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EFFECT OF GRAZING EXCLUSION ON SOIL BACTERIAL COMMUNITY LINKS WITH VARIATIONS IN SOIL PROPERTIES BUT NOT IN VEGETATION CHARACTERISTICS

QING-RONG HUANG[1], HAN-WEN DENG[1], LEI JI[2], YU-MENG LU[1], ZI-JIE WANG[1], YONG-CHUL PARK[3], YAN-LIN SUN[1*] AND SOON-KWAN HONG[3*]

[1]*School of Life Sciences, Ludong University, Yantai, 264025, PR China*
[2]*Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Key Laboratory of Grassland Ecology and Restoration, Ministry of Agriculture, 120 East Wulanchabu Street, Hohhot, 010010, PR China*
[3]*Department of Bio-Health Technology, Kangwon National University, Chuncheon 24341, South Korea*
**Corresponding authors' email: laddiya@hotmail.com; soonkwan@kangwon.ac.kr*

Abstract

Soil bacterial community plays an important role in soil nutrient cycling, and its diversity has become the key indicator for evaluating soil health, and understanding climate changes, ecosystem resistance, resilience, and functional redundancy. However, the effects of grazing exclusion, as the commonly used soil management strategy for degraded grassland restoration, on soil bacterial community often obtain inconsistent conclusions from different trials. In order to clarity the effects of grazing exclusion on soil bacterial community, we selected a 36 years grazing exclusion area (GE) and three free grazing areas (FGs) with different grazing intensities in Xilingol Grassland of Inner Mongolia, China, and examined the biomass, composition, and diversity of soil bacterial community, together with their corresponding vegetation and soil properties, to further understand the driving factors of soil bacterial community changes. Our results showed that grazing exclusion had no significant effect on the abundance and diversity of total soil bacterial community, but it did change the composition of soil bacteria, especially increasing the abundances of the phyla Diapherotrites and Gemmatimonadetes. In addition, grazing exclusion significantly increased vegetation aboveground biomass, litter mass, soil N stock and the availabilities of K and P, but reduced vegetation diversity. The correlation between soil bacterial communities and their environmental factors suggested that soil bacterial communities closely linked with soil available potassium (AK), and available phosphorus contents (AP), but not with vegetation characteristics. However, the soil properties were closely affected by vegetation characteristics. Therefore, our study suggested that soil bacterial communities mainly responded to the changes of soil properties directly, but also indirectly affected by the changes of vegetation diversity and biomass. Our results would provide new insights for the restoration evaluation of degraded grasslands.

Key words: Long-term grazing exclusion, Soil bacterial community, Vegetation diversity, Correlation analysis, Soil property.

Introduction

Grasslands occupy approximately 20% of the terrestrial surface and play an important role in providing multiple ecosystem and cultural services, and supporting peoples' livelihoods (Ebrahimi *et al*., 2016; Chai *et al*., 2019). In recent decades, because of climate deterioration, overgrazing, and other unreasonable human activities, grasslands are severely deteriorating worldwide (Li *et al*., 2015; Miao *et al*., 2015; Liu *et al*., 2017). Grazing exclusion is regarded as an effective way to restore degraded grasslands (Cheng *et al*., 2016), and its effectiveness on vegetation biomass and diversity, soil nutrient contents, and the abundance and diversity of soil microbial community have been proverbially approved (Jiang *et al*., 2009; Jing *et al*., 2014).

Soil bacterial community plays key roles in ecosystems and mediate many ecological processes (Balser & Firestone, 2005), and has become one of the important indicators to evaluate the degree of soil ecological health (Schloter *et al*., 2018). Many studies have focused on the impact of grazing exclusion on soil bacterial community, but there has been no unified understanding (Liu e*t al*., 2010; Sato *et al*., 2019). After all, soil bacterial communities are affected by various environmental factors. Zhang *et al*., (2019) reported that grazing exclusion could greatly improve soil microbial abundance and diversity, while other studies found that the grazing exclusion effects on soil microbial community had no clear pattern to follow (Aldezabal *et al*., 2015). Even if short-term grazing exclusion could increase the diversity of soil microbial community, long-term grazing exclusion would cause the decline in abundance and diversity of soil microbial community from a peak (Zhou *et al*., 2012). According to our previous understanding, spatial heterogeneity is always recognized as the most important factor causing the changes of soil bacterial community (Sayer *et al*., 2013). In addition, based on the theory of plant-soil feedback (Zak *et al*., 2003; Faucon *et al*., 2017), soil bacterial community could also be affected by vegetation, and soil properties (Ogram *et al*., 2006; Png *et al*., 2018). There have been many literatures about the positive or negative effects of grazing exclusion on vegetation biomass and diversity, soil nutrient contents, and soil physical properties (Fernández-Lugo *et al*., 2013; Miao *et al*., 2015; Zhou *et al*., 2017), and the vegetation characteristics and soil physicochemical properties of the grassland ecosystem under grazing exclusion have been synergistically changing (Marschner *et al*., 2001; Loranger-Merciris *et al*., 2006). Therefore, it was more complicated to explain the grazing exclusion effect on soil bacterial community, which might also be one of the reasons why previous studies had not obtained consistent response patterns of soil bacterial community to grazing exclusion.

In this study, we selected one 36 years continuous grazing exclusion area (GE) and three free grazing areas (FGs) of the typical steppe in Xilingol Grassland of Inner Mongolia, China to investigate soil bacterial abundance, diversity, composition, vegetation biomass and diversity,

and soil physical and chemical properties and across for GE and FGs. We aimed to comprehensively understand the response pattern of soil bacterial community to grazing exclusion, and its associations between vegetation and soil properties. This work would contribute to our understanding of the combined effects of long-term grazing exclusion on vegetation, soil properties, and soil bacterial community, and the driving forces of soil bacterial community variations, would help us build new insights for the restoration evaluation of degraded grasslands.

Materials and Methods

Study area: This study was conducted at the *Leymus chinensis* steppe (43°35'N, 116°44-45'E, 1168-1211 m above sea level) within Xilin Gol grassland of Inner Mongolia, China. This steppe belongs to the temperate and semi-arid continental climate (Chen, 1988). The annual mean temperature is 0.18°C, the annual mean precipitation is 349.6 mm with 70% falling during the growing season from June to August (Baoyin *et al*., 2003). The soil of this steppe is chestnut calcareous soil (Chen, 1988). The dominant plant species in this steppe are originally *Leymus chinensis* and *Stipa grandis*. However, due to high intensity of utilization, some areas in this steppe have seriously degraded (Bai, 1991), and then the dominant plant species of degraded areas have gradually changed to *Artemisia frigida* and *Cleistogenes squarrosa* (Wang *et al*., 1996). For preserving and recovering plant diversity and productivity, this seriously degraded areas have been fenced to be excluded from grazing and any human activities since 1983 (Wang *et al*., 1996). Grazing intensity restriction strategy has been implemented outside the fenced steppe by the government. In this study, GE was the area enclosed from 1983, and FGs were selected outside the fence to ensure that the precipitation, temperature, or other environmental factors, e.g. soil type and nutrient status were not too different. Based on the grazing intensity restriction strategy implemented outside the fenced steppe by the government, the grazing intensity in FG1 exhibiting slight degradation (Li, 1997) was 1 sheep per hectare, that in FG2 exhibiting moderate degradation (Li, 1997) was 5 sheep per hectare, and that in FG3 exhibiting severe degradation (Li, 1997) was 10 sheep per hectare. Considering the possible interference of spatial heterogeneity, we delineated three big enough sampling site (600×400 m^2) in each study area, and divided it into three small areas (400×200 m^2) on average, and set up 15 sampling points in each small area. The soil samples used for determination of soil physicochemical properties and soil bacterial communities were made by mixing soil samples from 5 sampling points. The vegetation characteristics were analyzed according to the statistical results of three quadrats (1×1 m^2) in each small area.

Soil sampling: Sampling took place in GE, and FGs in June 2019. The sampling days were selected on sunny days, and lasted five days. Before soil sampling, there had been no rain for more than one week. Soil was sampled by drilling a 7 cm diameter soil core into 0-10 cm, 10-20 cm, and 20-30 cm soil layer in each quadrat, respectively. Five drills with different soil depths were mixed into a soil sample of different soil depths. Each soil sample was divided into two sets. One set was preserved in -80°C liquid nitrogen for DNA extract. The other set was air-dried, ground, and sieved for 1 mm to measure the belowground biomass, physical, chemical, and enzymatic properties of soil samples.

DNA extraction and PCR amplification: The genomic DNA of each soil sample was extracted using the FastDNA^{TM} SPIN Kit for Soil DNA Extraction (MP Biomedicals, LLC, OH, USA) according to the manufacturer's instructions. The DNA quality was evaluated by detection with agarose gel electrophoresis (MultiDoc-It Digital imaging system, UVP, Cambridge, UK), concentration assay (NanoDrop2000, Thermo Fisher Scientifi, USA), and OD_{260}/OD_{280} ratio (NanoDrop2000, Thermo Fisher Scientifi, USA). The DNAs with satisfactory concentrations and good quality were used for subsequent high-throughput 16S rRNA PCR amplification (Genesky Biotechnologies Inc., Shanghai 201315, China). Bacterial V4V5 region was the objective fragment, and the universal primers of V4V5 region were used for PCR amplification. The library was sequenced by Illumina 2×250 bp double terminal sequencing strategy, and then bioinformatics analysis was carried out. RDP (Ribosomal Database Project) bioinformatics database was used for the sequencing identification.

Measurements: The aboveground biomass (AB, g m^{-2}) of different types of species was reported by the weight of aboveground parts of corresponding species in each quadrat. The species richness (SR) of different types of species was reported by the number of corresponding species in each quadrat. The vegetation belowground biomass (BB, g m^{-3}) was calculated by the weight of total vegetation roots in each layer soil taken by a cylindrical soil block with a diameter of 7 cm. The soil bulk density (SBD) was calculated by dividing the weight of a cylindrical soil block with a diameter of 5 cm and 5 cm in height by its volume. And then, the cylindrical soil block (fresh weight, FW) was oven dried weighted at 105°C for 24 h, and weighted again to get the dry weight (DW). The soil water content (SWC) was calculated as (FW-DW)·100%/DW. The pH value of the soil (PH) was determined with the potentiometry method. Seven soil nutrient parameters were measured in this study: total nitrogen, alkali-hydrolyzable nitrogen, total phosphorus, available phosphorus, total potassium, available potassium, and organic matter. The total nitrogen content (TN) was determined using the Kjeldahl nitrogen determination method (NY/T53-1987); the alkali-hydrolyzable nitrogen content (AHN) was determined using the alkaline hydrolysis diffusion method; the total phosphorus content (TP) was determined using the alkali fusion-Mo-Sb colorimetric method (NY/T88-1988); available phosphorus content (AP) was determined using the sodium bicarbonate leaching-Mo-Sb colorimetric method (LY/T1233-1999); the total potassium content (TK) was determined using HF-$HClO_4$ heating digestion method (Jackson, 1958); the available potassium content (AK) was determined using ammonium acetate extraction method (Jones, 1973); and the soil organic matter content (SOM) was determined with $K_2Cr_2O_7$ oxidation volumetric method (Li, 1983).

Data analysis: The Shannon-Wiener diversity (H) and the Pielou evenness (E) of vegetation species were calculated according to the methods of West (1993). The two-way analysis of variance (ANOVA) based on Duncan's multiple-range test in SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to examine differences among different study sites, and correlations between soil bacterial community and environmental factors. The high throughput absolute and relative quantitative Illumina MiSeq sequencing results have been uploaded and published in Sequence Read Archive (SRA) data of NCBI servers (https://www.ncbi.nlm.nih.gov/sra), with the accession number of PRJNA646998.

Results

Effect of grazing exclusion on soil bacterial community: Based on the high throughput 16S rRNA absolute quantitative sequencing results, there were higher abundance of soil bacterial community in FG1 than those in other areas (Fig. 1). Among the OTUs of the soil bacterial communities, most were common, but some were unique for each area (Fig. 2A). Because of the different effects of grazing exclusion and grazing intensity on soil bacterial community, the four study areas were separated from each other according to the PLS-DA result (Fig. 2B). In particular, the phylum Gemmatimonadetes showed significantly different abundance among four study areas, having the highest relative abundance in FG3 (P=0.002), and the phylum Diapherotrites showed significant different relative abundance (P=0.022) and absolute abundance (P=0.015) among four study areas (Fig. 2C).

Effect of grazing exclusion on vegetation biomass and diversity: Grazing exclusion significantly increased vegetation aboveground biomass, especially expressing on the increases in forbs and shrubs (Fig. 3A). There was no significant variation in gramineous biomass across for grazing exclusion and free grazing areas (Fig. 3A). However, due to the increase in total aboveground biomass, the ratios of gramineous biomass in GE were lower than those in FGs (Fig. 3B). In FGs, the annual and biennial species were widely existed, but because most species such as *Salsola collina*, *Lepidium apetalum*, have low plant heights and thin stems, their biomass accumulation was much lower than those of other types.

The belowground biomass in each soil layer decreased as the soil depth increasing (Fig. 3C). And the belowground biomass in 0-10 cm soil layer had the largest ones in each area, accounting for 49.23% (GE-3)-78.57% (FG2-2) of total belowground biomass in 0-30 cm soil layer (Fig. 3D). The belowground biomass in GE was significantly higher than that only in FG3, while it was higher than those in FG1 and FG2, but the differences were not significant. This indicated that long-term grazing exclusion promoted the accumulation of vegetation belowground biomass in this study. Considering the Pearson correlations between vegetation biomass indicators and grazing intensity, not only AB, LM, EAB, AAB, FAB, and SAB had significant correlations with grazing intensity, but GAB and BB also had significant correlations with grazing intensity (Table 1).

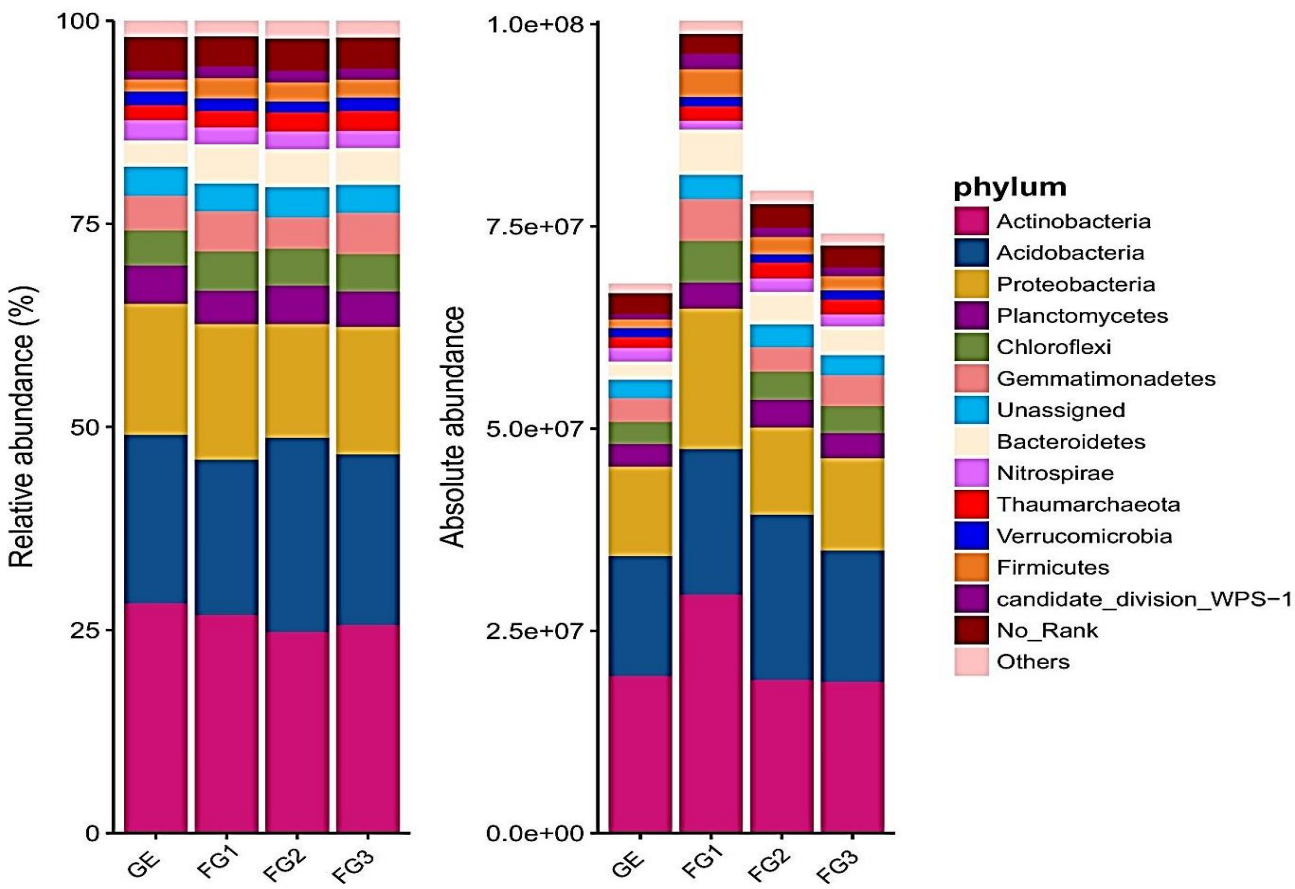

Fig. 1. Relative abundance and absolute abundance of different study areas across for grazing exclusion and free grazing in the level of phylum.

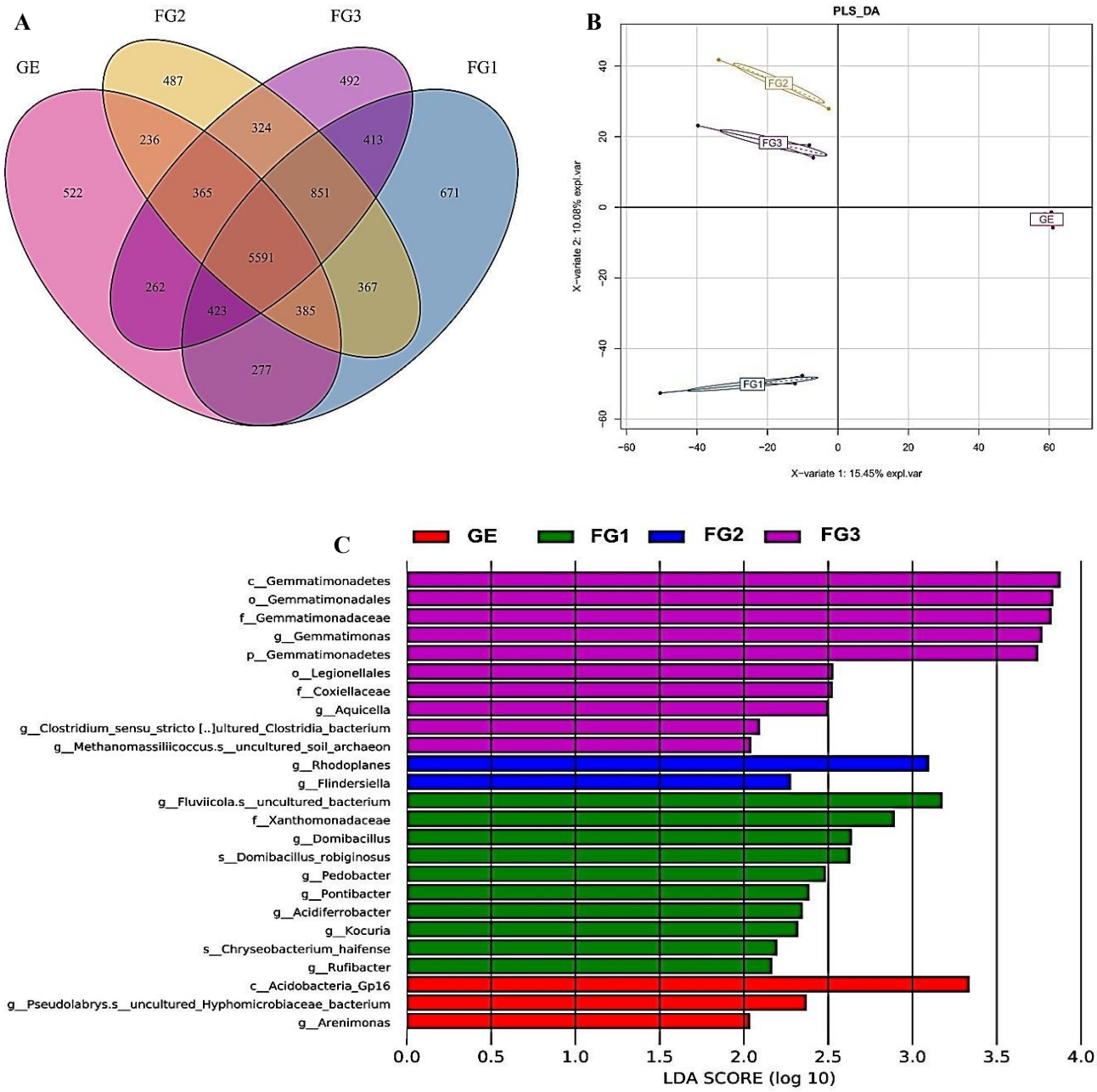

Fig. 2. Venn map (A) and Partial Least Squares Discriminant Analysis (PLS-DA) (B), the bacterial species showing significant differences among different study areas (C).

Based on the important values of each plant species appeared in each sampling plot, it suggested that grazing exclusion could affect the vegetation composition (Fig. 4). The important values of the gramineous species had a little decrease following grazing exclusion. The important values of the forb species were lower, especially in the FG3 than those in other areas, but those of the annual and biennial species were higher in this area than those in others. Following long-term grazing exclusion, the annual and biennia species were nearly gone (Fig. 3A, Fig. 4). In addition, except in the grazing exclusion areas, there were shrubs only in the FG3 area among the free grazing areas.

In view of vegetation diversity, grazing exclusion slightly decreased the Shannon-Wiener diversity index and Pielou evenness index (Fig. 5A). However, the species richness in GE and FG3 reached a relatively higher level than those in the other both free grazing areas (Fig. 5B), suggested that the level of species richness was closely related to the presence or absence of shrubs. Although only SR significantly responded to grazing exclusion, it did not have obvious correlation with grazing intensity. In addition, H, GSR, and ASR were also significantly correlated with grazing intensity (Table 1).

Effect of grazing exclusion on soil properties: Grazing exclusion significantly affected SWC, AHN, AP, AK, and TN, but had no significant effects on other soil properties in different study areas (Table 2). However, SBD, SOM, TP, and TK had no significant difference among the four study areas, but they had significant correlation with grazing intensity (Table 2). Although grazing exclusion could have a great impact on SWC, especially the SWC of the 0-10 cm soil layer, there was no obvious correlation between SWC and grazing intensity.

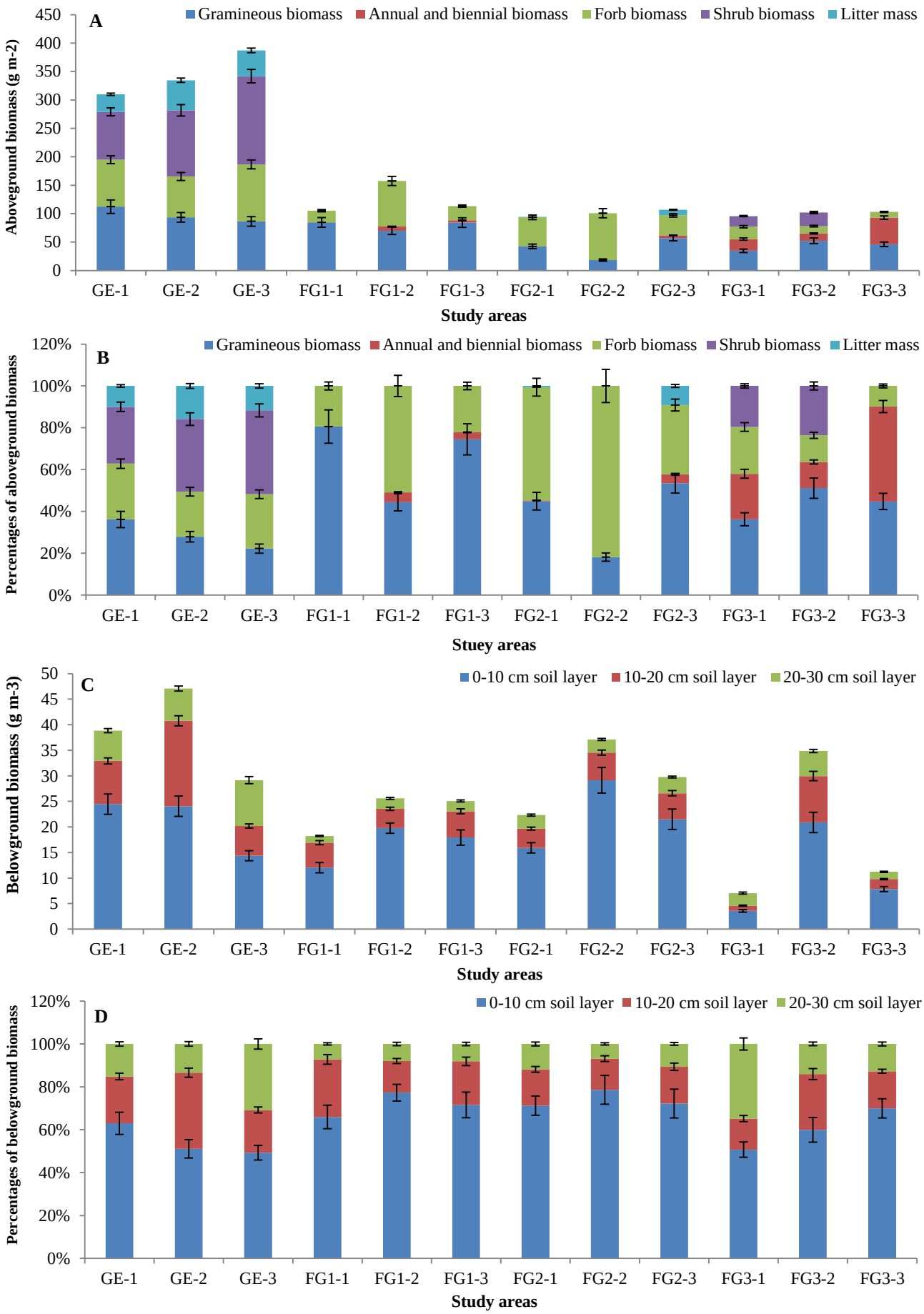

Fig. 3. Absolute (A) and relative species biomass (B) of different types and litter mass, and absolute (C) and relative belowground biomass in different soil layers (D).

Table 1. Pearson correlations between vegetation characteristics and grazing intensity.

Pearson correlations	SR	ESR	H	E	GSR	ASR	FSR	SSR
r	0.093	-0.093	0.585*	0.515	0.784**	0.577*	-0.472	-0.488
P	0.773	0.774	0.046	0.086	0.003	0.050	0.122	0.108
Pearson correlations	**AB**	**EAB**	**LM**	**BB**	**GAB**	**AAB**	**FAB**	**SAB**
r	-0.781**	-0.845**	-0.784**	-0.682*	-0.848**	0.709**	-0.637*	-0.667*
P	0.003	0.001	0.003	0.015	NT	0.010	0.026	0.018

SR: species richness; ESR: effective species richness; H: Shannon-Wiener diversity index; E: Pielou evenness index; GSR: gramineous species richness; ASR: annual and biennial species richness; FSR: forbs species richness; SSR: shrub species richness; AB: aboveground biomass; EAB: effective aboveground biomass; LM: litter mass; BB: belowground biomass; GAB: gramineous aboveground biomass; AAB: annual and biennial aboveground biomass; FAB: forbs aboveground biomass; SAB: shrub aboveground biomass. r indicated the corresponding Pearson correlations between the corresponding vegetation characteristic and grazing intensity. *P* stood for *P* value, indicating the significance of Pearson correlations. * and ** indicated the significant difference in the level of $p<0.05$ and $p<0.01$, respectively

Table 3. Pearson correlations of soil bacterial abundance and diversity with vegetation biomass, diversity, and soil property indices.

Pearson correlations		SR	ESR	H	E	GSR	ASR	FSR	SSR		
Soil bacterial abundance	**r**	-0.205	-0.209	-0.051	0.064	-0.035	-0.020	-0.155	-0.159		
	P	0.522	0.514	0.876	0.844	0.914	0.951	0.632	0.622		
Soil bacterial diversity	**r**	0.024	-0.220	0.360	0.362	0.421	0.495	-0.416	-0.221		
	P	0.940	0.491	0.250	0.248	0.173	0.101	0.179	0.490		
Pearson correlations		**AB**	**EAB**	**LM**	**BB**	**GAB**	**AAB**	**FAB**	**SAB**		
Soil bacterial abundance	**r**	-0.105	-0.092	-0.110	0.559	0.008	-0.040	-0.067	-0.141		
	P	0.744	0.776	0.733	0.059	0.979	0.901	0.836	0.663		
Soil bacterial diversity	**r**	-0.361	-0.405	-0.365	0.260	-0.291	0.479	-0.511	-0.316		
	P	0.249	0.191	0.243	0.415	0.358	0.115	0.089	0.317		
Pearson correlations		**SBD**	**SWC**	**PH**	**SOM**	**AHN**	**AP**	**AK**	**TN**	**TP**	**TK**
Soil bacterial abundance	**r**	-0.199	-0.286	-0.513	0.273	0.231	0.793**	0.600*	0.18	0.178	0.104
	P	0.535	0.367	0.088	0.391	0.469	0.002	0.039	0.575	0.581	0.748
Soil bacterial diversity	**r**	0.142	-0.275	-0.326	0.172	-0.118	0.348	0.241	-0.018	0.173	0.292
	P	0.660	0.387	0.301	0.594	0.714	0.268	0.450	0.955	0.590	0.357

SR: species richness; ESR: effective species richness; H: Shannon-Wiener diversity index; E: Pielou evenness index; GSR: gramineous species richness; ASR: annual and biennial species richness; FSR: forbs species richness; SSR: shrub species richness; AB: aboveground biomass; EAB: effective aboveground biomass; LM: litter mass; BB: belowground biomass; GAB: gramineous aboveground biomass; AAB: annual and biennial aboveground biomass; FAB: forbs aboveground biomass; SAB: shrub aboveground biomass; SBD: soil bulk density; SWC: soil water content; PH: soil pH value; SOM: soil organic matter content; AHN: alkali-hydrolyzable nitrogen content; AP: available phosphorus content; AK: available potassium content; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content. The ones in bold indicated the correlations were statistically significant at the $p<0.05$ level. * and ** indicated the significant difference in the level of $p<0.05$ and $p<0.01$, respectively

Discussion

Soil bacterial community directly responses to soil properties but not vegetation: Soil bacterial community was found to be largely influenced by grazing exclusion, along with vegetation and soil properties (Olivera *et al*., 2016). According to the previous understanding, whether the heterogeneity of grassland types, topography, climate, or human management strategies, the growth of soil bacteria finally seems to be directly affected by vegetation and soil properties (Chen *et al*., 2018). Thus, in order to clarify the grazing exclusion effects on soil bacterial community, we usually need to clarify the effects on vegetation and soil properties, and the relationships between these effects and variations in soil bacterial community. Cheng *et al*., (2016) have confirmed that grazing exclusion could improve the diversity and abundance of soil bacteria. And this similar response to grazing exclusion was also supported by a short-term trail in the typical steppe of Inner Mongolia, China (Zhou *et al*., 2012). However, only moderate grazing or short-term grazing exclusion showed improved effects on soil bacterial abundance and diversity (Zhou *et al*., 2012; Beneduzi *et al*., 2019), and long-term grazing exclusion would cause a negative effect (Zou *et al*., 2014; Cheng *et al*., 2016). In the present study, we found 36 years long-term grazing exclusion did not increase the abundance and diversity of soil bacterial community, but slight grazing (FG1 having high vegetation aboveground biomass) could promote them (Fig. 1).

By analyzing the correlations of soil bacterial community with vegetation and soil, we found vegetation biomass and diversity had no significant correlations with soil bacterial community (r=-0.10059, *P*=0.7447, Fig. 6A), and despite soil properties also showed no significant correlations with soil bacterial community (r=0.20232, *P*=0.1319, Fig. 6B) as a whole, among all soil indices, AK and AP were significantly correlated with soil bacterial community alone (r=0.600, *P*=0.039, and r=0.793, *P*=0.002, respectively) (Table 3, Fig. 6). This has also been verified previously that AK and AP have interaction with the relative abundances of some dominant groups along with SOC and TN (Cheng *et al*., 2016). In contrast to no significant correlations between soil bacterial community and vegetation in our study and some previous studies (Zak *et al*., 2003), some other studies demonstrated that plant diversity could significantly influence the activity and community composition of soil microbes (Loranger-Merciris *et al*., 2006). Soil bacterial community did not significantly respond to vegetation, but AK and AP were largely affected by vegetation diversity and biomass in our study (Table S1). This suggested that vegetation diversity was the primary driving force of soil bacterial variations directly regulated by AK and AP. Considering soil bacterial community might depend on both vegetation and soil status, which were able to be indefinitely affected by grazing exclusion due to multi-factors, the response of soil bacterial community to grazing exclusion needed to be further clarified in more grassland trials with different vegetation and soil status.

Table 2 Effects of grazing exclusion on soil properties, and the Pearson correlations between soil properties and grazing intensity

Study areas	SBD			SWC¶			SPH			SOM			AHN¶		
	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm
GE	1207.00±14.31	1467.45±65.06	1555.77±76.30	6.45±1.20	3.41±0.13	2.93±0.33	6.98±0.17	7.17±0.07	7.24±0.13	6.13±0.26	4.42±0.58	1.49±0.19	61.02±2.96	33.83±5.13	21.78±3.71
FG1	1565.79±34.02	1694.57±53.26	1726.44±43.26	2.90±0.62	3.94±0.31	3.87±0.18	7.12±0.06	7.35±0.04	7.48±0.02	2.87±0.44	2.42±0.32	1.75±0.13	28.47±1.09	18.80±1.31	11.47±2.52
FG2	1522.58±60.08	1664.82±9.92	1666.65±36.89	2.94±0.58	3.09±0.56	3.07±0.64	7.12±0.06	7.30±0.04	7.42±0.02	4.44±0.05	3.00±0.37	2.02±0.23	23.47±5.38	19.05±5.24	17.00±2.20
FG3	1628.74±64.00	1675.45±47.60	1657.04±54.74	3.64±0.46	3.78±0.53	3.99±0.62	7.12±0.05	7.30±0.08	7.49±0.10	3.77±0.71	2.38±0.54	2.37±0.20	21.93±5.52	13.54±3.11	9.92±1.09
r	0.811**	0.646*	0.414	-0.567	0.018	0.308	0.453	0.420	0.508	-0.566	-0.727**	0.358	-0.843**	-0.826**	-0.610*
P	0.001	0.023	0.181	0.054	0.955	0.329	0.139	0.174	0.092	0.055	0.007	0.253	0.001	0.001	0.035
Study areas	**AP¶**			**AK¶**			**TN¶**			**TP**			**TK**		
	0-10 cm	**10-20 cm**	**20-30 cm**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**0-10 cm**	**10-20 cm**	**20-30 cm**
GE	4.29±0.31	2.42±0.23	1.96±0.12	16.29±1.72	5.65±0.49	3.22±0.26	0.63±0.04	0.28±0.02	0.14±0.01	0.22±0.03	0.12±0.01	0.09±0.00	34.25±1.28	32.59±1.19	31.95±1.79
FG1	6.25±0.37	1.97±0.12	1.68±0.13	15.42±1.48	6.56±0.22	3.97±0.36	0.20±0.02	0.13±0.01	0.06±0.01	0.14±0.00	0.14±0.01	0.12±0.02	36.54±0.26	36.80±0.17	36.67±0.17
FG2	2.98±0.26	2.10±0.08	1.48±0.15	7.99±0.72	5.35±0.59	4.53±0.39	0.26±0.02	0.14±0.01	0.09±0.00	0.18±0.02	0.14±0.00	0.12±0.00	36.54±0.00	36.55±0.51	36.80±0.17
FG3	2.80±0.10	0.53±0.04	0.99±0.09	8.58±0.86	4.82±0.48	3.98±0.32	0.24±0.02	0.14±0.01	0.11±0.01	0.16±0.01	0.14±0.01	0.14±0.01	36.67±0.17	36.67±0.17	36.67±0.17
r	-0.527	-0.824**	-0.736**	-0.617*	-0.209	0.34	-0.739**	-0.725**	-0.287	-0.513	0.422	0.676*	0.616*	0.713**	0.688*
P	0.078	0.001	0.006	0.033	0.514	0.279	0.006	0.008	0.365	0.088	0.172	0.016	0.033	0.009	0.013

SBD: soil bulk density, g m^{-3}; SWC: soil water content, g g^{-1} soil fresh weight; SPH: soil pH value; SOM: soil organic matter content, mg g^{-1}; AHN: alkali-hydrolyzable nitrogen content, mg kg^{-1}; AP: available phosphorus content, mg kg^{-1}; AK: available potassium content, mg kg^{-1}; TN: total nitrogen content, g kg^{-1}; TP: total phosphorus content, g kg^{-1}; TK: total potassium content, g kg^{-1}. Indicated the values of corresponding soil properties in the same soil layer were significantly different among different study areas ($p<0.05$). r indicated the corresponding Pearson correlations between the corresponding soil property and grazing intensity. *P* stood for *P* value, indicating the significance of Pearson correlations. * and ** indicated the significant difference in the level of$p<0.05$ and $p<0.01$, respectively

Table S1 Pearson correlations between soil properties and vegetation characteristics.

Vegetation characteristics			SBD			SWC			SPH			SOM			AHN		
			0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm
Vegetation diversity index	SR	*r*	-0.299	-0.394	-0.424	0.586*	0.139	0.111	-0.068	-0.614*	-0.426	0.435	0.263	-0.194	0.325	0.241	0.239
		p	0.344	0.206	0.17	0.045	0.667	0.731	0.834	0.034	0.167	0.158	0.409	0.547	0.303	0.451	0.455
	ESR	*r*	-0.483	-0.501	-0.529	0.579*	-0.106	-0.166	-0.019	-0.646*	-0.495	0.586*	0.466	-0.467	0.420	0.351	0.570
		p	0.112	0.097	0.077	0.049	0.742	0.605	0.954	0.023	0.101	0.045	0.126	0.126	0.174	0.264	0.053
	H	*r*	0.510	0.588*	0.489	0.038	0.127	0.217	0.495	0.264	0.475	-0.261	-0.484	0.157	-0.553	-0.567	-0.460
		p	0.090	0.044	0.106	0.906	0.694	0.498	0.101	0.407	0.119	0.413	0.111	0.627	0.062	0.055	0.132
	E	*r*	0.643*	0.775**	0.699*	-0.207	0.136	0.214	0.529	0.497	0.630*	-0.478	-0.593*	0.153	-0.675*	-0.644*	-0.529
		p	0.024	0.003	0.011	0.519	0.672	0.504	0.077	0.100	0.028	0.116	0.042	0.634	0.016	0.024	0.077
	GSR	*r*	0.559	0.290	0.209	-0.364	0.142	0.329	0.185	-0.027	0.089	-0.266	-0.363	0.141	-0.540	-0.507	-0.171
		p	0.059	0.361	0.515	0.245	0.659	0.296	0.565	0.934	0.784	0.404	0.246	0.662	0.070	0.093	0.596
	ASR	*r*	0.478	0.402	0.198	-0.090	0.255	0.461	0.344	0.284	0.436	-0.311	-0.496	0.424	-0.446	-0.502	-0.680*
		p	0.116	0.195	0.538	0.782	0.425	0.132	0.273	0.372	0.156	0.326	0.101	0.169	0.146	0.096	0.015
	FSR	*r*	-0.701*	-0.570	-0.472	0.765**	-0.113	-0.330	-0.112	-0.637*	-0.526	0.598*	0.543	-0.589*	0.640*	0.576	0.602*
		p	0.011	0.053	0.121	0.004	0.727	0.295	0.729	0.026	0.079	0.040	0.068	0.044	0.025	0.050	0.038
	SSR	*r*	-0.721**	-0.823**	-0.704*	0.598*	0.050	-0.106	-0.625*	-0.799**	-0.842**	0.727**	0.746**	-0.194	0.826**	0.775**	0.666*
		p	0.008	0.001	0.011	0.040	0.878	0.742	0.030	0.002	0.001	0.007	0.005	0.546	0.001	0.003	0.018
Vegetation biomass index	AB	*r*	-0.913**	-0.770**	-0.536	0.846**	-0.164	-0.457	-0.430	-0.527	-0.598*	0.726**	0.708**	-0.338	0.923**	0.765**	0.576*
		p	NT	0.003	0.073	0.001	0.610	0.135	0.163	0.079	0.040	0.008	0.010	0.283	NT	0.004	0.050
	ESR	*r*	-0.942**	-0.760**	-0.53	0.819**	-0.197	-0.485	-0.383	-0.496	-0.592*	0.730**	0.729**	-0.393	0.930**	0.781**	0.622*
		p	NT	0.004	0.077	0.001	0.540	0.110	0.219	0.101	0.042	0.007	0.007	0.207	NT	0.003	0.031
	LM	*r*	-0.917**	-0.786**	-0.543	0.850**	-0.160	-0.457	-0.478	-0.566	-0.633*	0.752**	0.732**	-0.311	0.932**	0.794**	0.605*
		p	NT	0.002	0.068	NT	0.620	0.135	0.116	0.055	0.027	0.005	0.007	0.325	NT	0.002	0.037
	BB	*r*	-0.795**	-0.786**	-0.741**	0.254	-0.441	-0.508	-0.557	-0.352	-0.543	0.772**	0.795**	0.003	0.735**	0.723**	0.789**
		p	0.002	0.002	0.006	0.425	0.151	0.092	0.060	0.262	0.068	0.003	0.002	0.993	0.006	0.008	0.002
	GAB	*r*	-0.615*	-0.598*	-0.361	0.557	0.276	-0.025	-0.390	-0.432	-0.395	0.387	0.644*	-0.448	0.752**	0.653*	0.393
		p	0.033	0.040	0.248	0.060	0.385	0.939	0.210	0.161	0.203	0.214	0.024	0.144	0.005	0.021	0.206
	AAB	*r*	0.547	0.292	0.137	-0.191	0.130	0.265	0.117	0.231	0.450	-0.332	-0.441	0.425	-0.484	-0.557	-0.609*
		p	0.066	0.358	0.670	0.552	0.687	0.405	0.716	0.469	0.142	0.291	0.151	0.168	0.111	0.060	0.035
	FAB	*r*	-0.809**	-0.523	-0.430	0.438	-0.530	-0.656*	-0.086	-0.215	-0.464	0.524	0.465	-0.404	0.649*	0.564	0.573
		p	0.001	0.081	0.163	0.155	0.076	0.020	0.791	0.502	0.129	0.080	0.128	0.193	0.022	0.056	0.051
	SAB	*r*	-0.862**	-0.737**	-0.486	0.910**	-0.132	-0.425	-0.494	-0.586*	-0.630*	0.782**	0.684*	-0.200	0.887**	0.745**	0.571
		p	NT	0.006	0.110	NT	0.683	0.169	0.103	0.045	0.028	0.003	0.014	0.533	NT	0.005	0.052

SBD: soil bulk density; SWC: soil water content; SPH: soil pH value; SOM: soil organic matter content; AHN: alkali-hydrolyzable nitrogen content; AP: available phosphorus content; AK: available potassium content; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content; SR: species richness; ESR: effective species richness; H: Shannon-Wiener diversity index; E: Pielou evenness index; GSR: gramineous species richness; ASR: annual and biennial species richness; FSR: forbs species richness; SSR: shrub species richness; AB: aboveground biomass; EAB: effective aboveground biomass; LM: litter mass; BB: belowground biomass; GAB: gramineous aboveground biomass; AAB: annual and biennial aboveground biomass; FAB: forbs aboveground biomass; SAB: shrub aboveground biomass. r indicated the corresponding Pearson correlations between the corresponding soil property and vegetation characteristics. P stood for P value, indicating the significance of Pearson correlations. NT indicated the P value was less than 0.001. * and ** indicated the significant difference of the corresponding Pearson correlation in the level of $p<0.05$ and $p<0.01$, respectively

Table S1 (Cont'd.).

Vegetation characteristics			SBD			SWC			SPH			SOM			AHN		
			0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm
Vegetation diversity index	SR	*r*	-0.486	-0.357	-0.102	-0.058	-0.187	-0.186	0.423	0.395	0.394	0.325	-0.233	-0.141	-0.397	-0.339	-0.339
		p	0.109	0.255	0.753	0.858	0.560	0.563	0.170	0.204	0.205	0.302	0.466	0.663	0.202	0.281	0.282
	ESR	*r*	-0.520	-0.044	0.035	-0.239	-0.375	-0.256	0.505	0.423	0.428	0.405	-0.335	-0.401	-0.617*	-0.371	-0.386
		p	0.083	0.892	0.915	0.455	0.229	0.421	0.094	0.171	0.165	0.191	0.288	0.196	0.033	0.236	0.215
	H	*r*	-0.619*	-0.626*	-0.741**	-0.604*	-0.383	-0.147	-0.339	-0.371	-0.292	-0.425	-0.263	0.238	0.238	0.213	0.590*
		p	0.032	0.030	0.006	0.038	0.219	0.649	0.281	0.235	0.356	0.169	0.409	0.456	0.456	0.507	0.044
	E	*r*	-0.383	-0.451	-0.686*	-0.622*	-0.358	-0.132	-0.519	-0.547	-0.452	-0.592*	-0.249	0.218	0.376	0.365	0.713**
		p	0.219	0.141	0.014	0.031	0.253	0.682	0.084	0.066	0.140	0.043	0.436	0.496	0.228	0.244	0.009
	GSR	*r*	-0.502	-0.708*	-0.575	-0.638*	-0.504	-0.038	-0.435	-0.386	-0.109	-0.279	0.197	0.332	0.460	0.588*	0.361
		p	0.096	0.010	0.051	0.026	0.095	0.908	0.157	0.216	0.737	0.380	0.538	0.292	0.133	0.044	0.249
	ASR	*r*	-0.293	-0.752**	-0.562	-0.130	0.070	0.174	-0.340	-0.245	-0.121	-0.314	0.271	0.684*	0.345	0.285	0.481
		p	0.356	0.005	0.057	0.688	0.829	0.589	0.279	0.444	0.709	0.320	0.394	0.014	0.272	0.369	0.113
	FSR	*r*	-0.177	0.326	0.329	0.175	-0.025	-0.239	0.651*	0.493	0.287	0.435	-0.482	-0.636*	-0.800**	-0.655*	-0.533
		p	0.583	0.301	0.296	0.587	0.938	0.454	0.022	0.104	0.366	0.158	0.113	0.026	0.002	0.021	0.075
	SSR	*r*	-0.029	0.261	0.497	0.323	-0.055	-0.259	0.806**	0.827**	0.748**	0.753**	-0.312	-0.520	-0.515	-0.654*	-0.927**
		p	0.928	0.412	0.100	0.305	0.865	0.417	0.002	0.001	0.005	0.005	0.324	0.083	0.087	0.021	NT
Vegetation biomass index	AB	*r*	0.213	0.524	0.621*	0.633*	0.182	-0.270	0.884**	0.778**	0.417	0.627*	-0.503	-0.690*	-0.768**	-0.935**	-0.821**
		p	0.506	0.081	0.031	0.027	0.571	0.396	NT	0.003	0.178	0.029	0.095	0.013	0.004	NT	0.001
	ESR	*r*	0.261	0.612*	0.678*	0.621*	0.178	-0.269	0.882**	0.775**	0.380	0.612*	-0.507	-0.713**	-0.790**	-0.914**	-0.791**
		p	0.413	0.035	0.015	0.031	0.580	0.398	NT	0.003	0.224	0.035	0.093	0.009	0.002	NT	0.002
	LM	*r*	0.189	0.537	0.622*	0.623*	0.170	-0.285	0.904**	0.802**	0.438	0.663*	-0.515	-0.708**	-0.747**	-0.947**	-0.849**
		p	0.556	0.072	0.031	0.030	0.597	0.370	NT	0.002	0.155	0.019	0.087	0.010	0.005	NT	NT
	BB	*r*	0.210	0.674*	0.599*	0.281	-0.066	-0.156	0.719**	0.738**	0.561	0.825**	0.018	-0.377	-0.393	-0.481	-0.656*
		p	0.513	0.016	0.040	0.376	0.839	0.629	0.008	0.006	0.058	0.001	0.955	0.227	0.206	0.114	0.021
	GAB	*r*	0.528	0.499	0.527	0.552	0.073	-0.483	0.600*	0.671*	0.356	0.28	-0.441	-0.588*	-0.628*	-0.586*	-0.651*
		p	0.078	0.099	0.078	0.063	0.821	0.112	0.039	0.017	0.256	0.378	0.151	0.044	0.029	0.045	0.022
	AAB	*r*	-0.398	-0.788**	-0.695*	-0.241	-0.083	0.140	-0.411	-0.353	0.020	-0.271	0.322	0.551	0.333	0.303	0.327
		p	0.200	0.002	0.012	0.450	0.797	0.665	0.184	0.260	0.950	0.395	0.307	0.063	0.291	0.339	0.299
	FAB	*r*	0.243	0.717**	0.772**	0.526	0.403	0.227	0.574	0.377	0.099	0.473	-0.140	-0.482	-0.625*	-0.609*	-0.489
		p	0.447	0.009	0.003	0.079	0.194	0.477	0.051	0.227	0.760	0.120	0.664	0.112	0.030	0.036	0.106
	SAB	*r*	0.041	0.389	0.481	0.519	0.047	-0.373	0.927**	0.817**	0.447	0.686*	-0.610*	-0.701*	-0.668*	-0.970**	-0.825**
		p	0.900	0.211	0.114	0.084	0.885	0.232	NT	0.001	0.145	0.014	0.035	0.011	0.017	NT	0.001

SBD: soil bulk density; SWC: soil water content; SPH: soil pH value; SOM: soil organic matter content; AHN: alkali-hydrolyzable nitrogen content; AP: available phosphorus content; AK: available potassium content; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content; SR: species richness; ESR: effective species richness; H: Shannon-Wiener diversity index; E: Pielou evenness index; GSR: gramineous species richness; ASR: annual and biennial species richness; FSR: forbs species richness; SSR: shrub species richness; AB: aboveground biomass; EAB: effective aboveground biomass; LM: litter mass; BB: belowground biomass; GAB: gramineous aboveground biomass; AAB: annual and biennial aboveground biomass; FAB: forbs aboveground biomass; SAB: shrub aboveground biomass. r indicated the corresponding Pearson correlations between the corresponding soil property and vegetation characteristics. P stood for P value, indicating the significance of Pearson correlations. NT indicated the P value was less than 0.001. * and ** indicated the significant difference of the corresponding Pearson correlation in the level of $p<0.05$ and $p<0.01$, respectively

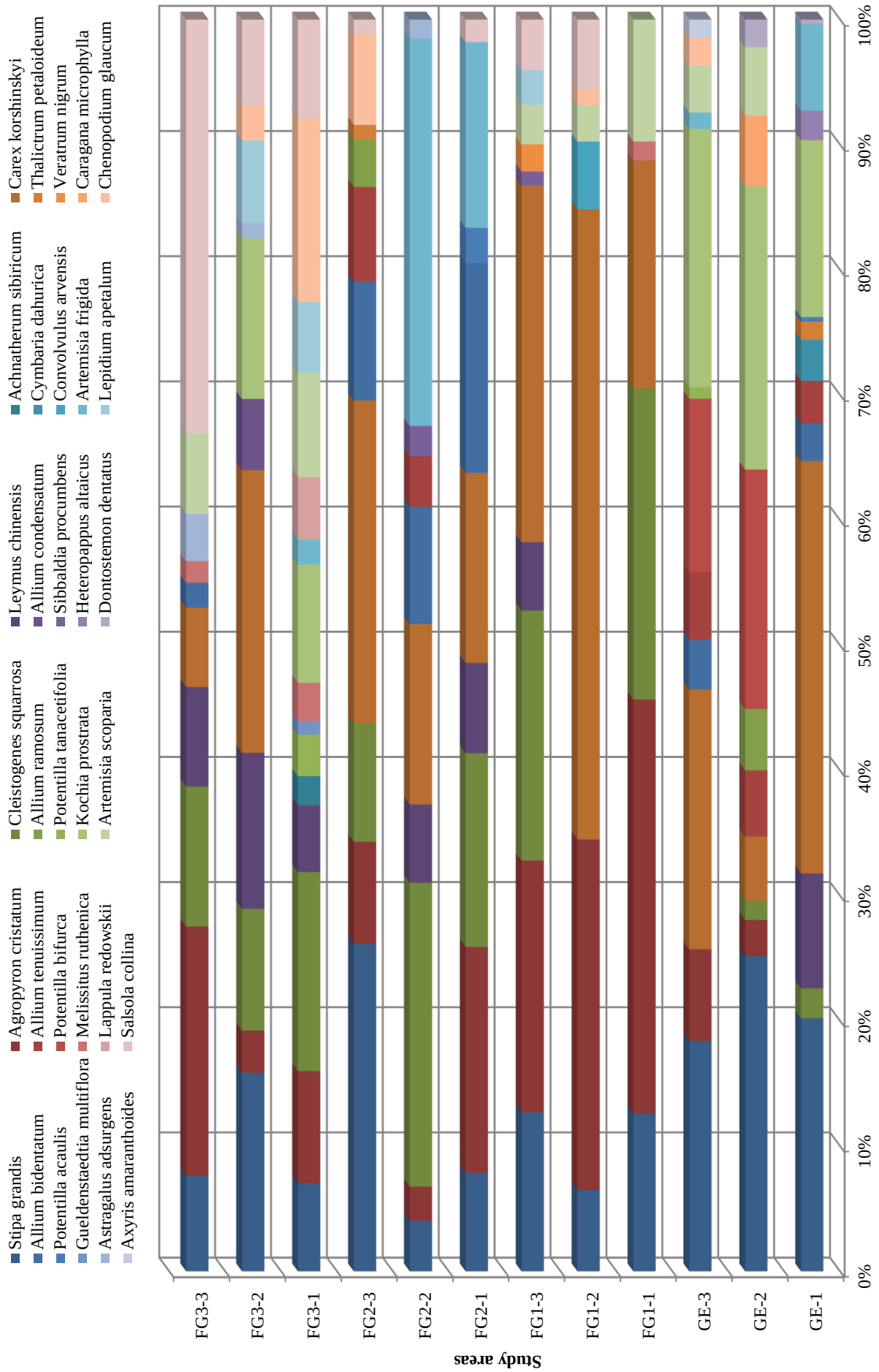

Fig. 4. Vegetation composition of grazing exclusion (GE) and free grazing areas (FGs).

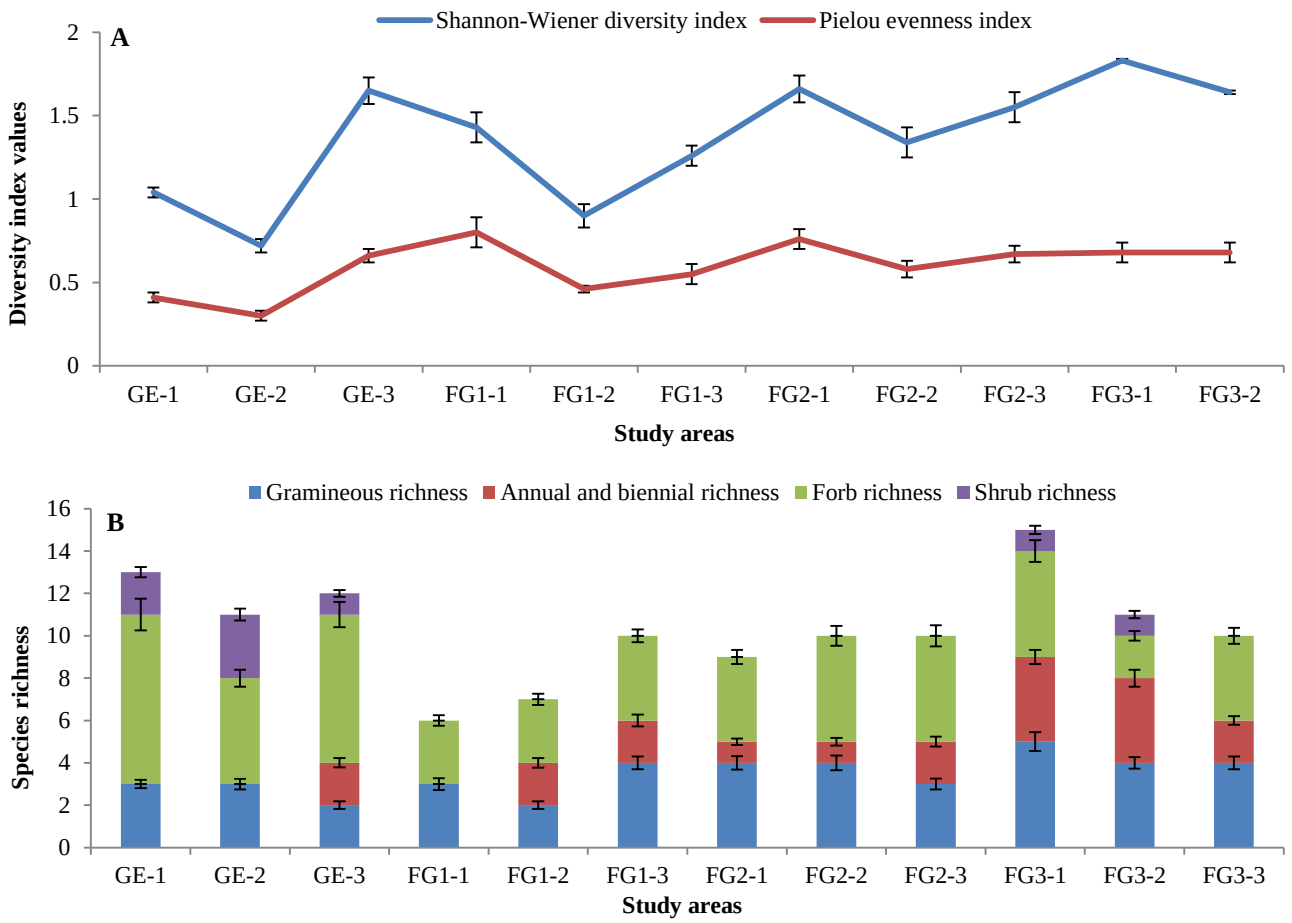

Fig. 5. Vegetation diversity indices (A) and species richness of different types (B).

Soil properties are improved by grazing exclusion directly affecting plant characteristics: Grazing exclusion is considered to be a critical restoration strategy for degraded grasslands. However, there has been no consistent effect on vegetation and soil (Cheng *et al*., 2016). Some studies reported that grazing exclusion did not significantly alter vegetation diversity and soil nutrient contents (Wu *et al*., 2014; Lu *et al*., 2015), while some studies showed grazing exclusion could significantly affect vegetation diversity and soil organic carbon (SOC) content (Fernández-Lugo *et al*., 2013; Ebrahimi *et al*., 2016). Recently, numerous studies found the effects on vegetation diversity and species composition were not invariable during grazing exclusion: in the beginning of grazing exclusion, vegetation diversity increased, and after a period of grazing exclusion, it reached a peak and then began to decline (Yuan *et al*., 2016; Yu *et al*., 2019). However, how long it took for vegetation diversity to reach the peak was equivocal due to various actual situations of different study sites. That was why the effects of grazing exclusion on plant diversity in the same study site even showed different results (Wang *et al*., 2019). That also was why the same grazing exclusion duration produced different effects on vegetation and soil in different geographical environments (Lu *et al*., 2015; Ebrahimi *et al*., 2016). Our study showed long-term grazing exclusion (36 years) increased vegetation aboveground biomass, litter mass, soil water content, soil organic matter, and nitrogen stocks, which were widely consistent with many existing results (Deng *et al*., 2014; Chai *et al*., 2019). However, the vegetation diversity was lower in the long-term grazing exclusion areas than the free grazing exclusion areas in our study (Fig. 5), which was, though, in line with the result of Chai *et al*., (2019), but contrary to the increased vegetation diversity result of Xiong *et al*., (2016). These diverse responses depended on the exclusion duration (Chai *et al*., 2019), and the actual status of each study site in the exclusion initiation, including vegetation composition, soil properties, and so on. In addition, the herbivore absence would gradually cause negative effects on vegetation diversity and community composition (Khishigbayar *et al*., 2015). Once vegetation diversity reduced, the natural stress resistance and grazing tolerance also decreased (Zou *et al*., 2014). Especially in the present study, the Pielou evenness index showed a downward trend after long-term grazing exclusion (Fig. 5), which suggested that the advantage of the dominant species would be more obvious, effectively controlling the reproduction and growth of the inferior species, and thus resulting in continual vegetation diversity loss. However, the improvement of soil properties in our present study was not achieved in a short time, it was the result of long-term soil management strategy. In particular, some important indicators, such as vegetation aboveground biomass, litter mass, belowground biomass, and the abundance and composition of soil bacterial communities, all directly affected soil properties (Liu *et al*., 2010). Our results also proved that vegetation biomass closely linked with SBD, SWC, SOM, AHN, AK, TN, TP, and TK, and vegetation diversity closely linked with AP, and AK (Table S1). The significantly increased N stock, and P and K availability (Table 2) following long-term grazing exclusion would in turn promote plant growth and development.

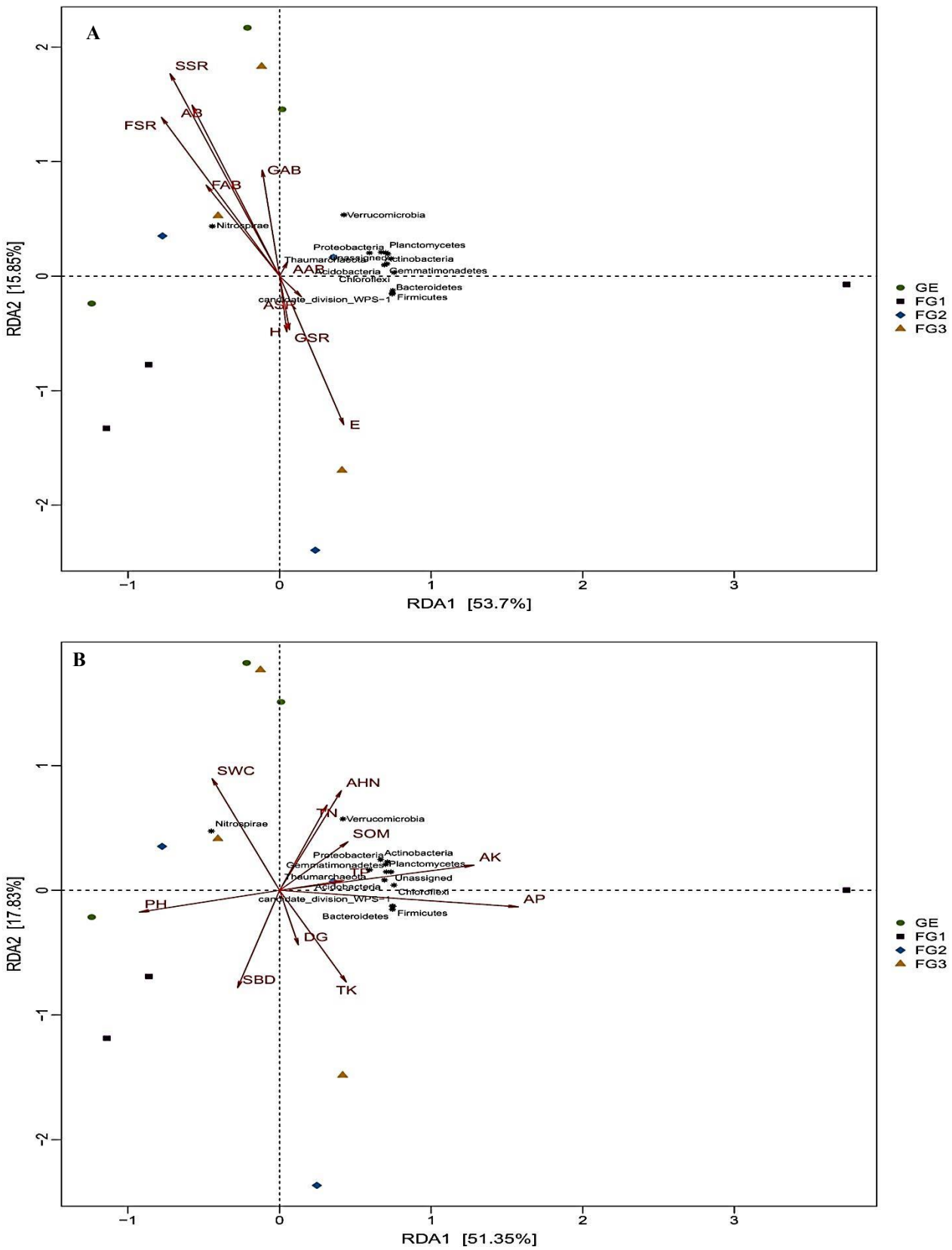

Fig. 6. Redundancy analysis (RDA) of soil bacterial communities with vegetation characteristic indicators (A) and soil property indicators (B).

SR: species richness; ESR: effective species richness; H: Shannon-Wiener diversity index; E: Pielou evenness index; GSR: gramineous species richness; ASR: annual and biennial species richness; FSR: forbs species richness; SSR: shrub species richness; AB: aboveground biomass; EAB: effective aboveground biomass; LM: litter mass; BB: belowground biomass; GAB: gramineous aboveground biomass; AAB: annual and biennial aboveground biomass; FAB: forbs aboveground biomass; SAB: shrub aboveground biomass; SBD: soil bulk density; SWC: soil water content; PH: soil pH value; SOM: soil organic matter content; AHN: alkali-hydrolyzable nitrogen content; AP: available phosphorus content; AK: available potassium content; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content.

Conclusions

Long-term grazing exclusion significantly changed the abundance of some dominant bacterial species, and the structure of soil bacterial community. The variations in soil bacterial abundance significantly linked with soil AK and AP, but not directly with vegetation. However, the restoration and reproduction of vegetation community was often faster than the improvement of soil properties, and the variations in soil properties were closely related with vegetation biomass and diversity. Especially, AK and AP were affected not only by other soil properties, but also by various vegetation biomass and diversity indicators. Thus, the soil bacterial community changes were directly related to the variations in soil properties, they were actually affected by the variations in vegetation characteristics caused by grazing exclusion in an indirect way. We agreed with the view that long-term grazing exclusion had negative effects on grassland ecosystem, mainly expressing on decreased vegetation diversity, shrunken soil bacterial abundance and diversity in our present study. This indicates that the restoration management of degraded grasslands needs to be actively adjusted according to the actual situation to maintain biodiversity, resistance, resilience and function redundancy of ecosystems. The more reasonable restoration and utilization strategy should be carried out when the biodiversity tends to decline, such as mowing or light grazing.

Declarations of interest: The authors declare that there is no competing interest for this paper.

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