Intermediate grazing intensities by sheep increase soil bacterial diversities in an Inner Mongolian steppe

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Abstract Ungulate grazing is known to play a crucial role in regulating nutrient cycling and controlling plant community structure and productivity in grassland ecosystems. However, little is known about the effects of grazing intensities on soil bacterial community structure and diversity, particularly at the long-term scale. In this study, we measured plant biomass and diversity, soil characteristics and bacterial community structure and diversity in a 16-year field experiment that had four grazing intensity treatments (non-grazed, CK; low-intensity grazing (LG), moderate-intensity grazing (MG) and high-intensity grazing (HG)) in an Inner Mongolian typical grassland. The CK, LG, MG and HG sites were grazed by 0.00, 1.33, 4.00 and 6.67 sheep ha^{-1}, respectively. Bacterial community structure and diversity under grazing intensity treatments were assessed with PCR amplification of DNAs extracted from soils and denaturing gradient gel electrophoresis (DGGE) separation. The results showed that the CK soil had higher moisture, organic C, NH_4^+-N and NO_3^--N concentrations than grazed soils, and the HG treatment had the lowest plant biomass and diversity across all the treatments. Principal components analysis of DGGE patterns showed that the LG and MG treatments were different from the CK and HG treatments. In addition, soil bacterial diversities in the LG and MG treatments were significantly higher than those in the other treatments. The relationships between environmental variables and soil bacterial community structure were assessed using redundancy analysis and we found that soil

moisture content, *Artimesia frigida* biomass and pH were the best indicator of the changes in soil bacterial community structure among all the treatments. Overall, our results indicated that intermediate grazing intensities (LG and MG) increased soil bacterial diversities and along with previous studies in this area, we suggested the MG treatment was the most suitable management practice in the Inner Mongolian steppe, not only supporting greater livestock amounts but also harboring greater bacterial diversity.

Keywords plant biomass · plant diversity · grazing intensity · bacteria · community structure · Inner Mongolia · grassland

Introduction

Soil microorganisms represent the world's largest reservoir of biological diversity with enormous diversities of 16S rRNA gene sequences amplified from soil DNA and RNA (McCaig et al. 1999; Sun et al. 2004). Numerous studies have characterized soil bacterial communities, attempting to draw links between plants and below-ground bacterial diversity. A number of factors were found to influence soil microbial community composition and diversity and these factors included plant species (Miethling et al. 2003; Smalla et al. 2001), soil organic C content and soil C:N ratio (Wieland et al. 2001; Graham and Haynes 2005). Previous studies have focused on the effects of plant communities on soil bacterial community structure and diversity in the rhizosphere of grasslands (e.g., Kowalchuk et al. 2002; McCaig et al. 1999, 2001). However, little has been known about the effects of grazing intensities on soil bacterial community structure and diversity at the long-term.

The Inner Mongolia steppe is an important, representative part of the Eurasian grasslands which are the largest contiguous grassland area in the world (Bai et al. 2004). Grazing is one of the most important land-uses on the Inner Mongolia steppe and has been so far for thousands of years. The effects of different grazing intensities on the plant community structure and productivity have been documented in this area (Chen and Wang 2000). However, only a few studies have investigated the effects of different grazing intensities on the culturable bacterial community composition (Liu and

Liao 1997).

It was well established that plate count cannot be used to study the community composition of soil microbes since the majority of soil microorganisms are not culturable (Amann et al. 1995). Molecular techniques have circumvented this problem (Muyzer et al. 1993). Usually these techniques are based on the extraction of genomic DNA from soil, followed by the PCR amplification of 16S rRNA genes and separation of amplicons with procedures such as denaturing gradient gel electrophoresis (DGGE). DGGE analysis of bacterial community structure has been widely applied to grassland soils (Kowalchuk et al. 2002; McCaig et al. 1999; Nunan et al. 2005).

The main objectives of this study were to (1) investigate the effects of different grazing intensities on the plant community structure and diversity, soil characteristics and associated bacterial community structure and diversity using PCR-DGGE, (2) quantify the relationships between environmental variables and community composition of bacteria in soil, and (3) determine which grazing intensities were more suitable for local grazing management in the Inner Mongolian steppe.

Materials and methods

Site description

The experiment was conducted within the grazing enclosures of the Inner Mongolia Grassland Ecosystem Research Station of Chinese Academy of Sciences, located in the central part of Inner Mongolia Autonomous Region. The latitude of the experimental site is 43°50′N and the longitude is 116°34′E, with an average elevation of 1,100 m above sea level. The climate is temperate and semi-arid with cold, dry winters and mild, wet summers. The mean annual temperature is -0.4 ℃ with mean monthly temperatures ranging from 17.9 ℃ in July to -23 ℃ in January. The mean annual precipitation is 350 mm with most rain events occurring in July and August. Prior to the experimental design, *Artemisia frigida*, a native grass, was dominant plant species. Vegetation within the experimental plots is dominated by *Artemisia frigida*, *Kochia prostrate* and *Potentilla acaulis*. Soils are coarse textured with a mean of 71% sand, 15% silt, and 9% clay across all

experimental plots (Chen and Wang 2000; Xu et al. 2007).

Grazing management experimental plots were established in 1989 using a randomized complete block design with six grazing intensities (0.00, 1.33, 2.33, 4.00, 5.33, 6.67 sheep ha^{-1}) with three replicates (Chen and Wang 2000). The plant biomass and soil properties (soil texture, structure and chemical characteristics) were relatively uniform among all experimental plots before the initiation of the grazing treatments. The grazing intensity treatments were maintained for 16 years from 1989 to 2004, at which point samples were obtained from this study. There were 0, 4, 8, 12, 16 and 20 Inner Mongolia fine wool sheep grazing rotationally in three replicated 1-ha plots, representing the grazing intensities of 0, 1.33, 2.67, 4.00, 5.33 and 6.67 sheep ha^{-1}, respectively. Four grazing intensity were examined in this study: non-grazed (CK; 0 sheep ha^{-1}), light-grazing intensity (LG; 1.33 sheep ha^{-1}); moderate-grazing intensity (MG; 4.00 sheep ha^{-1}) and heavy-grazing intensity (HG; 6.67 sheep ha^{-1}) (Zhou et al. 2008a). Every year, grazing started on May 20th and ended on October 5th. Each plot was rotationally grazed three times per year, each time for 15 days with a rotation interval of 30 days. The total grazing period of each grazed plot was thus 45 days per year. During grazing periods, the sheep were driven to the enclosures at 5:00 am and back home at 8:00 pm. The sheep drank water twice daily from the nearby Xilin River, once before being driven to the enclosures and once after being driven out of the enclosures. No fertilizer was used in any treatment.

Soil sampling and plant aboveground biomass and diversity

Within each replicated plot, five 1×1 m quadrats were randomly selected in early August 2004 and aboveground vegetation was clipped at ground level to determine plant community composition and diversity. Within each quadrat, five soil cores were taken at a depth of 0 to 10 cm with a 3 cm diameter auger. All soil cores were hand-sorted to remove stones and coarse root, cooled on ice in the field and transported into the laboratory. After sieving (2 mm), 25 soil cores within each plot were combined to form a single sample. All the samples were stored at 4 ℃ and soil characteristics were determined within two weeks. For plant biomass and diversity measurements, the plant samples were separated into different species and the weight of each species was determined prior

to oven-drying at 65 ℃ for 48 h (Chen and Wang 2000).

Measurement of environmental factors

Water content of the composite sample from each plot was determined gravimetrically by oven-drying at 105 ℃ for 24 h. Air-dried soil samples were used for measuring pH, organic C content and organic N content. The pH values were determined in water suspension (water/soil=2.5:1). Soil organic C contents were analyzed using a H_2SO_4-$K_2Cr_2O_7$ oxidation method (Nelson and Sommers 1982). Soil organic N contents were measured using the Kjeldahl digestion method (Kjektec System 1026 Distilling Unit, Sweden). A 5 g subsample from each composite sample was extracted with 30 ml of 2 M KCl on an end-to-end shaker for 1 h. The soil suspension was filtered through a Whatman no. 42 filter paper and the filtrate analyzed for NH_4^+-N and NO_3^-N on a flow injection auto-analyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark).

Extraction and PCR amplification of total DNA from soil

Nucleic acids were extracted and purified from 0.5g aliquots of each composite sample following the procedures described in Zhou et al. (2008b). Briefly, soil was placed in a 2 ml screw-cap tube containing a mixture of ceramic and silica particles (Bio101, Carlsbad, Calif.); the mixture was homogenized for 30 s in a FastPrep bead beater cell disrupter (Bio101). The nucleic acids were then precipitated and washed twice in 75% (v/v) ethanol, and the final DNA was re-suspended in 100 μl TE buffer. The crude extracts were purified with Qiagen gel extraction Kit (Qiagen, Inc.) and DNA concentrations were determined by nucleic acid analysis (Shimadzu, Kyoto, Japan).

PCR amplifications of bacterial 16S rRNA were carried out using the primers P338F (5’ ACTTCTACGGGAGGCAGCAG 3’) with 40 GC clamps and P518R (5’ ATTAC CGCGG CTGCT GG) (Muyzer et al. 1993). Amplification was performed in a 25μl (total volume) reaction mixture. The final concentration of different components in the mixture was as follows: ~ 50ng of purified DNA, 0.4 μM of each primer, 200 μM of each deoxynucleoside triphosphate, 1.5 μM $MgCl_2$, 1×

thermophilic DNA polymerase 10×reaction buffer ($MgCl_2$-free), 1.25U per 50 μl of Taq DNA polymerase (Promega, Madison, WI, USA), and DNAse and RNAse free filter sterilized water (LiYuan Apparatus Com., Beijing, China). The reaction was performed in a PTC-200 thermal cycler (MJ Research Com., USA) using 35 cycles consisting of 94 ℃ for 1 min, 56 ℃ for 1 min, and 72 ℃ for 1 min and a final extension step consisting of 72 ℃ for 10 min. After each PCR, the size of the amplification products was verified on a 1.2% agarose gel.

DGGE analysis

DGGE analysis was performed using a Dcode system (Bio-Rad, Hercules, CA, USA; Muyzer et al. 1993). Polyacrylamide gels (8% acrylamide, 0.5 × TAE (20mM Tris, 20 mM acetate, 1mM EDTA [pH 8.0]), 37:1 acrylamide: bisacrylamide) were cast using 40-60% denaturant; 100% denaturant was defined as 7M urea with 40% (vol/vol) formamide. Electrophoresis was run overnight for 12 h at a constant temperature of 60℃ at 110 V. After electrophoresis, the gels were soaked in SYBR green II nucleic acid gel stain (1:10000 dilution; FMC Bioproducts, Rockland, ME, USA) for 30 min. The stained gels were immediately photographed on a UV trans-illumination table with a Hewlett Packard Scanjet 5370C. The DGGE patterns were determined using the Labworks software (Labworks TM software version 4.0. UVP, UK). Band intensities of each lane were read based on peak heights in the densitometric curve. The Shannon index of general diversity (H) was used to measure the bacterial and plant diversity (Dengen et al. 2000); H was calculated based on the band intensity in the gel lanes as follows: $H = -\sum(n_i/N)\log(n_i/N)$, where n_i is the height of the peak and N the sum of all peak heights of the densitometric curve. Evenness (E) was calculated by the equation of $E=H/H_{max}$, where H_{max} was the largest H within a special sample (Dengen et al. 2000).

Cloning and sequencing analysis

The specific bands of the DGGE pattern were excised from the gel and re-amplified with the same reaction procedure described above. The PCR products were excised from an agarose gel, purified with Qiagen gel extraction kit (Qiagen Inc.) to remove primers and short oligonucleotides and then

cloned into plasmids with a pGEM-T easy vector kit (Promega). Plasmid DNAs were isolated from randomly selected clones and screened for inserts of the expected sizes. Following confirmation of their DGGE positions under the conditions described before, the plasmid DNAs were sequenced with primers (T7/sp6 forward/reverse) on an ABI PRISM 3700 DNA analyzer (ABI Biosystems, Inc.). The identities of the 16S rRNA gene sequences were confirmed by searching the international sequence NCBI database using the BLAST programs (Zhou et al. 2008b).

Statistical analysis

One-way analysis of variance (ANOVA) was employed to determine the effect of grazing intensities on the soil physical and chemical properties as well as on the plant biomass and diversity. Means and least significant differences were calculated at the 5% level. For DGGE patterns of soil bacteria, principal components analysis (PCA) was carried out with a correlation similarity matrix. All ANOVA, PCA and regression analyses were performed using SPSS 12.0 software (SPSS Inc., USA).

Redundancy analysis (RDA) was used to study the relationships between environmental variables (soil properties and plant biomass and diversity) and bacterial community structure based on DGGE profiles. Data was analyzed by detrended correspondence analysis using Canoco Software 4.5 (Microcomputer Power, USA), which revealed that the data exhibited a linear, rather than unimodal, response to the environmental variables. Redundancy analysis of the DGGE profiles in all the treatments were related to environmental variables by selecting the linear combination of environmental variables that gave the smallest residual sum of squares (Kennedy et al. 2004).

Nucleotide sequence accession numbers

Three nucleotide sequences obtained in this study were sent to NCBI database. The accession numbers for the excised band sequence are DQ420618 to DQ420620.

Results

Soil characteristics and plant biomass and diversity

The CK soil had significantly higher moisture, organic C, NH_4^+-N and NO_3^--N concentrations than the grazed soils (Table 1), while the MG soil had the highest pH. There were no significant differences in the soil organic N contents among all the treatments.

The dominant species, plant biomass and diversity index are shown in Table 2. The CK site was dominated by *Leymus chinensis*, *Artemisia frigida* and *Potentilla acaulis*, whereas, the grazing sites were dominated by *Artemisia frigida*, *Kochia prostrate* and *Carex duriuscula*. The plant biomass and diversity in the HG treatment were significantly lower than those in the other treatments.

PCR-DGGE analysis of bacterial community

Before PCR-DGGE analysis, DNA yields after extraction were examined and no significant differences were found among all the treatments. DGGE patterns of soil bacterial community under different grazing intensities are shown in Fig. 1. A great number of bands in DGGE patterns were detected and there were also many bands forming a smear, probably due to the abundant bacterial species present in the grassland soils. Clear differences were observed across sites with many bands absent in the CK and HG soils and some bands present in the LG and MG soils. To identify the specific dominant bacterial species in the DGGE pattern under different grazing intensities, three main bands were excised from the gel (Fig. 1), re-amplified and sequenced. The sequences of bands 1, 2 and 3 showed 98% similarity to *Intestinal bacterium D22* (AY374116), 97% to *Uncultured Acidobacteria bacteria* (AKYH520*)* and 98% to *Bosea minatitlanensis* (AF273081), respectively. These sequences fell into subtaxa of *Gamma-proteobacteria*, *Acidobacteria* and *Alpha-proteobacteria,* respectively.

The bacterial community structure among all the treatments was analyzed by PCA (Fig. 2). The PC1 and PC2 explained 49.68% and 13.16% of the variance of the data, respectively. The LG and MG soils differed from the CK and HG soils with high ordinate scores on the PC1. There was a clear separation between the CK and HG soils along the PC2. The LG and MG soils had higher bacterial diversities and Evenness than the CK and HG soils (Table 3).

Relationships between environmental variables and bacterial community structure

The relationships between environmental variables and soil bacterial community composition are shown in Fig. 3. Eigenvalues of RDA indicated that axes 1 and 2 explained 0.21 and 0.19 of the overall variance within the soil bacterial community in all the treatments, respectively. Species-environment correlation for both axes was 0.98, indicating that soil bacterial community composition was strongly correlated with environmental parameters. The plot can be interpreted quantitatively by noting that the length of an arrow indicates how much variance is explained by that factor. The direction of arrows for each environmental variable indicates an increasing concentration of that variable (the longer the arrow, the stronger the relationship; Kennedy et al. 2004). The arrows for soil moisture content, *A. frigida* biomass and pH were longer than those of the other variables, indicating that these factors accounted for the greatest proportion of variance in the soil bacterial community composition. The patterns of soil bacterial community structure in the treatments with approximately the same direction as the environmental variable arrow indicate a high positive correlation. The patterns of community composition of bacteria in the CK and HG treatments showed a positive relationship with soil moisture and organic C contents, while those in the LG and MG treatments were positively influenced by *A. frigida* biomass and pH, followed by soil organic N contents. Ammonium-N and NO_3^--N concentrations, and plant biomass and diversity contributed to some extent to the changes in community composition of bacteria under different grazing intensity treatments.

Discussion

Effects of grazing intensities on the bacterial community structure

Grazing represents the most important land-use in the Inner Mongolian steppe and can determine the grassland productivity and lifestyles of local residents (Chen and Wang 2000). The decrease in plant biomass along the grazing gradient is consistent with a previous study (Chen and Wang 2000).

In addition, there were considerable changes in dominant plant species with the increasing grazing intensities (Table 2). It has been shown that plant species had a major selective influence on the soil bacterial community of their rhizosphere (Smalla et al. 2001), which could result in the differences in the soil bacterial community. In this study, we found that intermediate grazing intensity treatments (LG and MG) considerably changed soil bacterial community structure and significantly increased bacterial diversities as compared to the CK and HG treatments (Fig. 2 and Table 3). Our results were in contrast to what reported by McCaig et al. (1999, 2001) and Clegg (2006), who have conducted their studies in the unimproved and improved grassland ecosystems in England and reported that there were no significant differences in soil bacterial diversity between both ecosystems using the methods of DGGE and clone library. However, Grayston et al. (2004) found that the improved grassland soil had greater functional diversity than the unimproved grassland soil using Biolog method.

These large variations of bacterial diversity in grazed and non-grazed treatments could be attributed to grazing intensity. In fact, grazing by ungulate animal affects soil bacterial diversity through various factors such as faecal and urine deposition (Ritz et al. 2004), shifts in rhizosphere exudation (Guitian and Bardgett 2000), shifts in aboveground plant community composition (Dorrough et al. 2004; Wang et al. 2001) and changes in soil texture and permeability (Jia et al. 1997). These factors could exert a direct or indirect influence on soil bacterial community composition and diversity (Nunan et al. 2005). In general, intermediate grazing intensities will negligibly affect or even benefit grassland ecosystems in terms of dry matter production, nutrient cycling and C and N storage, possibly due to increased nutrient availability and facilitated vegetation regeneration (Han et al. 2008). Previous studies revealed that intermediate grazing intensities increased plant productivity (plant biomass + biomass removal by grazing) (Chen and Wang 2000) and stimulated the cumulative net N mineralization, nitrification and ammonification (Xu et al. 2007) and potential denitrification (Brůček et al. 2009), which might support higher soil bacterial diversities. However, it has been shown that long-term heavy grazing significantly decreased the storage of soil C and N and caused grassland degradation (Cui et al. 2005; Han et al. 2008; Steffens et al. 2008), which might result in a decrease in bacterial diversity. Along with previous studies at the same experimental site (Chen and Wang 2000; Xu et al. 2007), we suggested that the MG treatment was the most suitable management practice in this area, not only supporting

greater livestock amounts and rapid N cycling but also harboring greater soil bacterial diversity.

What was driving changes in bacterial community structure?

The RDA can be a suitable method for quantifying the relationships between environmental variables and changes in soil bacterial community structure (Kennedy et al. 2004; Macdonald et al. 2009), aiming to determine the main factors affecting soil bacterial community structure among all the treatments. It has been shown that the soil microbial community were predominately affected by abiotic environmental factors, such as soil moisture content (McDonald et al. 2009), pH (Fierer et al. 2009), soil organic C content (Degens et al. 2000), and C:N ratio (Graham and Haynes 2005).

In grassland soils, Kowalchuk et al. (2002) found that soil bacterial community was driven by plant community composition and diversity. On the contrary, Nunan et al. (2005) reported that soil bacterial community structure in the natural grassland soil was subject to a wide range influences such as plant species, plant community composition and soil characteristics, and so on, none of which was found to be dominant. In this study, we found that soil moisture content, *A. frigida* biomass and pH were the best indicators of the changes in soil bacterial community structure under grazing intensity treatments. The *A. frigida* biomass contributed more to the changes in bacterial community structure compared to overall plant biomass and diversity. This result indicated that keystone plant species had a more important influence on bacterial community structure than plant biomass and diversity. The patterns of soil bacterial community structure in the LG and MG treatments were positively influenced by *A. frigida* biomass and pH (Fig. 3). The *A. frigida* species in the LG and MG treatments could affect soil bacterial communities through increasing root exudation, possibly due to the stimulation by intermediate grazing (Chen and Wang 2000).

Conclusions

In summary, grazing intensities over long-term periods (ca. 16 years) greatly influenced soil bacterial community structure and diversity. The non-grazed treatment had higher soil moisture, organic C, NH_4^+-N and NO_3^--N concentrations in comparison to the different grazing intensity treatments. Among the grazing intensity treatments, the HG treatment had the lowest plant biomass

and diversity. Intermediate grazing intensity treatments (LG and MG sites) considerably changed soil bacterial community structure and significantly increased bacterial diversities as compared to the CK and HG treatments. The relationships between environmental variables and soil bacterial community structure were analyzed using RDA and we found that soil moisture content, *A. frigida* biomass and pH were the best indicators of the changes in soil bacterial community structure among all the treatments. Overall, our results indicated that intermediate grazing intensities (LG and MG) increased soil bacterial diversities and along with previous studies in this area, we suggest the MG treatment is the most suitable management practice in the Inner Mongolian steppe, not only supporting greater livestock amounts and rapid N cycling but also harboring greater soil bacterial diversity.

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Table 1 Soil properties under different grazing intensity treatments in the Inner Mongolia steppe.

Treatment	Soil moisture (%)	pH	soil organic C (g kg^{-1})	NH_4^+-N (μg g^{-1})	NO_3^--N(μg g^{-1})	soil organic N (g kg^{-1})
CK	18.7±0.05a	6.65±0.02b	1.79±0.02a	4.34±0.07a	3.10±0.06a	0.71±0.05
LG	11.16±0.04b	6.65±0.03b	1.47±0.02b	3.33±0.02b	1.84±0.09b	0.69±0.05
MG	12.13±0.07b	6.73±0.02a	1.47±0.05b	3.34±0.03b	1.34±0.03b	0.66±0.08
HG	12.65±0.09b	6.58±0.04b	1.56±0.03b	3.25±0.08b	0.89±0.05c	0.55±0.09

CK, non-grazed; LG, low-intensity grazing; MG, moderate-intensity grazing; HG, heavy-intensity grazing. The CK, LG, MG and HG sites were grazed by 0.00, 1.33, 4.00 and 6.67sheep ha^{-1}, respectively. Each plot was rotationally grazed three times per year, each time for 15 days with a rotation interval of 30 days.

Each value represents the mean with standard errors (n=3).

Different letters within columns showed significant differences at $P<0.05$.

Table 2 Dominant species, total aboveground biomass and plant diversity Shannon index (H) under different grazing intensity treatments.

treatment		CK	LG	MG	HG
Dominant species aboveground biomass (g m^{-2})	*Artemisia frigida*	27.5±0.3c	36.5±0.2b	49.1±0.5a	12.1±0.6d
	Kochia prostrata		14.2±2.92b	21.1±1.8a	12.6±3.3b
	Potentilla acaulis	19.9±2.8b			29.5±2.1a
	Leymus chinensis	44.4±2.3			
	Carex duriuscula		16.6±5.49	23.7±6.09	
Total aboveground biomass (g m^{-2})		127.1±12.9a	98.3±12.1a	102.1±13.2a	56.7±12.6b
Plant community Shannon diversity H		1.48±0.11a	1.33±0.07a	1.23±0.12ab	1.10±0.06b

CK, non-grazed; LG, low-intensity grazing; MG, moderate-intensity grazing; HG, heavy-intensity grazing.

Each value represents the mean with standard errors (n=3).

Different letters within rows showed significant differences at $P<0.05$.

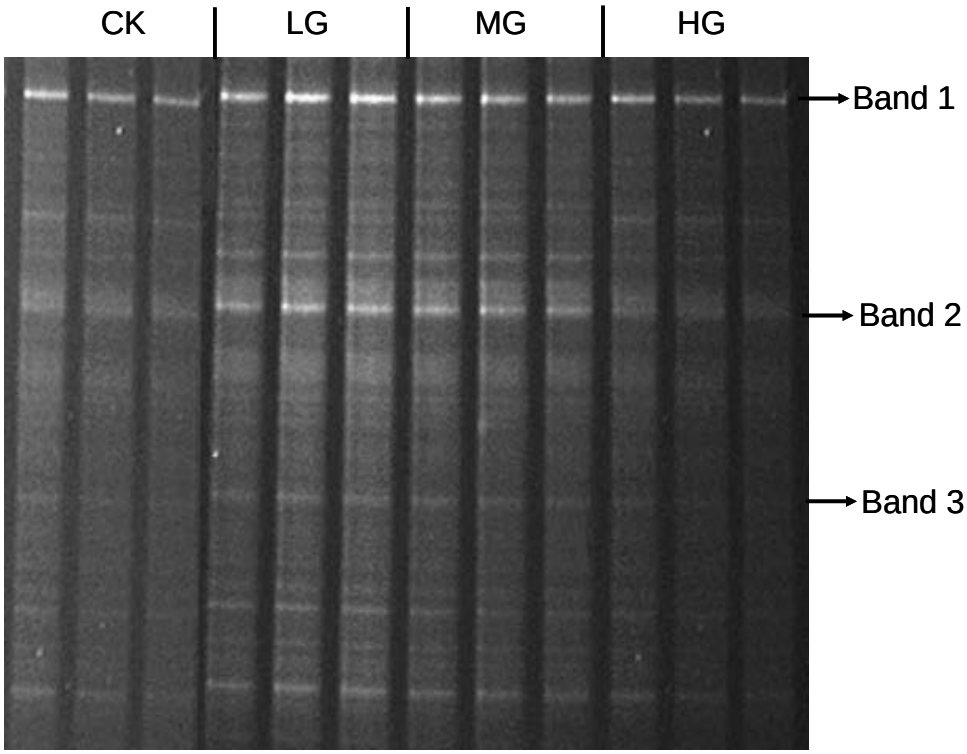

Fig. 1 DGGE patterns of soil bacterial community under different grazing intensity treatments. Bands 1, 2 and 3 were excised from the gel and were further sequenced to indentify the specific bacterial taxon. CK, non-grazed; LG, low-intensity grazing; MG, moderate-intensity grazing; HG, heavy-intensity grazing.

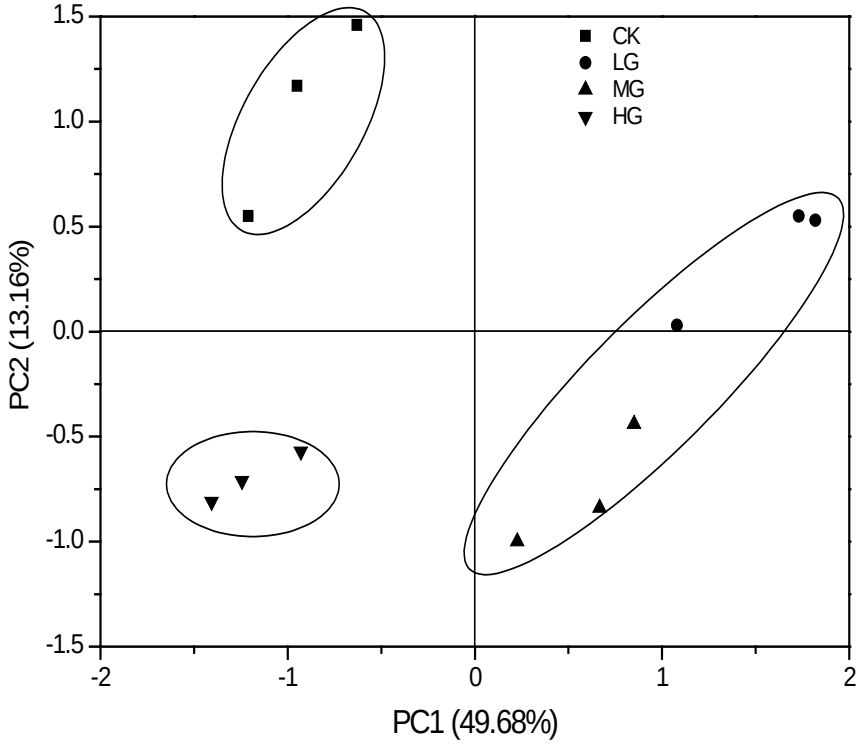

Fig. 2 Ordination plot of principal components analysis (PCA) of DGGE patterns in soils under different grazing intensity treatments. Numbers in parenthesis are percentage variance by each principal component (PC). CK, non-grazed; LG, low-intensity grazing; MG, moderate-intensity grazing; HG, heavy-intensity grazing.

Table 3 Shannon diversity index (H) and Evenness (E) of soil bacterial diversity based on the DGGE profiles under different grazing intensity treatments in the Inner Mongolian steppe

Treatment	H	E
CK	1.88±0.04b	0.84±0.01b
LG	2.39±0.05a	0.89±0.02a
MG	2.31±0.07a	0.89±0.01a
HG	1.76±0.09b	0.79±0.03b

Means within a column followed by the same letter indicate no significant differences at $P<0.05$. CK, non-grazed; LG, low-intensity grazing; MG, moderate-intensity grazing; HG, heavy-intensity grazing.

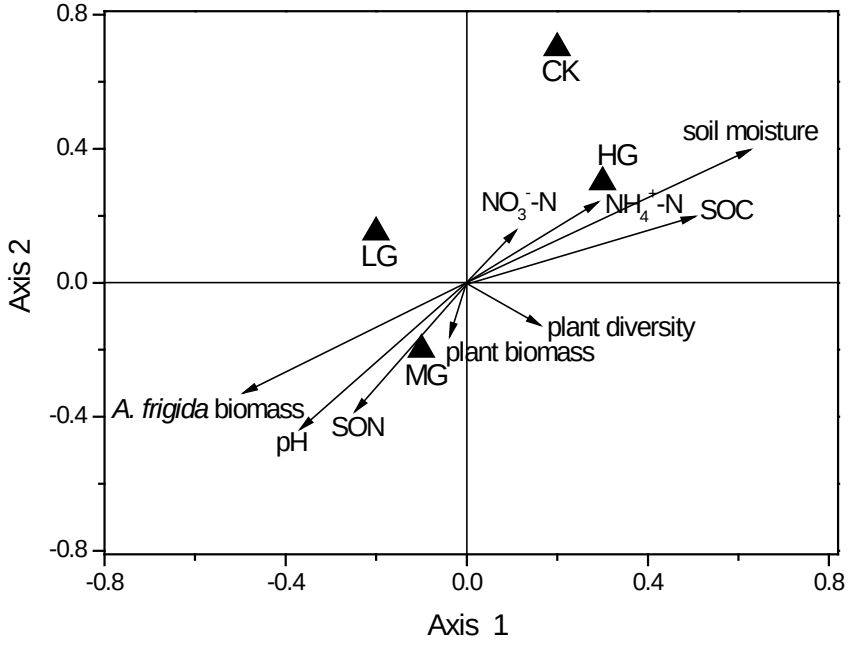

Fig. 3 Biplots of redundancy analysis (RDA) of the relationships between environmental variables and soil bacterial community structure based on DGGE profiles under different grazing intensity treatments. SOC, soil organic C and SON, soil organic N. CK, non-grazed; LG, low-intensity grazing; MG, moderate-intensity grazing; HG, heavy-intensity grazing.