Effects of grazing on the ecosystems multifunctionality of montane meadow-grassland on the northern slope of the Tianshan Mountains, China

Kangwei Jiang
Qingqing Zhang (✉ 108585302@qq.com)
Yafei Wang
Hong Li
Yongqiang Yang
Tursunnay Reyimu

Research Article

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Abstract

Ecosystem multifunctionality (EMF) plays an irreplaceable role in maintaining ecological balance and ensuring human survival and development. However, few studies have focused on the effects of different grazing intensities on EMF, and little is known about changes in the function of multiple ecosystems at different grazing intensities. The study investigated EMF of mountain meadow grasslands on the northern slopes of the Tianshan Mountains in China, by way of a plant community survey coupled with high-throughput sequencing technology. The study calculated the EMF using the mean value method and explore the effects of no grazing (CK), light grazing (LG), and heavy grazing (HG) on grassland EMF. Results showed that HG significantly improved moisture regulation (MR) function ($p < 0.05$), and decreased soil fertility (SF) ($p > 0.05$), soil carbon storage (SCS) ($p > 0.05$), nutrient conversion and cycling (NC) ($p > 0.05$), grassland productivity (GP) function ($p < 0.05$), and EMF ($p < 0.05$). The EMF index of the grassland ecosystem under grazing conditions ranged from 0.3328–0.6018. GP, SCS, and NC functions had the highest contribution to EMF under CK, LG, and HG conditions, respectively. Under grazing conditions, EMF showed a cooperative relationship with SF, SCS, and GP, and the correlation coefficient ($r$) was between 0.62–0.76 ($p < 0.05$). At the same time, EMF and grassland water MR showed a relationship of trade-offs ($r = 0.68$, $p < 0.05$). The results of structural equation models showed that grazing had a significant effect on EMF directly, and also indirectly through soil fungal diversity. Therefore, reasonable reduction of grazing intensity is the most effective management approach for maintaining ecosystem function. At the same time, grazing plays a key role in maintaining EMF by regulating both above- and below-ground ecosystem functions, primarily through soil fungal diversity. This study elucidates the response of mountain grassland EMF to grazing intensity and provides a theoretical basis for restoring degraded grassland and sustainable ecosystem development.

Introduction

Ecosystem multifunctionality (EMF) refers to the ability of an ecosystem to provide multiple services and functions (Perrings & Walker, 1997; Hector & Bagchi, 2007). It includes a variety of ecosystem functions such as net primary material production, carbon storage and sink, wind and sand control, soil and water conservation, and climate regulation. Grassland EMF determines the ecological processes and functions of grasslands that provide a variety of ecosystem services for human survival and development. These ecosystem service functions play a crucial role in ensuring food security, sustaining herders’ lives, and regulating ecological balance, and also play an irreplaceable role in the future survival and development of humans (Soliveres et al. 2016; Mori et al. 2017). Due to global climate change and human interference, ecosystems are gradually being destroyed (Nelson et al. 2013; Li et al. 2019a; Kang et al. 2020), and grassland degradation is becoming increasingly serious (Dong et al. 2021; Ren et al. 2021). The scientific and rational nature of grassland utilization has been neglected, which directly affects grassland ecosystem service functions such as decreased productivity, reduced biodiversity, and reduced system stability, and seriously threatens the sustainable development of human society. In the context of global climate change, the disturbance of human activities and climate change will at the same time provide
feedback to the ecosystem, and the ecosystem will reflect this feedback in the functions of ecosystem productivity, carbon sequestration, nutrient cycling, and ecosystem stability. Faced with unprecedented changes in the ecological environment, people have begun to think about how human activities and climate change will affect ecosystem functions and consider various ecosystem functions as a whole (ecosystem multifunctionality). Therefore, the impact of disturbance on ecosystem function is now reasonably assessed, and the research results can provide a scientific basis for the sustainable development and management of ecosystems.

Grasslands are considered to be one of the most widely distributed ecosystems in the world and an important component of terrestrial ecosystems in China (Dai et al. 2009; Gang et al. 2016; Bengtsson et al. 2019; Zhan et al. 2020). The quality of grassland ecosystems will directly affect national ecological security, the level of agriculture and animal husbandry, and regional stability. Human activities, mainly grazing, have become the main environmental factor of grassland ecosystem degradation (Tong et al. 2004; Fanselow et al. 2011). Grazing is one of the main modes of grassland utilization. Different livestock species, grazing intensity, years, history, and regimes affect soil physicochemical properties and the characteristics of grassland plant and microbial communities. The results of related studies have further shown that grazing affects the structure, function, and processes of grassland ecosystems (Wang et al. 2020a; Zhang et al. 2021a), with the ultimate feedback being the effect on EMF. Various behaviors of grazing livestock during grazing can affect plant communities and ecosystem function, such as selective feeding, trampling, and livestock excrement (Bardgett et al. 2003). In general, heavy grazing can damage grassland ecosystems. Previous studies have shown that increased grazing intensity leads to changes in dominant species thresholds and significantly affects soil nutrient content (Merdas et al. 2017; Zhou et al. 2017; Yang et al. 2018). Grazing significantly reduces soil C and N cycling and also alters the physical and chemical properties of soils (Wachendorf et al. 2008; Aarons et al. 2009; Bai et al. 2015; Wang et al. 2017), which suggests that grazing can significantly reduce ecosystem services and functions. However, some studies have also shown that moderate grazing can better sustain EMF (Ren et al. 2018). In contrast, grazing bans, natural grazing, and abandonment of tillage for grazing have lower effects on EMF than does moderate grazing (Li et al. 2016). Therefore, there is still a big controversy about the mechanism of grazing effects on EMF, and it is important to study the changes of EMF and its mechanisms of influence under different grazing conditions.

Although a large number of studies have been conducted in recent years on the driving factors affecting EMF, few studies have focused on the effect of grazing intensity on EMF. In addition, grassland EMF studies have mainly focused on alpine meadows on the Qinghai-Tibet Plateau and warm steppes in Inner Mongolia, while less research has been conducted on mountain meadows in Xinjiang under grazing disturbances. Mountain meadows in the middle part of the northern slope of the Tianshan Mountains are important summer and winter pastures in Xinjiang—the essence of grassland mowing and grazing in Xinjiang—and occupy a very important position in traditional seasonal livestock husbandry in Xinjiang (Hu et al. 2016). In recent years, studies on the northern slopes of Tianshan have gradually increased, but most of them have focused on the study of plant community characteristics and soil physicochemical properties on the northern slopes of Tianshan and the relationship between them, and few studies have
focused on the changes of grassland ecosystems on the northern slopes of Tianshan under grazing disturbances. Therefore, this paper investigates the changes in EMF under different grazing intensities and discusses the specific mechanisms by which grazing affects EMF. This work provides a theoretical basis for rational utilization and conservation of grassland ecosystems, and grassland restoration, in the arid areas of Xinjiang, China, and for answering the following scientific questions:

(1) What are the effects of different grazing intensities on EMF? What are the trade-offs between coordinating different ecosystem functions under grazing conditions?

(2) What are the specific mechanisms of grazing disturbance affecting EMF? In what ways is EMF affected (directly and indirectly)?

Materials And Methods

Study area

The study was conducted in the middle part of the Northern Slope of Tianshan Mountain, Changji City, Ashli Kazakh Ethnic Township (43°59´ ~ 43°60´N, 86°67´ ~ 86°71´E), at an altitude of 2200–2400 m (See Fig. 1 for location of the study site) (Li et al. 2021). The region has a dry, typical continental climate with more precipitation but only in summer, an annual rainfall of 450–500 mm, annual temperature of 6.5°C, and frost-free period of 100 d. The soil type is mountain chernozem soil, and the grassland type is mountain meadow-grassland. The common species mainly include *Stipa capillata*, *Carex stenocarpa*, *Festuca Ovina*, *Alchemilla Tianschanica*, *Potentilla Bifurca*, and *Heteropappus altaicus*. The grazing mode in the study area is mainly free grazing, and the main grazing animals are mixed sheep, cattle, and horses, supplemented by cattle and horses. The sheep breeds are mainly Xinjiang fine wool sheep or Kazakh sheep suitable for local nomads.

Experimental design

In this paper, six grazing sites were selected as replicates that were as similar as possible in terms of grassland topography, soil conditions, grassland area, and livestock species and numbers. Then, the grazing intensity was specifically classified according to the dominant plant community species in the grazed grasslands, and each grazing site was divided into three grazing experimental groups: lightly grazed grassland, heavily grazed grassland, and control group (ungrazed grassland) according to previous studies (Li et al. 1997; Yi et al. 2012; Wen et al. 2013). Among them, the dominant species of the grazed grassland plant community was *Achnatherum Inebrians*, and this grazing site was designated as a heavily grazed (HG) area. The dominant species of the main plant community of the grazing site were *Carex stenocarpa* and *Stipa capillata*, designating this grazing site as a light grazing (LG) area. Finally, the fenced grassland (ungrazed grassland) will be used as an anthropogenic control sample (CK) in this paper in 2018; the dominant species of plant communities in this grassland were *Stipa capillata*, *Festuca ovina*, and *Poa pratensis*. The type of grassland in the three grazing experimental groups mentioned
above was mountain meadow grassland, and the differences in the dominant species of plant communities in each experimental group were all caused by the differences in grazing intensity (Fig. 2).

**Sampling and measurement**

Parallel sampling strips were set up as grassland sampling points in each of the three grazing experimental groups in the above experimental area, seven 1×1 m sample squares (spaced about 50 m) were set up in each parallel sampling strip, and then followed by vegetation surveys in August 2020 and 2021. The sample plots were selected to avoid areas with livestock manure as much as possible to avoid experimental errors. After the sample plots were set up, the plant species composition of the grassland sample plots was recorded, and the natural height of the grassland plants, grassland cover (visual method) and the number of each species were measured. Finally, all plants in the grassland sample plots were cut flush to the ground, weighed fresh and placed in named envelope bags, then brought back to the laboratory to be dried at 65°C in order to determine the dry weight of above-ground biomass.

Meanwhile, based on the grassland vegetation survey, soil from 0–10 cm soil layer was collected by soil auger method (three replicates per sample square) in this paper. Then, the soil samples from seven sample squares were evenly mixed into one soil sample, and the soil was removed from the adulterated plant roots and gravels with a 2-mm sieve. Finally, the total soil sample was divided into two parts, one of which was placed in sterile centrifuge tubes, which in turn were then placed into an ice box. These boxes were immediately transported back to the laboratory and stored at -20°C to characterize the soil microbial community. The other soil samples were placed in sealed bags and labeled, and returned to the laboratory to assessing the physicochemical properties of the soil after natural air drying.

**Sample collection and index determination**

**Determination of soil physical properties**

According to the soil agro-chemical analysis undertaken by Bao (2000), the physical and chemical properties of soil such as organic carbon, soil total nitrogen, soil total phosphorus, soil total potassium, soil available nitrogen, soil available phosphorus, soil available potassium, and soil pH were typically measured (Table 1).
Table 1
Methods of determination of soil physical and chemical indexes

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic carbon</td>
<td>Potassium dichromate-concentrated sulfuric acid external heating method</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>Kjeldahl method</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>Sodium hydroxide alkali fusion-molybdenum antimony anti-colorimetry</td>
</tr>
<tr>
<td>Total potassium</td>
<td>Sodium hydroxide alkali melt-flame spectrophotometry</td>
</tr>
<tr>
<td>Available nitrogen</td>
<td>Sodium hydroxide alkaline diffusion method</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>Sodium bicarbonate leaching-molybdenum antimony anti-colorimetric method</td>
</tr>
<tr>
<td>Available potassium</td>
<td>Ammonium acetate extraction - flame photometry</td>
</tr>
<tr>
<td>pH value</td>
<td>Potentiometric method (water-soil ratio 5:1)</td>
</tr>
</tbody>
</table>

**Extraction and sequencing of soil microbial DNA**

In this study, total DNA was extracted from soil microorganisms using the MP-FAST DNATMSpin Kit for Soil (Omega Bio-Tek, Norcross, GA, USA). The extracted genomic DNA was detected with 1% agarose gel electrophoresis. The corresponding regions of the bacterial 16S rRNA gene and fungal ITS rRNA gene were amplified with PCR using respective primers. The reaction conditions were as follows: pre-denaturation at 95°C for 3 min, 27 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s), and a final extension at 72°C for 10 min. The PCR products were pooled and detected using 2% agarose gel electrophoresis. The PCR products were quantified using a QuantifluorTM-ST Blue Fluorescence Quantification System (Promega Company). The PE250 library was constructed from the purified amplified fragments according to an Illumina MiSeq platform (Illumina, San Diego, USA). Sequencing was undertaken with an Illumina Misqe PE250 platform. Shanghai Meiji Biomedical Technology Co., Ltd completed the library construction and sequencing tasks; Mothur software was used to calculate the α diversity index of the sample.

**Quantification of ecosystem multifunctionality**

Based on a previous system of evaluating EMF (Jing et al. 2015; Xiong et al. 2016; Wang et al. 2019), five types of functions (13 variable indicators) related to grassland ecosystem were selected in this paper, taking into account the regulatory, supply and support service functions of grassland ecosystems. These included water regulation (MR), soil fertility (SF), nutrient transformation and cycling (NC), soil carbon storage (SCS), and grassland productivity (GP). Finally, this study also established an evaluation system of mountain meadow-grassland EMF on the north slope of Tianshan Mountain (Table 2).
Table 2
Evaluation system of grassland ecosystem multifunctionality

<table>
<thead>
<tr>
<th>Type</th>
<th>Ecosystem functioning</th>
<th>Ecosystem function indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuning services</td>
<td>Moisture regulation (MR)</td>
<td>Soil bulk density; Soil moisture content</td>
</tr>
<tr>
<td>Supply services</td>
<td>Grassland productivity (GP)</td>
<td>Aboveground biomass; Underground biomass</td>
</tr>
<tr>
<td>Support services</td>
<td>Nutrient conversion and cycling (NC)</td>
<td>Soil carbon-nitrogen ratio; Soil pH</td>
</tr>
<tr>
<td></td>
<td>Soil fertility (SF)</td>
<td>Soil total nitrogen; Soil available nitrogen; Soil total potassium; Soil available potassium; Soil total phosphorus; Soil available phosphorus</td>
</tr>
<tr>
<td></td>
<td>Soil carbon storage (SCS)</td>
<td>Soil organic carbon</td>
</tr>
<tr>
<td></td>
<td>Soil nutrients (SS)</td>
<td>Soil conductivity</td>
</tr>
</tbody>
</table>

**Statistical analysis and data processing**

Calculation of diversity index of the plant community

Shannon-Wiener Diversity Index: \( H' = -\sum P_i \ln P_i \) \( (Pi = \frac{Ni}{N}) \) (1)

In the formula, \( P_i \) is the ratio of the number of individuals of the ith species to the total number of individuals of all species in the community, \( N \) is the total number of individuals of all species in the sample, and \( S \) is the number of species in the sample.

Soil microbial data processing

In this paper, the data were spliced using FLASH software and quality controlled using Trimmomatic software to obtain valid sequences. The obtained sequences were clustered according to a 97% similarity criterion. Then the corresponding diversity index of \( \alpha \) could be obtained by Mothur software. Comparisons were made with the Silva (Release128) database, species classification of different sequences were made, and the sequences annotated. Finally, the community composition of each sample was counted, and the species diversity in each sample was analyzed.

Calculation of the Ecosystem Multifunctionality Index

In this study, the averaging method was used to evaluate the electromagnetic fields as follows:
All indicators (Table 2) were standardized to the same order of magnitude using the min-max standardization method. The maximum value of an indicator is the average of the top 5% observed value of each indicator, and the minimum value of an indicator is the bottom 5% observed value of each indicator:

Min-max normalization calculation: 
\[ f_{ij} = \frac{x_{ij} - min_{ij}}{max_{ij} - min_{ij}} \]  

(2)

\( f_{ij} \) is the normalized value of the type j ecosystem function variable of sample i, \( x_{ij} \) is the measurement value of the type j ecosystem function variable of sample i, \( min_{ij} \) is the minimum value of the j ecosystem function variable across all plots of the same factor, and \( max_{ij} \) is the maximum value of the j ecosystem function variable across all plots of the same factor.

Ecosystem single-function index: Calculates the single-function index that reflects the different functions of the ecosystem (Table 2 for details).

Ecosystem Single Function Index F: 
\[ F_{ij} = \frac{\sum_{j}^n f_{ij}}{n} \]  

(3)

\( F_{ij} \) is the functional index of the j function of the sample i, and n is the number of ecosystem variable indicators contained in the function.

Index of EMF: 
\[ EMF_i = \frac{\sum_{j=1}^N f_{ij}}{N} \]  

(4)

\( EMF_i \) is the ecosystem multifunctionality index of sample i, calculated based on the standardized mean of all variable indicators in the sample, \( N \) is the number of ecosystem functions contained in sample i.

Analysis of trade-offs and coordination among ecosystem functions

This study used Pearson correlation analysis to study trade-offs and coordination between different functions. If there was a significant positive correlation between the two functions (\( r > 0, p < 0.05 \)), it indicated that the two functions are synergistic. If there was a significant negative correlation between the two functions (\( r < 0, p < 0.05 \)), it indicated that the two functions were a trade-off relationship. If they were not correlated, no association was present.

The data were classified and analyzed by using Excel 2019 software. To analyze the differences in the multifunctional index of lightly grazed, heavily grazed and ungrazed grassland ecosystems, this study used the Duncan's test in SPSS 26.0 software to test for significant differences between the single functional and EMF index. Analysis of Pearson correlations between ecological functions were undertaken in R 4.1.1 software. The relationship between the diversity of plant and soil microbial and ecosystem function was analyzed using Pearson correlation analysis. Finally, this study used structural equation modeling (SEM) to analyze the mediating effect of biodiversity on EMF under grazing.
Conditions. SEM fitting tests were performed on plant diversity, bacterial diversity, and fungal diversity under different grazing intensities to determine the direct and indirect effects of grazing intensity on EMF.

**Results**

**Effects of grazing on a single function index of grassland ecosystem**

Except for MR, the ecosystem function index of this grassland was the lowest under HG (Fig. 3). In addition, GP showed that CK was significantly higher than LG and HG, and LG was significantly higher than HG ($p < 0.05$). However, CK was significantly lower than LG and HG in MR ($p < 0.05$). There were no significant differences in SCS, SF, and NC functions under different grazing intensities.

**Effects of grazing on the multifunctional index of grassland ecosystems**

The grassland EMF distribution was $0.3328 - 0.6018$, and its median value was $0.5174$ (Fig. 4(a)). CK and LG were significantly higher than HG under different grazing intensities ($p < 0.05$). This study used the average value of the EMF index and single function index under different grazing intensity conditions for plotting (Fig. 4(b)). The results of the study showed that SCS contributed most to the EMF of LG, NC contributed most to the EMF of HG, and GP contributed most to CK.

**Coordination analysis of trade-offs between grassland ecosystem functions**

This study used interpersonal analysis to examine the trade-offs and coordination relationships between different functions. Coordination, trade-offs, and neutral relationships existed among 15 functional combinations consisting of six ecosystem functions (Fig. 5). Among them, EMF had a highly significant positive correlation with SF, SCS, and GP, showing coordination between functions, with correlation coefficients ($r$) ranging from $0.62 - 0.76$ ($p < 0.01$). In addition, EMF showed a significant negative correlation with grass MR ($r = -0.52$, $p < 0.05$), exhibiting a trade-offs relationship. A significant collocation relationship was exhibited between SCS and SF ($r = 0.68$, $p < 0.01$), but a significant trade-offs relationship existed between SCS and NC ($r = -0.55$, $p < 0.05$). A significant trade-offs relationship was also exhibited between GP and MR ($r = -0.56$, $p < 0.05$). The relationships between the remaining ecosystem functions were all non-significant.

**The mediating effect of grazing intensity on the multifunctional index of grassland ecosystems**
To further investigate the specific mechanism of grazing on EMF, this study selected plant and soil microbial diversity indices and used SEM to explore the direct and indirect effects of grazing on EMF. For a chi-square test, $p > 0.05$ which does not reach significance and thus the null hypothesis was not rejected. All the fitness indicators of SEM satisfy the model fitness criterion, indicating that this model fits well. Therefore, the results of this model can be used to explore the mediating role of biodiversity on EMF (Fig. 6). According to the SEM, grazing can directly affect EMF and explain 63% of its variation. In addition, grazing significantly impacted grassland EMF by reducing soil fungal diversity (total development: $-0.561 \times 0.372 = 0.209$). Although grazing negatively affected plant and soil bacterial diversity, the indirect impact on EMF through the two was not significant.

Discussion

Effects of grazing on grassland ecosystem functions

The use of grassland resources is mainly achieved through the grazing, and the variation in grazing intensity affects the physical and chemical properties of the soil and thus the stability of the grassland ecosystem (Rauber et al. 2021). Moreover, this feedback will impact ecosystem diversity. The trampling effect of livestock increases soil compactness, reduces capillary porosity, and reduces soil air permeability and water retention capacity (Snyman & Preez, 2005; Li et al. 2007), manifesting as a reduction in EMF during the evaluation process. In addition to the trampling effect of livestock, the excretory behavior of livestock during grazing also affects EMF. In the grazing process, livestock excreta gradually increase, resulting in a large number of nutrients returning to the soil (Moe and Wegge 2008; Aarons et al. 2009) thus changing the nutrient cycle of the grassland ecosystem. In this study, HG significantly reduced EMF compared to LG, mainly because HG reduced multiple ecosystem functions (Fig. 3). HG significantly reduced grassland productivity (GP), mainly by lowering the aboveground and belowground biomass of grassland plants, and excessive grazing by livestock slowed the compensatory growth of plants to some extent (Wang et al. 2020b). Moreover, This study shows that MR is significantly higher in moderate and HG than in CK and LG. The reason for this may be that the MR function in this paper is mainly composed of the soil bulk density and soil water content index. On the one hand, livestock grazing may decrease the height and coverage of grassland plants (Yuan and Hou 2015; Liu et al. 2017), further causing the grass to appear bare, leading to a decrease in soil water content. However, the HG grasslands in the study area did not appear to be bare, so the change in soil water content was not significant. On the other hand, the trampling behavior of livestock in the process of grazing will improve the soil compactness and soil bulk density (Hiernaux et al. 1999; Tate et al. 2004), thus improving water regulation function. However, there was no significant difference in SF, SCS, and NC under different grazing intensity conditions, mainly because these functions were composed of soil physical and chemical indicators. Grassland ecosystems have hysteresis and capacity, and the change of soil physical and chemical indicators is a relatively lengthy process (Basher and Lynn 1996; Reeder and Schuman 2002), which is comprehensively affected by climate, temperature, topography, soil vegetation type, grazing history, and other factors (Milchunas and Lauenroth 1993). In addition, from the perspective
of contributions of various ecosystem functions to EMF, GP, SCS, and NC were the highest contributions to EMF under CK, LG, and HG, respectively. These results indicate that each ecological function had different contributions to the EMF index under different grazing intensity conditions. With the increase of grazing intensity, ecosystem function would be affected to different degrees, and the degree of contribution of different ecosystem functions would eventually change.

Grassland ecosystem functions are not only affected by external environmental factors but also have regulatory effects among ecosystem functions (Manning et al. 2018). Previous studies have shown that under the action of external environmental factors there will be trade-offs and coordination between ecosystem functions (Mouillot et al. 2011; Soliveres et al. 2014). In the present study, EMF and SF, SCS, and GP showed a highly significant coordinated relationship, which suggests that the montane meadow-grassland ecosystem on the northern slopes of the Tianshan Mountains maintains and provides a variety of services and functions that need better soil quality to be supported. Moreover, there is a cooperative relationship between SF, SCS and GP, indicating that good soil quality can simultaneously increase grassland productivity, while higher grassland productivity can further increase EMF. In addition, there was a significant trade-off between EMF and MR function \( (p < 0.05) \). The reason for this may be the change of soil bulk density index in MR function. With the increase of grazing intensity, the soil bulk density index will also rise, leading to a change in soil physical and chemical properties and, finally, the decline of soil fertility and plant productivity (Yi et al. 2012; Sun et al. 2017; Zhang et al. 2019). At the same time, this also explains why there were significant trade-offs between GP and MR in the grassland under grazing conditions in the present study \( (p < 0.05) \). However, there were significant trade-offs between SCS and NC function \( (p < 0.05) \), because soil microorganisms impacted the soil carbon pool during nutrient cycle conversion. For example, arbuscular mycorrhizal fungi (AMFs) can regulate the nutrient cycling of aboveground and belowground ecosystems, and negatively affect the soil carbon pools (Kowalchuk 2012).

**Effects of biodiversity on ecosystem multifunctionality during grazing**

This study investigated the mediating effect of biodiversity (plants and microorganisms) on EMF under grazing conditions in the montane meadow-grassland ecosystem on the northern slope of the Tianshan Mountains. The results showed a positive correlation between plant diversity and soil microbial diversity and EMF (Fig. 7), similar to most previous results (Delgado-Baquerizo et al. 2016; Zhang et al. 2021b). Bacteria and fungi control key ecological processes in soil (van der Heijden et al. 2008), such as SF and NC. However, in the present study, the correlation between plant diversity and soil microbial diversity, and SF function and NC function was not significant (Fig. 6), and this result may be due to differences between study areas. Soil microbial diversity was positively correlated with plant diversity and grassland productivity \( (p < 0.05) \), which indicates that the increase in soil microbial diversity could promote the rise of aboveground and belowground biomass of plant communities. Most of the dominant plant populations in this study area are perennial grasses, whose rich and dense roots provide a large amount
of organic matter for microbial growth and development (Guo et al. 2019). At the same time, microorganisms also provide nutrients to plant communities by consuming organic materials, showing that soil microorganisms can communicate ecological functions aboveground and underground.

The results of this paper showed that grazing intensity can directly or indirectly affect EMF (Fig. 6). The structural equation model showed that grazing could significantly reduce EMF indirectly through fungal diversity \( (p < 0.01) \), which indicates that the maintenance of the mountain meadow-grassland EMF on the northern slope of Tianshan Mountain was more dependent on fungal species diversity. The increase in fungal diversity may improve the EMF, which has been confirmed to some extent in the study of Li et al. (2019b). On the one hand, some studies have shown that the mediating effect of soil microorganisms on EMF is relatively weak in desert steppes with low water and nutrient content (Hou et al. 2016; Zhao et al. 2017). In the present study, mountain meadow-grassland had higher water and nutrient levels, which may improve the mediating effect of soil microorganisms on EMF under grazing conditions. On the other hand, compared with soil bacteria, soil fungi have a higher utilization efficiency of organic matter (Mazzetto et al. 2016; He et al. 2021), and the input of plant organic matter will have a more significant impact on fungi (Delgado-Baquerizo et al. 2018; Wang et al. 2018). However, grazing, trampling, and excretion of livestock significantly affect the characteristics of plant communities. Under HG, vegetation is scarce, grassland productivity decreases, and plant organic matter input decreases (Mapfumo et al. 2002; Cheng et al. 2008; Peng et al. 2020). Therefore, grazing could regulate EMF through fungal diversity. The results also suggest that although plant, soil bacterial, and soil fungal diversity are critical for maintaining both individual ecosystem functions and EMF in mountain meadow-grasslands, fungal diversity plays a more crucial role in modulating EMF responses to grazing disturbances.

**Conclusion**

In this study, the effect of grazing on grassland EMF was studied using mountain grasslands as an example. This has provided an essential theoretical reference for the restoration, utilization, and management of the meadow grassland ecosystem on the north slope of Tianshan Mountain.

(1) The results showed that heavy grazing significantly decreased GP and significantly increased MR. In terms of ecosystem function trade-offs and coordination, EMF has a significant coordination relationship with SF, SCS, and GP, and a significant trade-offs relationship with MR.

(2) According to the structural equation model, increasing grazing intensity can directly reduce EMF, mainly by reducing GP and other ecosystem functions. The indirect effect of grazing on EMF is primarily due to a reduction in soil fungal diversity rather than through changes in plant diversity and soil bacterial diversity.

(3) The results highlight the obvious fact that heavy grazing significantly affects the EMF compared to light grazing and no grazing. As such, in terms of improving the grassland ecological environment, the author suggests that a reasonable and scientific reduction of grazing intensity is the most effective management approach to maintain meadow-grassland biodiversity and protect ecosystem functions.
The results also suggest that the effect of grazing disturbance on EMF can be explained by comparing the responses of different components of biodiversity (plant, bacterial and fungal diversity), but that fungal diversity is a more effective predictor of EMF. Soil fungal diversity plays an important role in the maintenance of EMF by regulating ecosystem functions above and below the ground.

Therefore, when assessing the impact of grazing on the EMF of grasslands, it is necessary to not only focus on the trade-off between ecosystem functions under grazing conditions, but also to consider the critical role of biodiversity in maintaining EMF. In addition, this study only discussed the mechanism of EMF effects on grazing disturbances, and did not discuss EMF and biodiversity in depth. Subsequent studies should reveal the underlying mechanisms of the relationship under grazing disturbance; these results would contribute to ecosystem management and the sustainable development of human society.

Declarations

Author contributions Qingqing Zhang designed the research. Kangwei Jiang, Qingqing Zhang, Yafei Wang, Hong Li, Yongqiang Yang, and Reyimu Tursunnay performed the experiments. Kangwei Jiang and Reyimu Zhang wrote the paper. All authors contributed to the article and approved the submitted version.

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Data availability The data will be available from the corresponding author upon reasonable request.

Conflict of interest The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the paper they submitted

References


Figures
Figure 1

Map of the study area.

Figure 2

Test layout diagram
Figure 3

The effects of grazing intensity on moisture regulation (MR) function, soil fertility (SF) function, soil carbon storage (SCS) function, nutrient conversion and cycling (NC) function, and grassland productivity (GP) function. The function index of each ecosystem is the function index after standardization. The lower case letters indicate significant differences ($p < 0.05$) among grazing treatments.
**Figure 4**

The effects of grazing intensity on grassland EMF (a) and a radar map of the relative contribution (b). The EMF index is the function index after standardization. Different lower-case letters indicate significant differences ($p < 0.05$) among grazing treatments.
Figure 5

Analysis of the trade-offs relationship between moisture regulation (MR) function, soil fertility (SF) function, soil carbon storage (SCS) function, nutrient conversion and cycling (NC) function, grassland productivity (GP) function, and EMF correlations in grassland ecosystems. The solid arrows represent standardized path coefficients with significance indicated as **$p < 0.01$ and *$p < 0.05$. 

<table>
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<th>GP</th>
<th>EMF</th>
<th>SCS</th>
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Figure 6

Structural equation model describing the effects of grazing intensity on ecosystem multifunctionality (EMF) via its effects on plant diversity and soil microbial diversity. Blue lines indicate positive effects, and red lines indicate negative effects. Numbers adjacent to arrows indicate the effect size of the relationship, and the solid arrows represent standardized path coefficients with significance indicated as ***(p < 0.001), **(p < 0.01) and *(p < 0.05).

\[ \chi^2 = 0.537 \quad p = 0.464 \quad \text{RMSEA} = 0.00 \quad \text{CFI} = 1.00 \]
**Figure 7**

Correlations between biodiversity and grassland ecosystem functions. The solid arrows represent standardized path coefficients with significance indicated as **$p < 0.01$** and *$p < 0.05$*. MR: moisture regulation function, SF: soil fertility function, SCS: soil carbon storage function, NC: nutrient conversion and cycling function, GP: grassland productivity function, PS: plant diversity, FS: soil fungal diversity, BS: soil bacterial diversity.