



Re-introducing mixed grazing benefits soil health in a passive restored grassland: Evidence from soil nematode functional status

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ABSTRACT

Re-introducing livestock grazing offers a promising management strategy for degraded grasslands that have sufficiently recovered; however, the optimal livestock assemblage remains uncertain. To address this gap, we conducted a field experiment by re-introducing different livestock assemblages into a grassland that had been fenced for passive restoration since 1993. From 2017–2020, we implemented a grazing experiment involving cattle and sheep, both individually and in mixed herds, at moderate intensity during the growing season. Using nematode-based ecological indices, we evaluated the effects of re-introducing different herbivore assemblage on the structure and functions of the soil food web. We found that re-introducing livestock grazing in a mixed regime preserved the soil food web structure and significantly enhanced its functions, as indicated by increases in nematode functional metabolic footprints and energy flux. The increase was attributed to the greater abundance and body size of bacterivores, fungivores, and omnivores-predators, primarily driven by enhanced soil microbial biomass and nutrient availability under mixed grazing. In contrast, cattle or sheep mono-grazing tended to have negative effects on the structure and functions of the soil food web. These findings provide valuable insights for grassland management, suggesting that re-introducing mixed grazing in restored grasslands could promote soil health while efficiently utilizing forage resources. Furthermore, our findings contribute to the growing body of evidence highlighting the unique benefits of mixed grazing on grassland ecosystems compared to single-species grazing.

1. Introduction

Grasslands constitute one of Earth's most extensive terrestrial ecosystems, providing multiple crucial ecosystem functions and services, such as food production, water regulation, carbon sequestration, and biodiversity conservation (Buisson et al., 2022; FAO, 2023). However, grassland degradation has become widespread and is accelerating across many regions of the world due to the impacts of anthropogenic disturbances (particularly livestock overgrazing) and climate change, with

approximately 49 % of global grassland areas showing varying degrees of degradation (Bardgett et al., 2021). For example, the Songnen Grassland, a crucial part of the Eurasian Steppe, has experienced severe degradation caused by long-term livestock overgrazing (Ma et al., 2024a). To combat grassland degradation, fencing to exclude livestock is commonly considered an effective passive restoration strategy (Török et al., 2021). While this approach has achieved notable success in increasing vegetation productivity and biodiversity, prolonged fencing can introduce new challenges, such as underutilization of grassland

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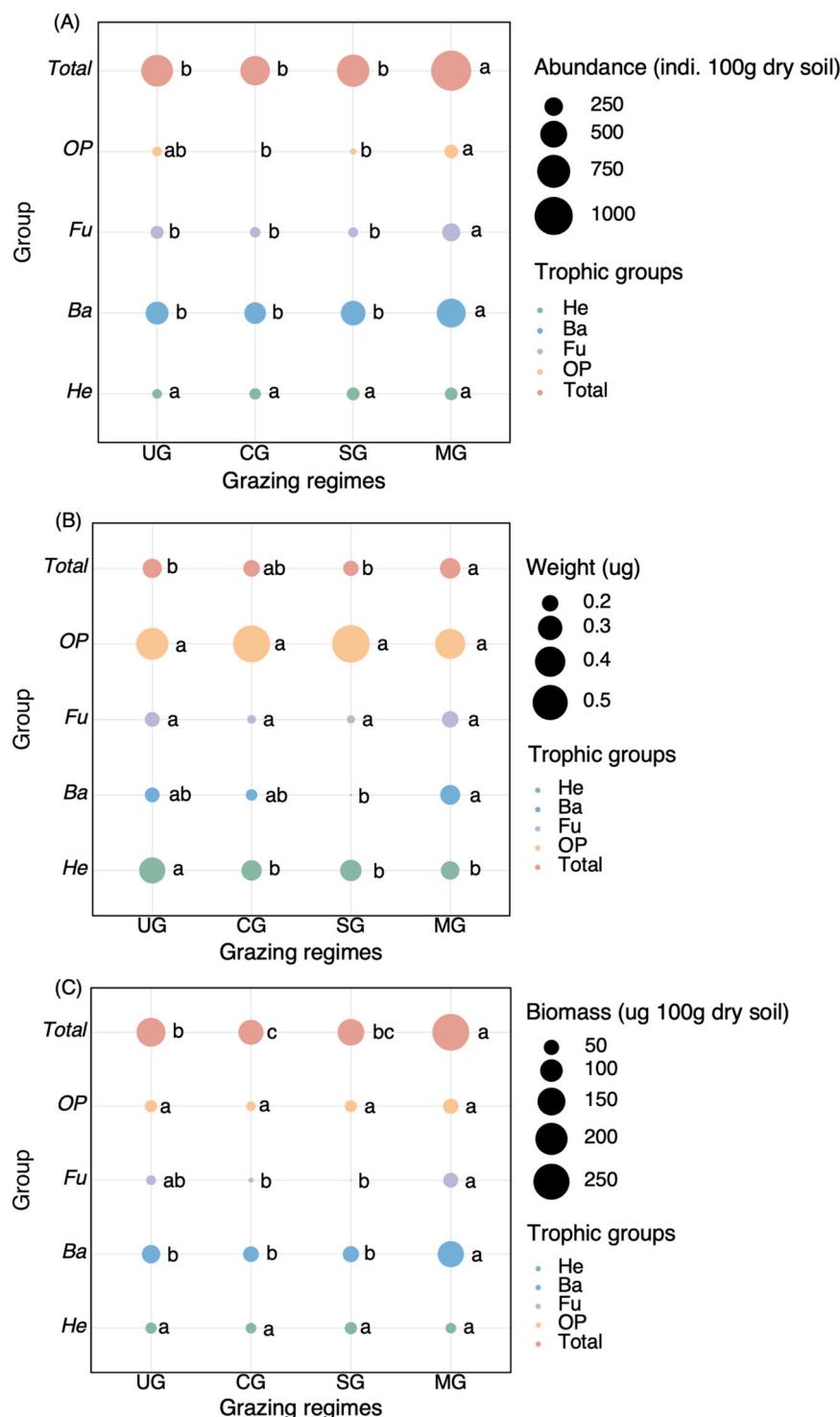


Fig. 1. Effects of grazing on the abundance (A), weight (B), and biomass (C) of total nematodes and trophic groups in the restored grassland. The significant differences among grazing regimes were labelled by different lowercases. The grazing treatments included ungrazing as a control (UG), sheep grazing (SG), and cattle grazing (CG). Soil nematodes were classified into four trophic groups (herbivores, He; bacterivores, Ba; fungivores, Fu; omnivores-predators, OP).

resources (Lyons et al., 2023). Thus, achieving a balance between grassland restoration and resource utilization remains a critical challenge requiring further attention.

The re-introduction of livestock grazing presents a viable management option once degraded grasslands achieve sufficient recovery. Substantial evidence indicates that re-introducing large herbivores at moderate intensities in restored grasslands helps reduce plant competition and creates ecological niches that enhance plant diversity (Kapás

et al., 2020; Török et al., 2021; Dvorský et al., 2022). Light to moderate grazing is widely adopted as an effective management practice for promoting sustainable grassland development (Metzger et al., 2010; Zhang et al., 2021). According to the intermediate disturbance hypothesis, moderate grazing can enhance or maintain plant diversity and productivity by stimulating plant compensatory growth (Milchunas et al., 1988). Nevertheless, grazing impacts largely rely on herbivore types, climatic aridity, and grassland status (Gao and Carmel, 2020).

Globally, mixed grazing involving multiple herbivore species is more common than mono-grazing (Wang et al., 2019). Previous research has demonstrated that mixed grazing with diversified livestock (e.g., cattle and sheep) can exert complementary effects on plant communities and reduce gastrointestinal parasite infections through a "dilution effect", thereby providing enhanced benefits for grassland biodiversity, ecosystem functionality, and livestock production (Fraser et al., 2014; Wang et al., 2019; Jerrentrup et al., 2020; Su et al., 2023). Additionally, mixed grazing may benefit soil fauna by increasing the diversity of animal dung and plant litter inputs (Wang et al., 2019). Despite these recognized benefits of grazing management, existing research on livestock grazing has predominantly focused on plant production, above-ground diversity, and soil microbial communities, while our understanding of soil fauna responses to grazing—particularly under mixed grazing systems—remains substantially limited. Given that soil fauna serve as critical regulators of soil microbial activity, nutrient cycling processes, and energy flux dynamics (Bardgett and Van Der Putten, 2014), it is essential to elucidate the impacts of grazing on soil fauna communities to develop comprehensive strategies for sustainable grassland management.

Among soil fauna, nematodes are the most abundant and diverse soil organisms, serving as reliable indicators of soil health and ecosystem functions due to their ubiquitous presence across all major trophic levels within soil food webs and their high sensitivity to environmental changes (Bongers and Ferris, 1999; Yeates, 2003; van den Hoogen et al., 2019). Over the past three decades, a series of nematode-specific indices have been developed to reflect soil food web structure and ecosystem functions, thereby informing optimal land management strategies (Zhao et al., 2019; Du Preez et al., 2022). Particularly noteworthy are nematode functional metabolic footprints and energy flux analyses across multi-trophic levels, which together offer powerful and universal metrics for quantifying the magnitude of ecosystem functions and soil health provided by various organisms within the soil food web (Ferris, 2010; Sikora et al., 2018; Wan et al., 2022). Studies examining the effects of livestock grazing on grassland soil nematode communities have shown that light grazing (e.g., 1.8 sheep ha^{-1}) or moderate grazing (e.g., 2 yaks ha^{-1}) can enhance soil nematode diversity and functional metabolic footprints, supporting sustainable grassland management practices (Gao et al., 2022; Zhang et al., 2022). In addition to grazing intensity, herbivore species and grassland degradation status should also be critically considered for grazing management (Wang et al., 2019; Niu et al., 2025). For instance, cattle and sheep exhibit distinct foraging preferences, trampling intensities, and excretion patterns, which can differentially alter soil basal resources and the soil environment, thereby leading to varied impacts on soil communities (Wang et al., 2019; Ma et al., 2024a). However, existing studies have reported divergent findings regarding the effects of different livestock species on soil nematode communities. While some research has documented significant negative impacts of sheep grazing (Zhou et al., 2023; Ma et al., 2024a), other studies have indicated that mixed grazing by cattle and sheep may have neutral or even beneficial effects (Wang et al., 2019; Sun et al., 2024). Despite these findings, a comprehensive understanding of species-specific impacts remains lacking. Notably, experimental evidence examining how soil nematode communities respond to grazing by different livestock species and their combinations, particularly in restored grassland ecosystems, remains substantially limited.

To address this knowledge gap, we designed a field grazing experiment in a passively restored grassland that has been protected by fencing since 1993. This long-term conservation approach has facilitated remarkable ecosystem recovery, characterized by substantial increases in plant biomass and biodiversity. From 2017–2020, we conducted a four-year grazing experiment involving cattle and sheep, both individually and in mixed herds, maintained at a consistent moderate intensity during the growing season. Utilizing nematode-based ecological indices, we systematically assessed the impacts of different grazing regimes on the structure and functions of the soil food web. Our primary objective

was to evaluate whether the re-introduction of livestock grazing benefits soil health in the restored grassland and to identify the optimal grazing regime. We hypothesize that 1) relative to ungrazed control, reintroducing livestock grazing at moderate intensity would increase soil nematode taxonomic diversity and energy flux by enhancing litter and dung inputs; and 2) mixed grazing by cattle and sheep will produce a more pronounced positive effect on nematode-indicated soil health compared to single-species grazing, as the complementary foraging behaviors and dung types of different herbivores increase soil resource heterogeneity.

2. Materials and methods

2.1. Study site

The study was conducted at the Jilin Songnen Grassland Ecosystem National Observation and Research Station ($44^{\circ}35'33''\text{N}$, $123^{\circ}30'47''\text{E}$; 138–167 m a.s.l.) in Changling County, northeastern China. This site exemplifies a semi-arid continental climate, with an average annual precipitation ranging from 280 mm to 430 mm, approximately 70 % of which occurs during the growing season from June to September. The average annual temperature ranges from 4.6°C to 6.4°C . The study area has historically experienced prolonged intensive livestock grazing, resulting in severe degradation. Recognizing the critical need for passive restoration, the area was fenced since 1993 to mitigate anthropogenic disturbances and facilitate natural regeneration processes. This long-term conservation strategy has yielded remarkable ecological outcomes, demonstrating significant improvements in vegetation biomass and species diversity over the past 25 years. The recovered vegetation is predominantly composed of resilient perennial grasses (*Leymus chinensis* and *Phragmites australis*), accompanied by salt-tolerant forb species (e.g., *Artemisia anethifolia* and *Kochia sieversiana*). According to the Chinese soil classification system, soils are categorized as meadow-chernozemic and saline-alkaline, with a pH of 8.0–10.0.

2.2. Experimental design

A randomized block experimental design was established in the restored area, comprising nine enclosures ($25 \text{ m} \times 25 \text{ m}$ each) distributed in three blocks (Fig. 1). Four grazing treatments were randomly assigned within each block: cattle-only (CG), sheep-only (SG), cattle-sheep combination (MG), and ungrazed control (UG). The grazing protocol, implemented from 2017 to 2020, involved biweekly sessions during each growing season (June–August), with grazing conducted in designated morning (06:00–08:00) and afternoon (16:00–18:00) periods. Each single-species grazing paddock housed either three cattle (mean weight: $350.0 \pm 10.5 \text{ kg}$) or fifteen sheep (mean weight: $35.0 \pm 1.6 \text{ kg}$), while each mixed grazing paddock accommodated three cattle and fifteen sheep, with the grazing duration halved relative to single-species grazing to equalize total grazing intensity. These stocking rates were calibrated through preliminary trials based on daily forage consumption, with individual cattle and sheep removing $7.5 \pm 0.9 \text{ kg}$ and $1.5 \pm 0.5 \text{ kg}$ of plant dry biomass, respectively. This established a cattle-to-sheep ratio of 1:5 to ensure equivalent forage utilization.

2.3. Data collection

Soil sampling was conducted in late August 2020, with six replicates established per enclosure. Each replicate comprised five soil cores (2.5 cm diameter, 0 – 10 cm depth) collected using an auger and put into a single sterile plastic bag. All soil samples were immediately placed in a cool box to suppress biological activity. In the laboratory, homogenized samples were subdivided for four distinct analyses: 1) nematode extraction and soil moisture determination; 2) microbial biomass and available nitrogen quantification (2 mm sieved); 3) physicochemical properties assessment: pH and electrical conductivity (2 mm sieved); 4)

total organic carbon (TOC), nitrogen, and phosphorus (0.15 mm sieved).

2.3.1. Nematode extraction and measurements

Nematode extraction was performed on 50 g of fresh soil (sieved to 5 mm) using modified Baermann funnels (Hallman and Viaene, 2013), and the extracted nematodes were preserved in 4 % formalin solution. Nematode abundance was determined using an inverted microscope (Nikon 80I, Japan) and expressed as individuals per 100 g of dry soil. For taxonomic analysis, 150 specimens per sample were identified to the genus or family level and photographed using an Olympus BX51 microscope equipped with a DP72 digital camera. Morphometric measurements (body length L and maximum diameter D) were obtained using Nikon NIS-Elements software, excluding specimens undergoing ecdysis to ensure measurement accuracy. Individual fresh biomass (W) was calculated using Andrassy's (1956) formula: $W = L \times D^2 \times 1.6^{-1} \times 10^{-6}$. Total nematode biomass was calculated by multiplying mean

individual weight by population density.

2.3.2. Nematode ecological indices

Nematode specimens were categorized by both trophic functions (herbivores-He, bacterivores-Ba, fungivores-Fu, omnivores-Om, predators-Pr) and colonizer-persister (cp 1–5) life-history strategies (Bongers, 1990; Yeates et al., 1993). Community analyses included community-weighted mean body weight (cwmW), abundance, and biomass metrics (total and trophic group-specific). The following ecological indices were calculated:

Diversity indices: Shannon-Wiener Index (H'): $-\sum pi (\ln pi)$, Trophic Diversity (TD): $1 / \sum Pi^2$, where pi represents genus proportion and Pi denotes trophic group proportion.

Feeding Preference (Bongers, 1990): Maturity Index (MI): $\sum vi \times fi$ (free-living nematodes), where v and f represent cp values and proportions, respectively.

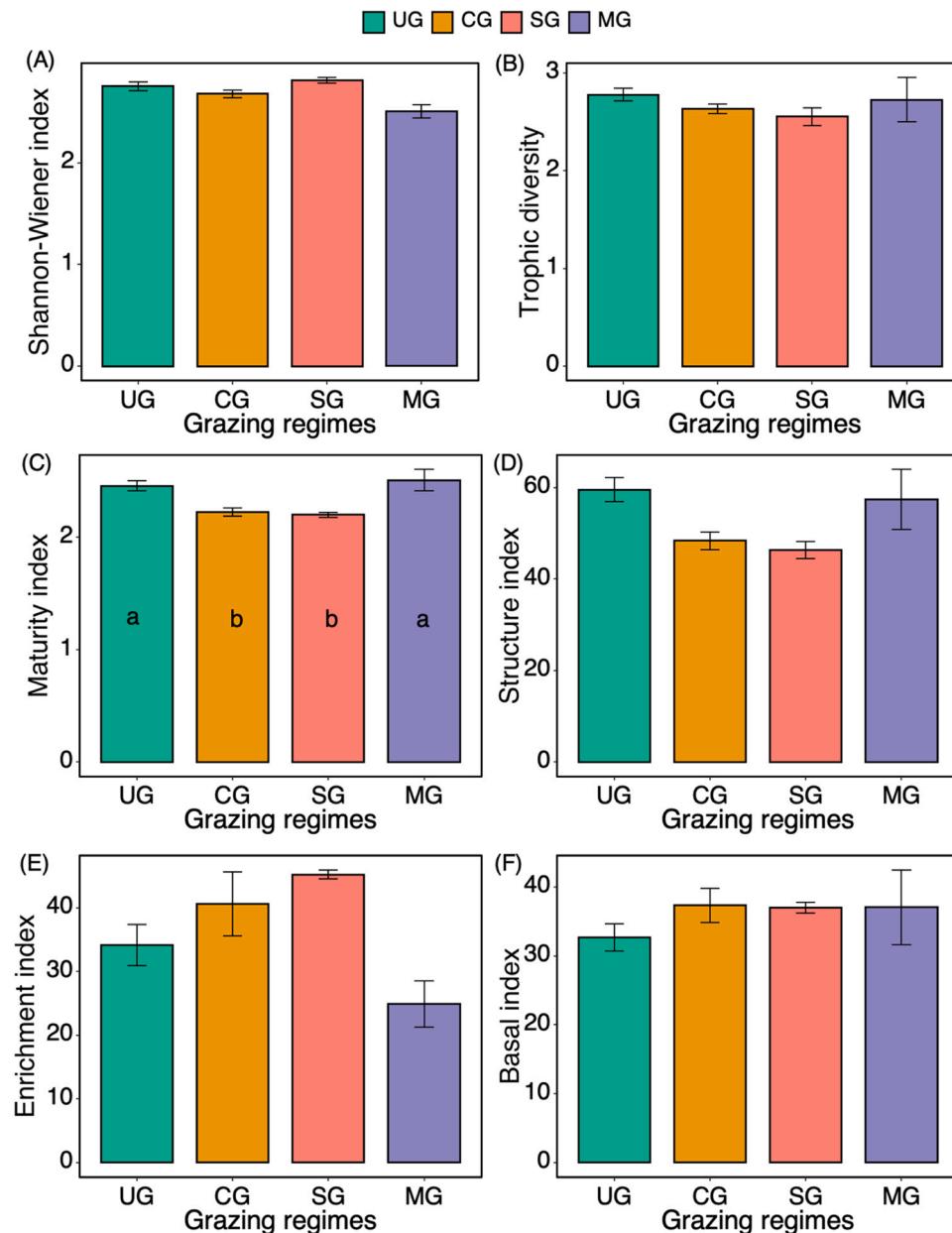


Fig. 2. The soil nematode maturity index (MI, A), structure index (SI, B), enrichment index (EI, C), and basal index (BI, D) under different grazing regimes in the restored grassland. The grazing treatments included ungrazing as a control (UG), sheep grazing (SG), and cattle grazing (CG). The significant differences among treatments were labelled by different lowercase.

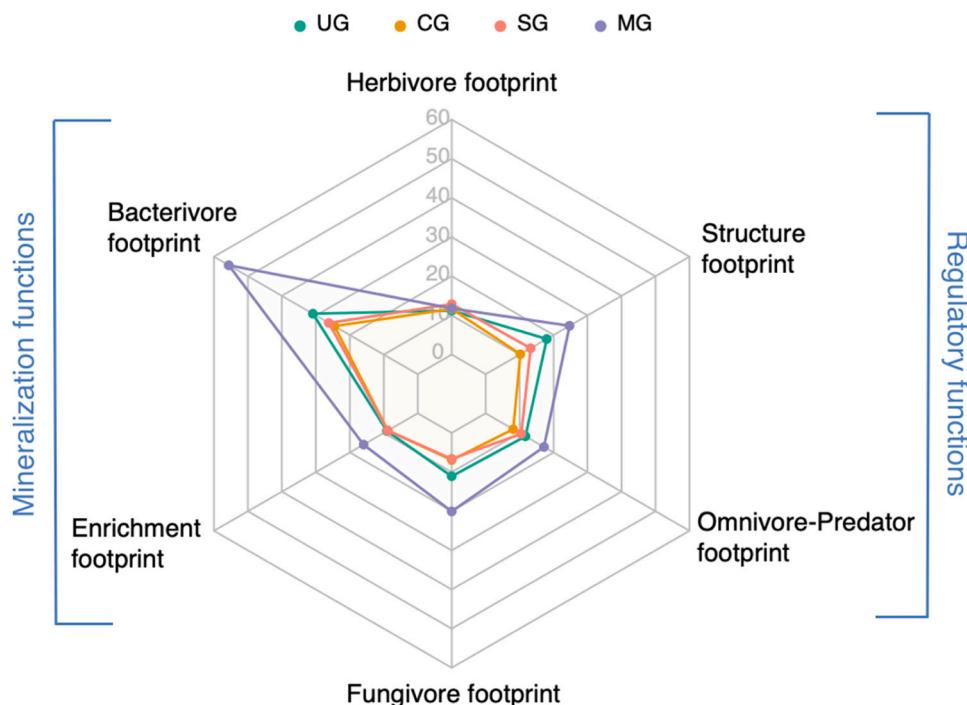


Fig. 3. Radar charts combined nematode metabolic footprints of different trophic groups and enrichment/structure components, which indicates the magnitude of ecosystem functions provided by the soil food web under different grazing regimes in the restored grassland. The grazing treatments included control ungrazing (UG), cattle grazing (CG), sheep grazing (SG), and mixed grazing (MG).

Food Web Indices (Ferris et al., 2001): Basal Index (BI): $100 \times b/(b + e + s)$, Enrichment Index (EI): $100 \times e / (b + e)$, Structure Index (SI): $100 \times s / (b + s)$, Components (b, e, s) were calculated as weighted sums ($\sum k \times n$) of respective guild abundances, representing basal (Ba₂, Fu₂), enrichment (Ba₁, Fu₂), and structure (cp 3–5 of Ba, Fu, Om, Pr) components.

Nematode metabolic footprints: The nematode metabolic footprints (F_p) were calculated as: $F_p = \sum (N_t (0.1 (W_t / m_t) + 0.273 (W_t^{0.75})))$, where N_t , W_t , and m_t are the abundance, biomass, and cp values of taxon t , respectively. The F_p of each trophic group and enrichment and structure components were calculated for conducting a radar chart to comprehensively mirror the magnitude of the ecosystem functions provided by the soil food web. The enrichment footprint reflects the metabolic footprint of nematodes that respond most rapidly to resource enrichment and indicates a mineralization function, while the structure footprint represents the metabolic footprint of higher trophic levels that represents a regulatory function in the food web (Sikora et al., 2018).

Nematode energy flux: Nematode-based food web energy flux were quantified through potential carbon flux calculations following Wan et al. (2022). The potential carbon flux (Fi) per trophic level was derived as: $Fi = (F + L) / ea$, where: F = Total energy consumed (respiration + production), L = Energy loss to higher trophic levels, and ea = Assimilation efficiency (herbivores: 0.25, bacterivores: 0.60, fungivores: 0.38, omnivore-predators: 0.50). Energy consumption (F) was calculated as: $F = \sum (N_t (0.1 (Wt / mt / 12) + 0.0159 (Wt^{0.75})))$, where N_t , W_t , and m_t represent abundance, biomass, and cp values of taxon t , respectively. The calculation sequence began with omnivores-carnivores (no higher trophic loss), followed by energy losses (L) from lower trophic groups: $L = Dio \times Fo$, where: Dio = Abundance-dependent feeding preference, Fo = Omnivore-predator energy demand. Energy flow uniformity was assessed as the ratio between mean total energy flux per channel and its standard deviation.

2.3.3. Soil properties

Soil physicochemical analyses were conducted using standardized methods. Soil water content (SWC) was determined by oven-drying at

105°C for 48 h until constant weight. Microbial biomass carbon (MBC) was measured using the fumigation-incubation method and quantified with an Elementar Vario TOC analyzer. Total organic carbon (TOC) and total nitrogen (TN) were quantified using an Elementar Vario MAX cube analyzer, while total phosphorus (TP) was measured with an AMS Smartchem 450 auto discrete analyzer. Soil ammonium nitrogen (NH_4^+ -N) and nitrate nitrogen (NO_3^- -N) were extracted with a 2 mol/L potassium chloride solution and measured using a continuous flow analyzer (SEAL AA3). Soil pH was determined in a 1:2.5 soil-to-water suspension using a Sartorius PB-10 m, and salinity was assessed by measuring electrical conductivity (EC) in a 1:5 soil-to-water suspension with a Mettler Toledo FE30 conductivity meter.

2.4. Statistical analyses

The effects of grazing regimes on nematode community metrics and soil properties were analyzed using linear mixed-effects ANOVA (the lme function in the nlme package). The model included grazing regimes as fixed effects and sample replicates nested within grazing treatments within blocks as random effects to account for pseudo-replication. Significant differences were identified using Tukey's post hoc test. Principal component analysis (PCA) was conducted to examine correlations among nematode energy flux-related metrics (total energy flux, flow uniformity, biomass, abundance, and cwmW) and soil properties (MBC, MBN, TOC, TN, TP, SWC, SBD, pH, EC, NH_4^+ -N, and NO_3^- -N). Additionally, path analysis was performed to evaluate the direct and indirect pathways influencing nematode total energy flux, mediated by nematode abundance and cwmW, as well as their determining factors. These factors were selected based on marginally significant differences identified by the ANOVA and PCA results. Pearson correlation analyses were used to assess relationships between soil nematode total energy flux, its components, flow uniformity, and nematode community structure indices (Shannon-Wiener index, trophic diversity, enrichment index, structure index, maturity index, and basal index). Statistical significance was set at $P < 0.05$. All analyses were conducted using R version 4.4.0 (R Core Team, 2024).

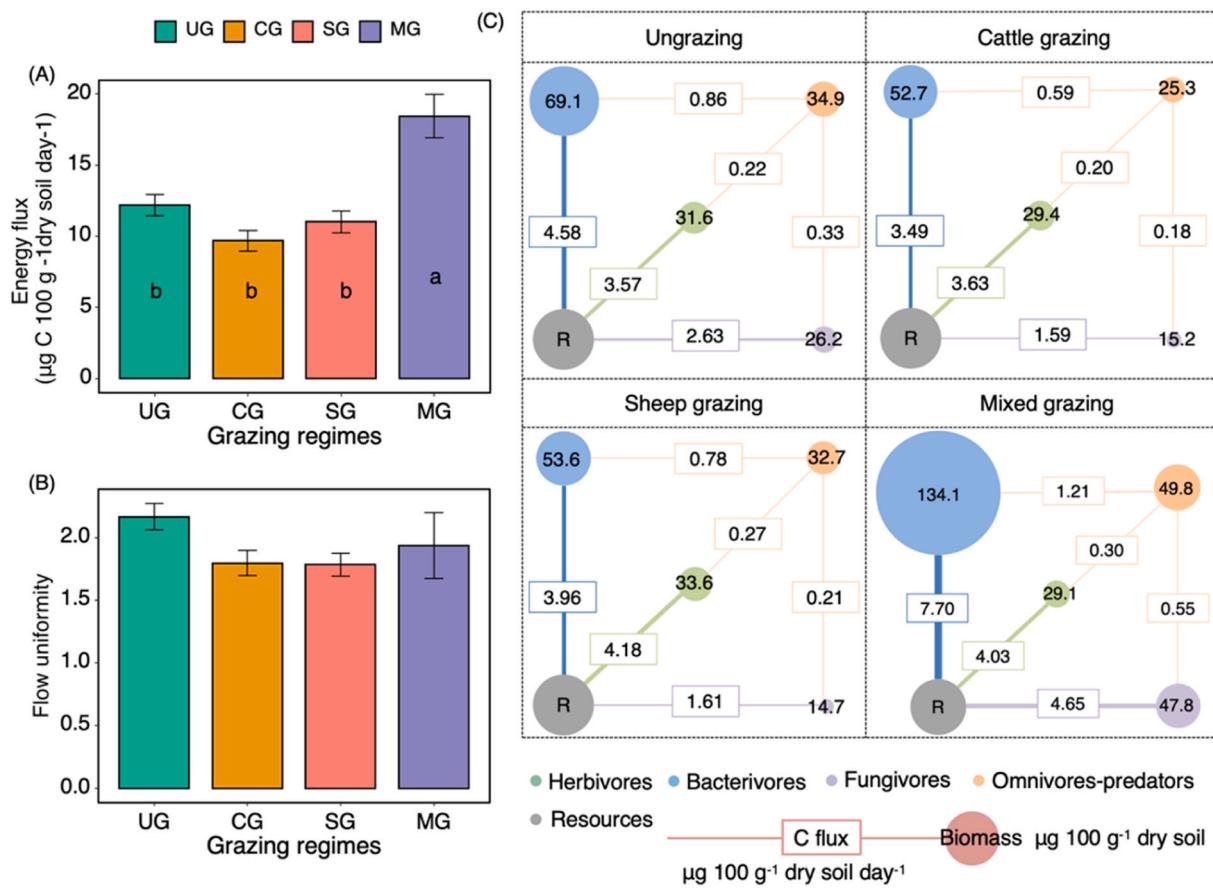


Fig. 4. The total energy flux (A), flow uniformity (B), and energetic structures (C) of the nematode food web under different grazing treatments. For each treatment, a five-node food web was constructed with herbivores (green), bacterivores (blue), and fungivores (purple) acquiring energy from basal resources (R), and omnivores-predators (orange) acquiring energy from other nodes. The size of the nodes corresponds to nematode fresh biomass ($\mu\text{g }100 \text{ g}^{-1}\text{ dry soil}$). The numbers along the lines and the line widths represent energy flux ($\mu\text{g C }100 \text{ g}^{-1}\text{ dry soil day}^{-1}$). Significant differences among treatments are indicated by different lower-case letters.

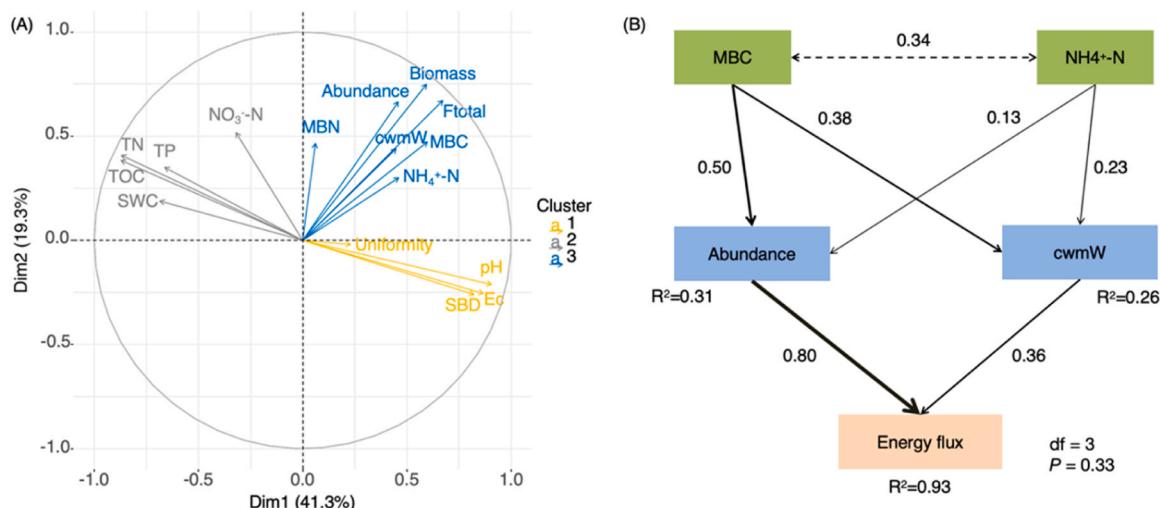


Fig. 5. (A) The principle component analysis (PCA) shows the correlations among nematode community indices and environmental factors. The nematode community indices include nematode total energy flux, flow uniformity, biomass, abundance, and cwmW; the environmental factors include soil microbial carbon (MBC), microbial carbon (MBN), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), soil water content (SWC), soil bulk density (SBD), soil pH (pH), electrical conductivity (Ec), ammonium nitrogen (NH_4^+ -N), and nitrate nitrogen (NO_3^- -N). (B) The path analysis diagram reveals the direct and indirect pathways through which nematode total energy flux is driven by MBC and NH_4^+ -N. The two factors closely relate to nematode abundance and cwmW, which are the two primary constructs influencing nematode total energy flux.

3. Results

3.1. Soil nematode abundance, weight, and biomass

Grazing had significant effects on the abundance, body weight, and biomass of total nematodes, as well as on the abundance and biomass of bacterivores and fungivores (Table S1). Specifically, mixed grazing significantly increased total nematode biomass, primarily driven by an increase in the abundance of bacterivores and fungivores rather than changes in their body weight (Fig. 1). In addition, the abundance of omnivores–predators was significantly higher under mixed grazing compared to cattle or sheep grazing, whereas their body weight tended to be lower under mixed grazing than under cattle or sheep grazing.

3.2. Soil nematode-based ecological indices and energy flux

Grazing significantly affected the maturity index, the metabolic footprints of bacterivores and fungivores, and total energy flux (Table S1). Notably, mixed grazing maintained trophic diversity, maturity index, and structure index at levels comparable to the ungrazed control, whereas cattle or sheep grazing reduced these indices (Fig. 2A–D). Conversely, cattle or sheep grazing increased the enrichment index, whereas mixed grazing decreased it (Fig. 2E). All three grazing regimes tended to increase the basal index (Fig. 2F). As illustrated in the radar chart, mixed grazing enhanced the metabolic footprints of bacterivores and fungivores, as well as the enrichment component, indicating heightened mineralization functions (Fig. 3). Moreover, mixed grazing increased the omnivore–predator and structure footprints, suggesting enhanced regulatory functions. In contrast, cattle or sheep mono-grazing diminished these critical ecosystem functions.

Moreover, mixed grazing significantly increased the total energy flux of the nematode-based soil food web, whereas cattle or sheep grazing tended to decrease both the energy flux and flow uniformity (Fig. 4A–B). The increase in total energy flux under mixed grazing compared to the ungrazed control was primarily due to increases in energy flux from resources to bacterivores (+68.1 %) and fungivores (+76.8 %), as well as from bacterivores (+40.7 %) and fungivores (+66.7 %) to omnivores–predators (Fig. 4C). Conversely, under cattle or sheep grazing, the energy flux from resources to the second trophic level and from the second to the third trophic level were lower than those under the ungrazed control.

3.3. Soil properties determining soil nematode energy flux

Soil MBC, pH, EC, NH_4^+ -N, and NO_3^- -N were higher under mixed grazing compared to the ungrazed control and cattle- or sheep-only grazing, while other soil properties showed no significant differences among the grazing treatments (Table S2). The ANOVA results showed marginally significant effects of grazing on MBC ($P = 0.07$) and NH_4^+ -N ($P = 0.03$). Furthermore, principal component analysis (PCA) showed that nematode total energy flux was closely associated with nematode biomass, abundance, and cwmW, all of which were positively correlated with microbial biomass carbon (MBC) and ammonium nitrogen (NH_4^+ -N; Fig. 5A). The subsequent path analysis indicated that nematode total energy flux was primarily driven by nematode abundance, which in turn was strongly influenced by MBC (Fig. 5B). A smaller contribution to the variation in nematode total energy flux was attributed to cwmW, which was jointly regulated by MBC and NH_4^+ -N. In addition, Pearson correlation analysis showed that soil nematode total energy flux, the energy flux from the second to the third trophic level, and flow uniformity were significantly positively correlated with nematode trophic diversity, structure index, and maturity index, while being negatively correlated with the nematode basal index (Fig. S1).

4. Discussions

To prevent ongoing grassland degradation, fencing to exclude large herbivores is widely regarded as an effective passive restoration strategy (Török et al., 2021). However, prolonged fencing can introduce new challenges, such as resource underutilization (Lyons et al., 2023). Re-introducing livestock grazing may be a potential solution, but the optimal grazing regime remains uncertain. In this study, we used nematode abundance-based structure indices and size-based functional indices to comprehensively assess the impacts of different grazing regimes on the soil food web structure and soil ecosystem functions in a long-term passively restored grassland. Our primary finding was that mixed grazing by cattle and sheep at moderate intensity helped sustain the soil food web structure, significantly enhanced its total energy flux, and improved the ecosystem mineralization and regulatory functions as indicated by increased nematode metabolic footprints. In contrast, single-species grazing tended to exhibit negative effects on the structure and functions of the soil food web. Therefore, re-introducing mixed grazing during the restoration of degraded grasslands may help balance grassland soil health and resource utilization, representing a more sustainable approach to grassland management.

The increase in total energy flux of the nematode-based soil food web under mixed grazing was attributed to the enhanced energy flux from resources to bacterivores and fungivores, as well as from these second trophic levels to omnivores–predators. These increases in energy flux were primarily driven by increasing the abundance of bacterivores, fungivores, and omnivores–predators, as well as by enhancing body sizes of bacterivores and fungivores under mixed grazing. Conversely, cattle or sheep mono-grazing reduced the abundance of omnivores–predators and the body sizes of bacterivores and fungivores, leading to subsequent decreases in nematode biomass and energy flux. Moreover, as shown by the radar chart depicting soil ecosystem functions based on nematode metabolic footprints, mixed grazing promoted both regulatory and mineralization functions, as indicated by increases in nematode enrichment and structure footprints, whereas cattle or sheep grazing diminished these critical ecosystem functions.

Our study provides experimental evidence that mixed grazing, rather than single-species grazing, has the potential to improve grassland soil health by enhancing soil nematode metabolic footprints and the energy flux of the soil food web. This is a valuable complement to previous research, which concluded that mixed grazing by multiple large herbivore species benefits grassland ecosystems by enhancing plant diversity, aboveground productivity, and soil carbon and nitrogen storage (e.g., Fraser et al., 2014; Liu et al., 2016; Chang et al., 2018; Jerrentrup et al., 2020). In particular, a study conducted in the Songnen Grassland found that mixed grazing by cattle and sheep enhanced multitrophic diversity and multifunctionality (Wang et al., 2019). However, the positive effect of mixed grazing on soil nematodes observed in this study does not align with some previous studies reporting negative impacts, although these effects were still less detrimental than those under single-species grazing (Chen et al., 2021; Zhou et al., 2023). The negative effects of cattle or sheep mono-grazing on nematode abundance and body size are consistent with most previous observations (e.g., Andriuzzi and Wall, 2018; Wang et al., 2020; Sun et al., 2024; Zhou et al., 2023). Nevertheless, some studies have found that grazing at moderate intensities can increase nematode abundance or taxonomic diversity, supporting the intermediate disturbance hypothesis (Wang et al., 2019; Zhang et al., 2022). The impacts of grazing on soil nematodes depend on grassland type, grazing intensity, grazing animals, and grazing duration (Sun et al., 2024; Zhou et al., 2023). Our study provides crucial experimental evidence for these context-dependent responses of soil nematodes to grazing practices and emphasizes the need for comprehensive long-term and large-scale assessments.

To elucidate the mechanisms driving the positive impacts of mixed grazing on grassland ecosystems, it is essential to consider the distinct dietary preferences and foraging behaviors of cattle and sheep. These

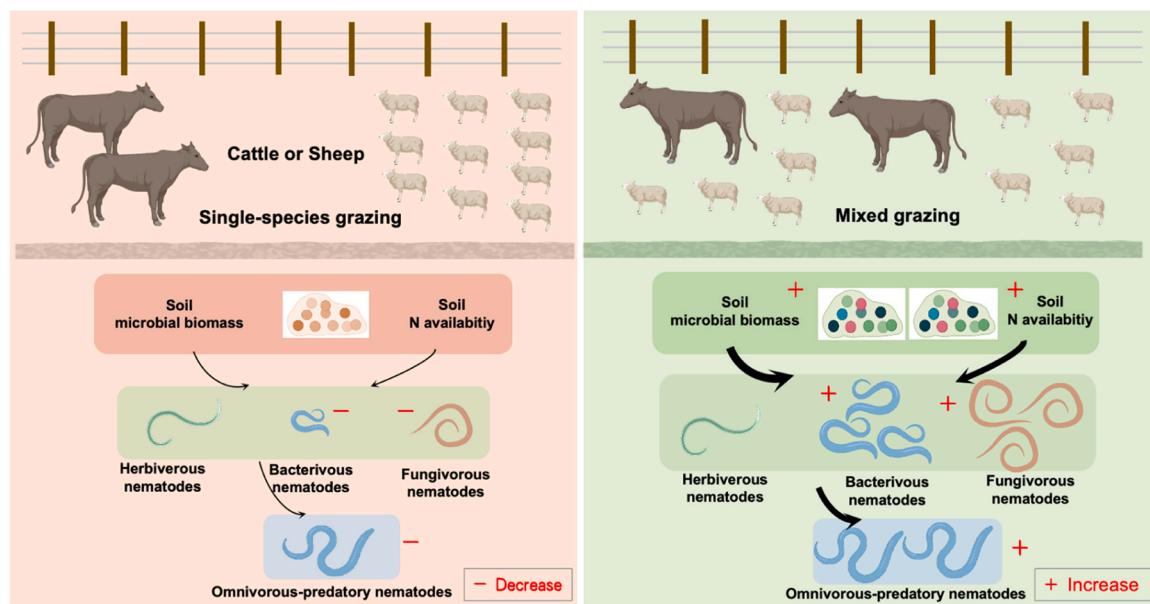


Fig. 6. Conceptual diagram illustrating the effects of single-species (cattle or sheep) grazing versus mixed grazing on the nematode-based soil food web. Compared to ungrazing, mixed grazing enhanced the abundance and body size of bacterivores, fungivores, and omnivores-predators, primarily driven by enhanced soil microbial biomass and nutrient availability. The positive effects of mixed grazing resulted in higher metabolic footprints and greater energy flux within the soil food web, contrasting with the detrimental impacts observed in single-species grazing.

differences can lead to synergistic and complementary effects on plant growth, biomass allocation, and root exudation (Ritchie and Olff, 1999; Liu et al., 2015). Moreover, grazing by a diverse mix of livestock may expand the range of niches available to soil organisms by increasing the variety of animal dung and plant litter inputs (Wang et al., 2019). These synergistic interactions and enhanced habitat diversity likely contribute to our finding that mixed grazing significantly enhanced soil nematode metabolic footprints and energy flux through multiple interconnected pathways. The primary pathway appears to be via elevated soil microbial biomass carbon (MBC), as evidenced by significantly higher values under mixed grazing (309.6 mg/kg) compared to other grazing systems (195.33–259.62 mg/kg). This enriched microbial biomass provides an abundant food source for bacterivores and frugivores, which subsequently supports higher trophic-level omnivores and predators through bottom-up effects (Wang et al., 2019; Ma et al., 2024b). This results in an increase in the abundance and body size of corresponding nematode trophic groups, establishing a strong foundation for energy transfer within the soil food web. Additionally, mixed grazing showed increased soil pH (9.28 vs. 8.65–8.76), suggesting favorable soil conditions for microbial activity (Pietri and Brookes, 2008; Wu et al., 2024). Moreover, mixed grazing resulted in higher NH₄⁺-N concentrations (6.07 mg/kg vs. 3.87–4.05 mg/kg in other treatments) and NO₃⁻-N levels (3.83 mg/kg vs. 2.57–3.54 mg/kg), indicating improved soil nutrient availability. This nutrient enhancement likely stems from the diverse inputs of livestock excreta and plant litter under mixed grazing. These improvements in soil attributes create a positive feedback loop: enhanced microbial biomass accelerates organic matter decomposition, thereby increases nutrient availability and cycling efficiency. This is further supported by the improved electrical conductivity under mixed grazing, which facilitates nutrient transport and availability (Eigenberg et al., 2002; Heiniger et al., 2003). Collectively, these findings suggest that mixed grazing enhances soil nematode functional footprints and energy flux primarily through increased microbial biomass, and secondarily through improved nutrient conditions, forming a self-reinforcing system that maintains higher energy flow within the soil food web. Nevertheless, microbial biomass and available nitrogen explained only part of the variation in soil nematode abundance and body size, highlighting the need for further studies involving additional factors and processes to

uncover the complex mechanisms underlying the effects of mixed grazing on soil nematodes.

In conclusion, our study demonstrates that re-introducing mixed grazing by cattle and sheep, rather than single-species grazing, in a long-term fenced and restored grassland can benefit soil health by enhancing soil functional metabolic footprints and energy flux, particularly by increasing soil microbial biomass and nutrient availability (Fig. 6). These findings offer valuable insights for grassland management, suggesting that the re-introduction of grazing by mixed livestock pattern in restored grasslands may serve as an effective strategy to balance grassland soil health and resource utilization. Furthermore, our findings add to the growing body of evidence that mixed grazing offers unique benefits to grassland ecosystems compared to single-species grazing. Nonetheless, as our study was conducted within a single grassland type and focused on short-term effects, further research is needed to assess the long-term sustainability and broader applicability of these findings across diverse grassland ecosystems.

CRediT authorship contribution statement

Quanhui Ma: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yao Wang:** Software, Resources, Investigation, Data curation. **Yonghuan Yue:** Resources, Investigation, Data curation. **Haoran Yu:** Resources, Investigation, Data curation. **Deli Wang:** Writing – review & editing, Validation, Supervision, Conceptualization. **Yu Zhu:** Writing – review & editing, Writing – original draft, Visualization, Software, Funding acquisition, Formal analysis. **Ling Wang:** Writing – review & editing, Visualization, Validation, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

All authors declare that they have no commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2025.109861](https://doi.org/10.1016/j.agee.2025.109861).

Data availability

Data will be made available on request.

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