Heavy Grazing Reduces the Diversity of Soil Microbial Communities in Meadow Grassland Under Long-Term Grazing

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Research

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Abstract

Background: Soil microorganisms are an important part of the grassland ecosystem and promote material transformation and energy flow in the entire ecological environment. Moreover, Hulun Buir grassland is the material basis for the development of animal husbandry. Therefore, it is of great scientific significance to study the changes of soil microbial community caused by grazing in Hulunbuir grassland for the sustainable and stable development of grassland ecosystem.

Methods: The present research used high-throughput sequencing of soil microorganism (bacteria and fungi) genes to compare microbial communities in 6 levels of grazing intensity (0.00, 0.23, 0.34, 0.46, 0.69, and 0.92 Au ha⁻¹) under the Hulun Buir structure and the diversity characteristics of *Leymus chinensis* meadow steppe.

Results: The 0-10 cm soil layer of the G0.34 test area had the highest content, and the content of the G0.92 test area was lower than the other grazing treatments. With increasing depth, the carbon and nitrogen contents of microorganisms decreased. The diversity of soil bacteria in the light grazing test area (0.23Au ha⁻¹) was higher than the heavy grazing area, and the diversity of fungi in the non-grazing area was higher than the specific grazing areas. Most bacterial species were enriched in the G0.00 grazing areas, and the other grazing intensities were less abundant. The underground biomass ($P = 0.039$) significantly influenced the bacterial community structure, and pH ($P = 0.032$), total nitrogen ($P = 0.011$) and litter ($P = 0.007$) significantly influenced the fungal community.

Conclusions: In conclusion, the structures of bacterial and fungal communities are very sensitive to grazing and varied with grazing intensity. Our findings demonstrated that a grazing intensity of approximately 0.23 Au ha⁻¹ was the most appropriate for the grassland of the meadow in Hulun Buir.

Introduction

Soil microorganisms are largest underground repository of grassland ecosystems and play an important role in the grassland ecological system in the promotion of the flow of material and energy transfer in the entire environment and the maintenance of the grassland ecological system [1]. Soil microorganisms are extremely sensitive to changes of environment [2]. Grazing is the main use of grassland ecological systems [3], and long-term unreasonable grazing affects the vegetation community structure and soil characteristics [4–6], which change the soil microbial community. Changes in the soil microbial community structure affect the vegetation in the community, which impacts the entire ecosystem [7]. Soil microbial diversity is an important index for evaluations of community characteristics and stability, which reflect the functional evolution of ecosystems and environmental changes. In general, greater microbial diversity indicates stronger soil biological activity, which is more conducive to plant growth [8]. Therefore, it is of great significance to examine the relationship between different grazing intensities and microbial community structure and diversity of grassland and soil and vegetation characteristics to reveal the mechanisms of succession direction of grazing grasslands.

To perform clear and reasonable grazing management of grasslands, increasing studies were performed in recent years on the impact of grazing on the soil microbial community, including soil microbial number [9], soil microbial quantity [10], soil microbial enzyme activity [11], soil microbial community structure [12] and soil microbial diversity [13]. These studies showed that light grazing significantly increased the soil microbial population and microbial biomass [14], but overgrazing reduced the soil microbial biomass carbon [15]. Moderate grazing changed the living environment of soil microorganisms, which was conducive to their growth [16], improved microbial diversity, changed the microbial community structure, improved soil quality and maximized grassland productivity [17]. Zhalnina et al. [18] found that soil pH determined microbial diversity and composition, and bacterial diversity was higher in neutral soil and weakly acidic soil. Sun Yifei et al. [19] showed that changes in usable nitrogen content caused by grazing led to significant changes in ammonia-oxidizing microbial community structure. Xun et al. [20] showed that grazing changed the microbial composition of meadow grasslands from slow-growing and fungi to fast-growing and bacteria-dominated communities. For the Qinghai-Tibet Plateau, the research results showed that soil bacterial diversity was the highest in moderate grazing areas, and the migration of soil bacterial diversity grazing changed [21–23]. Grazing may affect the distribution of alpine grassland microorganisms on a small spatial scale and changed the impact of water on the microbial community [24]. These results also reflect the inconsistent response mode of soil microorganisms to grazing due to different geographical locations and seasonal climate [25–26], the complex relationship between grazing and soil microorganisms, and different changing trends under the influence of environmental factors.

As an important part of the Eurasian steppe, Hulunbuir steppe is an important material basis for the development of animal husbandry and the basic means of production for the survival of farmers and herdsmen. Therefore, to study the relationship between grazing and microorganisms in this region and establish a reasonable grazing system is vital importance. In this study, high-throughput sequencing was used to sequence some bacterial 16S R RNA and fungal 18S R RNA genes, to evaluate the structure of soil microbial community under six grazing intensities. The microbial biomass carbon and nitrogen and soil physical and chemical properties were measured under different grazing intensities. The objectives of this study were to investigate grazing-induced changes in the composition, diversity, microbial biomass carbon and nitrogen, and their interaction with the physical and chemical properties of vegetation and soil.

Materials And Methods

Study site and sampling

The research area is located in the Inner Mongolia Autonomous Region, China. The experiment was based on the National Field Scientific Observation and Research Station of Hulun Buir Grassland Ecosystem (N 49°19' 349 " ~ 49°20' 173", E 119°56' 521 " ~ 119°57' 854", altitude of 666 ~ 680 m), which is a temperate semi-arid continental climate. The average annual temperature is -5 ~ -2°C, with the highest and lowest temperatures of 21.1°C and -27.2°C, respectively. The average annual precipitation is 350 ~ 400 mm, and the highest precipitation periods are from July to September (Fig. 1). The soil types of the

study sites were or chestnut soil, which corresponds to Castanozems in the soil taxonomic system of FAO and the calcic-orthic aridosol in the US soil classification system. The study regionalization was divided into 6 grazing areas with different grazing intensities, 0.00, 0.23, 0.34, 0.46, 0.69, and 0.92 Au ha⁻¹, named G0.00, G0.23, G0.34, G0.46, G0.69 and G0.92, respectively. For grazing, 500-kg beef cattle was used as the standard cattle beef unit (Fig. 2). Each grazing intensity was divided into three repetitions, and each plot area was 5 ha⁻¹. Since 2009, grazing began on June 1st and stopped on October 1st each year for 120 days. Samples were collected in the 10th year of grazing treatment. Soil samples were collected in August 2018 to determine the microbial community structure and soil physical and chemical properties and investigate the characteristics of the plant community.

Five quadrats (1 × 1 m) in each test area were randomly selected to measure the height, coverage and density of plants in each quadrat, and the aboveground parts of plants in the quadrat were divided and cut using the method of cutting the whole ground. Plants were put in a sample bag, numbered and transported to the laboratory. The fresh weight was measured, and plants were dried in a paper bag under a constant temperature (85°C) for 12 h. The dry weight was measured, and the aboveground biomass of the community was calculated. The underground biomass of community was determined using the excavation method of 5 topsoil samples (0–10 cm depth) that were randomly selected from each quadrat and immediately mixed into a single soil sample. Rocks and animal and plant residues were removed from the soil for the determination of soil chemical properties, soil physical properties, bacteria and fungi, microbial carbon and nitrogen.

Determination of Physical and Chemical Properties of the Soil

A specific method was used to determine the soil chemical properties that was different from the instrument instructions. Soil temperatures (ST) of the 0–10 cm soil layers were measured by portable thermometers that can be inserted down to different soil depths when collecting soil samples that need to determine the soil microbial community, and three replicate soil samples of each test area (0–10 cm soil layers) were collected simultaneously. Soil moisture (SM) was measured by the oven-drying method, and the gravimetric soil water content was determined by drying the sampled soil at 105°C for 24 h. Soil pH was measured in a 1:2.5 soil/water mixture by a multi-parameter water quality analyser. Soil total nitrogen (TN) concentration was measured the semi-micro Kjeldahl determination. The dichromate oxidation and sodium hydroxide alkali-molybdenum-antimony colorimetric methods were used to determine the soil organic carbon (SOC) concentration, and soil total phosphorus (TP) concentration. A spectrophotometer was used to measure soil total potassium (TK) concentration, soil available phosphorus (SAP) concentration and soil available potassium (SAK) concentration. Soil ammonium nitrogen and nitrate nitrogen (NH₄⁺-N and NO₃⁻-N) were determined using a flow injection auto-analyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured using fumigation-volumetric analysis and the fumigation-ninhydrin colorimetric method, respectively.

DNA collection and high-throughput sequencing

Genomic DNA was isolated from 0.5 g of each pooled soil sample from each sample plot (n = 36) using the Power Soil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA) per the manufacturer's instructions. The extracts of three technical repeats were mixed into a single DNA sample. Extracted genomic DNA was detected using 1% agarose gel electrophoresis. PCR was carried out on a Gene Amp 9700 PCR system (Applied Biosystems, Foster City, CA, USA). Based previous reports, the primers (Biosciences, Union City, CA, USA) were washed with Tris-HCl and verified using 2% agarose gel electrophoresis. PCR products were quantified using the Quanti Fluor TMs 338F-806R [27] and 817F-1196R [28] for the 16S rRNA and 18SrRNA genes, respectively. Amplified products were detected using 2% agarose gel electrophoresis and recovered from the gel using the Axy Prep DNA gel extraction kit (Axygen -ST Fluorometer, Promega Biotech, Beijing, China), and the samples were adjusted as needed for sequencing. Sequencing was performed by Shanghai Majorbio Bio-pharm Technology (Shanghai, China) using an Illumina MiSeq platform (San Diego, CA, USA).

Statistical analysis

One-way ANOVA of soil physical and chemical properties was performed using SPSS 21.0. Significance was calculated using Tukey's test ($P < 0.05$). Origin 2017 was used for figures of MBC and MBN. The relationship between soil microbial community structure and each affecting factor was analysed using RDA and variation partitioning. RDA eliminates redundant variables depending on the other measured variables (variables with large effects are and automatically selected) and on the variance inflation factor values to gradually remove redundant parameters, and the significance levels are based on 999 Monte Carlo permutations. Linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe) analysis was performed to search for significantly different biomarkers between groups [29].

Results

Soil and vegetation characteristics change under different grazing intensities

As can be seen from Table 1, the changes of soil physical and chemical properties under different grazing intensities were as follows: SM was significantly reduced with the increase of grazing intensities ($P < 0.05$), and the content of non-grazing plot was the highest. NO₃-N was significantly higher than other grazing intensity in the G0.69 plot ($P < 0.05$), while other factors showed no significant difference under different grazing intensity. Vegetation characteristics showed significant differences under different grazing intensities ($P < 0.05$) (Table 1): BGB and Coverage of non-grazing and light grazing (0.23, 0.34) were significantly higher than middle and heavy grazing (0.46, 0.69). With the increase of grazing intensity, Litter decreased significantly, which was highest in the non-grazing treatment, with no litter at the moderate and severe levels (0.46, 0.69, 0.92).

Changes in the soil microbial biomass carbon and nitrogen under different grazing intensities

Our results showed that the variation trends of microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) downstream of grazing treatment were the same (Fig. 3). The content in the G0.34 plots was the highest in the 0–10 cm soil layer, and the content in the G0.92 test plots was smaller than other

grazing treatments. Except for the area with a grazing intensity of 0.34, the greater the grazing intensity, the lower the MBC and MBN content. The G0.00 soil in soil layers 10–20 cm and 20–30 cm was higher than the other grazing intensities. The microbial carbon and nitrogen contents decreased with increasing depth. These results are consistent with the parallel and vertical distribution of microorganisms.

Composition of microbial community under different grazing intensities

Soil microbial diversity changes under different grazing intensities

The soil microbial diversity indexes of the 6 grazing treatments are shown in Fig. 4. Coverage represents the proportion of the sequence obtained by sequencing of the whole genome. The Shannon index reflects the variation or difference degree of the microbial community, which was affected by the total number of samples and uniformity. Higher values indicate higher microbial community diversity in the soil[30]. The Chao index uses the chao1 algorithm to estimate the number of operational taxonomic units in samples. Chao1 is often used to estimate the total number of species in ecology. The coverage value of each sample was higher than 0.97, which indicates that the sequence obtained by sequencing had a high coverage degree and good representativeness. This result reflects that the clone library constructed in this study represented the diversity of soil bacteria in this area.

The α diversity indexes of G0.23 and G0.34 bacteria were higher, and the α diversity indexes of G0.00 and G0.92 grasses were lower. These results are consistent with the intermediate disturbance hypothesis. The Chao of the fungus in the heavy grazing area was lower than the other treatments, but the diversity and uniformity of the fungal community was higher than the light grazing area.

Microbial community structures in soils with different grazing intensities

The histograms of dominant gates of soil bacteria and fungi (Fig. 5A and B) were obtained according to the rule that the proportion of microbial abundance was greater than 1%. There were 10 species abundance ratios of bacteria greater than 0.01, and the 10 dominant bacteria (abundance values greater than 1%) detected in 6 different grazing treatments were *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Bacteroidetes*, *Gemmatimonadetes*, *Nitrospirae*, *Firmicutes*, and *Plancomycete*. Among these bacteria, *Chloroflexi* differed significantly between different grazing intensities ($P < 0.05$), with the lowest *Chloroflexi* in non-grazing and heavy grazing intensities, and the highest amounts in a medium grazing area (G0.46). There were 4–5 phyla with fungal abundance values greater than 1% in different grazing intensities: *Ascomycota*, *Zygomycota*, *Unclassified_k_Fu*, *Basidiomycota*, and *Chytridiomycota*. The abundance of *Chytridiomycota* was greater than 1% only in non-grazed (G0.00) and moderately grazed (G0.46) grasslands. Bacteria had different abundances across the 6 grazing intensities. *Actinobacteria* and *Proteobacteria* were the absolute dominant bacterial species. *Ascomycota* was the fungus phylum with dominant abundance values in the 6 treatments, and G0.46 was higher than the other treatments.

Microbial communities with statistically significant differences

Microbial communities with statistically significant differences were determined using α - and β -diversities. Another primary goal of comparing microbial communities was to identify specialized communities within the samples using the LEfSe tool. This tool allows analyses of microbial community data at any clade. Because the analysis of the large number of OTUs detected in the present study would be computationally too complex, statistical analysis was performed only from the domain to the genus level. Groups are shown in cladograms, and LDA scores of 2 or greater were confirmed using LEfSe (Fig. 6,7).

Most bacterial species were enriched in G0.00 and G0.69 and less abundant in the other grazing intensities (Fig. 6). Fifteen bacteria are enriched significantly in G0.00, including p_Bacteroidetes, o_Sphingobacteriales and c_Sphingobacteriia, which were the most important (LDA > 3.5). Four species of bacteria were significantly enriched in G0.23, and f_Burkholderiaceae was the most important (LDA = 3.5). There were 4 bacteria with significant enrichment in G0.34, of which o_norank_c_Gemmatimonadetes, g_norank_c_Gemmatimonadetes and f_norank_c_Gemmatimonadetes were the most important (LDA = 2.5). Three species of bacteria were significantly enriched in G0.46, and p_Chloroflexi was the most important (LDA > 4.0). Thirteen species of bacteria were significantly enriched in G0.69, and f_Roseiflexaceae, c_Chloroflexia, o_Chloroflexales and g_Roseiflexus were the most important (LDA > 3.0). Only g_Hymenobacter microbes were enriched significantly in G0.92 (LDA > 2.5). Most fungal species were enriched in G0.00 and less enriched in the other grazing intensities (Fig. 7). Ten fungi were significantly enriched in G0.00 (Etes, o_unclassified_c_Lecanoromycetes, f_unclassified_c_Lecanoromycetes, g_unclassified_c_Lecanoromycetes, g_unclassified_c_Orbiliomycetes, g_unclassified_c_Orbiliomycetes, and g_unclassified_cetes), and F_unclassified_CetesOrbiliomycetes was more important (LDA > 3.0). Only c_Wallemiomycetes was enriched significantly in G0.23 (LDA > 3.0). Three species of fungi were significantly enriched in G0.34, and g_Stilbella, f_Porotheleaceae and g_Porotheleum were more important (2.5 < LDA < 3). Two fungi were enriched significantly in G0.46, and g_Penicillium was the most important (LDA > 3.5). Two fungi were significantly enriched in G0.69, and g_unclassified_f_norank_o_Pleosporales was the most important (LDA > 3.5). Two fungi were significantly enriched in G0.69, and g_Glitopilus was the most important (LDA > 4.0).

Relationship between microbial community structure and environmental characteristics

Correlation between environmental factors and the microbial community

Spearman correlation analysis was used to study the mutual changes between environmental factors and species and obtain the correlation and significance values between pair-wise comparisons. The bacteria and environmental parameters were significantly correlated (Fig. 8A). Norank_c_KD4-96 significantly positively correlated with pH. Norank_cacidobacteria significantly positively correlated with SAP. Rubrobacter significantly negatively correlated with TP, and norank_o_Gaiellales significantly negatively correlated with BGB. Spearman correlation analysis showed the following significant correlations (Fig. 8B). *Fusarium*, *Mortierella* and *SBD* were significantly positively correlated ($P < 0.05$). *Gibberella* significantly correlated with SM ($P < 0.05$), *SBD* ($P < 0.05$), TK ($P < 0.01$), NH4+ – N ($P < 0.05$), Mull ($P < 0.001$), and coverage ($P < 0.01$). These results showed a significant correlation between *Penicillium* and SM ($P < 0.05$), *SBD*

($P < 0.01$) and TK ($P < 0.05$). Unclassified_Fasiosphaeriaceae significantly correlated with SM ($P < 0.001$), Mull ($P < 0.01$) and coverage ($P < 0.05$). There was a significant correlation between Unclassified_f_Nectriaceae and SBD ($P < 0.05$), TK ($P < 0.05$), $\text{NH}_4^+\text{-N}$ ($P < 0.05$) and coverage ($P < 0.05$).

Environmental factors and the RDA of the microbial community

Grazing changes microbial community structures and environmental characteristics. The disrupting effect of grazing on microbial communities may primarily be mediated by aboveground plant and soil geochemical characteristics. Therefore, the present study investigated whether microbial community structure and environmental characteristics were related. RDA revealed that the microbial community structure was formed by primary environmental characteristics, including soil moisture (SM), soil bulk density (SBD), pH, soil organic carbon (SOC), total nitrogen (TN), TP, TK, SAP, SAK, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, vegetation belowground biomass (BGB), litter, and coverage. After removal of the redundant variables, 14 environmental characteristics were chosen for RDA. As shown in Fig. 7A and B, BGB ($p = 0.001$) significantly affected the bacterial community structure, and pH ($p = 0.032$) TN ($p = 0.011$) and litter ($p = 0.007$) significantly affected the fungal community structure. The first two axes of the RDA of the bacterial community (Fig. 9A) accounted for 71.56% of the total variation in bacterial community composition, and the first axis accounted for 60.33% of the variation. The G0.23 samples distributed together, but the sample distribution patterns of other grazing intensities were discrete. The first two axes of the RDA of the fungal community (Fig. 9B) accounted for 25.96% of the total variation in fungal community composition, and the first axis accounted for 22.73% of the variation. The G0.00 and G0.92 samples distributed together, but the sample distribution patterns of the other grazing intensities were discrete.

Variance partition analysis was performed to dissect the contributions of soil and plant characteristics to the microbial community structure. These selected characteristics together explained 46.98 and 61.77% of bacterial and fungal community changes, respectively (Fig. 9C,D). The contribution of soil and plant characteristics explained 45.35 and 0.75%, respectively, of bacterial community changes and 55.26 and 4.69%, respectively, of the fungal community changes. The combined contribution of soil and plant characteristics explained 0.88 and 1.82% of the bacterial and fungal community changes, respectively, which reveals a very close interaction between soil and plant characteristics.

Discussion

Vegetation, soil and microorganisms do not change in isolation, but form a feedback mechanism and interact with each other. Grazing livestock changes the growth of community plants, and soil trampling and excretion affect the nutrient cycle of the soil. Changes in plant growth patterns and the soil environment indirectly affect the microbial environment in the soil, which leads to changes in the microbial community. Soil provides a living environment for microbial communities, and soil characteristics affect microbial communities[31].

Soil water has a significant inhibitory effect on bacteria, and limits the nutrient uptake and production of bacteria, which leads to metabolism restriction due to nutrient deficiency[32]. Soil pH does not directly impact the microbial community, but it indirectly affects the aboveground vegetation, changes the community characteristics of the aboveground vegetation, and reduces soil microbial energy and organic matter content, which affects the microbial community[33].

Effects of grazing intensity on soil microbial community structure

The present study examined 18 sample plots of grassland with six grazing intensities in the Hulun Buir grassland to analyse the relationships between soil bacterial and fungal communities and grazing intensity, and the interaction between plant communities and soil physical and chemical properties resulting from grazing intensity and bacterial and fungal communities. The predominant flora in the bacterial communities of Hulun Buir grassland soil was generally consistent between the six grazing regimens, but there were differences in relative abundance, and each region had its own unique fungal populations. Bacterial and fungal diversity was highest in G0.23 and G0.00, respectively. Similarly, Lienhard et al. (2013) [34] found that maximum bacterial and fungal diversity occurred with different utilization schemes. Microbial biomass or diversity increases, decreases, or remains the same depending on the type of grassland, geographical location, and the grazing system and intensity [9–10, 13, 35–39].

Although the diversity of bacterial and fungal communities respond to grazing intensity in different ways, our study showed that the soil microbial community structure changed significantly along a gradient of grazing intensity, which was consistent with changes in soil and plant characteristics.

Relationship between microbial community and environment

We found that the environmental changes that occurred with changes in grazing intensity contributed differently to different microbial groups in the community (Fig. 9A,B). The results showed a significant correlation between changes in the bacterial community and the underground biomass of the community ($P < 0.05$), but this correlation was not found in the fungal community. There was a significant relationship between litter and the fungal community ($P < 0.05$), but no significant relationship with bacteria was observed. Zhou et al. [13] found that livestock feeding affected aboveground vegetation biomass and community structure, and indirectly changed soil physical and chemical properties. These changes were a result of the interaction of microorganisms with plants [40]. The fungal community structure in our study area was not as sensitive as the bacterial community structure to vegetation biomass changes. This difference may be because fungi are more likely to degrade lignocellulose from different plants than bacteria, which allows them to first obtain resources from many of the relevant available substances [41].

We also found significant direct relationships of bacterial and fungal community structures with soil total nitrogen. Soil nitrogen storage decreases with increasing grazing intensity[42–43]. Nitrogen is one of the most important nutrients for life. Therefore, plant and microbial activities may gradually reduce the content of nitrogen in soil [7]. However, correlations between microbial communities and environmental factors must be carefully explained because it is often very difficult to firmly establish the relationship between microbial communities and soil nutrient cycling [44].

We found that the soil characteristic factors evaluated explained over 45% of the shift in the microbial communities in this study, which suggests that soil characteristics factors are the primary factors influencing microbial community structure. However, 40–55% of the factors influencing the dynamic changes in the microbial community could not be determined. Multiple studies showed that the effect of mild or moderate grazing on grassland soil was relatively small, and these grazing intensities are beneficial to dry matter production, nutrient cycling, and carbon and nitrogen storage[45–46].

Grazing in G0.23 had relatively little effect on grassland soil and plant characteristics in the present study. For the bacterial communities, 0.23 Cow.Au ha⁻¹ may be considered a moderate stocking rate in the study region. According to the intermediate disturbance hypothesis[47], 0.23 Cow.Au ha⁻¹ may be close to the moderate-disturbance stocking rate in the Hulunbuir meadow grassland.

Conclusion

The present study examined the soil microbial structure and its relationship with the environment in 6 *leymus chinensis* meadow steppes with different grazing intensities. High-throughput sequencing results revealed that bacterial and fungal communities were extremely sensitive to grazing and varied with grazing intensity. The diversity of soil bacteria in the light grazing test area was higher than the heavy grazing area, and the diversity of fungi in the non-grazing area was higher than the specific grazing area. Heavy grazing-induced reduction the diversity of soil bacteria and fungi. The underground biomass of the community significantly influenced the bacterial community structure, and pH, total nitrogen and litter significantly influenced the fungal community. In conclusion, heavy grazing reduces the diversity of the community and is not conducive to the development of Hulunbuir meadow grassland.

Abbreviations

SM, soil moisture;

SBD, soil bulk density;

SOC, soil organic carbon;

TN, total nitrogen;

TP, total phosphorus;

TK, total potassium;

SAP, soil available phosphorus;

SAK, soil available potassium;

NH₄⁺-N, Soil ammonium nitrogen;

NO₃⁻-N, Soil nitrate nitrogen;

BGB, Belowground biomass.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publicaation

Not applicable

Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. The sequencing data in this article is available at the NCBI Sequence Read Archive under the accession numbers SUB8689126.

Competinginterests

The authors declare that they have no competing interests.

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Authors' Contributions

YZ participated in the design of the study ideas, carried out the diversity of soil microbial communities, performed the statistical analysis and drafted the manuscript. XPX participated in the analyzed the data. RQL participated in the design of the study ideas. WBX participated in the design of the study ideas. RFZ participated in the analyzed the data. LHL participated in the analyzed the data. XW participated in the analyzed the data. JQC participated in the analyzed the data. RY conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

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Tables

Table 1

Changes of Plant characteristics and soil physical and chemical properties under different grazing intensities in the growing seasons of 2018 ($M \pm s.e.$). Abbreviations: SM, soil moisture; SBD, soil bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; SAP, soil available phosphorus; SAK, soil available potassium; NH_4^+ -N, Soil ammonium nitrogen; NO_3^- -N, Soil nitrate nitrogen; BGB, Belowground biomass.

	factors	G0.00	G0.23	G0.34	G0.46	G0.69	G0.92
soil characteristics	SM(%)	20.81±1.00a	20.34±0.57ab	19.53±0.65ab	18.47±0.90bc	18.01±0.19c	17.94±0.59c
	SBD(g/cm ³)	0.96±0.02a	0.99±0.03a	1.01±0.01a	1.02±0.04a	1.03±0.01a	1.05±0.04a
	pH	6.94±0.45a	6.59±0.16a	6.59±0.12a	6.89±0.10a	6.47±0.09a	6.59±0.05a
	SOC(g/kg)	39.23±0.07a	41.26±1.14a	42.72±2.52a	44.07±3.15a	43.78±4.63a	38.32±1.80a
	TN(g/kg)	3.37±0.11ab	3.39±0.08ab	3.34±0.09ab	3.41±0.03a	3.18±0.04ab	3.13±0.09b
	TP(g/kg)	0.43±0.03a	0.38±0.01a	0.44±0.00a	0.41±0.04a	0.48±0.05a	0.46±0.08a
	TK(g/kg)	15.85±0.45b	17.28±0.44ab	17.62±0.28a	18.84±0.59a	18.93±0.89a	18.69±0.42a
	SAP(mg/kg)	3.73±0.17a	3.80±0.17a	3.74±0.55a	3.70±0.73a	3.46±0.28a	3.24±0.12a
	SAK(mg/kg)	309.80±77.40a	314.44±12.46a	279.32±35.12a	354.71±73.45a	307.49±56.78a	330.37±25.75a
	NH ₄ ⁺ -N mg/kg)	5.72±0.95a	5.38±0.66a	6.22±1.17a	5.85±1.48a	4.66±1.08a	3.22±0.00a
	NO ₃ ⁻ -N mg/kg)	20.09±3.21b	16.08±1.20b	18.91±2.28b	17.94±2.39b	34.97±8.63a	21.31±1.47b
Vegetation characteristics	BGB(g/m ²)	1,162.38±145.85a	972.48±69.38ab	1,011.36±112.08ab	605.31±59.90c	790.75±70.41bc	849.41±96.75abc
	Litter(g/m ²)	206.28±27.41a	135.48±15.77b	105.36±31.84b	67.02±27.94bc	0.00±0.00c	0.00±0.00c
	Coverage(%)	84.65±0.63a	80.25±1.16ab	80.12±1.80ab	78.01±1.34b	78.23±10.49b	68.84±2.87c

Figures

Figure 1

Monthly precipitation and temperature in 2018 for the experiment plot.

Figure 2

Geographic location of the study sites and design diagram of different cattle grazing intensities. The values 0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU ha⁻¹ correspond to different livestock rates (1 AU = 500 kg of adult cattle). The stocking rates were achieved using 0, 2, 3, 4, 6 and 8 young cattle (250-300 kg) per plot. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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Figure 4

Microbial carbon (MBC) and nitrogen (MBN) contents in August under different grazing intensities. Lower case letters represent significant differences between different grazing intensities ($P < 0.05$).

Figure 5

Estimated values of bacterial and fungal community α diversity indexes. Chao reflects the richness of the community. Shannon reflects the diversity of the community and is used to estimate one of the indexes of microbial diversity in the sample. Shannoneven reflects the homogeneity of the community. Significant differences are indicated by different letters.

Figure 6

According to the rule that the proportion of microbial abundance was greater than 1%, the dominant phylum histograms of soil bacteria (A) and fungi (B) were drawn. The abscissa is the group name, and the ordinate is the proportion of the species in the sample. The columns of different colours represent different species, and the length of the column represents the proportion of the species. Bacterial (C) and fungal (D) community heatmaps. The abscissa/ordinate is the sample name. In figure A and B, the vertical/horizontal coordinates are the proportion of species in this sample. Columns of different colours represent different species, and the length of columns represents the proportion of this species. In C and D, the ordinate is the species name, and the variation of abundance of different species in the sample is shown by colour gradient of the colour block. The value represented by the colour gradient is on the right of the figure. Colour gradients are used to represent the size of the data in a two-dimensional matrix or table and to present information about community species composition and species abundance. Clustering was carried out according to the similarity of the abundance between species or samples, and the results are presented in the community heatmap, which groups the species with high and low abundance clusters in blocks, and reflects the similarity and difference of community composition of different groups (or samples) at each taxonomic level through colour change and similarity degree.

Figure 7

Cladogram showing the phylogenetic distribution of the bacterial lineages associated with soils with different grazing intensities. Indicator bacteria with LDA scores of 2 or greater in bacterial communities associated with soils with different grazing intensities. Different-coloured regions represent different constituents. Circles indicate phylogenetic levels from domain to genus. The diameter of each circle is proportional to the abundance of the group.

Figure 8

Cladogram showing the phylogenetic distribution of the fungal lineages associated with soils with different grazing intensities. Indicator fungus with LDA scores of 2 or greater in fungal communities associated with soil with different grazing intensities. Different-coloured regions represent different constituents. Circles indicate phylogenetic levels from domain to genus. The diameter of each circle is proportional to the abundance of the group.

Bacteria Spearman Correlation Heatmap

Fungus Spearman Correlation Heatmap

Figure 9

Heatmap analyses of the correlations between bacterial and fungal species abundance and environmental factors. By calculating the Spearman correlation coefficient between the environmental factors of the top 10 species and the total abundance, the obtained numerical matrix is visually displayed in the heatmap. The X axis and the Y axis are the environmental factors and species, respectively. Colour changes reflect the data information in the two-dimensional matrix or table. The colour depth represents the size of the data value, which is directly represented by the defined colour depth. The legend on the right is the colour interval of different R values. * 0.01 < P ≤ 0.05, ** 0.001 < P ≤ 0.01, *** P ≤ 0.001.

Figure 10

Redundancy analysis (RDA) of MiSeq data (symbols) and environmental characteristics (arrows). Bacterial and fungal communities are shown in A and B, respectively. The values of axes 1 and 2 are the percentages explained by the corresponding axis. Dots of different colours or shapes in the figure represent groups of samples under different environments or conditions. The red arrows represent quantitative environmental factors, and the length of the arrows of environmental factors represent the impact of environmental factors on species data (interpretation). The angle between the arrows of environmental factors represents positive and negative correlations (acute angle: positive correlation; Obtuse angle: negative correlation; Right angle: no correlation). The distance from the projection point to the origin represents the relative influence of the environmental factors on the distribution of the sample community. Whether the point and the arrow are consistent represents the positive and negative correlation. Analysis of the level of contribution of soil and plant characteristics to changes in bacterial (C) and fungal (D) communities.