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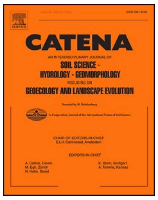

Soil carbon mineralization decreased in desert steppe by light grazing but not fencing management

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ABSTRACT

Fencing off grassland soils emits massive amounts of carbon into the atmosphere. Whether grazing management in desert steppes with fragile ecosystems can mitigate this trend remains highly uncertain. Here, we examined how soil carbon mineralization, as well as its underlying mechanisms, varied with grazing intensity by sheep in a long-term (17 – year) experiment in the desert steppe. Carbon mineralization decreased by 15 % – 55 % under different grazing intensities compared to fencing controls. Soil organic carbon (SOC) maintained high levels under light grazing, whereas it decreased with increasing grazing intensity. Reductions in plant carbon and absolute microbial abundance due to grazing, coupled with changes in soil carbon quality and the environment, drove the reduction in carbon mineralization. We suggest that mechanisms of carbon mineralization can be integrated into predictive modelling efforts to better understand the impact of grazing on carbon fluxes in ecologically fragile, but globally important, arid and semi-arid grasslands.

1. Introduction

Soil carbon mineralization is a crucial factor that determines the amount of carbon retained in, or released from, the soil, thereby significantly influencing the global carbon cycle (Zhou et al. 2019; Ganguli & O'Rourke 2022; He et al. 2023). While the majority of studies have focused on increases in carbon dioxide (CO_2) emissions from the soil due to global warming, anthropogenic disturbances can greatly influence fluctuations of soil carbon mineralization. Grasslands cover 40.5 % of terrestrial ecosystem area (Ganguli & O'Rourke 2022; Zhang et al. 2023) and high intensity grazing and other disturbances have been implicated as an important cause of the surge in soil carbon mineralization and organic matter loss (Zhou et al. 2019; Ganguli & O'Rourke 2022). In response, many grassland restorative strategies aim to reduce grazing intensity or used fencing for protection (Wigley et al. 2020; Chen et al. 2015). However, these strategies to reduce or completely eliminate grazing have been challenged in recent years despite studies that have found increased carbon mineralization rates due to higher root biomass in ungrazed systems when compared to grazed grasslands (Thorhallsdottir & Gudmundsson 2023; Sharkhuu et al. 2016). Our understanding of the factors underlying carbon mineralization, and how those factors are altered by anthropogenic factors, plays a critical role in developing accurate projections of feedbacks between global change and carbon feedbacks, which are needed to reduce the uncertainties in terrestrial ecosystem models (McSherry & Ritchie 2013; Zhou et al. 2017; Wang et al. 2022). For example, the large carbon stock in global grasslands is strongly influenced by livestock grazing via multiple pathways including aboveground plant production and litter decomposition, as well as by promoting or inhibiting belowground carbon processes, such as soil respiration and carbon mineralization (Allard et al. 2007; Kang et al. 2013; Sharkhuu et al. 2016).

Soil carbon mineralization rate, a key parameter of carbon

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decomposition, may be changed in response to environmental disturbances (Kang et al. 2013; Zhao et al. 2024a). Recent modelling efforts have suggested that grazing can promote soil carbon mineralization rates by fragmenting soil aggregates, and as a result, lead to higher rates of carbon decomposition by microbes (McSherry & Ritchie 2013; Zhou et al. 2019). Soil carbon mineralization is defined as the total amount of CO_2 released by a soil per unit time, and its fluctuation has been shown to be regulated by soil microorganisms (He et al. 2023; Zhao et al. 2024b). Results from some field trials suggested that grazing can reduce soil CO_2 emissions amount by reducing the carbon sources available to microorganisms (Kang et al. 2013; Machmuller et al. 2015; Yang et al. 2023). Collectively, these empirical models have elucidated the mechanisms by which variables can directly or indirectly drive carbon mineralization because of environmental factors (He et al. 2023). As a result, the direction by which different levels of grazing alter carbon mineralization and the consequent release of carbon into the atmosphere remains uncertain. Limiting our ability to accurately predict soil carbon dynamics and potential carbon sequestration capacity when the intensity of livestock grazing varies under different management scenarios.

Soil carbon mineralization is primarily influenced by the soil environment, the quality of the soil organic carbon (SOC), and the microbial community (Qin et al. 2019; Kooch et al. 2020; Reinhart et al. 2021). First, features of the soil environment that influence carbon mineralization include abiotic factors (e.g., pH, bulk density, and aggregate fraction) and biotic factors (plant carbon input and enzyme activity) (Paz-Ferreiro et al. 2012; Wu et al. 2012; Alvarez et al. 2018). Our previous study showed that long-term heavy grazing significantly diminished carbon sinks in desert steppe ecosystems and the amount of reduction was related to environmental conditions (Jin et al. 2023). The influence of the soil abiotic environment on soil carbon mineralization operates indirectly by changing physicochemical properties of dissolved organic matter as well as microbial respiration (Kooch et al. 2020; Reinhart et al. 2021; Liu et al., 2022a,Liu et al., 2022b). Plant carbon input, especially from roots, is the most prominent carbon source available for microbes and higher rates of plant carbon input and enzyme activity are usually accompanied by higher rates of mineralization (i.e., enzyme kinetic theory) (Averill et al. 2014; Alvarez et al. 2018; Bai & Cotrufo 2022). Second, the composition of SOC can strongly influence carbon mineralization (Qin et al. 2019). For example, recent methodological advances using solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy have shown that soil organic carbon containing more O-alkyl carbon (a type of labile substrate) is more easily mineralized (Qin et al. 2019). Third, the abundance, diversity and composition of soil microbes can play an important role in mediating soil carbon mineralization via their use of labile organic substrates (Alvarez et al. 2018).

Globally, ~70 % of grasslands are under varying degrees of degradation due to overgrazing, with dramatic fluctuations in soil carbon mineralization (Wu et al. 2012; Qin et al. 2019). This phenomenon is particularly pronounced in the fragile ecosystems of arid and semi-arid grasslands. Grazing activities, including defoliation, dung and urine deposition and trampling, can influence each of the above processes that influence soil carbon mineralization both directly and indirectly (Cao et al. 2004; Chen et al. 2015). For example, soil pH and bulk density tend to increase under moderate to intense livestock grazing (Chen et al. 2015; Jin et al. 2023). Likewise, soil aggregation can sometimes increase with grazing and other land-uses (Wu et al. 2012; Jiang et al. 2017), but can decrease with grazing in other cases (Dungait et al. 2012; Pu et al. 2022). The abundance and composition of soil microbial communities that decompose substrates can also be substantially altered by grazing activities (Jiang et al. 2017; Che et al. 2019). Understanding how grazing alters each of these processes, and how they regulate variation in carbon mineralization, will provide important information when developing modelling scenarios for soil carbon dynamics and fluxes under different land-use intensities.

Here, we examined the role of sheep grazing on carbon mineralization in a long-term experiment (17 year) in the desert steppe of Inner Mongolia, China. This area has a long history of grazing, and in some areas, recent intense overgrazing has caused large-scale grassland degradation (Sharkhuu et al. 2016; Oggioni et al. 2020). We examined the effects of grazing at different intensities on the direct effects of the soil environment, carbon quality and soil microbes as well as indirect effects mediated by plant carbon input on soil carbon mineralization. We hypothesize that light grazing management can reduce carbon mineralization as well as maintain a higher SOC compared to fencing or higher grazing intensity. Our study provides a more detailed understanding of the influence of livestock grazing on carbon dynamics in this fragile ecosystem that serves as an important carbon sink in the global carbon cycle.

2. Materials and methods

2.1. Site and experimental design

Our experiment took place in a desert steppe located at Siziwang Banner (41°46′44″N, 111°53′42″E, elevation 1456 m a.s.L.) in Inner Mongolia, China. The dominant plant species at the site include two perennial grasses (*Stipa breviflora* and *Cleistogenes songorica*) and a perennial shrub (*Artemisia frigida*) (Zhang et al. 2022).

In June 2004 the grazing experiment was established as a randomized complete block design with three blocks (replicates) to test the effects of four levels of sheep grazing intensity (12 plots total) (Zhang et al. 2018). Each replicate plot was 4.4 ha. The four grazing intensities were 'no grazing by fencing' (no sheep present), 'light grazing' (4 sheep present = 0.15 sheep ha^{-1} month^{-1}), 'moderate grazing' (8 sheep present = 0.30 sheep ha^{-1} month^{-1}), and 'heaving grazing' (12 sheep present = 0.45 sheep ha^{-1} month^{-1}). These grazing intensities were based on the design proposed by Wei et al. (2000) in a desert grassland of Inner Mongolia. We maintained the grazing period from June to November each year and from 6 am to 6 pm each day, with sheep penned in a separate enclosure at night. Initially, the grazing experiment indicators such as plant community diversity and soil organic carbon content was not different among treatments ($P>0.05$, Table S1).

2.2. Plant and soil sampling

In July 2021, we established three 100 m^2 plots within each of the sheep grazing plots and within those, established five 1 m^2 quadrats at the corners and center for plant and soil sampling (Figure S1). To sample aboveground plant material, we clipped plants in each quadrat to ground level after removing litter. We sampled root biomass at 0–10 cm using three soil samples per 1-m^2 quadrat. We removed roots from the soil by rinsing them in water and then oven-dried them at 65 °C for 48 h. Plant C inputs (g C/m^{-2}(−|-)) from aboveground (shoots) and belowground (roots) were calculated as follows (Liu et al., 2021): $\Delta Lc = Mx \times Cx$. Where ΔLc is the C input (g C/m^{-2}(−|-)) from both shoots and roots, Mx is the production of foliar shoots and roots (g/m^{-2}(−|-)) and Cx is the corresponding C content (g C/g) of shoots and roots. We determined C content of shoots and roots using a C/N elemental analyzer (Multi-N/C 2100, Analytik Jena AG, Jena, Germany).

To sample soil in the 0–10 cm layer, we used a 7.5 cm diameter core to collect five soil cores from each quadrat (the four corners and center of the quadrat) and combined them for subsequent analyses (Figure. S1, A). We kept the 36 soil samples on ice, transported them to the laboratory within 24 h, and sieved them through 2 mm mesh to remove root debris and stones. We then stored 100 g of each sample at −80 °C for DNA extraction for microbial analysis (see below), and air-dried the remaining sample prior to analyzing soil physicochemical properties.

To obtain *in situ* soil aggregates, we excavated a pit within each quadrat and used aluminum boxes (10 × 8 × 5 cm) to collect soil in the 0–10 cm layer. We then air-dried the samples in the laboratory to determine the aggregate fractions and for use in the incubation experiment (Figure. S1, B).

2.3. Incubation experiment to measure carbon mineralization

We used established methods to measure carbon mineralization (Chen et al. 2016; Sun et al., 2020; Liu et al., 2021) over a 105 − day incubation period in the laboratory according to the following protocol (Figure. S1, C) Our intention was to compare among treatments, not to develop predictions of actual carbon fluxes from the natural system. From each sample, we put fresh sieved soil (approximately 20 g dried basis) into 500 ml airtight jars, which we then incubated at 25 °C in the dark. We adjusted soil moisture to 60 % of water holding capacity using deionized water. Next, we used a flat-bottomed plastic cup containing 10 ml of 0.2mol l^{-1} NaOH solution to capture mineralized CO_2 from the soil in each jar (Sun et al., 2020). We incubated three additional jars containing the NaOH solution without soil in parallel to serve as a control to detect CO_2 captured from the air. Carbon dioxide that evolved during the incubation period was trapped in the NaOH solution, and excess CO_2 was then titrated with 0.1 mol/L HCl after adding $BaCl_2$. We changed the NaOH solution in the plastic cup every 1 to 4 days for the first two weeks and then once per week for the rest of the incubation period. We then calculated mineralized carbon as the cumulative CO_2 flux.

2.4. Measurements of abiotic and biotic variables associated with carbon mineralization

We isolated three soil aggregate fractions using the wet sieving technique (Qin et al., 2019): 0.25——0.2 mm (large aggregates), 0.2——0.053 mm (small aggregates) and < 0.053 mm (microaggregates). We transferred 100 g of air-dried bulk soil (<2 mm) to the top of a set of sieves (0.25 and 0.053 mm), pre-soaked the sample in distilled water for 10 min, and then vertically shook the sample for 10 min at 1 oscillation s^{-1} (accompanied by a 4 cm amplitude).

We measured the quality of SOC using solid-state ^{13}C-cross-polarization/total sideband suppression nuclear magnetic resonance (NMR). We first treated soil samples with 10 % hydrofluoric acid, and then rinsed samples with deionized water and freeze-dried them (Helfrich et al. 2006). We then used MestReNova 9.0 (Mestrelab Research S.L., Santiago de Compostela, Galicia, Spain) to integrate the spectra into four chemical shift regions and acquire the relative intensity of each region, i.e., 0–50 (alkyl-C), 50–110 (O-alkyl C), 110–165 (aromatic C), and 165–210 ppm (carboxylic C) (Qin et al. 2019).

2.5. DNA extraction, amplicon generation and quantitative PCR analyses

We extracted DNA from each of the 36 soil samples using the a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). We measured DNA concentration and purity using a NanoDrop One (Thermo Fisher Scientific, MA, USA). We amplified16S rRNA and ITS genes of distinct regions (Bac 16S: V3-V4; Fug 18S: ITS2, respectively) using specific primers (16S: 338F and 806R; 18S: ITS5-1737F and ITS2-2043R) with 12 bp barcode (Che et al. 2019).

We performed qPCR analyses to quantify the specific genes of the extracted DNA with three replicates using a StepOnePlusTM Real-Time PCR System instrument (Applied Biosystems, Foster City, CA, USA). We detected gene copy numbers of 16S rRNA and ITS using standard primers for bacteria and fungi. We calculated all gene copy numbers to generate the absolute abundance of microbes from the standard curves of 16S rRNA and ITS genes using the 1 Ct (cycle threshold) method. We conducted all qPCR reactions in triplicate.

2.6. High-throughput sequencing

We generated sequencing libraries using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA), and assessed the library quality on the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA). We sequenced the library on an Illumina Nova6000 platform and 250 bp paired-end reads (Guangdong Magigene Biotechnology Co. Ltd., Guangzhou, China). We then compared soil bacteria reads to the Silva database (https://www.arbsilva.de/) and fungal reads to the Unite database (https://unite.ut.ee/) using the UCHIME algorithm to detected chimera sequences (which we then removed to obtain the clean reads). We applied an integrated high-throughput absolute abundance quantification (iHAAQ) method to characterize soil microorganisms and their communities (Lou et al. 2018). The absolute abundance of each phylum was calculated by multiplying the total number of microorganisms, the copy number of microbial genes in qPCR, and the corresponding relative abundance in high-throughput sequencing.

2.7. Estimating microbial community properties

We estimated the absolute abundance of each phylum in the soil bacterial and fungi communities by multiplying total bacterial or fungi quantities, the copy number of 16S rRNA and ITS gene from qPCR, and the corresponding relative abundance from high-throughput sequencing (Lou et al. 2018). We assessed microbial diversity using the observed community richness, the Chao 1 species richness extrapolation, Shannon and Simpson's index, which emphasizes common species more than rare species in the *vegan* package (R Development Core Team, 2016). For species composition, we visualized differences using nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distance, and compared differences using Permutation Multivariate Analysis of Variance (PERMANOVA) in the *vegan* package.

2.8. Statistical analyses

Our analyses mainly included the following three procedures. First, we used mixed effect models to assess whether and how carbon mineralization was affected by different sheep grazing intensities, with grazing treatments as fixed effects and replicate as a random effect. Second, we analyzed the effects of different sheep grazing intensities on soil physicochemical and microbial properties using a linear mixed effects model where different grazing intensities was set as a fixed effect and replicate was treated as a random effect. A multiple comparison was performed by LSD test with a significance level of $P<0.05$. We fit this model using the *lme4* package (R Development Core Team, 2016). We performed all above analyses using R v4.1.2 (https://www.r-project.org/).

Finally, we used structural equation modelling (SEM) to evaluate the relative importance of the direct and indirect effects of soil environmental variables, plant carbon input, enzyme activities and microbial characteristics (abundance, diversity and composition) on soil carbon mineralization across different grazing intensities. Considering the complexity of carbon mineralization, the construction of an over-all variables correlation initial model is necessary (He et al. 2023, Initial model: Figure. S2). We only included variables significantly correlated with carbon mineralization in the PCA (Figure. S3). Because variables (i.e., soil environmental variables, plant carbon input, enzyme activities and microbial characteristics) were highly correlated in each group, we performed principal components analysis (PCA) (Lou et al. 2018) to create a new integrated metric to represent each type of factor. We introduced the first component (PC1) as a new variable in the subsequent SEM analysis. We evaluated the fit of the final model using the model's Chi-square test (χ^2) goodness of fit index (GFI) and root mean square error of approximation (Lou et al. 2018). We performed the SEM analyses using AMOS 21.0 (AMOS Development Corporation, Chicago, IL, USA).

3. Results

3.1. Soil carbon mineralization and its governing factors under grazing intensities

We found that the CO_2 release from the soil was 15——55 % lower with increasing grazing intensities compared to fencing control plots (Fig. 1). However, the SOC content was only lower with moderate and heavy grazing intensities (Figure S4, $P<0.05$), resulting in a positive correlation between SOC content and carbon mineralization (Figure S3, $P<0.05$). Notably, the SOC contents were numerically higher under light grazing when compared to fencing (Figure S4).

We measured several environmental factors that are associated with the regulation of carbon mineralization in the soil, including pH, bulk soil density, and the SOC in aggregates. While there was no significant changes in pH or soil density with light grazing, both increased in the higher grazing intensity (Table S2). At the same time, we found that the SOC contained within both large and small aggregates decreased with moderate and intense grazing (Table S2, $P<0.05$).

Using solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy, we found that O-alkyl C, a labile substrate, was lower with increasing grazing intensity (Fig. 2 A, B, $P<0.05$). In contrast, we found higher levels alkyl C, a more stable substrate, with light grazing (Fig. 2 A, C, $P<0.05$). As a result, the alkyl C/O-alkyl C ratio was also highest with light grazing (Fig. 2, D, $P<0.05$). According to the enzyme kinetic theory (Averill et al. 2014; Alvarez et al. 2018; Bai & Cotrufo 2022), substrates with more stable carbon tend to release less CO_2. Thus, our observations of changes in SOC quality could explain the reduction in CO_2 release under light grazing. However, because the alkyl C/O-alkyl C ratios under moderate and heavy grazing did not differ from the no grazing treatment, carbon quality cannot be the only explanation for the reduction in relative soil carbon mineralization under higher grazing intensities.

We found a positive correlation between the reduction in plant carbon inputs due to grazing and carbon mineralization (Figure. S3, $P<0.05$). Root C inputs (167——302 g C/m^{-2}(−|-)) were much higher than shoot C inputs (33.3——41.4 g C/m^{-2}(−|-); Table. S3) across grazing treatments, but increasing grazing intensity led to reduced plant C inputs overall by 26——41 % (Table. S2). In particular, grazing significantly reduced root C inputs compared to fencing (30——44 %, Table. S3, $P<0.05$). Root C inputs had no difference under light and moderate grazing (Table. S3, $P>0.05$), and was lowest under heavy grazing.

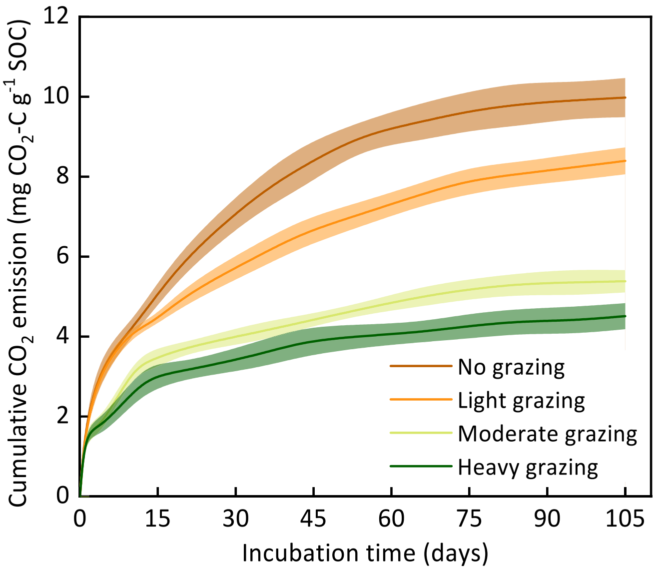

Fig. 1. Cumulative soil CO_2 emissions under different grazing intensities. The x-axis is the number of incubation days and the y-axis represents the cumulative CO_2 emissions. The curves and shadows represent the mean and standard error, respectively.

3.2. Microbial response to grazing intensity

Using high-throughput sequencing, we found that the absolute abundance of soil bacteria was 50 % − 65 % lower with moderate to heavy grazing compared to no grazing controls (Fig. 3, A, $P<0.05$). The two most abundant phyla, Actinobactera, Acidobacteria (>10 % of the community) largely mirrored these results, although Acidobacteria were more abundant with light grazing compared to moderate and heavy grazing (Fig. 3, B − C, $P<0.05$). In addition, we found that the abundance of fungi was reduced by about 50 % in the moderate and heavy grazing treatments (Fig. 3, D, $P<0.05$). The Ascomycota, which were the most abundant group of fungi, were also reduced with moderate to heavy (but not light) grazing (Fig. 3, E, $P<0.05$).

We found that soil carbon mineralization was positively correlated with microbial absolute abundance, diversity, and species composition (Figure. S3, $P<0.05$). Light grazing had no influence on any of the diversity metrics we analyzed including: species richness, extrapolated richness (estimated using the Chao1 extrapolation), and Shannon and Simpson indices, for both soil bacteria and fungi (Table S2; Table S4, $P<0.05$). However, the richness and extrapolated richness of bacteria and fungi were both lower with moderate and heavy grazing (Table S2; Table S4, $P<0.05$). Grazing also altered the composition of both soil bacteria (Fig. 4 A) and soil fungi (Fig. 4 B). Light, moderate and heavy grazing all changed the bacterial community structure compared to no grazing. Similarities in bacterial community composition existed under moderate and heavy grazing. In addition, heavy grazing changed the structure of the fungal community. There was similarity in fungal community structure under light and moderate grazing compared to fencing (Fig. 4).

To examine the role of microbes in regulating carbon mineralization, we measured the activity of three enzymes β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase and leucine arylamidase, which are known to involved in the organic matter decomposition (Yang et al. 2022a). We found that the activity of all three enzymes declined with increasing grazing intensity (Table S2) and were positively correlated with carbon mineralization.

3.3. Mechanisms controlling the level of carbon mineralization under grazing

We developed a structural equation modelling (SEM) approach to examine the interrelationships between the soil environmental factors, plant carbon input, the microbial community, and enzyme activities in driving the rates of soil carbon mineralization. We found that grazing directly affect the soil environment and plant carbon inputs, both of which are positively correlated and together drive changes in soil carbon mineralization (Fig. 5). We found that soil microbial community had the highest effect on carbon mineralization. Diminished soil enzyme activity and deterioration of the soil environment also directly decline carbon mineralization. Furthermore, we found that plant carbon input was positively associated with enzyme activity, which indirectly declining carbon mineralization (Fig. 5; Table S5). In all, we found that as grazing intensity increases, the soil environment degrades together with lower plant carbon inputs, leading to a decrease in microbial abundance and enzyme activity, which combine to decrease soil carbon mineralization (Fig. 5).

4. Discussion

4.1. Grazing declines carbon mineralization in desert steppe

Overall, our study showed that the cumulative effect of long-term

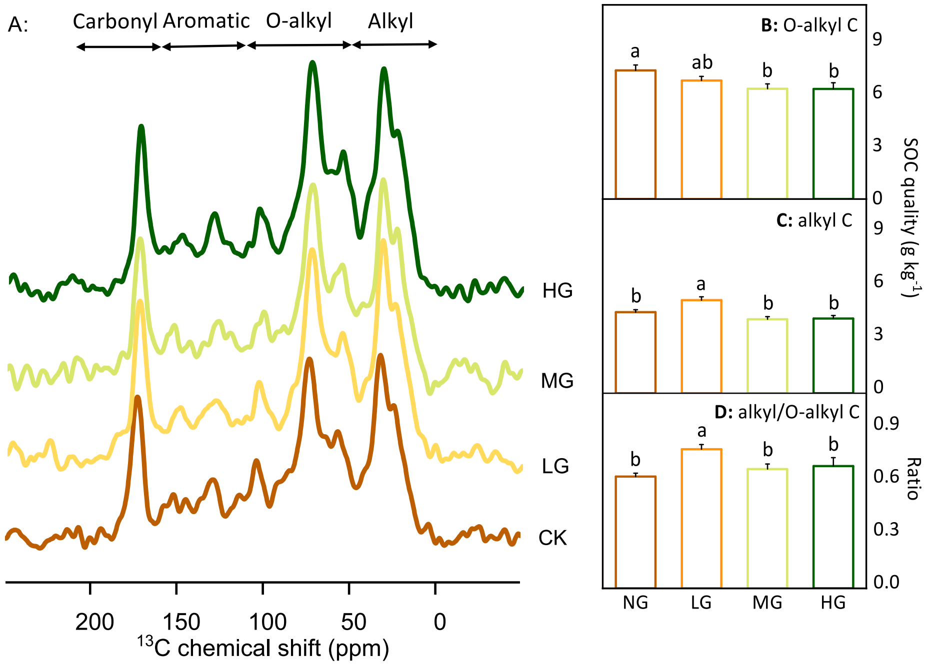

Fig. 2. **^{13}C NMR mapping at different grazing intensities** (means ± SE). (**A**)**:** The spectral chemical shift between 0–50 represents alkyl-C, between 50–110 represents aromatic C, between 110–165 represents O-alkyl C, and between 165–210 represents carboxylic C; (**B**)**:** the amount of alkyl-C at different stocking rates, and (**C**) represents the O-alkyl C at different stocking rates; (**D**)**:** the ratio of alkyl-C and O-alkyl C at different grazing intensities. The significance level is $P<0.05$. NG: no grazing, LG: light grazing, MG: moderate grazing and HG: heavy grazing.

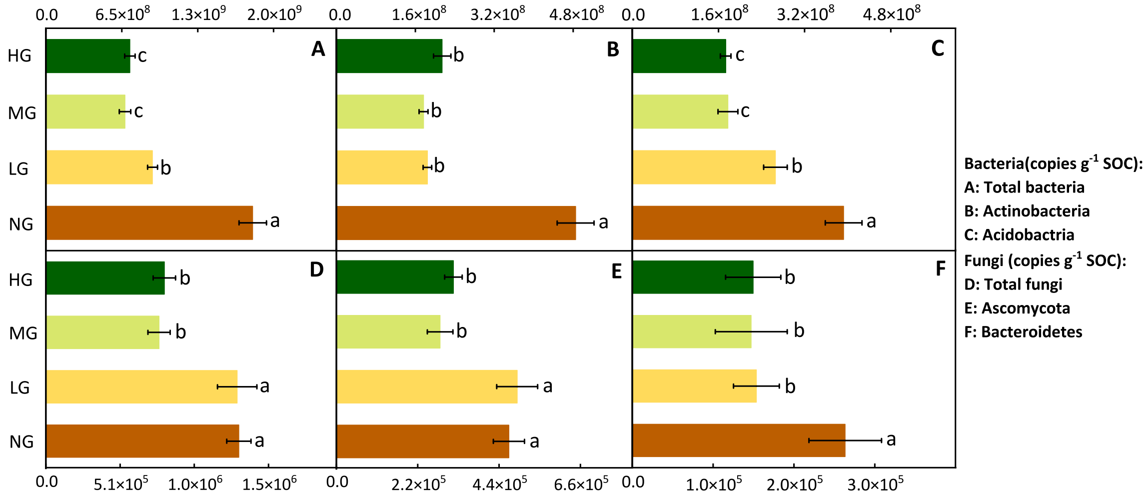

Fig. 3. **Effect of different grazing intensities on the abundance of soil bacteria (a-c) and fungi (d-f)** (means ± SE). NG: no grazing, LG: light grazing, MG: moderate grazing and HG: heavy grazing. Letters above each bar represent significant differences at the $P<0.05$.

sheep grazing in desert steppe of Inner Mongolia was the decline of soil carbon mineralization, which inhibiting CO_2 emissions (Fig. 6). Notably, our results also indicated that light grazing maintained higher SOC, but reduced carbon mineralization and CO_2 emissions, which was associated with reduced plant carbon inputs and bacterial abundance. The main reasons for the reduction in carbon mineralization was due to the dramatic reductions in plant carbon inputs, fungal, and bacterial abundance under both moderate and heavy grazing (Fig. 6), in line with our hypothesis. Similar patterns have been observed in other long-term grazing experiments conducted in arid and semi-arid areas, including the Loess (Chen et al. 2015) and Tibetan Plateau's (Cao et al. 2004) in China, as well as the shortgrass steppe of Colorado (LeCain et al. 2002) in the United States. These experiments concluded that the underlying cause of the decrease in carbon mineralization with grazing was a reduction in SOC, consistent with the results of our study. Consequently, our results indicate that by reducing the CO_2 emissions from soils, grazing can mitigate some of the positive feedbacks that influence climate change. Moreover, by combining previous studies, we further demonstrate that understanding the mechanisms of carbon mineralization requires an integrated consideration of the complex set of direct and indirect pathways between the soil environment, soil microbes, and carbon dynamics (Bai & Cotrufo 2022, Qin et al. 2019, Chen et al. 2016).

4.2. *Grazing affects carbon mineralization through changes in soil environment, plant carbon inputs, and microbial communities*

Changes in carbon mineralization, which decreased with increasing grazing intensity, involved interactions among changes in the soil

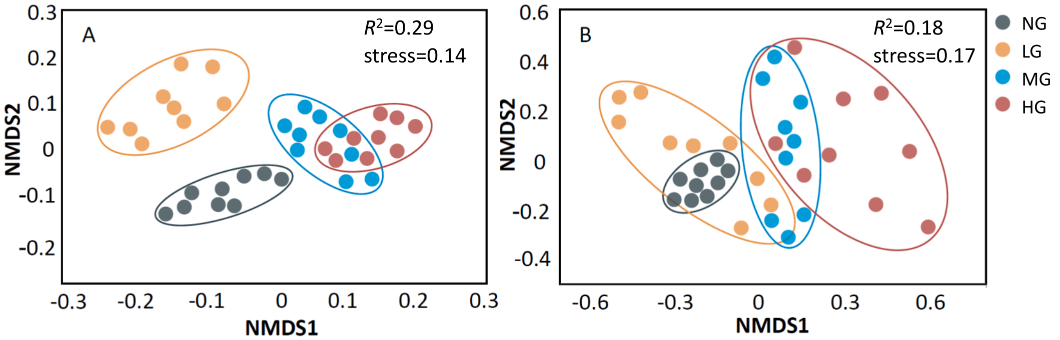

Fig. 4. The composition of soil bacterial (A) and fungal (B) communities under different grazing intensities. Points are projected in non-metric multidimensional space indicating the composition of each replicate community in a given treatment. The distance between points represents the Bray-Curtis similarity in composition between those communities (more distant communities are more different in composition). Ellipses surrounding each treatment represent the 95% confidence interval. NG: no grazing, LG: light grazing, MG: moderate grazing and HG: heavy grazing.

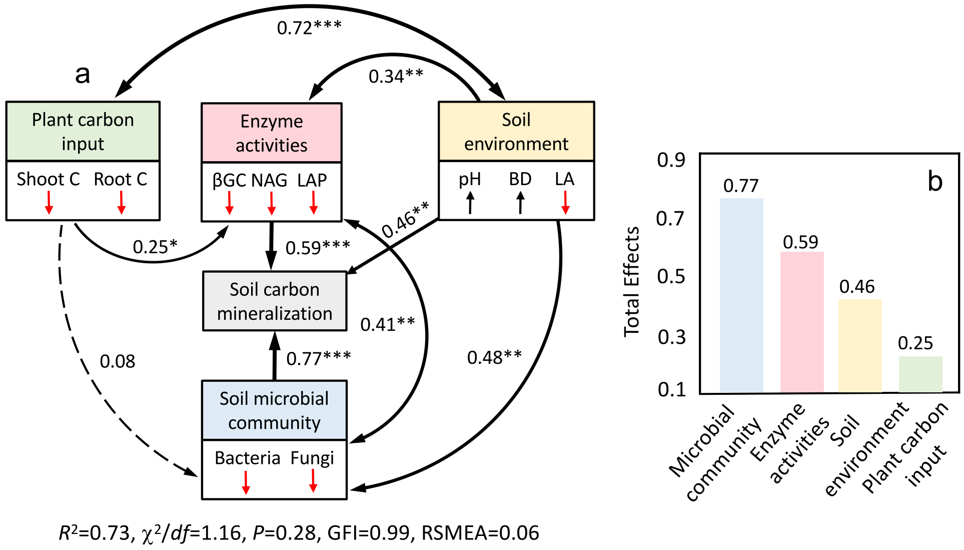

Fig. 5. Structural equation model (SEM) illustrating the direct and indirect effects of sheep grazing on soil carbon mineralization. Boxes represent measured variables in the model. Values adjacent to arrows are standardized path coefficients that indicate the strength of the relationship. The solid lines represent significant correlations, while dashed lines indicate no relationships between the two factors. Red arrows in the boxes indicate that the indicator increases with grazing intensity and black arrows indicate that it decreases with grazing intensity. BD: soil bulk density; LA: Large aggregates; βGC, NAG, LAP for β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase and leucine arylamidase respectively; Bacteria and Fungi: bacteria and fungi characteristics (abundance, diversity and composition). Significance levels: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

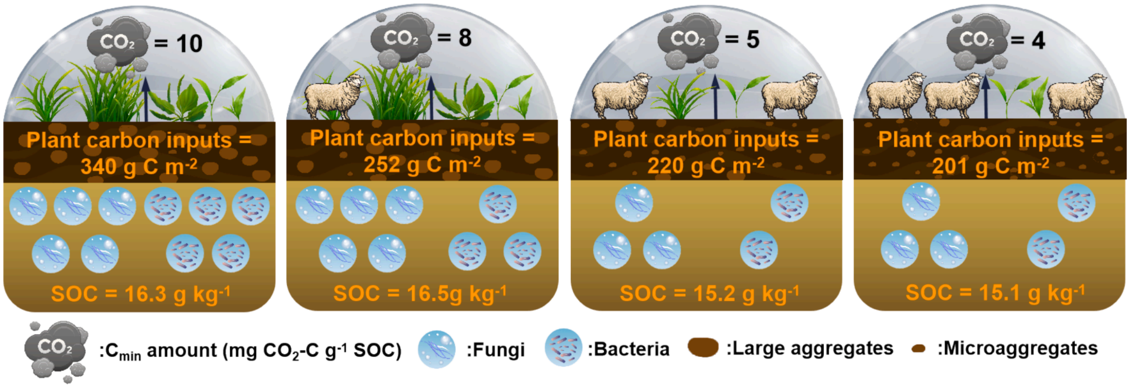

Fig. 6. Grazing-driven changes in soil carbon mineralization and their mechanisms. The SOC and carbon mineralization were highest under no grazing. As grazing intensity increased, trampling by sheep broke up large soil aggregates, leading to a loss of SOC and a decrease in carbon mineralization. Light grazing maintained high SOC content, but reduced carbon mineralization, which was associated with reduced plant carbon input and bacterial abundance. The main reasons for the reduction in carbon mineralization were due to the dramatic reductions in plant carbon input, fungal and bacterial abundance under both moderate and heavy grazing.

environment, plant carbon inputs, microbial abundances and enzyme activities. This effect can be divided into two processes. First, the degradation of the soil due to grazing results in structural damage to the soil via an increase in soil bulk density and a decrease of larger soil aggregates. In the absence of physical disturbances, the potential for C-mineralization may depend on the average 'random walk' of microorganisms through the tortuous soil pore space, which is influenced by soil bulk density (Dungait et al. 2012). However, when soil bulk density increases and soil aggregate size decreases, there can be a strong reduction of the digestion of organic C by enzymes, together with restricted oxygen diffusion that can decrease soil carbon mineralization (McSherry & Ritchie 2013; Chen et al. 2019). Our results are supported by a long-term grazing experiment in the North Great Plains, which showed that grazing-induced soil compaction decreased the amount of large aggregates and reduced soil carbon mineralization (Reinhart et al. 2021). Therefore, we suggest that the reduction in SOC in large and small aggregates in the face of grazing may play a role in the reduction in carbon mineralization. Second, the degradation of the soil environment (e.g., more compact soils, higher pH) can reduce soil carbon mineralization by affecting soil enzyme activity and microbial community characteristics (Jiang et al. 2017; Alvarez et al. 2018). Previous studies have shown that sheep excreta can lead to higher pH in grazed pastures than fenced, in the study it only averages to 0.3 pH unit difference but it was statistically significant (Hong et al. 2021; Fabian et al. 2014). Soil pH mainly controls the general pattern of soil microbial community structure, especially bacterial community (Ni et al. 2020). Although sheep excreta into the soil can increase carbon input, most of this carbon is poorly decomposed (Hong et al. 2021). In a study following heavy grazing in desert steppe, functional genes involved in the decomposition of labile carbon were found to be increased (Zhao et al. 2024b). This result confirms that more sheep excreta may cause carbon to become more resistant to mineralization from the perspective of microbial metabolism.

Decreases in plant carbon input with increasing grazing intensity, via a reduction of its regulatory effect on enzyme activity, led to a decrease in carbon mineralization. This is mainly due to the decline in the abundance of soil microbes. A positive feedback relationship between reduced microbial abundance and soil enzyme activity under high intensity grazing has been demonstrated (Prieto et al. 2011). Drivers of this relationship may be related to the amount of carbon input from roots (Zhu et al. 2021). Our previous study found that carbon input from below-ground (i.e., roots) was much higher than that from above-ground. Also, plant carbon inputs were reduced by almost 50 % under heavy grazing treatment (see also (Zhou et al. 2017; Zhang et al. 2018; Yang et al. 2022b)). This suggest that a substantial reduction of available carbon for microbes, which would weaken the *in vitro* modification of organic matter by soil enzymes and lead to a reduction in soil carbon mineralization (Alvarez et al. 2018; Bai & Cotrufo 2022; Yang et al. 2022a). Plant carbon and sheep excreta are mineralized with different characteristics as two available carbon sources for soil microbes (Oggioni et al. 2020; Bai & Cotrufo 2022). Generally, plant root secretions and humus from the decomposition of litter are more accessible as microbially available carbon (Alvarez et al. 2018; Yang et al. 2022a). However, only a small portion of sheep excreta is metabolized by microorganisms, and most of it accumulates in the soil for a long period of time or is converted to plant carbon to be mineralized by microorganisms (Zhu et al. 2021; Yang et al. 2023).

We found that the abundance of microbes was positively correlated with carbon mineralization, but was inhibited by grazing, as observed elsewhere (LeCain et al. 2002; Zhang et al. 2018). This was most pronounced in bacteria, and in particular, the dominant soil bacteria in the Actinobacteria and Acidobacteria, which likely played a strong role in carbon mineralization rates (Paz-Ferreiro et al. 2012). The weaker response in total fungal abundance, especially in the less intensely grazed treatments, may result from differences in their morphological structures (Wagg et al. 2019; Reinhart et al. 2021). Specifically, fungal mycelia can be disrupted by sheep trampling, weakening their ability to break down recalcitrant substrates (Reinhart et al. 2021), while bacteria present as mostly single cells that may be less physically disturbed (Reinhart et al. 2021; Yang et al. 2022b).

We found that grazing not only led to a reduction in the total abundance of microbes, but also decreased several measures of microbial diversity, likely because of reduced belowground biomass (Zhang et al. 2022). Furthermore, we found shifts in both bacterial and fungal community structure with grazing, consistent with previous studies (LeCain et al. 2002; Helfrich et al. 2006; Jiang et al. 2017). Indeed, grazing in the desert steppe inhibits microbial turnover and weakens microbial respiration, which underlies the positive correlation between microbial community diversity and structural composition and reduced carbon mineralization. However, bacterial and fungal communities responded differently to grazing (Reinhart et al. 2021). Specifically, the community structure of bacteria was more heterogeneous than that of fungi, which has all been attributed to the deterioration of the soil environment and the reduction of available carbon for microbes (Reinhart et al. 2021). Altogether, the effect of grazing on soil organic carbon content and carbon mineralization characteristics is a complex process, which is mainly modulated directly and indirectly by grazing effects. A field study demonstrated that as grazing intensities increased, the effect of environmentally driven organic carbon accumulation declined (40 % to 12 %), while the effect of microbes was increasing (32 % to 46 %) (Zhao et al. 2024a). This result indicates a strong indirect effect of light and moderate grazing on soil carbon cycling through the soil environment. Whereas, heavy grazing directly affects the organic carbon content and mineralization characteristics mainly by affecting the environment. Combined with the results of this study, whether grazing can promote soil carbon mineralization depends critically on if there is a positive feedback of environmental changes on microbial communities (Sharkhuu et al. 2016; Yang et al. 2022b; Zhao et al. 2024a).

4.3. *Light grazing potentially enhances soil carbon sink function*

Although a main result of our study is that heavy grazing leads to reduced soil carbon mineralization and decreased CO_2 emissions, we emphasize this does not necessarily imply that heavy grazing is the best land-use for achieving reduced carbon emissions goals. First, we found that the O-alkyl carbon was highest in ungrazed grasslands, indicating that labile carbon content was reduced by grazing (Helfrich et al. 2006). However, lightly grazed soil retained a large amount of alkyl carbon and had a higher alkyl/O-alkyl carbon ratio, suggesting that soil carbon was more stable when grazing was light, which could stimulate soil to sequester larger molecules with stable organic matter (e.g., cutin, waxes, etc.) and promote the formation of more stable carbon pools (Chen & Chiu 2003; Ussiri & Johnson 2003; Solomon et al. 2007; Pu et al. 2022). Indeed, previous studies using the same grazing experiment showed that light grazing can be associated with many positive aspects of grassland health, including soil microhabitat (Zhang et al. 2020), soil enzyme activity (Zhu et al. 2021) and grassland ecosystem multifunctionality (Zhang et al. 2022). Evaluating whether grazing enhances grassland carbon sinks function requires considering the cumulative effects of several factors, with the main focus being on whether carbon emissions from soil (soil animals and microorganisms) and livestock respiration are less than the amount of carbon sequestered by plants and soils (Allard et al. 2007; Bai & Cotrufo 2022). Appropriate grazing can promote soil carbon sequestration and higher stocking rates may lead to the conversion of grasslands from carbon sinks to sources have been demonstrated (Reinhart et al. 2021; Sharkhuu et al. 2016). Although the results of the present study can show that grazing in desert steppe can mitigate CO_2 emission from the perspective of soil carbon mineralization, the evaluation of the carbon sequestration capacity of desert steppe ecosystems in combination with the respiration of livestock still needs further exploration.

5. Conclusions

In summary, after 17 years of grazing, we found that carbon mineralization was strongly reduced by manipulating the intensity of sheep grazing in a desert steppe. This emerged from a combination of direct and indirect effects of grazing, trampling and excreta on plant carbon input, enzyme activity, microbial abundance, diversity, and composition. In this fragile grassland ecosystem, higher grazing intensity lead to a drastic loss of SOC and the application of light grazing management rather than fencing, not only contributed to the reduction of soil carbon mineralization with only a small effect on both the abundance and structure of the soil fungal community, but can also maintain high productivity and more stable carbon content. Thus, our study exemplifies the importance of incorporating soil carbon mineralization and appropriate levels of grazing into terrestrial ecosystem models and for optimizing grassland management policies in a changing world.

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CRediT authorship contribution statement

Tianqi Zhao: Writing – original draft, Investigation, Data curation, Conceptualization. **Ton Bisseling:** Writing – review & editing. **Yuanhe Yang:** Writing – review & editing, Investigation. **Mengli Zhao:** Writing – original draft, Supervision, Conceptualization. **Bin Zhang:** . **Guodong Han:** .

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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