Response of Soil Microbial Community to Grazing and Its Relationship with Environmental Factors: Postprint

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Abstract

To investigate the differential responses of soil microbial community characteristics to grazing intensity, grasslands with different grazing intensities in the middle section of the northern slope of the Tianshan Mountains were selected as the study area. Through field investigation and laboratory analysis, the variation patterns of soil microbial community characteristics along grazing intensity gradients and their intrinsic relationships with soil factors were explored. The results showed that Actinobacteria and Ascomycota were the dominant bacterial and fungal phyla, respectively. Compared with heavy grazing, light grazing significantly enhanced the α-diversity of microbial communities ($P<0.05$) and promoted the accumulation of soil microbial biomass carbon, nitrogen, and phosphorus. Redundancy analysis and Mantel tests indicated that soil microbial community characteristics were positively correlated with soil total nitrogen and negatively correlated with soil bulk density ($P<0.05$). Structural equation modeling revealed that grazing negatively affected microbial diversity, richness, biomass, and OTU characteristics by increasing bulk density and decreasing soil nutrient levels ($P<0.05$), with microbial community diversity showing higher sensitivity to grazing. In summary, light grazing benefits microbial communities, and rational regulation of grazing intensity is a viable strategy for ensuring their stable development.

Full Text

Abstract

To explore the differential responses of soil microbial community characteristics to varying grazing intensities, we selected grasslands with different grazing

intensities in the middle section of the northern slopes of the Tianshan Mountains as our study area. Through field investigation and laboratory analysis, we examined how soil microbial community characteristics change with grazing intensity and their intrinsic relationships with soil factors. The results showed that *Actinobacteria* and *Ascomycota* were the dominant bacterial and fungal phyla, respectively. Compared with heavy grazing, light grazing significantly enhanced the alpha diversity of microbial communities ($P<0.05$) and promoted the accumulation of soil microbial biomass carbon, nitrogen, and phosphorus. Redundancy analysis and Mantel tests indicated that soil microbial community characteristics were positively correlated with soil total nitrogen and negatively correlated with soil bulk density ($P<0.05$). Structural equation modeling revealed that grazing negatively affected microbial diversity, richness, biomass, and OTU characteristics by increasing bulk density and reducing soil nutrients ($P<0.05$). Moreover, microbial community diversity exhibited higher sensitivity to grazing responses. In summary, light grazing is beneficial for improving microbial communities, and reasonable regulation of grazing intensity represents a feasible strategy for ensuring the stable development of microbial communities.

Keywords: grazing intensity; soil fungi; soil bacteria; environmental factors; northern slopes of Tianshan Mountains

Introduction

Grassland ecosystems are highly susceptible to disturbance, and grassland quality directly affects national ecological security, agricultural and pastoral productivity, and regional stability. Currently, climate change and anthropogenic interference are severely impacting grassland ecosystems, leading to reduced productivity, deteriorated soil structure, stalled nutrient cycling, and diminished system resistance and resilience. These changes collectively cause increasingly severe grassland degradation that threatens ecological balance and biodiversity. When the scientific and rational use of grasslands is neglected, the stability of grassland ecosystems will be continuously undermined, interfering with the development of grassland animal husbandry and seriously threatening the sustainable development of human society.

Soil microorganisms play crucial roles in ecosystems, including maintaining soil structure, degrading pollutants, regulating ecological balance, and preserving biodiversity. The effects of grazing intensity changes on soil microbial communities can be divided into short-term and long-term effects. Short-term effects primarily occur through grazing stimulation of vegetation root exudates and the return of animal manure and urine, which influence the quantity and quality of nutrients available to soil microorganisms. Long-term effects arise from livestock feeding on plants, trampling, and excessive excretion, which alter plant communities and soil environments, ultimately leading to changes in microbial communities. Studies have shown that moderate grazing helps maintain soil microbial community stability, while heavy grazing may affect microbial survival and development. However, research on the Tibetan Plateau found no differ-

ences in soil microbial community diversity under different grazing methods, and studies in temperate grasslands of Inner Mongolia similarly found that only soil fungal community diversity showed significant differences between grazing and enclosure conditions, while bacterial community diversity showed no significant differences. Conversely, research in *Pinus sylvestris* var. *mongolica* forests in sandy lands indicated that grazing significantly affected soil bacteria but not fungi. These findings demonstrate that grazing effects on soil microbial communities are complex and uncertain, with fungal and bacterial communities responding inconsistently to grazing, and no universally accepted conclusions have yet been formed. Moreover, research results on how environmental factors influence soil microbial communities under grazing disturbance remain incomplete. Therefore, investigating grazing effects on microbial communities from multiple perspectives is essential for the rational utilization and protection of grassland resources.

Currently, studies on soil microbial communities in grazing contexts are increasing annually, focusing primarily on changes in microbial diversity and community composition under grazing conditions, as well as the influence of soil environmental factors on microbial communities. However, few studies have examined whether the responses of alpha diversity, beta diversity, richness, OTU characteristics, and microbial biomass to grazing show differences, and whether the effects of soil factors on microbial community characteristics are consistent remains unclear. Furthermore, the grasslands on the middle northern slopes of the Tianshan Mountains represent an important ecosystem in Xinjiang, particularly the mountain meadows, whose unique geographical and ecological environments provide rich habitats and food sources for wildlife in mid-altitude areas and serve as crucial summer pastures for the region. However, long-term overgrazing has led to grassland degradation, manifested by low vegetation coverage and poor usability, seriously affecting the sustainable development of grassland animal husbandry. Previous studies have shown that heavy grazing alters plant community structure and reduces its diversity and biomass, but relevant research on soil microbial communities is limited, and the effects of grazing on different microbial community characteristics remain unclear. Therefore, based on free-grazing mountain meadows on the northern slopes of the Tianshan Mountains, this study explored the relationship between soil factors and microbial communities under grazing disturbance, aiming to address the following scientific question: Do soil factors exhibit consistent effects on microbial community diversity, richness, microbial biomass, and OTUs under grazing disturbance? The findings will provide a scientific basis for the rational utilization and sustainable development of grassland resources on the northern slopes of the Tianshan Mountains.

1.1 Study Area Overview

The study area was selected in natural grazing grasslands in Ashili Township, Changji City, Xinjiang (43°10 ~44°10 N, 86°30 ~87°30 E). The altitude ranges

from 1900 to 2400 m, with a temperate continental arid climate and an annual precipitation of 400–500 mm. The grassland type is mountain meadow, and the soil type is mountain chernozem. Dominant species include *Stipa capillata*, *Carex stenocarpa*, and *Achnatherum inebrians*, with companion species such as *Phlomis pratensis*, *Alchemilla tianshanica*, *Heteropappus altaicus*, and *Potentilla bifurca*.

1.2 Experimental Design and Sample Collection

1.2.1 Experimental Design Three pastoral grasslands with similar topography, slope, grazing history, and grazing patterns were selected as grazing sample plots. Based on existing research and actual conditions in the experimental area, sample plots at 500 m from residential areas were designated as heavy grazing (HG) plots, with *Achnatherum inebrians* as the dominant plant, vegetation coverage below 30%, and utilization rate exceeding 85%. Sample plots at 2500 m from residential areas were designated as light grazing (LG) plots, with vegetation coverage above 85%, utilization rate below 35%, and dominant species of *Carex stenocarpa* and *Stipa capillata*. The differences between light and heavy grazing plots primarily resulted from herdsmen concentrating livestock near residential areas. Grazing plots were subjected to free grazing from June to September annually, with enclosure management during the remaining months. No-grazing (NG) plots were established in enclosed grasslands 2000 m from residential areas, used collectively for hay harvesting from early to late August. Differences in soil physicochemical properties among different plots are shown in . Variations in plant dominant species among plots were mainly caused by differences in grazing intensity rather than grassland type.

1.2.2 Plant and Soil Sample Collection Parallel transects were established in the aforementioned grazing plots ([Figure 1: see original paper]). Each plot contained three transects, with five 1 m × 1 m quadrats randomly arranged in an "S" pattern along each transect. Sample collection was conducted during the plant growth peak in July 2022. Soil samples were collected from each quadrat using a soil auger (diameter 5 cm) at depths of 0–10 cm. Soil samples from the same transect were mixed and passed through a 2 mm sieve. The sieved samples were divided into three portions: one for laboratory measurement of soil physicochemical properties, another sealed and labeled for determination of soil microbial characteristics, and the third portion placed in a cooler and sent to the laboratory for storage at -80°C.

1.3 Measurement Indicators and Methods

1.3.1 Soil Physicochemical Indicators Soil physicochemical indicators were measured according to Bao Shidan' s *Soil Agrochemical Analysis*. Measurements included organic carbon, total nitrogen, total phosphorus, total potassium, available nitrogen, available phosphorus, available potassium, bulk density, and water content.

1.3.2 Soil Microbial Biomass Measurement Soil microbial biomass was measured using the chloroform fumigation extraction method. Microbial biomass carbon was extracted with potassium chlorate and measured using the volumetric method, microbial biomass nitrogen was measured using the ninhydrin colorimetric method, and microbial biomass phosphorus was measured using the molybdenum-antimony anti-colorimetric method.

1.3.3 Soil Microbial Community Analysis Soil microbial total DNA was extracted using a kit, and the bacterial 16S rRNA V3–V4 region (primers 338F/806R) and fungal ITS1 region (primers ITS1F/ITS2R) were amplified. Reaction conditions were as follows: initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. PCR products were sequenced using Illumina MiSeq. Raw sequences were quality-filtered using Trimmomatic software, and effective sequences were screened. Valid sequences were aligned against the Silva 128 and Unite 6.0 databases for classification. Dominant phyla and genera (relative abundance > 1%) were defined, and unclassified taxa were grouped as "Others." Microbial coverage and diversity indices were calculated using Mothur software.

1.4 Data Processing and Statistical Analysis

SPSS 26.0 software was used for one-way ANOVA and Duncan' s test to explore differences in soil microbial community diversity under different grazing treatments. Beta diversity of soil microbial communities was characterized based on Bray–Curtis distance and analyzed through principal coordinates analysis (PCoA). The envfit test was used to analyze the response of different microbial community characteristics to soil factors. After excluding factor autocorrelation, redundancy analysis (RDA) and Mantel tests were used to analyze the correlation between soil factors and microbial communities. The "lavaan" package in R 4.1.2 was used to construct structural equation models (SEM) integrating soil microbial diversity, richness, biomass, and OTUs as latent variables. Referring to the calculation method for ecosystem multifunctionality, soil nutrient indicators were standardized to represent "soil nutrients." Model fit was evaluated using chi-square tests, comparative fit index (CFI), and root mean square error of approximation (RMSEA). If P(^{2}) > 0.05 and RMSEA < 0.05, the model was considered well-fitted. Based on previous theories, a priori models were constructed assuming that grazing intensity affects soil water content, bulk density, and nutrients, which in turn affect soil microbial communities.

2 Results

2.1 Effects of Grazing on Soil Microbial Community Diversity

2.1.1 Alpha Diversity Analysis of Soil Microbial Communities Under Grazing Disturbance The coverage of soil microbial communities in the

grasslands of the middle northern slopes of the Tianshan Mountains exceeded 99%. ANOVA results showed that bacterial and fungal coverage both followed the pattern of LG > NG > HG, with no significant differences between treatments. Shannon and Chao1 indices showed the same trend. Compared with HG, bacterial Shannon and Chao1 indices increased by 11.5% and 16.1% in LG, respectively, while fungal Shannon and Chao1 indices increased by 18.8% and 11.6%, respectively ($P<0.05$). No significant differences were found between LG and NG treatments.

2.1.2 Beta Diversity Analysis of Soil Microbial Communities Under Grazing Disturbance PCoA analysis revealed differentiation in bacterial and fungal communities under different grazing treatments ([Figure 4: see original paper]). ANOSIM and Adonis tests showed no significant differences in microbial community beta diversity among grazing treatments.

2.2 Effects of Grazing on Soil Microbial Community Composition

2.2.1 Analysis of Soil Bacterial Community Composition Under Grazing Disturbance A total of 37 bacterial phyla were identified in soil samples. *Actinobacteria* was the most abundant phylum, accounting for 43.07%, 41.55%, and 39.89% in NG, LG, and HG treatments, respectively. The relative abundance of *Proteobacteria* was higher in HG than in NG and LG, while *Acidobacteria* showed the opposite pattern. *Bacteroidetes* was significantly higher in HG than in NG and LG ($P<0.05$). Random forest analysis revealed that *Acidobacteria*, *Methylomirabilota*, *Gemmatimonadetes*, *Planctomycetota*, and *Cyanobacteria* were significantly correlated with grazing intensity changes ($P<0.05$). At the bacterial genus level ([Figure 5: see original paper]), 252 genera had relative abundance > 0.1%. Unclassified genera accounted for approximately 52.07%. *Sphingomonas*, *Microlunatus*, *Pseudonocardia*, and *Rubrobacter* showed more pronounced responses to grazing. *Pseudonocardia* was significantly higher in HG than in NG and LG, while *Nocardia* showed the opposite pattern ($P<0.05$).

2.2.2 Analysis of Soil Fungal Community Composition Under Grazing Disturbance At the fungal phylum level, *Ascomycota* had the highest relative abundance across all grazing treatments, at 77.95%, 81.40%, and 73.77% in NG, LG, and HG, respectively. This was followed by *Basidiomycota* at 9.29% and 18.56%. The relative abundance of *Glomeromycota*, *Rozellomycota*, *Chytridiomycota*, and *Olpidiomycota* was below 1%. Variance analysis showed that *Basidiomycota* was significantly higher in HG than in NG and LG ($P<0.05$), while *Preussia* showed the opposite pattern ($P<0.05$). At the genus level, genera with relative abundance > 1% included *Penicillium* and *Knufia*. Unclassified genera accounted for approximately 30.92%. Random forest results indicated that *Ascomycota*, *Basidiomycota*, *Penicillium*, and *Knufia* were sensitive to grazing intensity changes ($P<0.05$).

2.3 Effects of Grazing on Soil Microbial Biomass

Soil microbial carbon, nitrogen, and phosphorus under different grazing treatments all showed the pattern of LG > NG > HG, with the highest values in LG treatment ([Figure 7: see original paper]). Microbial carbon, nitrogen, and phosphorus were significantly higher in LG than in HG ($P<0.05$). Further analysis revealed that microbial carbon, nitrogen, and phosphorus increased by 65.03%, 139.42%, and 30.92%, respectively, in LG compared with HG.

2.4 Effects of Soil Physicochemical Factors on Soil Microbial Communities Under Grazing Disturbance

RDA was performed on soil physicochemical properties and soil microorganisms, using microbial community diversity, richness, biomass, and OTUs as response variables and soil factors as explanatory variables. Overall, the combined explanatory power of soil factors for variables exceeded 93.5%. Soil total nitrogen and bulk density significantly affected microbial community diversity, richness, biomass, and OTUs ($P<0.05$). After removing factor collinearity, envfit test results showed that total nitrogen and bulk density significantly influenced microbial community diversity, richness, biomass, and OTUs ($P<0.05$). Mantel test analysis of the correlation between soil factors and microbial communities showed that microbial diversity, richness, biomass, and OTUs were all significantly correlated with soil water content, bulk density, soil organic carbon, available nitrogen, total nitrogen, and total phosphorus ($P<0.05$). Soil organic carbon, water content, available nitrogen, available potassium, total nitrogen, total phosphorus, and total potassium were significantly positively correlated with various microbial community indicators, while bulk density was significantly negatively correlated ($P<0.05$).

To further explore the interactions between soil microbial communities and soil factors under grazing disturbance on the middle northern slopes of the Tianshan Mountains, SEM was used to reveal the effects of soil factors on microbial community diversity, richness, biomass, and OTUs. Overall, based on chi-square tests, CFI, and RMSEA, all models met the fitting criteria, indicating good model fit. Grazing directly exerted significant negative effects on soil microbial community diversity, richness, biomass, and OTUs ($P<0.05$). Additionally, grazing indirectly negatively affected soil microbial communities by increasing bulk density and reducing soil nutrients.

3 Discussion

3.1 Changes in Grassland Soil Microbial Communities Under Grazing Disturbance

Most studies have shown that the dominant bacterial groups in grassland ecosystems are *Actinobacteria*, *Proteobacteria*, and *Acidobacteria*, which aligns with our results and findings from Inner Mongolia grasslands and Qilian Mountain

grasslands. The relative abundance of *Actinobacteria* exceeded 39% under different grazing treatments. *Actinobacteria* functions by decomposing cellulose and chitin, and high-nutrient soils can promote its growth. The relative abundances of *Proteobacteria* and *Acidobacteria* both exceeded 10%, with the former having unique copiotrophic attributes and the latter showing strong environmental adaptability. Diverse survival strategies and metabolic pathways make these microbial groups dominant. At the bacterial genus level, *Sphingomonas*, *Microlunatus*, *Pseudonocardia*, and *Rubrobacter* showed obvious responses to grazing. Among them, *Microlunatus*, *Pseudonocardia*, and *Rubrobacter* belong to *Actinobacteria*, while *Sphingomonas* belongs to *Proteobacteria*, and both phyla significantly influence soil aggregate structure. However, heavy grazing severely damages the physical properties of plant root-zone soil, causing changes in soil aggregates and consequently altering the abundance of related bacterial groups.

Soil fungal dominant groups are *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*, with combined proportions exceeding 80%, consistent with previous studies in alpine and desert grasslands. *Ascomycota* drives nutrient cycling by decomposing recalcitrant organic matter, while *Basidiomycota* and *Mortierellomycota* decompose plant residues such as lignin and cellulose, making them dominant groups due to their unique physiological characteristics. Random forest results showed that *Ascomycota* and *Basidiomycota* were sensitive to grazing changes, both growing on lignin substrates and being sensitive to environmental changes. Heavy grazing alters the concentration of nutrient substrates they utilize, resulting in lower relative abundance. At the bacterial genus level, *Penicillium* and *Knufia* were sensitive to grazing intensity changes, both belonging to *Ascomycota* and capable of utilizing various soil nutrients for growth. During grazing, plant litter and dead roots increase due to livestock feeding, providing more nutrients and promoting the growth of these fungi. In summary, soil microbial communities in the middle northern slopes of the Tianshan Mountains may differ in distribution and composition from other regions, but the main dominant groups are similar.

This study found that compared with heavy grazing, light grazing increased microbial community alpha diversity (11.6%–18.8%) and microbial biomass (30.92%–139.42%), consistent with previous research. Increased vegetation litter decomposition and root exudate input under light grazing promote microbial growth. However, under heavy grazing, increased soil compaction and reduced nutrient return from plant communities inhibit microbial growth. Some studies suggest no significant differences in microbial diversity and biomass under different grazing treatments, indicating that grazing effects on microbial diversity are complex processes requiring comprehensive consideration of climate, land use, vegetation type, grazing method, and history, necessitating longer-term and broader regional studies. Notably, different grazing intensity classification standards may contribute to discrepancies in research results. Furthermore, no significant differences in beta diversity of soil bacterial and fungal communities under different grazing treatments indicate that grazing mainly affects microbial quantity (alpha diversity) rather than community

structure and composition.

Mantel test results showed that bulk density and total nitrogen were the main drivers of microbial community changes. Grazing alters nutrient content returned from plant communities to soil, limiting microbial nutrient acquisition and consequently causing community changes. Soil nitrogen cycling is a key process in grassland ecosystems, in which microorganisms play important roles. Grazing changes nitrogen mineralization rates and nitrification through livestock feeding, trampling, and excretion, thereby affecting soil nitrogen content and driving microbial community changes. Specifically, feeding changes plant root exudate content, trampling changes the amount and decomposition rate of residual roots and litter, and excretion directly alters soil nutrient content. Meanwhile, livestock trampling also changes the microenvironment where microorganisms reside, causing community changes. Additionally, this study found that fungal communities were more sensitive to soil factors than bacterial communities, mainly because fungi are more conducive to nutrient cycling. Changes in soil physical properties are closely related to microbial habitats; heavy grazing destroys soil structure, while low-intensity grazing helps protect soil and promotes the growth of specific microorganisms. Microorganisms show high sensitivity to their microenvironment, with soil mechanical composition, bulk density, water content, aeration, and pH all affecting their communities. Heavy grazing destroys soil aggregates and surface crusts, while low or no grazing promotes plant growth and reduces soil erosion through plant-soil feedback mechanisms, improving the microbial living environment and promoting the survival and reproduction of specific functional microbial communities.

Structural equation model analysis revealed that grazing can negatively affect microbial communities by increasing soil bulk density and reducing chemical nutrients. Soil nutrients and bulk density are important indicators for evaluating soil fertility and quality, respectively, indicating that the maintenance of soil microbial homeostasis in Tianshan grasslands may depend more on soil quality and fertility. As previously mentioned, various livestock behaviors can affect soil microbial communities. According to plant-soil feedback, plant communities provide nutrients to microorganisms through roots and exudates, while microorganisms support plant growth by decomposing organic matter. Livestock selective feeding, trampling, and excretion behaviors can alter plant communities and soil structure, disturbing the soil microenvironment and nutrient balance and consequently changing various aspects of microbial communities. Among all structural equation models, grazing explained microbial community diversity (97.7%) better than richness (98.4%) and biomass (93.5%), indicating that diversity is more sensitive to grazing disturbance. This sensitivity primarily arises because grazing significantly alters soil microbial diversity through indirect effects such as livestock feeding, trampling, and excretion. Although OTU results differ slightly, this finding still indicates that soil microbial community diversity is more sensitive to grazing disturbance than other variables and can better reflect the balance and integrity of grassland soil ecosystems. Therefore, soil microbial community diversity can be considered an important

indicator for evaluating soil quality and fertility in Tianshan grasslands, consistent with previous research conclusions. Future grazing management planning should fully consider changes in soil microbial community diversity to ensure grassland ecosystem sustainability.

4 Conclusions

Based on field investigation and laboratory soil and microbial measurements, this study analyzed the effects of different grazing intensities on microbial community characteristics in the middle section of the northern slopes of the Tianshan Mountains, further revealing the differential responses of different soil microbial community characteristics to grazing. The following conclusions were drawn:

1) Across the three grazing treatments, soil fungal and bacterial community alpha diversity showed the pattern of LG > NG > HG. Compared with heavy grazing, light grazing significantly increased the alpha diversity and biomass of soil bacterial and fungal communities but had no significant effect on beta diversity.

2) Compared with soil water content, soil nutrients and bulk density showed more significant mediating effects in regulating microbial communities, highlighting that soil microbial community stability under grazing disturbance depends more on soil quality and fertility.

3) Among all indicators, soil microbial community diversity was the most sensitive to grazing, making it a key indicator for evaluating the impact of grazing on grassland ecosystems in this region.

4) Light grazing is beneficial for improving microbial communities, and reasonable regulation of grazing intensity is a feasible strategy for ensuring the stable development of microbial communities.

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Note: Figure translations are in progress. See original paper for figures.