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RESEARCH ARTICLE

Plant and soil responses to grazing intensity drive changes in the soil microbiome in a desert steppe

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Abstract

Background and aims Grazing pressure can degrade environmental quality and disrupt ecosystem structure and functions, while its potential effect on the soil microbiome is unclear.

Method We evaluated the effects of grazing intensity (CK: no grazing, LG: light grazing, MG: moderate grazing, HG: heavy grazing, and OG: overgrazing) on soil microbial diversity and community composition in a desert steppe.

Results Different microbial communities were found under different grazing intensities, resulting from differences in soil moisture, nutrients and plant species. Alpha-diversity in the bacterial community was strongly correlated with soil organic content (SOC) and soil water content, while the alpha-diversity of the fungi depended on the SOC and pH of the soil. Grazing treatments LG, HG and OG caused strong shifts in bacterial and fungal community composition. Heavy grazing (HG and OG) significantly increased the relative abundances of Chloroflexi, Gemmatimonadetes, and Firmicutes bacteria, while light grazing (LG) significantly decreased the relative abundance of Actinobacteria. Grazing intensities HG and OG increased the relative abundances of certain fungi (e.g., Ascomycota). Co-occurrence network analysis indicated that bacterial communities had a more complex network than fungal communities. A multivariate regression tree demonstrated that the bacterial community responded to grazing via changes in the biomass of perennial plant species and SOC, whereas the SOC and pH value altered the fungal community composition.

Conclusions Our findings indicate that different grazing intensities can initiate different changes in the soil microbiome; sustainable grazing intensity over

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decades facilitates the recovery of primary productivity and ecosystem functions in a desert steppe.

Keywords Alpha diversity · Bacterial communities · Fungal communities · Soil-plant-herbivore system · Soil fertility · Stocking rate

Introduction

Grazing used to be a common land use strategy to maintain plant species diversity and the functioning of grassland ecosystems (Han et al., 2008; Wang et al. 2014; Lü et al. 2015). Grazing can affect the plant community through the consumption of plant material by livestock and can influence the soil microbial community structure and functioning by changing the soil's physicochemical properties (Huhe et al. 2017; Martensson and Olsson 2012). Improper land management, particularly overgrazing, has pronounced negative effects on the structure and functioning of grassland ecosystems (Bai et al. 2012). Overgrazing is widely recognized as the primary contributor to the degradation of arid and semi-arid grasslands worldwide (Zhang et al. 2021). Therefore, an increasing number of studies have focused on restoring grassland ecosystems by changing management practices (e.g., grazing intensity and grazing exclusion) (Hu et al. 2016; Wang et al. 2018). However, in the soil-plant-herbivore system, linkages between the responses of plants, soil and microorganisms to grazing intensity, especially the timing and duration of grazing, are still unclear.

Grazing intensity can directly and indirectly affect plant, soil and soil microbial communities through the deposition of faeces and urine, as well as the trampling and removal of plant parts. Deposition of animal excreta (faeces and urine) directly increases the soil carbon (C) content and enhances nitrogen (N) availability (Kohler et al. 2005). Animal trampling causes soil compaction, thus changing the soil air permeability and soil water potential, which subsequently affect soil microbial community composition and functioning (Yang et al. 2013). Previous studies showed that animal trampling has a major impact on denitrifying soil microbiota, which stimulates denitrification (Treweek et al. 2016). In addition, previous studies have demonstrated that the removal of aboveground plant parts through intake by livestock directly causes shifts in the plant community composition from dominant perennial grasses (e.g., C_3 plant species) to forbs (e.g., C_4 plant species). Such shifts change primary production (Augustine et al. 2017) and also influence the quantity and quality of root exudates and litter (i.e., C inputs into the soil) (Bardgett and van der Putten 2014). Thereby, these shifts directly affect the microbial community structure and functioning (Mueller et al. 2017). For example, proper grazing (e.g., light or moderate grazing) increases plant community production and accelerates nutrient cycling by stimulating compensatory growth, enhancing the dominance of species with higher nutrient concentrations and favoring soil bacteria (Bardgett et al., 1998). In contrast, overgrazing has pronounced negative effects on plant community composition, decreases species diversity and increases the abundance of species with low-quality litter, thus favoring soil fungi (Wang et al. 2014; Yang et al. 2013). The effects of grazing intensity on the extent to which changes in the plant community and soil properties mediate the soil microbiome under grazing are unknown. In addition, the cumulative effects of continuous heavy grazing or overgrazing change the soil microbial community (Wang et al. 2021), and there is little empirical information on the role of the soil microbial community in the temporal progression of recovery following a reduction in grazing intensity.

Grazed arid ecosystems comprise around 30% of the earth's terrestrial surface (Hille Ris Lambers et al. 2001). Desert grassland accounts for 39% of the native grassland area in Inner Mongolia, and most of this grassland has been degraded due to overgrazing (Li et al. 2000). Grazing-mediated effects on plant community characteristics and soil properties can in turn impact the microbial community composition (Egelkraut et al. 2017). Understanding the responses of desert steppes to grazing intensity is crucial for predicting future ecosystem services and for developing sustainable management practices. Here, we monitored vegetation change, soil physicochemical properties and soil microbial composition and functioning to evaluate the interaction between vegetation, soil chemistry and the soil microbial community under different levels of grazing intensity. We hypothesized that (1) grazing intensity changes the soil bacterial and fungal community compositions by altering the species diversity of microorganisms and the abundance of specific phyla or genera; and (2) the effect of grazing intensity on the soil microbiome is dependent

on the changes in soil properties and plant characteristics; and (3) appropriate grassland management can boost soil fertility and plant productivity by promoting particular groups of bacteria and fungi.

Materials and Methods

Site description

Our study was conducted in a desert steppe, located in Xisu Banner (42°47′N, 112°40′E), Inner Mongolia, P. R. China. The mean annual temperature at this site is 4.9°C, and the mean annual precipitation is 180.3 mm. More than 80% of the rainfall occurs from June to September. The soil type of the site is classified by the Food and Agricultural Organization (FAO) of the United Nations as a Haplic Calcisol with a sandy loam texture (Zhang et al. 2020). The basic soil fractions of the study areas were 8.6% clay, 21.6% silt, and 69.8% sand based on the hydrometer method (Kettler et al. 2001). Dominant vegetation species in this desert steppe are the C_3 grass *Stipa klemenzii* and the C_4 grass *Cleistogenes songorica* (Roshev.) Ohwi; moreover, 19 subordinate plant species were found at this site (Table S1).

Experimental design

The experiment was established in 2008 as a complete randomized block design with 5 treatments and 3 replicates. There were a total of 15 plots, and each plot was 16 hectares. The five grazing intensities were 0 sheep ha^{-1} (no grazing; CK), 0.375 sheep ha^{-1} (low grazing intensity; LG), 0.500 sheep ha^{-1} (moderate grazing intensity, MG), 0.625 sheep ha^{-1} (high grazing intensity; HG), and 0.750 sheep ha^{-1} (overgrazing; OG). Grazing occurred from May to October every year (from 2008 until 2020) on each day (the sheep stayed in the plots for the entire day) during the grazing period. Two-year-old wethers were selected for this study; every three years these wethers were replaced by new 2-year-old wethers.

Plant community and soil properties

In 2020, nine movable cages (1.5 m × 1.5 m) were placed randomly within each plot before grazing, and 1 m^2 quadrats (one quadrat per cage) were sampled to assess the plant community aboveground net primary productivity (ANPP), with a total of 9 × 5 × 3 = 135 quadrats. At the end of August, all herbaceous plants were harvested to ground level after litter removal within the 1 m^2 quadrat of each of the 135 cages, and then plant biomass was oven-dried at 65°C for 48 h (Wang et al. 2014). ANPP was calculated as the sum of the aboveground biomass for all plant species (Wang et al. 2016). Species richness was calculated as the number of species in a quadrat. The dominant species (DS) were assessed as the biomass of the dominant species in a quadrat, and the subordinate plant species biomass (SS) was assessed as the biomass of subordinate species in a quadrat (Zhang et al. 2018).

Soil samples (0–15 cm layer) were collected from nine different random sites per plot by collecting three soil cores with a diameter of 3.5 cm diameter per site. The nine soil samples per plot were combined to form one composite sample per plot. The roots, stones, litter, and debris from each composite soil sample were removed using a 2 mm sieve, stored in the field and transported to the laboratory on ice in a cooler. Each composite soil sample from each plot was then divided into three subsamples. One subsample was air dried to measure the physicochemical characteristics, the second subsample was stored at 4 °C and analyzed within one week to determine the soil ammonium (NH_4^+) and nitrate (NO_3^-) contents, microbial C and N biomass, and soil enzymatic activities, and the third subsample was immediately stored at −80 °C for DNA extraction. The soil pH was determined using a 1:2.5 soil: water mixture. The soil water content (SW) was determined using the gravimetric method. Soil organic carbon (SOC) was measured by the dichromate oxidation method (Nelson and Sommers, 1996), and total nitrogen (TN) was measured using an Elemental Analyzer (vario MACRO cube, Elementar, Germany). A 2 M KCl solution was used to extract soil ammonium (NH_4^+) and nitrate (NO_3^-), followed by analysis with an FIAstar 5000 (FOSS Analytical, Höganäs, Sweden). An Astoria auto-analyzer was used to measure the total P (TP) concentration (Clackamas, OR). The available phosphorus (AP) content was measured using the Olsen method (Yang and Jacobsen 1990). The fumigation extraction method was

used to measure the soil microbial C and N biomass (Vance et al., 1987).

Assays of soil enzyme activities

The potential activities of C-acquiring enzyme (BG: β-1,4-glucosidase), N-acquiring enzyme (NAG: β-1,4-N-acetyl-glucosaminidase), and P-acquiring enzyme (AKP: alkaline phosphatase) were determined in fluorometric assays according to the protocols proposed by Saiya-Cork et al. (2002). The activities of the three enzymes in one soil sample were determined in one black 96-well microplate. The enzyme activities were measured using 1 g of fresh soil, which was placed into 125 ml of deionized water and homogenized for 2 h at 180 r.min^{-1} on a rotary shaker (25 °C). Next, 200 µL of each sample was placed in a 96-well microplate (six parallel subsamples for each sample) before adding 50 µL of the 200 µmol L^{-1} substrate. In addition, 50 µL of deionized water and 200 µL of each sample were placed into blank microplates. We added 50 µL of standard substrate (4-MUB at 10 µmol L^{-1} for BG, , NAG, and AKP) and 200 µL of sample suspension to the quenched standard microplates. The negative control microplates contained 50 µL of substrate and 50 µL of deionized water. We added 50 µL of standard material and 200 µL of deionized water to the microplates. The blank and negative control for each sample comprised three parallel samples. The quenching standard and reference standard comprised six parallel samples for each micro-plate. The 96-well microplates were incubated in the dark at 25°C for 4 h. The 4-MUB fluorescence excitation and detection wavelengths were 365 and 450 nm, respectively (German et al. 2011).

The calculation formulas of soil enzyme activity are as follows (DeForest 2009):

$$\begin{aligned} A\,b &= F \times V/\left(e \times V_1\ V1 \times t \times m\right),\\ F &= \left(f - f_b\right)/q - f_s,\\ e &= f_r/\left(c_s \times V_2\right),\\ q &= \left(f_q - f_b\ \right)/f_r. \end{aligned}$$

A_b is the enzyme activity of the soil sample (nmol·g^{-1}·h^{-1}); F is the corrected sample fluorescence value; V is the total volume of the soil suspension (125 mL); V_1 is the volume of the sample suspension added to each well of the microplate (0.2 mL); t is the dark incubation time (4 h); m is the mass of the dry soil sample (the result of converting 1 g of fresh soil sample to dry soil sample); f is the fluorescence value of the microwell for the sample read by the microplate reader; f_b is the fluorescence value of the blank microwell; q is the quenching coefficient; f_s is the fluorescence value of the negative control microwell; e is the fluorescence release coefficient; f_r is the fluorescence value of the reference standard microwell; C_s is the concentration of the reference standard microwell (10 µmol·L^{-1}); V_2 is the volume added to the reference standard (0.05 mL); and f_q is the fluorescence value of the quenched standard microwell.

Soil DNA Extraction and Sequencing

Microbial community genomic DNA was extracted from soil samples using the Fast DNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions and stored at −20°C prior to further analysis. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The hypervariable V3–V4 region of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The fungal ITS1 region was amplified using the primers ITS5 (50-GGAAGTAAAAGTCGT AACAAGG-30) and ITS2 (50-GCTGCGTTCTTC ATCGATGC-30) (Bellemain et al. 2010). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 µl of Q5 reaction buffer (5×), 5 µl of Q5 High-Fidelity GC buffer (5×), 0.25 µl of Q5 High-Fidelity DNA Polymerase (5 U/µl), 2 µl (2.5 mM) of dNTPs, 1 µl (10 uM) of each forward and reverse primer, 2 µl of DNA template, and 8.75 µl of ddH_2O. Thermal cycling for both 16S and ITS consisted of initial denaturation at 98 °C for 2 min, followed by 25 cycles consisting of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 5 min at 72 °C. PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit

(Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and was quantified using a Quantus™ Fluorometer (Promega, USA).

Purified amplicons were pooled in equimolar amounts and were paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Shanghai Personal Biotechnology Co., Ltd (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Bacterial accession number: PRJNA779826; Fungal accession number: PRJNA779837). After sequencing, 250-bp paired-end reads were generated and assigned to the sample based on its unique barcode sequence, and then the barcode and primer sequence were cut. We used FLASH (version 1.2.7) to merge paired-end reads (Magoč and Salzberg 2011). According to the QIIME quality control process (version 1.7.0) (Caporaso et al. 2010), quality filtering was performed to obtain only high-quality clean tags (Bokulich et al. 2013). Operational taxonomic units (OTUs) with 97% similarity cut-off were clustered using UPARSE version 7.1 (Stackebrandt and Goebel 1994; Edgar 2013), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al., 2007) against the SILVA (bacteria; Edgar 2010) and UNITE (fungal; Zheng et al. 2018) databases using a confidence threshold of 0.7. We normalized the OTU abundance information using a standard sequence number, which corresponded to the sample with the lowest number of sequences (46,820 for bacteria and 47,432 for fungi).

Co-occurrence network analysis

We used genera to perform co-occurrence network analysis and key taxa selection for microbial (bacterial and fungal) communities as described previously (Banerjee et al. 2019). The co-occurrence pattern in the microbial community was evaluated by network analysis using the maximum information coefficient (MIC) scores in the MINE statistics of the *minerva* package (Reshef et al. 2011). MIC is an insightful score that shows the positive, negative, and non-linear relationships among genera. Network analysis was conducted only on genera that were present in at least two samples to minimize pairwise comparisons. MIC correlation is associated with the false discovery rate (FDR), and the final network construction has a relationship that is statistically significant ($p < 0.05$) after FDR correction. The networks were visualized using Cytoscape version 3.7.2 (Shannon et al. 2003). The network only showed the most important interactions, including strong positive ($r > 0.8$), strong negative ($r < -0.8$) and strong nonlinear (MIC > 0.8) relationships. The *NetworkAnalyzer* tool was used to evaluate the network topology parameters (Banerjee et al. 2019). The degree indicates the number of edges connected to the node. The highest connectivity among nodes in a specific area of the network is the clustering coefficient, while the shortest path represents the speed of information propagation between two nodes. The network diameter is the maximum distance between two nodes in the network. The genera with both the highest degree and highest closeness centrality were considered to be the keystone taxa (Berry and Widder 2014). Closeness centrality reflects the central importance of the node in disseminating information as the average shortest path. In addition, the centrality reveals the role of nodes as bridges between network components. For the overall network, the bacterial genera with the highest degree (≥ 14) and the highest closeness centrality (> 0.45) scores were considered to be the keystone bacterial taxa, and the fungal genera with the highest degree (≥ 5) and highest closeness centrality (> 0.29) scores were considered to be the keystone fungal taxa. For networks with different aggregation scores, the keystone taxa were selected for bacterial genera with a degree higher than 10 and close centrality higher than 0.35, and the keystone taxa were selected for fungal genera with a degree higher than 4 and close centrality higher than 0.24. A set of cut-off values was selected for consistent comparison across the aggregation network. We also calculated the proportional influence of various phyla by dividing the number of nodes belonging to a particular phylum by the number of connections it shared in the network structure (Banerjee et al. 2019).

Statistical Analysis

The plant characteristic data in the 9 quadrats of 1 m × 1 m per plot were averaged before statistical analysis. Soil samples were pooled per plot before analysis. All statistical analyses were conducted using R software (version 3.5.1). Univariate analysis of variance (ANOVA) was used to

determine the effects of different grazing intensities on plant characteristics (plant functional group, ANPP and diversity), soil physicochemical metrics (SOC, TN, TP, NH_4^+, NO_3^- and AP), soil enzymatic activities (β-1,4-glucosidase (BG), β-1,4-Nacetylglucosaminidase (NAG) and alkaline phosphatase (AKP)), soil microbial characteristics (C and N biomass, and diversity), bacterial 16S rRNA abundance and fungal ITS abundance. Microbial diversity was examined using diversity metrics to analyze species diversity with QIIME, including total OTUs, Chao1, Shannon's diversity, Simpson diversity, Pielou_e, and Goods_coverage, and was visualized using R software (version 3.5.1). Chao1 and Observed species indices were used to characterize richness and Shannon and Simpson indices were used to characterize diversity. Evolution-based diversity was characterized by Faith's PD index, evenness by Pielou's evenness index, and coverage by Good's coverage index. Tukey's honestly significant difference (HSD) was used to test mean comparisons. Statistical significance was defined as the *P* value in Tukey's HSD and was corrected for the Benjamini-Hochberg false discovery rate.

Multivariate PERMANOVAs were performed to examine the effect of grazing intensity on the soil bacterial and fungal community composition based on the Bray–Curtis distance. Principal coordinate analyses (PCoAs) based on the Bray–Curtis distance were performed to assess the differences in the composition of the microbial communities among different grazing treatments. Dissimilarity in the bacterial and fungal community composition between pairwise grazing intensities was examined by multivariate PERMANOVA using the VEGAN package in the R software (version 3.5.1). We uploaded the fungal OTU taxonomic information to FUNGuild for functional prediction (Nguyen et al. 2016). Pearson correlations between the abundance of soil properties and α-diversity, and between the abundance of arbuscular mycorrhizal fungi and perennial plant biomass and ANPP were calculated. Heat maps were used to show the relationships between the relative abundances of the different taxa in the soil bacterial and fungal communities and plant and environmental variables (plant functional groups, plant characteristics and soil properties) and microbial C and N biomass. A multivariate regression tree (MRT) analysis was used to evaluate the most important biotic and abiotic factors for bacterial and fungal alpha diversity pattern (total OTUs, Chao1, Shannon's diversity, Simpson diversity, Pielou_e, and Goods_coverage) and community composition using the mvpart package (De' Ath 2002).

Results

Plant and soil properties

Compared with the CK, the ANPP, dominant species biomass, and biomass of *S. klemenzii* and *C. songorica* and species richness under LG increased by 29.3%, 86.3%, 113.6%, 60.9%, and 9.3%, respectively ($P < 0.05$, Table 1). Both HG and OG significantly reduced the ANPP, dominant species biomass, biomass of *S. klemenzii* and of *C. songorica* and species richness, compared with no grazing ($P < 0.05$, Table 1). The biomass of *Salsola collina* was higher under MG, HG and OG than under CK ($P < 0.05$, Table 1).

SOC, TN and TP were lower under HG and OG than under CK ($P < 0.05$, Table 1). The SOC was 9.58 g kg^{-1} under CK, 21.2% higher under LG, 28.8% lower under HG and 19.9% lower under OG ($P < 0.05$, Table 1). Similarly, the TN was 0.76 g kg^{-1} and TP was 0.24 g kg^{-1} under CK; the respective TN and TP were 17.1% and 4.2% higher under LG, 15.8% and 9.2% lower under HG, and 9.2% and 8.3% lower under OG ($P < 0.05$, Table 1). The soil NH_4^+ content was lower in LG and HG than in CK, but the soil NO_3^- content was higher in HG than in CK ($P < 0.05$, Table 1). Compared with CK, MG significantly increased the soil TN content ($P < 0.05$, Table 1).

Soil microbial biomass and enzymatic activities

Soil microbial C biomass and soil microbial N biomass were higher under MG than under CK, but lower in both HG and OG ($P < 0.05$, Table 1). The soil enzyme activities, including those of BG, NAG, and AKP, were 25.9%, 67.3%, and 70.2% higher, respectively, under OG than under CK ($P < 0.05$, Table 1). Compared with CK, the BG and NAG decreased by 39.1% and 63.2%, respectively, under MG ($P < 0.05$, Table 1).

Table 1 Effects of different grazing intensities on plant, soil and microbial characteristics

	CK	LG	MG	HG	OG	*F*	*P* value
ANPP (g m^{-2})	78.91b	102.03a	76.32b	63.60c	65.02c	**54.29**	**<0.001**
Dominant species (g m^{-2})	27.76b	51.71a	27.78b	12.08c	9.74c	**86.11**	**<0.001**
Subordinate species (g m^{-2})	51.15	51.30	48.82	51.52	55.28	1.08	0.375
S. klemenzii (g m^{-2})	13.42b	28.66a	15.96b	6.60c	5.70c	**50.84**	**<0.001**
C. songorica (g m^{-2})	14.33b	23.05a	11.82b	5.48c	4.04c	**35.22**	**<0.001**
S. collina (g m^{-2})	22.16b	20.96b	30.57a	34.03a	36.43a	**8.27**	**<0.001**
Species richness	6.00b	6.56a	5.22bc	5.89bc	5.00c	**3.31**	**0.019**
SW (%)	2.17b	3.09a	2.03b	2.40b	2.36b	**6.75**	**<0.001**
pH	7.66c	8.04a	8.00b	7.83b	7.60c	**18.16**	**<0.001**
SOC (g kg^{-1})	9.58b	11.61a	9.89b	6.82c	7.67c	**17.67**	**<0.001**
TN (g kg^{-1})	0.76c	0.89a	0.82b	0.64c	0.69c	**19.05**	**<0.001**
C/N ratio	12.61b	13.06a	12.07b	10.57c	11.09c	**10.95**	**<0.001**
TP (g kg^{-1})	0.24b	0.25a	0.23b	0.21c	0.22b	**7.34**	**<0.001**
NH_4^+ (mg kg^{-1})	1.28a	0.77c	1.41a	0.77bc	1.21ab	**3.70**	**0.012**
NO_3^- (mg kg^{-1})	2.49b	2.50b	2.31b	3.94a	2.66b	**5.41**	**0.001**
AP (mg kg^{-1})	4.26b	3.81b	4.23ab	4.72a	4.37a	**3.13**	**0.025**
Microbial C (mg kg^{-1})	121.09b	129.83b	175.67a	87.07c	95.56c	**37.01**	**<0.001**
Microbial N (mg kg^{-1})	4.93b	5.11ab	5.56a	3.56c	3.28c	**22.17**	**<0.001**
BG (nmol g^{-1} h^{-1})	822.29b	471.23c	500.75c	880.62ab	1035.30a	**13.87**	**<0.001**
NAG (nmol g^{-1} h^{-1})	119.44b	70.94b	43.92c	171.65a	199.79a	**35.85**	**<0.001**
AKP (nmol g^{-1} h^{-1})	395.22bc	191.83d	309.98c	465.66b	672.80a	**19.78**	**<0.001**

For each parameter, a different letter indicates a significant difference at the 0.05 probability level ($P < 0.05$) based on Tukey's honest test. *F* and *P* values in bold show statistically significant differences.

CK: no grazing, LG: light grazing, MG: moderate grazing, HG: heavy grazing, OG: overgrazing.

Note: ANPP, above-ground net primary productivity; SW, soil water content; SOC, soil organic carbon content; TN, soil total nitrogen content; TP, soil total phosphorus content; AP, soil available phosphorus content; BG, β-1,4-glucosidase; NAG, β-1,4-Nacetylglucosaminidase; AKP, alkaline phosphatase.

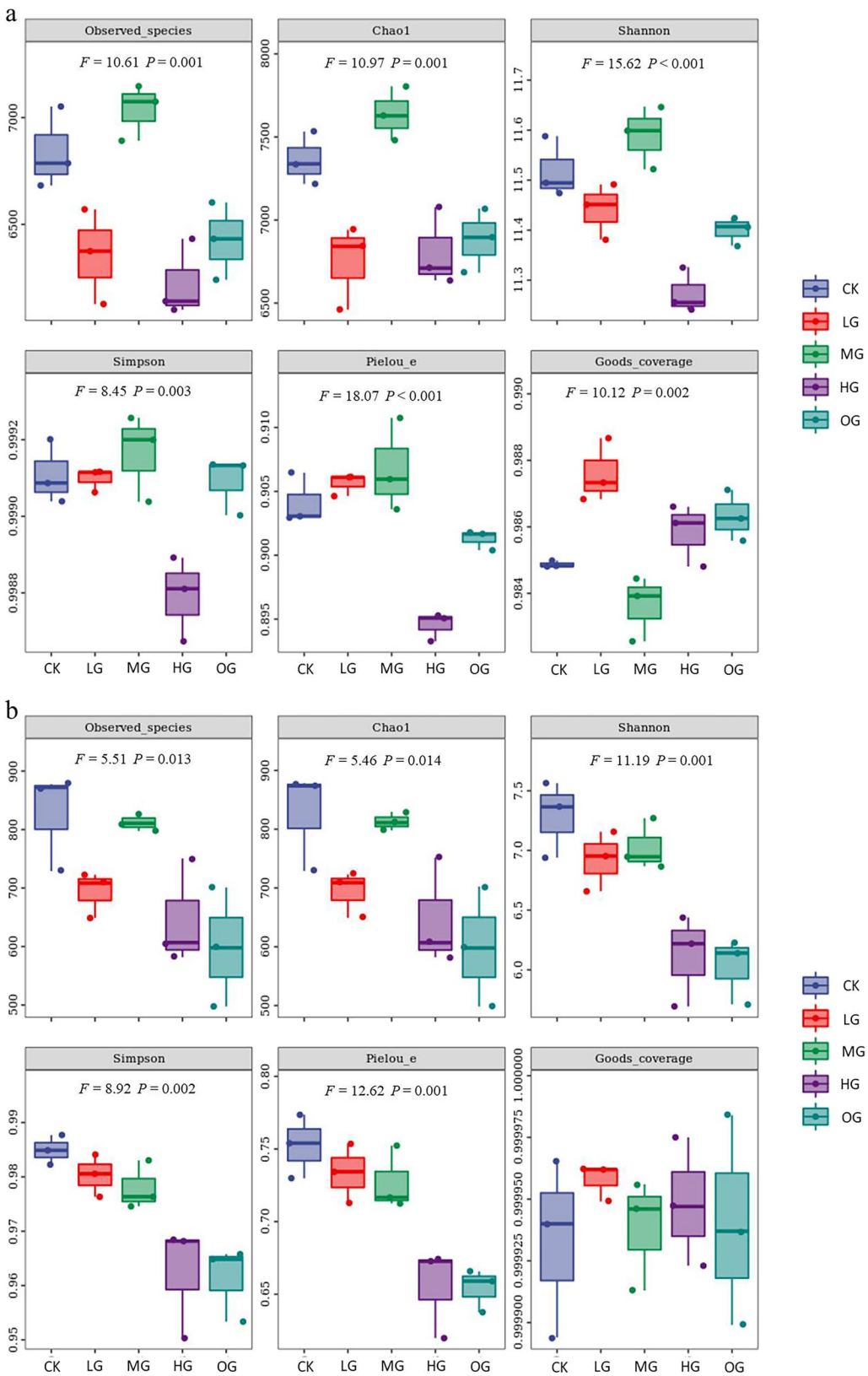
Soil microbial α-diversity

Bacterial alpha diversity was lower in HG than in CK, illustrated by lower OTU richness, Chao1, Shannon and Pielou_e diversity ($P < 0.05$, Fig. 1a). The Simpson index was lower under HG than under CK ($P < 0.05$, Fig. 1a). The fungal alpha diversity showed a similar pattern of changes: HG and OG had a lower fungal alpha diversity than CK, illustrated by lower values for OTU richness, Chao1, Simpson, Shannon and Pielou_e diversity ($P < 0.05$, Fig. 1b).

Soil bacterial community structure and composition

The PCoA ordination showed that the bacterial community composition (beta-diversity) was clearly different under the different grazing intensities (Fig. 2a), which was statistically supported by the Adonis (PERMANOVA) test ($R^2 = 0.55$, $P < 0.001$). Dominant bacterial phyla were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Firmicutes, Nitrospirae and Cyanobacteria, which accounted for more than 95% of the total relative bacterial abundance (Fig. 3). Compared with CK, the relative abundances of Actinobacteria and Cyanobacteria were 13.4% and 68.6% higher, respectively, under LG, whereas the relative abundance of Actinobacteria was 32.3% lower under CK than under LG ($P < 0.05$, Fig. 3). The relative abundance of Chloroflexi was higher under HG and OG than under CK ($P < 0.05$, Fig. 3). The relative abundance of Gemmatimonadetes was 15.5% lower in LG than in CK ($P < 0.05$, Fig. 3).

Compared with CK, the relative abundance of *Rubrobacter*, *67-14*, *Solirubrobacter*, and


a
Observed_species
F = 10.61 P = 0.001
Chao1
F = 10.97 P = 0.001
Shannon
F = 15.62 P < 0.001
Simpson
F = 8.45 P = 0.003
Pielou_e
F = 18.07 P < 0.001
Goods_coverage
F = 10.12 P = 0.002
CK
LG
MG
HG
OG
b
Observed_species
F = 5.51 P = 0.013
Chao1
F = 5.46 P = 0.014
Shannon
F = 11.19 P = 0.001
Simpson
F = 8.92 P = 0.002
Pielou_e
F = 12.62 P = 0.001
Goods_coverage
CK
LG
MG
HG
OG

◂**Figure 1** Responses of the alpha diversity of bacteria (Fig.1a) and fungi (Fig. 2b) to different grazing intensities. Results reported as the mean ± standard error (n = 3). For each parameter, a different letter indicates a significant difference at the 0.05 probability level ($P < 0.05$) based on Tukey's honest test. Alpha diversity indices include Observed_species, Chao 1, Shannon, Simpson, Pielou_e, and Good_coverage. CK: no grazing, LG: light grazing, MG: moderate grazing, HG: heavy grazing, OG: overgrazing

Conexibacter in LG had significantly increased by 19.4%, 41.6%, 18.0%, and 108.2%, respectively ($P < 0.05$, Table S2). The relative abundance of *Solirubrobacter*, *Streptomyces*, *Actinophytocola*, and *Mycobacterium* was higher in MG than in CK, while the MG significantly decreased the relative abundance of *Geodermatophilus*, *Modestobacter*, and *Craurococcus* ($P < 0.05$, Table S2). The relative abundance of *Blastococcus* was higher in both HG and OG than in CK ($P < 0.05$, Table S2). The CoNet results showed that the total bacterial network was composed of 48 nodes and 122 significant edges (Fig. 4a). The clustering coefficient was 0.332, and the average number of neighbors was 5.083 (Fig. 4a). Three keystone taxa included the genera *Rubrobacter* and *Geodermatophilus*, and *Gitt_GS_136*. The core groups included *Rubrobacter* and *Geodermatophilu*s belonging to Actinobacteria, and *Gitt_GS_136* belonging to Chloroflexi. Other taxa identified belonged to Actinobacteria, Proteobacteria, Acidobacteria, Rokubacteria, Gemmatimonadetes, Entotheonellaeota and Firmicutes.

Soil fungal community structure and composition

As was the case for the bacterial community composition, the PCoA also showed that the fungal community composition was clearly different for the different grazing intensities based on the Adonis (PERMANOVA) test ($R^2 = 0.53$, $P < 0.001$). Grazing intensities HG and OG had similar relative abundances of Ascomycota, which were higher than that of CK ($P < 0.05$, Fig. 3). The relative abundance of Mortierellomycota was 114.3% higher in HG than in CK, while the relative abundance of Glomeromycota was 47.0% higher in MG than in CK ($P < 0.05$, Fig. 3).

The relative abundance of *Didymella*, *Comoclathris* and *Filobasidium* was higher in LG than in CK (increased by 399.0%, 433.3%, and 533.3%, respectively), while LG significantly decreased the relative abundance of *Cercophora* and *Fusarium* by 72.6% and 49.5%, respectively ($P < 0.05$, Table S3), compared with CK. The relative abundance of *Darksidea* was the highest in MG ($P < 0.05$, Table S3). The relative abundances of *Cercophora*, *Fusarium*, and *Sporormia* were lower in the HG and OG treatments than in CK, but the relative abundance of *Alternaria* under HG and OG significantly increased by 39.2% and 47.0%, respectively ($P < 0.05$, Table S3). Compared with G0, G4 significantly increased the relative abundances of *Naganishia* and *Filobasidium* ($P < 0.05$, Table S3). The total fungal network consisted of 29 nodes, and 29 significant edges were detected by CoNet analysis (Fig. 4b). The clustering coefficient was 0.130, and the average number of neighbors was 2.0. *Umbilicaria*, *Cercophora* and *Didymella* were the core groups, which all belong to Ascomycota. Other taxa belong to the phyla Mortierellpmycota, Chytridiomycota and Basidiomycota.

Fungal functional groups

Most OTUs from the functional groups (animal pathogens, dung saprotrophic species, lichenized saprotrophic species, and plant pathogens) were identified to belong to the phylum Ascomycota using the FUNGuild database (Fig. 5). The relative abundance of the plant pathogen functional group was higher in HG (by 52.2%) and OG (by 30.4%) than in CK ($P < 0.05$, Fig. 5). Compared with CK, the relative abundance of the functional group animal pathogen was significantly higher under LG (by 27.7%), MG (by 22.8%), HG (by 48.3%), and OG (by 64.8%) ($P < 0.05$, Fig. 5). MG and OG significantly increased the relative abundance of endophytes by 115.9% and 83.9%, respectively ($P < 0.05$, Fig. 5). The relative abundance of arbuscular mycorrhizal fungi was 395.8% higher under LG than under CK ($P < 0.05$, Fig. 5).

Relationship between plant characteristics, soil properties and soil microbial community under different grazing intensities

A positive relationship was detected between *S. klemenzii* biomass ($F = 8.24$, $P = 0.013$), *C. songorica* biomass ($F = 23.87$, $P < 0.001$) and ANPP ($F = 18.67$, $P < 0.001$) and the relative abundance of arbuscular mycorrhizae in different grazing intensities (Fig. S1). SOC was strongly positively correlated with α-diversity, and enzymes activities (BG, NAG,

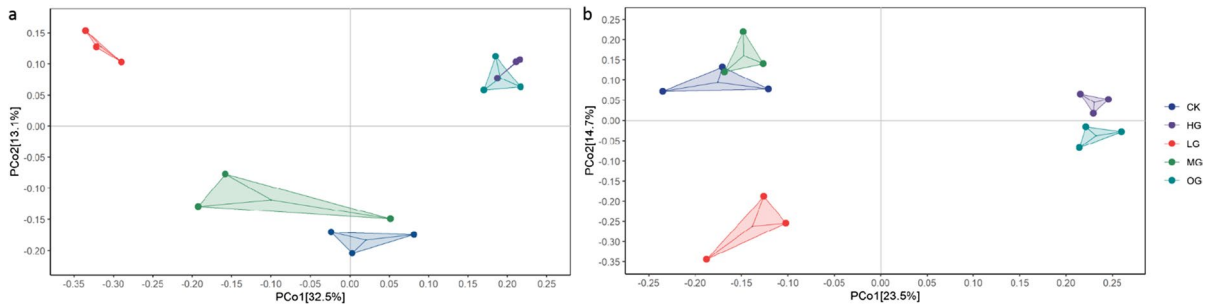


Figure 2 Principal coordinate analysis (PCoA) of the dissimilarity of bacteria (**a**) and fungi (**b**) based on the Bray–Curtis distance under different grazing intensities. CK: no grazing, LG: light grazing, MG: moderate grazing, HG: heavy grazing, OG: overgrazing

and AKP) were negatively correlated with most soil nutrients based on Pearson correlation analyses (Table S4). Results from the MRT showed that SW (60.10%) and SOC (21.76%) regulated the change in bacterial α-diversity, while SOC (49.95%) and pH were major drivers determining the change in fungal α-diversity (14.16%; Fig. 6). For bacterial communities, significant correlations based on heat map analyses were detected between soil or plant characteristics and bacterial taxa (Fig. 7a). For fungal communities, soil or plant characteristics and fungal phyla (e.g., Ascomycota, Chytridiomycota, and Glomeromycota) were correlated based on heat map analyses (Fig. 7b). MRT analysis showed three splits in both bacterial communities (cross-validated relative errors of 0.223 and 0.825, respectively) and fungal communities (cross-validated relative errors of 0.179 and 0.573, respectively) based on plant and soil properties (Fig. 7c, d). The MRT analysis showed that the bacterial communities were influenced by DS and SOC, which explained 65.20% and 12.51%, respectively, of the observed variation (Fig. 7c). The SOC and pH explained 69.11% and 12.96%, respectively, of the variation in the fungal communities (Fig. 7d).

Discussion

Effects of grazing intensity on plant and soil properties

Our results demonstrated that individual plant species, e.g., *Stipa klemenzii*, C. *songorica*, and *Salsola collina*, responded differently to the different grazing intensities. Species differences in physiological and morphological traits could contribute to those diverse responses to grazing (Wang et al. 2014; Zhang et al. 2018). *Stipa klemenzii* and *C. songorica* were preferentially foraged by sheep, and the biomass of *Stipa klemenzii* and *C. songorica* was higher under light grazing because of the compensatory effect. The compensatory effects of the dominant species, including *Stipa klemenzii* and *C. songorica* (C_4 grass), resulted in high biomass in the LG treatment through litter accumulation and the input of urine and faeces produced by the sheep. On the contrary, heavy grazing (e.g., HG and OG) increased livestock intake, reduced litter accumulation and decreased soil physical and chemical fertility (cf. Schönbach et al. 2011). Lower soil fertility inhibited the growth of the dominant species and enhanced competition by nutrient-poor species (e.g., *Salsola collina*) with a lower nitrogen concentration and higher fiber content. As a weedy annual, *Salsola* accounted for more than 50% of the total aboveground biomass under heavy grazing (HG and OG). Our results also suggested a shift to a plant community dominated by annual-perennial species, whereas the livestock would be better served with vegetation consisting of perennial grasses, given their importance for the ecosystem services of these grasslands (Han and Biligetu 2004). Our results further revealed that the grazing effect on plant community productivity was mainly due to the responses of three species, *Stipa klemenzii*, C. *songorica* and *Salsola collina*; together, they accounted for 70% of the total aboveground biomass. Thus, our results highlighted the importance of identifying key species in predicting community-level responses to grazing intensity in this desert steppe with low species diversity.

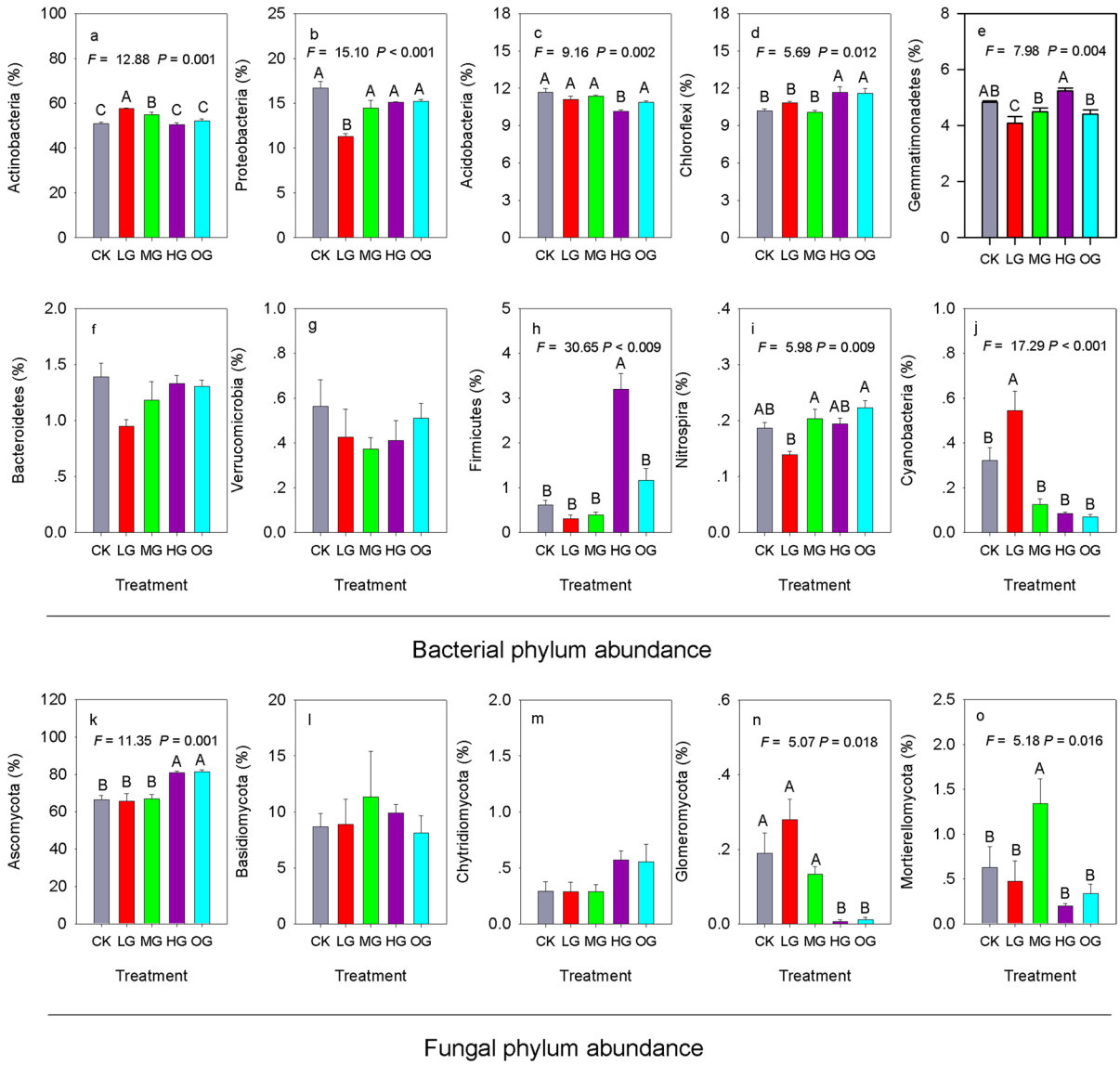


Figure 3 Response of bacterial and fungal phyla to different grazing intensities. Results reported as the mean ± standard error (n = 3). For each parameter, a different letter indicates a significant difference at the 0.05 probability level ($P < 0.05$) based on Tukey's honest test. Shown are the major bacterial phyla: Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Firmicutes, Nitrospirae and Cyanobacteria. Shown are the major fungal phyla: Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Mortierellomycota. CK: no grazing, LG: light grazing, MG: moderate grazing, HG: heavy grazing, OG: overgrazing

Our results indicated that SOC and TN were the highest under LG and were significantly lower when grazing intensity increased (Table 1). In our study, the higher SOC and TN contents in LG may be due to the input of readily decomposable faeces and urine by livestock and of organic matter inputs from plant litter accumulation and root exudates to the soil (Rossignol et al. 2006; Panayiotou et al. 2017; Zhang et al. 2020). On the contrary, our results are inconsistent with a previous study in an alpine meadow ecosystem in which heavy grazing increased the soil C and N contents (Li et al. 2011). The soil became bare and was exposed to wind erosion due to vegetation removal and decreased litter accumulation (cf. Su et al. 2005), which in turn

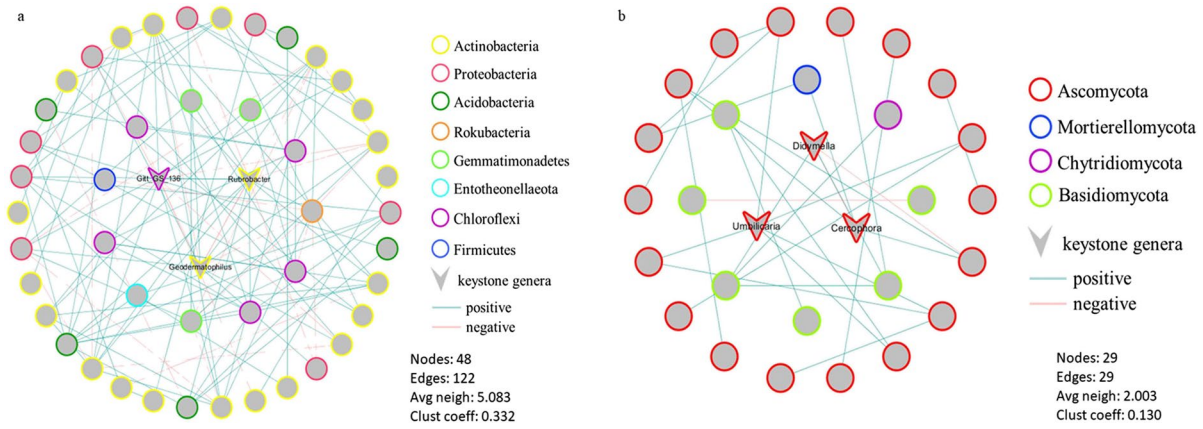


Figure 4 Overall network of bacterial communities (**a**) and fungal communities (**b**) across different grazing intensities. The overall network is arranged according to genus. Green and pink lines represent positive and negative relationships, respectively. Large triangular nodes indicate the keystone taxa in the network. The top three nodes with the highest degree, highest closeness centrality and lowest betweenness centrality were selected as the keystone taxa

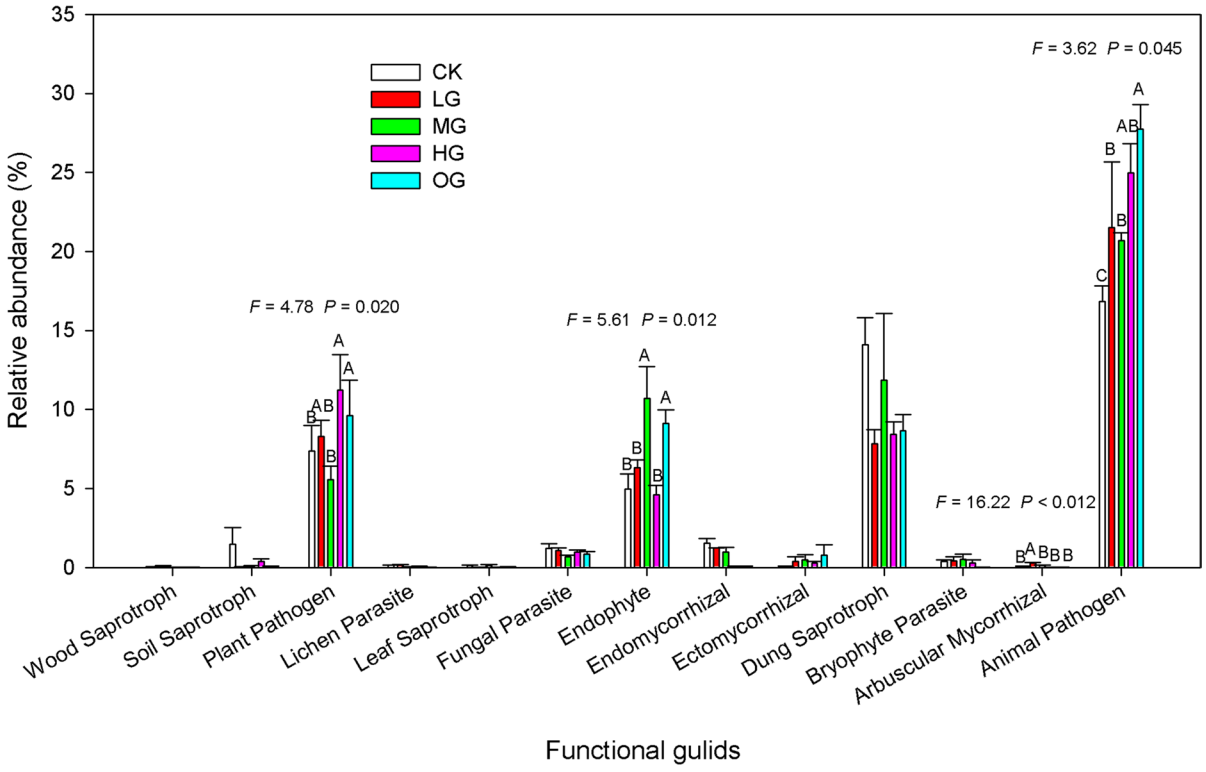


Figure 5 Effects of different grazing intensities on the relative abundances (%) of different fungal functional guilds. CK: no grazing, LG: light grazing, MG: moderate grazing, HG: heavy grazing, OG: overgrazing

resulted in the loss of C and N under HG and OG. In addition, the greater net loss of SOC under overgrazing might have caused the soil organic matter to decompose faster than the soil organic carbon accumulated because overgrazing resulted in higher soil emissions of CO_2 (cf. Morgan et al. 2004). The nutrient

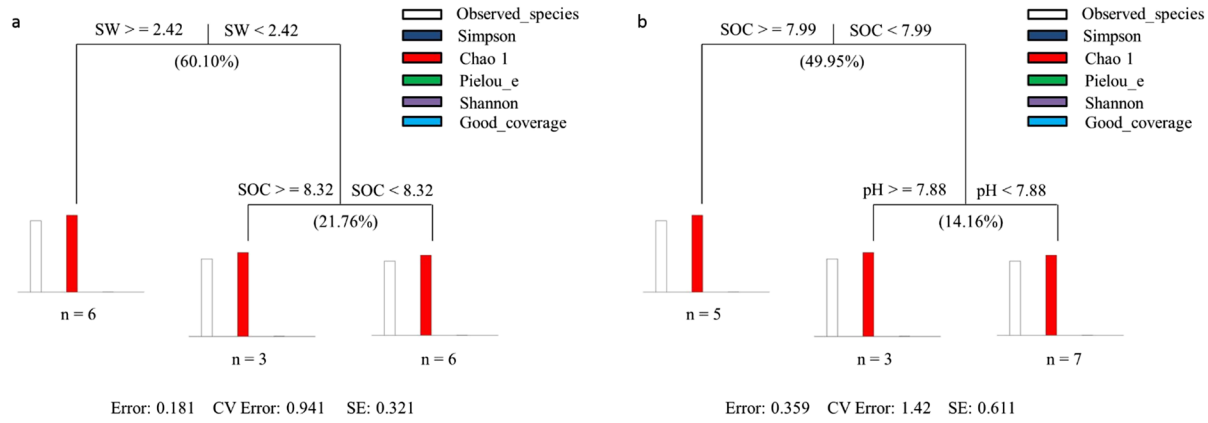


Figure 6 Multivariate regression tree analysis of the effects of environmental factors on the patterns in soil bacterial (**a**) and fungal (**b**) alpha diversity. The alpha diversity includes total OTUs, Chao1, Shannon's diversity, Simpson diversity, Pielou_e, and Goods_coverage

conservation strategy of different plant species affects the soil P content under grazing (Lü et al. 2013; Lü et al. 2015). The differences in the abundance of perennial plant species (i.e., those with high-quality litter) with high nutrient resorption and annual-biennial plant species (i.e., those with a low nitrogen concentration and high fiber content) with low nutrient resorption led to a high P content in LG and a low P content in HG and OG. We also found that a large amount of litter of annual-biennial plants (easily moved by wind) produced under heavy grazing could not be incorporated into the soil, which contributed to the low TP content in HG and OG. Our study provides support that appropriate grazing can improve primary production by compensatory growth and by increasing the soil nutrient content (McNaughton 1979).

Effects of grazing intensity on the α-diversity of microbes

Heavy grazing (HG and OG) significantly reduced the α-diversity of bacteria and fungi (as indicated by Chao1, Shannon, and Pielou_e biodiversity indices). The negative effects of overgrazing on the α-diversity of bacteria are in agreement with previous studies (Eldridge et al. 2017; Xun et al. 2018) in which overgrazing modified the diversity of soil microbes by changing the competitive abilities of different microbial phyla. In addition, the harsh habitats under overgrazing affect the α-diversity of microbes (Zhang et al. 2020), such as the low soil nutrient content of SOC, TN and TP, and poor soil physical properties (SW) (Table 1). The bacterial MRT results also indicated that SW and SOC were the main factors driving the change in the α-diversity of bacteria under the different grazing intensities. Moreover, a previous study showed that soil SW and pH are important factors affecting the fungal community composition (Rousk and Bååth 2011). Observed variations in the α-diversity of fungi might have been the result of variations in soil SOC and pH associated with differences in grazing intensity (Fig. 6b). The fungal α-diversity was significantly lower under heavy grazing (HG and OG), in line with the fact that the fungal community is sensitive to pH (Yang et al. 2013). The change in fungal α-diversity was also linked with enzymes (BG, NAG, and AKP) (Table S4). The strongly negative correlation between the fungal α-diversity and enzymes (BG, NAG, and AKP) (Table S4) suggests that fungi can secrete more enzymes to meet nutrient requirements with a low nutrient content and a large amount of difficult-to-degrade substrates in the ecosystem (Sinsabaugh et al. 2008; Averill et al. 2014; Crowther et al. 2019), and the soil nutrient content can have a large impact on fungal alpha diversity by altering the concentration of readily available substrates (Kivlin and Treseder 2014).

Effects of grazing intensity on the bacterial community composition

The change in the dominant phyla and genera associated with different grazing intensities affected

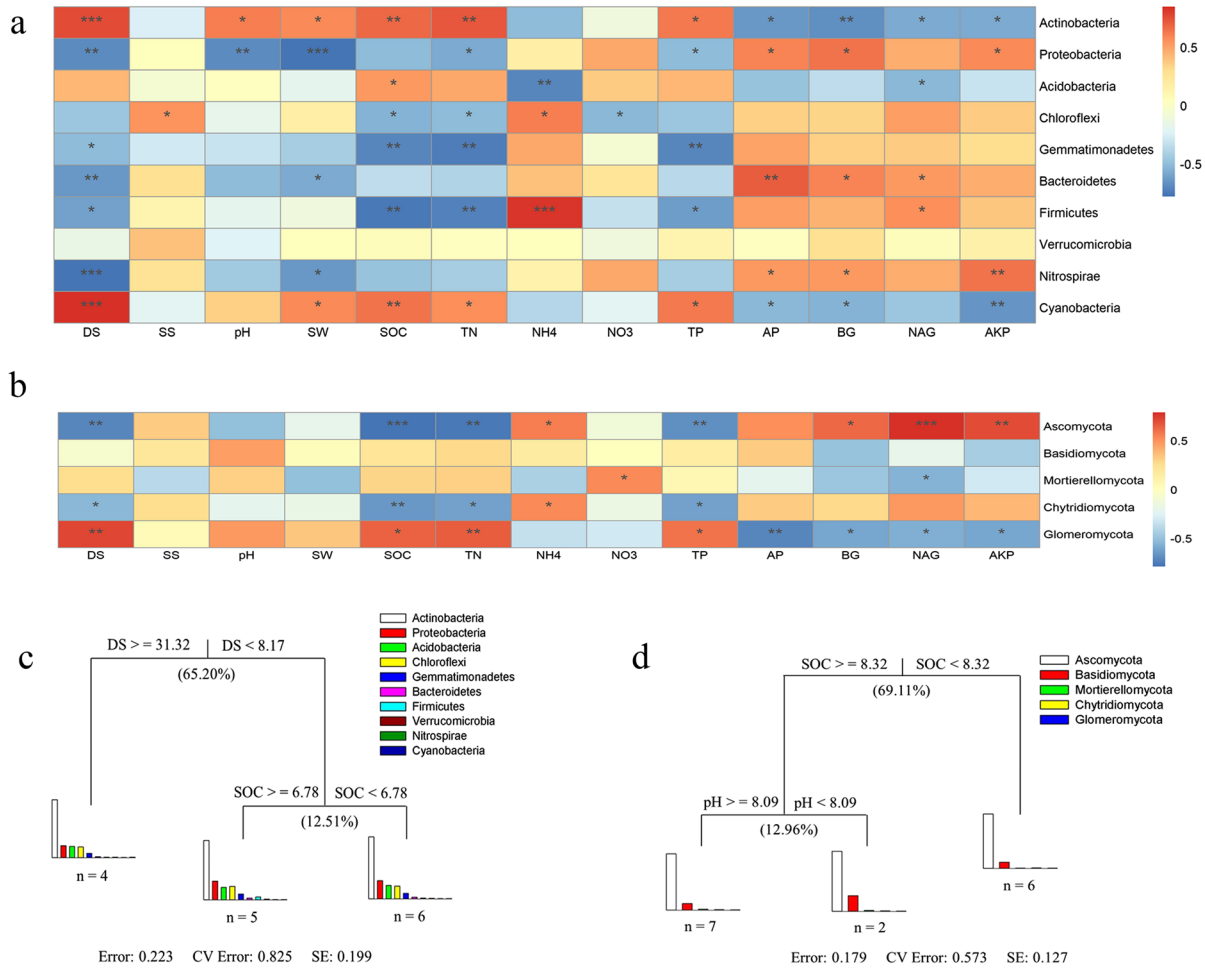


Figure 7 Correlations between biotic and abiotic factors and dominant bacterial (**a**) and fungal (**b**) phyla. Multivariate regression tree analysis of the effect of environmental factors on the patterns in the soil bacterial (**c**) and fungal (**d**) community composition. The number of soil samples included in the analysis are shown beneath the bar plots. Plant community characteristics include the DS (dominant plant species biomass), and SS (subordinate plant species biomass). Soil properties include the pH (soil pH value), soil water content (SW), SOC (soil organic carbon content), TN (soil total nitrogen content), TP (soil total phosphorus content), NH4 (soil NH_4^+ content), NO3 (soil NO_3^- content) and AP (soil available phosphorus content). Soil enzyme activities include BG (β-1,4-glucosidase), NAG (β-1,4-N-acetyl-glucosaminidase) and AKP (alkaline phosphatase). Significance levels in heat maps analysis are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

the bacterial community composition in our study (Fig. 3; Table S2). We expected that increasing grazing intensity could change soil properties and plant characteristics (Table 1), which altered the bacterial community composition (Fig. 2). We found a greater abundance of the copiotrophic Actinobacteria and Cyanobacteria under LG with a high nutrient content (Leff et al. 2015; Liu et al. 2017), while the oligotrophic Choroflexi and Gemmatimonadetes were more abundant under heavy grazing (HG and OG) because of low-quality resources (Bergmann et al. 2011). Our heat map results indicated that the abundance of the phylum Actinobacteria was positively correlated with DS, SOC and TN, while the abundances of the phyla Choroflexi and Gemmatimonadetes were negatively correlated with soil nutrients. Previous studies indicated that rich organic matter was favorable for the growth of Actinobacteria (Goodfellow and Williams 1983; Wang et al. 2021). The phylum Cyanobacteria is an important

contributor to carbon and nitrogen fixation (Munoz-Martin et al. 2019); this was visible in the increase in relative abundance under LG. The higher relative abundance of Choroflexi and Gemmatimonadetes under HG and OG might have been due to a lower dependence on the readily available substrates provided (Xu et al. 2016). In addition, the MRT results further showed that DS was a key factor controlling the bacterial community composition. In our study, the change in the biomass of perennial plant species directly affected the high-quality litter accumulation under different grazing intensities. The higher biomass of perennial plants under LG enhanced high-quality litter accumulation and increased nutrient resorption, subsequently enhancing the soil nutrient content (i.e., C and N storage).

At the genus level of the bacteria, the change of dominant genera, including *Rubrobacter*, *67-14*, *Solirubrobacter*, *Subgroup_6*, *Geodermatophilus*, *Blastococcus*, *Pseudonocardia*, *Gaiella*, *Nocardioides*, *Modestobacter*, *Conexibacter*, *Virgisporangium, Streptomyces, Actinophytocola, Cellulomonas*, *Kribbella* and *Iamia*, all belonging to the Actinobacteria phylum, altered the community composition of the bacteria (Table S2). Our results are in agreement with previous studies on Australian and northern Antarctic soils, i.e., representative microorganisms under different grazing intensities adapt to extreme conditions and are able to metabolize different substrates (e.g., SOC, TN, TP and pH) (Siciliano et al. 2014; Ferrari et al. 2016). Our co-occurrence network results also indicated that the change in the relative abundance of the *Rubrobacter* and *Geodermatophilus* genera (keystone taxa) affected the bacterial community composition. Although co-occurrence network inference as well as ordination methods can be sensitive to sampling size, many small-sample studies have used network analysis (e.g., Sengupta and Dick 2015; Dong et al. 2017; Li et al. 2021). In addition, in a similar desert steppe in northern China, a co-occurrence network analysis of small samples (n = 12) was used to analyze the effect of grazing intensity on the microbial community composition of original soil aggregates (Fan et al. 2021). Moreover, the low number of samples taken (n = 15) was due to the randomized block design employed, while the nine soil samples per plot were combined to form one composite sample per plot. Therefore, we considered the results of the co-occurrence network for the 15 mixed samples reasonable in our study.

Within the Proteobacteria phylum, most drought-resistant OTUs were classified as Alphaproteobacteria and Deltaprotebacteria, and were mainly represented by the genera *Microvirga*, *Sphingomonas*, *Craurococcus*, *Rubellimicrobium*, *Haliangium* and *Mycobacterium* (Santos-Medellín et al. 2017), which also resulted in changes in the community composition of bacteria under different grazing intensities. In addition, the genera *Gemmatirosa* and *Gemmatimonas* were the main components of the Gemmatimonadetes phylum, which were detected under HG and OG with resource scarcity and drought stress, indicating that they are adapted to low soil nutrient and moisture conditions (DeBruyn et al., 2011). Moreover, the increasing relative abundance of *Bacillus* (Firmicutes) under heavy grazing (HG and OG) suggested that *Bacillus* can promote the drought resistance of plants (Zhou et al. 2016). Therefore, the change in microbial communities from slow-growing oligotrophic bacteria under LG to fast-growing copiotrophic bacteria under heavy grazing also suggests that proper grassland management approaches can facilitate ecosystem recovery and monitoring for ecosystem resilience.

Effects of grazing intensity on the fungal community composition

The grazing-induced significant changes in the community composition of soil fungi were mainly related to the Ascomycota phylum (Figs 3 and 4). In our study, the relative abundance of the Ascomycota phylum (dominant phylum) exceeded 60% under different grazing intensities, especially under heavy grazing (HG and OG), and the relative abundance of the Ascomycota phylum exceeded 80%. With increasing grazing intensity, the increased relative abundance of Ascomycota was mainly due to the adaptation to drought conditions as well as scarce resources (Sterkenburg et al. 2015; Chen et al. 2017). In addition, these functional groups (e.g., plant pathogens, endophyte and animal pathogens) all belong to the Ascomycota phylum. The higher relative abundance of these functional groups under heavy grazing (HG and OG) than under CK resulted in a marked shift in the fungal community composition due to the dominance of Ascomycota in this desert steppe. Moreover, the

change in the relative abundance of the Glomeromycota phylum under different grazing intensities also influenced the community composition. We found that the higher relative abundance of the Glomeromycota phylum under LG was associated with the higher perennial plant biomass and plant community biomass (Fig. S1), which provide more organic substrates (as energy) to meet the availability of fungi due to the higher levels of plant detritus production (Sterkenburg et al. 2015; Wang et al., 2020).

At the genus level of the fungi, the change in the relative abundance of *Alternaria*, *Didymella*, *Darksidea*, *Cercophora*, *Fusarium*, *Comoclathris* and *Sporormia* (all belonging to the Ascomycota phylum) under the different grazing intensities altered the community composition of the fungi (Table S3). The change in the abundance of the dominant genera affected the fungal functional groups, e.g., plant pathogens (*Alternaria*, *Didymella* and *Fusarium*), endophytes (*Darksidea*), and dung saprotrophic species (*Sporormia* and *Cercophora*), which changed the fungal community composition. Our co-occurrence network results also showed that the dominant genera *Didymella* and *Cercophora* were the main components of Ascomycota and have been detected in various environments, especially in dry soils (Chen et al. 2017), indicating their adaptation to low soil moisture. In addition, the relative dominance of arbuscular mycorrhizal fungi under LG changed the aboveground plant biomass with increasing soil fertility, which also changed the composition of the fungal community.

Conclusions

Plant production and soil nutrients showed a parallel increase under LG and a decrease under HG and OG compared with CK. Our results indicated a shift in the soil (bacterial and fungal) microbiome via changes in the dominant phyla due to altered plant functional and soil properties under different grazing intensities. The overgrazing treatment had higher proportions of oligotrophs (phyla Chloroflexi, Gemmatimonadetes, and Firmicutes), whereas a higher proportion of copiotrophs (phylum Actinobacteria) was found under light grazing. The alterations in resource availability also affected the fungal community composition, such as a higher relative abundance of Ascomycota and a lower relative abundance of Glomeromycota under HG and OG than under no grazing. Bacteria possess a more complex network of co-occurrences than fungi, suggesting that bacteria are more resistant to environmental stress because different taxa can complement one other. Our results indicated a shift in the soil (bacterial and fungal) microbiome via changes in the dominant phyla or genera due to altered plant functional and soil properties under different grazing intensities. Proper grazing may be a sustainable grazing system in desert steppes, as it is beneficial to the maintenance of plant ANPP and soil nutrients, while having higher microbial diversity, indicating a high potential for microbial function. LG may be an appropriate management system for the restoration of grassland ecology in our study. Light grazing increases plant productivity and soil fertility, which enhance the abundance of some phyla (e.g., Actinobacteria, Cyanobacteria, and Glomeromycota) and genera (e.g. *Rubrobacter* and *Solirubrobacter*). These phyla or genera are associated with enhanced plant growth. Our results highlight the importance of different plant functional groups and soil properties in modulating the microbial diversity and community composition under different grazing intensities. Proper grazing (e.g., LG) is beneficial to the long-term sustainable development of desert grasslands.

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Declarations

Ethics approval and consent to participate This article does not contain any studies with human participants. The sheep used for the grazing experiments were exposed to different levels of forage availability, but otherwise their well-being was not affected in any way. Ethical approval was not necessary for our study.

Conflict of interests The authors declare that they have no competing interests.

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