Effects of grazing intensity on richness and composition of rhizosphere and non-rhizosphere microbial communities in a semiarid grassland

Yang Yang[1], Zhang Hao[2], Liu Wei[3], Sun Jiamei[3], Mengli Zhao[2], Guodong Han[2], and Qingmin Pan[4]

[1]Chinese Academy of Sciences
[2]Inner Mongolia Agricultural University
[3]Institute of Botany Chinese Academy of Sciences
[4]Institute of Botany, Chinese Academy of Sciences

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Abstract

1.Overgrazing-induced grassland degradation has become a severe ecological problem worldwide. The diversity and composition of soil microbial communities are responsive to grazing disturbance. Yet, our understanding is limited with respect to the effects of grazing intensity on bacterial and fungal communities, especially in plant rhizosphere. 2.Using a long-term (15 years) grazing experiment, we evaluated the richness and composition of microbial communities in both rhizosphere and non-rhizosphere regions, under light, moderate, and heavy intensities of grazing, in a semiarid grassland. We also examined the relative roles of grazing-induced changes in some abiotic and biotic factors in affecting the richness and composition of microbial communities. 3.Our results showed that the responses of soil bacteria to grazing intensity differed greatly between rhizosphere and non-rhizosphere, and so did soil fungi. Specifically, the bacterial richness decreased markedly under moderate and heavy grazing in rhizosphere soil, whereas little impact on the fungal richness was observed. For microbial composition, with the increase in grazing intensity, an increase in dissimilarity among bacterial communities was observed, and this trend also held true for the fungal communities. Hierarchical partitioning analyses indicated that the bacterial composition in rhizosphere was primarily driven by root nitrogen and soil nitrogen concentrations while that in non-rhizosphere by soil available phosphorus. In addition, soil available phosphorus played an important role in affecting the fungal composition in both rhizosphere and non-rhizosphere regions. 4.Synthesis: This study provides direct experimental evidence that the richness and composition of microbial communities were severely altered by heavy grazing in a semiarid grassland. Thus, to restore the grazing-induced, degraded grasslands, we should pay more attention to the conservation of soil microbe in addition to vegetation recovery.

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Yang Yang [a, b, c], Hao Zhang [c], Mengli Zhao [c], Guodong Han [c], Qingmin Pan[a, b*], Wei Liu [a], Jiamei Sun[a],

[a]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, the Chinese Academy of Sciences, Beijing 100093, China;

[b] University of Chinese Academy of Sciences, Beijing 100049, China;

[c] Inner Mongolia Agriculture University, College of Grassland, Resources and Environment, Hohhot 010019, China

Corresponding author:

Prof. Qingmin Pan

State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, the Chinese Academy of Sciences, Beijing, 100093, China.

Tel.: +86-10-6283-6977

Fax: +86-10-6283-6279

E-mail: *pqm@ibcas.ac.cn*

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2. Using a long-term (15 years) grazing experiment, we evaluated the richness and composition of microbial communities in both rhizosphere and non-rhizosphere regions, under light, moderate, and heavy intensities of grazing, in a semiarid grassland. We also examined the relative roles of grazing-induced changes in some abiotic and biotic factors in affecting the richness and composition of microbial communities.
3. Our results showed that the responses of soil bacteria to grazing intensity differed greatly between rhizosphere and non-rhizosphere, and so did soil fungi. Specifically, the bacterial richness decreased markedly under moderate and heavy grazing in rhizosphere soil, whereas little impact on the fungal richness was observed. For microbial composition, with the increase in grazing intensity, an increase in dissimilarity among bacterial communities was observed, and this trend also held true for the fungal communities. Hierarchical partitioning analyses indicated that the bacterial composition in rhizosphere was primarily driven by root nitrogen and soil nitrogen concentrations while that in non-rhizosphere by soil available phosphorus. In addition, soil available phosphorus played an important role in affecting the fungal composition in both rhizosphere and non-rhizosphere regions.
4. *Synthesis* : This study provides direct experimental evidence that the richness and composition of microbial communities were severely altered by heavy grazing in a semiarid grassland. Thus, to restore the grazing-induced, degraded grasslands, we should pay more attention to the conservation of soil microbe in addition to vegetation recovery.

KEYWORDS:bacterial diversity, bacterial composition, fungal diversity, fungal composition, grassland degradation, grazing intensity, non-rhizosphere, rhizosphere

1 | INTRODUCTION

Grasslands cover about 40% of Earth's land surface and are mostly used for livestock grazing (Suttie, Reynolds, & Batello, 2005). While light and/or moderate grazing can improve the diversity and productivity of grasslands, heavy grazing usually causes severe grassland degradation (Kemp et al., 2013; McNaughton, 1979). By far, overgrazing-induced grassland degradation has become a major ecological problem worldwide (Dlamini, Chivenge, & Chaplot, 2016; Eldridge et al., 2017; van de Koppel, Rietkerk, & Weissing, 1997). This is especially the case in China, where more than 90% of grasslands are in a degraded status (Kemp et al., 2013).

Soil microbes play important roles in regulating grassland structure and functioning (Bardgett & van der Putten, 2014). However, soil microbial communities are prone to be affected by heavy grazing (Hu et al., 2017; Lame, Kennedy, & Siciliano, 2011; Wang et al., 2021a; Zhang et al., 2020). Grazing can influence the soil microbe through several mechanisms. First, herbivory on plants can have dual effects on soil microbial communities. On the one hand, removal of above-ground biomass decreases the litter input into the soil, which reduces the supply of carbon resource to soil microbe, and thus causes reduction in soil microbial

richness (Dwivedi et al., 2017). On the other hand, removal of above-ground parts may also stimulate the allocation of organic nutrients to belowground organs (Mueller et al., 2017), resulting in enrichment of root exudates (Hamilton & Frank, 2001), which in turn affects the structure of microbial community (Hu et al., 2018). Second, manure from livestock may affect soil pH values (Wang et al., 2021a; Yang et al., 2018) and improve the available soil nitrogen (Han et al., 2008). As a result, the relative abundance of copiotrophic taxa were enhanced while that of oligotrophic taxa was reduced (Nie et al., 2018). Finally, livestock trampling decreases soil moisture due to increased soil compactness (Kobayashi, Hori, & Nomoto, 1997), which in turn affects the soil microbial properties (Chen et al., 2019). These studies provide important insights into grazing impacts on soil microbial communities, however, most studies did not distinguish the microbial responses between non-rhizosphere and rhizosphere regions. Given that plant rhizosphere is a micro-region that plant-soil-microbe interacts directly (Curl & Truelove, 1986), the influence of grazing on microbial communities in this region is likely stronger than those in non-rhizosphere.

Here we examined the impacts of grazing intensity on the richness and composition of microbial communities in rhizosphere vs. non-rhizosphere by a 15-year grazing intensity experiment in a semiarid grassland. We focused on three specific questions: i) How do microbial communities differ in richness and composition between rhizosphere and non-rhizosphere? ii) How do bacterial and fungal communities respond to grazing intensities? iii) What are the relative roles of abiotic and biotic factors in affecting bacterial and fungal responses to grazing intensity?

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted in a semiarid grassland on the Inner Mongolia Plateau (41°46'43.6" N, 111deg53'41.7" E; elevation 1456 m). This area has a temperate continental grassland climate, with annual mean temperature and precipitation of 3.6 degC and 214 mm, respectively (Wang et al., 2014). About 80% of precipitation occurs during the growing season (May to September). The vegetation in the experiment site was dominated by*Stipa breviflora* and *Cleistogenes songorica* . The soil is a Kastanozem soil (FAO soil classification) with a sandy loam texture. This area is traditionally used as pastures for free grazing and 90 % of them have been degraded due to overgrazing (Kemp et al., 2013).

2.2 | Experimental design

A long-term grazing intensity experiment was established in 2004. We had four grazing intensities: control (zero sheep-unit ha^{-1} $month^{-1}$, G0), light (0.15 sheep-unit ha^{-1} $month^{-1}$, G0.15), moderate (0.30 sheep-unit $ha^{-1}month^{-1}$, G0.30), and heavy grazing (0.45 sheep-unit ha^{-1} $month^{-1}$, G0.45) (Zhang et al., 2021). Each treatment had three replicates. Under a complete randomized block design, 12 plots, each with an area of 4.4 ha, were separated by fences. Sheep with similar size were selected in advance and the grazing period lasted from June 1 to November 30 every year.

2.3 | Field sampling and laboratory analysis

We randomly harvested 30 bunches of *C. songorica* in each plot in late August of 2018, corresponding to peak biomass time. Each bunch of plants with soil were dug out to a depth of 15 cm, with approximately 90% of the roots harvested. The non-rhizosphere soil was defined as those shaken off from the roots while the rhizosphere soil as those that adhere to the roots tightly and cannot be shaken off (Chaudhary et al., 2015). Plants of *C. songorica* were separated into shoots and roots, and oven-dried at 65degC for 72 h and weighed. Soil samples from the same plot were mixed to form a composite sample, thus, we obtained 12 rhizosphere and 12 non-rhizosphere composite soil samples (4 treatments x 3 replications).

Each composite soil sample was equally separated into three portions: one air-dried, one kept fresh at 4degC, and one stored at -80 degC. The fresh sample was used to determine the soil moisture and inorganic nitrogen concentration. Concentrations of ammonium nitrogen and nitrate nitrogen were measured using a continuous flow analyzer (Auto Analyzer III, Bran+Luebbe, Germany), and the inorganic nitrogen was their sum. The air-dried samples were used to determine the concentrations of total carbon, total nitrogen, total

phosphorus, available phosphorus, and the pH values. Concentrations of total carbon and total nitrogen in soil/root samples were determined by an element analyzer (Elementar Vario Micro Cube, Germany), whereas soil total phosphorus and available phosphorus by a spectrophotometer. Soil pH was measured by a pH meter. The frozen samples were used to extract DNA for evaluating the richness and composition of microbial communities.

2.4 | DNA extraction, PCR amplification, and sequencing

For each sample, DNA was extracted from 0.25 g thawed soil by a DNeasy(r) PowerSoil(r) DNA Isolation Kit (Qiagen, Germany). The quality of isolated DNA was determined by electrophoresis on 1% agarose gel. The purity was examined using NanoDrop. In total, 12 rhizosphere and 12 non-rhizosphere DNA samples were prepared, respectively.

The 16S rRNA gene was amplified to estimate the relative abundance of soil bacteria while internal transcribed spacer (ITS) region for the fungi. The paired primers, 341F and 806R, were used to target the hypervariable V3+V4 region of the 16S rRNA gene; while the paired primers, ITS5-1737F and ITS2-2043R, for the ITS1 regions of fungi. The primers in each sample were tagged with unique barcodes. All PCRs were carried out in 30-μL reactions with10 ng of template DNA, 0.2 μM of forward and reverse primers, and 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs). For thermal cycles, the initial denaturation was at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s. Then annealing was at 55 °C for 30 s, and elongation at 72 °C for 30 s. Finally, an extension was at 72 °C for 5 min.

The PCR products of each sample and 1X loading buffer (with SYBR green) were mixed with equal volumes and then electrophoresed on a 2% agarose gel to examine the successful amplification of target DNA fragments. The PCR products for each sample were quantified, and mixed with 80ng of DNA in equal proportions. The resultant mixed PCR products were electrophoresed by 2% agarose gel, and the fragments of DNA ranging from 400 - 450bp were excised and then purified with a GeneJET^{TM} Gel Extraction Kit (Thermo Scientific).

Following the manufacturer's recommendation, Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) was used for sequencing libraries preparation. Qubit@ 2.0 fluorometer (Thermo Scientific) and Agilent Bioanalyser 2100 system (Agilent, USA) were used to evaluate the library quality. The libraries were sequenced on IonS5^{TM}XL platform, generating 400-bp/600-bp single-end reads.

2.5 | Bioinformatics analyses

Based on the unique barcode of each sample, single-end reads were identified and truncated by shearing off the barcode and primer sequences. The Cutadapt (Martin, 2011) quality controlled process was used to filter the raw reads under specific filtering conditions, we obtained the high-quality clean reads. The reads were compare with the reference database (Silva and Unite database) (Kõljalg et al., 2013; Quast et al., 2013) using UCHIME (Edgar, et al., 2011) algorithm to detect chimera sequences, and removed them. Then final clean reads were generated. We did sequence analysis using Uparse software (Uparse v7.0.1001) (Edgar, 2013). Sequences with a similarity higher than 97% were assigned to the same operational taxonomic unit (OTU). Representative sequence for each OTU was screened for further annotation. The taxonomic information of bacterial representative sequences was annotated using the Silva132 SSUrRNA database (Quast et al., 2013) based on Mothur algorithm. For fungal representative sequences, we annotated taxonomic information using the Unite (v7.2) database (Kõljalg et al., 2013) based Blast algorithm which was calculated by QIIME software (Version 1.9.1).

In total, we obtained 947,987 and 958,264 bacterial high-quality sequences from rhizosphere and non-rhizosphere samples, respectively. These reads were clustered into 23,160 and 37,278 OTU. For fungi, we obtained 961,649 and 928,290 high-quality sequences from rhizosphere and non-rhizosphere samples, respectively, which were clustered into 11,695 and 17,424 OTU (Table S1).

2.6 | Statistical analysis

All statistical analyses were conducted in R 4.0.2 (R Core Team, 2020). We used microbial OTU richness as an index of microbial diversity. A t-test was used to evaluate the differences in microbial richness between rhizosphere and non-rhizosphere. Differences in microbial community composition between the two regions were evaluated using permutational multivariate analysis of variance (PERMANOVA) with the function "adonis" in the package "vegan" with 999 permutations (Oksanen et al., 2020), and visualized by the principal coordinate analysis (PCoA). One-way ANOVA followed by Tukey-Test was used to assess the effects of grazing intensity on microbial richness, composition (i.e., the relative abundance of main microbial taxa), biotic variables (shoot biomass, root biomass, root total carbon, and root total nitrogen of *C. songorica*), and abiotic variables (concentrations of total carbon, nitrogen, and phosphorus, concentrations of available nitrogen and phosphorus, soil pH and soil moisture). Prior to analyses, Shapiro-Wilks was used for normal distribution evaluation, while Bartlett's test for homogeneity of the data. The dissimilarity in microbial community composition was determined by the Bray-Curtis distance among treatments, performed with the "vegdist" function in package "vegan" (Oksanen et al., 2020).

A Mantel test was used to evaluate how changes in microbial community diversity or composition are related to biotic and abiotic variables by the "mantel test" function in the package "vegan", and visualized by the package "linkET" (Huang et al., 2021). The multicollinearity of variables was assessed based on variance inflation factors (VIF), and those with a VIF value less than 10 were regarded as variables with low collinearity. The relative importance of variables in affecting microbial richness and composition was evaluated with the package of "rdacca.hp" (Lai et al., 2021).

3 | Results

3.1 |Microbial richness and composition in rhizosphere vs. non-rhizosphere

Microbial communities differed markedly between rhizosphere and non-rhizosphere regions in OTU richness (Figure 1). The bacterial richness was significantly lower in rhizosphere than in non-rhizosphere (Figure 1a), and so was the fungal richness (Figure 1b).

The bacterial community composition differed greatly between the two regions (Figure 1c). This was more evident in the relative abundances of three dominant bacterial phyla, *Proteobacteria* ,*Acidobacteria,* and *Actinobacteria* . For example, the relative abundance of *Proteobacteria* was 74.4% in rhizosphere, but decreased to 23.6% in non-rhizosphere (Figure 1e, Table S2). In contrast, the relative abundances of *Acidobacteria*and *Actinobacteria* were 6.4 % and 5.9 % in rhizosphere, but increased to 21.2% and 26.7 % in non-rhizosphere, respectively (Figure 1e, Table S2). The fungal community composition also differed between the two regions (Figure 1 d). For example, the relative abundance of*Ascomycota* was 48.8% in rhizosphere but decreased to 38.6% in non-rhizosphere (Figure 1f, Table S2).

3.2 | Effects of grazing intensity on microbial richness and composition

Grazing intensity had significant impacts on rhizosphere bacterial richness but not on that in non-rhizosphere (Figure 2a, b). However, grazing intensity had little impact on fungal richness in both regions (Figure 2c, d).

For microbial composition, the dissimilarity in bacterial community exhibited an increasing trend with the increase in grazing intensity in both regions (Figure 3a). In rhizosphere, the relative abundance of*Proteobacteria* increased significantly under moderate and heavy grazing while that of *Acidobacteria* decreased (Figure 3b; Table S3). In non-rhizosphere, the relative abundances of*Proteobacteria* and *Acidobacteria* declined with the heavy grazing while that of*Actinobacteria*increased (Figure 3c; Table S3). The dissimilarity in fungal community also displayed an increasing trend with the increase in grazing intensity in both regions (Figure 3d). Specifically, the dominant taxa,*Ascomycota* , displayed a marked increase in relative abundance under moderate and heavy grazing (Figure 3e, f; Table S3).

3.3 | Biotic and abiotic drivers for microbial richness

In general, all examined biotic and abiotic variables, except for soil total phosphorus and soil pH, significantly affected soil bacterial richness in rhizosphere, whereas that in non-rhizosphere was not affected by these

variables (Figure 4). The fungal richness in both regions was not affected by examined biotic and abiotic variables (Figure 4).

3.4 | Biotic and abiotic drivers for microbial community composition

In general, all examined biotic and abiotic variables, except for soil total phosphorus and soil pH, affected the composition of bacterial or fungal communities in both regions (Figure 5a). Moreover, among six variables with low collinearity, the rhizosphere bacterial composition was mainly affected by root total nitrogen and soil total nitrogen, whereas that in non-rhizosphere by soil available phosphorus (Figure 5b, c). In addition, soil available phosphorus was consistently the primary variable affecting the fungal composition in both rhizosphere and non-rhizosphere (Figure 5d, e).

4 | Discussion

4.1 | Differences in microbial richness and composition between rhizosphere and non-rhizosphere

Our results demonstrated that the microbial communities differed markedly between rhizosphere and non-rhizosphere in richness and composition in this grassland ecosystem. The rhizosphere microbial richness was lower than that in non-rhizosphere (Figure 1), which is in line with previous findings that the microbial diversity decreased with decreasing distance to plant roots (Donn et al., 2015; Fan et al., 2017). Such a difference can be explained by a mechanism that rhizosphere microbiomes are derived from non-rhizosphere microbiomes under selection effect of host plants (Hartmann et al., 2009; Nan et al., 2020).

The microbial composition also differed greatly between the two regions (Figure 1c,d). This was especially evident for the relative abundances of dominant microbial taxa. The relative abundance of*Proteobacteria* , a dominant bacterial phylum, was 23.6% in non-rhizosphere, but increased to 74.4 % in rhizosphere (Figure 1e; Table S2), likely due to its strategy in nutrient use.*Proteobacteria* is a representative copiotrophic bacteria phylum (Nie et al., 2018; Zhang et al., 2018; Zhang et al., 2020). Increased availability of nutrient in rhizosphere due to the acid activation by root exudates (Cesco et al., 2010) fosters its growth, because more nutrients facilitate the copiotrophic bacteria but restrain the oligotrophic bacteria according to the oligotroph-copiotroph hypothesis (Fierer, Bradford, & Jackson, 2007; Leff et al., 2015). Similarly,*Ascomycota* is a dominant fungal phylum, and its relative abundance increased from 38.6% in non-rhizosphere to 48.8% in rhizosphere (Figure 1f; Table S2). Such a difference in relative abundance of *Ascomycota* between two regions has also been observed in an alpine grassland (Jiang et al., 2021). Collectively, these results suggest that the plant rhizosphere microenvironment may facilitate the flourishing of certain bacterial and fungal taxa.

4.2 | Responses of bacterial and fungal communities to grazing intensity

An intriguing result from our experiment was that the rhizosphere bacterial richness was more sensitive to grazing intensity than that in non-rhizosphere (Zhang et al., 2019). As indicated in Figure 2, the rhizosphere bacterial richness sharply declined under heavy grazing intensity, whereas that in non-rhizosphere remained unchanged (Zhang et al., 2020). Two mechanisms may underlie grazing-induced decline in rhizosphere bacterial richness. First, herbivory removes aboveground organs of a plant, resulting in a reduction in assimilated carbon allocated to belowground organs, and thus a decline in the quantity of carbon sources available for bacteria in plant rhizosphere (Aldezabal et al., 2015; Mueller et al., 2017; Byrnes et al., 2018), which in turn reduces rhizosphere bacterial richness. In this study, the rhizosphere bacterial richness was significantly affected by root carbon (Figure 4), providing support for this mechanism. Second, plant diversity is usually coupled with microbial diversity in a community (De Deyn & van der Putten, 2005). Thus, grazing-induced reduction in plant diversity may decrease the diversity of carbon sources for bacterial taxa (Dwivedi et al., 2017), as a consequence, some bacterial taxa cannot grow well or disappear and the rhizosphere bacterial diversity declines.

In contrast to the response of rhizosphere bacterial richness, the fungal richness was not significantly affected by grazing intensity in both regions (Figure 2c,d), which is consistent with results from grazing experiments

(Zhang & Fu, 2020; Yang et al., 2021). The weak response of fungal richness may be due to the fact that fungi were more tolerant to environmental stresses than bacteria (Rousk & Bååth, 2011). For example, fungi often exhibit more resistance to soil drought or acidity than bacteria (Rousk & Bååth, 2011). In this study, the fungal richness was not significantly affected by examined biotic and abiotic factors (Figure 4), implying that grazing-induced changes in these variables did not exert significant impact on fungal richness. Overall, our results suggest that grazing in this grassland has little impact on soil fungal richness.

For bacterial composition, previous studies have almost exclusively concentrated on non-rhizosphere bacteria (Hu et al., 2017; Zhang et al., 2020; Wang et al., 2021a). Our results suggest that grazing intensity has stronger impacts on bacterial composition in rhizosphere than that in non-rhizosphere. For example, heavy grazing resulted in a 6.55-fold increase in community dissimilarity in rhizosphere while a 1.89-fold increase in non-rhizosphere (Figure 3a). An impressive result from this experiment was that, with the increase of grazing intensity, the relative abundance of a dominant bacterial phylum,*Proteobacteria* , decreased in non-rhizosphere but increased in rhizosphere (Figure 3b, c). Such opposite responses to grazing intensity were likely due to its copiotrophic strategy in nutrient use (Leff et al., 2015). Increased grazing intensity led to a relatively higher plant root nutrient (Tables S4, S5), which facilitates the growth of copiotrophic bacteria. This is supported by our results that the relative abundance of *Proteobacteria* in rhizosphere was positively related to plant root nitrogen (Figure S1a). By contrast, grazing-induced decrease in the relative abundance of*Proteobacteria* in non-rhizosphere was related to the reduction in soil total nitrogen (Figure S1b; Tables S4, S5).

Our results also demonstrated strong impacts of grazing intensity on fungal composition in this grassland. As indicated by the increased dissimilarity of fungal community with grazing intensities (Figure 3d), the fungal composition was dramatically altered by heavy grazing in both regions. A significant increase in relative abundance was observed for a dominant taxon, *Ascomycota* , under heavy grazing intensity (Figure 3e,f). This phenomenon could be explained by its oligotrophic strategy, which facilitates its growth in relatively low carbon environment (Sterkenburg et al., 2015). As indicated in this experiment, heavy grazing resulted in a great reduction in carbon concentrations in plant roots and soils (Tables S4, S5), and the relative abundance of*Ascomycota* was negatively related to these carbon concentrations (Figure S2a, b).

4.3 | The roles of biotic and abiotic factors in affecting microbial responses to grazing intensity

In this experiment, grazing-induced decrease in rhizosphere bacterial richness was related to changes in 9 of 11 variables (Figure 4), consistent with a previous study in alpine meadow finding that soil microbial richness was affected by multiple biotic and abiotic factors (Zhang & Fu, 2020). Moreover, we found that all examined biotic variables were closely related to rhizosphere bacterial richness (Figure 4), highlighting the impacts of plant selection effect on rhizosphere bacterial richness (Hartmann et al., 2009; Nan et al., 2020). However, the examined variables had little effect on fungal richness in both regions (Figure 4), consistent with previous results (Yang et a., 2018). This may be due to the fact that fungal are more resistant to environmental variability relative to bacteria (Rousk & Bååth, 2011).

Previous studies have highlighted the role of soil pH in affecting microbial richness (Qu et al., 2016; Yang et al., 2018; Zhang & Fu, 2020). However, in this study, the changes in bacterial and fungal richness were not affected by soil pH in both regions. The weak effect of soil pH in our study could be explained by the results that the soil pH in this experiment was not significantly altered by grazing intensity (Table S4, S5).

Our results indicated that the bacterial and fungal community compositions in both regions were co-affected by several biotic and abiotic variables (Figure 5a). For bacteria, the rhizosphere community composition was primarily affected by plant root biomass and root carbon concentration, highlighting the importance of plant-related resources in affecting rhizosphere bacterial communities (Costa et al., 2006; Berg & Smalla, 2009). In contrast, the bacterial community composition in non-rhizosphere was primarily affected by soil available phosphorus, highlighting the role of abiotic factors in affecting non-rhizosphere bacterial composition (Tian et al., 2017; Schöps et al., 2018). Moreover, soil available phosphorus was also the primary factor affecting the fungal composition in both regions (Figure 5d,e). The important role of soil available phosphorus in affecting

microbial composition may be due to the fact that the grasslands in northern China have a relatively lower soil phosphorus than other grasslands (Han et al., 2005). For example, the soil total phosphorus concentration (515 mg kg^{-1}) in this experiment site (Table S5) was much lower than the mean value (699 mg kg^{-1}) in grasslands of Northern America (US Geological Survey, 2001). Also, soils in this region are rich in Ca^{2+} and Mg^{2+}, and phosphorus is usually bounded to these metal ions, further constraining the available phosphorus for soil fungi (Wang et al., 2021b). Importantly, the lack of soil available phosphorus was exacerbated by heavy grazing in this experiment (Table S4, S5). This is likely due to the grazing-induced strong wind erosion that the nutrient-rich, top-layer soils were removed from the grassland (Giese et al., 2013). Given that some arbuscular mycorrhizal fungi can help plants efficiently exploit and absorb soil phosphorus (van der Heijden et al., 1998), the strong relationship between soil available phosphorus and fungal community composition also suggests that some fungal taxa may play important roles in mediating the availability of soil phosphorus in this grassland. Overall, our study provides experimental evidence that grazing intensity caused changes in abiotic and biotic factors, and these changes in turn altered the soil microbial composition.

5 | Conclusion

Our results demonstrated that rhizosphere bacterial richness was greatly reduced by heavy grazing. At the same time, the bacterial and fungal compositions were greatly altered in both rhizosphere and non-rhizosphere regions. These findings highlight that overgrazing not only causes severe vegetation degradation (Wang et al., 2014; Zhang et al., 2021) but also great decrease in diversity and strong alteration in composition of soil microbial communities, which may greatly demote the functioning of the whole ecosystem (Bardgett & van der Putten, 2014). Given that soil microbes play an important role in the restoration of degraded ecosystems (Averill et al., 2022; Coban, De Deyn, & van der Ploeg, 2022), we suggest that, along with vegetation conservation, motoring and restoring microbial communities are important for the restoration of extensive degraded grasslands in China.

AUTHOR CONTRIBUTIONS

Qingmin Pan, Hao Zhang, and Mengli Zhao conceived the ideas and designed the study. Mengli Zhao and Guodong Han designed the grazing experiment. Yang Yang and Hao Zhang conducted the field sampling and laboratory analysis. Yang Yang, Wei Liu, and Jiamei Sun analyzed the data. Yang Yang, Hao Zhang, and Qingmin Pan led writing of this manuscript. All authors contributed to the draft and revision and gave the final approval for publication.

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CONFLICT OF INTERSET

Authors declare that they have no competing interests.

DATA ACCESSIBILITY STATEMENT

We are willing to upload our experimental data to Dryad Digital Repository, if our manuscript are accepted.

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Supplementary information includes Tables S1-S5 and Figs. S1-S2.

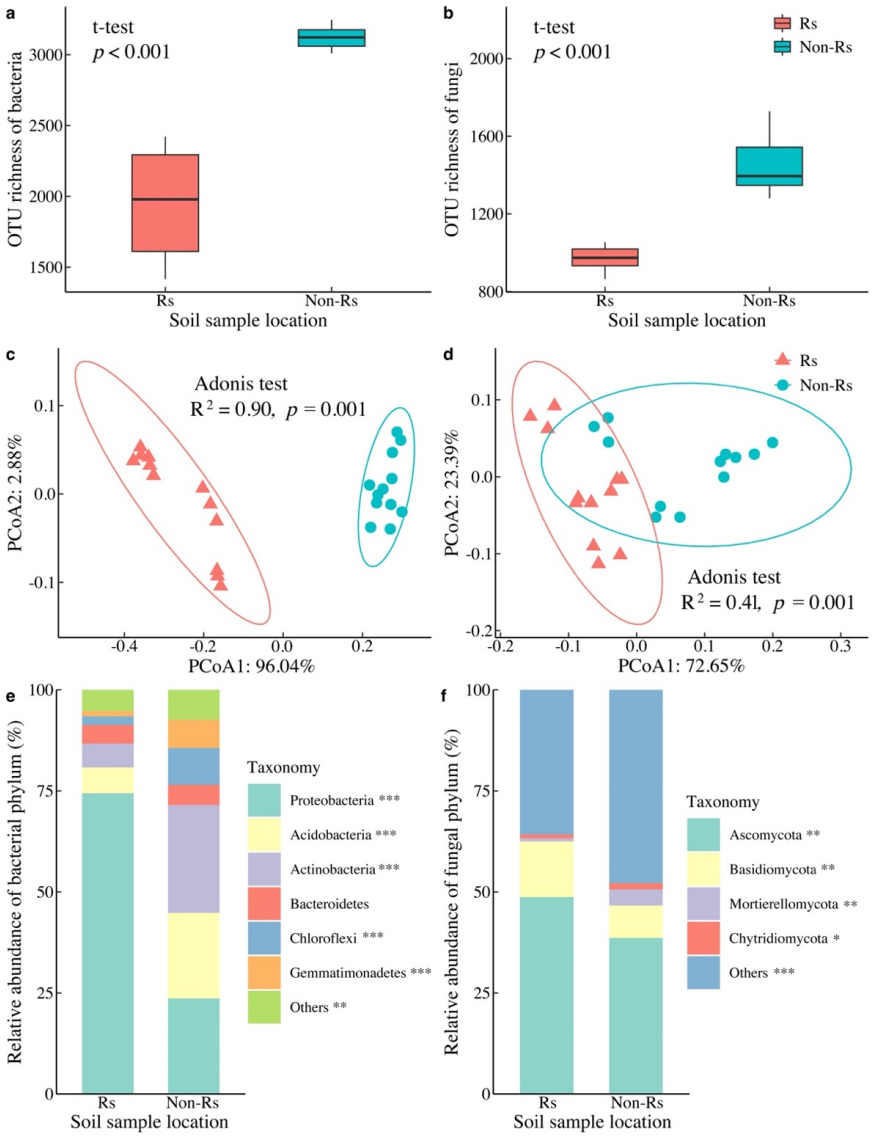

FIGURE 1 Comparisons in microbial richness and composition between rhizosphere (Rs) and non-rhizosphere (Non-Rs) regions . Notes: Bacterial **(a)** and fungal **(b)** richness; Difference in bacterial **(c)** and fungal **(d)** community composition; Relative abundance of main bacterial **(e) and** fungal**(f)** taxa. The taxa with a relative abundance $< 1\%$ were assigned into "Others". *, **, and *** indicated significant difference between two regions by t-test at $p = 0.05$, 0.01, and 0.001, respectively.

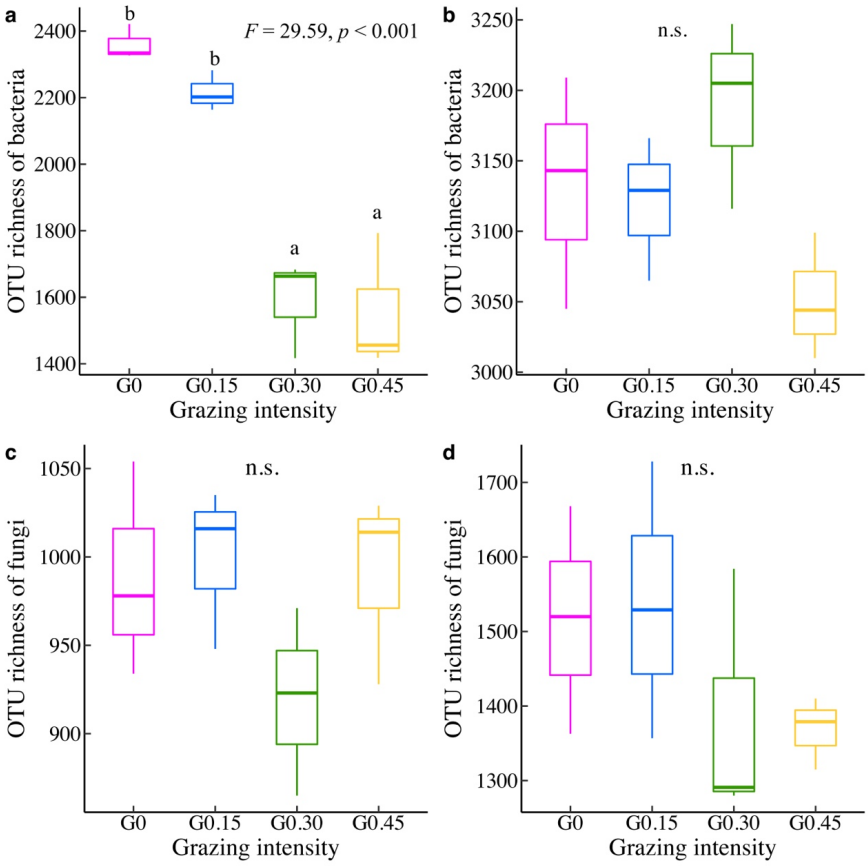

FIGURE 2 Effects of grazing intensity on microbial richness in rhizosphere and non-rhizosphere. Notes: rhizosphere **(a)** and non-rhizosphere **(b)** bacterial richness; Rhizosphere**(c)** and non-rhizosphere **(d)** fungal richness. G0, G0.15, G0.30, and G0.45 refer to control, light, moderate, and heavy grazing, respectively. Different letters indicate significant difference among treatments at $p = 0.05$, while "n.s." indicated no significant difference among treatments

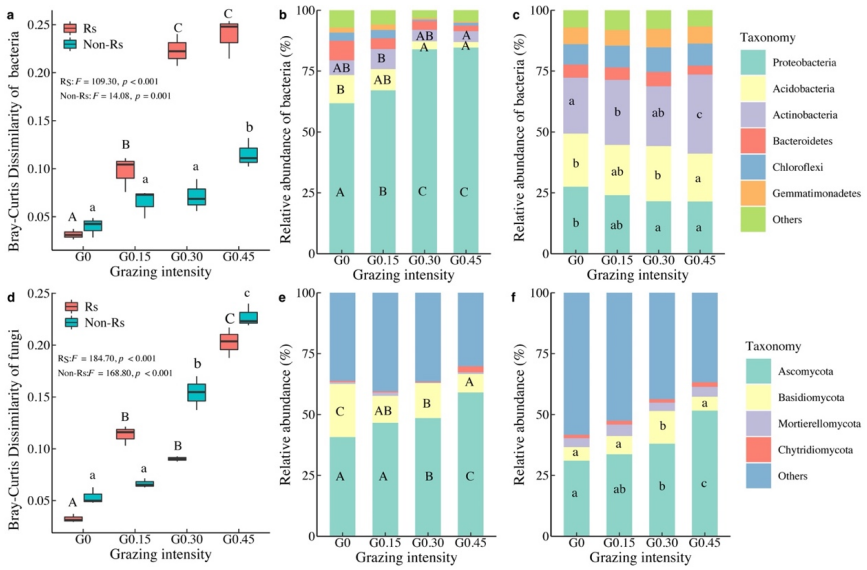

FIGURE 3 Effects of grazing intensity on dissimilarity of microbial community composition and on relative abundance of microbial taxa in rhizosphere (Rs) and non-rhizosphere (Non-Rs) regions . Notes: Bacterial **(a)** and fungal **(d)** community dissimilarity; Relative abundance of bacterial phyla in rhizosphere **(b)** and non-rhizosphere **(c)** ; Relative abundance of fungal phyla in rhizosphere **(e)** and non-rhizosphere**(f)** . Different letters indicate significant differences among treatments. G0, G0.15, G0.30, and G0.45 are the same as in Figure 2.

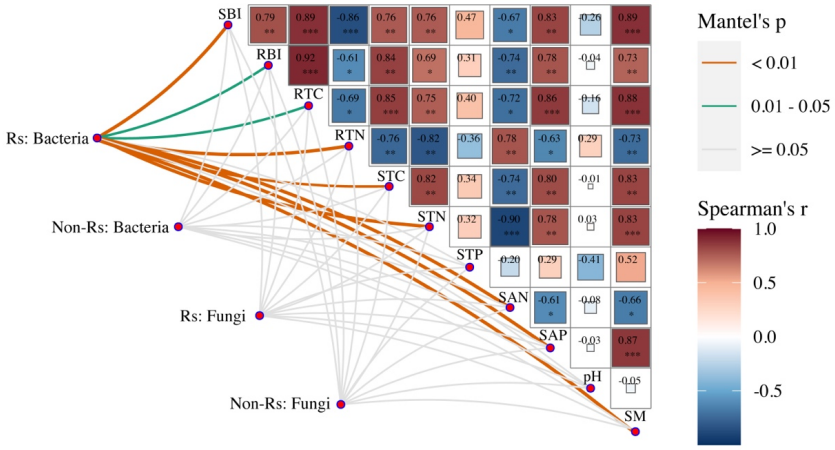

FIGURE 4 Impacts of abiotic and biotic factors on bacterial and fungal richness in rhizosphere (Rs) and non-rhizosphere (Non-Rs) . SBI, shoot biomass; RBI, root biomass; RTC, root total carbon; RTN, root total nitrogen; STC, soil total carbon; STN, soil total nitrogen; STP, soil total phosphorus; SAN: soil available nitrogen; SAP: soil available phosphorus; SM: soil moisture. Overall R^2indicates the total explained fraction by the six variables.

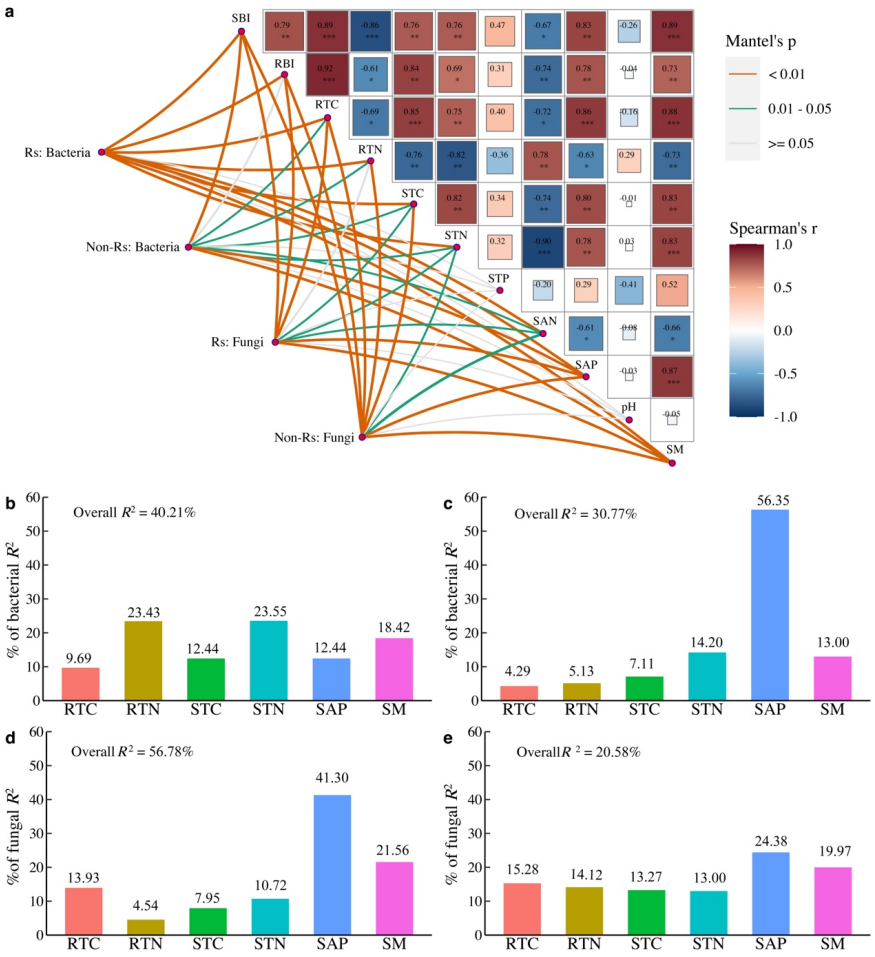

FIGURE 5 Impacts of abiotic and biotic factors on microbial compositions in rhizosphere (Rs) and non-rhizosphere (Non-Rs) . Notes: Mantel results on the impacts of examined variables on bacterial and fungal compositions **(a)** . Relative roles of six variables with low-collinearity on rhizosphere **(b)** and non-rhizosphere bacterial compositions **(c)** . Relative roles of six variables with low-collinearity on rhizosphere **(d)** and non-rhizosphere fungal compositions **(e)** . SBI, RBI, RTC, RTN, STC, STN, STP, SAN, SAP, and SM are the same as in Figure 4. OverallR^2 indicates the total explained fraction by the six variables.