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Grazing greatly reduces the temporal stability of soil cellulolytic fungal community in a steppe on the Tibetan Plateau

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ABSTRACT

Excessive livestock grazing degrades grasslands ecosystem stability and sustainability by reducing soil organic matter and plant productivity. However, the effects of grazing on soil cellulolytic fungi, an important indicator of the degradation process for soil organic matter, remain less well understood. Using T-RFLP and sequencing methods, we investigated the effects of grazing on the temporal changes of cellulolytic fungal abundance and community structure in dry steppe soils during the growing months from May to September, on the Tibetan Plateau using T-RFLP and sequencing methods. The results demonstrated that the abundance of soil cellulolytic fungi under grazing treatment changed significantly from month to month, and was positively correlated with dissolved organic carbon (DOC) and soil temperature, but negatively correlated with soil pH. Contrastingly, cellulolytic fungal abundance did not change within the fencing treatment (ungrazed conditions). Cellulolytic fungal community structure changed significantly in the growing months in grazed soils, but did not change in fenced soils. Grazing played a key role in determining the community structure of soil cellulolytic fungi by explaining 8.1% of the variation, while pH and DOC explained 4.1% and 4.0%, respectively. Phylogenetically, the cellulolytic fungi were primarily affiliated with Ascomycota (69.65% in relative abundance) and Basidiomycota (30.35%). Therefore, grazing substantially reduced the stability of soil cellulolytic fungal abundance and community structure, as compared with the fencing treatment. Our finding provides a new insight into the responses of organic matter-decomposing microbes for grassland managements.

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Introduction

Excessive livestock grazing has caused severe grassland degradations worldwide and reduced ecosystem functioning, stability, and sustainability (Klein et al., 2005; Wang et al., 2011). China's grassland accounts for approximately 40% of the national land area and 6%-8% of the world's total grassland area (Ni, 2002). However, the long-term effects of grazing on soil carbon and nitrogen cycling remain largely elusive. This fundamental understanding is particularly essential to achieve the goal of "4 per mille Soils for Food Security and Climate". The 4 per mille initiative aspires to increase global soil organic matter stocks by 4 per 1000 (or 0.4%) per year as a compensation for the global emissions of greenhouse gases by anthropogenic sources (Minasny et al., 2017).

Animal grazing substantially degrades grasslands by reducing soil productivity and sustainability and soil nutrients (Fan et al., 2020; Li et al., 2013). Previous studies have shown that grazing can decrease soil organic carbon by 23.04% compared with fenced in meadow (Wu et al., 2010), and a similar effect was observed in sandy steppe (Li et al., 2006). A meta-analysis revealed that grazing decreased soil carbon stock in China's grasslands by 0.09 Mg/(ha•yr) (Deng et al., 2017). Grazing also substantially decreased total nitrogen and phosphorus by 27.4% and 10.5%, respectively, in alpine meadow soils (Ma et al., 2016). Grazing can increase other soil physicochemical properties, such as soil pH (Liu et al., 2019; Molaeinasab et al., 2018). Grazing-induced changes in soil physicochemical properties could collectively alter soil microbial compositions and functions. For instance, the relative abundance of bacteria (such as Bacteroidetes, Chlorobi, and Nitrospirae) and fungi decreased with increasing nutrient availability in grazed soils (Wang et al., 2019; Zhang et al., 2020). Correspondingly, soil microbial respiration, which is closely related to the quality and quantity of soil organic carbon, was significantly decreased by grazing (Li et al., 2013). Grazing also shifted the dominant microbial compositions from fungi degrading recalcitrant carbon compounds to bacteria decomposing more labile compounds (Xun et al., 2018). However, the effects of grazing on functional microbial groups associated with carbon cycling remain less understood.

Soil microbes play a dominant role in carbon cycling, particularly in fragile ecosystems (Khan et al., 2019; Liang et al., 2019). Cellulose, a major component of various plant litters (Corona et al., 1998), is decomposed by a group of enzymes, among which fungal glycoside hydrolase family 7 cellobiohydrolase I (encoded by *cbhI*) catalyze the rate-limiting step in the cellulose degradation (Edwards et al., 2008). Soil cellulolytic fungi are phylogenetically affiliated with Ascomycota, Basidiomycota and Chytridiomycota (Edwards et al., 2008), whose abundance range from 10^5 to 10^9 per gram dry soil (Chen et al., 2018; Fan et al., 2012; Zhang et al., 2017). Soil cellulolytic fungal abundance is influenced by the composition and concentration of both labile and total organic carbon (Li et al., 2017; Zhang et al., 2017). The abundance and richness of soil cellulolytic fungi increases with enhanced soil pH and decreased nitrate (Chen et al., 2018; Fan et al., 2012). These studies focused on the change of soil cellulolytic fungi at a specific time point, but less knowledge about the temporal variations of cellulolytic fungi is available (Grover et al., 2020; Weber et al., 2011, 2012; Zhang et al., 2017). Given that grazing changes soil physicochemical properties and plant biomass (Deng et al., 2014), and that grazing effects are dependent on growing months (Fan et al., 2020), the quantity and quality of soil cellulose are expected to change with grazing and months. However, the grazing effects on the cellulolytic fungal community and its temporal variation remain largely unknown.

In this study, we investigated the effects of grazing on the cellulolytic fungal community by targeting the *cbhI* gene in alpine steppe on the Tibetan Plateau. The Tibetan Plateau (> 4000 m a.s.l.) represents an ecologically fragile ecosystem, characterized by drought, year-round low temperatures, high ultraviolet irradiation, and short growing months, that stores 33.52 Pg organic carbon in its soils (Wang et al., 2002). It is estimated that over 110,636 thousand hectares of grassland on the Tibetan Plateau are experiencing severe grazing (Wu and Yang, 2000), leading to an unstable pool of soil organic carbon (Yang et al., 2010). The aim of this study was to reveal how the management of grazing influences cellulolytic fungi, an indicator of the degradation process for soil organic matter, and the key soil properties that drive the cellulolytic fungal community in alpine steppe soils during the growing months (from May to September). For this purpose, we investigated the temporal patterns of soil cellulolytic fungal abundance and community structure in grazed and fenced steppe. We hypothesized that (1) Grazing would decrease soil cellulolytic fungal abundance and change their community structure, (2) Grazing would weaken the effect of nutrients on soil cellulolytic fungal community, and the key soil properties that drive the cellulolytic fungi would vary in grazed and fenced steppe soils, and (3) The grazing effect on the cellulolytic fungal community was dependent on growing months.

1. Materials and methods

1.1. *Field experimental setup*

The study was conducted at the Nam Co Monitoring and Research Station for Multi-Sphere Interactions, Chinese Academy of Sciences (36°46′N, 90°59′E, 4730 m a.s.l). This station is located on the southeast shore of Nam Co Lake, northern slope of the Mt. Nyenchen Tanglha. The local climate is characterized by long, cold winters and short, mild summers, with a mean annual temperature of -0.6°C. The region is semiarid with an annual precipitation of about 414.6 mm, which mainly occurs between May and September (Wei et al., 2014).

The field experiment contains two treatments (grazing and fencing treatments). The grazing treatment was a typical native spring and autumn pasture for yaks and sheep, and the fencing treatment had been performed in the alpine steppe since May 2011. Both treatments contained five randomly distributed plots (1 m × 1 m), with a 2 m buffer between them. Within each plot, five soil cores (0-10 cm), free of animal dung and plant debris, were randomly collected using a soil core (2.5 cm in diameter) and were mixed as a composite sample. The sampling sites were marked to avoid being repeatedly sampled in next month. Soil samples were collected in each plot in the middle of each month from May to September in 2016

(5 years after set-up of the fencing treatment). Each soil sample was sieved (< 2 mm), transported to laboratory in coolers with ice bags. Soil samples for DNA extraction were stored at -80°C, and the remaining soils, for physicochemical analyses, were air-dried.

1.2. Soil properties analysis

Soil properties were measured as in previous studies (Li et al., 2019; Zhao et al., 2018). Soil pH was determined in 1:2.5 (soil:water, *m*/V) by using a pH meter (PB-10, Sartorius, Germany). Soil water content (SWC) was gravimetrically determined after drying at 105°C for 12 hr. Soil ammonium and nitrate were extracted with 2 mol/L KCl and determined using Automated Discrete Analyzer (AQ2+, SEAL Analytical Inc., England). Soil total organic carbon (TOC) was measured in solid state using a TOC analyser (TOC-VCPH, Shimadzu, Japan). Soil dissolved organic carbon (DOC) was measured using freeze-dried soil (3 g), which was mixed with 15 mL Milli-Q water (solid/liquid, *m*/*V*, 1/5), and then shaken at 200 r/min and centrifuged at 8000 r/min for 10 min (Li et al., 2018). The supernatant was filtered through 0.45 μm and determined by a TOC analyser (TOC-VCPH and TNM-1, Shimadzu, Japan). Soil temperature (0-10 cm) was recorded by HOBO weather station (Onset Inc., Bourne, MA, USA).

1.3. DNA extraction and real-time quantitative PCR

Soil DNA was extracted using the PowerSoil® DNA Isolation Kit (MOBIO, USA) following the manufacturer's instructions. The purity and quantity of DNA were determined using the NanoDrop ND-1000 spectrophoto meter (NanoDrop Technologies Inc., Wilmington, DE, USA). The *cbhI* gene was quantified using the primer set fungcbhIF and fungcbhIR (Edwards et al., 2008) on a LightCycler 480II thermo cycler (Roche, Switzerland). Each PCR reaction (10 µL) contained 5 µL of SYBR® Green Premix (Takara Bio Inc, Shiga, Japan), 0.5 µL (10 µmol/L) of each primer, 1 µL DNA template. PCR amplification program was as follows: 94°C for 3 min, followed by 35 cycles of 30 sec at 94°C, 45 sec at 50°C, 90 sec at 72°C, and the fluorescence data were collected at 72°C. PCR product specificity was checked by the melt curve analysis and agarose gel electrophoresis (1%). The *cbhI* gene numbers were determined using a standard curve, which was generated by purified template plasmid DNA with a 10-fold dilution.

1.4. T-RFLP analysis of cellulolytic fungal community

The cellulolytic fungal community structure was screened by terminal restriction fragment length polymorphism (T-RFLP) profiles. The primer set and PCR conditions of the *cbhI* gene amplification were the same as described above, except that the forward primer fungcbhIF was labelled with 6-carboxyfluorescein (FAM) at the 5′ end. The PCR products were gel-purified using an AxyPrep DNA purification kit (AxyGen, USA). After gel purification, a 20 µL volume system was constructed, containing approximately 200 ng labelled DNA and 10 U of the enzyme (MspI). The digested products were purified using Sigma-Aldrich Spin Post Reaction Clean-Up columns (Sigma, USA), and then mixed with deionized formamide and the internal standard GeneScan-1000 LIZ (Applied Biosystems, USA). The mixtures were denatured for 3 min at 95°C, and the DNA fragments were screened using a 3130xL Genetic Analyzer (Applied Biosystems, USA). The peak areas of the terminal restriction fragments (T-RFs) that differed by ±1 bp were summed and considered to be a single fragment. The relative abundance of each T-RF was calculated as described as our previous study (Guo et al., 2015).

1.5. Cloning, sequencing and phylogenetic analysis of cellulolytic fungi

To identify the soil cellulolytic fungal taxa, clone libraries were generated from fencing treatment soils collected in July and September in fencing. A new PCR amplification was performed using the same primer set without FAM label and the same PCR amplification program as described above. Subsequently, PCR products were purified, ligated into the pGEM-T Easy Vector System I (Promega, Madison, WI, USA) and transformed into Escherichia coli JM109 competent cells according to manufacturer's instruction. Positive clones were screened by PCR amplification with the vector-specific primer set M13F and M13R (Zhao et al., 2018). For clone libraries of *cbhI* gene, 56 positive clones were randomly picked and sequenced using an ABI model 3730xL DNA analyser (Applied Biosystems, CA, USA).

Sequence introns were predicted and excised from *cbhI* sequences using Genewise 2.2.0, based on the hidden Markov model (HMM) for glycosyl hydrolase family 7 (PF00840). To use the newest database from PFAM (http://pfam.xfam.org), we constructed the HMM using HMMER version 2.3 (https://hmmer.janelia.org), so as to allow for compatibility with Genewise (Birney, 2004). Inferred amino acid sequences predicted by Genewise were imported to MOTHUR v.1.34.3 (Schloss et al., 2009), and operational taxonomic units (OTUs) were delineated at 93% amino acid similarity. BLASTP (www.ncbi.nlm.nih.gov/BLAST/) was employed to search GenBank for sequences closely related to each OTU. Phylogenetic tree was constructed using neighbour-joining method with a bootstrap of 1000 iterations in MEGA7.0 (Kumar et al., 2016). The phylogenetic tree was visualized using the online tool ITOL (https://itol.embl.de/). Sequences generated in this study have been deposited in the National Center for Biotechnology Information GenBank database under accession numbers MN399338-MN399372.

1.6. Statistical analysis

Statistical analyses were performed using R software (version 3.6.1, https://www.r-project.org). The temporal stability of cellulolytic fungal abundance was quantified as the ratio of mean abundance to its temporal s.d. in each plot over the five months of the experiment (Ma et al., 2017; Yang et al., 2017). All environment data were summarized by R package plyr. The differences in physicochemical properties and the *cbhI* gene abundance between fencing and grazing treatments of the same month were measured by Wilcox-test using R package ggpubr. Linear regressions were calculated by R packages basicTrendline and Hmisc . Redundancy analysis (RDA)

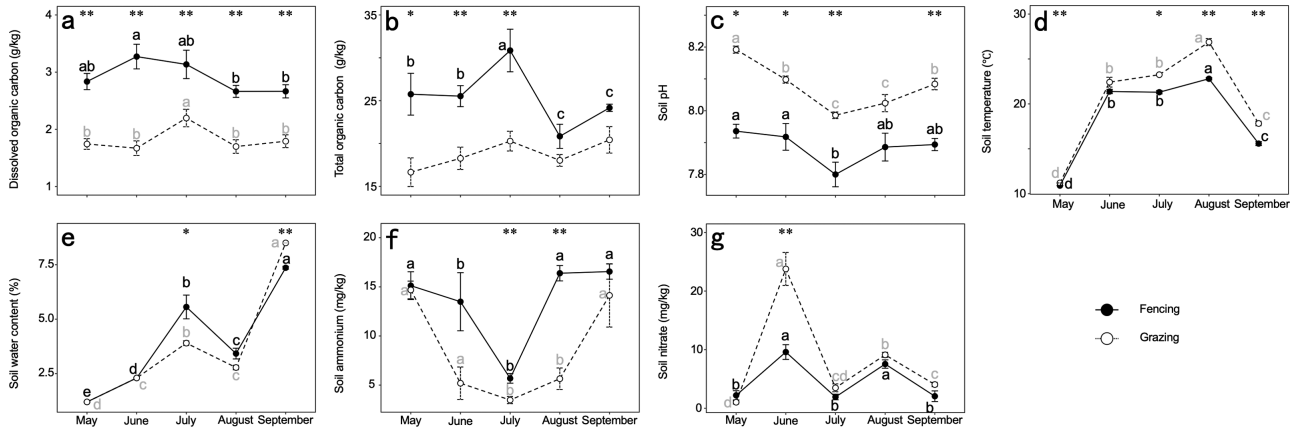

Fig. 1 – Changes in soil properties change over the growing months in grazing and fencing treatments, including (a) dissolved organic carbon, (b) total organic carbon, (c) soil pH, (d) soil temperature, (e) soil water content, (f) ammonium and (g) nitrate. The differences of soil properties between growing months were analyzed by ANOVA, and grey and black letters on plots represent the ANOVA results in grazing and fencing treatment, respectively. The difference within an individual month between treatments was analyzed by Wilcox-test: *$P < 0.05$, **$P < 0.01$.

was performed by R package vegan to compare the differences of the cellulolytic fungal community structure. Permutational multivariate analysis of variance (PERMANOVA) was used to determine the differences of the cellulolytic fungal community structure between consecutive months. To identify the contribution of soil properties on cellulolytic fungal community structure, we conducted hierarchical partitioning using R package rdacca.hp (Lai et al., 2021). Structural equation modeling (SEM) was performed using R package lavaan to explore the relationships among soil physicochemical factors, cellulolytic fungi community, fencing, and growing month (Rosseel, 2012). The community structure of cellulolytic fungi was represented by the NMDS1 of Non-metric Multidimensional Scaling (NMDS) analysis based on Bray-Curtis distance. We first considered a full model that incorporated all reasonable pathways based on linear regression and RDA results, and then sequentially removed non-significant relationships until all model parameters fit the criterions. The SEM fitness was evaluated by X^2 test ($P > 0.05$), $X^2/df < 3$, goodness of fit index (GFI), comparative fit index (CFI) > 0.90 and root mean square error of approximation (RMSEA) < 0.1 (Guo et al., 2015). All continuous variables were centralized and normalized to nondimensionalize. All the figures in this study were made using R packages ggplot2 and cowplot.

2. Results

2.1. *Effects of grazing on soil properties*

Grazing decreased DOC and TOC by 43%-63% and 16%-55% (**Appendix A Figs. S1a and b**), respectively, and the extent of decrease was dependent on the month (**Figs. 1a and b**). In both grazing and fencing, DOC and TOC both gradually increased and then decreased over the growing months, with the highest values being observed in July. Fencing significantly decreased soil pH (**Appendix A Fig. S1c**), from 7.94 and 8.19 in May to 7.80 and 7.99 in July, followed by a gradually increase to 7.89 and 8.08 in September in fencing and grazing treatments, respectively (**Fig. 1c**). Grazing significantly increased soil temperature (**Appendix A Fig. S1d**), which gradually increased from May to August, and then decreased in both grazing and fencing treatments (**Fig. 1d**). Soil water content (SWC) did not show significant differences between grazing and fencing treatments (**Appendix A Fig. S1e**). During growing months, SWC consistently increased, except for a slight reduction in August (**Fig. 1e**). Soil ammonium gradually decreased from May to July and then significantly increased from 3.48-14.69 mg/kg in the grazing treatment and from 5.68-16.56 mg/kg in fencing treatment between July and September (**Fig. 1f**). Soil nitrate did not show significant differences between grazing and fencing treatments (**Appendix A Fig. S1g**). During the growing months, nitrate fluctuated, with two spikes observed in June and August (**Fig. 1g**). Positive relationships between soil pH, DOC, and TOC were observed in both grazing and fencing treatments (all $P < 0.05$) (**Appendix A Fig. S2**).

2.2. *The* cbhI *gene abundance and their correlations with soil physicochemical properties*

Grazing significantly decreased *cbhI* gene abundance during the growing months ($P < 0.05$) (**Fig. 2a**), in particular, by 55% in May and by 58% in September (both $P < 0.05$) (**Fig. 2b**). Soil *cbhI* gene abundance gradually increased from May to July and thereafter decreased from July to September in grazing, whereas there was no significant change during growing months in the fencing treatment. Linear regression analysis demonstrated that the *cbhI* gene abundance in grazing positively correlated with DOC and soil temperature, but negatively with soil pH (all $P < 0.05$) (**Fig. 3**). In contrast, *cbhI* gene abundance did not correlate with any soil physicochemical properties in the fencing treatment. To further disentangle the relationships between the cellulolytic fungal community and environmental properties, an SEM was constructed. Consistent with the linear regressions, the SEM result demonstrated that DOC exerted a direct and positive influence on *cbhI* gene

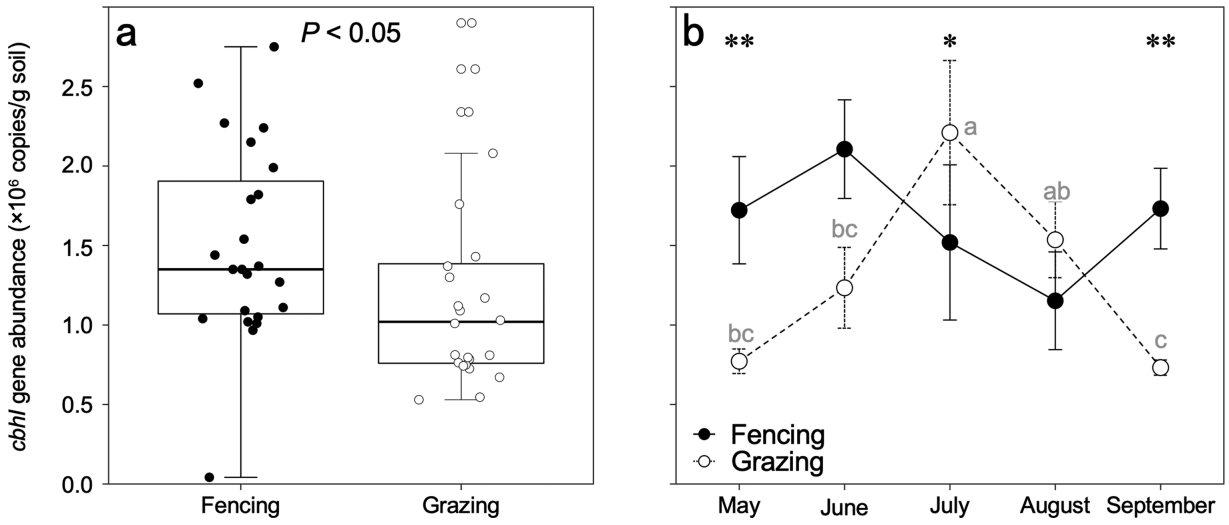

Fig. 2 – Cellulolytic fungal abundance in grazing and fencing treatments, including (a) the average abundance of the entire growing months and (b) during each growing month. The differences of cellulolytic fungal abundance between growing months were analyzed by ANOVA, and grey letters on panel (b) represent the ANOVA results in grazing treatment. The difference within an individual month between treatments was analyzed by Wilcox-test: *P < 0.05, **P < 0.01.

Table 1 – Monthly differences in soil cellulolytic fungal community structure in grazing and fencing treatments.

Test group		*P* value
Grazing treatment	May and June	**0.014**
	June and July	**0.003**
	July and August	0.893
	August and September	0.065
Fencing treatment	May and June	0.954
	June and July	0.052
	July and August	0.090
	August and September	0.069
Grazing and fencing		**0.001**

Results are based on Permutation multivariate analysis of variance: significance was assessed by randomized permutations procedure. Bold font indicates statistical significance ($P \leq 0.05$).

abundance, while grazing indirectly decreased the gene abundance by reducing DOC (both $P < 0.05$) (**Appendix A Fig. S3**).

2.3. *Cellulolytic fungal community structure and its relations with soil physicochemical properties*

A total of 18 *cbhI* T-RFs were detected across all soils (**Appendix A Fig. S4**). PERMANOVA analysis showed that the management of grazing significantly altered the community structure of cellulolytic fungi in soils ($P < 0.001$) (**Table 1 and Fig. 4a**). Redundancy analysis (RDA) revealed that the community structure variation was significantly explained by the management of grazing (8.14%), soil DOC (4.01%), and soil pH (4.09%) (all $P < 0.001$) (**Table 2**). In agreement with the RDA results, the SEM results further supported that grazing indirectly influenced the cellulolytic fungal community structure by enhancing soil pH (all $P < 0.05$) (**Appendix A Fig. S3**). Under grazing conditions, cellulolytic fungal community structure differed significantly between May and June as well as between June and July (both $P < 0.05$) (**Table 1 and Fig. 4b**). In contrast, no significant differences were detected in the cellulolytic fungal community structure between adjacent months in the fencing treatment (**Table 1 and Fig. 4c**). Additionally, the driving factors of cellulolytic fungal community structure were different under the two treatments. In the fencing treatment, the cellulolytic fungal community structure was primarily explained by soil SWC (6.15%) and pH (2.72%) (**Table 2 and Fig. 4b**). Under grazing conditions, however, the cellulolytic fungal community structure was primarily explained by nitrate (6.79%), ammonium (4.48%), and soil temperature (4.40%) (**Table 2 and Fig. 4c**).

2.4. *Taxonomic composition of cellulolytic fungal community*

To identify the taxonomic composition of the cellulolytic fungi, *cbhI* PCR products were cloned and sequenced for soils from the fencing treatment. A total of 56 clones were recovered from the soil samples, and were phylogenetically assigned to three groups, including Dothideomycetes, Sordariomyteces (both belong to Ascomycota), and Agaricomycetes (belong to Basidiomycota) (**Appendix A Fig. S5a**). All of these clones exhibited high sequence similarity with those from desert and forest soils (Weber et al., 2011). The relative abundances were 62.51%, 30.35%, and 7.14% for Dothideomycetes, Agaricomycetes, and Sordariomyteces, respectively (**Appendix A Fig. S5b**).

3. Discussion

3.1. *Grazing changed soil physicochemical properties*

We demonstrated that grazing significantly reduced soil DOC and TOC (**Fig. 1 and Appendix A Fig. S1**), implying reduced or-

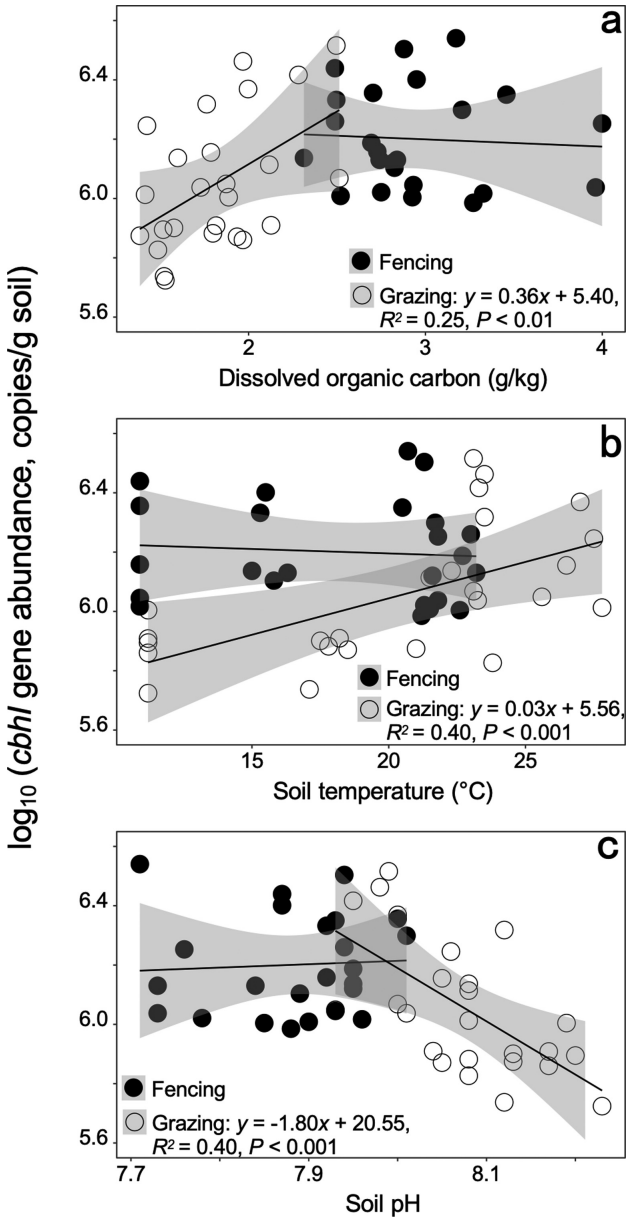

Fig. 3 – Linear regressions between cellulolytic fungal abundance and (a) dissolved organic carbon, (b) soil temperature and (c) soil pH in grazing treatment. Shadow areas indicate the 99% confidence interval. Cellulolytic fungal abundance is log-transformed.

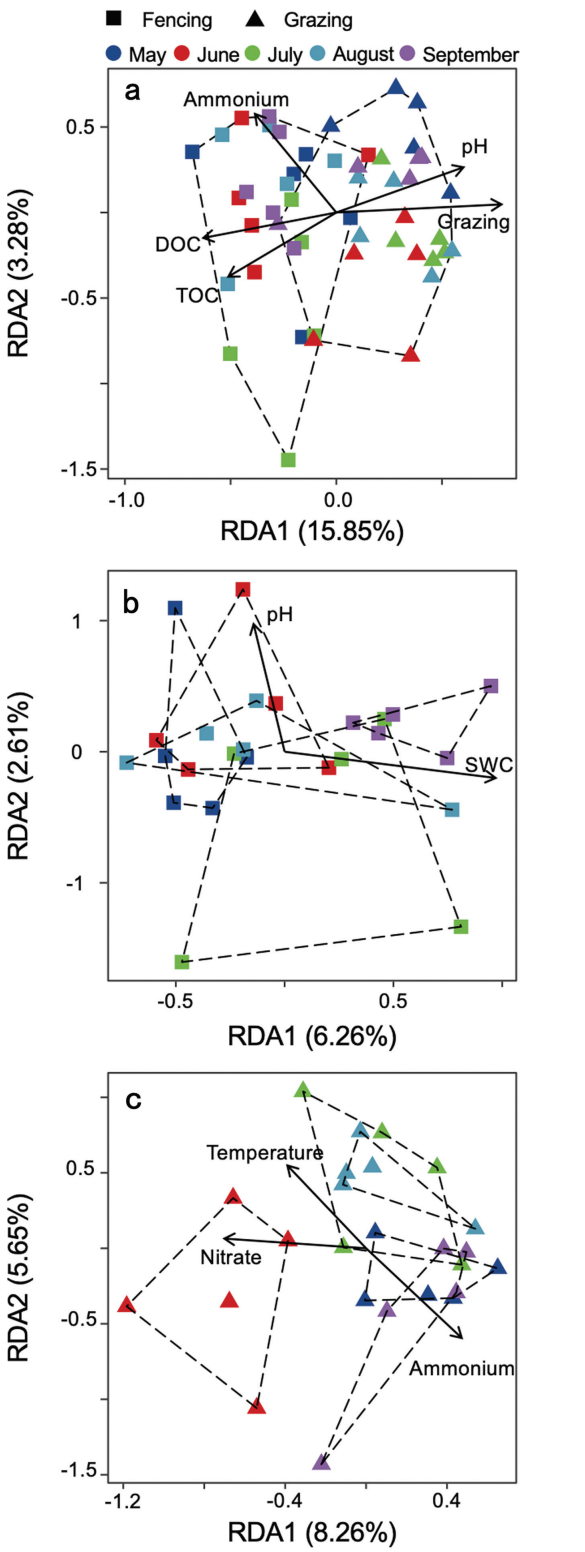

Fig. 4 – The redundancy analysis of cellulolytic fungal community composition of (a) grazing and fencing, (b) fencing treatment and (c) grazing treatment. DOC: dissolved organic carbon, TOC: total organic carbon, SWC: soil water content.

ganic matter stock in grassland soils on the Tibetan Plateau. The reduction of soil DOC and TOC could be attributed to the grazing-induced decrease in both plant litters and root exudates (Sun et al., 2018), both of which are crucial substrate resources for soil microbes (Don and Kalbitz, 2005; Ingelög and Nohrstedt, 1993; Iqbal et al., 2010; Lauber et al., 2009; Ren et al., 2018). Our results also demonstrated that grazing significantly enhanced soil pH (**Fig. 1c, Appendix A Figs. S1c and S3**), which resulted from the decreased DOC and TOC contents, as indicated by the negative correlations of soil pH with both DOC and TOC (**Appendix A Fig. S2**). This was in agreement with previous research showing that increased organic carbon gener-

Table 2 – Proportion of soil cellulolytic fungal community structure explained by the individual factors involved in redundancy analysis.

Properties	All treatments		Fencing		Grazing	
	Contribution	*P* value	Contribution	*P* value	Contribution	*P* value
Grazing	**8.14%**	**0.001**	–	–	–	–
Dissolved organic carbon	**4.01%**	**0.015**	–	–	–	–
pH	**4.09%**	**0.017**	2.72%	0.773	–	–
Ammonium	3.54%	0.062	–	–	4.48%	0.385
Nitrate	–	–	–	–	**6.79%**	**0.006**
Total organic carbon	2.86%	0.140	–	–	–	–
Soil temperature	–	–	–	–	4.40%	0.352
Soil water content	–	–	6.15%	0.111	–	–

The relative importance of different factor was calculated by hierarchical partitioning. Significance was assessed by randomized permutations procedure. Bold font indicates statistical significance ($P \leq 0.05$). "–" indicates that the factor was not included in redundancy analysis.

ally reduces soil pH (Wu et al., 2010). Thus, soil organic carbon plays a central role in driving soil fungal community through both direct (substrates) and indirect (changing soil pH) ways (**Appendix A Fig. S3**), which is consistent with previous studies (Kooijman and Cammeraat, 2010; Lucas and Davis, 1961; Rukshana et al., 2014). Additionally, grazing substantially increased soil temperature, due to soils with decreased vegetation coverage receiving greater amounts of solar radiation (Facelli and Pickett, 1991; Yan et al., 2018). Soil temperature regulates microbial activity and physiology by affecting enzyme kinetics (Guo et al., 2017). Our results indicate that grazing profoundly changes soil physicochemical properties, which collectively play a key role in shaping soil microbial community associated organic matter decomposition.

3.2. *Grazing reduced soil cellulolytic fungal abundance and its stability*

Our results demonstrated that grazing significantly reduced the abundance of cellulolytic fungi (**Fig. 2a**), indicating a decrease in the amount of available cellulose for these organisms in grazing treatment. This is consistent with previous observations that grazing greatly decreased plant biomass inputs into soils, and consequently reduced soil microbial abundance (Cheng et al., 2016; Jing et al., 2014). The lower *cbhI* gene abundance in the grazed soil point to the importance of the fungi community in cellulose degradation in the alpine steppe. Interestingly, our results showed that the *cbhI* gene abundance substantially changed over the course of the growing months in the grazing treatment, but did not change within the fencing treatment, suggesting that grazing changed the cellulolytic fungal abundance to an unstable state. This reduction of the monthly stability within the grazing treatment could be due to the relatively lower soil nutrients and resources (Ghimire et al., 2019; Shade et al., 2012), compared with the fencing treatment. This hypothesis is supported by the positive correlation between soil DOC and *cbhI* gene abundance in the grazing treatment, consistent with a previous study that increased carbon availability enhanced *cbhI* gene abundance in soils (Li et al., 2017). By stark contrast, the loose (non-significant) correlation between *cbhI* gene abundance and soil DOC in the fencing treatment was detected, suggesting that the abundance of cellulolytic fungi was not constrained by soil nutrients and energy (De Vries and Shade, 2013; Shade et al., 2012). In combination, the constraint of cellulolytic fungal abundance by nutrients and energy under grazing conditions illustrates the ecological significance of cellulolytic fungi in organic matter decomposition and soil carbon cycling.

The positive correlation between soil temperature and *cbhI* gene abundance in the grazing treatment suggested that the cellulolytic enzyme activity could be enhanced by soil temperature (Guo et al., 2017). Soil temperature is one of the key soil properties that regulate microbial metabolism (Guo et al., 2015; Wang et al., 2006), and has been shown to increase the abundance of Ascomycota and Basidiomycota (Baldrian et al., 2012; Zhang et al., 2016). The negative correlation between *cbhI* gene abundance and soil pH was contrasted with observations in upland-paddy soil (pH 5.0-5.9) (Chen et al., 2018). Such a discrepancy could be explained by the difference in soil pH between the two ecosystems. The optimum pH of cellobiohydrolase ranges from 3.0 to 6.0 (Wahab et al., 2019). The steppe soils in this study were alkaline (**Fig. 1c**), thus grazing-induced pH increase may reduce the abundance of cellulolytic fungi. These results collectively indicate that grazing destabilized the abundance of soil cellulolytic fungi.

3.3. *Grazing reduced the stability of soil cellulolytic fungal community structure*

Our results revealed that the management of grazing played a more important role in driving cellulolytic fungal community structure than soil properties, including DOC, TOC, nutrients, and pH (**Table 2, Fig. 4a and Appendix A Fig. S3**). High resource availability and favorable abiotic conditions usually facilitate the survival and growth of soil microbes (Wallenstein and Hall, 2012; Yuan et al., 2012; Zhang et al., 2018). The changes of these factors also hint at a varied potential for cellulolytic fungi to decompose cellulose in the face of environmental change scenarios. The key soil properties driving the community structure substantially differed between grazing and fencing treatments. Soil temperature played an important role in influencing cellulolytic fungal community structure in grazing treatment, likely due to the accelerated metabolic

activities from increased temperature (Marañón et al., 2018). This is consistent with other microbial communities in Tibetan Plateau soils, highlighting the temperature effect under the year round low temperature conditions (Guo et al., 2015; Zhao et al., 2018).

The monthly variation of soil cellulolytic fungal community structure was substantially greater under grazing treatment than fencing. Shifts in cellulolytic fungal community structure are linked to resource availability under land management (Li et al., 2017). Thus, such a discrepancy may result from the differences in resource availability and abiotic conditions between grazing and fencing, consistent with that the soil properties driving community structure differed substantially. In grazing treatment, the variation in community structure of cellulolytic fungi was mainly driven by soil nutrients (nitrate and ammonium) and soil temperature (**Table 1, Fig. 4c**). Soil nutrients are well documented as the driver of microbial community structure (Allison et al., 2007; Hu et al., 2014). In barren soils, nutrient and resource additions often inhibit the growth of oligotrophic fungi, but accelerate the growth of saprotrophic fungi (Malik et al., 2020; Shade et al., 2012). Soil cellulolytic fungi are primarily saprotrophic and prefer soils with higher plant litters and organic carbon containing greater aromaticity (Colpaert and Van Tichelen, 1996; Crowther et al., 2012; Li et al., 2017; Zhang et al., 2017). Grazing generally reduces plant litters and thus altered the quantity and quality of organic carbon in soils (Araújo et al., 2013). Thus, grazing provides fewer nutrients and resources to soils and strengthens the nutrient limitations on cellulolytic fungi, which is in agreement with decreased plant-derived nutrient inputs could reduce the resilience and stability of soil microbial communities (De Vries et al., 2012). This is partly consistent with the important roles soil ammonium and nitrate played in driving cellulolytic fungal community structure in the grazing treatment, while soil pH and SWC played key roles in the fencing treatment (**Fig. 4**). This result implied that grazing-driven changes of abiotic properties reduced the temporal stability of the cellulolytic fungal community structure during the growing months.

3.4. *Cellulolytic fungi were affiliated with Ascomycota and Basidiomycota*

Cellulolytic fungal taxa were phylogenetically affiliated with Ascomycota and Basidiomycota in this study. The clones were phylogenetically closely related to those isolated from aspen plantation, pine plantation, and American desert soils (**Appendix A Fig. S5**). Fungal decomposers have been found to be dominated by Ascomycota and Basidiomycota in aquatic environments (Jones et al., 2015), semiarid Loess Plateau soils (Tian et al., 2017), and alpine soils (Xiong et al., 2014). The relative abundances of Ascomycota and Basidiomycota in soil are highly influenced by plants. For example, in high-elevation grasslands with little vegetation, Basidiomycota dominated soil cellulolytic fungal community, whereas Ascomycota dominated low altitude sites with abundant vegetation (Cui et al., 2019). Our results also demonstrated a higher relative abundance of Ascomycota than Basidiomycota in the fencing treatment, which contained more plants, consistent with that the relative abundance of Basidiomycota decreased with increasing plant detritus (Hanson et al., 2008). Overall, the varied cellulolytic fungi in steppe soils reveal their ecological significance with respect to the cellulose degradation, decomposition of organic matter, and cycling of carbon in the context of land management.

4. Conclusions

In conclusion, our results demonstrated that grazing decreased soil nutrients and resources, but increased soil pH and temperature. These changes of abiotic soil properties consequently reduced the temporal stability of cellulolytic fungal abundance and community structure during the growing months. We thus speculated that grazing strengthens the nutrient limitations on cellulolytic fungi and enhances the correlations between the cellulolytic fungal community and soil properties. In contrast, resources and nutrients exhibited insignificant influences on cellulolytic fungi present in the fencing treatment, where resources and nutrients were relatively abundant. Therefore, our results highlight the negative role of grazing on dry steppe restoration by reducing soil nutrients and the stability of the organic matter-degrading microbial community.

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.09.023.

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