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Effects of Seasonal Grazing on Microbial Community Structure in Rhizosphere Soil of Stellera chamaejasme in Gannan Alpine Meadow

Ke Xi[1, a], Minxia Liu[1, b, *], Mingxing Wang[1, c], Cheng Liu[1, d], Fengling Yin[1, e]

[1]College of Geography and Environmental Science, Northwest Normal University, Lanzhou 730070, China

[a]1290175053@qq.com, [b]xiaminl@163.com, [c]2206196519@qq.com, [d]1097699341@qq.com, [e]2529861461@qq.com

Abstract

Stellera chamaejasme has become a constructive species in degraded grassland. In this paper, the phospholipid fatty acid method (PLFA) was used to determine the PLFA value of the microbial community in the rhizosphere soil of Stellera chamaejasme under seasonal grazing, and the relationship between the soil microorganisms and the physicochemical properties of it was analyzed using redundancy analysis (RDA) and variance partitioning analysis (VPA). Then the results showed that soil water content, soil organic carbon, and available nitrogen would be the main environmental factors affecting soil microorganisms in Stellera chamaejasme. According to VPA analysis, among the three environmental factors, VPA analysis indicated that the effect of available nitrogen on microbial changes in the rhizosphere soil of Stellera chamaejasme was the largest, with 37.31%. And the second is soil water content, while the third is soil organic carbon. Under the influence of multiple factors, it is mainly the combined effect of available nitrogen and soil water content on the microbial changes in the rhizosphere soil of Stellera chamaejasme.

Keywords

Stellera chamaejasme; Soil microbial community structure; Phospholipid fatty acids (PLFA); Grazing method.

1. Introduction

Grassland is the largest terrestrial ecosystem in China, with rich biodiversity and important ecological functions. However, it is also one of the terrestrial ecosystems most affected by human activities. And grazing is the most important use of grassland ecosystems. Also, grass-eating livestock would affect the physical structure of grassland soil and the circulation of grassland nutrients through their grazing behaviors, including trampling, grazing, and excretion, thereby causing changes in the chemical composition of grassland soil [2]. While unreasonable grazing intensity and grassland management system could not only affect the community structure and function of grasslands, the physical and chemical properties of soil, but also lead to a decline in grassland productivity. What's more, overgrazing is one of the most important factors leading to grassland degradation. Therefore, the degradation of grassland soil caused by grazing and its impact on grassland ecosystems would have received increasing attention and become the important content of relevant research [3].

In fact, soil is not only the most important substrate for biomass production in grassland ecosystems, but also the site for the decomposition and material cycling of animals and plants. And it is composed of minerals, organic matter, water, air, etc., which would constitute a culture medium for microorganisms. Generally, there are hundreds of millions of microbial cells in one

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gram of soil [4]. Then the root system is the main organ for plants to absorb nutrients, and the rhizosphere is the most active area for the plant and microbial interactions, which can multiply the efficiency of plant activation and utilization of soil nutrients [5]. In addition, soil microorganisms are closely related to plant nutrition and soil fertility, and are important indicators for evaluating ecological restoration and changes in physical and chemical factors in soil ecosystems [6]. Besides, research on soil microorganisms has attracted the most attention to their diversity, especially in terms of the relationship between microbial community structure and distribution and soil environment [7],. Meng Lingjun [8] and other researchers found that neutral phosphatase was the preferred soil enzyme index in the rhizosphere and nonrhizosphere soils of six Chinese herbal medicines in the Taibai Mountains of the Qinling Mountains. While Luo Dan [9] and other researchers found that soil bacterial abundance was mainly affected by total nitrogen, available nitrogen, total phosphorus, and available potassium. Then Shao Wei [10] and other researchers found that the metabolism of rhizosphere microbial communities was positively correlated with bacterial communities, and negatively correlated with fungal communities.

Stellera chamaejasme is a perennial herb belonging to the genus Stellera of the Daphne family. And it grows at an altitude of 2300-4200m and is mainly distributed on dry sunny hillsides with an annual average temperature of around 0 ℃, as well as on alpine and subalpine grasslands. On the degraded grassland of type, the excellent forage population gradually declines after being ingested in large quantities. Also, it begins to breed and spread with strong adaptability and competitiveness, gradually developing into a dominant species. Focusing on the impact of Stellera chamaejasme on natural grasslands, existing studies have mainly focused on the biological characteristics, toxicity, allelopathy, chemical control, and comprehensive utilization. Jin Hui [11] and others have found that allelochemicals from Stellera chamaejasme can affect the composition and diversity of bacterial communities in rhizosphere soil. While Ma Jianguo [12] and others have studied the significant impact of Stellera chamaejasme on grassland productivity. And Huang Ruike [13] and others studied that the growth of it has a significant impact on the composition of its rhizosphere fungal community. Then Chen Xiaopeng [14] and other researchers have found that chemical eradication is an effective means of controlling the degraded grassland of its type. Last but not least, An Dongyun [15] and other researchers have found that it can improve soil physical and chemical properties and improve soil biological activity to a certain extent. However, there is still a lack of sufficient understanding and systematic research on the relationship between grazing methods and rhizosphere microorganisms of Stellera chamaejasme. Based on this, this paper conducted a systematic study on the microbial community of Stellera chamaejasme rhizosphere distributed on the grassland under seasonal grazing in Gannan alpine meadow, and combined with soil physical and chemical properties and enzyme activity, analyzed the correlation between microorganisms and ecosystem elements. Using redundancy analysis and variance decomposition analysis, the paper attempted to explore the distribution of microbial community in soil of Stellera chamaejasme rhizosphere and the changes in the physical and chemical properties of it under seasonal grazing, By analyzing the internal relationship between changes in soil physical and chemical properties and the distribution of soil microbial communities, it is hoped that through studying the above issues, the impact of microorganisms and the environment will be explored, and it is hoped to provide a research basis for exploring the root ecosystem of the toxic plant Stellera chamaejasme and the rational management methods of pasture.

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2. Research Area and Research Method

2.1. Overview of the research area

The study area is located in Maqu County, the junction of Gansu, Qinghai, and Sichuan provinces, in the southwest of Gannan Tibetan Autonomous Prefecture, with an average altitude of 3600 m. The climate is a plateau continental high cold and humid climate zone with an average annual temperature of 1.2 ℃, average sunshine of 2531.9 hours, annual precipitation of 151 days, and annual precipitation of 611.9 mm. The climate is cold and humid, and there is no absolute frost-free period. The soil type is alpine meadow soil.

2.2. Experimental Design and Sample Collection

In July 2021, a mountainous area with the same direction (south slope) was selected in Maqu County (slope orientation was determined by using a 360° electronic compass) at an altitude gradient of 3000 m. And five samples of 0.5 m × 0.5 m were randomly selected from the enclosed grazing area (enclosed for 5 years, NG), summer grazing (May to September is the grazing period, and other times are the rest grazing period, SG), and winter grazing (October April is the grazing period, and other times are the rest grazing period, WG). Then 5 plants of Stellera chamaejasme with relatively consistent growth are selected in each sample, and the shaking method was used to collect the rhizosphere soil of Stellera chamaejasme plants [16]. Then the soil samples are sealed in a sterile sealed bag prepared in advance and quickly returned to the laboratory in an ice box. Some of the soil samples are to be naturally dried, ground, and screened for the determination of soil physical and chemical properties, while the other part is to be placed in a refrigerator at - 70 ℃ for the determination of soil microorganisms.

2.3. Determination of soil microbial community

The soil microbial community was determined to use the phospholipid fatty acid method. We would weigh 5 g of fresh soil sample and fully mix it with methanol solution, also place it in a constant temperature (37 ℃) shaking bed for 1 hour, and then vortex oscillate. Next, we would add acetic acid solution to adjust pH, extract and centrifuge, extract the supernatant, combine the hexane solution containing fatty acids dissolved twice, and blow dry with nitrogen. Later, we could add a 1:1 n-hexane methyl butyl ether solution, allow it to fully dissolve after standing, filter it into a GC vial, add an internal standard (i19:0), and use gas chromatography mass spectrometry (GC-MS) for PLFA detection.

According to the PLFA characteristic maps of different soil microbial groups, the PLFA labeling method was used to classify their groups into five categories: gram-positive bacteria (G+), gram-negative bacteria (G −), bacteria, fungi, and actinomycetes. Then the peak surface method and GC-MS method were used to calculate phospholipid fatty acids, and the content was expressed in nmol/g.

2.4. Determination of soil physical and chemical properties

The physical and chemical properties of soil were measured using the following methods. And soil temperature was measured using a multi-function thermometer (DT-131), while bulk density was measured using the cutting ring method. Then soil water content was measured using the drying method (repeated for three times), while pH value was extracted with water using the potential method, also organic carbon was digested with sulfuric acid - potassium dichromate external heating method. Besides, total nitrogen was determined using a semi micro Kjeldahl method, while total phosphorus was digested with sulfuric acid - perchloric acid, and available nitrogen was determined using the alkali hydrolysis diffusion method. Finally, the activity of soil urease (URE) was measured using phenol sodium hypochlorite colorimetry [17], and the activity of catalase (CAT) was measured using potassium permanganate titration [18].

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2.5. Data Analysis

We would calculate the Shannon Wiener diversity index, Simpson diversity index, and Pielou evenness index based on the experimentally measured PLFA data, using the following formula.

$$H = -\sum (P_i)(\ln P_i)$$

$$D = 1 - \sum (N_i/N)^2$$

$$J = H/\ln S$$

$$P_i = N_i/N$$

In the formula, H is the Shannon Weiner diversity index. D is the Simpson diversity index. And J is the Pielou uniformity index, while S is the total species number of phospholipid fatty acids measured in the community. Then Ni is the content of the ith phospholipid fatty acid, and N is the total amount of phospholipid fatty acid markers.

2.6. Data processing

Excel 2010 was used to do statistics and calculations on the experimental data, and Origin 9.0 was used for plotting. SPSS 18.0 was used for correlation analysis and one-way analysis of variance, CANOCO 4.5 software was used for redundancy analysis (RDA), and the varpart function in the R software (Version 2.15.3) vegan package was used for variance partitioning analysis (VPA) analysis.

3. Results and Analysis

3.1. Effects of seasonal grazing on the physicochemical properties of soil from Stellera chamaejasme

According to Table 1, there are significant differences in the physical and chemical properties of soil from Stellera chamaejasme under the three grazing methods. The pH value of the soil in the three sample plots was significantly different (P<0.05). The pH of the NG sample plot was neutral, and the soil water content and soil organic carbon content were both NG>SG>WG. The soil bulk density of grazing sample plots was higher than that of enclosed plots. The variation trend of soil total nitrogen, available nitrogen, urease and catalase was WG>SG>EN. The soil total phosphorus content of sample plot WG was the highest, 4.19 g/kg, and there was a significant difference in total phosphorus content between sample plot WG and sample plots SG and NG (P<0.05).

Table 1. Changes of environmental factors under different grazing methods

Sample Site	Soil water content	Soil bulk density	Soil organic carbon	Soil total nitrogen	Available nitrogen	Soil total phosphorus	PH	Urease	Catalase
NG	14.78±0.12a	0.96±0.13c	72.87±1.43a	4.69±0.21c	18.26±0.91c	4.10±0.17b	7.12±0.21c	0.37±0.06c	0.53±0.11c
SG	8.98±0.14b	0.99±0.08a	60.49±1.82c	5.03±0.58b	21.73±1.02a	4.09±0.25b	6.54±0.37a	0.41±0.04b	0.72±0.08b
WG	8.41±0.07b	0.98±0.06b	70.38±0.94b	5.32±0.37a	19.28±0.78b	4.19±0.34a	6.21±0.03b	0.47±0.03a	0.80±0.05a

Note: NG grazing method is enclosure grazing, SG grazing method is summer operation grazing, and WG grazing method is winter operation grazing. Different letters in the same column indicate significant differences (P<0.05).

3.2. Changes in PLFA content of soil microorganisms from Stellera chamaejasme under seasonal grazing

From Table 2, it can be seen that due to different factors such as plant growth characteristics, climate, and the management and utilization of grasslands by herdsmen in the study area, the

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distribution pattern of microbial groups in the rhizosphere soil is as follows: SG has the largest content of total phospholipid fatty acids and bacteria, while the content of gram-positive bacteria, gram negative bacteria, fungi, and actinomycetes varies as follows: WG>SG>NG, with significant differences in phospholipid fatty acid content among various soils (P<0.05).

Table 2. Distribution characteristics of soil microorganisms in different grazing methods

Sample Site	Total PLFAs	Bacteria	gram-positive	Gram negative	Fungal	Actinomyces
NG	28.97±1.82c	14.03±1.23b	7.63±1.75c	5.34±0.95c	9.38±1.21c	5.56±0.85c
SG	36.91±0.96b	19.75±1.56b	8.78±0.69b	6.52±1.67b	10.68±1.76b	6.48±0.32b
WG	35.99±1.14a	17.56±2.14b	9.48±1.15a	7.72±1.09a	11.04±1.28a	7.39±1.22a

Note: NG grazing method is enclosure grazing, SG grazing method is summer operation grazing, and WG grazing method is winter operation grazing. Different letters in the same column indicate significant differences (P<0.05).

3.3. Effects of seasonal grazing on soil microbial community structure of Stellera chamaejasme

Bacteria/fungi and gram-positive bacteria G+/gram-negative bacteria G - are important indicators for evaluating the structure of microbial communities. There are differences between soil microbial bacteria/fungi (B/F) and gram-positive bacteria G+/gram negative bacteria G - (G+/G -) under different grazing patterns. From Table 3, it can be seen that the changing trend of B/F is: SG>WG>NG, and the changing trend of G+/G is: NG>SG>WG.

Table 3. Soil microbial community structure in different grazing methods

Sample Site	B/F	G+/G-
NG	1.4957±0.02c	1.49±0.02a
SG	1.7921±0.03a	1.35±0.03b
WG	1.5979±0.02b	1.28±0.03c

Note: NG grazing method is enclosure grazing, SG grazing method is summer operation grazing, and WG grazing method is winter operation grazing. Different letters in the same column indicate significant differences (P<0.05).

3.4. Effects of Seasonal Grazing on the Diversity of Soil Microbial Communities in Stellera chamaejasme

From Table 4, it can be seen that under different grazing methods, the variation trend of Shannon Wiener diversity index and Simpson diversity index is: NG>WG>SG, and the variation trend of Pielou evenness index is: NG>SG>WG. There are significant differences among the three sample plots in Shannon Wiener diversity index, Simpson diversity index, and Pielou evenness index (P<0.05).

Table 4. Soil microbial community diversity in different grazing methods

Sample Site	Shannon-Wiener diversity index	Simpson diversity index	Pielou evenness index
NG	2.8394±0.5716a	0.9041±0.0684a	0.9572±0.3572a
SG	1.9743±0.6703c	0.7841±0.0195c	0.8548±0.1478c
WG	2.3841±1.0241b	0.8346±0.372b	0.8738±0.0472b

Note: NG grazing method is enclosure grazing, SG grazing method is summer operation grazing, and WG grazing method is winter operation grazing. Different letters in the same column indicate significant differences (P<0.05).

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3.5. Correlation analysis of soil microbial communities of Stellera chamaejasme under seasonal grazing

RDA analysis shows that (Figures 1), the black arrow represents different microbial groups, and the red arrow represents various environmental factors; The angle between the lines represents the correlation between various environmental factors and soil microorganisms; The difference between them can be expressed by the length of the connection.

The analysis results are shown in Figure 1. The interpretation rates of the changes in bacteria, fungi, and actinomycetes in the rhizosphere soil microbial community of Stellera chamaejasme by the two sequencing axes reached 98.389%, with the first and second constraint axes accounting for 93.82% and 4.569% of the changes, respectively.

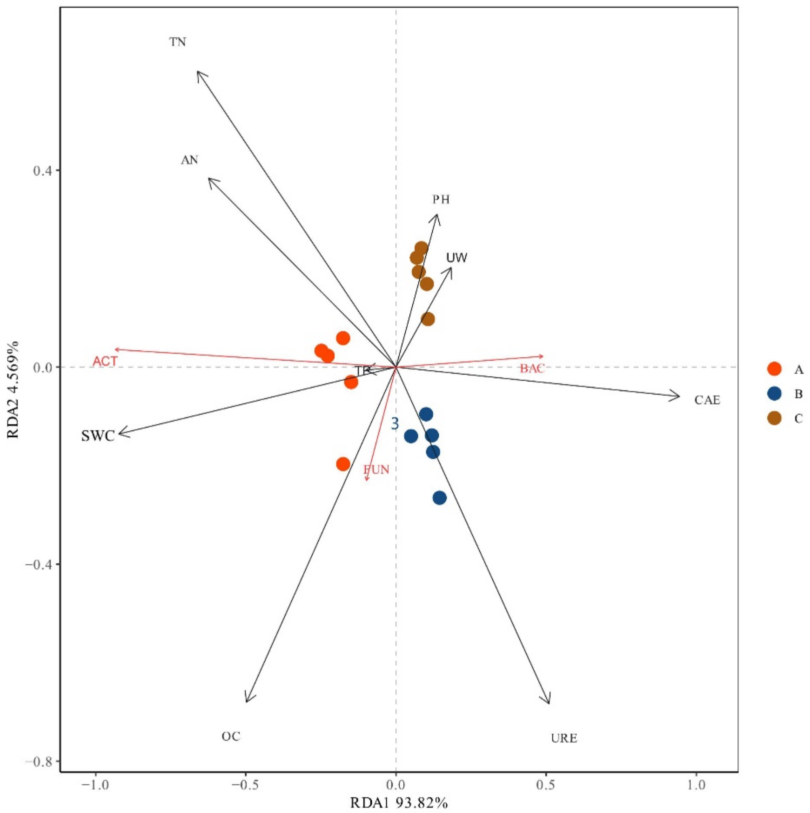

Figure 1. Redundancy Analysis of Soil Microbes and Environmental Factors

Note: BAC: Bacterium Bacteria; FUN: Fungus; ACF: Actinomycetes; SWC: Soil water content; UW: Soil bulk density; OC: Soil organic carbon; TN: Soil total nitrogen; AN: Available nitrogen; TP: Soil total phosphorus; URE: Urease; CAE: Catalase

3.6. Variance decomposition analysis of soil microorganisms under different grazing methods

VPA analysis can determine the interpretation ratio of specified environmental factors to changes in community structure, and can be used in conjunction with RDA to quantitatively analyze the interpretation degree of different environmental factors on the impact of microbial community species.

According to RDA analysis, three environmental factors, soil water content, soil organic carbon, and available nitrogen, were selected for the rhizosphere soil microorganisms of Stellera chamaejasme. The analysis results are shown in Figure 2. Among the three influencing factors, available nitrogen has the greatest ability to explain the microbial changes in the rhizosphere

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soil, with 37.31%. Secondly, the explanation of soil water content for the changes of soil microorganisms was 31.11%, and the explanation of soil organic carbon for the changes of soil microorganisms in the rhizosphere of Stellera chamaejasme was 14.60%. Under the influence of multiple factors, the explanation amount of available nitrogen and soil water content for the changes of soil microorganisms is 22.78%, the explanation amount of available nitrogen and soil organic carbon for the changes of soil microorganisms in the rhizosphere of Stellera chamaejasme is 11.68%, and the common explanation amount of available nitrogen, soil water content, and soil organic carbon is 6.80%.

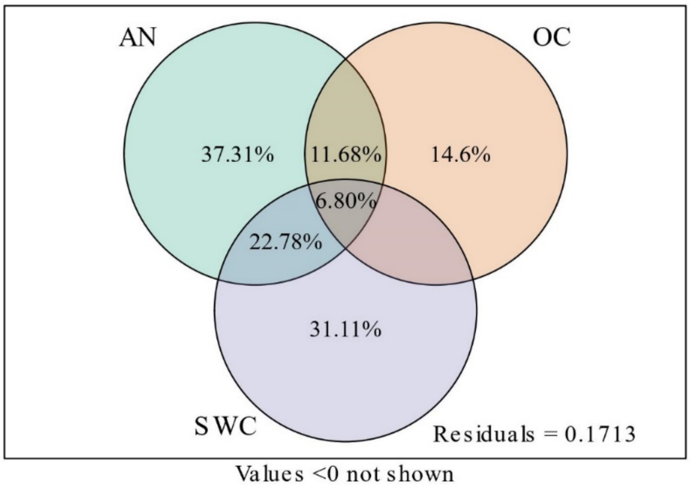

Figure 2. Variance decomposition analysis of soil microorganisms and environmental factors
Note: OC: Soil organic carbon; SWC: Soil water content; AN: Available 6nitrogen

4. Discussion

As an important factor affecting the geochemical cycle, soil microorganisms not only have important significance in changing soil physical and chemical properties and promoting plant growth and development, but also can participate in the nutrient cycle in the soil as a reservoir of nutrients. Therefore, they are important indicators of soil development and nutrient cycle.

4.1. Effects of grazing methods on soil physical and chemical properties

Due to the trampling behavior of livestock during grazing, grazing can improve the compactness of soil, increase soil bulk density, and at the same time reduce soil water content and reduce soil permeability [19], which is the same as the results of this experimental study. In this study (Table 1), under the three grazing methods of enclosure, summer grazing, and winter grazing, soil water content decreased in turn, and soil bulk density increased in turn. In this study, the pH value of the enclosed land is neutral, and the soil pH value in the study conducted by Yang Pengnian [20] will first decrease and then increase with the extension of the enclosure time. Within a certain enclosure time, the pH value is neutral; The pH value of operating grazing in summer is lower than that of operating grazing in winter, which may be due to the fact that the sampling time is during the summer grazing period and the winter grazing period. The grazing and trampling of livestock lead to a decrease in the coverage of surface plants and an increase in soil moisture evaporation. Therefore, under operating grazing in summer, the soil pH value decreases [21].

Grazing can also change the chemical elements in the soil through behaviors such as livestock excretion, thereby changing the chemical properties of the soil. The trampling of livestock can also change the transfer and fixation of chemical elements, thereby changing the spatial

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redistribution of chemical elements, ultimately affecting the chemical properties of natural grassland systems. Grazing leads to changes in soil organic carbon patterns, which decrease with the increase of soil depth, due to the influence of it and grazing conditions [22]. The content of soil organic carbon decreases with the increase in grazing intensity, and the content of surface soil's organic carbon changes significantly [23]. In this study, the highest organic carbon content in enclosed land may be due to the fact that under enclosed conditions, more above-ground vegetation increases the accumulation of organic carbon, while under summer grazing, the soil organic carbon content is the smallest. Some studies have also shown that grazing can promote the decomposition of soil organic carbon, as animal manure can bring a large number of microorganisms and nutrients into the soil, This increases the richness and diversity of microbial communities in the soil [24].

Nitrogen is a component of many important organic compounds in plants, which affects the metabolic process and growth and development of plants in many ways [25]. The content of nitrogen in the soil is directly or indirectly affected by a series of behaviors of grazing livestock such as feeding, trampling, and excretion. Wang Yuhui [26] pointed out that the content of nutrients such as nitrogen in soil gradually decreases with the increase in grazing intensity. And plants require a large amount of nitrogen during their growth process, and then the total nitrogen is converted into quick-acting components. In this study, it is possible that during the growing period of Stellera chamaejasme plants under summer grazing, the content of available nitrogen is higher, so the content of available nitrogen under this method is greater than that under the other two grazing methods. Phosphorus is one of the essential nutrients for plant growth, and soil is the main way for plants to absorb phosphorus. Phosphorus has a direct impact on processes such as photosynthesis, respiration, and biosynthesis in plants [27]. In this study, the effects of three grazing methods on soil total phosphorus were not significant. Rong Yuping [28] believes that grazing intensity does not have a significant impact on soil phosphorus content.

Soil enzyme activity is an important indicator of microbial community [29]. Soil urease plays an important role in the decomposition and transformation cycle of nitrogen compounds. In this study, it was found that grazing can appropriately increase the urease content, consistent with Zhu Lin et al. (Effects of different stocking rates on the activities of three soil enzymes in the desert steppe). It was found that the urease activity of Stipa breviflora desert steppe in Inner Mongolia was higher under grazing utilization than in the grazing area. Soil catalase provides oxygen for aerobic microorganisms, which is related to soil fertility and can reflect the accumulation of soil organic matter and the intensity of soil humification. It is one of the indicators for evaluating the quality of the soil. In this study, it was found that grazing can appropriately increase the catalase content, consistent with the results of Yan Ruirui et al. [30] (Effects of different stocking rates on the activities of three soil enzymes in the desert steppe)'s research on the Hulunbeir grassland. It was found that soil urease and catalase activities were higher under light grazing than in non-grazing and heavy grazing areas.

4.2. Impact of grazing methods on soil microbial communities

In degraded grassland ecosystems, the community structure of rhizosphere soil microorganisms is tolerant and sensitive to changes in external resources, and can accurately and quickly reflect changes in soil microdomains. It is one of the important indicators to measure soil health. In this study (Table 2), under the three grazing methods of enclosure, summer grazing, and winter grazing, the content of total phospholipid fatty acids, bacteria, and fungi decreased sequentially; The content of gram-positive bacteria, gram-negative bacteria, and actinomycetes decreased in turn. Moderate grazing can promote the growth of above-ground plants and further improve the efficiency of nutrient turnover, which is conducive to promoting the growth and reproduction of underground microorganisms, leading to an increase in the content of PLFA in microorganisms. Consistent with Wang Deping's research (the impact of grassland utilization methods on the microbial community structure of Hulunbuir grassland), soil microorganisms are crucial to maintaining the stability, health, and quality of the soil system, The structure and composition of soil microbial communities directly affect the functioning of the soil. Bacteria/fungi in soil can reflect the response of the structure and function of soil microbial communities to different soil conditions. The higher the ratio, the more stable the ecosystem is., In this study (Table 3), the bacteria/fungi in the enclosed land are the smallest, which can be inferred to be beneficial to maintaining the stability of the ecosystem. G-bacteria prefer to use fresh organic carbon, while G-bacteria prefer to use soil organic matter to derive carbon. The ratio can be used to indicate the availability of soil carbon or soil nutrient status [31]. In this study (Table 3)

In this study, G+/G − significantly decreased ($P<0.05$), indicating a shift in the soil microbial community towards decomposing fresh organic carbon during grazing. This may be due to the increase in soil organic carbon content through enclosure, which is conducive to the growth and reproduction of G-bacteria.

Under the three grazing methods in this study, the rhizosphere microorganisms of Stellera chamaejasme are mainly affected by soil water content, soil organic carbon, and available nitrogen (Figure 2). It may be because in the rhizosphere soil of Stellera chamaejasme, soil water content has a greater impact on the growth environment of microorganisms, and microorganisms require a large amount of organic carbon to provide energy due to their own survival needs, so they are more susceptible to the impact of soil organic carbon. During the grazing process, livestock returns most of the plant nutrients consumed to the soil through high-nitrogen excreta, increasing the soil nitrogen content [33]. Therefore, under the three grazing methods, the rhizosphere microorganisms of Stellera chamaejasme are also affected by available nitrogen.

5. Conclusion

(1) Under the three grazing modes of the enclosure, summer grazing and winter grazing, the content of total phospholipid fatty acids, bacteria, and fungi decreased sequentially; The content of gram-positive bacteria, gram-negative bacteria, and actinomycetes decreased in turn.

(2) The microbial community structure in the rhizosphere soil of Stellera chamaejasme is mainly influenced by three environmental factors: soil water content, soil organic carbon, and available nitrogen. Among the three influencing factors, the ability to interpret the microbial community structure is available nitrogen>soil water content>soil organic carbon. Under the influence of multiple factors, it is mainly the combined effects of available nitrogen and soil water content on the microbial changes in the rhizosphere soil of Stellera chamaejasme.

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