

Combination of grazing exclusion and grassland type altered soil properties and fungal β -diversity but not α -diversity.

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Abstract

Soil fungi are involved in the decomposition of organic matter, alter soil structure and physicochemical properties and drive the material cycle and energy flow in terrestrial ecosystems. Grazing exclusion is one of the most common measures to restore degraded grasslands worldwide. However, changes in soil fungal community characteristics during grazing exclusion in different types of grasslands are unknown, and in particular, there is a lack of experimental evidence on the effects of grassland type and grazing exclusion on soil fungal diversity. Here, we investigate the effects of a 9-year grazing exclusion on soil properties, fungal community composition and diversity in three grassland types (Temperate desert, Temperate steppe and Mountain meadow). The results showed that soil fungal community composition differed between grassland types, and that grazing exclusion, grassland type and the interaction between the two did not significantly affect soil fungal α -diversity and composition, but significantly altered fungal β -diversity. Overall, our results highlight the importance of soil nutrient content on fungal diversity, particularly soil total nitrogen, total phosphorus and organic carbon, and provide key evidence to reveal the mechanisms by which soil fungi respond to grazing exclusion in different grassland types.

Combination of grazing exclusion and grassland type altered soil properties and fungal β -diversity but not α -diversity11**Fund Project:** Supported the 2022 National Natural Science Foundation(32260355).* **Corresponding author:** DONG Yiqiang (1989-), male, native of Pizhou, Jiangsu, lecturer, master supervisor, doctor, mainly engaged in grassland resources and ecological research, E-mail: xjdyq1210@163.com

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Abstract: Soil fungi are involved in the decomposition of organic matter, alter soil structure and physicochemical properties and drive the material cycle and energy flow in terrestrial ecosystems. Grazing exclusion is one of the most common measures to restore degraded grasslands worldwide. However, changes in soil fungal community characteristics during grazing exclusion in different types of grasslands are unknown, and in particular, there is a lack of experimental evidence on the effects of grassland type and grazing exclusion on soil fungal diversity. Here, we investigate the effects of a 9-year grazing exclusion on soil properties, fungal community composition and diversity in three grassland types (Temperate desert, Temperate steppe and Mountain meadow). The results showed that soil fungal community composition differed between grassland types, and that grazing exclusion, grassland type and the interaction between the two did not significantly

affect soil fungal α -diversity and composition, but significantly altered fungal β -diversity. Overall, our results highlight the importance of soil nutrient content on fungal diversity, particularly soil total nitrogen, total phosphorus and organic carbon, and provide key evidence to reveal the mechanisms by which soil fungi respond to grazing exclusion in different grassland types.

Keywords: Grazing exclusion; Grassland type; Fungal diversity; Fungal communities; Soil properties

Introduction

Grasslands cover 20% of the land surface and play an important role in preventing soil erosion and supporting livestock farming in semi-arid areas (Jing et al., 2013; Jing et al., 2014). In recent decades, grassland degradation has become widespread globally due to increased human activity and climate change (O'Mara, 2012; Jalaludin et al., 2020). China has about 400 million hectares of grasslands of various types, accounting for about 41.7% of the country's land area (Xu et al., 2016), and more than 90% of the grasslands are in a degraded state (Wang et al., 2020). This will lead to a severe weakening of grassland ecological functions, degradation of soil nutrients (Jing et al., 2014), and disruption of the original balance of the system, transforming it into a fragile ecosystem (Zheng et al., 2012). How to effectively rehabilitate degraded grasslands is a huge scientific and technical challenge for humans (Wang et al., 2020). The most widely used technical measure for ecological management of degraded grasslands is grazing exclusion, which can restore vegetation biomass (Su et al., 2005), improve soil fertility (Mekuria et al., 2007) and increase grassland use efficiency (Yang et al., 2005). Previous studies have focused on the effects of grazing exclusion on plant communities and soil properties (Wu, 2021; Dong et al., 2016), while research on the effects of soil fungal communities has been limited.

Soil fungal communities are sensitive to environmental change and have been recognised as early warning and important indicators of change in soil ecosystems (Kennedy et al., 1995; Somova et al., 2001) and play a key role in regulating material cycling in terrestrial ecosystems (Bais et al., 2006). The structure and function of soil fungal communities in degraded grasslands are often disintegrated and limited (Zhou et al., 2011). Therefore, rebuilding soil microbial communities is the primary task in restoring degraded grasslands. Furthermore, soil fungal communities play an essential role in reflecting environmental change (Chen et al., 2020). The composition and diversity of soil fungal communities have been extensively used as an important biological indicator for restoring soil quality and ecosystem function in degraded grasslands (Gao et al., 2021). Therefore, it is critical to assess changes in soil fungal communities under grazing exclusion conditions for soil and vegetation restoration and to predict changes in grassland ecosystem dynamics under environmental change.

Different types of grasslands have an integral part in maintaining the stability and diversity of grassland ecosystems (Yang et al., 2018). Numerous studies have shown that the response of soil microorganisms to grazing exclusion is not consistent across different grassland types. In alpine grasslands, soil microbial richness indices were significantly higher than in grazed samples, grazing changed the structure of soil microbial communities (Jing et al., 2021), while in alpine meadows, the total number of soil microorganisms was significantly lower after grazing (Xie et al., 2017). Sun et al. found that grazing exclusion had no significant effect on the overall numbers of soil bacteria and fungi (Sun et al., 2018). Hence, the response pattern of soil microorganisms to grazing exclusion still needs further study. Fungi can be closely associated with host plants through symbiosis or parasitism (García et al., 2014) and play a dominant role in soil nutrient cycling (Mcguire et al., 2010; Treseder et al., 2013). However, former studies have largely shown that grazing exclusion does not significantly affect the diversity of fungal communities, probably due to the greater stability of fungi compared to bacteria (Cheng et al., 2016; Zhang et al., 2018). The results of studies on the effects of grazing exclusion on the characteristics of soil fungal communities in different grassland types are still highly controversial, which limits our understanding of the changes in soil fungal communities after grazing exclusion. The total area of natural grassland in Xinjiang is about 5.7×10^7 hm^2 , accounting for 34.4% of the land area (Xu et al., 1993), which is a valuable resource for the development of the livestock industry and national economy in Xinjiang. Among them, temperate desert, temperate steppe and mountain meadow grasslands account for 48.28% of the total grassland area in Xinjiang (Wang et al., 2006). Therefore,

it is vital to understand the changes in soil fungal communities of different types of grassland in Xinjiang during grazing exclusion in order to assess the factors that influence the fungi communities' characteristics in different grassland types of exclusion, this has great significance for grassland managers and policymakers.

Here, we conducted a regional field experiment to assess the effects of grazing exclusion, grassland type and their interaction on soil physicochemical properties, soil fungal community composition and diversity in combination with plant community characteristics and soil properties. We attempted to answer three questions: 1) Does fungal composition and diversity respond consistently to grazing exclusion in different grassland types? 2) Do grazing exclusion and grassland types alter fungal communities and diversity? 3) Is soil fungal diversity indirectly driven by plant and abiotic factors under the grazing exclusion and grassland type treatments? This study contributes to the understanding of changes in soil nutrients, especially soil fungi in different grassland types and provides new ideas for grassland management in the semi-arid zone.

Materials and methods

2.1. Study area

The study area is located in Changji Hui Autonomous Prefecture (43.62°-44.39°N, 88.15°-90.25°E, 514-2611m elevation) in the eastern section of the northern slope of the Tianshan Mountains in Xinjiang. The region has a typical temperate continental arid climate, with high temperatures and rainy summers and cold, dry winters, with an average annual precipitation of 150-300 mm and an average annual temperature of 5-6°C. In the temperate desert zone(Fukang City), the main dominant plants are *Haloxylon ammodendron* and *Seriphidium santolinum* ; in the temperate steppe zone(Mubi County), the main dominant plants are *Festuca ovina* and *Carex liparocarpos* ; in the mountain meadow zone(Qitai County), the dominant plants are mostly grasses and weedy grasses.

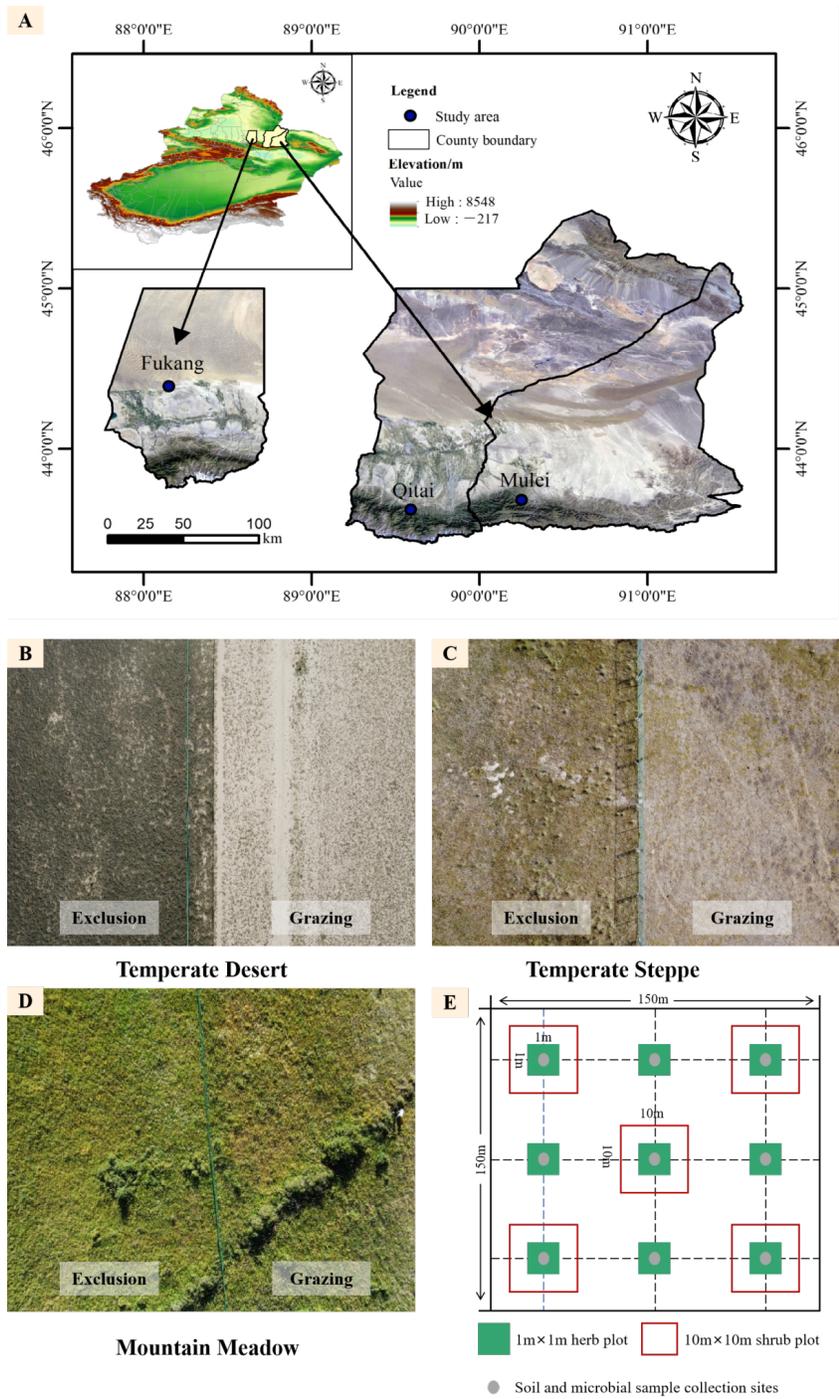


Fig. 1. Location of sampling points (Fig. A). Each grassland type includes a paired no-grazing and grazing site. Fig B shows a temperate desert sample site; Fig C shows a temperate grassland sample site; Fig D shows a montane meadow sample site. Fig E shows the sample layout.

2.2. Experimental design and field sampling

In July 2021, three areas (Fukang City, Mubi County and Qitai County) on the eastern section of the northern

slope of Tianshan were selected, each area corresponds to one grassland type: temperate desert, temperate steppe and mountain meadow. The test sites are all located within the national fixed monitoring sites and have been fenced since 2012. The fenced areas are surrounded by spring and autumn grazing areas for sheep, and the grazing intensity is medium (0.6-1.0 sheep/hm²). At each site, paired plots were sampled, which were long-term grazing plots and nearby grazing exclusion plots. All paired plots had the same soil type and similar geographical conditions including slope, elevation and topography. Three typical sample lines were set up at 50 m intervals in each of the grazing exclusion and grazing areas, three 1 m x 1 m herbaceous samples were laid out at approximately 50 m intervals in each sample line (Total of 54 small samples). If there are shrubs in the plot, 5 additional shrub plots (10m×10m) are measured. Field vegetation collections were carried out to record the species present in each square and to determine cover, height, density and biomass by species. Plant coverage was measured by the projection method; natural heights were measured with the help of a ruler, and individual quantities (densities) of each specie were recorded with the help of statistical methods. Above ground biomass of each specie (only the green plant parts) were figured out by clipping the whole plant from the soil surface using scissors in each sampling plot and brought back to the laboratory for treatment(dried at 80°C for 24 h to constant weight). The specific plant data have all been obtained (Supplementary Table 1)

Soil samples were taken in layers of 0-5 cm and 5-10 cm depth, and each sample line was mixed separately and placed in sealed bags. Some of the samples were stored in a refrigerator at 4degC, while the remainder was dried indoors by picking out plant roots, gravel and other debris, and then ground and mixed, and stored in 1 mm and 0.25 mm sieves for indoor analysis. Soil microbial samples were collected from 0-5 and 5-10 cm soil layers in the sample plots, and each sample line was evenly mixed in a sealed bag and taken back to the laboratory in a vehicle refrigerator (-20degC).

2.3. Soil property analysis

The soil pH was determined using a ratio of 2.5:1 water to soil with a standard pH meter (Cao et al., 2017); soil water content(SWC) was determined gravimetrically by drying the soil samples (105°C, 24 h); and bulk density(BD) was measured gravimetrically after oven-drying (105 , 24 h). The soil organic carbon(SOC) was determined using the dichromate oxidation method (Walkley and Black, 1934). Total phosphorus (TP) was measured with Mo-Sb colorimetricmethod using a spectrophotometer (Lambda25 UV-visspectrometer, United States). Total nitrogen(TN) was determined using the Kjeldahl method.

2.4. DNA extraction and high-throughput sequencing

High-throughput sequencing was performed using the Illumina MiSeq PE 300 platform to analyse the soil fungal community. Total DNA was extracted from the samples using the macrogenomic DNA extraction kit; the variable region sequence of the ITS1-1F region of the fungal ITS rRNA gene was used as the target, and the fungal primers were ITS1-1F-F and ITS1-1F-R. PCR amplification was performed to obtain the PCR products; the PCR products were quantified and the library was constructed to obtain the fungal variable region base sequence information. The microbial sequencing analyses in this study were all done on the biochemical cloud platform of Shenzhen Microcomputer Technology Group Co.(<https://bioincloud.tech/pipelines>)

2.5. Statistics and analysis

All data are shown as mean and standard error. Data on soil physicochemical properties and microbial diversity indices were subjected to two-way variance comparisons and Pearson correlations using SPSS 26.0. Bar graphs were generated in OriginPro 2021 (originlab Corporation, USA). Principal Coordinate Analysis (PCoAs) from BrayCurtis distances were used to visualise the effects of grazing exclusion and grassland type on fungal community composition. PERMANOVA was performed in the 'vegan' package using the ANOSIM function to test for significance of differences in community composition and plotted using the 'ggplot2' package in R 4.2.1. Finally, we implemented structural equation modelling (SEM) based on the lavaan package (Rosseel., 2011) to assess the effects of grazing exclusion and grassland type on fungal diversity through changes in plant and soil abiotic variables, the statistical analyses of which were carried out using R version 4.2.1.

Results

3.1. Soil physicochemical properties as affected by grazing exclusion and grassland type

In the 0-5 cm soil layer (Table 1), grazing exclusion significantly increased 139.34% of SOC and 36.36% of TP in temperate desert ($P < 0.05$), while significantly increasing temperate steppe SWC, SOC and mountain meadow TN ($P < 0.05$), but grazing exclusion significantly reduced 16.67% of BD in temperate steppe ($P < 0.05$). Before the grazing exclusion, soil pH and BD were significantly higher in temperate desert than in temperate steppe and montane meadow, while the differences between temperate steppe and montane meadow were not significant. C:N did not differ significantly between the three grassland types. All other soil physicochemical properties were significantly higher in temperate steppe and mountain meadow than in temperate desert. After exclusion, grazing exclusion significantly increased the differences in SWC, SOC, TN and N:P between the three grassland types, while the other soil chemistry properties were consistent with the results before exclusion. The interaction of grazing exclusion and grassland type significantly altered TN and N:P ($P < 0.05$; Supplementary Table 2). Soil physicochemical properties were all highly significantly influenced by different grassland types in general compared to grazing exclusion, while grazing exclusion, grassland type and the interaction between the two did not significantly alter C:N.

In the 5-10 cm soil layer, grazing exclusion significantly increased TP in temperate deserts by 34.1%, while significantly decreasing BD by 9.8% and N:P by 47.1%. In temperate steppes, none of the effects of grazing exclusion on the measured soil physicochemical properties were significant. In mountain meadows, grazing exclusion resulted in a significant increase in SWC and SOC. BD and SOC were significantly different between the three grassland types before the grazing exclusion, while C:N was not significantly different. All other soil physicochemical properties were significantly different between temperate desert and temperate grassland, mountain meadow, while the differences between temperate steppe and mountain meadow were not significant. After grazing exclusion, C:N was significantly higher in temperate deserts than in temperate steppe and mountain meadows, while SOC and BD were not significantly different between temperate steppe and mountain meadows. The interaction of grazing exclusion and grassland type significantly altered BD, SOC, TP and C:N ($P < 0.05$; Supplementary Table 3).

Table 1 Soil physicochemical properties as affected by grazing exclusion and grassland type.

Index	Treatment	Temperate Desert	Temperate Desert	Temperate Steppe	Temperate Steppe	Mountain M
		0-5cm	5-10cm	0-5cm	5-10cm	0-5cm
pH	Garzing	9.59±0.12Aa	9.62±0.17Aa	6.66±0.09Ab	6.73±0.07Ab	6.78±0.10Ab
	Exclusion	9.39±0.23Aa	9.67±0.21Aa	6.66±0.04Ab	6.64±0.04Ab	6.98±0.07Ab
SWC(%)	Garzing	2.00±1.00Ab	2.00±0.00Ab	17.00±2.00Ba	19.00±2.00Aa	18.00±2.00Aa
	Exclusion	2.00±0.00Ac	2.00±0.00Ab	23.00±0.00Aa	18.00±1.00Aa	18.00±2.00Aa
BD(g·cm ³)	Garzing	1.48±0.07Aa	1.53±0.02Aa	0.78±0.02Ab	0.78±0.02Ac	0.82±0.05Ab
	Exclusion	1.37±0.06Aa	1.38±0.04Ba	0.65±0.01Bb	0.78±0.01Ab	0.74±0.02Ab
SOC(g·kg ⁻¹)	Garzing	1.22±0.15Bb	0.87±0.17Ac	98.44±1.47Ba	88.05±8.33Aa	84.92±12.60
	Exclusion	2.92±0.62Ac	1.27±0.08Ab	119.88±6.87Aa	70.57±3.98Aa	81.60±10.36
TN(g·kg ⁻¹)	Garzing	0.13±0.06Ab	0.07±0.00Ab	8.25±1.18Aa	8.75±0.73Aa	10.00±0.19Aa
	Exclusion	0.32±0.08Ac	0.05±0.01Ab	10.89±0.39Aa	7.60±0.31Aa	8.41±0.10Bb
TP(g·kg ⁻¹)	Garzing	0.44±0.01Bb	0.44±0.01Bb	0.85±0.09Aa	0.95±0.04Aa	0.99±0.02Aa
	Exclusion	0.60±0.03Ab	0.59±0.02Ab	0.98±0.03Aa	0.86±0.02Aa	0.93±0.05Aa
C:N	Garzing	14.40±6.32Aa	11.78±2.12Aa	12.50±2.01Aa	10.05±0.21Aa	8.45±1.12Aa
	Exclusion	9.34±0.89Aa	24.80±4.53Aa	11.00±0.44Aa	9.28±0.15Ab	9.72±1.34Aa
C:P	Garzing	2.81±0.40Ab	2.00±0.37Ab	118.06±13.26Aa	92.34±5.84Aa	86.00±11.92
	Exclusion	4.80±0.83Ab	2.16±0.17Ab	122.35±6.85Aa	81.57±3.35Aa	88.63±11.99
N:P	Garzing	0.30±0.14Ab	0.17±0.01Ab	9.58±0.41Aa	9.17±0.44Aa	10.16±0.12Aa
	Exclusion	0.53±0.11Ac	0.09±0.02Bb	11.11 ±0.20Aa	8.79±0.23Aa	9.15±0.48Ab

The means (\pm SE) for each variable followed by different uppercase letters indicate significant differences between grazing and exclusion sites ($P < 0.05$). The lowercase letters indicate significant difference among different grassland types ($P < 0.05$). SWC, soil water content; BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; C:N, carbon:nitrogen; C:P, carbon:phosphorus; N:P, nitrogen:phosphorus.

3.2. Soil fungi community OTU numbers and composition as affected by grazing exclusion and grassland type

The Wayne analysis (Fig. 1) showed that in the 0-5 cm soil layer (Fig. 2A), the number of fungal OTUs in mountain meadows (619) accounted for 38.98% of the total OTUs; the number of fungal OTUs in temperate steppe (512) accounted for 32.24%; and the number of fungal OTUs in temperate deserts (457) accounted for 28.78%. In addition, mountain meadows and temperate steppes shared 12.15% of fungal OTUs (193); mountain meadows and temperate deserts shared 0.56% of OTUs (9); temperate steppes and temperate deserts shared 1.32% of fungal OTUs (21) and the three grasslands shared 1.95% of fungal OTUs (31). After exclusion (Fig. 2B), the number of fungal OTUs in mountain meadows and temperate steppes decreased by 18.1% and 18.36%, respectively, compared with those before exclusion, but the number of fungal OTUs in temperate deserts increased by 38.51% compared with those before exclusion.

In the 5-10 cm soil layer (Fig. 2C), the number of fungal OTUs in mountain meadows (512) accounted for 32.04% of the total OTUs; the number of fungal OTUs in temperate steppes (596) accounted for 37.30%; and the number of fungal OTUs in temperate deserts (490) accounted for 30.66%. After exclusion (Fig. 2D), the number of fungal OTUs in temperate steppes decreased by 20.47% compared with that before exclusion, while the number of fungal OTUs in mountain meadows and temperate deserts increased by 2.7% and 15.71% respectively, compared with that before exclusion.

At the phylum level (Fig. 3A), *Ascomycota* and *Basidiomycota* were the dominant fungi in the three grassland types in the 0-5 cm soil layer before exclusion, accounting for 94.03% and 5.67% of the total fungi in temperate deserts, 80.76% and 18.51% of the total fungi in temperate steppes, and 78.43% and 18.52% of the total fungi in mountain meadows respectively. After exclusion, the *Ascomycota* decreased by 14.72% and 9.74% in temperate deserts and mountain meadows respectively, and increased by 4.82% in temperate steppes. In contrast to *Basidiomycota*, increased by 238.8% and 47.68% in temperate deserts and mountain meadows respectively, but it decreased by 23.12% in temperate steppes. In addition, significant differences were found between the three grassland types for the *Glomeromycota* and *Mucoromycota* ($P < 0.05$; Supplementary Table 4). The interaction between grazing exclusion and grassland type had no significant effect on the other fungi. In the 5-10 cm soil layer (Fig. 3B), the main fungi of the three grassland types were the same as in the 0-5 cm soil layer, and the analysis revealed that the *Mortierellomycota* was not affected by either grazing exclusion or grassland type, but the interaction between the two had a significant effect on the *Mortierellomycota* ($P < 0.05$; Supplementary Table 5). In addition, grassland type significantly influenced the *Blastocladiomycota* ($P < 0.05$), whereas grazing exclusion had no significant effect on any of the fungi measured ($P > 0.05$).

At the phyla level in the 0-5 cm soil layer (Fig. 3C), the dominant fungi in the three grassland types before and after exclusion included *Dothideomycetes*, *Archaeorhizomycetes*, *Sordariomycetes* and *Agaricomycetes*, while in the temperate desert *Archaeorhizomycetes* almost disappear in temperate deserts. Grassland type had a significant effect on *Glomeromycetes*, *Orbiliomycetes* and *Tremellomycetes* ($P < 0.05$; Supplementary Fig. 6). In the 5-10 cm soil layer (Fig. 3D), *Sordariomycetes* increased significantly by 279.32% in the temperate desert before exclusion compared to the 0-5 cm soil layer, and the interaction of grazing exclusion, grassland type and both significantly altered *Sordariomycetes* and *Pezizomycetes* ($P < 0.05$; Supplementary Fig. 7). Grassland type had a highly significant effect on *Ustilaginomycetes* ($P < 0.001$) and a significant effect on *Eurotiomycetes* and *Orbiliomycetes* ($P < 0.05$). In addition, grassland type had a highly significant effect on *Dothideomycetes* and *Archaeorhizomycetes* in both the 0-5 cm and 5-10 cm soil layers ($P < 0.001$; Supplementary Tables 6 and 7).

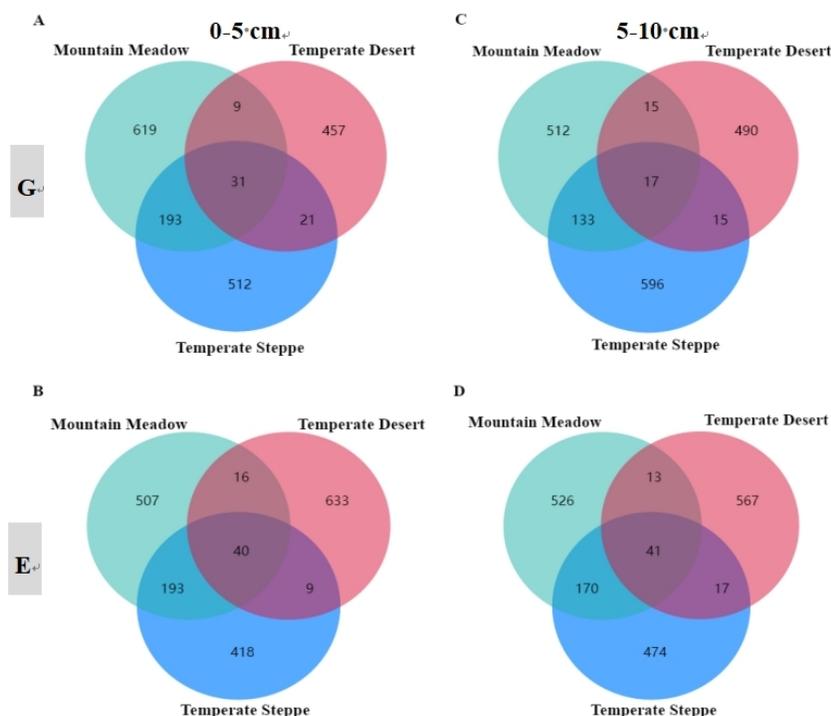


Fig. 2. Venn diagram of Fungal communities in different grassland types based on OTUs abundance. Different color represents grassland Types. The letter G denotes grazing and the letter E denotes grazing exclusion.

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Fig. 3. Effect of grazing exclusion and grassland type on soil fungi phylum and phyla community composition. Fig(A) shows the composition of the soil fungal phylum in the 0-5cm soil layer; Figure (B) shows the composition of the soil fungal phylum in the 5-10cm soil layer; Figure (C) shows the composition of the soil fungal phyla in the 0-5cm soil layer; Figure (D) shows the composition of the soil fungal phyla in the 5-10cm soil layer. TD, temperate desert; TS, temperate steppe; MM, mountain meadow.

3.3. Soil fungal community diversity as affected by grazing exclusion and grassland type

Soil fungal diversity was not significantly altered by grazing exclusion, grassland type or the interaction between the two in either the 0-5 cm or 5-10 cm soil layers ($P > 0.05$; Fig 4). In addition, the Chao1 index was significantly higher ($P < 0.05$) in mountain meadows than in temperate deserts in the 0-5 cm soil layer.

A principal coordinate analysis of soil fungal communities based on Bray-Curtis distances showed that PCoA1 and PCoA2 explained 27.0% and 13.1% of the variation in soil fungal communities in the 0-5 cm soil layer, with a cumulative contribution of 40.1% (Fig. 5a). Further Anosim analysis showed significant differences soil fungal communities ($R=0.527$, $P < 0.001$). In the 5-10cm soil layer, PCoA1 and PCoA2 explained 22.7% and 10.5% of the variation in soil fungal communities respectively, with a cumulative contribution of 33.2%

(Fig. 5b). Further Anosim analysis showed significant differences soil fungal communities ($R=0.475$, $P < 0.001$).

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Fig. 4. Soil fungal -diversity as affected by grazing exclusion and grassland type. Error bars indicate standard errors (3 replicate sites). The lowercase letters indicate significant difference among different grassland types ($P < 0.05$). TD, temperate desert; TS, temperate steppe; MM, mountain meadow.

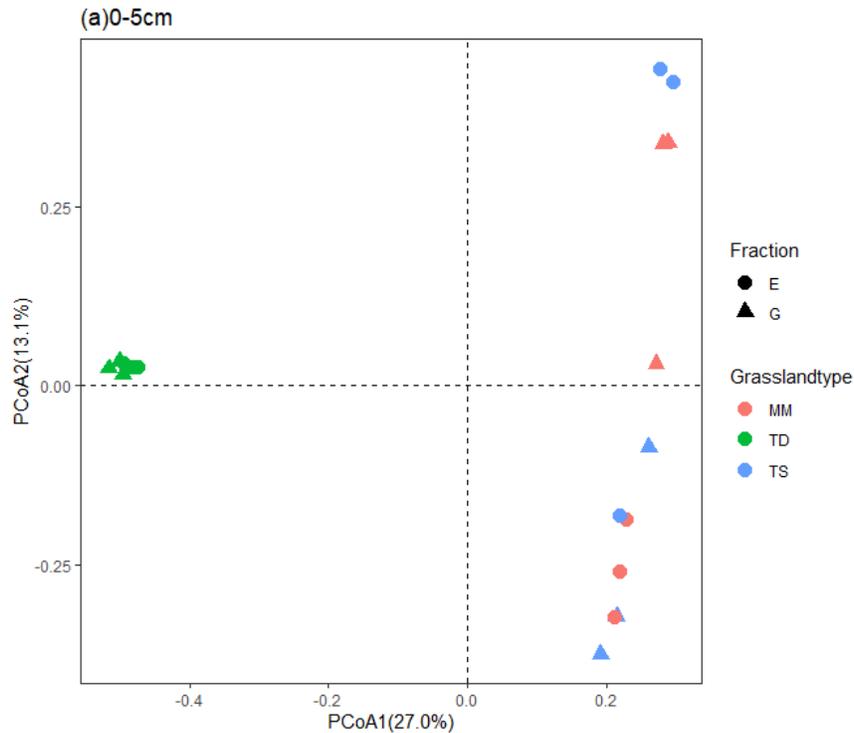


Fig. 5. Fungal community structure assessed by -diversity patterns using the principal coordinate analysis plots of Bray-Curtis distances. Different shape represents exclusion or grazing soils and color represents grassland types. E,exclusion; G,grazing; TD, temperate desert; TS, temperate steppe; MM, mountain meadow. ANOSIM similarity analysis was used to test the significance between groups.

3.4. Correlation of grazing exclusion and grassland type with plantsoil and fungal diversity

The correlations between grazing exclusion and grassland type with plantsoil and fungal diversity are shown in Figure 6. Fraction was only significantly negatively correlated with vegetation coverage, and all indicators were highly significantly correlated with grassland type, except for the vegetation Pielou index and the microbial Shannon and Chao1 indices. In addition, the microbial Simpson index showed a highly significant negative correlation with SOC, and a significant negative correlation with TN, TP, C:P and N:P.

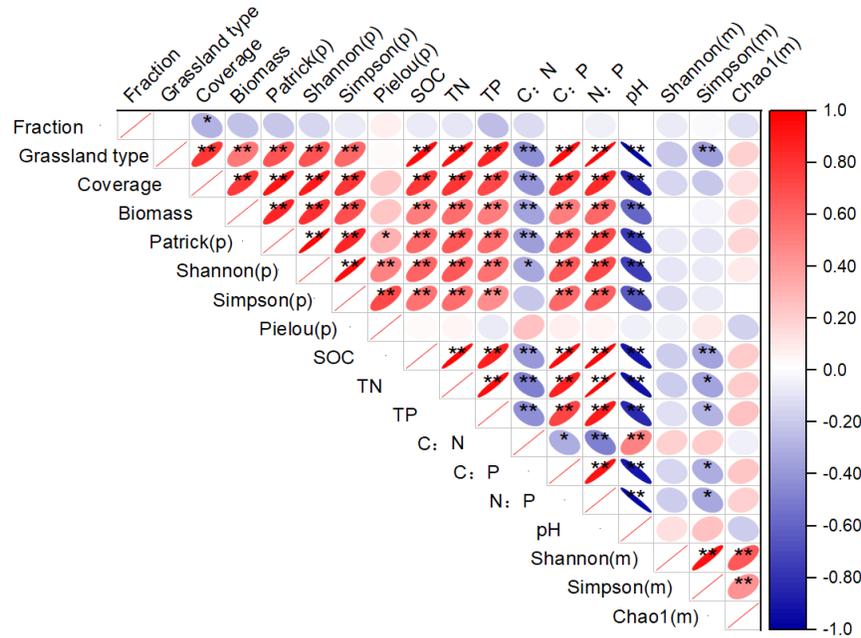


Fig. 6. Correlation of grazing exclusion and grassland type with plantsoil and fungal diversity. Fraction represents grazing exclusion and grazing; Grassland type represents temperate desert/temperate steppe and mountain meadow. (p), plant; (m), microorganism. Circle size and color represent the Pearson correlation coefficient.

3.5. Potential drivers of soil fungal community diversity

SEM analysis showed that total soil nitrogen and total soil phosphorus had a direct effect on fungal community diversity, while grazing exclusion, grassland type and soil organic carbon had an indirect effect on fungal community diversity. Fraction had a significant effect on fungal community diversity by directly affecting total soil phosphorus ($P < 0.05$) and grassland type had a significant effect on fungal community diversity by directly affecting soil organic carbon, which in turn affected total nitrogen and phosphorus ($P < 0.05$). These variables explained approximately 40% of the variation in fungal community diversity (Fig. 7a). In addition, combining direct and indirect effects revealed that soil total nitrogen was the most important predictor of change in fungal community diversity (Fig. 7b).

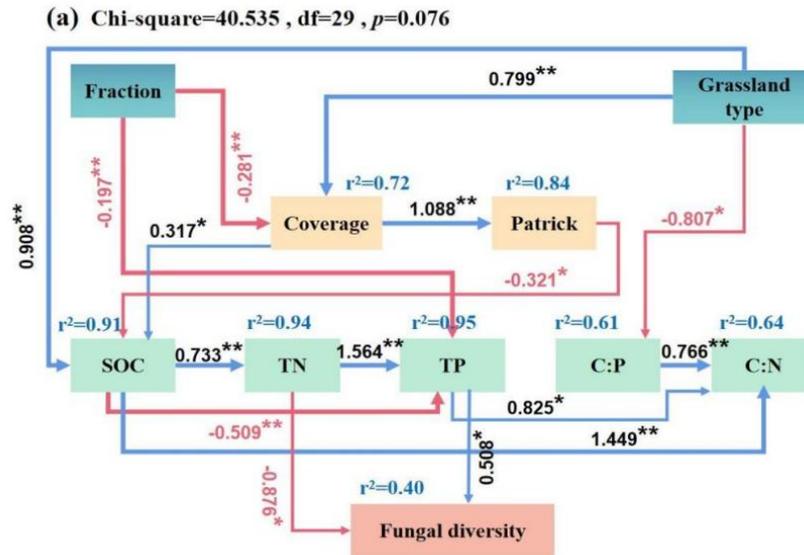


Fig. 7. (a) Structure equation modeling exploring direct and indirect effects of fraction (grazing and grazing exculsion), grassland type, plant index (coverage and Patrick index), and soil chemical properties in fungal diversity and (b) standardized effects of fraction, grassland type, plant index, and soil chemical properties in fungal diversity. Line thickness indicates the correlation strength: the thicker the line, the stronger is the correlation. The red and blue arrows indicate negative and positive relationships, respectively. The width of the arrow is proportional to the strength of the relationship. The numbers adjacent to the arrows represent standardized path coefficients. The proportion of variance explained (R^2) appeared alongside each response variable in the model.

Discussion

4.1. Effect of grazing exclusion on soil physicochemical properties in different grassland types

Whether grazing exclusion can change the diversity and composition of soil fungal communities, soil chemistry is the dominant factor (Li et al., 2019; Qin et al., 2021). There is growing evidence that fungal communities are more sensitive to carbon and nitrogen sources than bacterial communities (Zhang et al., 2018). Our study found that C:N did not differ significantly among the three grassland types in either 0-5 cm or 5-10 cm soils before grazing exclusion, probably because grazing can promote root secretions and fallen leaves decomposition through trampling, which can increase the amount of C and N entering the soil and keep soil C and N in dynamic equilibrium (Bi et al., 2018). Whereas C:N was significantly higher in temperate deserts than in temperate steppes and mountain meadows after grazing exclusion, the interaction of exclusion and grassland type significantly altered C:N, indicating that grazing exclusion reduces soil C and N pools (Sun et al., 2020), and is prominent in steppes and meadows. In addition, we found that grazing exclusion significantly increased SOC in all three grassland types and SWC in both temperate steppes and mountain meadow, due to higher soil fertility associated with good plant cover and greater apoplastic accumulation (Marzaioli et al., 2010; Jing et al., 2014). Above-ground biomass has a direct effect on apoplastic accumulation, which increases soil moisture. It explains the increase in SWC in both temperate steppes and mountain meadow. At the same time, apoplastic decomposes more rapidly in moist soils, and large amounts of decomposing apoplastic can increase soil nutrients (Jing et al., 2014).

Secondly, soil TP showed a close correlation with temperate desert, and grazing exclusion significantly increased the content of temperate desert TP, which is inconsistent with previous studies (Yang et al., 2018), it may be due to the fact that soil nutrients still had not returned to their original levels after 9 years of

grazing exclusion, but were in an unstable and fluctuating state, this may be related to the change in the main vegetation types on the surface, the rate of decomposition and the amount of nutrient uptake and return by plant roots. The effects of grazing exclusion on pH, C:N, C:P and N:P in the three grasslands were not significant regardless of the soil stratum. The reasons for this phenomenon may be: (1) the removal of grazing pressure after grazing exclusion in degraded grasslands, the disappearance of conditions that maintain the spatial pattern of soil nutrients or vegetation, the increase in spatial heterogeneity of soil nutrients, and the increase in the proportion of stochastic factors, making the research results more stochastic and the study results appearing differences (David, 1988); (2) the grazing areas have a higher soil nutrient content due to the trampling of livestock, which leads to the crushing of apoplastic and its entry into the soil, thus accelerating the circulation of organic matter, as well as the excrement of grazing livestock; and (3) the regional differences in the soil itself may also be responsible for this difference.

4.2. Effects of grazing exclusion on the composition and diversity of soil fungi

Our results go further than the commonly reported differences in fungal composition between grassland types (Zhang et al., 2010; Yang et al., 2022; Wang et al., 2021). Consistent with previous results (Maestre et al., 2015; Ren et al., 2017), we found that at the phylum level, the pre-grazing exclusion *Ascomycota* and *Basidiomycota* were the dominant fungi in all three grassland types. After the grazing exclusion *Ascomycota* decreased by 14.72% and 9.74% in temperate deserts and mountain meadows, respectively, while it increased by 4.82% in temperate steppes. In contrast to the *Ascomycota*, the *Basidiomycota* increased by 238.8% and 47.68% in temperate deserts and mountain meadows, respectively, while it decreased by 23.12% in temperate steppes. This may be due to the higher soil pH in temperate desert and the soils of temperate steppes and mountain meadows is acidic, and the positive response of *Ascomycota* and *Basidiomycota* fungi to changes in soil pH, the higher abundance of *Ascomycota* in soils with higher pH (Tederloo et al., 2014), which also explains the higher relative abundance of *Ascomycota* in temperate deserts. Additionally, studies of soil fungi have found that the highest diversity in soils is found in *Ascomycota* and *Basidiomycota* fungi in recent years, while later studies analysing the occurrence patterns of globally dominant soil fungal taxa have found a strong spatial structure of soil fungal communities along ecological gradients, but also signs of mass dispersal and the ability of some fungi to dominate many environments, with wind-dispersed *Ascomycota* taxa dominating the soil fungal communities, followed by *Basidiomycota* and other fungi (Bucbe et al., 2009; Egidi et al., 2019). Next, significant differences were found between the three grassland types for the *Glomeromycota* and *Mucoromycota*, and it was found that the *Mortierellomycota* was not influenced by either grazing exclusion or grassland type, but the interaction between the two had a significant effect on the *Mortierellomycota*. It has been shown that the activity of *Glomeromycota* is reduced in high acidity environments and is not efficient at taking up nitrogen or phosphorus from organic matter and converting it to inorganic components for plant use, eventually plants gradually form new symbiotic relationships with other organic matter mycorrhizal fungi and replace the *Glomeromycota* (Smith et al., 2008). In this study, the low organic matter content of the temperate desert soil and the lower relative abundance of *Glomeromycota* compared to the other two types of grassland. Validating the findings of most studies on the relationship between *Glomeromycota* and soil physicochemical properties, and support previous studies suggesting a suppressive effect of soil organic matter content on *Glomeromycota* (Pellissier et al., 2015; Smith et al., 2008). Furthermore, at the phyla level, it was found that grazing exclusion, grassland type and the interaction between the two significantly altered *Sordariomycetes* and *Pezizomycetes*, with grassland type having a highly significant effect on *Ustilaginomycetes* and a significant effect on *Eurotiomycetes* and *Orbiliomycetes*. This represents the different responses of various fungal phylas to environmental change, reflecting the role of environmental niche separation in selecting fungal communities (Pellissier et al., 2014).

Unlike the effect of grazing exclusion on soil fungal composition, grazing exclusion, grassland type and the interaction between the two did not significantly alter soil fungal -diversity in either 0-5 cm or 5-10 cm soils, similar to previous findings (Hao et al., 2020; Yang et al., 2019; Ding et al., 2020). The insignificant response of fungal alpha diversity to grazing exclusion and grassland type may be due to that fungi are less sensitive than bacteria (Cheng et al., 2016; Zhang et al., 2018). These results are consistent with previous findings that soil fungi are more stable than bacteria (Hamonts et al., 2017; Wang et al., 2019). Although there

was no effect on fungal -diversity, we found that the interaction of grazing exclusion and grassland type significantly altered fungal -diversity. This may be the ability of many soil microorganisms to colonise the inter-rhizosphere, where root biomass and plant C:N ratios may influence the utilisation of inter-rhizosphere resources by altering root turnover or root secretions, which may in turn strongly alter the composition of the microbial community (Chase, 2010; Philippot et al., 2013). Moreover, other studies have detected significant effects of grazing exclusion on soil fungal -diversity (Chen et al., 2020). In the current study, the strong link between soil microbial diversity and grazing exclusion and grassland type can be explained by the fact that microbial communities are often directly influenced by above-ground plant biomass and community structure (de Vries et al., 2012). For example, grazing exclusion increases above-ground plant biomass and apoplastic content, thus increased fungal diversity (Hamonts et al., 2017). The increase in species richness induced by grazing exclusion suggests that high plant diversity results in a greater variety of organic matter ultimately flowing into the subsurface, thereby generating more ecological niches for use by different species of microorganisms and altering the diversity of microbial communities (Lodge, 1997; Brodie et al., 2003). The results indicated that there was no significant change in soil fungal alpha diversity but significant differences in fungal beta diversity under the influence of both grazing exclusion and grassland type.

4.3. Potential drivers of soil fungal community diversity

SEM analysis showed that grazing exclusion had a significant effect on fungal community diversity by directly affecting total soil phosphorus, and that grassland type had a significant effect on fungal community diversity by directly affecting soil organic carbon, which in turn affected total nitrogen and phosphorus. In line with our findings, other studies have also shown that soil total phosphorus and nitrogen are closely related to soil fungal diversity (Lindahl et al., 2015; Lauber et al., 2008). In contrast, a recent experiment showed that soil fungal abundance and diversity differed between grassland habitats, but that this difference did not correlate with geographical distance, and that certain environmental factors, including climate, soil pH, nitrogen and phosphorus, were the main influences on the distribution, abundance and diversity of soil fungal communities (Pellissier et al., 2014). In our study, combining direct and indirect effects reveals that soil total nitrogen is the most important predictor of changes in fungal community diversity, the strong association between soil total nitrogen and fungal diversity is justified because total nitrogen is the main source of energy for soil microorganisms (Lindahl et al., 2015). For example, nitrogen provides an adequate source of nitrogen for fungal symbioses, and plants no longer import too much carbon into the subsurface for nitrogen acquisition, which can have an impact on fungal composition and biomass (Norris et al., 2013). However, it has been shown that fungal diversity does not correlate with total soil nitrogen and phosphorus, suggesting that environmental factors may not be a limiting factor in the structural diversity of soil fungal communities at the larger scale studied (Li., 2015). In the present study, soil organic carbon had an indirect effect on fungal community diversity, similar to the results of previous studies (Li., 2015). Soil organic carbon provides nutrients and energy for plant growth and soil microbial life (Li et al., 2006), and its content reflects the effectiveness of limiting resources in the soil and is the 'source' of nutrients for soil fungal community growth, the level of which affects soil fungal community structure and diversity. In addition, the natural environment in different regions can also affect soil fungal diversity, harsh environments such as drought can reduce fungal diversity and abundance (Maestre et al., 2015). In summary, soil nutrient content is the main factor influencing fungal diversity, especially total soil nitrogen, total phosphorus and organic carbon.

Conclusions

As revealed by the data from our study, the composition of soil fungal communities differed between grassland types. Under the influence of both grazing exclusion and grassland type, there was no significant change in soil fungal alpha diversity, but significant differences in fungal beta diversity. The changes in soil total nitrogen and phosphorus caused by grazing exclusion were closely related to soil fungal diversity. These results suggest that soil nutrient content is the main factor influencing fungal diversity, especially soil total nitrogen, total phosphorus and organic carbon.

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Data availability statement

Hereby affirm. Primary data including total data will be deposited in the Dryad Repository when the paper is accepted.

Conflict of Interest

The authors declare that they have no competing interests.

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