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Livestock grazing strengthens the effect of vole activity on the soil microbial community

Yunqi Xiong [a,1], Zhenzhen Zheng [a,1], Baofa Yin [b], Guoliang Li [c], Xinrong Wan [d], Ruyan Qian [a], Linfeng Li [e], Shuntian Guan [a], Yuan Liu [a], Yanfen Wang [e,f], Xiaoyong Cui [a,f], Jianqing Du [f], Kai Xue [e,f], Yanbin Hao [a,f,g,*]

[a] College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
[b] College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China
[c] Jiangxi Provincial Key Laboratory of Conservation Biology, College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China
[d] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China
[e] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China
[f] Beijing Yanshan Earth Critical Zone National Research Station, University of Chinese Academy of Sciences, Beijing 101408, China
[g] Key Laboratory of Earth System Numerical Modeling and Application, University of Chinese Academy of Sciences, Beijing 10049, China

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ABSTRACT

Livestock grazing may affect small mammalian herbivore-soil microbe interactions and their association with the structure and functions of the ecosystem. However, the role of factors such as vegetation and soil nutrients in regulating these impacts is not clear. Here we conducted a 9-year experiment in temperate steppe to study how Brandt's vole (*Lasiopodomys brandtii*) affects the soil microbial community under different livestock grazing intensities. This experiment contained 12 field enclosures with three livestock grazing intensities: control (CK), light grazing (LG), and moderate grazing (MG). We found that vole activity does not significantly change soil microbial diversity under non-grazing conditions. However, under livestock grazing conditions, vole activity led to a significant reduction in soil bacterial diversity and an increase in fungal diversity, demonstrating the impacts of livestock grazing on rodents-soil microbe interactions. The activity of voles significantly altered soil bacterial community composition, with changes primarily attributed to variations in the relative abundance of the phyla Actinobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes, and Proteobacteria. The soil fungal community remained relatively stable despite vole activity, which can be attributed to the richness of fungal colonies in mycelium and their low sensitivity to changes in external conditions. Vole activity also influenced soil microbial functional groups, and the variations in these groups were further amplified by livestock grazing. Furthermore, the shift in the microbial community composition and diversity induced by vole activity were mainly associated with the reduction of plant aboveground biomass. Overall, our study suggested that livestock grazing enhanced the changes in the soil microbial community induced by rodents, underscoring the importance of managing livestock grazing regimes for grassland conservation.

1. Introduction

Activities of small mammalian herbivores, such as rodents, can affect the topography, plant community and soil structure, and then have further influence on ecosystem functions, such as primary production, nutrient cycling, and habitat provisioning (Crooks, 2002; Qin et al., 2015; Su et al., 2020). Small mammals could cause soil disturbance via increasing plant diversity (Bagchi et al., 2006). Furthermore, the impact of browsing activities on the soil community is also critical for maintaining feedbacks between soil subsystems and plant–herbivore subsystems (Wardle et al., 2001; Bardgett and Wardle, 2003; Risch et al., 2015). However, the ecological consequences for burrowing effects are too diverse to predict (Wilske et al., 2015), and can be influenced by other co-occurring large mammalian herbivores.

Understanding the mechanisms by which small mammalian herbivores interact with other ecological members is a key issue in

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* Corresponding author at: College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China.
E-mail address: ybhao@ucas.ac.cn (Y. Hao).

[1] These authors contributed equally to this work.

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maintaining ecosystem structure and function (Zhang et al., 2003a; Qin et al., 2015; Lacher et al., 2019). Grazing by livestock, the most widespread land use in grassland, can directly alter the structure and diversity of plant communities by targeting high-quality forage (Anderson and Briske, 1995; Hurtt et al., 2020). This alteration in plant communities then change the resource availability for rodents, affecting their population abundance and activity (Li et al., 2016; Li et al., 2019a). Furthermore, the deposition of antibiotic-treated livestock manure demonstrated to alter nutrient cycling and the structure of the soil microbial community (Wepking et al., 2019). Some studies found that the diet of livestock generally differs from that of rodents, and grazing can lead to the vegetation structure and composition more favorable to rodents (Wangdwei et al., 2013; Su et al., 2017). In contrast, Li et al. (2016) revealed that successive grazing by sheep in the Inner Mongolia Plateau led to a decrease in vole population density by decreasing the quantity and quality of food. Livestock and rodents commonly co-exist and play important roles in shaping grassland ecosystems (Davidson et al., 2010; Eldridge et al., 2016; Yang et al., 2022). Although the complementary ecological roles between these two groups are well-demonstrated, less is known how rodent-induced ecosystem effects vary depending on livestock grazing regimes (Davidson et al., 2010; Yoshihara et al., 2010).

Soil microorganisms is critical in terrestrial ecosystem processes associated with the cycling of carbon, nitrogen, phosphorus and organic matter decomposition (Falkowski et al., 2008; Eldridge et al., 2016). Variations in environmental factors, such as biotic components and soil physicochemical properties, strongly affect the composition and diversity of soil microbial communities (Delgado-Baquerizo et al., 2017; Wei et al., 2019; Tajik et al., 2020). Accordingly, grazing by livestock and rodents can induce alterations in vegetation and soil nutrients, ultimately resulting in changes in the diversity and structure of soil microorganisms (Chen et al., 2021; Wu et al., 2022). Though some studies have examined the factors that alter the influence of herbivores on the composition and diversity of plant communities (Bakker et al., 2006), factors that influence the combined effects of livestock and small mammalian herbivores on the soil microbial community need more experimental evidence.

Brandt's vole (*Lasiopodomys brandtii*) is one of the typical rodent species in the grasslands of Inner Mongolia (Zhong et al., 1999; Zhang et al., 2003b). Numerous studies have examined the influence of rodents on soil physicochemical properties and plant characteristics (Pizzeghello et al., 2015; Tuomi et al., 2019; Cui et al., 2020). However, our understanding of the impact of vole activity on soil microorganisms is still limited. The feeding behavior and movement patterns of voles can modify soil habitat, nutrient availability, and plant community composition (Sun et al., 2015; Yang et al., 2022), thereby influencing soil microbial communities and activities (Rinnan et al., 2009; Moorhead et al., 2017). Nonetheless, the extent to which these ecological consequences are influenced by livestock grazing remains largely unclear (Wardle et al., 2001; Bardgett and Wardle, 2003).

So far, few studies have combined livestock grazing and vole activity, and investigate their effects on soil microbial communities (Aguilera et al., 2016, Eldridge et al., 2016), hindering a comprehensive understanding of the dynamics and functioning of the semi-arid steppe. In this study, we aimed to reveal the influence of vole activity on soil microorganisms under long-term grazing conditions. Specifically, we investigated how vole activities affect temporal stability on plant and soil communities. Furthermore, we tested whether and how the degree to which vole activities alter the plant and soil communities under the influence of concurrent livestock grazing. We asked (i) whether vole activities alone influence soil microbial diversity and composition; (ii) does grazing exacerbate the influence caused by vole activities; and (iii) which factors mediate these influences.

2. Materials and methods

2.1. Study site and experimental design

Our experiment was conducted from 2010 to 2018 at the Joint Research Station of Animal Ecology (44°18′N, 116°45′E, 1079 m a.s.l.) in Inner Mongolia, China. The study area experiences a temperate semi-arid climate, with the mean annual temperature of −1.4 °C and the mean annual precipitation of 276 mm. Around 68 % of the precipitation occurs between May and August (Li et al., 2020). The soil type here is chestnut, consisting of 60 % sand, 18 % clay and 17 % silt (Hao et al., 2018). Three dominant plant species in this area are *Cleistogenes squarrosa*, *Stipa krylovii*, and *Leymus chinensis*. Prior to this study in 2008, livestock was excluded from this area, which has a long history of sheep grazing.

Our experiment consists of four blocks, each containing three enclosures which were randomly assigned to control (CK; 0 sheep ha^{-1}), light grazing (LG; 2 sheep ha^{-1}), or moderate grazing treatment (MG; 4 sheep ha^{-1}), resulting in a total of 12 enclosures (Fig. 1). The size of each enclosure was 0.48 ha (80 m × 60 m) and it was surrounded by galvanized iron sheets, which were 2.4 m wide. These sheets were 1.4 m aboveground and extended 1 m belowground to prevent voles from entering or escaping. Each enclosure was covered with 50-cm-high wire netting (1 cm mesh size) on the four sides, and 10-cm mesh size nylon netting on the top to avoid avian predators (for more detailed description of the enclosures see Li et al. (2016)). The control treatment excluded sheep from the enclosures.

In late April of each year during 2010–2018, 26 new adult Brandt's voles with a 1:1 sex ratio were randomly assigned to each of the 12 enclosures. Before the start of the experiment in early May, Brandt's vole abundance was checked in each enclosure using live trapping to ensure that the founder populations consisted of 13 pairs of voles. If the vole abundance in any enclosure was lower than 13 pairs, new adult voles captured from nearby grassland were introduced to that enclosure (Li et al., 2020). To minimize the impacts caused by differences in the founder population of voles, all over-wintering voles were removed from the enclosures in early spring each year.

2.2. Field sampling

During the experiment, the plant composition structure of each enclosure was monitored. From 2010 to 2018, every year in late September, five replicate 1 m × 1 m quadrats were randomly set in each enclosure. The relative cover, frequency, and aboveground dry biomass of each plant species was recorded (for more details, see Li et al. (2016)).

In September 2018, to evaluate the impacts of vole activities on plants and soil microbes under continuous grazing treatments, a total of six 1 m × 1 m quadrats were established in both Brandt's vole colony and off-colony grasslands whin each enclosure. Within each quadrat, the frequency of plant species and total plant cover were counted, while the cover, abundance, and mean plant height of each species were also calculated. The aboveground parts of each species were harvested and dried at 55 °C for 48 h until reaching a constant weigh to obtain the aboveground dry biomass (AGB). To assess the influence of grazing and vole activity on the plant community, importance values (IV) were calculated for each plant species. Subsequently, Shannon-Weiner indexes (*H'*) were calculated based on these values. The formulas are as follows (Whittaker, 1975; Taft et al., 2006):

$$IV = (RD + RH + RC + RB)/4 \times 100\%$$

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where RD, RH, RC, and RB represent the relative abundance, height, cover, and aboveground dry biomass of each species, respectively; S

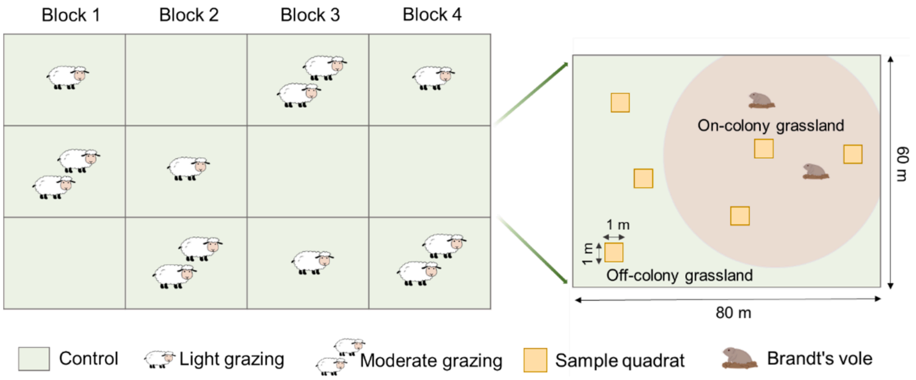

Fig. 1. Scheme of the experimental design. Twelve enclosures with three levels of grazing (control, CK; light grazing, LG; moderate grazing, MG) and four blocks were involved in this experiment. Each enclosure is 80 m long and 60 m wide. Three sample quadrats were established for on-colony grassland and off-colony grassland in each enclosure, respectively.

means the total number of species in each quadrat, p_i is the important values of species i.

After all plants were harvested, using a 3 cm diameter auger to collect a soil core from a depth of 0 to 5 cm from the center of each quadrat. These soil cores from three quadrats from the on-colony grassland and off-colony grassland were then mixed up to form a single sample, respectively. The mixed samples were sieved through a 2 mm mesh to remove stones and coarse roots. The collected roots were dried at 55 °C for 48 h, and the weight of the roots for three soil cores were converted to belowground dry biomass (BGB) for the 1 m × 1 m quadrat. We then calculated the root-shoot ratio by dividing the belowground biomass by the aboveground biomass. Each soil sample was divided into two parts: one air-dried for physicochemical analysis, and the other frozen at −20 °C for DNA extraction and sequencing.

Given that livestock manure have influences on various soil characteristics, such as bulk density, infiltration, soil fertility, water-holding capacity, and biological properties (Rayne and Aula, 2020), it is plausible that soil microbial communities may also be affected by livestock manure. As a potential factor in soil microbial alteration, we analyzed livestock manure within the enclosures. The fresh sheep manure samples were randomly collected from enclosures in 2018 and thoroughly mixed for microbiological composition analysis.

2.3. Analysis of soil properties and microbes

For each soil samples, we measured soil water content (SWC), soil pH, soil organic carbon (SOC), soil dissolved organic carbon (DOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), soil NH_4^+-N and NO_3^--N, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) (see supplementary material for details about the methods).

Soil DNA was extracted from 0.5 g of soil per sample using a MoBio PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. The 16S rRNA encoding gene 341F and 806R were targeted to amplify the V3-V4 region of the bacteria (Xiang et al., 2021). Fungal ITS genes were amplified using the primers ITS1F (Gardes and Bruns, 1993). Sequencing of amplicons on the IonS5™XL platform (Thermo Fisher Scientific, USA).

The sequences were quality-filtered, demultiplexed, and processed into operational taxonomic units (OTUs) using the EasyAmplicon pipeline (Liu et al., 2021). Alpha diversity (Shannon index and richness) of bacteria and fungi was then calculated for each treatment. Prokaryotic sequences were classified through the SILVA 138.1 database (Quast et al., 2012), and fungal sequences were grouped using the UNITE database (Version 16.10.2022) (Kessy et al., 2022).

The 16S rRNA gene was assigned to functional groups using FAPROTAX, which can analyze the functional groups for bacteria (Louca et al., 2016). Additionally, FUNGuild v1.0 (https://github.com/UMN-FuN/FUNGuild) was applied to estimate the functional group of fungi (Nguyen et al., 2016). Sequences that could not be identified or had multiple functional assignments in FUNGuild were unified as 'undefined'. To avoid excessive interpretation of fungi functions, we removed the confidence level of 'possible', and retained only two confidence levels: 'highly probable' and 'probable'.

2.4. Statistical analysis

Linear mixed models (LMMs) were conducted using 'lme4′ R package (https://github.com/lme4/lme4/) to investigate the effects of grazing treatment and vole activity on various factors such as plant community factors, soil physicochemical properties, soil microbial diversity (both bacterial and fungal), and the relative abundance of most abundant microbial phyla (with relative abundance > 1 %). The models included grazing treatment, vole activity and their interactions as fixed factors, with blocks being considered as random effects. Multiple comparisons using Tukey's post hoc test by 'multcomp' R package (Hothorn et al., 2008). The normality of distribution and homogeneity of variables and the residuals of the models were checked with the Shapiro-Wilk normality test and Levene's test, respectively. The log-transformation was applied as needed.

To visualize changes in microbial community composition in response to vole activity, we performed non-metric multi-dimensional scaling (NMDS) analysis at OTU level based on Bray-Curtis dissimilarity matrices using 'vagan' R package (https://github.com/vegandevs/vegan). A stress level under 0.20 was considered satisfactory (Kuczynski et al., 2011). The Adonis analysis (999 permutations) was used by 'adonis2′ function to test the significance of differences in microbial community composition between treatments. We also used 'betadisper' function to analyze the dispersion in microbial community composition among treatment groups.

The Pearson correlation and Mantel test were used to test the relationship of soil and plant variables with alpha diversity (Shannon index and richness) of soil bacterial and fungal communities using the 'linkET' R package (https://github.com/Hy4m/linkET). The variables that showed significant correlations with bacterial and fungal alpha diversity were included in structural equation models (SEMs) to examine the relationships between treatments, influential factors, and alpha diversity of soil bacteria and fungi. This analysis was carried out by 'piecewiseSEM' R package (Lefcheck, 2016). In the piecewise SEM, mixed models that included the block as a random effect was used by 'lmer' function. The 'scale' function was used to standardize all variables and the alpha diversity was constructed by taking the average of the standardized Shannon index and richness. The goodness of the modelling results was assessed by the Fisher's C test ($0 \leq$ Fisher's $C/df \leq 2$ and

$0.05 < P \leq 1.00$) and Akaike Information Criterion (AIC) (Liu et al., 2020). We excluded non-significant pathways based on the significance ($P < 0.05$) and the goodness of the model, unless they were biologically informative, or added pathways based on the residual correlations. Additionally, we utilized the 'psych' R package (https://CRAN.R-project.org/package=psych) to conduct Spearman correlation analysis and evaluate the relationships between influential factors and soil microbial phyla, as well as functional groups/guilds (with relative abundance > 1 %).

3. Results

3.1. Response of soil properties and plant communities to grazing and vole activity

Vole activity had a significant negative effect on soil dissolved organic carbon, NH_4^+-N, NO_3^--N, and plant aboveground biomass ($P < 0.05$; Table S1, Fig. 2). Additionally, a significant positive effect of vole activity on root-shoot ratio was performed ($P < 0.05$; Table S1, Fig. 2). The interaction of grazing and vole activity had a marginally significant effect on soil total nitrogen and available phosphorus ($P < 0.1$), and a significant effect on microbial biomass nitrogen ($P = 0.038$; Table S1). In the CK treatment, vole activity significantly increased available phosphorus, whereas it decreased in the LG and MG treatments (Table S1, Fig. 2f).

We also assessed the variation in plant community among treatments. We detected a non-significant increasing trend ($P < 0.1$) in Shannon-wiener diversity (Table S1, Fig. 2m). The dominant grass species in all treatments were *Cleistogenes squarrosa*, *Stipa krylovii*, and *Leymus chinensis* (Fig. S1a, Fig. S3). With grazing, the relative biomass of *C. squarrosa* tended to decrease slightly, while the relative biomass of *S. krylovii* showed an increasing trend (Fig. S1a). Vole activity slightly increased the relative biomass and important values of *S. krylovii*, while reducing that of *C. squarrosa*, especially in the MG treatment (Fig. S1).

3.2. Impacts of grazing and vole activity on microbial biodiversity

After quality filtering, 1,872,085 effective sequences were grouped into 16,042 bacterial OTUs and 1,860,470 effective sequences were grouped into 9,569 fungal OTUs. The effects of livestock grazing and vole activity on soil microbial alpha diversity varied between bacteria and fungi. Furthermore, grazing treatments interacted with vole activity, especially for fungal alpha diversity ($P < 0.05$; Table S1, Fig. 3). In the control treatment, no significant variations in soil bacterial and fungal diversity were observed between on-colony and off-colony grasslands (Fig. 3). However, vole activity had a significant effect on the diversity of soil microbes under livestock grazing ($P < 0.05$). Under LG and MG treatment, vole activity had significant negative effects on Shannon index and richness of bacteria. The bacterial Shannon index was reduced by 12.14 % and 23.14 % and bacterial richness was reduced

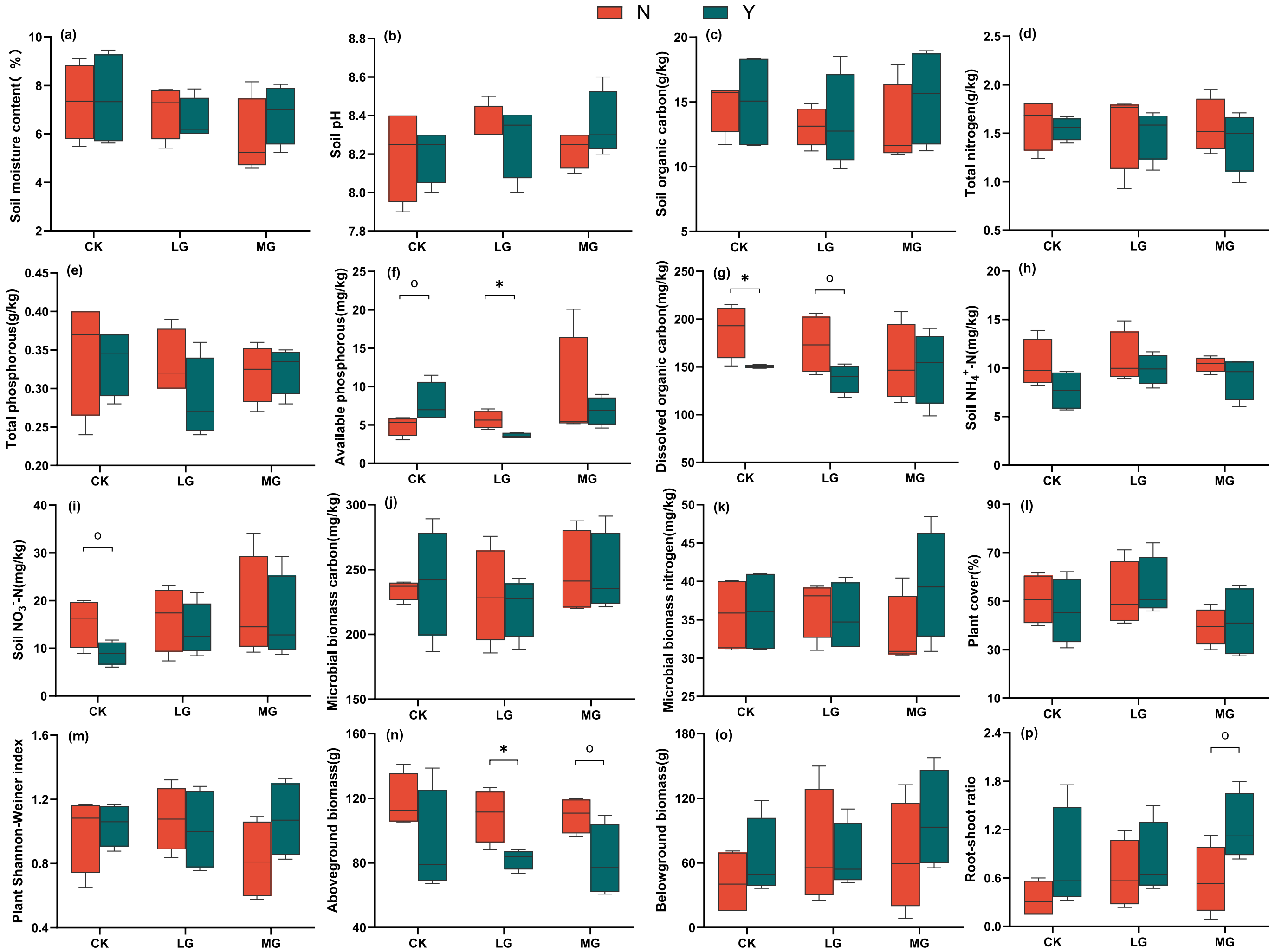

Fig. 2. The effects of vole activity, grazing treatments and their interactions on soil physicochemical properties (a-k) and plant traits (l-p). $^{o}P < 0.1$, $^{*}P < 0.05$, N: off-colony; Y: on-colony.

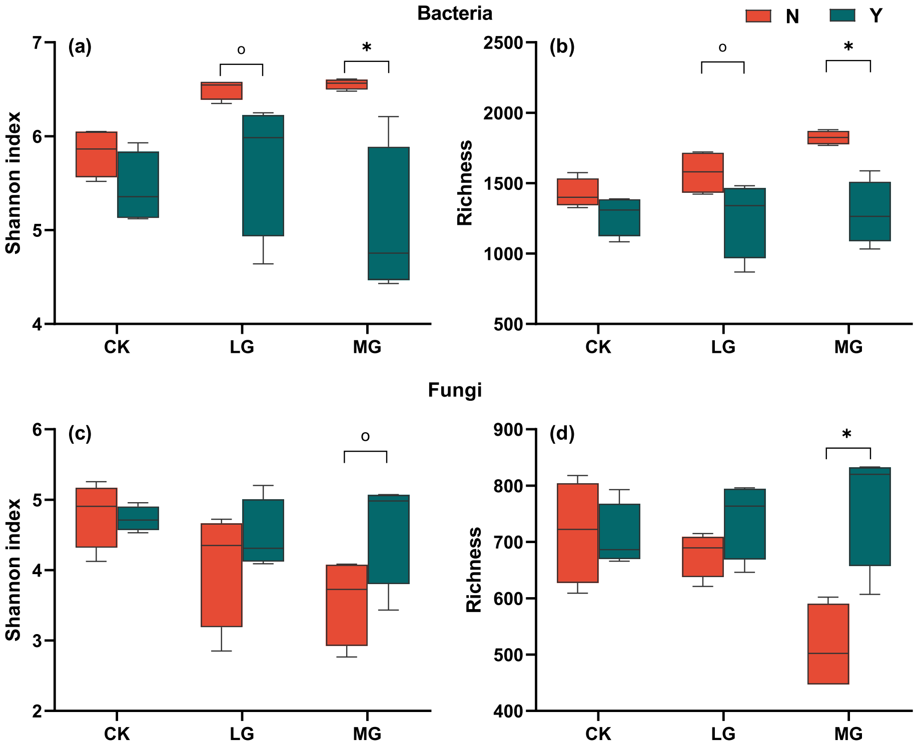

Fig. 3. The effects of vole activity, grazing treatments and their interactions on Shannon's diversity and richness for bacteria (a, b) and fungi (c, d). $^{o}P < 0.1$, $^{*}P < 0.05$, N: off-colony; Y: on-colony. CK: control, LG: light grazing, MG: moderate grazing.

by 20.21 % and 29.42 %, respectively (Fig. 3a-b). Conversely, a significant increase in Shannon index (29.11 %) and richness (50.02 %) of fungi induced by the vole activity was observed under MG treatment (Fig. 3c-d).

3.3. *Microbial community composition in soil and livestock manure*

Livestock grazing and vole activity had different effects on the composition of the soil bacterial and fungal community. In the on-

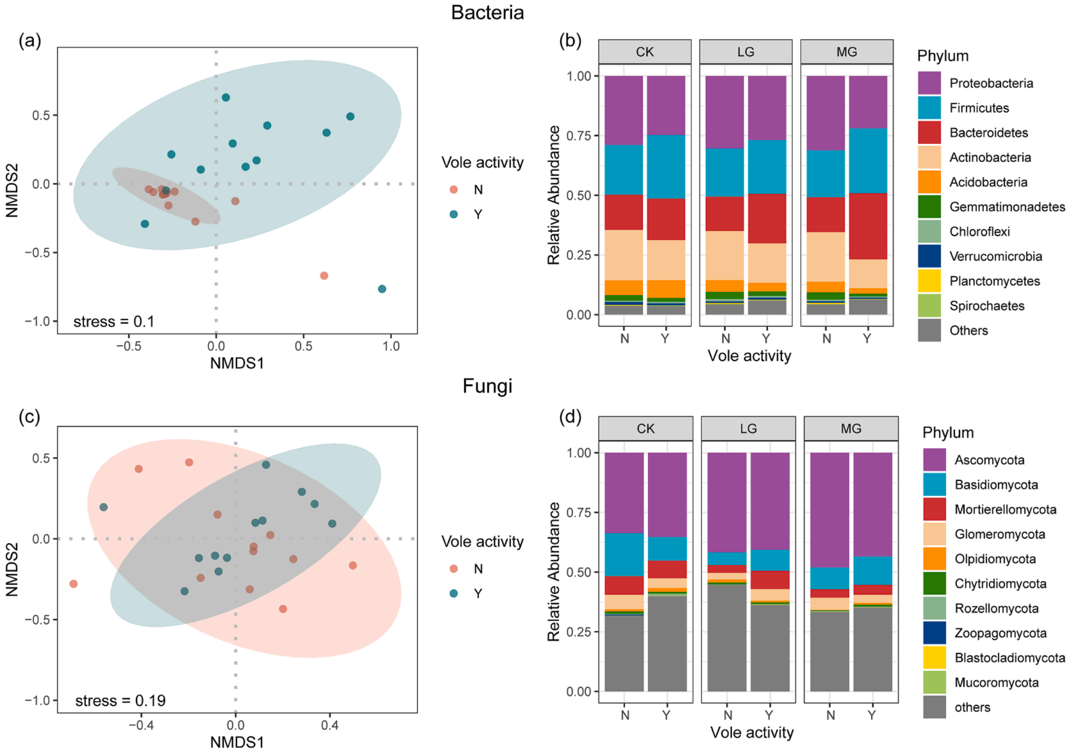

Fig. 4. Community structure and composition for soil bacteria (a, b) and fungi (c, d) in grazing treatments. N: off-colony; Y: on-colony. CK: control, LG: light grazing, MG: moderate grazing.

colony grassland, the soil bacteria community was distinct from that of the off-colony grassland (Adonis, $P < 0.05$, $R^2 = 0.139$; Fig. 4a), whereas the composition of fungal communities was not significantly different among the vole activity (Adonis, $P = 0.11$, $R^2 = 0.051$; Fig. 4c). Bacterial communities showed more highly homogeneous groupings for off-colony grassland (average distance to centroid = 0.343) and more dissimilarity in on-colony grassland (average distance to centroid = 0.436, $P = 0.028$; Table S2). No difference in dispersion between on-colony and off-colony grassland for soil fungal communities ($P = 0.410$; Table S2).

The dominant bacteria at the phylum level (relative abundance > 1 %) included Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, Gemmatimonadetes, and Campilobacterota (Fig. 4b). Livestock grazing significantly decreased the relative abundance of Acidobacteria ($P = 0.022$; Fig. 4b, Table S3). Vole activity, in all grazing treatments, decreased the relative abundance of Proteobacteria, Actinobacteria, and Gemmatimonadetes, while significantly increasing the relative abundance of Firmicutes and Bacteroidetes ($P < 0.05$; Fig. 4b, Table S3). The dominant fungal phyla (relative abundance > 1 %) included Ascomycota, Basidiomycota, Mortierellomycota, and Glomeromycota (Fig. 4d). The relative abundance of these fungal phyla was not significantly changed by grazing and vole activity, except for Glomeromycota (Table S3).

In our experiment, the bacterial taxa found in livestock manure were predominantly Bacteroidetes and Firmicutes, accounting for 57 % of the relative abundance (Fig. S4). Additionally, Fibrobacteres accounted for 8 %, Proteobacteria for 5 %, Spirochaetes for 3 %, and Euryachaeota for 2 %.

3.4. Differences between functional groups of the soil microbial community

In our study, a total of 92 bacterial functional groups were identified for 3,583 OTUs (22 % of the total OTUs) according to FAPROTAX. However, only the ten most redundant groups, with relative abundance exceeding 1 %, were displayed (Table S4). Among these groups, chemoheterotrophy was found to be the dominant metabolic strategy across all bacterial communities, accounting for 30 % to 40 % of the relative abundance. In the LG treatment, vole activity had a marginal-significant effect on reducing the relative abundance of animal parasites or symbionts ($P < 0.1$; Fig. 5). On the other hand, in the MG treatment, fermentation and animal parasites or symbionts showed a significant positive response to vole activity, while aerobic chemoheterotrophy and sulfate respiration showed a significant negative response (Fig. 5). It is worth noting that these ten bacterial functional groups did not show any variation between vole activity conditions in the control treatment.

Stemming from unambiguous guild-annotations in FUNGuild with confidence levels of ‘highly probable’ and ‘probable’, 3,480 (36 %) out of the 9,569 fungal OTUs were categorized into three primary trophic modes (saprotroph, symbiotroph, and pathotroph) and 578 (6.0 %) were assigned to ‘unassigned’. In all treatments, saprotroph (30 %-44 %) was the most dominant group, while pathotroph (9 %-21 %) exhibited the lowest relative abundances. Vole activity significantly increased the relative abundance of pathotrophic fungi in the control and MG treatment, but decreased in the LG treatment (Fig. 5, Table S4).

3.5. Relationship of microbial communities and plant and soil variables

The results of mantel test showed that bacterial diversity was highly correlated with soil water content and plant aboveground biomass, while fungal diversity was closely related to soil water content and plant Shannon diversity ($P < 0.05$, Fig. S2). Then, these three factors were maintained in our structural equation models (SEMs). The final model demonstrated a strong fit to the data, with a model Fisher’s C = 23.217, $P = 0.057$, AIC = 13.752 (Fig. 6a). Our piecewise SEM explained 48 % and 45 % of the variation in bacterial and fungal alpha diversity, respectively. Plant aboveground biomass, which was negatively affected by vole activity (standardized path coefficient $b = -0.62$, $P = 0.002$), played a significant role in directly shaping bacterial alpha diversity ($b = 0.53$, $P = 0.008$). Additionally, fungal diversity was negatively correlated with bacterial diversity ($b = -0.39$, $P = 0.032$), and fungal diversity was also significantly influenced by soil water content ($b = 0.54$, $P = 0.024$). Besides, livestock grazing had non-significant negative impacts on soil water content, plant aboveground biomass, and plant Shannon diversity ($P > 0.05$).

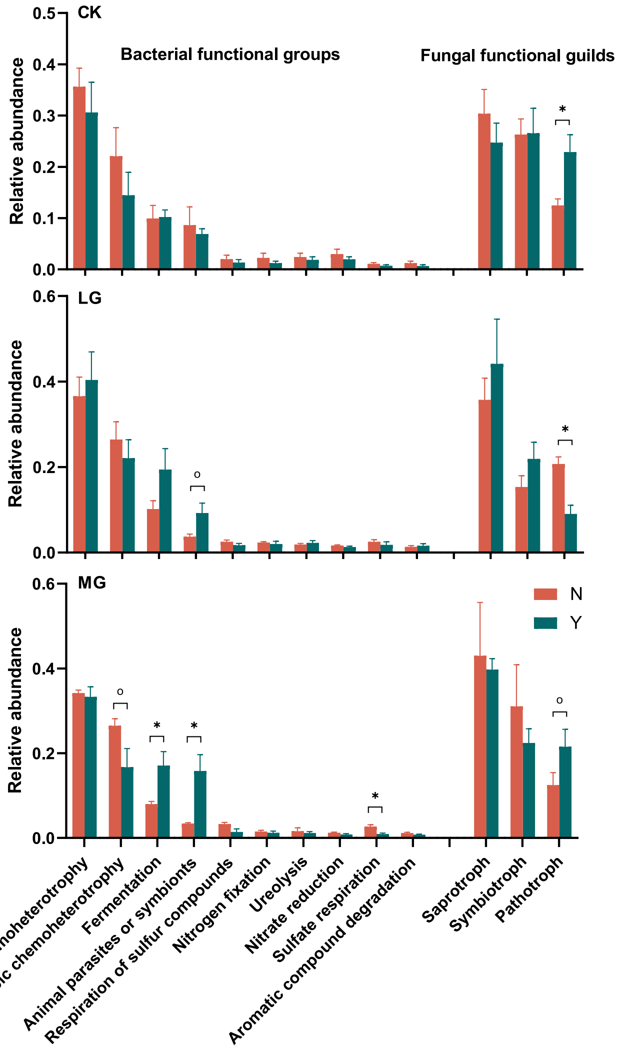

Fig. 5. The relative abundance of the dominant bacterial and fungal functional groups across treatments (mean values ± SE). The asterisk denotes significant differences between vole activity treatments ($^{o}P < 0.1$, $^{*}P < 0.05$). N: off-colony; Y: on-colony. CK: control, LG: light grazing, MG: moderate grazing.

Most bacterial phyla and functional groups showed a strong correlation with plant aboveground biomass (Fig. 6b, Table S5). Specifically, the relative abundance of Campilobacterota negatively associated with several environmental factors, such as soil water content, organic carbon, microbial biomass carbon and microbial biomass nitrogen. Conversely, it exhibited a positive relationship with soil NH_4^+-N, NO_3^--N, and plant aboveground biomass (Fig. 6b, Table S5). The relative abundance of most fungal phyla did not correlate with soil and plant factors, except for phyla Ascomycota and Mortierellomycota (Fig. 6b, Table S5).

4. Discussion

Our results reveal that livestock grazing have a dramatic impact on

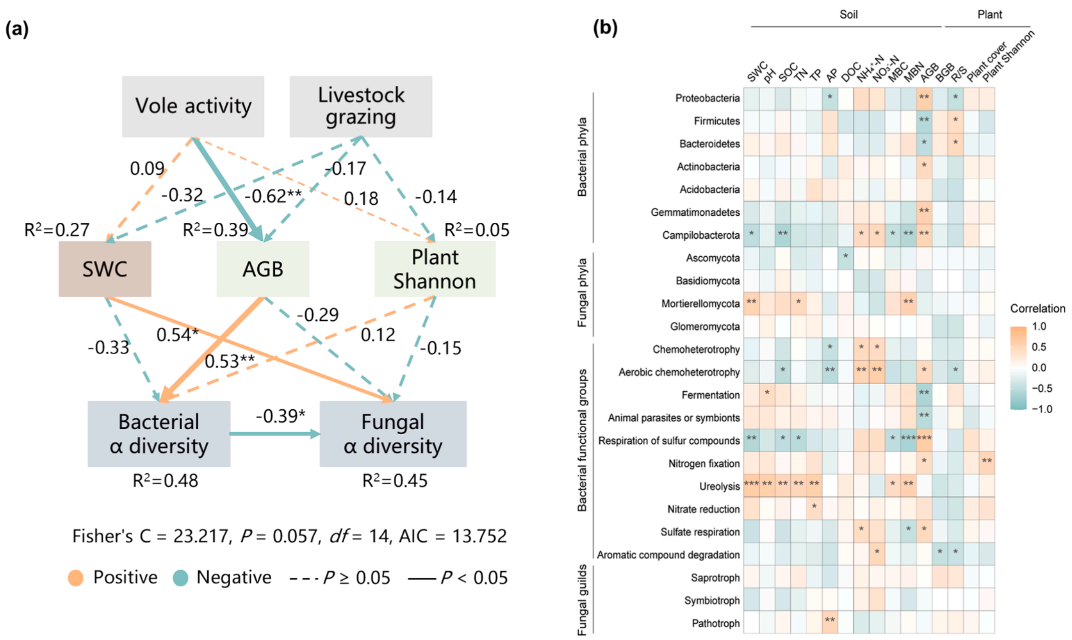

Fig. 6. Driving factors of the soil microbial community under vole activity and grazing treatments. (a) Structural equation models (SEMs) showing the effects of livestock grazing and vole activity on soil bacterial and fungal alpha diversity (include Shannon index and richness) via its effects on soil water content (SWC), plant aboveground biomass (AGB), and plant Shannon-wiener index. Positive effects are indicated by orange lines, and negative effects are indicated by green lines. Solid and dashed lines represent significant ($P < 0.05$) and non-significant ($P \geq 0.05$) relationships, respectively. Numbers adjacent to arrows indicate the standard path coefficients. R^2 represents the proportion of variance explained for every dependent variable. (b) Correlations between plant and soil variables and relative abundance of microbial phyla. Variables include: soil water content (SWC), soil pH, soil organic carbon (SOC), soil total nitrogen (TN), soil total phosphorus (TP), soil available phosphorus (AP), soil dissolved organic carbon (DOC), soil NH_4^+-N and NO_3^--N, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), plant aboveground biomass (AGB), belowground biomass (BGB), plant cover, plant Shannon-wiener index and root-shoot ratio (R/S). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

rodents-induced soil microbial community changes in the temperate steppe. After nine years of manipulative enclosure experiments, the plant community was changed significantly. The dominance of *C. squarrosa* in moderate degraded successional grasslands in the Inner Mongolia Plateau can be weakened by increasing grazing intensity (Hilbig, 1995; Wang and Li, 1999). We observed that vole activity had a negative impact on soil bacterial diversity and positive impact on soil fungal diversity mainly through the reduction of plant aboveground biomass. These changes were more pronounced in the livestock grazing conditions compared to the non-grazing treatments. Grazing also altered the impact of vole activity on microbial functional groups. Additionally, the response of the soil fungal community to vole activity showed greater stability compared to the bacterial community. This study emphasizes the synergistic roles played by the co-existence of livestock and small mammalian herbivores in shaping the structure and function of grassland ecosystems.

Our findings support the idea that small mammalian herbivores exert a significant influence on the distribution and abundance of soil microbes (Aguilera et al., 2016; Eldridge et al., 2016; Yang et al., 2022), specifically under livestock grazing. Bacterial diversity decreased significantly due to vole activity in the LG and MG treatments, while soil fungal diversity was positively affected by vole activity, particularly in the MG treatment (Table S1, Fig. 3). Such different response of microbiota to different livestock grazing intensities suggests that livestock grazing may amplify the effects caused by vole activity on soil microbes. Interestingly, this interactions between voles and livestock on microbial diversity appeared to be driven by the effects of livestock grazing on microbial diversity in the off-colony grasslands. In other words, livestock grazing led to an increase in soil bacterial diversity and a decrease in fungal diversity in off-colony grasslands, while microbial diversity in on-colony grasslands was relatively stable. These results indicated that barrowing rodents and livestock may have antagonistic effects on soil microbial diversity. Rodents, as ecosystem engineers, could mitigate or offset the changes caused by livestock grazing (Zhang et al., 2003a; Yoshihara et al., 2010).

Our piecewise SEM analyses revealed that vole-induced reduction in the bacterial alpha diversity were mainly associated with plant aboveground biomass (Fig. 6a). Notably, vole activity significantly decreased the plant aboveground biomass under livestock grazing (Table S1, Fig. 2n). Burrowing and digging activities of mammalian herbivores can bury vegetation and decrease plant aboveground biomass. Additionally, their foraging for food can also reduce plant biomass (Hagenah and Bennett, 2013). Plant aboveground biomass is an indicator of plant detritus production and significantly affects soil nutrient availability (Hooper et al., 2000; Liu et al., 2010), which in turn influences the diversity of soil microbial communities (Schlatter et al., 2015). In line with this, a reduction in soil nutrient availability, such as soil dissolved organic carbon, NH_4^+-N, and NO_3^--N, was observed (Fig. 2). Soil nutrients are closely linked to bacterial growth, and a reduction in soil nutrients can lead to a decrease in the diversity of soil bacteria (Yang et al., 2019). Furthermore, soil bacterial alpha diversity was negatively related to fungal alpha diversity (Fig. 6a), which could explain the opposite trends in the soil bacterial and fungal diversity under livestock grazing and vole activity. Soil fungi and bacteria may compete for the same resources (Mille-Lindblom et al., 2006), such as nutrients and organic matter, leading to the negative correlation between bacterial and fungal diversity.

As for the soil microbial community structure, the presence of voles significantly increased spatial heterogeneity of soil bacterial communities, as indicated by a higher average distance to centroid for on-colony grassland than off-colony grassland (Fig. 4, Table S2). Specifically, the vole activity caused a decrease in the relative abundance of Actinobacteria, Gemmatimonadetes, and Proteobacteria among bacteria, and an increase in the relative abundance of Bacteroidetes and Firmicutes (Fig. 4b, Table S3). One explanation for these changes in bacterial abundance may be due to the reduction in plant aboveground biomass induced by vole activity. Plant aboveground biomass was positively correlated with the abundance of Actinobacteria, Gemmatimonadetes, and Proteobacteria, while it was negatively related to the abundance of Bacteroidetes and Firmicutes (Fig. 6b). Another explanation could be dependent on the trophic strategies of the microbial phyla (Fierer et al., 2007; Qi et al., 2022). The copiotrophic microbial taxa are usually r-strategists with high nutritional requirements, while oligotrophic microbial taxa usually adopt K-strategy and exhibit slower growth rates (Fierer et al., 2007). Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes were generally considered to be copiotrophs (Fierer et al., 2007; Li et al., 2019b), which have rapid carbon cycling and growth rates, and whose structure and function can be easily affected by disturbance (Qi et al., 2022). Additionally, Firmicutes and Bacteroidetes were found to be the dominant phyla in the gut microbiota of Brandt's vole (Li et al., 2020) and in sheep manure (Fig. S4). This could explain the increased abundance of Firmicutes and Bacteroidetes in response to vole activity, and the slight intensification of rodents-induced changes in these two phyla under grazing conditions. On the other hand, Actinobacteria, which play vital roles in the decomposition of organic matter (Strap, 2011), was found to be more abundant in soils without vole activity. Proteobacteria are involved in autotrophic processes related to sulfur, methane, as well as hydrogen oxidation and sulfate reduction (Zhou et al., 2020). These groups of bacteria were more sensitive to Brant's vole activity in our study, and changes in the abundance of these bacteria can cause variations in microbial function.

We observed that soil dominant microbiome exhibited significant potential in decomposing or degrading various compounds such as carbon, nitrogen, sulfur compounds, and aromatic compounds (Fig. 5, Table S4). The primary functions carried out by bacteria were chemoheterotrophy and aerobic chemoheterotrophy, suggesting that microorganisms primarily obtained nutrition through decomposition of organic matter. Under the non-grazing treatment, vole activity did not induce changes in microbial functional groups. However, significant alterations in the composition of microbial functional groups were observed under grazing conditions. The reason for this inconsistency may be that soil bacteria possess functional redundancy, enabling them to adapt to a certain level of environmental change (Banerjee et al., 2016; Moya and Ferrer, 2016). Nevertheless, the environmental disturbances caused by grazing and vole activity exceeded this threshold, thereby resulting in changes in bacterial functional groups (Shade et al., 2012).

The composition of soil fungal community remains stable despite vole activity, which reinforce the idea that the soil fungal community is more resistant to disturbance compared to soil bacterial community (Fry et al., 2016; Ai et al., 2018). The limited impacts of grazing and vole activity on fungal phyla can be attributed to the rich mycelium of fungal communities, which make them less sensitive to changes from the external environmental, as Schleuss et al. (2019) have suggested. The most dominant fungal phyla in our study were Ascomycota, whose members are predominantly copiotrophic r-strategists and saprophytic organisms capable of decomposing soil organic matter (Yao et al., 2017; Li et al., 2022). In accordance with the predictions made by FUNGuild, most of the fungi observed in our study were also saprophytic organisms (Fig. 5, Table S4), which can play important roles in the soil nutrient acquisition and organic decomposition (Nguyen et al., 2016). Moreover, most of the fungal phyla showed no changes in response to soil and plant factors (Fig. 6a), indicating that the fungal community is relatively stable in this ecosystem. FAPROTAX and FUNGuild annotates bacterial and fungal functions based on information in the literature and databases, so they cannot predict the function of all detected taxa (Sansupa et al., 2021). A low match rate would occur when samples contain many microorganisms that are either not included in the databases or whose functions remain unknown. However, many studies have validated the applicability of FAPROTAX and FUNGuild for functional prediction in soil bacteria and fungi (Liu et al., 2020; Jiang et al., 2021; Wu et al., 2022). The predictive accuracy is subjected to data quality and quantity, and further experimental research is necessary to validate these findings.

5. Conclusion

Our study suggested that livestock grazing exacerbated the influence of vole activity in the grassland ecosystem. Specifically, we found that vole activity reduced soil bacterial diversity and increased fungal diversity, although the significant differences were only observed under grazing conditions. We also found that the soil bacterial community was more sensitive to disturbance than the fungal community. Additionally, the variation in the soil microbial community appeared to be driven by the plant aboveground biomass. Therefore, future increases in livestock grazing intensity in grasslands may exacerbate the loss of soil bacterial diversity induced by rodents, which may have profound impacts on ecosystem functioning. Lower livestock grazing intensities should be adopted to achieve biodiversity conservation and sustainability of ecosystem services and functions. Taken together, this research contributes to a greater understanding of the impact of land-use changes on the interactions between rodents and soil microorganisms in the grassland ecosystem.

CRediT authorship contribution statement

Yunqi Xiong: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Zhenzhen Zheng:** Writing – review & editing, Methodology, Investigation, Data curation. **Baofa Yin:** Project administration, Methodology, Conceptualization. **Guoliang Li:** Writing – review & editing, Supervision, Data curation. **Xinrong Wan:** Supervision, Data curation, Conceptualization. **Ruyan Qian:** Investigation, Data curation. **Linfeng Li:** Writing – review & editing, Formal analysis. **Shuntian Guan:** Investigation, Data curation. **Yuan Liu:** Investigation, Data curation. **Yanfen Wang:** Supervision, Funding acquisition. **Xiaoyong Cui:** Supervision, Project administration, Methodology. **Jianqing Du:** Supervision, Methodology. **Kai Xue:** Supervision, Methodology, Formal analysis. **Yanbin Hao:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2024.117070.

Data availability

The data associated with the manuscript are available in Figshare at https://doi.org/10.6084/m9.figshare.23593617. Soil microbial sequences have been archived in NCBI's SRA database (PRJNA1139399).

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