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Long-term grazing exclusion effects on vegetation characteristics, soil properties and bacterial communities in the semi-arid grasslands of China

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ABSTRACT

Grazing exclusion is regarded as an effective way to restore degraded grasslands. However, it remains unclear if grazing exclusion could improve soil bacterial communities and how the soil bacteria affect soil organic carbon (SOC) in semi-arid grasslands over 33 years of continuous grazing exclusion. We studied the effects of 33 years of grazing exclusion on vegetation characteristics, soil properties, and the soil bacterial communities in the semi-arid grasslands. Our results showed that grazing exclusion significantly increased species diversity and richness, coverage, above- and belowground biomass and litter biomass. Total nitrogen (TN), soil organic carbon (SOC), total phosphorus (TP), soil available potassium (AK), and soil available phosphorus (AP) significantly increased. Grazing exclusion also improved the diversity and abundance of soil bacteria, which had a significant positive correlation with SOC. The dominant taxonomic groups of soil bacteria in grazed and grazing exclusion grasslands included *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Chloroflexi*, *Gemmatimonadetes* and *Bacteroidetes*. There was an interaction between SOC, TN, AK, AP and the relative abundances of some dominant groups. Long-term grazing exclusion had a negative effect on diversity and the abundance of soil bacteria. Our results may provide new insights for grassland management in the semi-arid regions.

1. Introduction

Grasslands cover 20% of the terrestrial surface and play an important role in preventing soil erosion and supporting animal husbandry in semi-arid regions (Jing et al., 2013, 2014). Owing to human disturbance (especially over-grazing) and climate change, grasslands are severely deteriorating, which further accelerates soil nutrient degradation (Jiao et al., 2011), ecological function loss and social-economic damage (Jing et al., 2014). China has ~400 million hectares of various grasslands that account for approximately 41.7% of the country's land area (Ren et al., 2008). Recently, grassland degradation has become a serious problem in China (Ren et al., 2008; Jing et al., 2013, 2014). Therefore, restoration of degraded grassland ecosystems has become a central scientific issue in ecological engineering (Bai et al., 2004; Jing et al., 2013).

Grazing exclusion is regarded as the most effective method for restoring the degraded semi-arid grasslands of China (Jing et al., 2013). Many recent studies have addressed the influence of grazing exclusion on vegetation (Jing et al., 2013, 2014), soil properties (Jiao et al., 2011; Shi et al., 2013; Jing et al., 2014) and soil microbes (Jiang et al., 2009). However, these studies were based on short-term methods. Soil microbial communities play an important role in most soil nutrient transformations and influence plant diversity and productivity (Will et al., 2010). Furthermore, the variability of soil bacterial communities has been correlated with different soil properties (Lauber et al., 2008). For example, soil pH was found to determine microbial diversity and composition (Zhalnina et al., 2013), and bacterial diversity was higher in neutral soils and lower in acidic soils (Fierer and Jackson, 2006). While several studies have shown that soil bacterial structure and diversity are influenced by management practices (such as tillage, fertilization, and fire) (Mbuthia et al., 2015; Mikita-Barbato et al., 2015), there are minimal long-term effects of continuous grazing exclusion on the soil bacterial composition and activity. Therefore, understanding

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the changes in the soil bacterial community is crucial for soil and vegetation restoration.

This study aimed to analyze the effects of 33 years of continuous grazing exclusion on plant diversity, productivity, soil properties and soil bacteria in the semi-arid grasslands of China. We proposed the following hypotheses: (1) long-term grazing exclusion significantly increases plant diversity, productivity, and soil nutrients and further improves soil bacterial diversity and abundance; (2) the changes in the dominant phylum of the soil bacterial community may support or limit some soil properties after 33 years of continuous grazing exclusion. To test our hypotheses, we selected the largest semi-arid grassland regions of the Loess Plateau to investigate the characteristics of vegetation and soil. This study contributes to our understanding of restoration of vegetation and soil nutrients, especially soil bacteria, and provides new insights for grassland management in semi-arid regions.

2. Materials and methods

2.1. Study area

The study area is located on the largest typical grasslands of the Loess Plateau at Yunwu Mountain (106°21′–106°27′E, 36°10′–36°17′N) in the Ningxia Hui Autonomous Region of China (Fig. 1). Yunwu Mountain has been protected as a long-term monitoring site since 1982. The temperatures range from 22 to 25 °C in July, and the mean annual temperature is 7.01 °C. The mean annual rainfall is 425.42 mm, with approximately 60%–75% of the annual rainfall falling between July and September. The cumulative temperature (≥0 °C) is 2847 °C–3592 °C, and the annual daylight hours range from 2300 to 2500. Annual evaporation ranges from 1017 to 1739 mm and the frost-free season averages 137 days (from 1981 to 2015). The vegetation types mainly include *Stipa grandis*, *S. przewalskyi*, *S. bungeana*, *Artemisia sacrorum*, and *Thymus mongolicus*. The vegetation consists of 297 plant species. Gentianaceae, *Stipa* and *Potentilla* are important plant components, and the main dominant species include *S. bungeana*, *S. grandis*, *T. mongolicus*, *A. sacrorum*, *Potentilla acaulis*, *and Androsace erecta*. The soil is a montane grey-cinnamon soil, Calci-Orthic Aridisol, according to the Chinese taxonomic system, which is equivalent to a Haplic Calcisol in the FAO/UNESCO system. The area is located at an elevation of 1800–2100 m and has a total area of 6660 ha. The grassland protection areas include core conservation areas (1000 ha), buffer conservation areas (1300 ha) and experimental areas (4360 ha). Starting in 1982, different grazing exclusion treatments were established at different times in the grassland protection areas, and no agricultural activities (such as fertilization or crop cultivation) have been carried out in these grazing exclusion regions since 1982. The climate of the study area is semi-arid within the middle temperate zone (Jing et al., 2013, 2014).

2.2. Study design and sample collection

2.2.1. Experimental design

This experiment includes two parts: grazing exclusion grasslands and grazing grasslands. An *S. bungeana* grassland, *T. mongolicus* grassland, and *A. sacrorum* grassland were established for grazing exclusion in core conservation areas in 1982. Before grazing exclusion, these grasslands were grazed by sheep, and the stocking rates were at a heavy density (>50 sheep/ha); since 1982, these grasslands have been excluded from livestock grazing. Three grazing grasslands were selected as controls in the experimental area, and these grazing grasslands have a medium density of sheep during the whole year (8 sheep/ha), and the dominant species is *S. bungeana* perennial bunchgrass.

2.2.2. Vegetation sampling and analysis

The vegetation investigations were conducted in grazing exclusion areas and grazing grasslands when aboveground biomass reached a peak value in 2015. Using the line transect method, three equal-sized replicated blocks (50 m × 10 m) were established in each grassland. Then, nine 1 m × 1 m quadrats were established with three replicates in three blocks, and a total of twenty-seven quadrats were established for grazing exclusion and grazing grasslands. The distance between quadrats was at least 15 m. In each quadrat, plant species identifications were completed in situ, and plant species numbers, plant coverage, plant height and plant biomass were measured separately for each species. The aboveground biomass was clipped and measured by drying at 80 °C for 48 h to a constant weight for each species, and the sum of all species of aboveground biomass was used to represent the whole community. According to Bai et al. (2004) methods, we classified the species into the following five plant functional groups: perennial rhizome grass (PR), perennial bunchgrasses (PB), perennial forbs (PF), shrubs and semi-shrubs (SS), and annual and biennials (AB). And Jing et al. (2013) methods were used to classify the species into four functional groups: Poaceae, Fabaceae, Asteraceae and weeds.

In this study, the richness index (R), Shannon-Wiener diversity index (H) and Evenness index (E) were calculated for grazing exclusion and grazing grasslands according to the methods of Jing et al. (2013, 2014).

The Richness Index (R) was $R = S$;

The Shannon-Wiener diversity index (H) was calculated as follows:

$$H = -\sum_{i-1}^{s} P_i \ \ln P_i$$

The Evenness index(E) was calculated as : $E = \dfrac{H}{\ln S}$

with S representing the total species number of the community, and Pi representing the relative importance value of species i.

2.3. Soil sampling and analysis

2.3.1. Soil sampling

After vegetation sampling, the topsoil litter was removed before soil sampling. The soil samples were taken at five points; that is, four corners along the diagonal and the center of the quadrat by bucket auger at three different depths: 0–20, 20–40 and 40–60 cm. Fifteen soil samples of three quadrats were mixed as a soil sample of block, and three soil samples were collected for each grassland. A total of nine soil samples per depth were collected for grazing exclusion and grazing grasslands. All samples were sieved quickly on-site through 1.4 mm mesh. The samples were divided into two parts: 20 g was transported on dry ice to the laboratory and then stored at −80 °C for DNA extraction, and the remaining parts were air-dried and used to analyze the physical and chemical properties.

2.3.2. Physical and chemical properties analysis

From each sampling quadrat, a section (1 m × 0.8 m × 1.2 m) was dug to measure the soil bulk density (BD), and the soil cores were dried at 105 °C for 24 h and then weighed for calculating BD. All of the soil was air-dried and passed through a 1.4 mm sieve for soil nutrients analysis. The soil pH was measured using a 1:5 soil/water suspension. The soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), and available potassium (AK) were analyzed according to the methods of Jing et al. (2014).

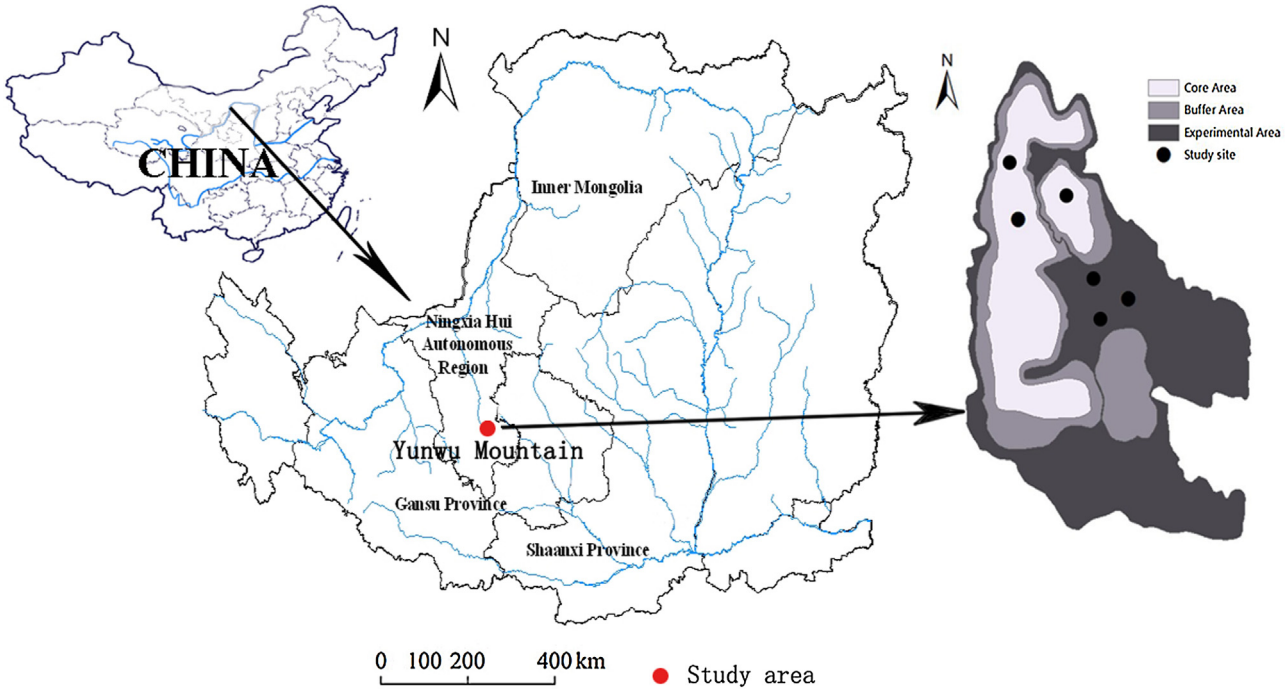

Fig. 1. Location of the study site.

2.3.3. Soil bacterial community analysis

The genomic DNA of the soils was extracted at 0.5 g using an E.Z.N.A soil DNA Kit (OMEGA, USA) according to the manufacturer's specifications. The quality and quantity of DNA was tested using 1% agarose gel electrophoresis and a spectrophotometer (ND-1000, Thermo Scientific, Wilmington, USA).

The V1-V3 region of the 16S rRNA gene was amplified using PCR. Two sets of primers were selected: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′, *Escherichia coli position* 9–27) and 515R (5′ −TTACCGCGGCTGCTGGCAC-3′, *E. coli* position 515–533) (Liu et al., 2012). The primers contained Roche 454 pyrosequencing adaptors A and B (Wu et al., 2013). The 20 μl PCR reaction system contained 4 μl 5× FastPfu Buffer, 2 μl mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl FastPfu polymerase (TransStart Fastpfu DNA Polymerase, TransGen) and 10 ng template DNA. The PCR amplification was completed using ABI GeneAmp® 9700. The following thermal cycling program was used: initial denaturation at 95 °C for 2 min, 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s and then a final extension at 72 °C for 5 min. All samples were amplified in triplicate, and the equivalent samples were pooled. The PCR products were purified with an AxyPrep DNA gel Extraction Kit (Axygen) according to the manufacturer's instructions. The concentrations of amplicons were quantified using PicoGreen® dsDNA Quantitation Reagent and QuantiFluor™-ST (Promega) as recommended by the manufacturers. EmPCR (emulsion-based clonal amplification) was performed using the Roche emPCRAmp-Lib_L Kit (Roche). Sequences of 16S rRNA were obtained using a GS FLX 454 system (454 Life Sciences, Roche Applied Science).

The quality of all sequence reads was determined using Mothur software v.1.31.2 (Schloss et al., 2009). The reads of <200 bp and average quality score <25 were filtered out, then forward and reverse primers and barcodes were removed after binning. The taxonomic identities of the 16S rRNA genes were determined using the SILVA 115 database, and the taxonomic levels were selected at a level of 97% sequence similarity (Pruesse et al., 2007; Quast et al., 2013). The operational taxonomic units (OTUs, 97% similarity) and rarefaction analysis, Good's coverage, richness indices (Chao and Ace) and diversity indices (Shannon and Simpson) were calculated using Mothur software v.1.31.2 (Roesch et al., 2007; Schloss et al., 2009). Based on the relative abundance of the bacterial phyla, a PCA was performed using R project for statistical computing. Spearman's rank correlation between the relative abundance of the dominant taxonomic phyla and soil properties was performed using SPSS 13.0 (SPSS Inc., IL). The 16 rRNA gene sequences derived from pyrosequencing were deposited in the NCBI Sequence Read Archive under accession number SRP030213.

2.4. Statistical analysis

All of the data were presented as the mean ± standard error. A one-way analysis of variance (ANOVA) was used to test the differences in vegetation characteristics, above- and belowground biomass, functional groups, soil properties, and soil bacteria between grazing exclusion and grazing grasslands. The significant differences of all statistical tests were estimated at a significant level of $P < 0.05$. The correlations between soil parameters and abundant bacterial phyla were calculated using a linear mixed model. All of the statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Vegetation characteristics

ANOVA analyses indicated that the Shannon-Wiener index ($P < 0.05$), coverage ($P < 0.001$), and species richness ($P < 0.01$) in grazing exclusion grasslands were significantly higher than those in grazing grasslands (Fig. 2a, c and d). Compared to grazing grasslands, grazing exclusion grasslands had a higher Evenness index, with no significant difference ($P > 0.05$) (Fig. 2b).

3.2. Plant community and function group biomass

ANOVA analyses indicated that grazing exclusion grasslands had higher aboveground biomass ($P < 0.001$), belowground biomass

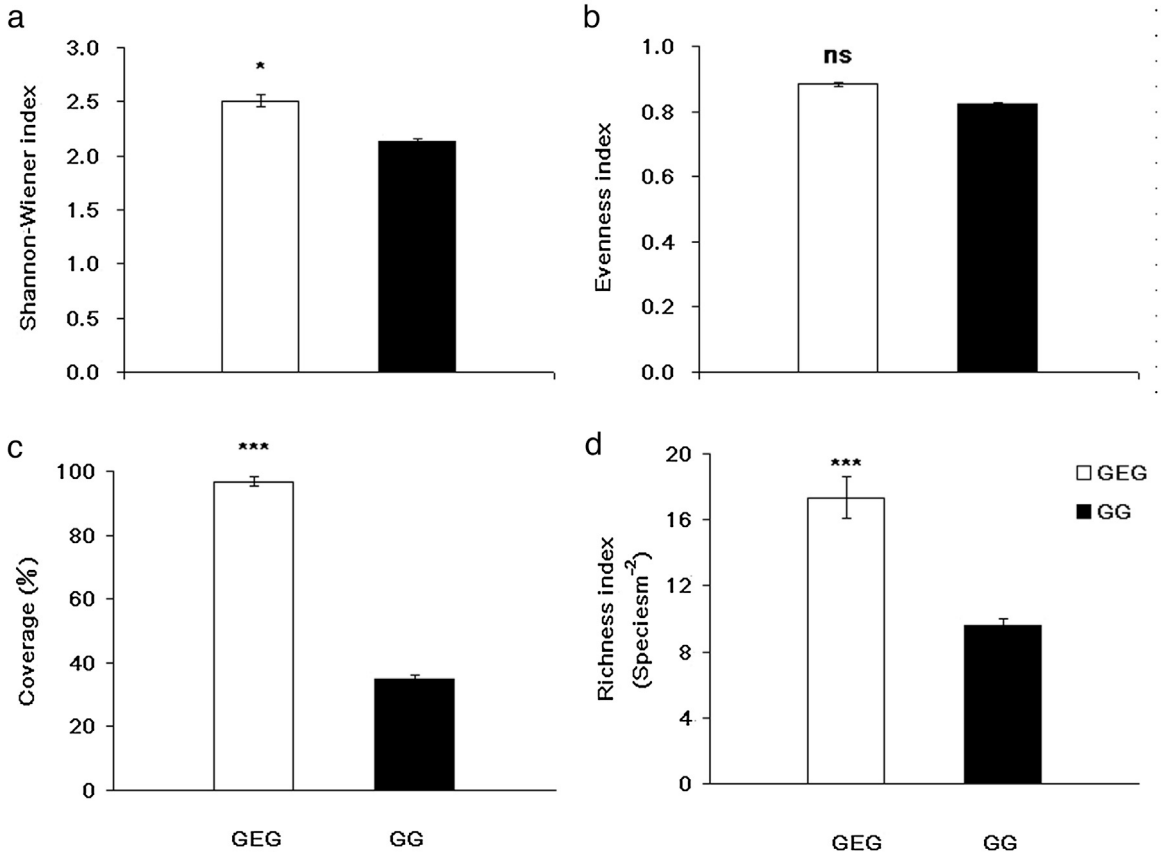

Fig. 2. Effects of grazing exclusion and grazing on Shannon-Wiener index (a), evenness index (b), coverage (c), and richness index (d). ***, * and ns indicated significant difference between grazing exclusion and grazing grasslands, ***$P < 0.001$, *$P < 0.05$; ns, no significant difference.

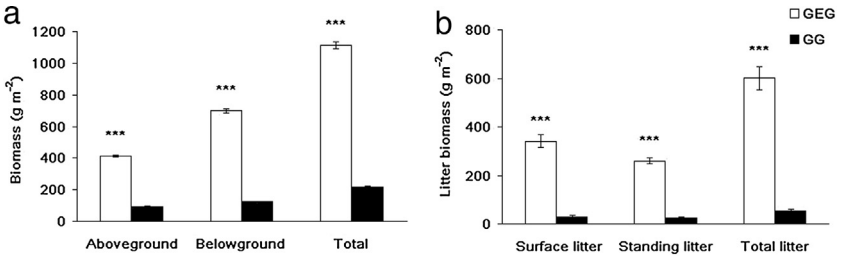

Fig. 3. Effects of grazing exclusion and grazing on above- and belowground biomass, total biomass (a); surface litter biomass, standing litter biomass and total litter biomass (b). *** indicated significant difference between grazing exclusion and grazing grasslands, ***$P < 0.001$.

($P < 0.001$), and total biomass ($P < 0.001$) compared to grazing grasslands (Fig. 3a). The surface litter biomass, standing litter biomass, and total litter biomass in grazing exclusion grasslands were also higher than those in grazing grasslands ($P < 0.001$) (Fig. 3b). Grazing exclusion increased the PB ($P < 0.001$) and SS ($P < 0.05$), but decreased the AB ($P < 0.01$), PR ($P < 0.01$) and PF ($P < 0.001$) (Fig. 4a). Grazing exclusion could increase Poaceae ($P < 0.001$) but reduce weeds ($P < 0.001$). The biomass of Fabaceae and Asteraceae exhibited no significant difference between grazing exclusion grasslands and grazing grasslands (Fig. 4b).

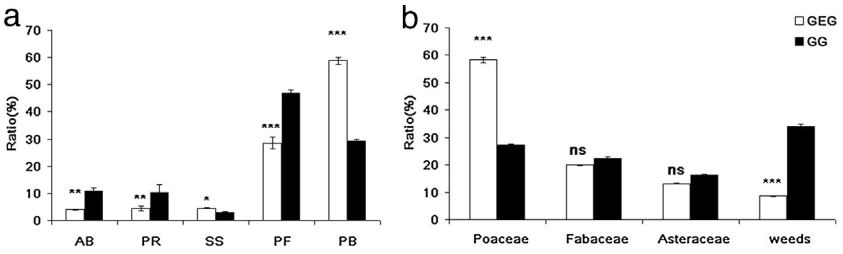

Fig. 4. Effects of grazing exclusion and grazing on relative biomass of perennial rhizome grass (PR), perennial bunchgrasses (PB), perennial forbs (PF), shrubs and semi-shrubs (SS), and annual and biennials (AB) (a); Poaceae, Fabaceae, Asteraceae and weeds (b). ***, **, * and ns indicated significant difference between grazing exclusion and grazing grasslands, ***$P < 0.001$, ** $P < 0.01$, *$P < 0.05$; ns, no significant difference.

3.3. Soil physical and chemical properties

Grazing exclusion grasslands had higher soil pH ($P<0.05$) (except 0–20 cm) than grazing grasslands. Long-term grazing exclusion significantly increased TN ($P<0.01$), SOC ($P<0.01$), TP ($P<0.001$), AK ($P<0.001$), and AP ($P<0.001$) in the 0–10 cm, 10–20 cm, 20–40 cm, and 40–60 cm layers (Fig. 5a–g). The soil C/N and C/P exhibited a significant difference between grazing exclusion and grazing grasslands ($P<0.05$) (Fig. 5h and i).

3.4. Soil bacterial community

Changes in the soil bacterial communities in grazing and grazing exclusion grasslands were amplified by using the universal primer pairs 27F and 515R. A total of 114,685 valid bacterial sequence reads were generated from the 454 pyrosequencing. The 75,290 valid sequences (≥200 bp) were obtained after removing the ambiguous and homologous sequences. There were 6371 sequences per sample, with an average sequence length of 443 nucleotides, excluding the barcode and adaptor primer sequences. And these trimmed sequences were used to analyze the phylogenetics.

The diversity and richness indices were calculated at a 97% sequence identity threshold. As shown in Table 1, the coverage values ranged from 65.11 to 84.13 and 59.80 to 82.00 for grazing and grazing exclusion grasslands, respectively, which indicated that the rarefaction curves were not saturated but averaged almost 75% of taxonomic diversity. The Shannon and Simpson indices were used to characterize the structures of the soil bacterial communities. Compared with grazing grasslands, the diversity of soil bacteria (Shannon and Simpson indices) was higher in grazing exclusion grasslands. However, the results indicated that no significant difference existed ($P>0.05$). The Shannon index and Simpson index decreased with soil depth (Table 1). The abundance of soil bacteria (Chao and Ace richness indices) also exhibited a similar tendency.

Table 1
Diversity indices of bacterial communities at different soil depth.

	No. of reads[a]	OTU[b]	Chao	Ace	Shannon	Simpson	Coverage (%)
G-1	4520	2335	5676[a]	11,437[a]	7.31[a]	0.0011[a]	65.11
G-2	5744	2067	6744[a]	4375[a]	6.90[a]	0.0023[a]	78.15
G-3	5836	1649	4575[a]	3265[a]	6.52[a]	0.0037[a]	84.13
F-1	4203	2390	9756[a]	6258[a]	7.42[a]	0.0008[a]	59.80
F-2	6227	2330	7059[a]	4829[a]	7.08[b]	0.0020[a]	77.63
F-3	7202	2297	6246[a]	4584[a]	6.96[a]	0.0027[a]	82.00

[a] The reads are the trimmed reads.
[b] The operational taxonomic units (OTU) were determined at 97% similarity. G-1, G-2, G-3 indicating soil depth 0–20 cm, 20–40 cm, 40–60 cm for grazing grassland; F-1, F-2, F-3 indicating the different soil depth 0–20 cm, 20–40 cm, 40–60 cm for grazing exclusion grassland. Means with the same letters in the same soil depth are not significantly different at the 0.05 level (LSD).

A phylogenetic analysis of phylotype was performed according to the best sequence matches based on the SILVA database. *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Chloroflexi*, *Gemmatimonadetes*, *Bacteroidetes*, *Nitrospirae*, Candidate_division_TM7 and *Cyanobacteria* were the predominant phyla in all samples. The relative abundance of class in each soil sample is shown in Fig. 6. *Thermoleophilia*, *Actinobacteria*, *Alphaproteobacteria*, *Gemmatimonadetes*, *Clostridia*, *Acidobacteria*, *Acidimicrobiia* and *Deltaproteobacteria* were relatively high (>5%). *Planctomycetacia*, SL56_marine_group, *Phycisphaerae*, *Betaproteobacteria*, *Gammaproteobacteria*, *Sphingobacteriia* and *Bacilli* were all relatively low (>1%). *Holophagae*, *Chloroflexi*, *Nitriliruptoria*, *Cytophagia*, *Cyanobacteria*, *Anaerolineae*, *Dehalococcoidetes*, *Nitrospira and Caldilineae* appeared in a very low proportion (>0.4%). An average of 6.44% of sequences were unclassified (no-rank sequences), ranging from 5.24% to 7.59%.

To compare the differences in the bacterial community composition between grazing and grazing exclusion grasslands, a differentially abundant features analysis at the genus level was

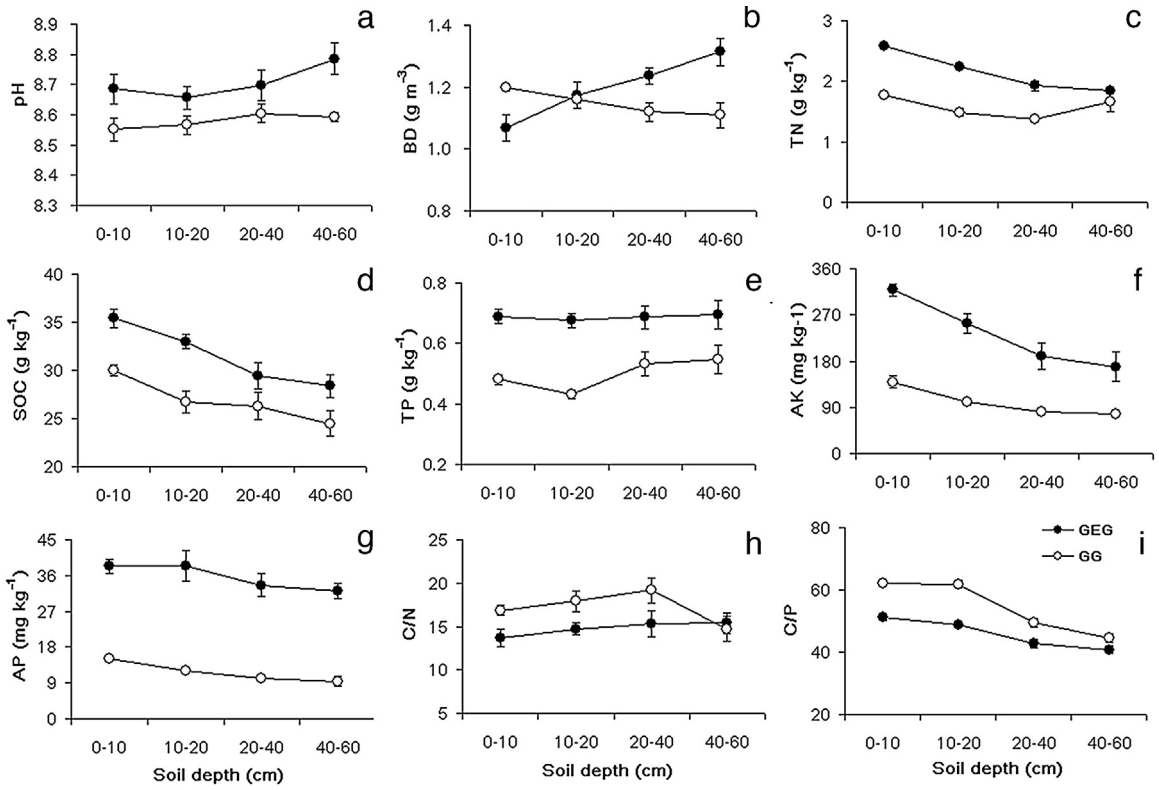

Fig. 5. Effects of grazing exclusion and grazing on pH (a), BD (b), TN (c), SOC (d), TP (e), AK (f), AP (g), C/N (h), C/P (i).

Table 2
Differentially abundant features analysis of genus level at 0–60 cm soil depth for grazing and grazing exclusion grasslands.

Abundant phyla	Grazing grasslands			Grazing exclusion grasslands				
	Mean	Variance	Standard	Mean	Variance	Standard	p value	q value
Acidimicrobiaceae_uncultured	0.0006	1.04E-09	1.86E-05	0.0003	2.79E-08	9.65E-05	0.034	1
Acidobacterium	0.0033	6.34E-07	0.0005	0.0058	1.71E-06	0.0008	0.040	1
Actinophytocola	0.0013	7.41E-08	0.0002	0.0004	2.97E-08	9.96E-05	0.008	1
Atopobium	0.0004	8.93E-09	5.46E-05	0.0001	1.21E-08	6.36E-05	0.022	1
Bauldia	0.0001	1.88E-10	7.92E-06	0	0	0	0.000	0.49
Candidatus_Kuenenia	0	0	0	0.0002	3.72E-09	3.52E-05	0.005	1
Candidatus_Rhodoluna	0.0016	6.60E-08	0.0001	0.0010	3.61E-08	0.0001	0.027	1
Citricoccus	0.0007	7.15E-08	0.0002	0.0001	3.23E-09	3.28E-05	0.018	1
Cobetia	0	0	0	0.0002	2.41E-08	8.96E-05	0.044	1
Comamonadaceae_uncultured	0.0014	1.66E-07	0.0002	0.0036	7.87E-07	0.0005	0.016	1
Coriobacteriaceae_uncultured	0.0014	3.77E-08	0.0001	0.0004	2.94E-08	9.90E-05	0.002	0.89
Cryptosporangium	0.0090	7.18E-06	0.0015	0.0034	1.61E-07	0.0002	0.019	1
Desulfotomaculum	0	0	0	0.0003	2.88E-09	3.10E-05	0.001	0.73
Desulfovibrio	0.0005	9.29E-08	0.0002	0.0010	1.82E-08	7.78E-05	0.048	1
Devosia	0.0001	2.24E-08	8.64E-05	0.0009	1.56E-07	0.0002	0.030	1
Edaphobacter	0.0007	1.74E-07	0.0002	0.0018	2.16E-07	0.0003	0.034	1
Ferruginibacter	0.0008	7.80E-07	0.0005	0.0026	5.73E-07	0.0004	0.046	1
Flexibacter	0.0024	1.15E-06	0.0006	0.0065	3.39E-06	0.0011	0.024	1
Friedmanniella	0.0024	9.35E-07	0.0006	0.0088	1.33E-06	0.0007	0.002	0.82
Gemmatimonas	0.0743	1.22E-04	0.0064	0.0531	4.35E-05	0.0038	0.042	1
Geobacter	0.0107	2.49E-06	0.0009	0.0215	2.99E-05	0.0032	0.026	1
Hyphomicrobium	8.59E-05	5.60E-09	4.32E-05	0.0005	3.12E-08	0.0001	0.021	1
Ideonella	0.0003	2.48E-08	9.09E-05	0.0011	1.25E-07	0.0002	0.019	1
Jiangella	0.0002	6.53E-09	4.66E-05	0.0018	9.39E-07	0.0006	0.038	1
Lishizhenia	4.55E-05	6.21E-09	4.55E-05	0.0003	1.25E-08	6.45E-05	0.020	1
Luteimonas	0.0002	4.30E-08	0.0001	0.0017	4.36E-07	0.0004	0.015	1
Lutispora	0.0011	1.77E-08	7.69E-05	0.0031	1.45E-07	0.0002	0.001	0.73
Lysobacter	0.0005	1.01E-07	0.0002	0.0058	1.22E-06	0.0006	0.001	0.73
Marinithermus	0	0	0	0.00010	1.06E-11	1.88E-06	0.000	0.36
Mesorhizobium	0.0011	6.70E-08	0.0001	0.0027	1.05E-06	0.0006	0.045	1
Microbispora	0.0001	1.88E-10	7.92E-06	3.63E-05	3.96E-09	3.63E-05	0.049	1
Microlunatus	0.0010	4.10E-07	0.0004	0.0024	3.95E-08	0.0001	0.017	1
Mucilaginibacter	0	0	0	0.0007	6.27E-08	0.0001	0.009	1
Niabella	0.0003	7.73E-08	0.0002	0.0017	1.73E-07	0.0002	0.008	1
Nitrosomonadaceae_uncultured	0.0013	5.75E-07	0.0004	0.0037	1.11E-06	0.0006	0.027	1
Nordella	0.0011	4.41E-07	0.0004	0.0028	1.81E-08	7.77E-05	0.011	1
Ornithinimicrobium	0.0002	6.70E-09	4.73E-05	3.63E-05	3.96E-09	3.63E-05	0.028	1
Rhodanobacter	0	0	0	0.0001	4.11E-09	3.70E-05	0.014	1
Rhodomicrobium	0	0	0	0.0002	3.72E-09	3.52E-05	0.005	1
Sanguibacter	0.0018	2.38E-07	0.0003	0.0006	3.24E-07	0.0003	0.043	1
Saprospiraceae_uncultured	0.0001	1.87E-08	7.90E-05	0.0023	1.58E-06	0.0007	0.037	1
Serinibacter	0.0019	4.82E-07	0.0004	0.0007	3.50E-09	3.41E-05	0.039	1
Solirubrobacter	0.1268	2.27E-04	0.0087	0.0672	9.02E-05	0.0055	0.004	1
Sphingosinicella	0	0	0	0.0004	3.15E-09	3.24E-05	0.001	0.73
Steroidobacter	0.0029	9.97E-08	0.0002	0.0054	2.15E-06	0.0008	0.043	1
Stigmatella	0.0005	8.30E-09	5.26E-05	0.0009	1.17E-08	6.24E-05	0.006	1
Streptacidiphilus	0.0001	3.38E-09	3.35E-05	3.67E-05	4.04E-09	3.67E-05	0.043	1
Streptoalloteichus	0.0005	3.04E-08	0.0001	0.0002	4.24E-11	3.76E-06	0.031	1
Urania-1B-19_marine_sediment_group	0.0028	5.47E-08	0.0001	0.0058	1.81E-06	0.0008	0.016	1
Victivallaceae_uncultured	0.0005	1.48E-08	7.02E-05	0.0008	1.94E-08	8.04E-05	0.048	1
Yonghaparkia	0	0	0	0.0003	1.29E-08	6.56E-05	0.012	1

performed (Table 2). A total of 747 bacterial genera were identified in the soil samples. As shown in Table 2, 51 bacterial genera exhibited significant differences ($p < 0.05$ or $p < 0.01$) between grazing exclusion and grazing grasslands. The dominant taxa in grazing exclusion soils were *Actinobacteria* (30.44 ± 3.09%), *Proteobacteria* (20.91 ± 4.71%), *Firmicutes* (10.91 ± 2.48%), *Acidobacteria* (7.37 ± 1.49%), *Gemmatimonadetes* (6.76 ± 0.98%), *Planctomycetes* (6.27 ± 1.09%), *Chloroflexi* (6.85 ± 0.79%) and *Bacteroidetes* (3.54 ± 0.32%), whereas in grazed soil, the dominant taxa were *Actinobacteria* (45.91 ± 3.74%), *Proteobacteria* (12.71 ± 3.51%), *Gemmatimonadetes* (9.17 ± 1.91%), *Firmicutes* (8.97 ± 0.75%), *Chloroflexi* (6.24 ± 0.49%), *Planctomycetes* (5.06 ± 0.56%), *Acidobacteria* (4.39 ± 2.34%), and *Bacteroidetes* (1.54 ± 0.74%).

3.5. Relationships among soil properties and the relative abundance of bacterial phyla

The correlation results indicated that the Chao community richness index had a significant positive correlation with SOC ($R = 0.90$; $p < 0.01$), and the Shannon community diversity index also had a significant positive correlation with SOC ($R = 0.84$; $p < 0.05$). At the phylum level (Table 3), the relative abundances of *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* were significantly correlated with pH, SOC, TN, AK and AP; *Acidobacteria* was significantly correlated with pH, SOC, AK and AP; *Planctomycetes* was correlated with SOC, TN, AK and AP; *Gemmatimonadetes* was significantly correlated with SOC, AK and AP; Candidate_division_TM7 was only correlated with TN; *Firmicutes*, *Chloroflexi*, *Nitrospirae* and *Cyanobacteria* were not significantly correlated with any soil properties.

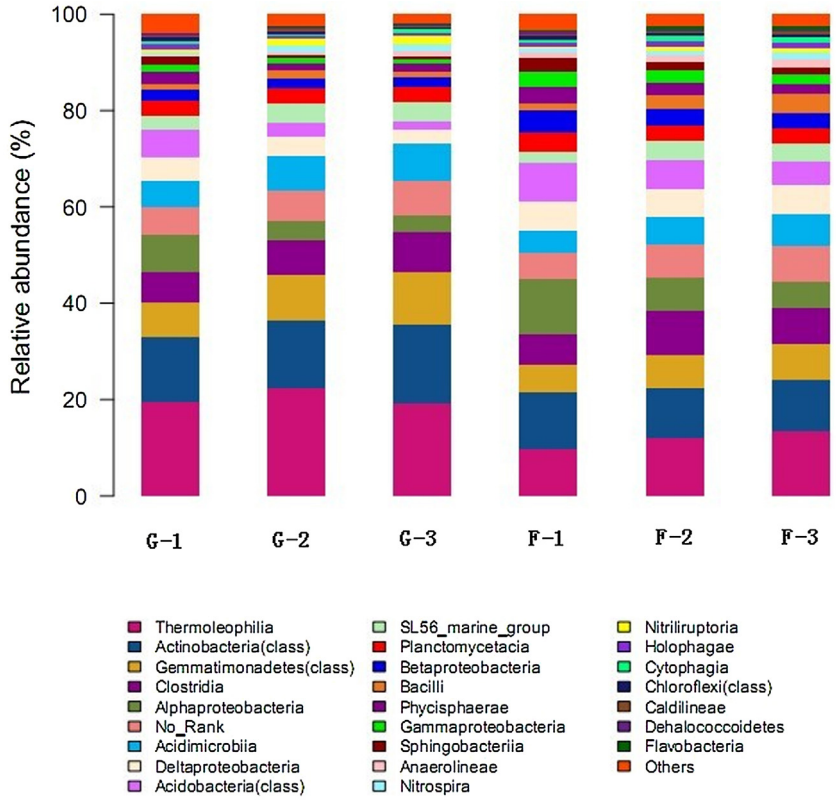

Fig. 6. Relative abundance of major taxonomic groups at the class level across for grazing and grazing exclusion grasslands.

4. Discussion

The restoration of degraded grasslands is a complex and long-term process in semi-arid areas, and grazing exclusion management is regarded as the most effective method for controlling degradation and improving vegetation restoration (Spooner and Briggs, 2008; Jing et al., 2013, 2014). The species richness, coverage, Shannon-Wiener index and Evenness index, were adopted as vegetative ecological characteristics to evaluate the restoration process of degraded grasslands (Wilkins et al., 2003; Jing et al., 2014). Similar to the success of farmland abandonment on the Loess Plateau (Wang et al., 2009), 32 years of grazing exclusion in our study significantly increased the Shannon-Wiener diversity, coverage and richness. The above- and belowground biomass in grazing exclusion grasslands were obviously higher than in grazing grasslands. This phenomenon indicates that long-term grazing accelerated leaf and shoot losses due to sheep ingestion, which would reduce the species richness, coverage, and aboveground biomass (Jing et al., 2014). In contrast, some studies found that richness and evenness declined with litter cover (Tilman et al., 2006; Lamb, 2008) and that grazing exclusion also significantly increased litter biomass. This may be due to litter cover in grazing exclusion grasslands improving the soil moisture and nutrients, which is conducive to plant growth. Therefore, our results agree with the first hypothesis that long-term grazing exclusion supports higher species diversity and productivity and further improves ecosystem function.

Plant functional groups could represent the ecological structure of the flora and be used to predict a general level of species assemblage (Santiago do Vale et al., 2010; Fry et al., 2013; Jing et al., 2014). In this study, Poaceae, Fabaceae and Asteraceae, especially xerophytic perennial herbaceous plants, played important roles in maintaining the community structure, succession process, and ecological characteristics of grasslands, which could be explained by the habitat suitability of the plants (Gurnell et al., 2002). The habitat suitability of plants is important for evaluating the environment supporting plant growth, such as rainfall and soil (Jing et al., 2013). Our study sites were located in typical semi-arid and arid climate zones, and water was the main limiting factor for plant growth. Perennial herbaceous plants are more drought-resistant due to a small leaf area and morphology and anatomy characteristics supporting typical drought adaptability. Therefore, we suggest that *Poaceae*, *Fabaceae* and *Asteraceae*, especially perennial bunchgrasses, should be selected as cultivated herbage for the construction of artificial grasslands in semi-arid areas of China, similar to the results on Loess Plateau (Jing et al., 2013). Furthermore, long-term grazing exclusion affected the vegetation succession process, which showed that mesophytic shrubs occurred on semi-shady slopes and in gullies after 32 years. This result may indicate

Table 3
Spearman's rank correlation between abundant phyla with soil properties for grazing and grazing exclusion grasslands. Only relative abundances of phylum that represented >0.45% were considered.

Abundant phyla	pH	BD	SOC	TN	TP	AK	AP
Actinobacteria	0.94**	−0.60	−0.97**	−0.83*	−0.54	−0.99**	−0.96**
Proteobacteria	−0.90**	0.18	0.99**	0.90*	0.59	0.97**	0.88*
Acidobacteria	−0.83*	0.49	0.93**	0.71	0.26	0.92**	0.94**
Firmicutes	0.14	0.43	−0.08	0.09	0.60	−0.09	−0.09
Planctomycetes	−0.77	0.43	0.87*	0.83*	0.31	0.89*	0.89*
Chloroflexi	−0.14	0.49	0.09	0.14	0.77	0.09	0.09
Gemmatimonadetes	0.83	−0.49	−0.94**	−0.71	−0.26	−0.93**	−0.95**
Bacteroidetes	−0.87*	0.54	0.91*	0.94*	0.60	0.93**	0.89**
Candidate_division_TM7	−0.66	0.20	0.54	0.83*	0.66	0.54	0.54
Nitrospirae	0.60	−0.49	−0.66	−0.31	0.14	−0.66	−0.66
Cyanobacteria	−0.37	0.14	0.20	−0.09	−0.37	0.20	0.20

BD soil bulk density, *SOC* soil organic carbon, *TN* soil total nitrogen, *TP* soil total phosphorus, *AK* soil available potassium, *AP* soil available phosphorus.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level.

that the tendency of grassland succession followed a trajectory of herbs to shrubs to forests. Our study also indicated that the restoration of plant community diversity and productivity improved soil fertility, such as TN, SOC, TP, AK, and AP, similar to previous studies (Wang et al., 2009; Jing et al., 2014). This is because high soil fertility is related to larger plant coverage and greater litter accumulation (Marzaioli et al., 2010; Jing et al., 2014). Therefore, the aboveground biomass had a direct effect on litter accumulation, and the litter accumulation may increase soil moisture. Meanwhile, litter decomposition was more rapid in moist soil, and soil nutrients could increase from the larger amounts of decomposed litter (Jing et al., 2014).

Bacterial microbial communities play an important role in grassland ecosystems, such as driving geochemical processes and performing biochemical reactions, ultimately improving soil quality (Bastida et al., 2013; Buckley and Schmidt 2003). Until now, in arid and semi-arid areas, bacterial communities have been relatively poorly understood compared to moist environments (Kim et al., 2013). Our study is the first to assess the soil bacterial diversity after 33 years of grazing exclusion on grasslands in semi-arid areas of China using high-throughput 454 pyrosequencing methods. Our results indicate that the diversity of soil bacteria (Shannon and Simpson indices) in grazing exclusion grasslands was higher than in grazing grasslands at a 97% similarity level, though with no significant difference. Furthermore, *Actinobacteria* and *Proteobacteria* were the most abundant bacterial phyla in the soil of these semi-arid grasslands. Our results are consistent with previous studies where *Actinobacteria* and *Proteobacteria* as dominant groups were used to degrade organic compounds and environmental contaminants in semi-arid and desert soils (Bastida et al., 2013; Fierer et al., 2012; Li et al., 2012). Compared to *Actinobacteria* and *Proteobacteria*, *Acidobacteria*, *Planctomycetes* and *Bacteroidetes* constituted a minor group of the bacterial community. These dominant taxa were similar to other studies of soil bacterial community composition (Bastida et al., 2013; Nacke et al., 2011). However, the relative abundances of these dominant bacterial phyla were significantly different between grazing exclusion and grazing grasslands. Differences in the dominant bacterial phyla between grazing exclusion and grazing grasslands were also found at different soil depths. For example, *Actinobacteria* exhibited the highest relative abundance of sequences in grazing grasslands, composing up to 41.63%, 47.60% and 48.51% in the 0–20, 20–40 and 40–60 cm soil depths, respectively, whereas grazing exclusion grasslands exhibited lower numbers (27.23%, 30.17% and 33.39% in the 0–20, 20–40 and 40–60 cm soil depths, respectively). However, for *Proteobacteria*, the relative abundance was higher ($p < 0.05$) in grazing exclusion grasslands (26.20%, 19.34% and 17.19% in the 0–20, 20–40 and 40–60 cm soil depths, respectively), compared to grazing grasslands (16.64%, 11.62% and 9.88% in the 0–20, 20–40 and 40–60 cm soil depths, respectively). At the phylum level, differentially abundant features analysis indicated that there were significant differences among *Actinobacteria*, *Bacteroidetes*, *Lentisphaerae*, *Thermotogae*, BD1-5 and Candidate_division_OD1 ($p < 0.01$) of grazing exclusion and grazing grasslands (data not shown). At the genus level, a comparison of the relative abundances of the bacterial community also revealed significant differences between grazing exclusion and grazing grasslands (Table 2).

Many studies have shown that soil properties such as pH, soil water content, and soil texture affect the composition and diversity of the soil bacterial community (Fierer and Jackson, 2006; Hartman et al., 2008; Lauber et al., 2009; Rousk et al., 2010). We used Spearman's rank order correction to assess the relationships between abundant phyla and soil properties (Table 3). We found that the relative abundance of *Acidobacteria*, *Proteobacteria* and *Bacteroidetes* phyla was negatively correlated with soil pH, whereas the abundance of *Actinobacteria* was positively correlated with soil pH. These results are consistent with studies in North and South America (Jones et al., 2009), in a German forest, and on grassland soils (Nacke et al., 2011). However, the results are quite contradictory to the studies of Li et al. (2012) in different farming systems. Furthermore, the soil nutrient contents (such as SOC, TN, AP and AK) were found to be the main drivers influencing the bacterial phyla. For example, soil nitrogen availability affects the structure of the soil microbial community (Frey et al., 2004) or the composition of dominant bacterial species (Zhang et al., 2013), and the total organic carbon accounted for the greatest proportion of variability in the bacterial community (Bai et al., 2012). Our research indicated that the relative abundance of *Actinobacteria* and *Gemmatimonadetes* was negatively correlated with SOC, TN, AP and AK, whereas *Proteobacteria*, *Acidobacteria*, *Planctomycetes* and *Bacteroidetes* were positively correlated with SOC, TN, AP and AK. We also found that grazing exclusion decreased *Actinobacteria* and *Gemmatimonadetes*, but increased *Proteobacteria*, *Acidobacteria*, *Planctomycetes* and *Bacteroidetes*, which indicates that soil nutrients, in particular soil C and N content, might be regulated by these dominant bacterial communities. These results support our second hypothesis that the changes of bacterial dominant phyla could increase the soil C and N content. However, different bacterial phyla had different correlations. The differences among soil properties and the abundance of bacterial phyla may be because they regulate the nutrient contents, rates of aerobic or anaerobic activity, and acidity in the soil (Wu et al., 2013).

5. Conclusions

Long-term grazing exclusion significantly increased species diversity, richness, coverage, above- and belowground biomass, and litter biomass, but did not affect evenness. The increase in species richness and aboveground biomass was mainly due to *Poaceae*, *Fabaceae*, PB and PF. Grazing exclusion could decrease the soil pH and had a favorable effect on the TN, SOC, TP, AK and AP. The diversity and abundance of soil bacteria could be enhanced by reasonable grazing exclusion in semi-arid grasslands. The relative abundances of some bacterial phyla were correlated with soil pH, SOC, TN, AK and AP. Long-term grazing exclusion has a negative effect on grassland renewal due to excess litter accumulation. Therefore, we suggest that reasonable and effective utilization should be carried out when community productivity reaches a peak during the grazing exclusion process, such as with rotational grazing or cutting.

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