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Soil Bacterial and Fungal Composition and Diversity Responses to Seasonal Deer Grazing in a Subalpine Meadow

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Abstract: Soil microbial composition and diversity are widely recognized for their role in ecological functioning. This study examined the differences of soil microbial communities between two seasonally grazed grasslands. The study area was in the Gansu red deer farm located on the Qilian Mountain range in the Gansu province of northwestern China. This farm adopted a seasonal rotation grazing system whereby grasslands at higher altitudes are grazed in summer (SG), whilst grasslands at lower altitudes are grazed in winter (WG). The soil bacterial and fungal communities were examined by Illumina MiSeq sequencing. We found that soil water content (SWC), organic carbon (OC), total carbon (TC), and total nitrogen (TN) were significantly higher, whereas the C/N ratio was significantly lower in SG than WG pastures. The α -diversity of bacteria was greater than that of fungi in both pastures, while both bacterial and fungal α -diversity were not significantly different between the pastures. The bacterial β -diversity was significantly different between the pastures, but fungal β -diversity was not. The bacterial phylum Actinobacteria and fungal phylum Ascomycota were dominant in both pastures. The relative abundance of Actinobacteria in soil was significantly higher in WG pastures, whereas the relative abundance of Proteobacteria in soil was significantly higher in SG pastures. Significant correlations between bacterial and fungal phyla and soil properties were observed, but this varied between the two grasslands. This study showed that distinct microbial community structures developed in two pastures within the same geographic location that were grazed in different seasons.

Keywords: Gansu red deer; seasonal grazing; metabarcoding



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1. Introduction

Most grasslands in the world are grazed by livestock. It is widely accepted that animal grazing is beneficial to grassland nutrient cycling [1,2] and biodiversity [3,4]. Yet, many recent studies have warned that grassland degradation is induced by grazing animal activities such as defoliation, treading, and excretion [5–7]. Excessive foraging by grazing animals can reduce vegetation cover [8,9], creating bare patches within the pasture and increasing vulnerability to soil erosion. Additionally, livestock trampling on the soil surface can cause soil compaction and pugging, which can increase the bulk density of the soil, affecting soil aeration [10,11]. These changes in soil physical properties can promote surface runoff. To overcome these detrimental effects of continuous grazing, some herders employ a grazing system based on seasonal rotation. This practice is common in some alpine grasslands in the Qinghai Tibetan Plateau (QTP) in China, where a seasonal rotation between higher-altitude grasslands is utilized in summer, and lower-altitude grasslands is utilized in winter [12].

Animal grazing behaviors can have a significant impact on soil physical properties, nutrient cycling [13], plant community characteristics [14,15], litter distribution and decomposition [16,17], and the distribution of urine and faeces. These grazing-induced impacts

can lead to changes in the soil microbial community [18]. For example, several studies have found that grazing intensity has a strong relationship with soil microbial biomass and diversity [19,20]. Higher microbial diversity was associated with intermediate grazing intensity [21,22], while light and moderate grazing intensities responded differently to microbial α - and β -diversities [23]. Qu et al. [24] found that soil bacterial diversity indices varied under different plant species growing under different grazing intensities. Other studies have indicated that bacterial and fungal community diversity can be differently impacted under long-term grazing exclusion, where plant and bacterial succession was seen to evolve more rapidly and in parallel to each other compared to fungal succession [25]. Though there are numerous studies on the impact of grazing on soil microbial communities, information on how seasonal grazing impacts soil microbial communities is lacking despite being vital for policy designs promoting best grazing management strategies that help maintain healthy grassland systems. This paper aims to address this research gap.

The study area was a subalpine meadow located on the Qilian mountain range in the Gansu province of northwestern China. Livestock grazing has been the main land use type in this area for many years [12]. The study area was a part of 5900 ha of grassland managed by a single commercial entity engaged in farming Gansu red deer. This farm adopted the seasonal rotation grazing system where grasslands at high altitudes approximately 2930–2834 m a.s.l. are grazed in summer, and grasslands around 2802–2745 m a.s.l. are grazed in winter. In this study, we hypothesized that seasonal grazing differences might impact soil microbial community structure. The aim of this paper is to describe the differences between the effects of summer and winter grazing on soil properties and soil bacterial and fungal community structure in two pastures within the same geographic location.

2. Materials and Methods

2.1. Research Site

The soil samples for this study were collected from two separate grassland areas grazed by Gansu red deer (*Cervus elaphus kansuensis*) in summer and winter at the Sunan Deer Farm, situated in an subalpine meadow north of Qilian Mountain Range, Sunan Yugur Nationality Zizhi county, Gansu province, China (38° 87' N, 99° 65' E) (Figure 1). The two grasslands will hereafter be referred to in this paper as the summer grazed pasture (SG) and winter grazed pasture (WG).

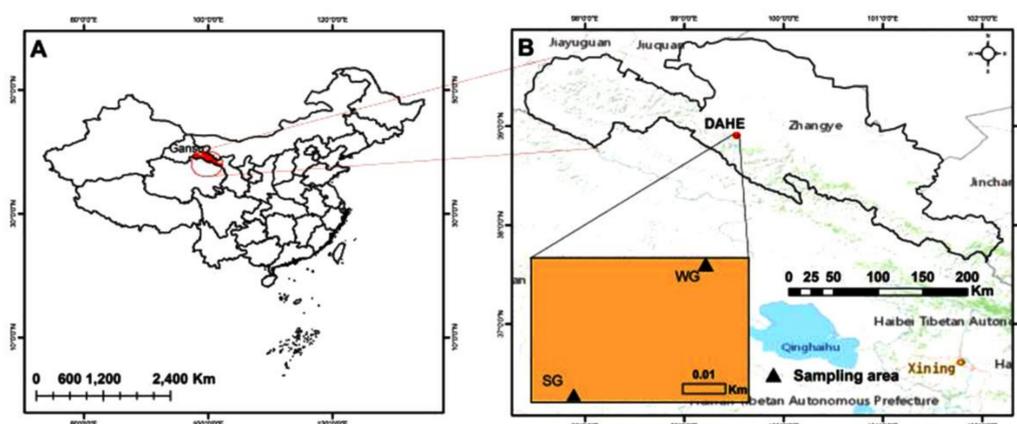


Figure 1. The experimental sitemap illustrating the location of Gansu Province in China (A) and the geographical locations of the summer (SG) and winter (WG) grazing pasture areas at Dahe, Sunan county in Gansu Province (B).

The summer season is May to July, while the winter season is October to April. The average annual rainfall is about 253.7 mm where the rainy season is mostly spread between the May to October period. The average annual temperature is 3.6 °C with a low

temperature of $-18.4\text{ }^{\circ}\text{C}$ in January and a high temperature of $13.9\text{ }^{\circ}\text{C}$ in July. The plant growth period is generally from April to September. Plants return to green in late April, optimum growth is in July, and withering is in September. The plant community in the SG pastures was dominated by *Caragana jubata* (Pallas) Poiret in Lamarck, *Potentilla fruticosa* Linnaeus, *Poa poiphagorum* Bor, *Stipa purpurea* Grisebach, *Poa alpigena* (Fr.) Lindm., *Leymus secalinus* (Georgi) Tzvelev, *Carex kansuensis* (Nelmes) Bull., *Potentilla anserina* Linnaeus, *Thalictrum alpinum* Linnaeus, *Artemisia annua* Linnaeus, *Polygonum viviparum* Linnaeus, *Oxytropis kansuensis* Bunge, and *Anaphalis bulleyana* (J. F. Jeffr.) Chang [26,27], whereas the WG pasture was dominated by *Stipa purpurea* Grisebach, *Leymus secalinus* (Georgi) Tzvele, *Poa annua* Linnaeus, *Achnatherum inebrians* (Hance) Keng ex Tzvelev, and *Carex atrata* subsp. *aterrima* (Hope) S. Yun Liang [27,28].

2.2. Sample Collection and Processing

In October 2018, four replicate soil samples were collected from each of the SG and WG pastures (Table 1). Soil samples were collected to a depth of 10 cm using a 10 cm diameter soil corer. In each pasture, soil samples were collected from 4 areas (10 m \times 10 m), and within each sampling area, five soil cores approximately 5 m apart were collected, pooled, and mixed thoroughly, resulting in a total of 8 soil samples (2 sites \times 4 replicates) for the study.

Table 1. Soil sampling locations and geographic information.

Sampling Location ID	Latitude (N)	Longitude (E)	Altitude (m)	Location
SG ^a	38°54.401'	99°31.586'	2834	Dahe, Sunan, Gansu, China
SG ^b	38°54.332'	99°31.526'	2866	Dahe, Sunan, Gansu, China
SG ^c	38°54.251'	99°31.457'	2907	Dahe, Sunan, Gansu, China
SG ^d	38°54.181'	99°31.388'	2930	Dahe, Sunan, Gansu, China
WG ^a	38°55.587'	99°32.528'	2745	Dahe, Sunan, Gansu, China
WG ^b	38°55.151'	99°32.465'	2756	Dahe, Sunan, Gansu, China
WG ^c	38°54.582'	99°32.410'	2773	Dahe, Sunan, Gansu, China
WG ^d	38°54.468'	99°32.262'	2802	Dahe, Sunan, Gansu, China

Note: ^a, ^b, ^c, and ^d are sampling points within the SG and WG pastures.

First, visible roots or other debris were removed from the soil samples. Then the samples were passed through a 2 mm sieve. Each sample was divided into three subsamples. One sub-sample was transported to the laboratory in a portable freezer and stored at $-20\text{ }^{\circ}\text{C}$ for microbial community analysis. Another subsample was used to analyze the soil water content (SWC). The remaining soil was air-dried beforehand in order to measure soil physicochemical properties, including pH, total nitrogen (TN), total carbon (TC), total sulfur (TS), and organic carbon (OC).

2.3. Soil Physicochemical Analysis

Soil water content (% *w/w*) was determined by oven drying (DHG-9240, Shanghai Jing Macro, Shanghai, China) at $105\text{ }^{\circ}\text{C}$ to a constant weight. Soil pH was determined by a pH meter (PE-10, Sartorius, Germany) using a slurry with a 1:5 soil-to-water ratio. Total soil C, TN, and TS were determined using an elemental analyzer (Elementar Vario EL/micro cube, Hanau, Germany). Soil organic carbon (OC) was determined by the Walkley–Black wet digestion method with $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ [29].

2.4. Microbial Community Analysis

Soil DNA was extracted using a FastDNA[®] spin kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. DNA quantity and purity were determined using the Nanodrop[®] ND-1000 UV-visible spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and stored at $-20\text{ }^{\circ}\text{C}$ until further use. The Illumina MiSeq sequencing was carried out at Shanghai Personal Biotechnology Co., Ltd. (Shanghai,

China). The MiSeq sequencing library preparation, PCR amplicon purification, Illumina MiSeq sequencing, sequencing data processing, and operational taxonomic unit (OTU) generation were carried out as described in Kang et al. [30]. The relative abundance of phyla and genera in each sample was used to describe the microbial community composition and diversity. The relative abundance of each taxonomic group was calculated by dividing the number of reads for any given group by the total number of reads obtained in that sample. The Chao 1 species richness index and the Shannon–Wiener (H') community evenness index were used to estimate the alpha diversity (diversity within a sample) of soil bacterial and fungal communities. The Chao 1 index was calculated as proposed by Chao [31], where the number of OTUs detected only once and twice in the community (i.e., “singleton” and “doubleton”) is used to estimate the number of species in the community using Equation (1). The Shannon–Wiener (H') estimates were calculated using Equation (2), as demonstrated by Shannon [32].

$$Chao\ 1 = S_{obs} + \frac{F_1^2}{2F_2}, \quad (1)$$

where S_{obs} is the observed number of species, F_1 and F_2 are the count of singletons and doubletons, respectively.

$$H = - \sum_{i=1}^{\delta} (P_i \log_2 P_i), \quad (2)$$

where δ is the number of OTUs and P_i is the proportion of the community represented by OTU i .

Non-metric multidimensional scaling (NMDS) analysis was carried out using a weighted UniFrac distance matrix [33] using R software [34] to compare the soil microbial community dissimilarity between the samples (beta diversity).

2.5. Statistical Analysis

Analysis of variance (ANOVA) was carried out to test the statistical significance of the effects of grassland type on α -diversity, the relative abundance of bacterial and fungal phyla and genera, and soil properties. The statistical significance of the means was compared using Duncan’s multiple range test. Statistical analysis was carried out using Statistical Product and Service Solutions (SPSS) software version 23.0 (SPSS Inc., Chicago, IL, USA). The normal distribution of the data was assessed by the Shapiro–Wilk test. Microbial community differences between the two grassland sites were statistically tested by analysis of similarities (ANOSIM) with 999 permutations using QIIME software. In all analyses, statistical significance was determined at $p \leq 0.05$. The linkage between microbial communities and soil physicochemical properties was examined using redundancy analysis (RDA) using the Canoco 4.5 software (Microcomputer Power, Ithaca, NY, USA). The relationships between dominant bacterial and fungal phyla and soil physicochemical properties were examined by Pearson correlations using SPSS software (IBM® SPSS® Statistics Version 23)

2.6. Nucleotide Sequence Accession Numbers

The nucleotide sequences retrieved from this study have been deposited in the NCBI GenBank under submission number SUB8164136 and the BioProject accession number PRJNA663459 (bacteria) and SUB8164136 and BioProject accession number PRJNA664266 (fungi).

3. Results and Discussion

This study investigated differences in soil properties and the structure of soil microbial communities between two pastures located within the same geographic location (Figure 1 and Table 1), grazed by Gansu red deer in different seasons. We found that soil physicochemical properties between the SG and WG pastures were different (Table 2). Total carbon, TN,

OC, and SWC were significantly higher in SG than in WG pasture, while the C/N ratio was significantly lower in SG pasture (Table 2). There was no significant difference in TS and soil pH between the pastures. The observed soil physicochemical differences can be attributed to the differences in vegetation characteristics [35]; animal grazing behaviors [36–39]; and the complex interactions of vegetation, grazing, and seasonal impacts. Ingram et al. [40] found that seasonal shifts in C3 and C4 plant species due to different grazing levels contributed to organic carbon and total nitrogen accumulation and losses, which in turn affected the soil microbial community. The soil physicochemical differences between SG and WG pastures may have led to the establishment of different bacterial and fungal composition between the two pastures.

Table 2. Differences in soil physicochemical properties at summer grazed (SG) and winter grazed (WG) pastures. The values are mean \pm standard error. Bold *p*-values indicate statistically significant differences between means from one-way ANOVA at $p \leq 0.05$.

Soil Properties	Summer Grazing	Winter Grazing	<i>p</i> -Value
Total Nitrogen (g kg ⁻¹)	4.6 \pm 0.29	2.8 \pm 0.13	0.002
Total Carbon (g kg ⁻¹)	45.3 \pm 2.78	31.3 \pm 0.46	0.003
Total Sulfur (g kg ⁻¹)	0.4 \pm 0.14	0.5 \pm 0.02	0.401
pH	7.47 \pm 0.120	7.59 \pm 0.055	0.372
Organic Carbon (g kg ⁻¹)	41.4 \pm 3.18	26.9 \pm 1.89	0.008
C/N	9.95 \pm 0.040	11.14 \pm 0.356	0.016
Water Content (%)	13.74 \pm 2.43	7.16 \pm 0.748	0.042

Twenty-six bacterial phyla were identified in this study. The most dominant five phyla in both pastures (94.8% of the total sequences) were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, and Gemmatimonadetes (Figure 2A). These phyla were found to be dominant in several other alpine grasslands [41–43]. The Actinobacteria abundance was significantly higher in WG pastures, while the abundance of Proteobacteria was significantly higher in SG pastures (Figure 2A). The differences in the abundance of these phyla reflect the soil fertility status of the two pastures. Proteobacteria exhibit a copiotrophic life strategy, generally thriving with high resource availability—a feature observed in SG pasture compared to the WG pasture. Actinobacteria were often dominantly observed in arid environments and are generally characterized by an oligotrophic life strategy, growing under low resource availability [44,45]. This was evident in our study; soil fertility was lower in WG pastures compared to SG, and the Actinobacteria abundance was greater in WG pastures. The relative abundances of Bacteroidetes and Rokubacteria were also significantly different between the two pastures (Figure 2A). In our study, Verrucomicrobia phyla showed a strong significant positive correlation with TC and TN in SG pastures (Table 3), which might support the fact that their abundances were higher in SG compared to WG pastures.

There were 381 bacterial genera identified in this study. The distribution of the most dominant 20 genera, which represented 98.7% of total sequences, is shown in Figure 2B. Among the most dominant 20 genera, the relative abundance of nine genera was significantly different between the pastures (Figure 2B). Here, bacterial genera *Conexibacter*, *Gemmatirosa*, and *Blastococcus* significantly differed between the two pastures at $p \leq 0.01$, while *Kribbella*, *Rhodoplanes*, *Bradyrhizobium*, *Rubrobacter*, *Nocardioidea*, and *Actinoplanes* significantly differed at $p \leq 0.05$ (Figure 2B). The *RB41* genus was the most abundant among the top 20 dominant bacterial genera in both pastures, and its abundance was not significantly different between pastures (Figure 2B). Ma et al. [46] found that *RB41* was dominant in several alpine grasslands on the Tibetan Plateau, and its abundance did not vary significantly with soil physicochemical properties.

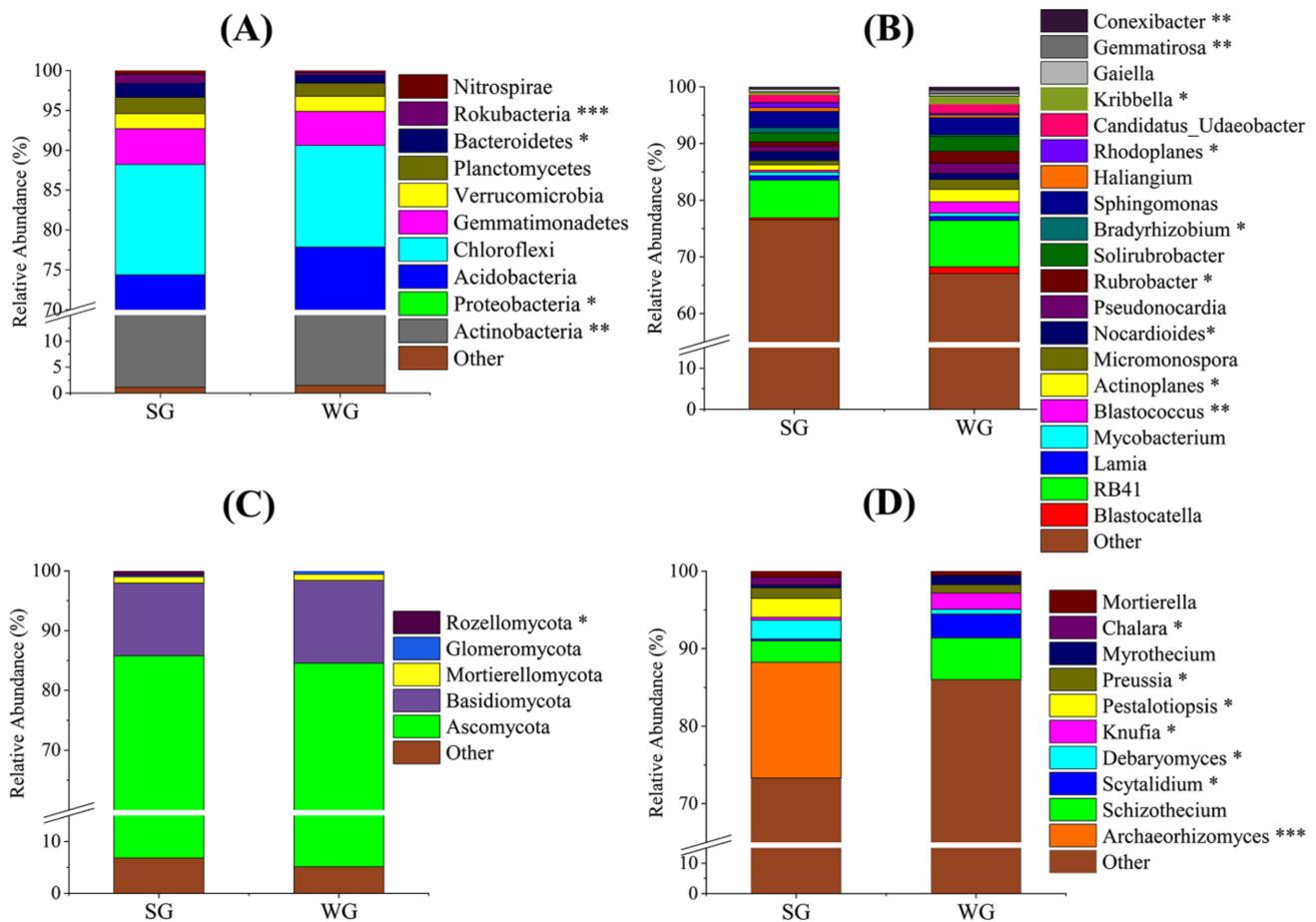


Figure 2. Relative abundances of dominant bacterial phyla (A), bacterial genera (B), fungal phyla (C), and fungal genera (D) between two different grazing scenarios—summer (SG) and winter (WG). Legends marked *, **, and *** are significantly different groups at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Eight fungal phyla were identified in this study. The five most dominant phyla in both pastures were Ascomycota, Basidiomycota, Mortierellomycota, Glomeromycota, and Rozellomycota (Figure 2C). These five phyla represented more than 90% of the fungal sequences in all the samples from both pastures. Fungal phyla Ascomycota and Basidiomycota represented 80% of sequences, but the relative abundance of these phyla was not significantly different between the two pastures. These fungal phyla are globally dominant [47], where Ascomycota show a greater rate of evolution than Basidiomycota [48], potentially explaining their higher abundance in both SG and WG pastures. There were 251 fungal genera identified in this study. There were significant differences between the relative abundance of 7 out of the top 10 abundant fungal genera. The fungal genus *Archaeorhizomyces* was significantly higher in SG pastures and significantly differed between the two pastures ($p \leq 0.001$). At the same time, *Chalara*, *Preussia*, *Pestalotiopsis*, *Knufia*, *Debaryomyces*, and *Scytalidium* significantly differed at $p \leq 0.05$ (Figure 2D).

There was no significant difference between species richness, indicated by Chao 1, or evenness, indicated by the Shannon index, between the pastures for both bacteria and fungi, indicating that the α -diversity was similar in the two pastures (Figure 3).

Nevertheless, α -diversity was higher for bacteria than fungi in both pastures, indicating that these soils are more bacteria-dominant. Greater fungi:bacteria ratios are generally linked to higher carbon storage in soil [49], meaning that both soils in our study may possess lower carbon storage capacity. The β -diversity examined by the NMDS analysis (Figure 4) indicated distinct soil bacterial communities in each of the SG and WG pastures (ANOSIM $R = 0.8$, p -value = 0.02), but fungal communities were not significantly different

(ANOSIM $R = 0.0$, p -value = 0.488). Zhong et al. [50] reported that the variation in plant species affects soil bacterial and fungal community diversity. The difference in bacterial β -diversity in our study may be linked to the difference in plant species composition between SG and WG pastures. By examining a range of grasslands around the world, Prober et al. [51] demonstrated that differences in plant diversity mostly predict bacterial β -diversity rather than fungal β -diversity. This is consistent with our findings, where we only observed significant differences between bacterial β -diversity (Figure 4).

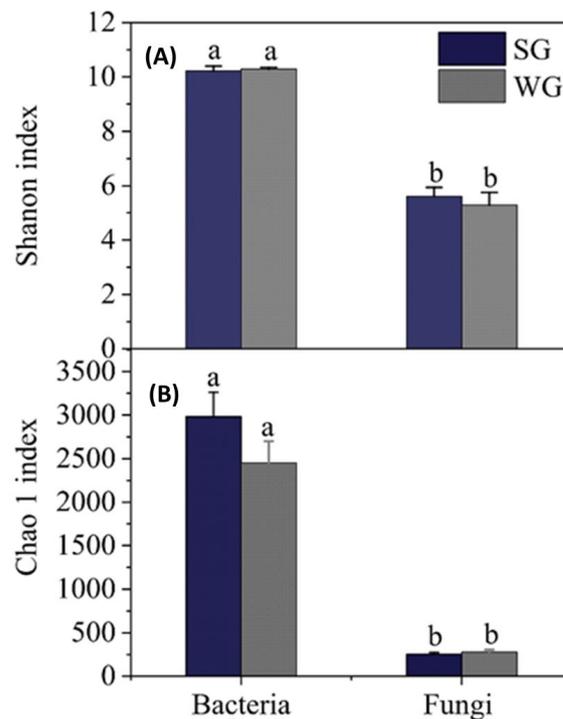


Figure 3. Microbial α -diversity differences in summer (SG) and winter (WG) grazing pastures. (A) Microbial evenness indicated by Shannon–Wiener index. (B) Microbial species richness indicated by Chao 1. Different lowercase letters indicate a statistical significance at $p \leq 0.5$ within a microbial group.

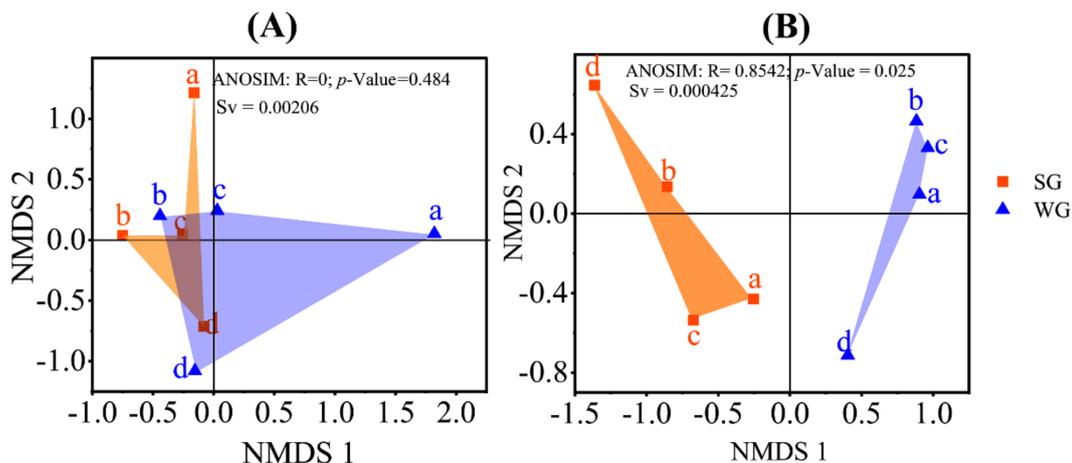


Figure 4. Non-metric multidimensional scaling (NMDS) plots to illustrate fungal (A) and bacterial (B) beta diversities as impacted by seasonal summer (SG) and winter (WG) grazing. Letters a, b, c, and d indicate replicates. Sv are stress values indicating the goodness of fit.

The importance of soil nutrients in shaping soil microbial abundance has been demonstrated in several previous studies [42,52,53]. Strong and significant correlations between soil nutrients and microbial abundances were observed in our study as well. RDA ordination biplots evaluated the linkage between fungi (Figure 5A,B) and bacteria (Figure 5C,D) and soil properties. Soil organic carbon, TN, TC, and SWC were strongly associated with the SG pasture bacterial community, whereas soil pH and C/N ratio were strongly associated with the WG pasture community. Soil pH has been widely reported as the dominant factor in determining soil microbial structure [54–56]; this was evident in our study, where fungal phyla, Glomeromycota, and about 12 dominant bacterial genera showed a strong positive relationship with soil pH (Figure 5A,D, Tables 3 and 4). Most bacterial phyla showed a strong link to the SG pasture bacterial community, while the phylum Actinobacteria was strongly linked to the WG pasture bacterial community (Figure 5C). In contrast, the most dominant bacterial genera found in this study were mostly associated with WG pastures (Figure 5D).

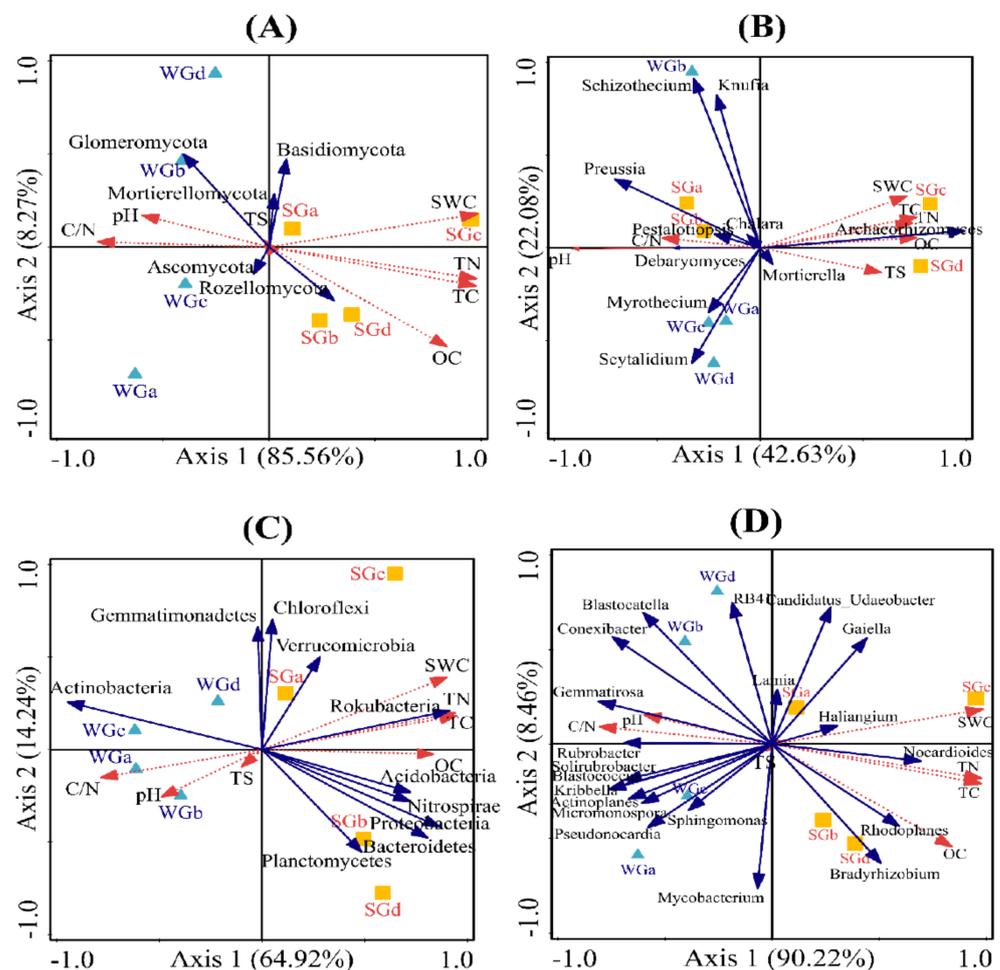


Figure 5. Redundancy analysis (RDA) demonstrating the relationships between dominant fungal phyla (A), fungal genera (B), bacterial phyla (C), and bacterial genera (D) and soil properties. The two axes (Axis 1 and 2) explain the microbial community and soil properties variation between SG and WG pastures. Solid blue and dashed red vectors indicate the microbial taxa and soil parameters, respectively. Yellow squares and blue triangles represent the SG and WG pastures, respectively.

Many studies indicate that *Archaeorhizomyces* is a widespread fungal genus that appears in many soil habitats, dominant in the rhizosphere [57] and Arctic soils [58]. Their significant, strong, positive relationships with SWC, TC, TN, and OC (Figure 5C) can be attributed to their higher abundances in SG compared to WG pastures, as evidenced in a

study by Pinto-Figueroa et al. [59]. This result indicates that *Archaeorhizomyces* are highly sensitive to edaphic factors. The fungal phylum Rozellomycota was only present in SG pastures, perhaps reflecting its potential sensitivity to seasonal variability as indicated by Tedersoo et al. [60].

In the SG pastures, bacterial phyla Verrucomicrobia and Planctomycetes showed opposite relationships to soil properties. Whereas Verrucomicrobia was significantly and positively correlated with TN, TC, and SWC with a strong negative correlation to soil pH, Planctomycetes responded negatively to TN, TC, and SWC and positively to soil pH (Table 3). These relationships were different in WG pastures. Apart from this, several other bacterial and fungal phyla also showed significant and strong correlations with soil physicochemical properties (Tables 3 and 4) differently between the SG and WG pastures. This also reflects the importance of seasonal grazing to alter soil properties, affecting microbial community responses. Dominant fungal phyla in our study also showed different strong correlations with different soil chemical properties between SG and WG grazed pastures. Similar observations were reported in Siles et al.'s study [61]. They reported seasonal patterns of soil chemical composition as being linked to soil fungal communities in alpine forest soils.

Table 3. Pearson correlation coefficients (r) between bacterial and fungal phyla and soil properties in summer grazed (SG) pastures. Strong correlations ($r > 0.7$ or $r < -0.7$) are indicated in bold (* $p < 0.05$; ** $p < 0.01$).

Classification	Taxonomy	TN	TC	TS	pH	OC	C/N	SWC
Fungi	Ascomycota	0.04	0.039	-0.519	0.094	-0.441	-0.01	0.381
	Basidiomycota	0.126	0.112	0.728	-0.387	0.519	-0.342	-0.19
	Mortierellomycota	-0.135	-0.091	-0.252	0.522	0.149	0.772	-0.284
	Glomeromycota	-0.136	-0.165	-0.314	-0.073	-0.561	-0.341	0.169
	Rozellomycota	0.05	0.027	0.693	-0.397	0.396	-0.421	-0.228
Bacteria	Actinobacteria	-0.097	-0.098	-0.619	0.211	-0.558	0.08	0.252
	Proteobacteria	0.209	0.206	0.722	-0.346	0.637	-0.218	-0.136
	Acidobacteria	-0.37	-0.372	0.256	0.166	0.109	0.18	-0.663
	Chloroflexi	-0.136	-0.147	-0.553	0.136	-0.603	-0.055	0.215
	Gemmatimonadetes	0.223	0.241	-0.465	0.113	-0.184	0.158	0.498
	Verrucomicrobia	0.938 *	0.941 *	0.488	-0.723	0.67	-0.555	0.990 **
	Planctomycetes	-0.675	-0.643	-0.532	0.843	-0.337	0.918 *	-0.79
	Bacteroidetes	0.174	0.201	0.388	0.003	0.605	0.267	-0.143
	Rokubacteria	0.69	0.716	0.068	-0.268	0.452	-0.042	0.778
Nitrospirae	-0.448	-0.406	-0.536	0.78	-0.192	0.940 *	-0.531	

Table 4. Pearson correlation coefficients (r) to illustrate relationships between bacterial and fungal phyla communities and soil properties in winter grazed (WG) pastures. Strong correlations ($r > 0.7$ or $r < -0.7$) are indicated in bold (* $p < 0.05$; ** $p < 0.01$).

Classification	Taxonomy	TN	TC	TS	pH	OC	C/N	SWC
Fungi	Ascomycota	-0.866	-0.724	0.776	-0.726	0.375	0.913	-0.779
	Basidiomycota	0.701	0.599	-0.573	0.498	-0.539	-0.744	0.849
	Mortierellomycota	0.092	-0.006	0.075	0.098	-0.879	-0.165	0.867
	Glomeromycota	0.103	-0.201	-0.128	0.787	-0.315	-0.237	0.354
Bacteria	Actinobacteria	0.223	0.291	-0.381	0.141	0.888	-0.151	-0.721
	Proteobacteria	-0.394	-0.666	0.396	0.4	-0.532	0.249	0.312
	Acidobacteria	0.024	-0.002	0.14	-0.104	-0.78	-0.066	0.74
	Chloroflexi	-0.399	-0.144	0.45844	-0.881	0.003	0.491	-0.207
	Gemmatimonadetes	-0.665	-0.479	0.543	-0.679	0.648	0.748	-0.936 *
	Verrucomicrobia	0.351	0.101	-0.221	0.639	-0.872	-0.48	0.994 **
	Planctomycetes	-0.678	-0.879	0.687	0.066	-0.532	0.554	0.171
	Bacteroidetes	-0.651	-0.846	0.625	0.105	-0.362	0.537	0.025
	Rokubacteria	0.211	0.391	-0.104	-0.41	-0.153	-0.137	0.24
Nitrospirae	-0.242	-0.343	0.398	-0.075	-0.931 *	0.155	0.753	

As found in several previous studies [43,62,63], the differences in bacterial community structure in our study were most likely driven by soil properties. In contrast, the fungal community structure was not perturbed by these factors. Fungi are generally considered to have a competitive advantage over bacteria under low organic conditions [64,65]. In this study, SG and WG pastures differed in plant community composition, which may be one of the factors that contributed to differences in soil properties, and thereby microbial composition, between these two pastures. Altitude may impact soil microorganisms [66,67], but those impacts were likely through altering of soil properties via vegetation changes.

Other studies have identified that animal grazing affects soil productivity by regulating the soil microbial community and nutrient turnover [23], and can disturb the microbial diversity associations with biogeochemical processes [68], consequently altering grassland productivity [69]. Our results indicate that microbial responses to grazing can be further complicated by seasonal impacts.

4. Conclusions

This study showed that distinct microbial community structures were developed in two pastures, located within reasonable proximity to each other but at different altitudes, grazed by Gansu red deer in two different seasons. Microbial diversity and composition are widely recognized as key factors in driving ecological functions, and this study provides evidence that seasonal rotational grazing can impact these key ecosystem drivers. These results will contribute to understanding soil microbial responses to seasonal grazing.

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Data Availability Statement: The nucleotide sequences retrieved from this study are openly available in the NCBI GenBank. See Section 2.6 for details.

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