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RESEARCH ARTICLE

Fungal but not bacterial β-diversity decreased after 38-year-long grazing in a southern grassland

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Abstract
Aims Livestock grazing greatly affects the soil environments and soil microbial community, potentially driving significant biodiversity losses. Yet, how soil microbial communities respond to grazing remains relatively unknown in southern grasslands. This study hypothesized that long-term grazing alters soil microbial community composition and reduces microbial diversity by changing underlying soil properties.

Methods To assess the impact of long-term grazing on soil properties, bacterial and fungal diversity and microbial community composition were investigated in replicate grazed (38 years of moderate intensity grazing) and ungrazed plots in a subtropical grassland, China.

Results Fungal β-diversity was more sensitive to long-term grazing than bacterial β-diversity, with fungal β-diversity decreasing by 28.8%. No significant differences in soil bacterial or fungal α-diversity were detected between grazed and ungrazed plots. Additionally, long-term grazing altered microbial community composition, altering the relative abundance of specific microbial taxa. For bacteria, the relative abundance of *Actinobacteriota* (41.9%) increased and *Acidobacteriota* decreased (−22.3%). For fungi, grazing increased the relative abundance of *Mortierellomycota* (108.1%) and decreased *Basidiomycota* (−79.5%). Changes in both bacterial and fungal community composition were well explained by available phosphorus, dissolved organic nitrogen, dissolved organic carbon, soil organic carbon, total soil nitrogen, and NH_4^+-N.

Conclusions Our study showed that fungal β-diversity decreased after long-term grazing, necessitating changes to grazing management practices to foster soil biodiversity conservation and functions in southern grasslands.

Keywords Long-term grazing · Microbial diversity · Microbial community composition · bacteria · fungi

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Juan Zhou and Meiyan Zhang contributed equally to this work.

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Introduction

Grassland ecosystems provide important functions and services, such as biodiversity conservation and carbon (C) sequestration (Filazzola et al. 2020; Koerner et al. 2018; Lorenz and Lal 2018). However, grasslands are increasingly subject to grazing worldwide (Fetzel et al. 2017; Kemp et al. 2013; Zhang et al. 2022a). Intensive, long-term grazing can lead to vegetation and soil degradation (Gao and Carmel 2020; Pulido et al. 2018; Zhang et al. 2022b). Degraded grasslands are not only incapable of meeting the needs of livestock and their human keepers, but can also show altered soil community functions (Ren et al. 2017; Stark et al. 2015; Zhao et al. 2017), threatening soil biodiversity and ecosystem functions (Buisson et al. 2022; Eldridge et al. 2016; Zhang et al. 2022a).

Soils harbor an enormous diversity of microorganisms, among which bacteria and fungi play critical roles in ecosystem functions, including organic matter decomposition and soil C dynamics, as well as mediating nutrient cycling (Bahram et al. 2018; Yang et al. 2022). Generally, soil bacterial and fungal diversity promote plant nutrient uptake via accelerating nutrient mineralization, potentially enhancing plant productivity (Delgado-Baquerizo et al. 2020; Wagg et al. 2019). Given the enormous diversity and importance of soil microbial communities to ecosystem functions, understanding the response of microbial diversity and composition to grazing is crucial for conserving soil biodiversity (Delgado-Baquerizo et al. 2016; Garcia-Palacios and Chen 2022).

Grazing can influence soil microbial communities in multiple ways (Fig. 1). First, grazing may modify soil conditions via animal trampling, which then directly affect soil microbial communities (Liu et al. 2015; Yang et al. 2013). Also, animal trampling results in soil compaction, disturbance, and erosion and increased soil pH (Bagchi et al. 2017; Qu et al. 2021). Previous studies have demonstrated that soil pH is an important factor altering soil microbial diversity and community composition (Rousk et al. 2010; Zhalnina et al. 2015). Second, grazing can also indirectly affect soil microbial communities by increasing soil nutrient availability via the deposition of urine and feces (Liu et al. 2018). Soil nutrients are another vital driver of

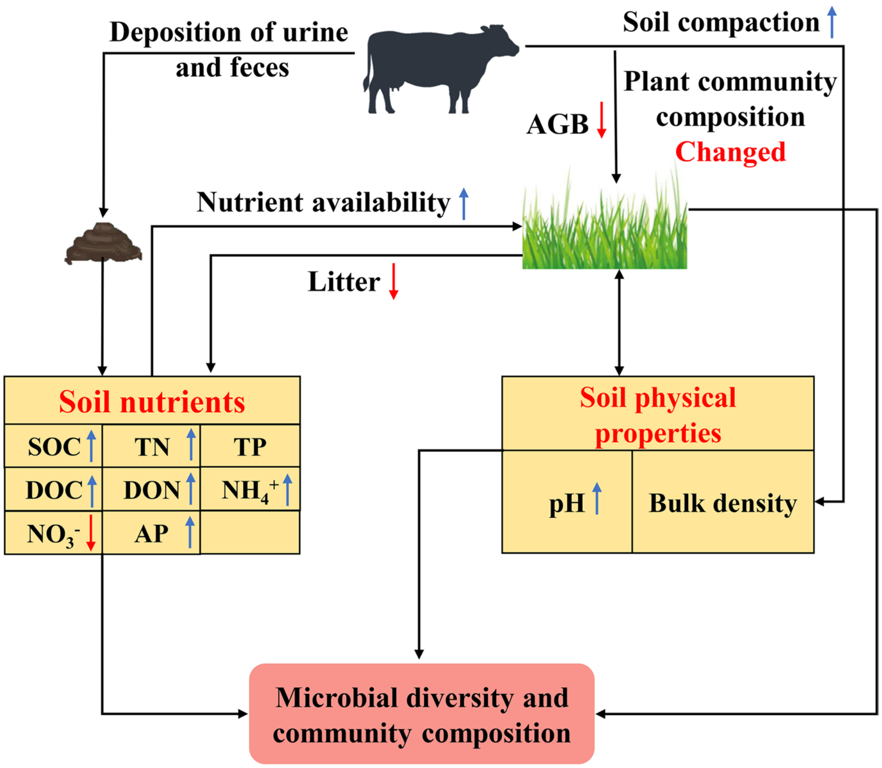


Fig. 1 Conceptual diagram showing the potential mechanisms of microbial diversity and community composition in response to grazing. AGB, aboveground biomass; SOC, soil organic carbon; TN, total soil nitrogen; TP, total soil phosphorus; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; AP, available phosphorus

microbial diversity and community composition (Koyama et al. 2014; Pan et al. 2014). For example, nitrogen and phosphorus additions have been reported to reduce microbial diversity (Leff et al. 2015; Ling et al. 2017). Third, grazing can influence soil microbial communities by changing the plant productivity and community composition (Chen et al. 2021). Grazing can greatly reduce the biomass of aboveground vegetation (Hao et al. 2018; Li et al. 2017; Yang et al. 2018) and result in less carbon allocated to roots and the soil (Bai et al. 2015). The carbon limitation then decreases soil microbial biomass and diversity (Veen et al. 2014). Low microbial diversity is an "early warning sign" of biodiversity losses and is therefore important to future biodiversity conservation (Wang et al. 2022). Though many studies have investigated the mechanisms underlying grazing effects on microbial community composition and diversity, most have only focused on short-term grazing effects (Bezemer et al. 2006; Zhao et al. 2019). The lack of research on long-term grazing effects limits our understanding of grazing effects on grassland ecosystems.

Grasslands in southern China are located in temperate and subtropical regions influenced by the monsoon climate, unlike the radiation-driven seasonal climates of northern China. Southern grasslands also have different vegetation and soils compared to alpine meadows and temperate steppes (Wang et al. 2019b). Thus, they may respond differently to environmental disturbance. Most studies of the southern grasslands to date have focused only on how grazing affects plant and soil properties, with relatively few studies examining how grazing affects soil microbial community composition and diversity, despite the importance of soil microbes for ecosystem functions (Chen and Sinsabaugh 2021; Delgado-Baquerizo et al. 2016). In this study, soil samples were collected from grazed soils (grazed continuously for 38 years) and adjacent non-grazed soils to explore how long-term grazing affects soil microbial diversity and community composition in the southern grasslands of China. Based on previous studies, the following hypotheses were tested: (1) long-term grazing alters microbial diversity and community composition and results in lower microbial diversity in southern grasslands; and (2) soil microbial community responses to long-term grazing may be mediated by soil properties in southern grasslands.

Materials and methods

Site description

This experiment was conducted at the Yunnan Academy of Grassland and Animal Science in Kunming City, Yunnan Province, China. The study site is located at 25°21′N, 102°58′E and has an elevation of 1962 m a.s.l. The area experiences a subtropical plateau monsoon climate with a mean annual temperature of 13.4 °C (minimum average temperature of 6 °C) and mean annual precipitation of 990 mm (Gao et al. 2013). Most precipitation occurs during the monsoon months of June to October. The precipitation is particularly low in winter and spring (Tang et al. 2013). Soils are silty loam mountain red soils with pH of 5.0, organic matter content of 2.28%, available nitrogen content of 23.10 mg kg^{-1}, and available phosphorus content of 7.58 mg kg^{-1} (Wu et al. 2013). Before 1983, the vegetation was dominated by *Pteridium revolutum*, *Rubus pectinellus*, *Arundinella hookeri*, and *Heteropogon contortus*. An artificial grassland was established in 1983. Woody plants and most of the understory community were clear cut, and grass species (i.e., *Trifolum repens*, *Setaria sphacelate*, *Dactylis glomerate*, *Penniselum elandestinum*, and *Lolium multiflorum*) were broadcasted. After 38 years of grassland managements, the vegetation is now dominated by *Pennisetum clandestinum* cv. Whittet, *Setaria sphacelata* (Schum) Stapf ex Massey cv. Narok and *Trifolium repens* L. cv. Haifa (Gao et al. 2013).

Experimental design, soil sampling and measurements

From 1983 until April 2021, the study area has been continuously grazed by cattle (throughout the year). Stocking rates were 2 AU ha^{-1} (moderate intensity). Paddocks adjacent to grazed plots, where no grazing had taken place for more than 38 years, were selected as controls. A random block design was used with ten replicates of both grazed and ungrazed plots. A total of ten blocks and 20 plots were established with an area of 10 × 10 m, and the distance among blocks was more than 50 m. A composite soil sample consisting of five cores (depth 0-10 cm) was randomly obtained from each plot using a soil auger. Each composite soil sample was mixed thoroughly and sieved and then divided into two subsamples. Fresh soil was used to determine soil ammonium nitrogen (NH_4^+ -N), soil nitrate nitrogen (NO_3^- -N), dissolved organic carbon, dissolved organic nitrogen, soil moisture, and soil microbial community. Air-dried soil was used to determine soil organic carbon, soil pH, total soil nitrogen, total soil phosphorus, and available phosphorus.

DNA extraction and high-throughput sequencing

Bacterial 16S rRNA sequences were amplified with the primer pair 515F and 806R (Caporaso et al. 2011), and fungal 18S rDNA was amplified with the primer pair ITS5-1737F and ITS2-2043R (Jiao et al. 2018). Soil microbial alpha (α)-diversity was quantified as the observed amplicon sequence variants (ASV) diversity, the Chao1 index, Shannon-Wiener index, and Simpson index; beta (β)-diversity were also recorded.

Statistical analyses

Soil properties and microbial alpha (α) diversity were compared using *t*-tests. Microbial community composition was estimated using non-metric multidimensional scaling (NMDS) based on the Bray–Curtis distances among samples; NMDS was performed using the "vegan" package (Oksanen et al. 2020). PERMANOVAs were then performed to test how long-term grazing affected soil bacterial and fungal community composition. Random forest (RF) analyses were used to estimate the relative importance of grazing-induced changes in soil bacterial and fungal communities. Relative importance was expressed as percentage increases in the mean squared error (MSE). The significance of each predictor was assessed via the "rfPermute" package (Archer 2021). All statistical analyses were conducted in R 4.0.2.

Results

Soil physicochemical properties

Long-term grazing had a positive effect on soil moisture and pH. Long-term grazing increased most soil nutrient concentrations (soil organic carbon, total soil nitrogen, available phosphorus, NH_4^+-N, dissolved organic carbon, and dissolved organic nitrogen). In contrast, NO_3^--N was significantly lower in grazed plots. Total phosphorous and soil bulk density were not affected by long-term grazing (Table 1).

Soil microbial diversity and community composition

Almost all alpha-diversity indicators for soil bacterial and fungal communities did not respond to long-term grazing, except that the Simpson index decreased for bacterial communities (Fig. 2). Fungal communities showed lower β-diversity in grazed plots versus control plots, while the β-diversity of bacterial communities did not differ between grazed and control plots (Fig. 3).

Across the soil samples, bacterial sequences primarily comprised the phyla *Proteobacteria* (30.9%), *Actinobacteria* (18.4%), *Acidobacteria* (15.4%), *Firmicutes* (6.9%) and V*errucomicrobiota* (7.9%) (Fig. 4). The most abundant fungal phyla were *Ascomycota* (50.4%), *Basidiomycota* (19.3%) and *Mortierellomycota* (18.1%) (Fig. 4). As shown in the NMDS for the ASV data, long-term grazing significantly affected both bacterial and fungal community composition (Fig. 4a and c). Grazing altered bacterial community composition by increasing the relative abundance of *Actinobacteriota* (41.9%) and *Firmicutes* (179.7%), and by decreasing the relative abundance of *Verrucomicrobiota* (−57.7%), *Acidobacteriota* (−22.3%), and *Planctomycetota* (−40.8%) (Fig. 4b). Similarly, grazing altered fungal community composition by increasing the relative abundance of *Mortierellomycota* (108.1%) and by decreasing the relative abundance of *Basidiomycota* (−79.5%) (Fig. 4d).

Edaphic factors shaped soil microbial community composition

In Mantel tests, multiple soil properties were significantly correlated with soil microbial community composition (Fig. 5). Increases in the bacterial taxa *Actinobacteriota* and *Firmicutes* were mainly driven by soil organic carbon and soil pH, respectively. *Acidobacteriota* was correlated with soil organic carbon and dissolved organic carbon. *Verrucomicrobiota* was correlated with soil organic carbon, available phosphorus, dissolved organic carbon, dissolved organic nitrogen, and pH (Fig. 5a). Additionally, significant

Table 1 Effects of grazing on soil properties

Treatment	pH	Moisture	Soil BD (g cm^{-3})	SOC (g kg^{-1})	TN (mg kg^{-1})	TP (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	AP (mg kg^{-1})	DOC (mg kg^{-1})	DON (mg kg^{-1})
Control	5.70 ± 0.06a	0.27 ± 0.01a	0.84 ± 0.03	44.40 ± 1.57a	3.66 ± 0.43a	1.04 ± 0.12	6.42 ± 0.24a	2.34 ± 0.77b	2.45 ± 0.39a	49.34 ± 8.25a	7.90 ± 3.17a
Grazed	5.95 ± 0.05b	0.34 ± 0.02b	0.74 ± 0.05	79.79 ± 4.38b	8.06 ± 0.52b	1.16 ± 0.17	18.28 ± 2.63b	0.80 ± 0.13a	48.5 ± 7.33b	151.6 ± 18.59b	145.70 ± 22.84b

SOC soil organic carbon, *TN* total soil nitrogen, *TP* total soil phosphorous, *NH_4^+-N* ammonium nitrogen, *NO_3^--N* nitrate nitrogen, *AP* available phosphorous, *DOC* dissolved organic carbon, *DON* dissolved organic nitrogen. Different lowercase letters indicate a statistically significant difference between grazed and control treatment ($P < 0.05$)

correlations were found between the fungal taxa *Basidiomycota* and soil organic carbon, total soil nitrogen, available phosphorus, dissolved organic carbon, soil moisture, and pH. Changes in soil moisture and soil organic carbon explained the positive response of *Mortierellomycota* to grazing (Fig. 5b).

The random forest (RF) analysis found that changes in both bacterial and fungal community composition were best explained by available phosphorus. Other important variables for predicting soil microbial community composition were dissolved organic nitrogen, dissolved organic carbon, soil organic carbon, total soil nitrogen, and NH_4^+-N (Fig. 6).

Discussion

Grazing has a major impact on microbial communities, but there is a lack of consistency in the existing reports on the actual effects of grazing on microbial diversity due to diverse study areas, herbivore types, grazing intensities and regimes. Our study provided a clear evidence that long-term grazing reduced fungal β-diversity, but did not change the microbial α-diversity in southern grasslands. Overall, this study highlights the risk of grassland microbial community homogenization with biodiversity reduction due to grazing disturbance.

Grazing effects on soil properties and microbial α-diversity

Our results showed that long-term grazing resulted in increased nutrient concentrations, suggesting that this grassland management practice may affect nutrient cycling in southern grasslands. However, previous studies have found grazing decreased soil nutrients (Wu et al. 2022; Yang et al. 2019). Two pathways may primarily determine how grazing affects soil nutrient concentrations. In the first pathway, grazing directly increases carbon, nitrogen, and phosphorus concentrations due to accumulation of dung and urine (Bai et al. 2012; Chen et al. 2021). In the second pathway, grazing indirectly decreases nutrient concentrations by reducing above- and belowground plant biomass (Chen et al. 2021). Our findings may largely result from the first pathway.

Our study suggested that long-term grazing did not change microbial α-diversity, consistent with

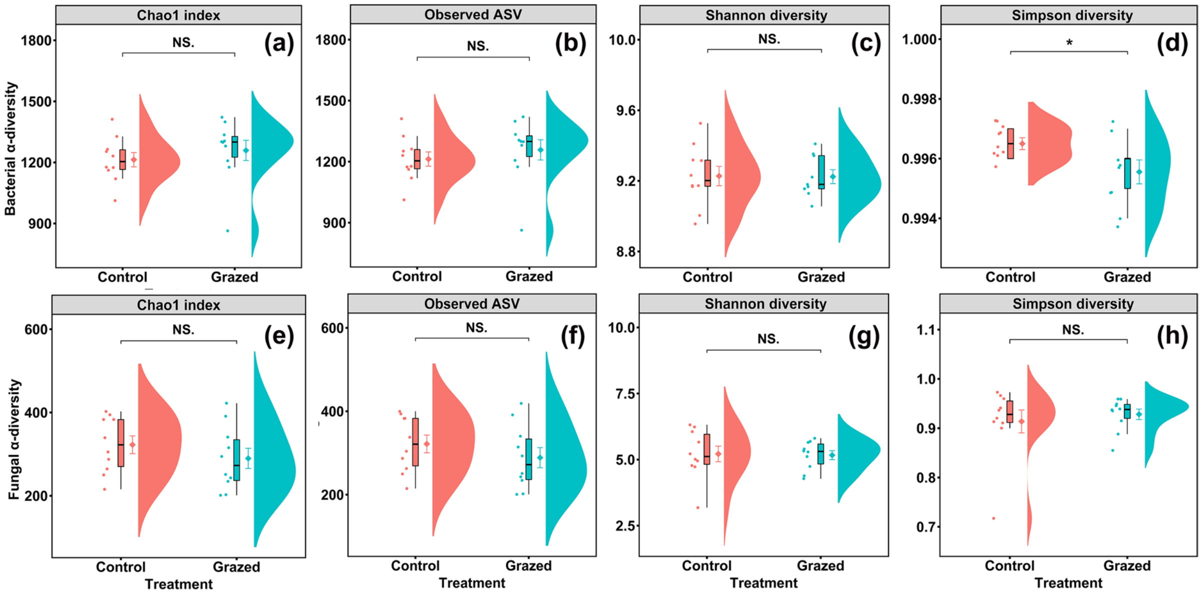
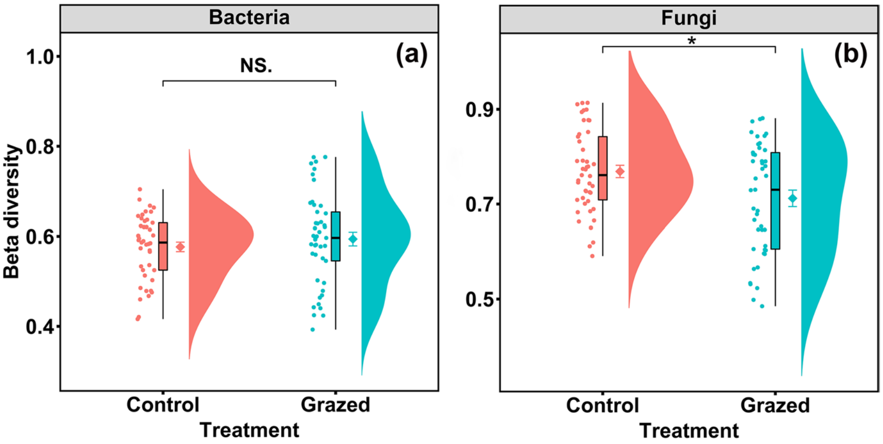


Fig. 2 Effects of grazing on soil bacterial and fungal α-diversity. NS, not significant. *, **, and *** indicate $P<0.05$, $P<0.01$, and $P<0.001$, respectively

Fig. 3 Effects of grazing on soil bacterial and fungal β-diversity. NS, not significant. *, **, and *** indicate $P<0.05$, $P<0.01$, and $P<0.001$, respectively



previous findings from Atlantic mountain grasslands (Aldezabal et al. 2015) and Qinghai-Tibetan Plateau grasslands (Qin et al. 2021). Grazing also had only marginal effects on bacterial or fungal α-diversity, which might be explained by the resilience of microbial communities to environmental disturbances, thus sustaining a certain α-diversity (Brown and Jumpponen 2015; Qin et al. 2021). However, previous studies have found that grazing significantly increased (Wang et al. 2019a; Wu et al. 2022) or decreased (Zhang and Fu 2021) bacterial α-diversity, inconsistent with our findings. This discrepancy may result from the following reasons. First, it is due to the variation in study climate and vegetation type. The study area used here has a monsoon climate, unlike the radiation-driven seasonal climate of northern China. Also, southern grasslands have different vegetation and soils compared to alpine meadows and temperate steppes (Wang et al. 2019b). Second, the minor changes in bacterial or fungal α-diversity may result from grazing intensity (Delgado-Baquerizo et al. 2016). The effects of grazing on soil microbial community were largely depended on the grazing intensity. A global meta-analysis suggested that light and

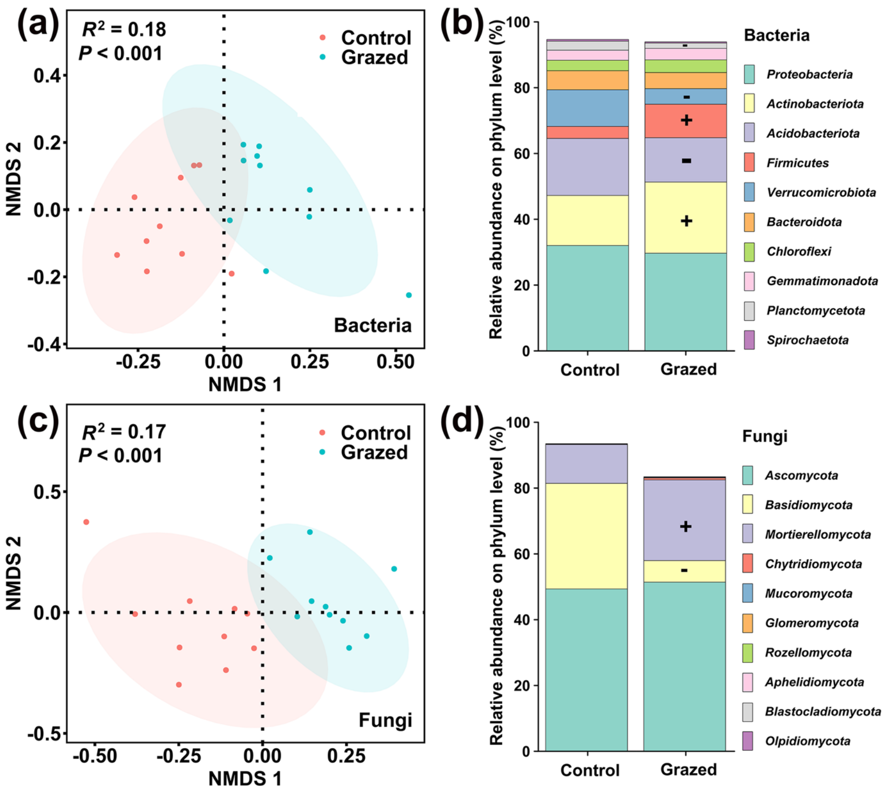


Fig. 4 Effects of grazing on soil bacterial and fungal community composition. The symbols '+' and '-' indicate significant ($P < 0.05$) increases and decreases, respectively

moderate grazing intensity did not significantly affect soil bacterial and fungal community, but heavy grazing intensity had negative on soil bacterial and fungal community (Zhao et al. 2017). In our study, the grazing intensity was moderate. Thus, no significant grazing effect on bacterial or fungal α-diversity was observed. Third, grazing regime (i.e., seasonal grazing or continuous grazing) may drive this discrepancy. For example, soil microbial community was more stable in continuous grazing than winter grazing (Yang et al. 2019). Our study provides evidence for how soil microbial α-diversity responds to grazing in southern grasslands, broadening our understanding of the effects of grazing on grassland ecosystems.

Grazing effects on soil microbial β-diversity and community composition

Grazing had different effects on bacterial and soil fungal β-diversity. Our study showed that long-term grazing caused a decline in soil fungal β-diversity, but not soil bacterial β-diversity. This suggests that soil fungi may favor less perturbed ecosystems than soil bacteria (Chen et al. 2021; Tolkkinen et al. 2015). Soil fungi have a faster growth rate, while bacteria are more resilient to disturbances (Wardle 2002); therefore, fungi might respond more rapidly to grazing, maintaining relatively stable communities. Meanwhile, filamentous fungi have more limited dispersal owing to their larger size, and therefore disturbed environments can reduce soil fungal diversity (Dassen et al. 2017). Here, fungal communities became more similar in composition over time due to grazing. Additionally, the decline in fungal β-diversity might have resulted from a concomitant decline in plant productivity; fungal and plant communities are often tightly linked, especially for arbuscular mycorrhizal fungi (Chen et al. 2018; Yang et al. 2019).

Grazing significantly altered microbial community composition, in large agreement with the first study hypothesis. Consistent with previous studies, grazing resulted in increases in the relative abundance of *Actinobacteriota* and *Firmicutes*, and decreases in the relative abundance of *Acidobacteriota* and *Verrucomicrobiota* (Qin et al. 2021; Wu et al. 2022). As a dominant bacterial phylum,

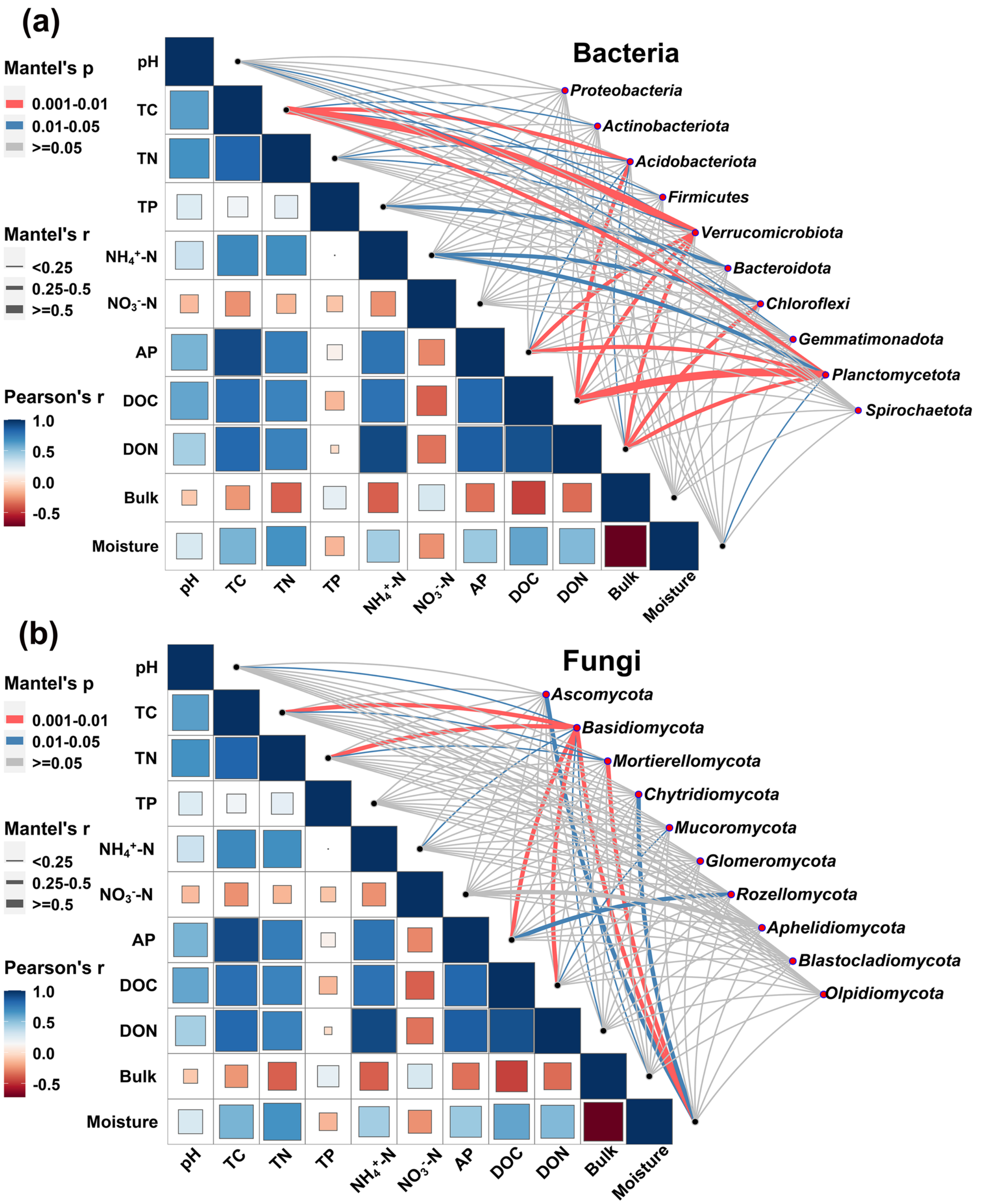


Fig. 5 Relationships between dominant microbial community phyla and soil properties. SOC, soil organic carbon; TN, total soil nitrogen; TP, total soil phosphorous; NH$_4^+$-N, ammonium nitrogen; NO$_3^-$-N, nitrate nitrogen; AP, available phosphorous; DOC, dissolved organic carbon; DON, dissolved organic nitrogen

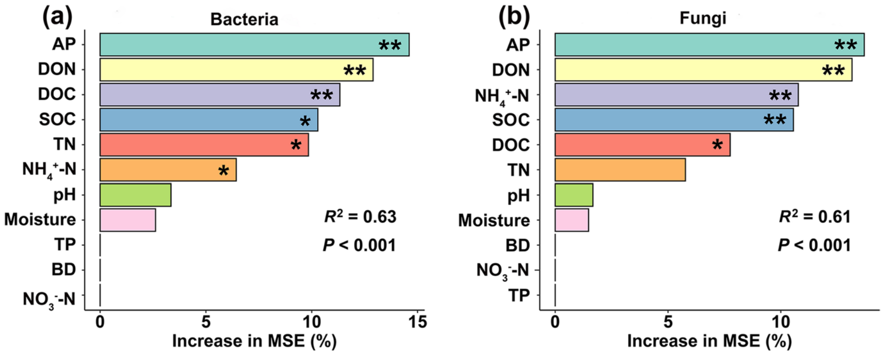


Fig. 6 Relationships between microbial community composition and soil properties. SOC, soil organic carbon; TN, total soil nitrogen; TP, total soil phosphorous; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorous; DOC, dissolved organic carbon; DON, dissolved organic nitrogen. *, **, and *** indicate $P<0.05$, $P<0.01$, and $P<0.001$, respectively

Actinobacteriota are widely distributed gram-positive bacteria that play key roles in microbial metabolism and organic matter turnover (Campbell et al. 2010; Qin et al. 2021; Stach et al. 2010). Increases in the relative abundance of copiotrophic *Actinobacteriota* may suppress subdominant microbial phyla, such as *Acidobacteriota* and *Verrucomicrobiota*, due to competitive exclusion. In addition, oligotrophic *Acidobacteriota* and *Verrucomicrobiota* prefer low nutrient environments (Eldridge et al. 2017; Maestre et al. 2015; Trivedi et al. 2012). Therefore, these phyla may be adversely affected by increases in soil nutrients (carbon, nitrogen and phosphorus) caused by grazing.

Additionally, grazing reduced the relative abundance of *Basidiomycota*, but increased the relative abundance of *Mortierellomycota*, thus resulting in changes in fungal community composition. This finding is supported by previous global studies (Angel et al. 2013; Manzoni et al. 2012). *Basidiomycota* are particularly sensitive to environmental disturbances and could be regarded as a fungal indicator of disturbance (Xun et al. 2018). Previous studies have identified soil fungal phyla, such as *Basidiomycota*, that favor resource-poor environments (Zechmeister-Boltenstern et al. 2015). Grazing-related increases in soil nutrients may be the main reason for the observed decrease in *Basidiomycota* abundance (Chen et al. 2021). Moreover, the increase in soil pH caused by grazing might impose physiological constraints on some soil fungi (e.g., *Basidiomycota*) (Maestre et al. 2015), as suggested by the Mantel tests here.

Changes in soil nutrient variables (i.e., available phosphorus, dissolved organic nitrogen, dissolved organic carbon, soil organic carbon, total soil nitrogen and NH_4^+-N) well explained the responses of bacterial and fungal community composition to grazing. These findings support the second study hypothesis that grazing-induced shifts in soil properties play an essential role in shaping soil microbial communities. The significant increase in soil nutrients under grazing might result from the direct effect of cattle dung and urine inputs, accelerating nutrient cycling and ultimately affecting soil microbial community composition (Bai et al. 2012; Chen et al. 2021). Although some studies have reported that soil pH was important factor affecting how microbial community composition responds to grazing (Qin et al. 2021; Zhalnina et al. 2015; Zhao et al. 2019), here, grazing-induced changes in pH had a negligible effect on microbial community composition. The reason for the lack of effect may be that grazing indirectly rather than directly altered soil microbial community composition via alterations to the plant community due to increasing pH (Wang et al. 2020).

Conclusions

This study provides insights into how microbial diversity and community composition react to long-term grazing in southern grasslands. First, our results found that grazing altered soil properties and soil microbial community composition, while decreasing microbial β-diversity (fungal β-diversity) but not α-diversity in the southern grasslands of China. Second, by altering soil nutrient availability, grazing shifted the relative abundance of dominant microbial phyla and altered soil microbial community composition. These findings suggest that long-term grazing could alter belowground microbial communities; as such, grazing management practices should consider soil biodiversity conservation and functions in southern grasslands.

Although this study provided important insights into microbial performance within a long-term grazing ecosystem, there are some uncertainties and limitations. First, the study area experiences a subtropical plateau monsoon climate, with obvious wet and dry seasons. As such, the microbial community may respond differently to grazing in different seasons. Thus, sampling across seasons should be considered in future studies. Second, grazing can affect soil microbial community composition by reducing plant species diversity. Unfortunately, the plant community was not surveyed here. Plant data should be included in further work to better understand the mechanisms underlying grazing effects on microbial communities.

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Author contributions Jianping Wu and Meiyan Zhang contributed to the study conception and design. Material preparation and data collection were performed by Meiyan Zhang, Juan Zhou, Syed Turab Raza, Shiming Yang, Junhua Liu, Ming Cai, and Shiming Xue. Analyses were conducted by Juan Zhou. The first draft of the manuscript was written by Juan Zhou. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated by the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Conflict of interest Authors declare that they have no known conflict of interest.

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