diversity, particularly the presence of nitrogen-fixing plants like legumes. Moderate grazing can also aid to maintain the microbial populations that are responsible for biological nitrogen fixation (BNF). On the other side, increased grazing intensity can result in overgrazing, which reduces plant cover, changes species composition, and depletes the soil's nitrogen-fixing capability. This degradation has an impact not just on the grassland's immediate productivity, but also on its long-term ecological health and resilience (Kakraliya et al., 2018).

Conclusion and Recommendation

This reduces the amount of soil nitrogen since intensive grazing is normally associated with overgrazing. Diminished plant biomass and reduced root system from overgrazing limit the input of organic matter, thus soil degradation and lowering the efficiency of such key processes of nitrogen cycling. High grazing pressure results in soil compaction with minimal aeration and thereby inhibited microbial activity. On the other hand, moderate grazing may positively impact soil nitrogen dynamics by enhancing plant growth and root turnover, which increases organic matter inputs. At this level of grazing, microbial activity is enhanced, leading to greater nitrogen mineralization and higher nitrogen availability to plants.

Minimal or no grazing can negatively impact the nitrogen cycle by reducing plant diversity and altering soil structure, leading to suboptimal nutrient cycling. Soil nitrogen content and its components under different grazing intensities follow quite different paths. During the years of major rainfall, moderate grazing positively correlated with increased soil nitrogen while overgrazing caused negative changes. Although the main effects of interannual variability, soil depth, and their interactions were large, the effects of grazing on nitrogen fractions were generally not very important. However, grazing played a notable role in altering soil nitrogen storage and composition, though hydrothermal conditions emerged as the dominant factor shaping these dynamics. Grazing has both positive and negative impacts on grassland ecosystems. It can accelerate soil salinization, increase soil evaporation, and hasten the decomposition of organic matter, leading to a decline in surface biomass. In addition, urination by grazing animals can contribute to soil acidification, which may disrupt nitrogen cycling processes. These findings explain why soil nitrogen performs differently under different grazing intensities. The current research also underlines the pivotal importance of grazing management for governing soil nitrogen cycling. Improved content in soil nitrogen results not only from sustainable grazing practice, especially moderate intensity rotational grazing, but also more robust plant and microbial diversity. In this context, healthy long-term functioning of alpine grasslands, preserving ecosystem resilience, could be ensured. Hence, higher intensities of grazing caused an increase in soil N, and rates of nitrogen transformations accelerated with heavy grazing in the alpine meadows of the Tibetan Plateau. The results highlighted the ability of short-term heavy grazing to accelerate soil nutrient cycling, potentially leading to increased forage quality and productivity and significant commercial gains for livestock industries that rely on alpine grasslands. This research introduces a novel perspective by linking grazing intensity to soil nutrient management, hence its potential in optimizing land use for sustainable agricultural practices. On an ecological and economic timescale, adaptive grazing regimes periods of heavy grazing combined with light or moderate intensities ensure sustainable alpine meadow ecosystem management but also increase productivity and profitability, making them more resilient to climate and land-use changes.

- Implement rotational grazing strategies in alpine grasslands to improve soil nitrogen dynamics and soil health.
- Overgrazing should be avoided to minimize soil compaction, loss of plant diversity, and nitrogen degradation.
- Monitor and modify grazing intensity to ensure long-term management of soil resources and ecosystem health.
- Encourage research and monitoring of how grazing affects soil nitrogen fixation dynamics to establish optimum methods for sustainable grassland management.

Acknowledgments

This study was funded by National Key R&D Program of China (2021YFE0112400; 2023YFF1304303), and National Science Foundation of China (32361143870; 32101315).

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: This study was funded by National Key R&D Program of China (2021YFE0112400; 2023YFF1304303), and National Science Foundation of China (32361143870; 32101315).

Ethical Approval Statement

All applicable national, international, as well as institutional guidelines mentioning the care and use of animals were strictly followed.

Contribution of Authors

Nawaz A: Conceived the idea, collected data, performed experimental analysis and wrote the original draft.

Afzal MK: Analyzed and verified all data, revised and edited article and approved the final draft.

References

Abdalla M, Hastings A, Chadwick DR, Jones DL, Evans CD, Jones MB and Smith PETE, 2018. A critical review of the impacts of grazing intensity on soil organic carbon storage and other soil quality indicators in extensively managed grasslands. *Agriculture, Ecosystems & Environment*, *253*, 62-81.

Barron AR, Wurzburger N, Bellenger JP, Wright SJ, Kraepiel AM and Hedin LO, 2009. Molybdenum limitation of a symbiotic nitrogen fixation in tropical forest soils. Nature Geoscience, 2(1), 42-45.

Bott RC, Greene EA, Koch K, Martinson KL, Siciliano PD, Williams C and Swinker A, 2013. Production and environmental implications of equine grazing. Journal of Equine Veterinary Science, 33(12), 1031-1043.

Bronick CJ and Lal R, 2005. Soil structure and management: a review. Geoderma 124: 3-22. DOI: 10.1016/j.geoderma.2004.03.005.

Canfield DE, Glazer AN and Falkowski PG, 2010. The evolution and future of Earth's nitrogen cycle. science, 330(6001), 192-196.

Chang X, Zhu X, Wang S, Luo C, Zhang Z, Duan J, Bai L and Wang W, 2012. Temperature and moisture effects on soil respiration in alpine grasslands. Soil science, 177(9), 554-560.

Cheng-Jim JI, Yuan-He YANG, Wen-Xuan HAN, Yan-Fang HE, Smith J and Smith P, 2014. Climatic and edaphic controls on soil pH in alpine grasslands on the Tibetan Plateau, China: a quantitative analysis. Pedosphere, 24(1), 39-44.

Chen X, Wang X, Song Y and Chi Y, 2024. A Review of Studies on the Effects of Anthropogenic Disturbances on Plant–Soil–Microorganism Interactions in Grassland Ecosystems: Based on Grazing and Tourism Perspectives. *Agronomy*, *14*(12), 2890.

Degens BP, Schipper LA, Sparling GP and Vojvodic-Vukovic M, 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry, 32(2), pp.189-196.

Dong S, Shang Z, Gao J and Boone RB, 2020. Enhancing the sustainability of grassland ecosystems through ecological restoration and grazing management in an era of climate change on Qinghai-Tibetan Plateau. Agriculture, Ecosystems & Environment, 287, 106684.

Eldridge DJ, Poore AG, Ruiz-Colmenero M, Letnic M and Soliveres S, 2016. Ecosystem structure, function, and composition in rangelands are negatively affected by livestock grazing. Ecological Applications, 26(4), 1273-1283.

Gao YH, Luo P, Wu N, Yi SL and Chen H, 2007. Biomass and nitrogen responses under different grazing intensity in an alpine meadow on the eastern Tibetan Plateau. Pol. J. Ecol. 55:469-479.

Gupta VVSR, Kroker SJ, Hicks M, Davoren CW, Descheemaeker K and Llewellyn R, 2014. Nitrogen cycling in summer active perennial grass systems in South Australia: non-symbiotic nitrogen fixation. Crop and Pasture Science, 65(10), 1044-1056.

Hao Y and He Z, 2019. Effects of grazing patterns on grassland biomass and soil environments in China: A meta-analysis. *PloS one*, *14*(4), e0215223.

Hao J, Dickhoefer U, Lin L, Müller K, Glindemann T, Schönbach P and Susenbeth A, 2013. Effects of rotational and continuous grazing on herbage quality, feed intake and performance of sheep on a semi-arid grassland steppe. Archives of Animal Nutrition, 67(1), 62-76.

Jain AK, Meiyappan P, Song Y and House JI, 2013. CO2 emissions from land-use change affected more by nitrogen cycle, than by the choice of land-cover data. Global change biology, 19(9), 2893-2906.

Jiang Y, Song M, Zhang S, Cai Z and Lei Y, 2018. Unravelling community assemblages through multi-element stoichiometry in plant leaves and roots across primary successional stages in a glacier retreat area. Plant and soil, 428, 291-305.

Kakraliya SK, Singh U, Bohra A, Choudhary KK, Kumar S, Meena RS and Jat ML, 2018. Nitrogen and legumes: a meta-analysis. Legumes for soil health and sustainable management, 277-314.

Knudsen T and Johansen T, 1989. The mode of inhibition of the Na+-K+ pump activity in mast cells by calcium. British journal of pharmacology, 98(4), 1119-1126.

Kooch Y, Moghimian N, Wirth S and Noghre N, 2020. Effects of grazing management on leaf litter decomposition and soil microbial activities in northern Iranian rangeland. *Geoderma, 361*, 114100.

Kleppel GS and Frank DA, 2022. Structure and functioning of wild and agricultural grazing ecosystems: A comparative review. *Frontiers in Sustainable Food Systems*, *6*, 945514.

LeRoux X, Bardy M, Loiseau P and Louault F, 2003. Stimulation of soil nitrification and denitrification by grazing in grasslands: Do changes in plant species composition matter? Oeocologia 137:417-425.

Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y and Feng Z, 2020. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. New England journal of medicine, 382(13), 1199-1207.

Lu X, Kelsey KC, Yan Y, Sun J, Wang X, Cheng G and Neff JC, 2017. Effects of grazing on ecosystem structure and function of alpine grasslands in Qinghai–Tibetan Plateau: A synthesis. Ecosphere, 8(1), e01656.

Niu K, He JS and Lechowicz MJ, 2016. Grazing-induced shifts in community functional composition and soil nutrient availability in Tibetan alpine meadows. J. Appl. Ecol. 53, 1554–1564.

Oelofse M, Markussen B, Knudsen L, Sørensen P and Schjønning P, 2015. Do soil organic carbon levels affect potential yields and nitrogen use efficiency? An analysis of winter wheat and spring barley field trials. European Journal of Agronomy, 66, 62-73.

Olofsson J, Kitti H, Rautiainen P, Stark P and Oksanen L, 2001. Effects of summer grazing by reindeer on composition of vegetation, productivity and nitrogen cycling. Ecography 24:13-24.

Qin R, Wei J, Ma L, Zhang Z, She Y, Su H and Zhou H, 2022. Effects of Pedicularis kansuensis expansion on plant community characteristics and soil nutrients in an alpine grassland. Plants, 11(13), 1673.

Rawls WJ, Pachepsky YA, Ritchie JC, Sobecki TM and Bloodworth H, 2003. Effect of soil organic carbon on soil water retention. Geoderma, 116(1-2), pp.61-76.

Reed SC, Cleveland CC and Townsend AR, 2011. Functional ecology of free-living nitrogen fixation: a contemporary perspective. Annual review of ecology, evolution, and systematics, 42(1), 489-512.

Schleuss PM, Widdig M, Biederman LA, Borer ET, Crawley MJ, Kirkman KP, Seabloom EW, Wragg PD and Spohn M, 2021. Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils. *Soil Biology and Biochemistry*, *155*, 108168.

Yang ZA, Xiong W, Xu Y, Jiang L, Zhu E, Zhan W and Chen H, 2016. Soil properties and species composition under different grazing intensity in an alpine meadow on the eastern Tibetan Plateau, China. Environmental Monitoring and Assessment, 188, 1-12.

Zhang G, Nan Z, Hu N, Yin Z, Zhao L, Cheng G and Mu C, 2022. Qinghai-Tibet Plateau permafrost at risk in the late 21st century. Nature Communications, 13(1), 1785.

Zhang M, Li X, Wang H and Huang Q, 2018. Comprehensive analysis of grazing intensity impacts soil organic carbon: A case study in typical steppe of Inner Mongolia, China. Appl. Soil Ecol.

Zheng M, Zhang W, Luo Y, Li D, Wang S, Huang J and Mo J, 2018. Stoichiometry controls asymbiotic nitrogen fixation and its response to nitrogen inputs in a nitrogen-saturated forest. Ecology, 99(9), 2037-2046.

Zhong L, Du R, Ding K, Kang X, Li FY, Bowatte S and Wang S, 2014. Effects of grazing on N_2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China. Soil Biology and Biochemistry, 69, 1-10.

Zhou W, Li C, Wang S, Ren Z and Stringer LC, 2023. Effects of vegetation restoration on soil properties and vegetation attributes in the arid and semi-arid regions of China. Journal of Environmental Management, 343, 118186.