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CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Effect of different grazing intensities on bacterial community composition and diversity in rhizosphere and non-rhizosphere soils in desert steppe of China

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Received: 1 February 2019; Accepted: 15 June 2019; doi:10.4067/S0718-58392019000400531

ABSTRACT

Degradation and desertification are extremely significant environmental problems in arid and semi-arid grassland ecosystems. Long-term overgrazing is the most fundamental cause of grassland degradation. We investigated relationships between grazing intensity and bacterial communities in non-rhizospheric and rhizospheric soils in desert steppe, including 0-10, 10-20 and 20-30 cm depth soils, as well as *Stipa breviflora* Griseb., *Cleistogenes songorica* (Roshev.) Ohwi, *Artemisia frigida* Willd. and plant community rhizospheric soils. This involved simulating grazing intensities in a long-term localization experiment, using a randomized block design. The effects of grazing on non-rhizospheric soil bacterial abundance were reflected in the 0-10 cm layer, increasing under light grazing and decreasing rapidly under moderate and heavy grazing, mainly related to *Bacillus*. Bacterial abundance in dominant plant rhizosphere responded differently. In *A. frigida* Willd. Rhizosphere, it decreased with increasing grazing intensity (a trend repeated in mixed rhizosphere). Bacterial abundance in *S. breviflora* and *C. songorica* rhizosphere increased under light and decreased under moderate and heavy grazing. Thus, changes in the dominant plant rhizospheric bacterial community did not significantly affect bacterial abundance in mixed rhizosphere. Changes in the rhizospheric bacterial abundance mainly resulted from levels of the dominant species, *Streptomyces* and *Arthrobacter*. There were significantly different results for bacterial community structure. Specifically, grazing had a nonsignificant and significant impact on bacterial community structures in non-rhizospheric ($F_{PERMANOVA} = 1.38$, $p = 0.199$) and rhizospheric ($F_{PERMANOVA} = 2.03$, $p = 0.012$) soil, respectively, varying significantly among plants ($F_{PERMANOVA} = 1.9$, $p = 0.022$). In conclusion, bacterial communities in rhizosphere were mainly affected by plant species and were more sensitive to changing grazing intensity than in non-rhizospheric soil.

Key words: Desert steppe, grazing intensity, non-rhizosphere, rhizosphere, soil bacteria.

INTRODUCTION

The desert steppe ecosystem of Inner Mongolia, which comprises 10.7% of the region's grassland, is important for both livestock production and preservation of biodiversity (Jia et al., 2017a). Grazing has been one of the most important land-use methods across all Inner Mongolian steppe types for thousands of years. However, overgrazing has caused

severe land degradation and desertification in the Inner Mongolian steppe. Compared to other types of grasslands, desert steppe accounts for 39% of total native grassland in Inner Mongolia (Lin et al., 2010a). Therefore, determining a suitable grazing intensity is of great significance in preventing soil degradation and the sustainable development of desert steppe.

Previous studies have considered the impact of grazing on desert steppe ecosystems. The effects of different grazing intensities on soil nutrients (Zhou et al., 2017), soil respiration (Zhao et al., 2017) and gas exchange (Li et al., 2018), plant community structure and productivity (Deng et al., 2014) have been widely studied. A study of the impacts of grazing on C components and total ecosystem organic C in the desert steppe of northern China found that grazing changed the relative distribution of C components in this arid desert grassland (Wang et al., 2017); the same study determined that light to moderate grazing (0.15-0.30 sheep ha^{-1} mo^{-1}) was beneficial for soil nutrient accumulation in the desert steppe. Grazing intensity has been shown to alter fine-scale processes in desert steppe and cause divergent responses in the spatial distribution of vegetation and soil fertility (Lin et al., 2010b). Grazing also enhances the suppression effect of climatic aridity on seed production in *Caragana stenophylla* (Xie et al., 2016). Appropriate and efficient grazing exclusion has been found to cause desirable transitions in the plant communities of desert steppe rangelands; this is an available method for counteracting local grassland degradation and promoting rangeland sustainability (Deng et al., 2014).

Soil microorganisms represent the world's largest reservoir of biological diversity: abundant 16S rRNA gene sequences have been amplified from soil DNA and RNA. They also constitute the most important and sensitive bioactive factor in soil, where they perform an irreplaceable role in the maintenance of healthy desert grassland ecosystems and the restoration of vegetation. Increased soil bacterial diversity not only improves soil ecosystem stability, but also helps to mitigate deterioration of the soil ecological environment (Gao et al., 2017). However, fewer studies have been reported concerning the effects of different grazing intensities on non-rhizospheric and rhizospheric bacterial communities in desert steppes (Eldridge et al., 2017).

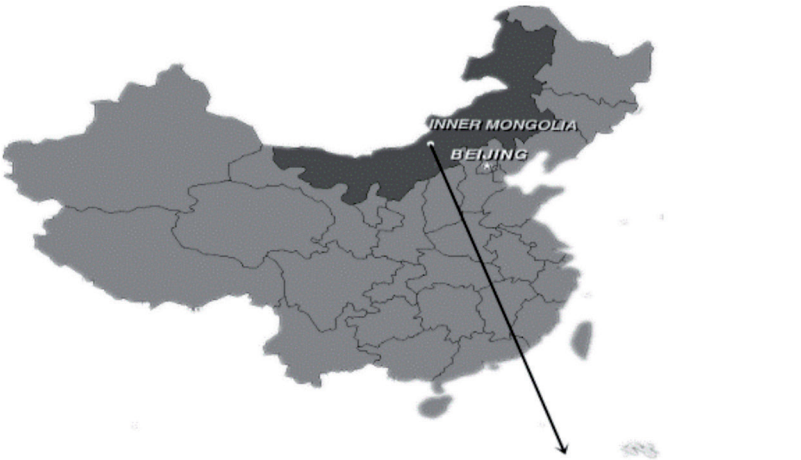
A gradient of grazing intensity can represent desert steppe ecosystems with different desertification potentials, given the lack of a common method for assessing desertification (Lin et al., 2010a). Therefore, the aim of this study was to investigate the relationship between soil degradation and the bacterial communities of non-rhizospheric and rhizospheric soils in desert steppe. Based on observation of artificial controlled grazing sample plots for 13 consecutive years, the results may contribute to the restoration of soil ecological environment and functioning in degraded grassland, and to the optimization of grazing methods. The findings may also lay a theoretical foundation for the sustainable development of grassland soil ecosystems (Gao et al., 2017).

MATERIALS AND METHODS

Study site

The experiment was conducted in the Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, located in Shiziwang Banner (41°47'17" N, 111°53'46" E; 1450 m a.s.l.), Inner Mongolia Autonomous Region (IMAR), northern China (Figure 1) (Lin et al., 2010b; Cao et al., 2013). The steppe is characterized by a semiarid continental monsoon climate with a short plant-growing period from May to September (Jia et al., 2017a). The mean annual temperature is 3.4 °C, and the three highest monthly mean temperatures are 21.5, 24.0, 23.5 °C in June, July and August, respectively (Lin et al., 2010b). The annual precipitation is approximately 280 mm with about more than 80% received from May to September (Cao et al., 2013). Annual average total sunshine time is 3118 h, and the annual mean amount of evaporation is 2300 mm (Cao et al., 2013). Soil in this area was classified as light chestnut soil (Haplic Calcisols, according to the FAO classification). The soil pH was 8.16, soil organic C was 16.29 g kg^{-1}, total N was 1.49 g kg^{-1}, available N was 24.57 mg kg^{-1}, and available P was 3.15 mg kg^{-1}. The plant composition of the study site, *Stipa breviflora* Griseb. desert steppe community, mainly comprising *S. breviflora* Griseb., *Artemisia frigida* Willd., and *Cleistogenes songorica* (Roshev.) Ohwi, accompanied by *Convolvulus ammannii* Desr., *Aster altaicus* Willd., *Neopallasia pectinata* (Pall.) Poljakov, *Kochia prostrata* (L.) Schrad., *Caragana stenophylla* Pojark., and *Leymus chinensis* (Trin.) Tzvelev. The plant photosynthesis type and composition percentage of the study site is showed in Table 1.

Figure 1. Study site location, and two-dimensional schematic diagram of experimental treatments.



CK: Non-grazed enclosure; LG: light grazing; HG: heavy grazing; MG: moderate grazing.

Table 1. Plant composition (%) on different types of grazing treatment in the study site.

Functional group	Species	Photosynthesis type	Grazing treatment			
			CK	LG	MG	HG
Perennial grasses	*Stipa breviflora* Griseb.	C3	24.47 ± 6.65	24.04 ± 7.91	30.98 ± 8.62	52.7 ± 3.61
	Cleistogenes songorica (Roshev.) Ohwi	C4	11.78 ± 5.99	22.11 ± 7.23	32.59 ± 8.07	32.52 ± 4.07
Perennial rhizome grasses	*Leymus chinensis* (Trin.) Tzvelev	C3	6.52 ± 4.00	6.73 ± 6.57	1.64 ± 0.97	0.12 ± 0.10
	Agropyron cristatum (L.) Gaertn.	C3	0.14 ± 0.11	1.81 ± 1.81	0.68 ± 0.49	1.91 ± 1.05
Perennial forbs	*Convolvulus ammannii* Desr.	C3	7.89 ± 0.30	4.3 ± 1.70	2.85 ± 0.20	3.74 ± 0.63
	Allium tenuissinum L.	C3	1.56 ± 0.24	0.46 ± 0.10	0.15 ± 0.08	0.04 ± 0.02
	Allium mongolicum Regel	C3	0 ± 0	0.07 ± 0.05	0 ± 0	0 ± 0
	Astragalus galactites Pall.	C3	0.77 ± 0.39	0.79 ± 0.26	0.48 ± 0.22	0.66 ± 0.11
	Heteropappus altaicus (Willd.) Novopokr.	C3	8.01 ± 1.64	6.37 ± 3.96	0.15 ± 0.10	0.31 ± 0.16
	Lagochilus ilicifolius Bunge	C3	0.77 ± 0.40	1.79 ± 1.79	0.45 ± 0.25	0.29 ± 0.14
	Potentilla bifurca Linn.	C3	0.79 ± 0.79	0.21 ± 0.17	2.66 ± 2.66	0.39 ± 0.39
	Haplophyllum dauricum (L.) G. Don	C3	0.03 ± 0.02	0 ± 0	0 ± 0	0 ± 0
	Phlomis mongolica Turcz.	C3	0.02 ± 0.02	0 ± 0	0 ± 0	0 ± 0
Shrubs and semi-shrubs	*Caragana microphylla* Lam.	C3	0 ± 0	4.06 ± 3.89	0 ± 0	0 ± 0
	Caragana stenophylla Pojark.	C3	1.82 ± 0.56	0.65 ± 0.08	0.83 ± 0.33	0.43 ± 0.07
	Kochia prostrata (L.) Schrad.	C4	11.87 ± 3.37	7.88 ± 1.7	2.66 ± 1.12	0.83 ± 0.45
	Ceratoides latens Reveal & N.H. Holmgren	C3	7.19 ± 3.93	0.16 ± 0.16	0.77 ± 0.77	0 ± 0
Annual and biennials	*Artemisia frigida* Willd.	C3	26.3 ± 3.41	29.11 ± 4.12	27.35 ± 12.44	7.83 ± 3.26
	Neopallasia pectinata (Pall.) Poljakov	C3	4.07 ± 1.51	9.97 ± 1.80	1.73 ± 0.93	0.18 ± 0.15
	Salsola collina Pall.	C4	0.21 ± 0.21	0.04 ± 0.03	0.17 ± 0.13	0 ± 0
	Tripolium vulgare Nees	C3	2.59 ± 1.27	0.23 ± 0.06	0.5 ± 0.49	0 ± 0

CK: Non-grazed enclosure; LG: light grazing; HG: heavy grazing; MG: moderate grazing.

Experimental design

The steppe desert where the study site is located has been grazed continuously since 1988, when herdsmen began to settle permanently in the area. A lack of effective management measures prior to the introduction of enclosures led to the steppe desert beginning to degrade (Cao et al., 2013). In May 2004, grazing management experimental plots were established in degraded pasture inside enclosures near the residential area, and in the unenclosed free-grazing area, using a randomized complete block design of four grazing intensities with three replicates (Figure 1). The area of each plot was 4.4 ha. A total of 12 plots were fenced and stocked with sheep at rates of 0, 0.15, 0.30, and 0.45 sheep ha^{-1} mo^{-1}, defined as non-grazing (CK), light grazing (LG), moderate grazing (MG), and heavy grazing (HG), respectively (Wang et al., 2011a).

The grazing animals were 2-yr-old local Mongolian sheep, which were allowed to graze from 15 May to 15 November in each year. Sheep were not randomly reassigned each year but were retained in their originally assigned plots during the experimental period (May 2004 to date). The sheep were kept in the experiment for 3 yr, and then were replaced by 2-yr-old animals from the same flock (Wang et al., 2017). The daily grazing schedule extended from 06:00 to 18:00 h. During the grazing period, the test sheep were herded to the different sample plots each morning, and then returned to the sheepfold each evening (Cao et al., 2013). Water was provided to the sheep twice per day (early morning and dusk) in their pen, and salt was provided *ad libitum* over the entire grazing period. During the winter, all experimental sheep were kept in the enclosure and supplied with hay and grains in order to meet their energy maintenance requirements (Wang et al., 2011a; 2017).

Sampling

Five soil cores were randomly selected from each replicated plot and collected using a soil drill (5 cm diameter) in August of each of the years (2013-2016) (this article is based on 2013 data). Each soil core was divided into three layers, namely, non-rhizospheric soil depths of 0-10 (A), 10-20 (B) and 20-30 cm (C). Part of each sample was thoroughly mixed into one composite sample. Three plants representative of the study site (*S. breviflora*, *C. songorica*, and *A. frigida*) and plant community rhizospheric soil were also collected. The method of collecting rhizospheric samples was to uproot the plants, quickly shake off the loose soil around the roots, and then use a blade to gently peel off the soil attached to the roots. When collecting the three dominant plant rhizospheric soils, sampling cores were selected from multiple areas of a single plant species, in order to avoid contamination by other plant species. A 1 m × 1 m square in the study site was randomly selected, and a 10 cm × 10 cm wire box was then used to randomly select a sample square. The plant rhizosphere was then collected by scraping as described above and mixed together as plant community rhizospheric soil. The collected soil was stored in a clean, numbered, sealed plastic pocket, and the soil samples were then quickly transferred to the laboratory and stored at -20 °C for later use.

Isolation and culture of bacteria from soil

Soil cultivable bacteria were cultured with beef protein medium and isolated using the dilution plate method. DNA was extracted using a soil DNA kit (Sangon Biotech Co. Ltd., Shanghai, China) according to the manufacturer's instructions. A polymerase chain reaction (PCR) was performed on PCR equipment (TC-960F; Blue Marlin, Zurich, Switzerland). The PCR reaction mixtures (50 μL) contained 5 μL 10 × Ex Buffer (plus Mg^{2+}), 4 μL 2.5 mmol L^{-1} deoxynucleotide (dNTP), 2 μL each of forward and reverse primers (341F: 5'-TACGGGAGGCAGCAG-3'; 534r: 5'-ATTACCGCGGCTGCTGG-3'), 0.5 μL Taq Ex DNA polymerase (5 U μL^{-1}), 10 μL DNA template (10 ng μL^{-1}), and 26.5 μL sterile and DNA-free water.

The PCR protocol was as follows: 94 °C for 5 min (initial denaturation); 30 cycles of 94 °C, 94 °C for 1 min (denaturation), 55 °C for 45 s (annealing), and 72 °C for 45 s (elongation); and 72 °C for 10 min (elongation). Amplified PCR products were detected using 1.5% agarose gel electrophoresis and sent for Sanger sequencing (BGI, Beijing, China). After sequence alignments with reference sequences in GenBank (http://www.ncbi.nlm.nih.gov), we downloaded high-quality sequences for phylogenetic analysis.

Statistical analysis

One-way ANOVA and least significant difference (LSD) multiple comparison tests were used in post-hoc analysis of significant differences among the factors, using SPSS 22.0 (Qu et al., 2016). Origin 2017 (https://www.originlab.com/2017) was used for data visualization. Two-way ANOVA was used to analyze the effects of grazing or soil depth on bacterial abundance in non-rhizospheric soils, and of grazing or plant species on rhizospheric soil bacterial abundance. Principal coordinate analysis (PCoA) based on the Bray-Curtis matrix was used to analyze changes in the bacterial community structure. Permutational multivariate ANOVA (PERMANOVA) was used to test dissimilarities among different non-rhizospheric soil bacteria at different soil depths or under different grazing treatments. PERMANOVA was also used to test rhizospheric soil bacteria from different plants or different grazing treatments using both the Bray-Curtis and Jaccard distance methods via the vegan package (v. 2.5.2) in R software (v. 3.5.1) (The R Foundation for Statistical Computing, Vienna, Austria). Three non-parametric multivariate statistical methods were used to examine the effects on rhizospheric or non-rhizospheric bacterial communities of different grazing regime, soil depth, or plants, including

analysis of similarities (ANOSIM), PERMANOVA, and the multiple response permutation procedure (MRPP) (Liu et al., 2015). The Pearson index was applied to calculate correlation among grazing intensity, plant abundance, litter, and bacterial species abundance using the vegan (v. 2.5.2) and corrplot (v. 0.84) packages in R (v. 3.5.1).

RESULTS

Bacterial abundance in non-rhizospheric and rhizospheric soils

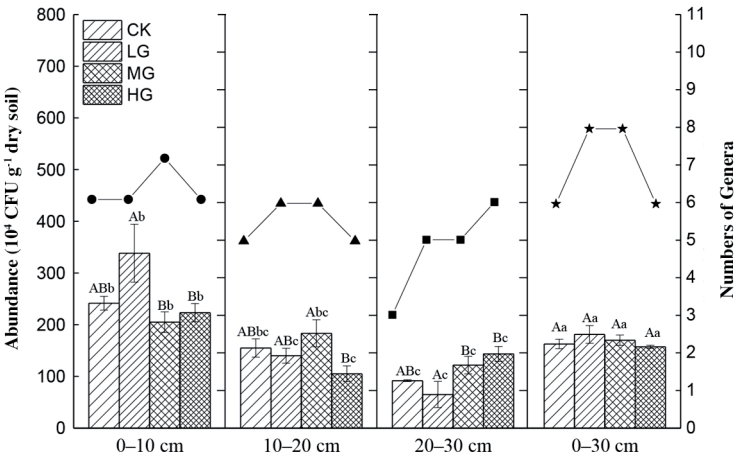
The quantity of cultivable bacteria at 0-30 cm depth in the LG plot was 181.11 × 10^4 colony-forming units (CFU) g^{-1}; this result was 11.26%, 6.54%, and 15.19% higher than in the CK, MG, and HG plots, respectively. The difference among the four treatments was nonsignificant. This result was more marked in the surface soil. The abundance of soil bacteria at 0-10 cm depth in the LG plot was 338.33 × 10^4 CFU g^{-1}, which was 40% higher than in the CK plot, and 65.04% and 51.49% higher than in the MG and HG plots, respectively, which showed a significant decrease in soil bacterial abundance (Figure 2, Table 2).

Bacterial abundance was much lower at 10-20 and 20-30 cm soil depths than at 0-10 cm depth, and the results were different when compared with the composite 0-30 cm depth (Figure 2). Therefore, changes in bacterial abundance in the 0-10 cm surface soil determined the trend shown in the 0-30 cm soil depth. The study results also indicated that current grazing intensities are significantly affecting the abundance of bacteria in the surface soil (0-10 cm). Specifically, the abundance of non-rhizospheric soil bacteria in the LG plot was higher than at other grazing intensities or in the CK plot.

The bacterial abundance of *S. breviflora* (Stibre) and *C. songorica* (Cleson) rhizosphere (RS, RC) was the highest in the LG plot, at 428.33 and 303.33 × 10^4 CFU g^{-1}, respectively (Figure 3, Table 3). Abundance then began to decrease with increasing grazing intensity. In particular, bacterial abundance of the Stibre rhizospheric soil in the LG plot was significantly higher than in the CK plot and other grazing intensities. *Artemisia frigida* (Artfri) rhizospheric soil (RA) bacterial abundance was insensitive to changes in grazing intensity compared with Stibre and Cleson rhizospheric soils. Compared with the CK plot, its bacterial abundance increased in the MG plot but decreased in the LG and HG plots; the difference was however nonsignificant ($p > 0.05$).

Compared with samples from the three dominant plants, bacterial abundance in the mixed rhizospheric soil (RCom) revealed a significantly different response to changes in grazing intensity. The abundance of RCom bacteria, when compared with the CK plot, gradually decreased with increasing grazing intensity; in the HG plot, this difference was significant ($p < 0.05$).

Figure 2. Abundance of cultivable bacteria measured in colony-forming units (CFU) in plots under four treatments at different non-rhizospheric soil depths.



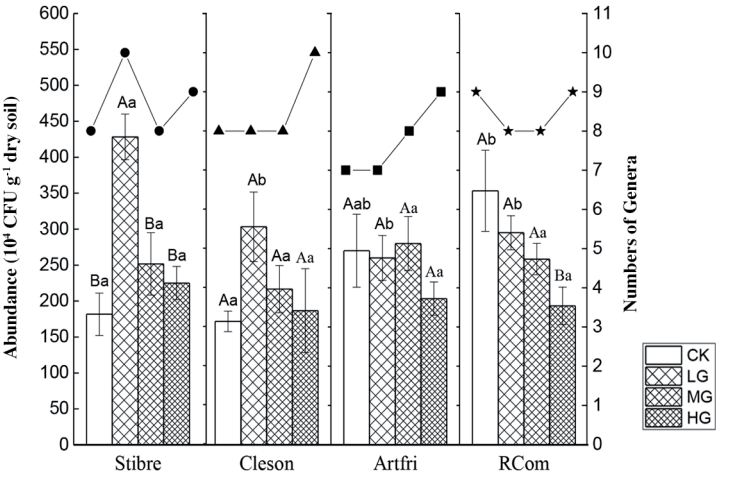
CK: Non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing.
The line graph shows the number of bacteria genera in each soil depth. Each value refers to the average of three replicates, and error bars represent the standard error (SE). Different uppercase letters at the same depth indicate significant differences at the $p < 0.05$ level; different lower-case letters for the same treatment indicate significant differences at the $p < 0.05$ level, based on a protected least significant difference (LSD) test.

Table 2. Composition and abundance of cultivable bacteria at different non-rhizospheric soil depths and under different grazing intensities (1×10^4 CFU g^{-1} dry soil).

Grazing intensity	Soil layers (cm)	*Bacillus*	*Jeotgalicoccus*	*Oceanobacillus*	*Arthrobacter*	*Streptomyces*	*Rhodococcus*	*Kocuria*	Unknown genus
CK	0-10	193.33 ± 4.41Aa	3.33 ± 3.33Aa	18.33 ± 8.82Aa	11.67 ± 9.28Aa	5.00 ± 2.89Aa	0 ± 0Aa	0 ± 0Aa	10.00 ± 7.64Aa
	10-20	131.67 ± 11.67Ab	0 ± 0Aa	8.33 ± 8.33Aa	3.33 ± 3.33Aa	5.00 ± 2.89Aa	0 ± 0Aa	0 ± 0Aa	6.67 ± 4.41Aa
	20-30	85.00 ± 2.89ABc	0 ± 0Aa	0 ± 0Aa	5.00 ± 2.89Aa	1.67 ± 1.67Aa	0 ± 0Aa	0 ± 0Aa	0 ± 0Aa
	0-30	136.67 ± 3.47Ab	1.11 ± 1.11Aa	8.89 ± 5.64Aa	6.67 ± 3.47Aa	3.89 ± 2.00Aa	0 ± 0Aa	0 ± 0Aa	5.56 ± 1.47Aa
LG	0-10	226.67 ± 34.20Aa	13.33 ± 3.33Aa	16.67 ± 3.33Aa	31.67 ± 14.24A	15.00 ± 2.89Aab	0 ± 0Aa	0 ± 0Aa	35.00 ± 30.14Aa
	10-20	113.33 ± 12.02ABa	3.33 ± 3.33Aa	0 ± 0Aa	10.00 ± 5.77Aa	10.00 ± 5.00Aab	0 ± 0Aa	1.67 ± 1.67Aa	1.67 ± 1.67Aa
	20-30	56.67 ± 19.22Aa	0 ± 0Aa	1.67 ± 1.67Aa	3.33 ± 3.33Aa	0 ± 0Aa	1.67 ± 1.67Aa	0 ± 0Aa	1.67 ± 1.67Aa
	0-30	132.22 ± 8.94Aa	5.56 ± 1.11ABa	6.11 ± 1.47Aa	15.00 ± 4.41Aa	8.33 ± 0.96Ab	0.56 ± 0.56Aa	0.56 ± 0.56Aa	12.78 ± 9.64Aa
MG	0-10	156.67 ± 31.80Aa	3.33 ± 3.33Aa	16.67 ± 10.14Aa	5.00 ± 5.00Aa	13.33 ± 8.82Aa	5.00 ± 2.89Ba	0 ± 0Aa	5.00 ± 0Aa
	10-20	141.67 ± 15.90Aa	0 ± 0Aa	8.33 ± 4.41Aa	15.00 ± 10.00Aa	13.33 ± 8.82Aa	0 ± 0Ab	1.67 ± 1.67Aa	3.33 ± 1.67Aa
	20-30	115.00 ± 13.23Ba	0 ± 0Aa	3.33 ± 3.33Aa	1.67 ± 1.67Aa	1.67 ± 1.67Aa	0 ± 0Ab	0 ± 0Aa	0 ± 0Aa
	0-30	137.78 ± 9.88Aa	1.11 ± 1.11Aa	9.44 ± 4.75Aa	7.22 ± 4.75Aa	9.44 ± 3.09Aa	1.67 ± 0.96Aab	0.56 ± 0.56Aa	2.78 ± 0.56Aa
HG	0-10	183.33 ± 11.67Aa	13.33 ± 13.33Aa	1.67 ± 1.67Aa	11.67 ± 1.67Aa	1.67 ± 1.67Aa	0 ± 0Aa	0 ± 0Aa	11.67 ± 11.67Aa
	10-20	78.33 ± 7.260Bb	8.33 ± 4.41Aa	0 ± 0Aa	5.00 ± 5.00Aa	8.33 ± 6.01Aa	0 ± 0Aa	0 ± 0Aa	5.00 ± 2.89Aa
	20-30	128.33 ± 16.41Bc	1.67 ± 1.67Aa	3.33 ± 3.33Aa	1.67 ± 1.67Aa	6.67 ± 3.33Aa	0 ± 0Aa	0 ± 0Aa	1.67 ± 1.67Aa
	0-30	130.00 ± 5.00Ac	7.78 ± 2.78Ba	1.67 ± 0.96Aa	6.11 ± 2.00Aa	5.56 ± 1.47Aa	0 ± 0Aa	0 ± 0Aa	6.11 ± 4.55Aa

CK: Non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing; CFU: colony-forming units.
Each value refers to the average of three replicates, and shows the standard error (SE). Different capital letters at the same depth indicate significant differences at the $p < 0.05$ level; different lower-case letters for the same treatment indicate significant differences at the $p < 0.05$ level, based on a protected least significant difference (LSD) test.

Figure 3. Abundance of cultivable bacteria measured in colony-forming units (CFU) in plots under four treatments in the rhizospheric soil of different plants.



CK: Non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing.
Stibre: *Stipa breviflora*; Cleson: *Cleistogenes songorica*; Artfri: *Artemisia frigida*; RCom: plant community rhizospheric soil.
The line graph shows the number of bacteria genera in each plant rhizosphere. Each value refers to the average of three replicates, and error bars represent the standard error (SE). Different uppercase letters for the same plant indicate significant differences at the $p < 0.05$ level; different lower-case letters for the same treatment indicate significant differences at the $p < 0.05$ level, based on a protected least significant difference (LSD) test.

Structure and composition of bacterial communities in desert steppe

Tables 2 and 3 show the bacterial genera identified in the present study and their abundance in non-rhizospheric and rhizospheric soil. A total of 16 bacterial genera were identified, seven belonging to *Firmicutes* and *Actinobacteria* in non-rhizospheric soil, and nine belonging to *Proteobacteria*, *Firmicutes*, and *Actinobacteria* in rhizospheric soil. The genera common to both rhizospheric and non-rhizospheric soils were *Bacillus*, *Arthrobacter*, and *Streptomyces* (Tables 2 and 3).

Bacillus had an overall relative abundance of over 10% in non-rhizospheric soil treatments and could therefore be regarded as a dominant genus. The quantity of bacteria in non-rhizospheric soils was the largest in the 0-10 cm soil layer;

Table 3. Composition and abundance of cultivable bacteria in different plant rhizospheric soil under different grazing intensities (1×10^4 CFU g^{-1} dry soil).

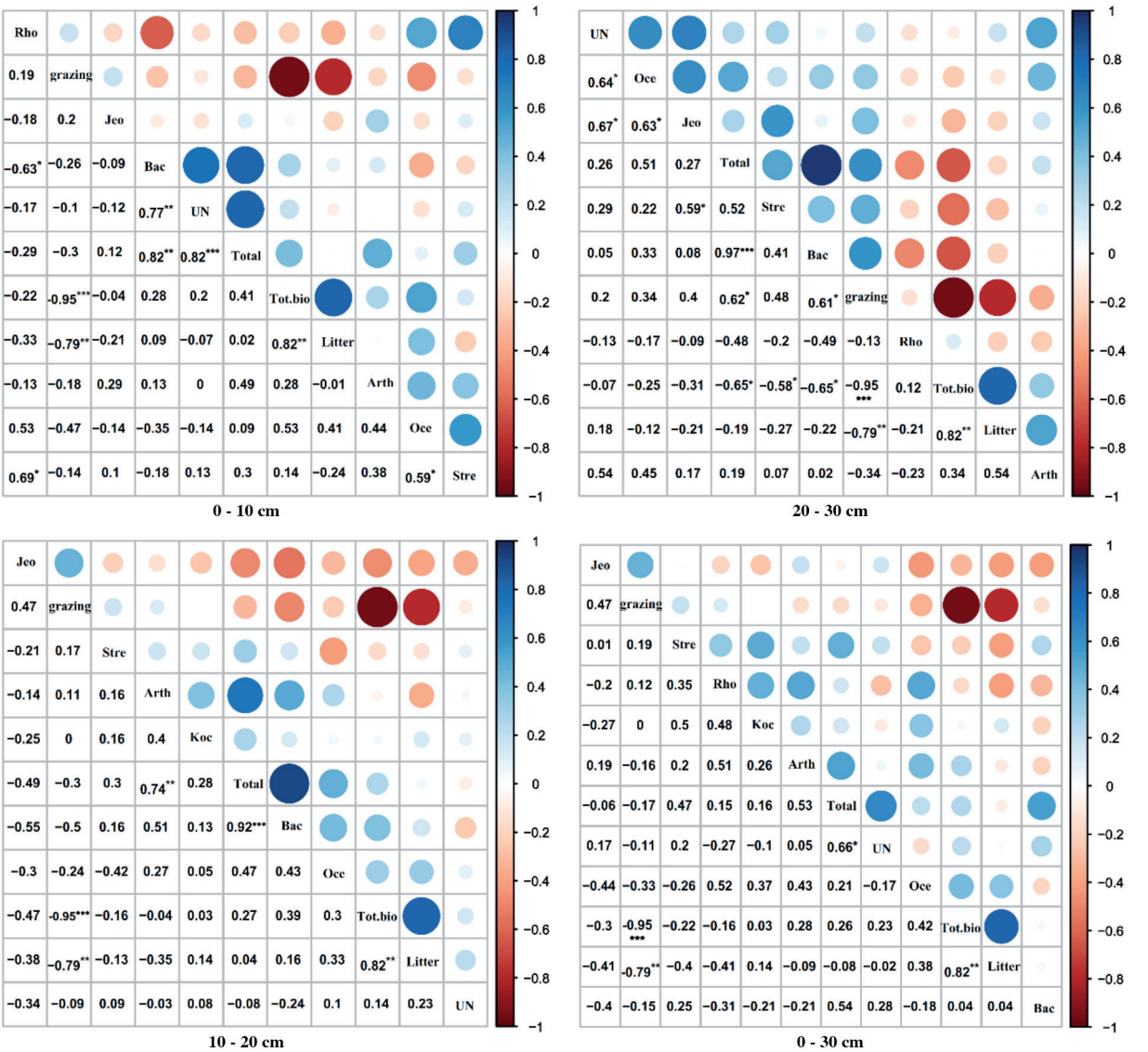
Plant	Grazing intensity	*Streptomyces*	*Rhizobium*	*Arthrobacter*	*Sphingopyxis*	*Bacillus*	*Massilia*	*Janthinobacterium*	*Paenibacillus*	*Aquincola*	Unknown genus
Stipa breviflora	CK	36.67 ± 4.41Aac	10.00±5.00Aa	66.67±16.67Aab	26.67±6.67Aab	1.67±1.67Aa	5.00±5.00Aa	3.33±3.33Aab	0±0Aa	0±0Aa	31.67±7.26Aa
	LG	105.00 ± 10.41Ba	10.00±5.00Aa	186.67±55.25Aa	25.00±11.55Aa	8.33±6.01Aa	46.67±24.21Aa	3.33±3.33Aa	1.67±1.67Aa	5.00±2.89Aa	36.67±20.48Aa
	MG	86.67 ± 13.02BCa	11.67±9.28Aa	80.00±5.00Aa	31.67±6.67Aa	11.67±11.67Aa	18.33±6.01Aa	0±0Aa	0±0Aa	1.67±1.67Aa	10.00±7.64Aa
	HG	53.33 ± 13.02ACa	6.67±1.67Aa	70.00±35.12Aa	23.33±10.93Aa	5.00±0Aa	8.33±6.01Aa	16.67±8.33Aa	0±0Aa	1.67±1.67Aa	40.00±30.00Aa
Cleistogenes songorica	CK	63.33 ± 11.67Ac	3.33±1.67Aa	31.67±18.78Aa	48.33±18.78Aa	1.67±1.67Aa	5.00±5.00Aa	5.00±5.00Aab	0±0Aa	0±0Aa	13.33±8.82Aa
	LG	130.00 ± 37.75Aa	1.67±1.67Aa	58.33±24.21ABa	41.67±18.78Aa	8.33±4.41Aa	0±0Aa	8.33±4.41Aa	6.67±6.67Aa	0±0Aa	48.33±19.22Ab
	MG	58.33 ± 12.02Aa	3.33±3.33Aa	101.67±4.41Ba	21.67±14.81Aa	10.00±5.77Aa	3.33±3.33Aa	0±0Aa	1.67±1.67Aa	0±0Aa	16.67±8.82Aa
	HG	68.33 ± 20.48Aa	5.00±2.89Aa	50.00±27.54ABa	20.00±5.77Aa	1.67±1.67Aa	6.67±6.67Aa	10.00±5.77Aa	1.67±1.67Aa	5.00±2.89Aa	18.33±7.26BAa
Artemisia frigida	CK	96.67 ± 6.67Abc	13.33±8.82Aa	128.33±31.8ABb	10.00±2.89Ab	8.33±1.67Aab	1.67±1.67Aa	0±0Ab	0±0Aa	0±0Aab	11.67±6.67Aa
	LG	61.67 ± 27.74Aa	8.33±3.33Aa	143.33±6.67Aa	13.33±4.41Aa	6.67±6.67Aa	3.33±3.33ABa	0±0Aa	0±0Aa	0±0Aa	23.33±14.53Ab
	MG	78.33 ± 19.22Aa	5.00±2.89Aa	96.67±14.53ABa	23.33±4.41Aa	16.67±4.41Aa	11.67±3.33Ba	13.33±3.33Aa	0±0Aa	0±0Aa	35.00±5.77Aa
	HG	81.67 ± 6.67Aa	6.67±6.67Aa	63.33±1.67Ba	15.00±5.00Aa	5.00±5.00Aa	3.33±3.33ABa	8.33±4.41Aa	1.67±1.67Aa	0±0Aa	18.33±1.67Aa
Community	CK	141.67 ± 21.67Ad	16.67±4.41Aa	125.00±30.41Ab	28.33±3.33Aab	11.67±4.41Ab	20.00±2.89Ab	1.67±1.67Abc	0±0Aa	1.67±1.67Aa	6.67±1.67Aa
	LG	98.33 ± 20.28Aa	8.33±1.67ABa	88.33±10.14ABa	36.67±15.9Aa	6.67±1.67Aa	31.67±10.14Aa	6.67±1.67AB	0±0Aa	0±0Aa	18.33±9.28Ab
	MG	95.00 ± 24.66Aa	3.33±1.67Ba	61.67±27.74ABa	36.67±16.67Aa	13.33±3.33Aa	13.33±13.33Aa	8.33±6.01ABa	0±0Aa	0±0Aa	26.67±3.33Aa
	HG	83.33 ± 6.01Aa	8.33±1.67ABa	30.00±11.55Ba	8.33±3.33Aa	15.00±15.00Aa	15.00±7.64Aa	13.33±1.67Ba	0±0Aa	3.33±3.33Aa	16.67±1.67Aa

CK: Non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing; CFU: colony-forming units.
Each value refers to the average of three replicates, and shows the standard error (SE). Different uppercase letters for the same plant indicate significant differences at the $p < 0.05$ level; different lower-case letters for the same treatment indicate significant differences at the $p < 0.05$ level, based on a protected least significant difference (LSD) test.

relative abundance of *Bacillus* was 80.56%, 67.57%, 76.09%, and 82.83% in the CK, LG, MG, and HG plots, respectively (Table 2). The change in *Bacillus* abundance was consistent with that in the non-rhizospheric surface soil (Figure 4; R = 0.82, $p < 0.01$), indicating that the change in bacterial abundance in the surface soil was mainly caused by the dominant genus, *Bacillus*. In addition, compared with CK, new genera, *Streptomyces* and *Rhodococcus*, emerged in LG and MG but disappeared in HG.

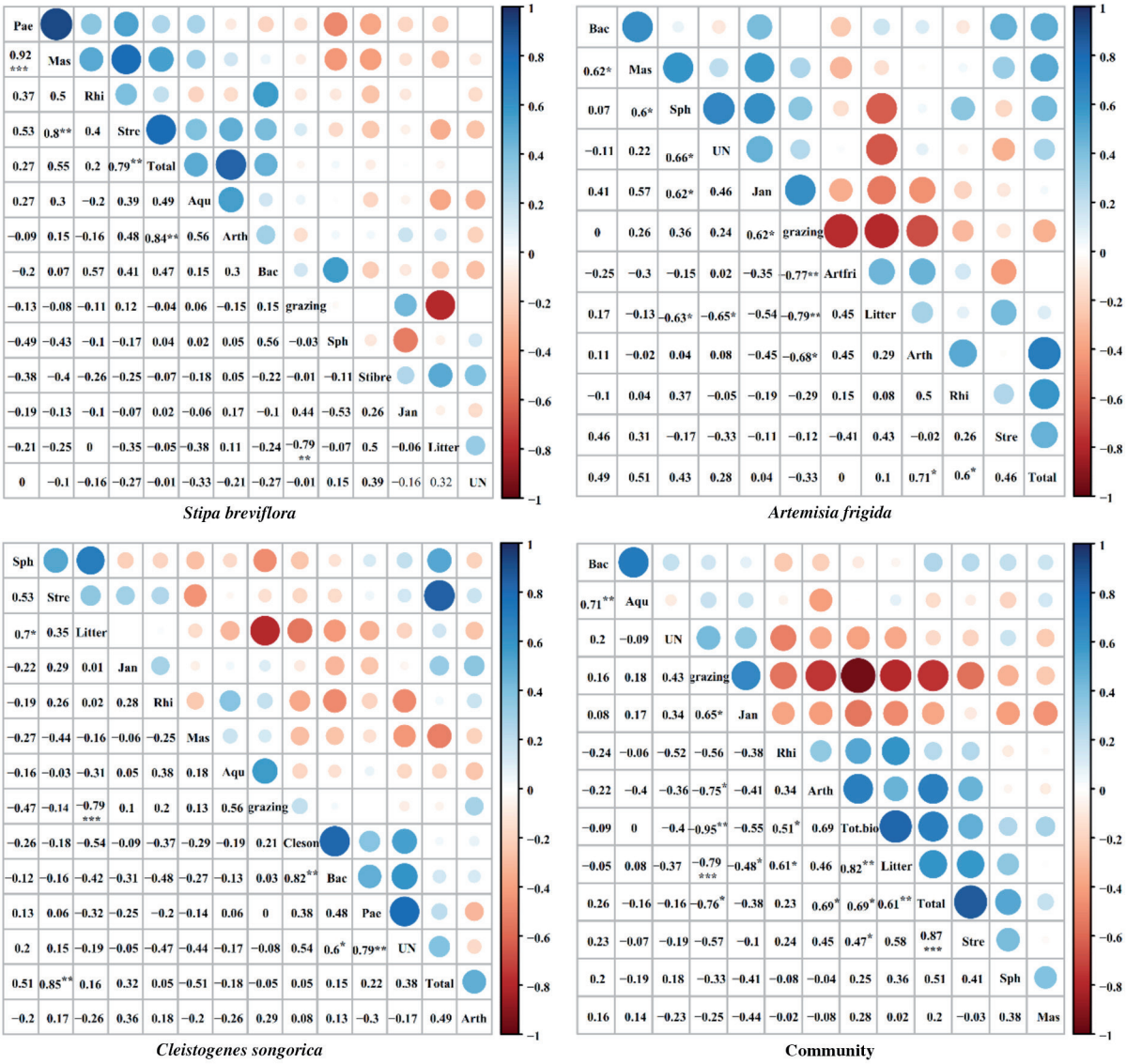
According to the same standard analysis methodology (Table 3), the dominant genera in the rhizospheric soil were *Streptomyces* and *Arthrobacter*. For example, the relative abundance of *Streptomyces* in the CK, LG, MG, and HG plots of the plant community rhizospheric soil was 40.17%, 32.68%, 35.66%, and 44.13%, respectively. The relative abundance of *Arthrobacter* was 34.4%, 30.85%, 25.68%, and 16.83%, respectively (Table 3). These changes in *Streptomyces* or *Arthrobacter* with different grazing intensities were significantly correlated with changes in bacterial abundance in different rhizospheric soils (Figure 5; $p < 0.05$), indicating that the change in bacterial abundance in rhizospheric soil was also mainly caused by changes in the abundance of dominant genera.

Figure 4. Correlation analysis of grazing intensity, plant abundance, and bacterial abundance in non-rhizospheric soil.



*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively.
Tot.bio: Aboveground plants; Total: bacterial abundance; Arth: *Arthrobacter*; Bac: *Bacillus*; Jeo: *Jeotgalicoccus*; Koc: *Kocuria*; Oce: *Oceanobacillus*; Rho: *Rhodococcus*; Stre: *Streptomyces;* UN: Unknown genus.

Figure 5. Correlation analysis of grazing intensity, plant abundance, and bacterial abundance in rhizospheric soil.

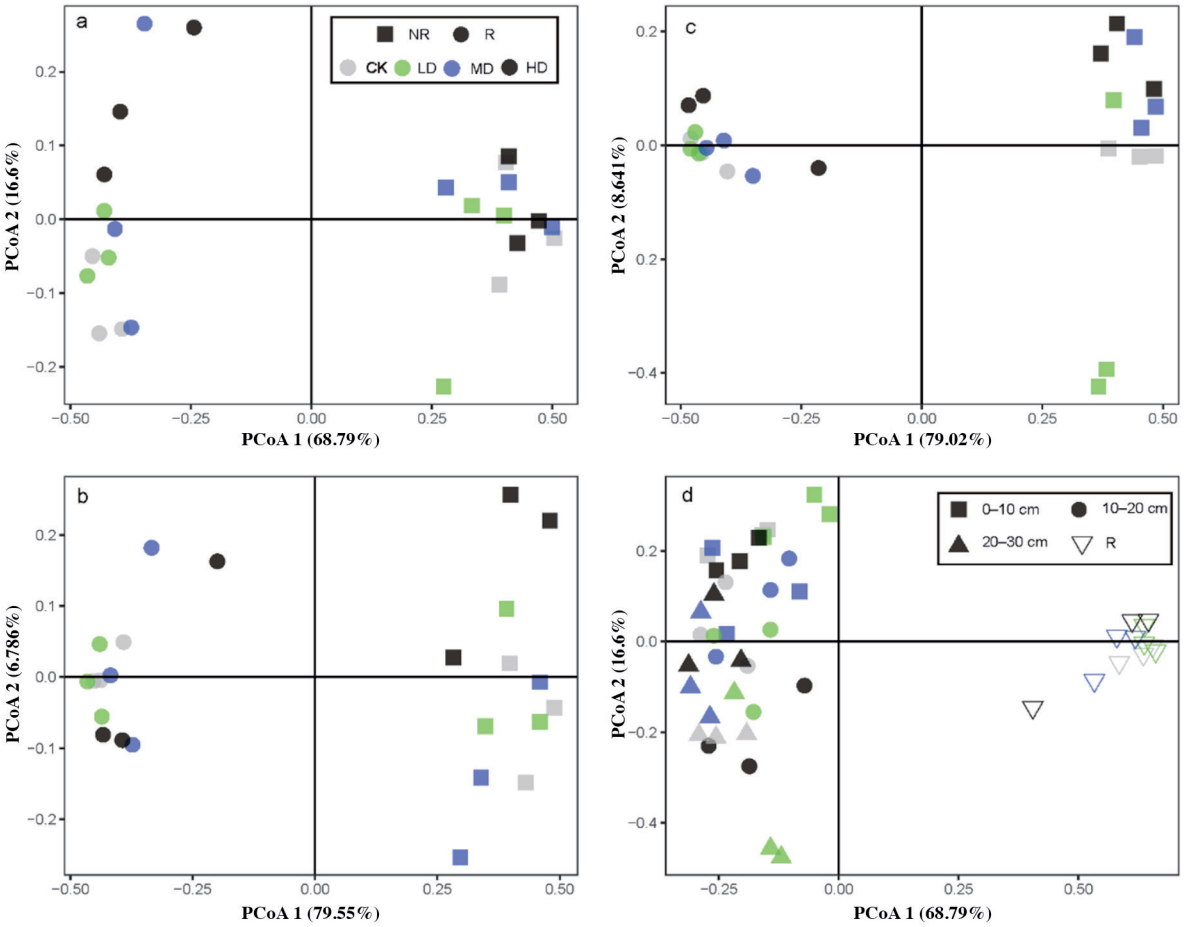


*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively.
Tot.bio: Aboveground plants; Total: bacterial abundance; Artfri: *Artemisia frigida*; Cleson: *Cleistogenes songorica*; Stibre: *Stipa breviflora*; Aqu: *Aquincola*; Arth: *Arthrobacter*; Bac: *Bacillus*; Jan: *Janthinobacterium*; Mas: *Massilia*; Pae: *Paenibacillus*; Rhi: *Rhizobium*; Sph: *Sphingopyxis*; Stre: *Streptomyces;* UN: Unknown genus.

β-Diversity-based statistical tools were employed to explore structural variances in non-rhizospheric and rhizospheric soil bacterial communities. According to the PCoA, there were significant differences in the bacterial community structure of rhizospheric soil compared with non-rhizospheric bacterial communities at different soil depths and when mixed (Figure 6; PERMANOVA, $p = 0.001$). Further dissimilarity tests revealed significant differences in bacterial communities between non-rhizospheric and rhizospheric soil. PERMANOVA tests, based on both Jaccard and Bray-Curtis distances, were used to confirm the significance results (Table 4). This method showed that differences among grazing treatments and plant species were significant and non-significant in rhizospheric and non-rhizospheric bacterial communities, respectively, which indicated that the disturbance effect of grazing on the former was greater.

On the other hand, dissimilarity tests for each treatment using MRPP, ANOSIM, and PERMANOVA methods based on Bray-Curtis distance, conducted to compare differences among soil depths or plant species under the same grazing

Figure 6. Principal coordinate analysis (PCoA) ordination of bacterial communities based on Bray-Curtis distances.



NR: Non-rhizosphere; R: rhizosphere; CK: non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing. Plots a-d respectively show the differences in bacterial community structure between non-rhizospheric (a: 0-10 cm; b: 10-20 cm; c: 20-30 cm; d: three layers combined) and rhizospheric (Com: compound plant community rhizosphere) soils.

treatment, showed a significant difference between non-rhizospheric and rhizospheric bacterial communities, in that the former decreased from CK ($F_{PERMANOVA}$ = 11.36, p = 0.003) to MG ($F_{PERMANOVA}$ = 1.12, p = 0.389), but the latter decreased from CK ($F_{PERMANOVA}$ = 3.12, p = 0.005) to HG ($F_{PERMANOVA}$ = 0.68, p = 0.858) (Table 5). As Table 5 shows, the bacterial community structure in the rhizospheric soils of different plants differed among different grazing intensities. The results of the three test methods showed that a change in grazing intensity had a nonsignificant and significant effect on changes in the bacterial community structure Cleson and Artfri rhizospheric soils, respectively. The MRPP and ANOSIM results revealed a significant difference among different grazing intensities in the bacterial community structure of the Stibre and Com rhizospheric soils (Table 5).

DISCUSSION

The purpose of this study was to determine the effects of different grazing intensities on bacterial community abundance and structure in different plant rhizospheric soils and in different depths of non-rhizospheric soil. Our results showed that bacterial abundance and community structure were significantly different among different depths, but that grazing intensity had no significant impact. On the other hand, bacterial abundance and community structure were significantly different among the plant rhizospheric soils, and showed a significant response to grazing intensity. These results partially supported previous reports that changes in ecological processes caused by large herbivore grazing (Qu et al., 2016; Zhao et al., 2017) and changes in the soil microbial community appear to be more dependent on soil attributes or specific plant species (Aldezabal et al., 2015).

Table 4. Dissimilarity tests of soil bacteria communities using permutational multivariate analysis of variance (PERMANOVA) based on Jaccard and Bray-Curtis distances.

		Jaccard		Bray-Curtis	
Soil	PERMANOVA test	F	p	F	p
NR	Layer (A, B, C)	9.08	0.001***	13.83	0.001***
	Treatment (CK, LG, MG, HG)	1.36	0.190	1.38	0.199
	Layer × Treatment	2.27	0.005**	2.82	0.002**
R	Plant (S, C, A, Com)	1.74	0.012*	1.90	0.022*
	Treatment (CK, LG, MG, HG)	1.93	0.001***	2.03	0.012*
	Plant × Treatment	1.46	0.010**	1.45	0.045*
Between non-rhizosphere and rhizosphere					
	NR vs. R	22.65	0.001***	46.36	0.001***
	0-10 cm vs. R	37.16	0.006***	80.36	0.006***
	10-20 cm vs. R	34.72	0.006***	75.06	0.006***
	20-30 cm vs. R	35.56	0.006***	75.59	0.006***
	CK (NR vs. R)	16.38	0.001***	38.06	0.001***
	LG (NR vs. R)	7.68	0.001***	14.58	0.001***
	MG (NR vs. R)	5.36	0.005 **	10.64	0.007**
	HG (NR vs. R)	9.06	0.001 ***	19.01	0.001***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively.
NR: Non-rhizosphere; R: rhizosphere; A: 0-10 cm; B: 10-20 cm; C: 20-30 cm; CK: non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing; S: *Stipa breviflora*; C: *Cleistogenes songorica*; A: *Artemisia frigida*; Com: compound plant community rhizosphere.

Table 5. Dissimilarity tests for each treatment using multiple response permutation procedure (MRPP), analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) methods based on the Bray-Curtis distance.

		MRPP		ANOSIM		PERMANOVA	
Soil	Treatment	Delta	p	R	p	F	p
NR	CK	0.29	0.008**	0.88	0.003**	11.36	0.003**
	LG	0.46	0.005**	0.63	0.003**	6.82	0.009**
	MG	0.25	0.321	0.03	0.348	1.12	0.389
	HG	0.32	0.003**	0.70	0.004**	5.84	0.002**
	0-10 cm	0.235	0.169	0.083	0.205	1.433	0.164
	10-20 cm	0.239	0.090	0.216	0.111	2.271	0.083
	20-30 cm	0.214	0.028*	0.303	0.029*	3.253	0.047*
R	CK	0.39	0.008**	0.46	0.007**	3.12	0.005**
	LG	0.38	0.028*	0.28	0.020*	2.00	0.029*
	MG	0.29	0.488	-0.02	0.544	1.06	0.414
	HG	0.38	0.431	0.02	0.402	0.68	0.858
	Stibre	0.353	0.029*	0.318	0.018*	1.654	0.09
	Cleson	0.428	0.403	0.054	0.321	1.050	0.44
	Artfri	0.235	0.029*	0.383	0.028*	2.924	0.017*
	Com	0.296	0.038*	0.036	0.023*	1.841	0.063

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively.
NR: Non-rhizosphere; R: rhizosphere; CK: non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing; Stibre: *Stipa breviflora*; Cleson: *Cleistogenes songorica*; Artfri: *Artemisia frigida*; Com: compound plant community rhizosphere.

Variation in bacterial community in relation to grazing intensity

Grazing is one of the most extensive grassland management strategies. It has the potential to change the availability of the soil matrix utilized by bacteria, and so has an important impact on soil bacterial communities (Stark et al., 2015; Zhao et al., 2017). Grazing livestock and plants can affect soil bacterial ecology in desert steppe regions through a range of specific factors, including plant community composition and biomass, feces and urinary sedimentation, rhizosphere exudation, soil texture, and soil physical and chemical properties (Liu et al., 2015; Abdalla et al., 2018). These factors

may have positive, neutral, or negative effects on bacterial community structure (Wang et al., 2011b; Hu et al., 2015; Liu et al., 2015; Mueller et al., 2017). Numerous studies have shown that properly managed grazing has a positive impact on soil bacterial diversity by altering soil physical and chemical properties in grassland ecosystems. However, when grazing intensity exceeds grassland limits, there are negative impacts on grassland aboveground vegetation or underground organisms (Xie et al., 2014; Qu et al., 2016).

The present study showed that bacterial abundance in non-rhizospheric soils at different depths did not vary consistently with increasing grazing intensity. Bacterial abundance at a depth of 0-10 cm responded more obviously to changes in grazing intensity. Compared with the CK plot, the LG treatment tended to increase bacterial abundance, while the MG and HG treatments reduced soil bacterial abundance (Figure 2). This indicated that the impact of grazing on the soil bacterial community mainly depends on grazing intensity (Zhao et al., 2017). In addition, *Bacillus* was clearly dominant in the non-rhizospheric soil bacterial composition, and was the main reason for the changes in soil bacterial abundance. The bacterial abundance of non-rhizospheric soil at the 0-10 cm depth increased in LG, probably because grazing had a significant effect on topsoil quality (Wang et al., 2018). We found that differences in grazing intensity had nonsignificant effect on the bacterial community structure of non-rhizospheric soil. Moreover, the response of non-rhizospheric soil bacterial communities to changes in grazing intensity was complex, and was rarely significantly correlated with aboveground plant responses (Figure 4). Our analysis indicated that non-rhizospheric soil bacterial communities were mainly affected by the properties of vertically distributed soils (Liu et al., 2015; Stark et al., 2015; Zhong et al., 2016).

Variation in the rhizospheric bacterial community in relation to grazing intensity and different plants

Most soil bacteria are heterotrophic, using plant exudates or decomposing plant material for growth (Bais et al., 2006; Bulgarelli et al., 2013; Edwards et al., 2015; Beckers et al., 2017). Therefore, plant species can affect bacterial community structures in the rhizosphere. The abundance and composition of rhizospheric microorganisms can vary among different plant species, resulting in rhizospheric bacterial communities with their own unique features (Rosenzweig et al., 2013; Murphy et al., 2016). Our study showed that plant species, grazing intensity, and the interaction between plants and grazing had significant impacts on the size and structure of rhizospheric bacterial communities (Tables 4 and 6). There were significant negative correlations between grazing intensity and the abundance of aboveground plants, the abundance of Artfri, and the bacterial abundance of both. Moreover, the abundance of Stibre and Cleson, their rhizospheric bacterial abundance, and the abundance of any genus in the rhizospheric soil was significantly related to changes in grazing intensity (Figure 5). In summary, the results showed that grazing may inhibit the abundance of aboveground plants, the abundance of Artfri, and the rhizospheric bacterial abundance of both in desert steppe; this would not be conducive to the development of desert steppe plants and rhizospheric bacterial diversity.

Our study showed that there was nonsignificant difference in rhizospheric bacterial abundance among the three dominant plants ($p > 0.05$). On the other hand, bacterial abundance in the Stibre and Cleson rhizosphere and in the plant community rhizosphere showed significant differences in the CK plot ($p < 0.05$). In addition, LG promoted bacterial abundance in the Stibre and Cleson rhizosphere. Particularly, rhizospheric bacterial abundance was significantly increased in Stibre, the most important species in this study site, primarily because of the obvious increase of *Streptomyces* in the LG plot (Table 4). The abundance of *Arthrobacter* and *Streptomyces*, dominant genera in the rhizospheric soil, significantly increased at 0-10 cm depth in non-rhizospheric soil in the LG plot (Table 2), which may be one of the reasons for the observed increase in bacterial abundance at this depth in the LG plot (Figures 2 and 3). In summary, these results indicated that the non-rhizospheric and rhizospheric bacterial communities interacted with each other through common genera that were dominant in their various communities.

Table 6. Two-way analysis of variance of the effects of grazing intensity and plant composition, grazing intensity, and soil depth on soil bacterial abundance.

	Layers		Plants		Grazing		Layers × Grazing		Plants × Grazing	
Abundance	F	p	F	p	F	p	F	p	F	p
R	-	-	2.5	0.08*	7.78	0.001**	-	-	2.95	0.014*
NR	168.80	0**	-	-	1.72	0.186	2.43	0.035*	-	-

*, **Significance at the 0.05 and 0.01 probability levels, respectively.
R: Rhizospheric soil; NR: non-rhizospheric soil.

The decrease in bacterial abundance in Artfri rhizospheric soil after grazing was consistent with the results of a study by Zang et al. (2017). In addition, there was a gradual decreasing of RA and RCom bacterial abundance with grazing intensity; this may be affected by grazing, the suppression of aboveground plants, and Artfri quantity (Figure 5). The rhizospheric bacterial community structure of Artfri was significantly different from that of Cleson and Com (Table 7). The effects of grazing on rhizospheric bacterial community structure varied with plant species. For example, although RA, RS, and RCom bacterial community structures varied significantly with grazing intensity, bacterial community structure changes in RC were limited, without significant changes (Table 5). The effect observed in this study seemed to indicate that the roots of different desert steppe plants may affect their rhizospheric bacterial communities in different ways, and there may also be differences in their response to external environmental changes and the corresponding results. Similar

Table 7. Comparison of similarity index between each layer or plant across four grazing intensities based on the Bray-Curtis and Jaccard indices.

			Layers		Treatments		Plants	
Distance	Soil	Layer/plant	R^2	p	R^2	p	R^2	p
Bray-Curtis	NR	A *vs*. B	0.32	0.001***				
		A *vs*. C	0.45	0.001***				
		B *vs*. C	0.11	0.049*				
		CK *vs*. LG			0.04	0.518		
		CK *vs*. MG			0.02	0.779		
		CK *vs*. HG			0.01	0.954		
		LG *vs*. MG			0.07	0.322		
		LG *vs*. HG			0.03	0.749		
		MG *vs*. HG			0.06	0.392		
	R	Stibre *vs*. Artfri					0.07	0.167
		Stibre *vs*. Cleson					0.02	0.747
		Stibre *vs*. Com					0.07	0.141
		Artfri *vs*. Cleson					0.10	0.042*
		Artfri *vs*. Com					0.12	0.021*
		Cleson *vs*. Com					0.06	0.242
		CK *vs*. LG			0.05	0.279		
		CK *vs*. MG			0.02	0.732		
		CK *vs*. HG			0.05	0.282		
		LG *vs*. MG			0.06	0.263		
		LG *vs*. HG			0.14	0.001***		
		MG *vs*. HG			0.10	0.03*		
Jaccard	NR	A *vs*. B	0.25	0.001***				
		A *vs*. C	0.36	0.001***				
		B *vs*. C	0.08	0.096*				
		CK *vs*. LG			0.05	0.473		
		CK *vs*. MG			0.03	0.747		
		CK *vs*. HG			0.01	0.967		
		LG *vs*. MG			0.08	0.214		
		LG *vs*. HG			0.04	0.649		
		MG *vs*. HG			0.05	0.479		
	R	Stibre *vs*. Artfri					0.07	0.124
		Stibre *vs*. Cleson					0.03	0.667
		Stibre *vs*. Com					0.06	0.164
		Artfri *vs*. Cleson					0.08	0.026*
		Artfri *vs*. Com					0.09	0.031*
		Cleson *vs*. Com					0.06	0.184
		CK *vs*. LG			0.05	0.343		
		CK *vs*. MG			0.04	0.447		
		CK *vs*. HG			0.06	0.162		
		LG *vs*. MG			0.06	0.198		
		LG *vs*. HG			0.12	0.002**		
		MG *vs*. HG			0.09	0.02*		

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively.
A: 0-10 cm; B: 10-20 cm; C: 20-30 cm; NR: non-rhizosphere; R: rhizosphere; CK: non-grazed enclosure; LG: light grazing; MG: moderate grazing; HG: heavy grazing; Stibre: *Stipa breviflora*; Cleson: *Cleistogenes songorica*; Artfri: *Artemisia frigida*; Com: compound plant community rhizosphere.

studies were conducted by Cleary et al. (2012), who observed that the variation in rhizospheric bacterial communities with depth in a salt marsh historically contaminated by mercury was mainly affected by plant species.

Different impacts of grazing intensity on soil bacterial communities of non-rhizospheric or rhizospheric soil

In this study, PCoA showed significant differences in the bacterial community structure in rhizospheric and non-rhizospheric soil along grazing intensities (Figure 6). A total of 13 genera were isolated from the soil of the study site, belonging to three major groups: Proteobacteria, Firmicutes and Actinobacteria (Tables 2 and 3). This finding was consistent with the study of Jia et al. (2017b). The bacterial community species composition of rhizospheric soil was more abundant than that of non-rhizospheric soil. A possible reason for this was that high plant diversity could improve soil bacterial activity (Lange et al., 2015). Therefore, a reduced plant diversity at the study site due to grazing explained why RCom bacterial abundance gradually decreased with enhanced grazing intensity (Figure 3).

The PERMANOVA testing based on different beta diversity distance matrices (Bray-Curtis, Jaccard) was used to examine differences in bacterial communities at different soil depths and grazing intensities (Abdalla et al., 2018). The smaller the Bray-Curtis dissimilarity coefficient, the more similar was the relationship between the two bacterial community structures. The effect of grazing on soil bacterial community structure was similar to that for bacterial abundance. The bacterial community structures in non-rhizospheric soils were significantly different among soil depths, but grazing intensity did not significantly contribute to differences in bacterial community structure. On the other hand, grazing intensity, plant species, grazing intensity, and the combination all three had significant impacts on bacterial community structures in the rhizospheric soil. Previous studies and the present study revealed that variations in soil bacterial community structure are closely related to changes in the soil environment, plant species, and grazing intensity. Additionally, grazing affects soil bacterial abundance and community structure by affecting the major bacterial groups (Figures 4 and 5) (Eldridge et al., 2017).

CONCLUSIONS

Our study showed that the effect of grazing on bacterial abundance in non-rhizospheric soil was mainly reflected in the surface soil. Bacterial abundance increased in the light grazing (LG) plot, and decreased rapidly in the moderate (MG) and heavy grazing (HG) plots, mainly related to the abundance of *Bacillus*. The differences in how rhizospheric bacteria responded to changes in grazing intensity were mainly reflected in the decrease in bacterial abundance in the mixed rhizospheric soil with increasing grazing intensity. This was consistent with variations in bacterial abundance in the *Artemisia frigida* rhizosphere; bacterial abundance in the *Stipa breviflora* and *Cleistogenes songorica* rhizosphere increased in the LG plot and began to decrease in the MG and HG plots. Thus, the response of bacterial abundance to grazing intensity in the mixed rhizospheric soil was not significantly affected by changes in the rhizospheric bacterial community of the dominant plants. Variations in bacterial abundance in the rhizospheric soil with grazing intensity were mainly caused by changes in the abundance of the dominant *Streptomyces* and *Arthrobacter* species. In addition, the effects of grazing intensity on bacterial community structures in the non-rhizospheric and rhizospheric soils were significantly different: grazing had nonsignificant impact on bacterial community structures in non-rhizospheric soil, but had a significant effect on rhizospheric soil. There were also significant variations in bacterial community structures among plant species. In conclusion, bacterial communities in rhizospheric soil were mainly affected by different plant species, and were also more susceptible to changes in grazing intensity than those in non-rhizospheric soil.

ACKNOWLEDGEMENTS

Jing Huang contributed equally to this work and should be regarded as co-first authors. The study was supported by grants from National Natural Science Foundation of China (31770499, 31100330, 31500365, 31270502, 41403082, 31300386), Tianjin Science and Technology Support Program (15ZCZDSF00410) and Tianjin Natural Science Foundation (12JCYBJC19700, 18JCYBJC96500). We gratefully thank Guodong Han, Guogang Zhang, Meiqing Jia and many others for setting up the experiment. We also thank Wei Huang and Shuai Xu for DNA extraction and soil microbe culture.

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