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Research

Ungulate herbivory affects grassland soil biota β-diversity and community assembly via modifying soil properties and plant root traits

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Summary

- Ungulate herbivory, a widespread and complex disturbance, shapes grassland biodiversity and functions primarily through three mechanisms: defoliation, trampling, and excreta return. However, the specific effects of these mechanisms on soil biodiversity and community assembly remain unclear.
- We conducted a 4-yr factorial experiment in the Eurasian steppe to investigate how defoliation, trampling, and excreta return influence soil bacterial, fungal, and nematode β-diversity and community assembly under moderate- and high-density ungulate grazing scenarios.
- Our findings reveal that herbivores affect soil biota through multiple pathways at different grazing intensities. Specifically, selective defoliation in the moderate-density scenario promoted stochastic community assembly of nematodes and fungi by increasing the specific root length of plant communities. Excreta return encouraged stochastic bacterial communities by carbon input, while urine-induced acidification and elevated ammonium levels promoted environmental filtering of bacteria and nematodes. In the high-density scenario, non-selective defoliation and heavy trampling created harsher soil conditions, reducing bacterial and nematode β-diversity via habitat filtering and diminishing association of soil biota with plant roots.
- This study explored how different components of ungulate behaviour influence soil community assembly and highlighted the crucial role of root traits in mediating soil biota responses, providing insights into the mechanisms of soil biodiversity maintenance under complex disturbances.

Introduction

Ungulate herbivores, including livestock and wildlife, function as ‘ecosystem engineers’ in grasslands by modifying plant community and soil properties (Cline *et al*., 2017; Maestre *et al*., 2022). Grassland soils host an unparalleled diversity of organisms (Anthony *et al*., 2023). Soil bacteria, fungi, and nematodes have complex interactions between plants and soil abiotic factors, contributing to grassland ecosystem functions (Bardgett & van der Putten, 2014). Ungulate herbivores shape soil biodiversity by influencing these interactions (Wang *et al*., 2020). Ungulate grazing affects local soil biodiversity (α-diversity), showing declining or unimodal patterns with increasing grazing intensity (Xu *et al*., 2023; Sun *et al*., 2024). However, there is limited understanding of how ungulates affect differences between local soil communities (β-diversity). Previous studies have yielded mixed results (Seaton *et al*., 2023; Zhou *et al*., 2023), likely due to the complex disturbances ungulates create through the joint effects of defoliation, trampling, and excreta return (Liu *et al*., 2015; Zhang *et al*., 2022; Guo *et al*., 2024a). Elucidating how soil organisms’ β-diversity responds to ungulate herbivores is essential for understanding the changes in grassland ecosystems and community assembly under complex disturbances (Mori *et al*., 2018).

Community assembly of soil organisms is regulated by deterministic and stochastic processes. Deterministic processes involve environmental filtering and biotic interactions, while stochastic processes are linked to random events, such as birth, death, and dispersal (Tripathi *et al*., 2018). Ungulate behaviours, such as defoliation, trampling, and excreta return, could alter the balance between deterministic and stochastic processes by affecting vegetation and soil properties (Liu *et al*., 2015). Also, the density of ungulates could be crucial in regulating the effects of these behaviours. At moderate densities, where forage is more available, ungulates tend to forage on higher energy and higher quality plants. On the other hand, at high densities, ungulates graze more intensively and unselectively, and cause more severe soil compaction (Onatibia *et al*., 2020). Although previous studies have sought to explain the effects of ungulates on soil organisms through these three key behaviours, direct evidence elucidating how defoliation, trampling, and excreta return specifically affect soil biodiversity remains scarce.

First, defoliation is a primary behaviour of ungulate herbivores. Moderate defoliation can enhance plant productivity (Wilson *et al*., 2018) and root exudates (Bardgett & Wardle, 2003), thereby alleviating resource limitations for soil organisms and strengthening stochastic processes (Zhang *et al*., 2017b). Selective defoliation at moderate grazing can increase plant α- and β-diversity (Guo *et al*., 2024a), offering diverse resources or hosts for soil organisms (Prober *et al*., 2015) and potentially enhancing soil β-diversity. By contrast, heavy defoliation may limit plant-derived carbon resources (Jiang *et al*., 2020; Qu *et al*., 2024), limiting the growth of soil organisms and resulting in a more homogeneous community. Defoliation also limits soil moisture by reducing surface cover (Veldhuis *et al*., 2014), affecting the dispersal and growth of water-sensitive bacteria and nematodes (Zhao *et al*., 2024) more than those of drought-resistant fungi (Canarini *et al*., 2017).

Second, excreta return affects soil organisms by altering soil properties. Nitrogen from ungulate urine is converted to ammonium nitrogen in the soil, which lowers pH (Liu *et al*., 2015), acting as a key environmental filter on soil organisms (Liu *et al*., 2021). Urine return could increase microbial activity by enhancing nutrient availability (Boon *et al*., 2014; Shen *et al*., 2023), potentially reducing habitat filtering. Also, faeces can provide organic matter and alleviate carbon limitation (Bardgett *et al*., 1998). Nutrient enrichment may increase the stochasticity of community assembly (Zheng *et al*., 2021).

Third, trampling is another non-negligible aspect of grazing, although its effects on soil biota community assembly remain underexplored. Trampling can increase soil bulk density and decrease soil pore size, acting as environmental filtering on soil organisms (Tang *et al*., 2020). Larger soil organisms with higher oxygen requirements, such as fungi and nematodes, may be more vulnerable to trampling than bacteria (Li *et al*., 2017; Erktan *et al*., 2020). However, moderate trampling (MT) can promote adequate mixing of litter or excreta with soil (Wei *et al*., 2021), enhancing nutrient inputs and thus increasing the role of stochastic processes. Collectively, all three factors (defoliation, excreta return, and trampling) can influence both deterministic and stochastic processes of grassland soil community assembly.

Most previous studies have focused on aboveground plant community structure, productivity, and soil abiotic factors to explain changes in soil organisms. However, roots, which interact directly with soil organisms, may have a stronger influence on soil biota than aboveground parts (Lopez-Angulo *et al*., 2020). For instance, specific root length (SRL) determines the dependence on arbuscular mycorrhizal fungi (AMF; Bergmann *et al*., 2020) and serves as an important factor influencing the rhizosphere's nematode communities (Zhang *et al*., 2022). Ungulate disturbance can change community-level SRL by altering vegetation composition and intraspecific trait variation (Cai *et al*., 2024). Liu *et al*. (2023) highlighted the ability of defoliation to increase SRL and reduce root biomass and root tissue density (RTD), influencing soil microbes. However, how changes in root traits under ungulate disturbance affects β-diversity and community assembly of soil organisms remains largely unknown.

To address this research gap, we aimed to investigate how defoliation, trampling, and excreta return influence the β-diversity and community assembly of soil organisms (bacteria, fungi, and nematodes) under different intensity scenarios (moderate and high ungulate densities) through a field experiment. We also investigated how these three aspects of grazing influence soil biota β-diversity and community assembly by affecting plant aboveground properties, root traits, and soil abiotic properties. We tested the following four hypotheses: (1) Defoliation of ungulates has a greater impact on soil biotic β-diversity and community assembly than trampling and excreta return. It affects soil biota via plant diversity, root traits, and soil moisture, whereas excreta return influences assembly by enhancing soil nutrient availability and trampling by soil compaction. Interactions among these aspects may further modulate their individual effects. (2) The effects of different intensity levels on soil biota are mainly driven by defoliation type. The selective defoliation (SD) under moderate scenarios promotes β-diversity and stochastic assembly by enhancing plant diversity and modifying root traits, whereas non-selective defoliation (NSD) under intense scenarios leads to deterministic assembly by degrading plant communities and soil conditions. (3) Root traits play a key role in shaping soil biotic community assembly, potentially exceeding the influence of aboveground plant properties and soil abiotic factors. (4) Different soil biotic groups respond variably to ungulate herbivory. Fungi are more resistant than bacteria, while larger nematodes are more sensitive than microbes.

Materials and Methods

Study site

The study was conducted at the HulunBuir Grassland-Agro-Ecosystem Station (49°28′N, 120°10′E, 703 m above sea level), located in the east of the Eurasian steppe in China. The climate is temperate continental monsoon, with mean annual precipitation of 352 mm, *c*. 75% falling from June to September. The mean annual temperature is −0.6°C, ranging from −26.1°C (January) to 19.8°C (July, monthly averages from 1981 to 2022). The soil is chernozem with a clay loam texture. The experimental site was sown with *Bromus inermis* Leyss. in 1997 and was managed for annual haymaking until 2019. The plant community is dominated by *Leymus chinensis* (Trin.) Tzvel and *B. inermis*, followed by *Potentilla reptans* L., *Carex pediformis* C. A. Mey., and *Potentilla bifurca* L. Locally common ungulates are Holstein cattle with an average weight of *c*. 400 kg. The local average stocking rate is 0.46 animal units per hectare (1 animal unit = 500 kg adult cattle; Hou *et al*., 2023).

Experiment design

The design of the simulated ungulate herbivory experiment was described by Guo *et al.* (2024a) and is described briefly here. In 2019, the experiment was established in an area with uniform vegetation. It simulated two ungulate herbivory scenarios: moderate ungulate density (Moderate scenario) and high ungulate density (Intense scenario). Each scenario followed a randomised block design with three treatment factors (defoliation, trampling, and excreta return) and their combinations (Supporting Information Fig. S1). The Moderate scenario included two levels of defoliation (no defoliation and selective defoliation (SD)), two levels of trampling (no trampling and MT), and two levels of excreta return (no excreta return and excreta return (E)). Similarly, the Intense scenario included two levels of defoliation (no defoliation and NSD), two levels of trampling (no trampling and heavy trampling (HT)), and two levels of excreta return (no excreta return and excreta return (E)). If all factors are at the level of zero (no defoliation, no trampling, and no excreta return), it is regarded as control (CK). It is worth noting that previous studies have shown a weak linear relationship between grazing intensity and the quantity of excreta returned (Dorji *et al.*, 2013) and that urination and defecation by ungulates typically occur in resting areas (Auerswald *et al.*, 2010), implying that the quantity of excreta return may not vary significantly between moderate and intense grazing. Consequently, the same excreta return dose was applied to both scenarios. Then, eight treatments were set up in each of the two scenarios to represent the separate or combination of the three factors: (1) series of treatments simulating the moderate grazing scenario, including CK, SD, E, MT, SD + E, SD + MT, MT + E, and SD + MT + E and (2) series of intense grazing scenarios, including CK, NSD, E, HT, NSD + E, NSD + HT, HT + E, and NSD + HT + E. It is important to note that since the CK and E treatments were identical in both scenarios, there were 14 unique treatments in this experiment instead of 16 ($2 \times 2 \times 2 \times 2$). Each treatment had six replicates, that is six blocks, resulting in 84 plots ($3 \times 3\ \mathrm{m}^2$ each; Fig. S1).

For the SD treatment, high-yield grasses (mainly *B. inermi* and *L. chinensis*) and high-quality legumes (mainly *Astragalus* and *Vicia*) were targeted based on the diet preferences of local Holstein cattle. In all Moderate scenario plots, including SD treatments, these two functional groups were cut to a stubble height of 5–6 cm using scissors, while other plants were left untreated. In the Intense scenario, NSD was simulated by mowing all plants to 5–6 cm stubble height. From 2019 to 2022, defoliation treatments were conducted three times annually, on 1 June, 1 July, and 1 August, to simulate short-duration grazing. To simulate ungulate trampling, a person wearing hoof-shaped shoes and carrying a counterweight walked in the plots after each defoliation treatment. The trampling aimed to simulate the effects of a 400-kg locally bred cow, assuming that the cow's weight was evenly distributed over four hooves. This resulted in an estimated hoof pressure of *c.* $1.65\ \mathrm{kg\ cm^{-2}}$ ($= 100\ \mathrm{kg}/60.6\ \mathrm{cm}^2$ per hoof). Moderate trampling involved walking evenly through the plots once a month, while HT involved walking twice. The response of soil bulk density to the Moderate and Intense scenarios (Fig. S2) was consistent with findings of previous studies on moderate (*c.* 0.46 animal units per hectare) and heavy (*c.* 0.92 animal units per hectare) grazing in the steppe (Ren *et al.*, 2018; Zhang *et al.*, 2022). After trampling, a mixture of cattle manure and urea solution was applied to simulate the excreta return. The quantities of dung and urea were calculated based on the intake, dry matter digestibility, urine excretion, and urinary nitrogen content of Holstein cattle. Annually, a total of $3\ \mathrm{l\ m^{-2}}$ of the mixture was applied to plots receiving excreta return treatment. From 2019 to 2022, the average annual inputs of carbon, nitrogen, and phosphorus to the plot with E treatment were 30.94, 5.44, and $0.75\ \mathrm{g\ m^{-2}}$, respectively (Guo *et al.*, 2024a). During the experimental, all plots were not subjected to livestock grazing or other anthropogenic disturbances.

Soil sampling

Soil samples were collected in late August 2022. Four randomly located soil cores (5 cm in diameter and 10 cm deep) were taken within each plot and combined into one composite sample. Then, all the composite soil samples were separated into three subsamples for different analyses. Two subsamples were sieved through a 2-mm mesh, with all visible stones and plant residues carefully removed. The first subsample was stored at 4°C to determine soil physicochemical properties (pH, $\mathrm{NH_4^+}$–N, $\mathrm{NO_3^-}$–N, and SOC). The second subsample was preserved at −80°C for molecular analysis. The remaining subsample was sieved through a 5-mm mesh to remove large stones and roots, and this sample was used immediately for nematode extraction. Soil bulk density was measured using three stainless steel cutting rings (5 cm diameter, $100\ \mathrm{cm^3}$) in each plot. Soil moisture was monitored from 1 June to 25 August 2022 (end of soil sampling) using TEROS 12 sensors (Meter, Pullman, WA, USA).

Soil physicochemical properties

Soil pH was determined in a 1 : 5 (soil : water) suspension using a pH/conductivity Benchtop Meter (ORION STAR A215; Thermo Scientific, Waltham, MA, USA). The concentrations of soil $\mathrm{NH_4^+}$–N and $\mathrm{NO_3^-}$–N were determined from 2 M KCl extracts using a continuous flow analyser (AA3; SEAL Analytical, Norderstedt, Germany). Soil organic carbon (SOC) was determined after the removal of inorganic carbon by HCl fumigation. This was followed by dry combustion measurements using an elemental analyser (Vario TOC; Elementar, Langenselbold, Germany).

Soil biota community

Soil nematodes were extracted from 50 g of fresh soil using a modified Baermann wet funnel technique and fixed in a 4% formalin solution. The first 100 fixed nematode specimens encountered per sample were identified to genus using a microscope (BX5; Olympus, Tokyo, Japan). If the number of nematodes was < 100 individuals in a sample, all individuals were identified. All nematodes were classified into functional groups based on their feeding habits (Table S1): bacterivores, fungivores, herbivores, and omnivores-predators (Zhang *et al*., 2017a). Nematodes were also classified according to the coloniser–persister (c–p) scale, and then, the maturity index (Methods S1) of each plot was calculated (Ferris *et al*., 2001).

Soil microbial diversity and community composition were assessed using high-throughput sequencing. Soil DNA was extracted using the Powersoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the standard protocol. The DNA quality was evaluated with a NanoDrop spectrophotometer (Nd-2000; Thermo Fisher Scientific, USA). To characterise the biodiversity of fungi and bacteria, eukaryotic 18S rRNA and prokaryotic 16S rRNA genes were amplified with the primer set F1391 (5′-GTACACCGCCCGTC-3′)/EukBr (5′-TGATCCTTCTG CAGGTTCACCTAC-3′) and 515F (5′-GTGCCAGCMGCCG CGGTAA-3′)/806R (5′-GGACTACVSGGGTATCTAAT-3′) (Delgado-Baquerizo *et al*., 2019). Purified amplicons were pooled in equimolar and paired-end sequences (2 × 300) on an Illumina MiSeq platform. After demultiplexing and quality-filtering, the acquired raw sequence data were sorted into valid reads using a combination of QIIME, USEARCH, and UNOISE3 (Zhang *et al*., 2021). Phylotypes (i.e. amplicon sequence variants, ASVs) were identified at the 100% identity level. The ASV matrices were rarefied to 30 000 and 12 000 sequences per sample for soil bacteria and fungi, respectively. The relative abundances of AMF and saprotroph fungi in soils were obtained from amplicon sequencing analyses and were inferred by parsing soil phylotypes using FUNguild (Nguyen *et al*., 2016; Feng *et al*., 2024).

Plant sampling and analysis

A 1 × 1-m fixed quadrat was randomly established in each plot to assess plant cover. The plant cover, that is the relative projected area covered by all species, was measured in the middle of August 2022 by the pinpoint method using a square frame of 100 grid points in the fixed quadrats, and plants were classified into five functional groups (grass, sedge, legume, perennial forb, and annual/biennial forbs), consistent with a previous study at this site (Guo *et al*., 2024a). In August 2022, a 0.5 × 0.5 m quadrat was randomly placed in each plot for aboveground biomass and root sampling. The dry weight of all live plants in the random quadrat was used to estimate standing biomass for the no-defoliation plots. In defoliated treatment plots (both SD and NSD), we recorded the biomass removed after each defoliation treatment by drying and weighing it. The total plant biomass for these plots was calculated by summing the removed biomass and the biomass measured in mid-August.

To measure the root functional traits, three soil cores (depth 10 cm; diameter 70 mm) were collected within the 0.5 × 0.5 m random quadrat from each plot after aboveground sampling. Three soil cores from each plot were mixed into a combined sample. Fine roots (root diameter (RD) < 2 mm) from the combined samples were cleaned and scanned using a PER-V700P scanner (Epson, Suwa, Japan). For the scanning, we spread roots on a transparent acrylic tray filled with water and set a 16-bit greyscale of 600 dpi (dots per inch) resolution. All digital images were analysed with the WinRHIZO software (Regent Instrument, Quebec City, QC, Canada) to obtain RD, root length, and root volume. After analysis, roots were then dried at 60°C for 48 h before dry weight was also determined. Specific root length was calculated as the ratio of root length to root dry mass, and RTD as the ratio of root dry biomass to root volume. Root nitrogen content (RNC) was determined on the ball-mill ground (MM400; Retsch, Haan, Germany) and dry root material with an elemental analyser (Elementar vario MICRO cube, Elementar; Xu *et al*., 2024).

Statistical analyses

All calculations and statistical analyses were performed in R 4.3.1. Following previous theoretical (Wang & Loreau, 2016) and empirical studies (Zhang *et al*., 2019; Liu *et al*., 2021), we defined α-diversity as the species richness within a plot (local community) and β-diversity as the difference in community composition between replicate plots of the same treatment. Soil microbial and nematode α-diversity were assessed using ASV richness and generic richness, respectively. β-diversity for all soil microbial and nematode was calculated using the abundance-based Bray–Curtis dissimilarity (*vegdist*, VEGAN package). To assess the relative roles of deterministic and stochastic processes in community assembly, we used a null model approach. We generated 999 permutations for randomised local communities within each plot (null communities) using the function *commsim* in VEGAN package by randomly placing individuals into each local community (plot) with the probabilities proportional to the relative abundance of each ASV (or genus for nematode), while maintaining local α-diversity (Liu *et al*., 2021). We calculated the standard effective size of β-diversity (hereafter 'β-deviation') as follows (Myers *et al*., 2013; Liu *et al*., 2021):

$$\beta\text{-deviation} = \frac{\beta_{\mathrm{obs}} - \beta_{\mathrm{null}}}{\mathrm{SD}\ (\beta_{\mathrm{null}})}$$

where β_{obs} is the observed β-diversity, β_{null} is the mean β-diversity of the null communities, and SD (β_{null}) is the SD of null β-diversity. Values close to zero indicate stochastic processes, while positive or negative values suggest deterministic processes driving higher or lower β-diversity. Two-tailed *t*-tests were used to assess whether β-deviation for each treatment was significantly different from the null model.

To test the effect of plant community composition on soil organism β-diversity and β-deviation, we also calculated abundance-based plant β-diversity using a similar approach. Soil heterogeneity was quantified as the pairwise Euclidean distance among six replicates within each treatment, based on six soil physicochemical variables (bulk density, soil moisture, pH, SOC, NH_4^+–N, and NO_3^-–N concentrations). These six soil physicochemical variables were standardised using the *Z*-score before the calculation.

The effects of defoliation, trampling, excreta return, and their interaction on α-diversity, β-diversity, β-deviation, soil physicochemical variables, plant standing biomass, root biomass, and root traits were analysed using a three-way ANOVA. The models were run separately for the Moderate and Intense scenarios with block

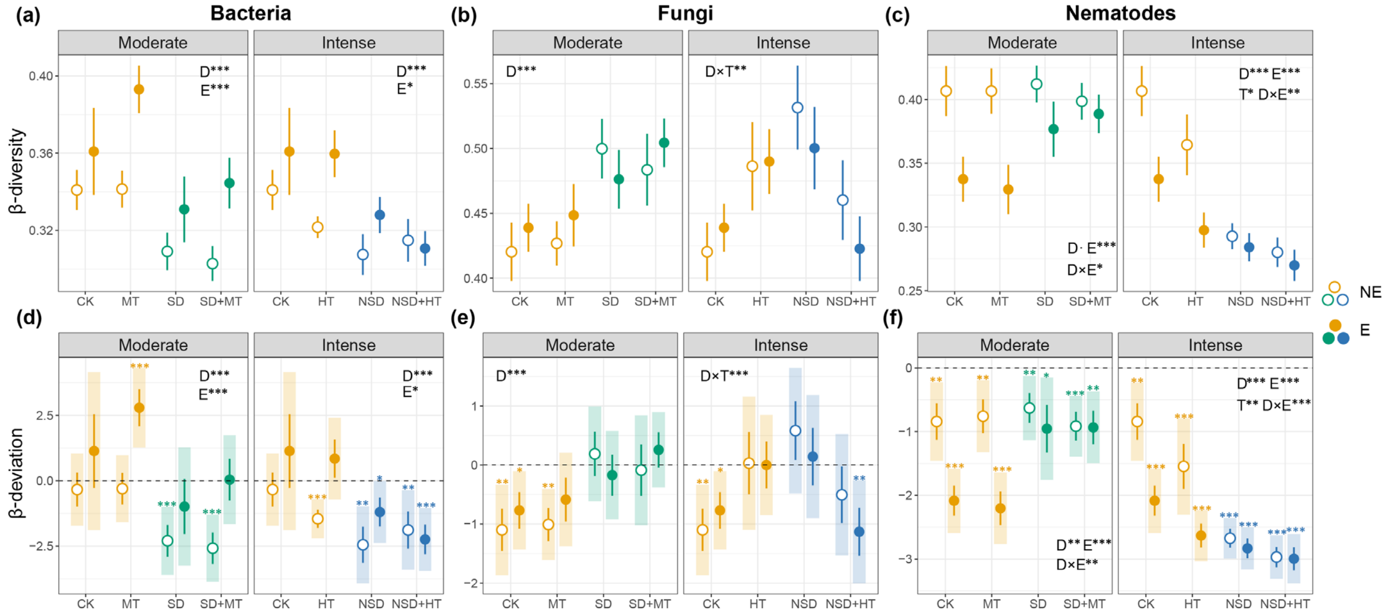

Fig. 1 Effects of defoliation (D), trampling (T), and excreta return (E) on β-diversity (a–c) and β-deviation (d–f) of soil bacteria, fungi, and nematodes under moderate-density (Moderate scenario) and high-density ungulate herbivores (Intense scenario). Data are mean ± SE ($n = 6$). Colours indicate different defoliation treatments: yellow for no defoliation, green for selective defoliation (SD), and blue for non-selective defoliation (NSD). Closed/open circles indicate different excreta return treatments: open circles for no excreta return (NE) and closed circles for excreta return (E). The effects of D, T, E, and their interaction on soil biota β-diversity and β-deviation were analysed using a three-way ANOVA (analysis of variance). An asterisk indicates significant effects of D, T, E, and their interactions: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. '·' indicates marginally significant ($0.05 < P < 0.1$). In (d–f), the shaded box indicates the confidence band (95%), and asterisks above the boxes indicate significant differences (via two-tailed *t*-tests) between the β-deviation and the null model (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). CK, control; HT, heavy trampling; MT, moderate trampling.

as a random factor. To explore whether plant and soil abiotic variables potentially affect the relative importance of deterministic and stochastic processes, the relationships between the β-deviation of soil organisms and the following variables significantly affected by treatments were examined using multiple linear regression analysis. Specifically, we considered plant standing biomass as an indicator of plant resource input; plant β-diversity as an indicator of plant diversity or community composition; RD, SRL, RTD, and RNC as root traits indicators; bulk density, soil moisture, and soil pH as soil environment indicators; SOC, NH_4^+–N, and NO_3^-–N concentrations as soil substrate quantity indicators; and soil heterogeneity as an indicator of environmental heterogeneity. Before the analyses, we examined the multicollinearity among these variables with the *VIF* function in the FMSB package, and the variance inflation factors were < 3 for all models. We also assessed the relative importance of plant and soil abiotic variables for β-deviation of soil microbial and nematode communities using the Lindeman–Merenda–Gold method, an approach that quantifies the relative importance of predictor variables by decomposing the variance explained by each variable, with the package RELAIMPO.

Results

Effects of ungulate herbivory behaviours on soil biota β-diversity and community assembly

Simulated moderate ungulate herbivory (SD + MT + E in the Moderate scenario) significantly increased soil fungal β-diversity ($P < 0.05$) but had no effect on bacteria or nematode β-diversity. By contrast, intense herbivory (NSD + HT + E in the Intense scenario) decreased the β-diversity of bacteria ($P < 0.05$) and nematodes ($P < 0.001$) but did not affect fungal β-diversity (Fig. S3a–c). The patterns of β-deviation mirrored those of β-diversity. In both control (CK) and moderate herbivory treatment, the β-deviations of soil bacteria and fungi were close to zero, indicating that stochastic processes dominate community assembly. However, under intense herbivory, bacterial and fungal β-deviations were significantly negative, suggesting that deterministic processes promoted more homogenous communities (Fig. S3d,e). In terms of soil nematodes, β-deviation was consistently lower than the null model across all treatments ($P < 0.01$), with the most pronounced deviation under intense herbivory (Fig. S3f).

In the Moderate scenario, SD significantly reduced bacterial β-diversity ($F = 14.82$, $P < 0.001$; Fig. 1a) and β-deviation ($F = 14.49$, $P < 0.001$; Fig. 1d) while increasing fungal β-diversity ($F = 13.65$, $P < 0.001$) and β-deviation ($F = 13.60$, $P < 0.001$), promoting stochastic fungal community assembly (Fig. 1b,e). Selective defoliation also marginally increased β-diversity ($F = 3.68$, $P < 0.1$) and significantly increased β-deviation ($F = 10.23$, $P < 0.01$), reducing the role of deterministic processes (Fig. 1c,f). Excreta return (E) counteracted the negative effects of SD on bacteria (Fig. 1a,d), increasing bacterial β-diversity ($F = 12.22$, $P < 0.001$) and β-deviation ($F = 12.59$, $P < 0.001$), but reduced nematode β-diversity ($F = 14.69$, $P < 0.001$) and β-deviation ($F = 15.61$, $P < 0.001$),

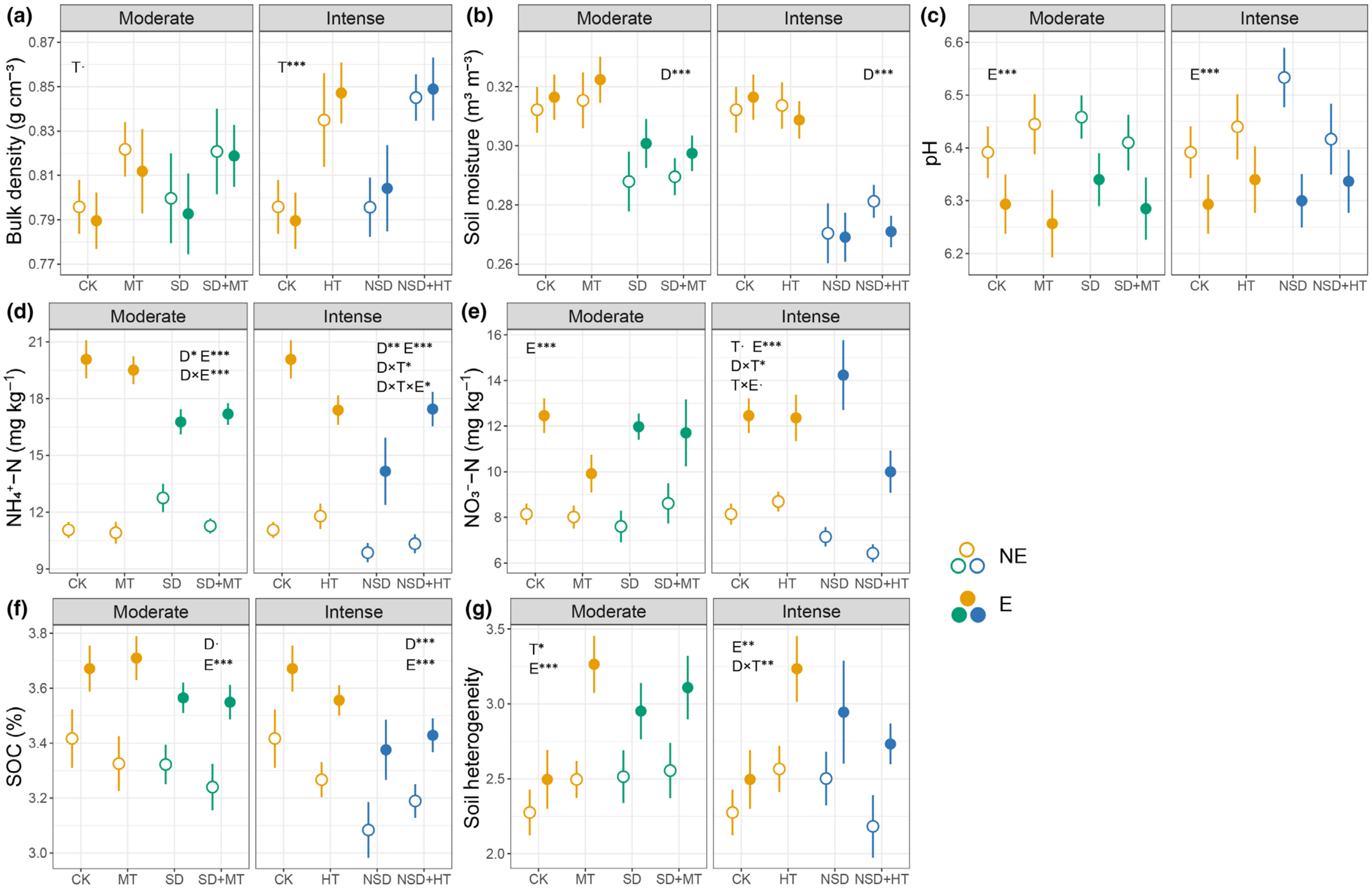

Fig. 2 Effects of defoliation (D), trampling (T), and excreta return (E) on soil bulk density (a), soil moisture (b), soil pH (c), soil NH_4^+–N concentrations (d), NO_3^-–N content (e), soil organic carbon (SOC, f), and soil heterogeneity (g) under moderate-density (Moderate scenario) and high-density (Intense scenario) ungulate herbivores. Data are mean ± SE ($n = 6$). Colours indicate different defoliation treatments: yellow for no defoliation, blue for selective defoliation (SD), and green for non-selective defoliation (NSD). Closed/open circles indicate different excreta return treatments: open circles for no excreta return (NE) and closed circles for excreta return (E). The effects of D, T, E, and their interaction on soil properties were analysed using a three-way ANOVA (analysis of variance). An asterisk indicates significant effects of D, T, E, and their interactions: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. '·' indicates marginally significant ($0.05 < P < 0.1$). CK, control; HT, heavy trampling; MT, moderate trampling.

outweighing the positive effect of SD (β-diversity: $F = 3.68$, $P < 0.1$; β-deviation: $F = 10.23$, $P < 0.01$). Significant interactions between SD and E were observed for soil nematode β-diversity ($F = 4.09$, $P < 0.001$) and β-deviation ($F = 9.32$, $P < 0.001$), where SD mitigated E's negative effects. Moderate trampling had no significant effects on β-diversity and β-deviation (Fig. 1).

In the Intense scenario, NSD decreased bacterial β-diversity ($F = 12.51$, $P < 0.001$) and β-deviation ($F = 13.65$, $P < 0.001$), reinforcing deterministic processes (Fig. 1a,d). Excreta return had a weaker positive effect on bacterial β-diversity ($F = 4.63$, $P < 0.05$) and β-deviation ($F = 4.69$, $P < 0.05$) than in the Moderate scenario (β-deviation: $F = 12.59$, $P < 0.001$) and could not fully offset NSD's negative impact (Fig. 1a,d). For fungi, none of the main effects of NSD, E, or HT significantly influenced the β-diversity or β-deviation, although the interaction between NSD and HT ($F = 11.35$, $P < 0.01$) indicated that NSD diminished HT's positive effect on β-diversity and β-deviation ($F = 11.81$, $P < 0.001$; Fig. 1b,e). For soil nematodes, NSD, HT, and E significantly decreased β-diversity and β-deviation, reinforcing the dominance of deterministic processes (Fig. 1c,f).

Effects of herbivory behaviours on soil properties

Ungulate herbivory behaviour affected soil physicochemical properties differently under the Moderate and Intense scenarios. Soil bulk density marginally increased by moderate trampling ($F = 3.69$, $P < 0.1$) and significantly increased under HT ($F = 20.68$, $P < 0.001$; Fig. 2a). In both scenarios, SD ($F = 15.70$, $P < 0.001$) and NSD ($F = 60.10$, $P < 0.001$) significantly reduced soil moisture (Fig. 2b). Soil pH, organic carbon, and available nitrogen concentrations were primarily affected by excreta return. Specifically, E significantly increased SOC and available nitrogen content while decreasing soil pH (Fig. 2c–f). Defoliation decreased SOC and soil NH_4^+–N concentrations, with greater reductions under NSD (SOC: $F = 13.03$, $P < 0.001$; NH_4^+–N: $F = 11.30$, $P < 0.01$) than under SD

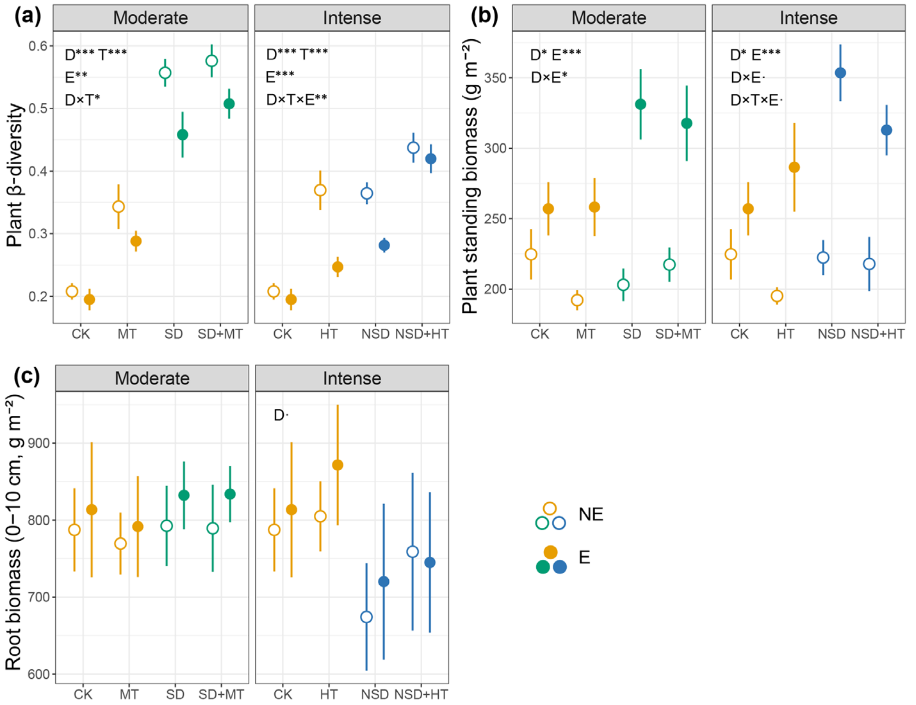

Fig. 3 Effects of defoliation (D), trampling (T), and excreta return (E) on plant β-diversity (a), plant standing biomass (b), and root biomass (c) under moderate-density (Moderate scenario) and high-density (Intense scenario) ungulate herbivores. Data are mean ± SE ($n = 6$). Colours indicate different defoliation treatments: yellow for no defoliation, blue for selective defoliation (SD), and green for non-selective defoliation (NSD). The shapes of circles indicate different excreta return treatments: open circles for no excreta return (NE) and closed circles for excreta return (E). The effects of D, T, E, and their interaction on plant β-diversity, plant standing biomass, and root biomass were analysed using a three-way ANOVA (analysis of variance). An asterisk indicates significant effects of D, T, E, and their interactions: *, $P < 0.05$; **, $P < 0.01$; ***; $P < 0.001$. '·' indicates marginally significant ($0.05 < P < 0.1$). CK, control; HT, heavy trampling; MT, moderate trampling.

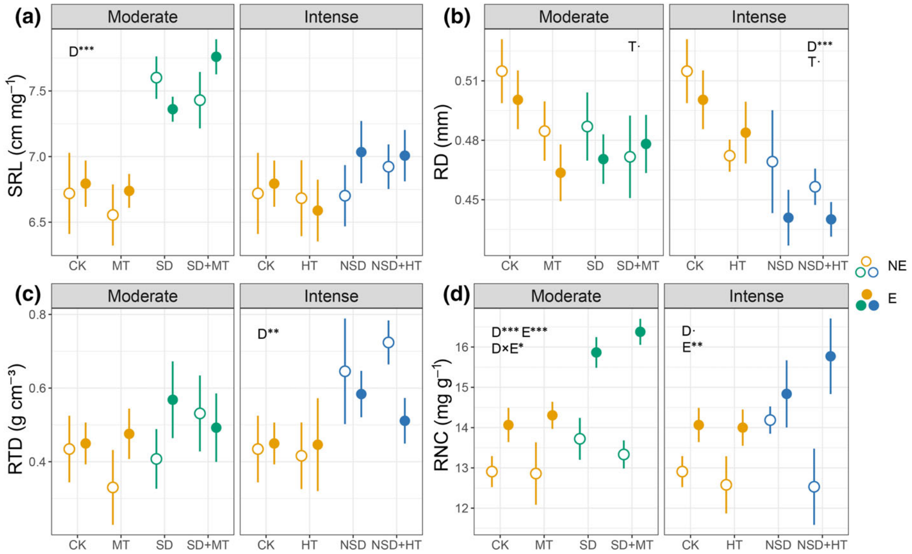

Fig. 4 Effects of defoliation (D), trampling (T), and excreta return (E) on specific root length (SRL, a), root diameter (RD, b), root tissue density (RTD, c), and root nitrogen content (RNC, d) under moderate-density (Moderate scenario) and high-density (Intense scenario) ungulate herbivores. Data are mean ± SE ($n = 6$). Colours indicate different defoliation treatments: yellow for no defoliation, blue for selective defoliation (SD), and green for non-selective defoliation (NSD). Closed/open circles indicate different excreta return treatments: open circles for no excreta return (NE) and closed circles for excreta return (E). The effects of D, T, E, and their interaction on root traits were analysed using a three-way ANOVA (analysis of variance). An asterisk indicates significant effects of D, T, E, and their interactions: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. '·' indicates marginally significant ($0.05 < P < 0.1$). CK, control; HT, heavy trampling; MT, moderate trampling.

(SOC: $F = 3.99$, $P < 0.1$; NH_4^+–N: $F = 4.21$, $P < 0.05$; Fig. 2d,f). Additionally, excreta return was the primary factor contributing to increased soil heterogeneity in both scenarios (Fig. 2g).

Effects of herbivory behaviours on plant communities and root traits

Defoliation and trampling significantly increased plant β-diversity in both the Moderate and Intense scenarios ($P < 0.001$), while excreta return reduced it ($P < 0.01$; Fig. 3a). Excreta return also drove plant standing biomass increases ($P < 0.001$; Fig. 3b). Additionally, we found a significant effect of defoliation and its interaction with E on standing biomass in the Moderate scenario ($F = 5.76$, $P < 0.05$). Specifically, in the presence of SD, E enhanced standing biomass more than in plots without defoliation. Root biomass remained unchanged in the Moderate scenario but was marginally reduced by NSD in the Intense scenario ($F = 3.09$, $P < 0.1$; Fig. 3c).

For root traits, SD significantly increased SRL in the Moderate scenario ($F = 44.66$, $P < 0.001$), whereas herbivory behaviour had no significant effect on SRL in the Intense scenario (Fig. 4a).

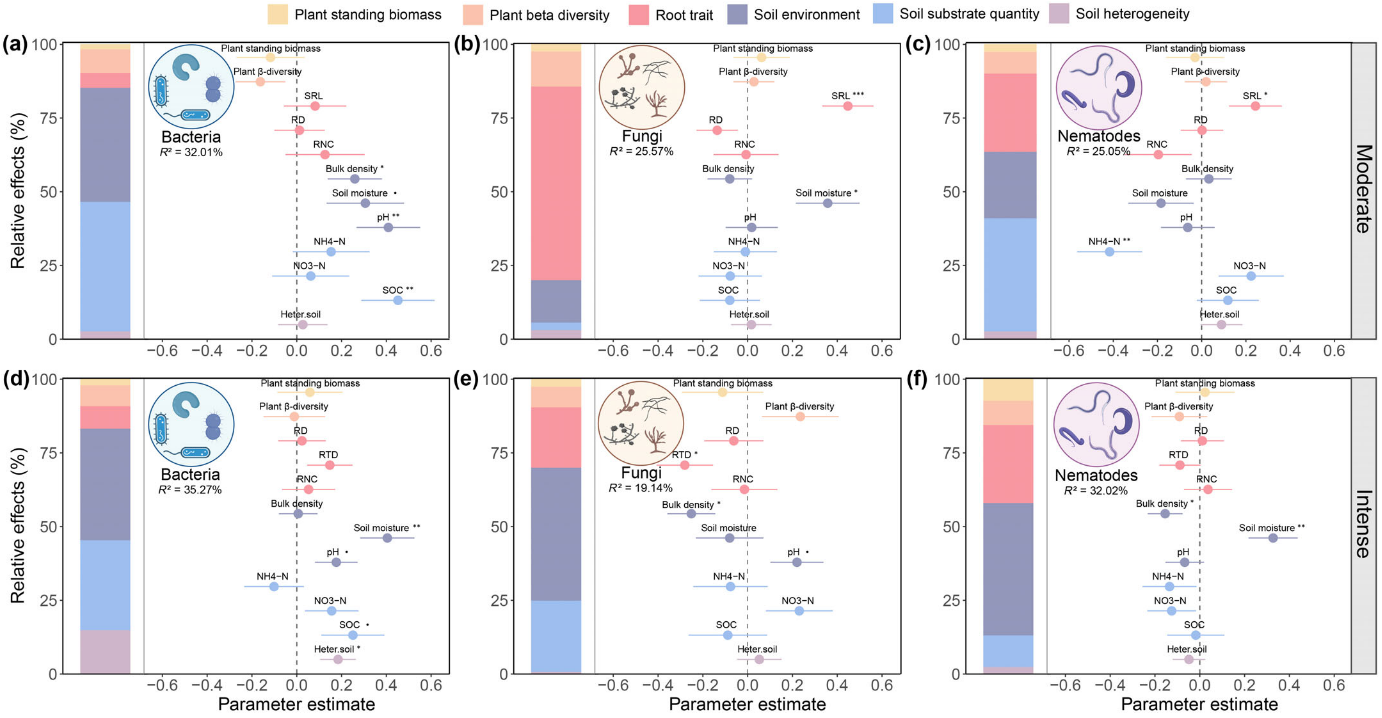

Fig. 5 Relative effects of multiple predictors (including plant community properties, root functional traits and soil abiotic variables) on β-deviation of soil bacteria, fungi, and nematodes under moderate-density (Moderate scenario, a–c) and high-density scenarios (Intense scenario, d–f). The stacked bar chart of each panel shows the relative importance of each group of predictors, expressed as the percentage of explained variance, and the right-hand side of each panel shows the average parameter estimates (standardised regression coefficients) of the predictors and their SE derived from multiple linear regression models. An asterisk indicates significant effects of predictors: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. '·' indicates marginally significant ($0.05 < P < 0.1$). Different colours indicate different groups of predictors (see the top of the figure for coding, e.g. yellow for plant biomass). heter.soil, soil heterogeneity; RD, root diameter; RNC, root nitrogen content; RTD, root tissue density; SOC, soil organic carbon; SRL, specific root length.

Both moderate and HT marginally ($P < 0.1$) reduced RD (Fig. 4b). In the Intense scenario, NSD significantly reduced RD ($F = 16.40$, $P < 0.001$). Root tissue density was unaffected in the Moderate scenario but increased by NSD in the Intense scenario ($F = 8.17$, $P < 0.01$; Fig. 4c). Selective defoliation ($F = 17.65$, $P < 0.001$) and E ($F = 40.24$, $P < 0.001$) significantly increased RNC in the Moderate scenario, with a positive interaction between SD and E ($F = 4.43$, $P < 0.05$; Fig. 4d). In the Intense scenario, E also increased RNC ($F = 10.87$, $P < 0.01$), and NSD had a marginally significant positive effect on RNC ($F = 3.70$, $P < 0.1$).

Predictors of soil biota β-deviation (community assembly) under ungulate herbivory

We selected plant and soil abiotic variables significantly influenced by ungulate herbivory as predictors in each of the two scenarios to explore the contribution of these variables to soil organisms' β-deviation. In the Moderate scenario, bacterial β-deviation was mainly positively driven by soil environmental variables (including bulk density, pH, and soil moisture) and SOC ($P < 0.05$; Fig. 5a). Fungal β-deviation was primarily explained by SRL ($P < 0.05$, 52% contribution to the explained variability) and was also positively influenced by soil moisture ($P < 0.05$; Fig. 5b). Nematode β-deviation was negatively influenced by NH_4^+–N content but positively associated with SRL (Fig. 5c).

In the Intense scenario, bacterial β-deviation was jointly affected by soil environmental variables (including pH and soil moisture), SOC, and soil heterogeneity (Fig. 5d). Fungal β-deviation was primarily driven by environmental variables (particularly bulk density and soil pH; Fig. 5e) with RTD also playing a significant role. However, root traits explained fungal β-deviation in the Intense scenario (20%) less than in the Moderate scenario (66%). Nematode β-deviation was negatively influenced by bulk density but positively affected by soil moisture (Fig. 5f). Plant β-diversity and standing biomass had no significant effect on soil biota β-deviation in either scenario. To eliminate potential covariance between predictors and β-deviation on the treatment response, we constructed multiple regression models using the residuals from the linear model analysis of the independent and dependent variables, which found similar patterns (Fig. S4).

Discussion

A mechanistic understanding of soil biodiversity requires identifying the factors that influence the balance between stochastic and deterministic processes in community assembly. However, how ungulate herbivory, the most prevalent disturbance in

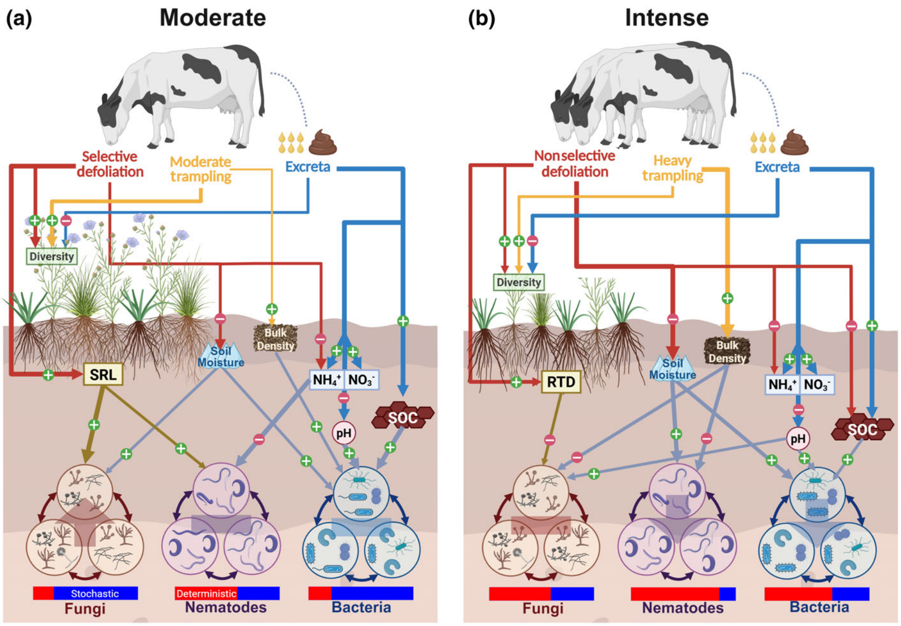

Fig. 6 Conceptual diagram summarising the effects of ungulate herbivores (including defoliation, trampling, and excreta return) on β-diversity and community assembly of soil bacteria, fungi, and nematodes under moderate (Moderate scenario, a) and high (Intense scenario, b) ungulate densities. In the Moderate scenario, fungal β-diversity increased and community assembly was predominantly stochastic, while soil bacteria and nematode β-diversity remained unchanged, with community assembly processes similar to the control (stochastic for bacteria and deterministic for nematodes). Specifically, the increase in fungal β-diversity was primarily due to increased specific root length (SRL) resulting from selective defoliation (SD). By contrast, soil bacteria were more influenced by soil abiotic factors. Decreased soil moisture due to SD and increased soil organic carbon (SOC) due to excreta return had opposite effects on bacterial β-diversity, ultimately resulting in insensitive bacterial communities in response to moderate grazing. Soil nematodes were affected by a combination of SRL and ammonium nitrogen (NH_4^+–N). Intense ungulate herbivores significantly reduced β-diversity and stochasticity in community assembly of soil bacteria and nematodes. In this scenario, ungulate herbivory reinforced the environmental filtering effect by lowering soil moisture and pH and increasing soil bulk density, while weakening the influence of plant-mediated pathways on community assembly. However, fungi were less sensitive to these soil environment changes than bacteria and nematodes. Red, bright yellow, and dark blue arrows indicate the effect of defoliation, trampling, and excreta return on plant and soil properties, respectively. Brown arrows show the influence of plant attributes on soil biota, while light blue arrows indicate the effects of soil abiotic factors on soil biota. Circles indicate local communities of soil fungi, nematodes, or bacteria. Variation in the three local communities represents β-diversity. The '↑', '↓', or '–' symbols within the groups of circles indicate positive, negative, or no effect of ungulate herbivory on β-diversity compared with the control, respectively. Bar charts below the circles illustrate the contribution of deterministic (red) and stochastic (blue) processes to community assembly according to Supporting Information Fig. S3. '+' and '–' above the arrow indicate the positive and negative effects, respectively. This figure was created in BioRender (BioRender.com/cka6j78). RTD, root tissue density.

grasslands, shapes these processes remains poorly understood. Our study revealed how different aspects of ungulate effects influence soil biota community assembly under different grazing intensities (Fig. 6) using a field-simulated ungulate herbivory experiment. We found that defoliation and excreta return were primary mechanisms by which ungulates shaped soil bacterial, fungal, and nematode community assembly. As hypothesised, our results highlighted the important role of plant root traits in mediating fungal and nematode community assembly, though this effect decreased as grazing intensity increased.

Opposing effects of ungulate defoliation and excreta return drive bacterial community assembly via soil abiotic factors

As human populations increase and demand for animal products grows, grasslands world-wide are progressively more affected by ungulates, with significant consequences for soil biodiversity (Michalk *et al.*, 2019). We found that simulated moderate-density ungulate herbivory did not significantly alter bacterial β-diversity or community assembly compared with control (Fig. S3). This can be attributed to the opposing effects of defoliation and excreta return (Fig. 1a,d). Mechanistically, defoliation decreased soil moisture (Fig. 2b) by reducing ground cover, which restricts bacterial movement and colonisation (habitat filtering), limiting stochastic processes in community assembly (Fig. 5a; Wang *et al.*, 2024). Excreta return had both positive and negative effects on bacterial communities (Figs 5, 6). Soil acidification, potentially linked to ammonium nitrogen in urine, may cause habitat filtering, aligning with previous studies that identified pH as a key predictor of bacterial community assembly (Fierer, 2017). Simultaneously, organic matter in excreta provided bacteria with additional carbon sources (Bardgett *et al.*, 1998), which promotes bacterial growth and colonisation. These combined effects, pH-mediated filtering and carbon-driven fast growth, likely shifted the balance between deterministic and stochastic processes. Ultimately, the positive effects of excreta return on stochasticity outweighed the negative effects, leading to more stochastic bacterial community assembly (Figs 1, 6). This aligns with evidence that excreta inputs enhance bacterial diversity through carbon and nutrient enrichment (Zhang *et al*., 2025a).

By contrast, high-density ungulate herbivory significantly reduced bacterial β-diversity, with deterministic processes dominating community assembly (Fig. S3). Unlike the Moderate scenario, excreta return could not offset the harsher habitat filtering on bacteria in the Intense scenario (Figs 1, 5), where soil moisture reduction from NSD likely limited substrate availability from excreta, amplifying environmental stress (Zhang *et al*., 2025b). However, since the same excreta return dose was used in both scenarios, and real-world heavy grazing likely produces more excreta (Dorji *et al*., 2013), these results may slightly underestimate the contribution of excreta to the stochastic process in the Intense scenario. Interestingly, increased soil environmental heterogeneity due to excreta return positively influenced bacterial β-diversity in the Intense scenario, similar to findings by Liu *et al*. (2021). Nevertheless, we are still unclear on how nutrient addition increases soil heterogeneity. In both scenarios, we found no evidence that ungulate behaviours affected bacterial community assembly by altering plant properties. This aligns with a previous study suggesting that soil bacteria can be associated with a wide range of plant species, meaning the effects of vegetation on bacterial communities are context-dependent and may take longer to become evident (Fierer, 2017). Our single-timepoint sampling may not capture slow shifts in bacteria–plant associations.

Selectivity of ungulate defoliation under different herbivory intensities drives fungal community assembly via reshaping root traits

Simulated ungulate herbivory had a weaker negative impact on fungal β-diversity than bacteria (Fig. S3), likely due to the resistance of fungi to adverse environmental conditions, such as soil acidification (Herold *et al*., 2012) and low soil moisture (de Vries *et al*., 2018). In the moderate-density scenario, fungal β-diversity increased, and stochastic processes dominated community assembly (Fig. S3), with SD playing a key role (Fig. 1e). Multiple regression analyses identified increased SRL as a significant driver of this stochasticity (Fig. 5b). Selective defoliation increased the relative abundance of sedges with high SRL compared with no-defoliation treatments (Fig. S5). Selective defoliation also alleviated light competition and expanded niche dimension by reducing the dominance of grasses (Guo *et al*., 2024a), allowing other species to invest more in root growth and development (Moehl *et al*., 2020; Wang *et al*., 2025). As a result, some sedges and forbs in SD treatment exhibited higher SRL than in no defoliation treatment (Fig. S6). This aligns with findings from northern China, where grazing-induced increasing SRL was linked to both community structure shifts and intraspecific trait variation (Cai *et al*., 2024). According to the root economics spectrum, plants with higher SRL favour a 'do it yourself' strategy, reducing reliance on specific symbiotic associations such as AMF (Fig. S7b; Bergmann *et al*., 2020; Sweeney *et al*., 2020). Although the primers used in our amplicon sequencing may not estimate the relative abundance of AMF as accurately as some specific primers, this result still, to some extent, explains the stochastic fungal community assembly under SD treatments. Our study found a significant reduction in AMF abundance under SD compared with that under no defoliation (Fig. S7a,b), supporting this explanation.

However, in the Intense scenario, an antagonistic interaction between defoliation and trampling constrained changes in fungal β-diversity and β-deviation (Fig. 1). As a result, the NSD + E + HT treatment (simulating high-density ungulates herbivory) exhibited no significant changes in fungal β-diversity or community assembly compared with that in the control, where deterministic processes dominated (Fig. S3). Heavy trampling increased soil bulk density and thus might restrict fungal dispersal by limiting oxygen diffusion and inhibiting mycelial growth (Nadian *et al*., 1998; Puschel *et al*., 2024). Additionally, increased RTD under NSD may have suppressed saprotrophic fungi by reducing root turnover and litter decomposability (Fig. S7d; Duchesneau *et al*., 2024). We found that NSD resulted in a lower abundance of saprotrophs than ND, although statistically insignificant ($P = 0.1645$, Fig. S7c). Notably, in both scenarios, fungal community assembly was unaffected by excreta return, which may be attributed to two fungal characteristics. First, fungi are more resistant to soil pH reductions due to nitrogen inputs than bacteria (Herold *et al*., 2012). Second, fungal spores typically spread shorter distances than bacteria (Peay *et al*., 2010; Adams *et al*., 2013), and thus, fungal community assembly is more affected by dispersal constraints. Consequently, although excreta inputs increased soil available nutrients, fungi were unable to colonise and spread as rapidly as bacteria, limiting their response to excreta return.

Ungulate herbivory behaviours alter deterministic nematode community assembly by influencing both root traits and soil abiotic factors

Nematode community assembly was consistently dominated by deterministic processes across all treatments, highlighting the greater sensitivity of larger soil organisms to environmental changes than of microbes (Luan *et al*., 2020). In the Moderate scenario, excreta return had the greatest impact on nematode β-diversity, likely due to the toxicity of increased soil ammonium content (Fig. 5c) caused by urine input (Zhou *et al*., 2022). However, SD not only enhanced soil nematode β-diversity and β-deviation but also mitigated the negative effect of excreta returns (Fig. 1c,f), which explains the nonsignificant differences in soil nematode community assembly between SD + E + MT and CK (Fig. S3f). Selective defoliation increased SRL significantly, promoting stochastic nematode community assembly (Fig. 5c). Higher SRL has been proposed to improve root water-use ability and promote water availability in the rhizosphere (Fort *et al*., 2017). The high SRL, in turn, benefits high-trophic level nematodes (higher relative abundance of omnivores-predators) and increases food web complexity (higher maturity index; Fig. S8) by creating thicker water films around soil particles for high-trophic level nematodes movement, predation, and reproduction (Wallace, 1968; Xiong *et al*., 2020). Moreover, defoliation-induced biomass removal and increased SRL may prompt plants to absorb nitrogen rapidly (Guo *et al.*, 2024b), reducing soil ammonium levels (Fig. 2d) and alleviating the environmental stress caused by urine on nematodes.

By contrast, NSD significantly reduced nematode β-diversity by intensifying habitat filtering, primarily through reduced soil moisture. Heavy trampling significantly reduced nematode β-diversity and β-deviation in the Intense scenario, possibly due to trampling-induced reduction in pore size limiting nematode movement and dispersal (Erktan *et al.*, 2020; Ma *et al.*, 2024). Due to multiple stresses caused by NSD, E, and HT, simulated high-density ungulate herbivory resulted in lower nematode diversity and a more deterministic community assembly.

Notably, root traits (SRL) significantly affected nematode community assembly in the Moderate scenario, but their influence diminished in the Intense scenario (Fig. 5). Similarly, root traits explained less variation in fungal community assembly in the Intense scenario (20.46%) than in the Moderate scenario (65.67%). These findings align with our hypothesis (2) and suggest that root traits are decoupled from soil organisms under heavy grazing, likely due to harsher soil conditions (e.g. lower soil moisture and higher bulk density) caused by more severe defoliation and trampling. As hypothesised, plant aboveground properties had a weaker influence (9.66–15.65%) on nematode, as well as fungal, β-deviation than root traits (20.46–65.67%) in both scenarios (Fig. 5). In water-limited Eurasian steppes, herbaceous plants typically increase their investment in root than those in wetter ecosystems, resulting in higher root-to-shoot ratios (Schenk & Jackson, 2002). This suggests that plant roots offer more substantial resources and have a more direct impact on soil organisms than aboveground parts, which primarily affect soil organisms through litter inputs (Villarino *et al.*, 2021; Liu *et al.*, 2024).

Caveats

Unlike β-diversity and community assembly patterns, simulated ungulate herbivory had a weaker effect on soil organism α-diversity (Fig. S9), especially on soil microbes. Many previous studies have also found that soil microbial α-diversity is much less sensitive to ungulate herbivory compared with β-diversity, even under long-term grazing (Qin *et al.*, 2021; Qiqige *et al.*, 2023; Usman *et al.*, 2024). The resistance of soil microbial richness could be attributed to the broad range of physiologies that soil microbes possess, which allows them to adapt to environmental changes (Qin *et al.*, 2021) and to recover quickly after disturbances (Comer & Perkins, 2021). Furthermore, horizontal gene transfer allows microbial communities to adjust their adaptations to environmental change without major shifts in taxonomic composition (Jain *et al.*, 1999; Ma *et al.*, 2019). Nonetheless, based on the microbial community assembly patterns observed in this study (Fig. 6b), sustained high-density ungulate herbivory could potentially lead to decreased microbial diversity and increased biotic homogenisation in the future.

While our study used simulated ungulate behaviour, which may not fully capture the natural complexity of herbivory, it offers valuable insights into how defoliation, trampling, and excreta return influence soil organisms' community assembly. For example, given the high labour intensity of the field experiment, we did not simulate the saliva secreted by herbivores during defoliation, which may have underestimated the effects of defoliation on soil organisms. Several studies have pointed out that saliva could stimulate plant regrowth and alter resource inputs from plants to the soil (Parnell *et al.*, 2024), potentially influencing soil community assembly. Additionally, our small-scale field experiment may not fully capture the spatial heterogeneity of natural grazing, such as patchy excreta return. Despite these limitations, the simulating experiment still allows for controlled observations of key mechanisms influencing soil β-diversity. Moreover, we believe that this study will inspire further research into the effects of complex disturbances on soil biota across different ecosystems and larger spatial scales.

Conclusion

Here, we provide empirical evidence that different aspects of ungulate herbivory alter β-diversity and community assembly of soil organisms, including bacteria, fungi, and nematodes, through a 4-yr field experiment. In high-density ungulate scenarios, harsher soil conditions caused by defoliation and tramping increased the contribution of deterministic processes in community assembly. Conversely, SD maintained stochastic community assembly for nematodes and fungi by increasing the SRL of the plant community. Excreta return had dual effects: Organic matter from faeces promoted bacterial community stochasticity by carbon input, while urine-induced acidification and increased ammonium levels drove environmental filtering for soil organisms. To our knowledge, this is the first study to examine the effects of defoliation, trampling, and excreta return of ungulates on diversity and community assembly of multiple soil organisms, although previous field-grazing studies often attempted to explain changes in soil organisms via these three aspects. Moreover, we highlight the critical role of root traits in shaping soil community assembly processes, enhancing our understanding of the mechanisms that maintain soil biodiversity under complex disturbances.

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Competing interests

None declared.

Author contributions

YZ and NL conceived the idea and designed the study. TG and MG performed field and laboratory work. TG, NL and MG performed data analyses. TG and NL drafted the manuscript. MR, MCR, MG and YZ revised the manuscript.

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Data availability

The data supporting the results in this paper are available from Figshare (doi: 10.6084/m9.figshare.27683403). The raw reads of soil samples are deposited in the SRA of the NCBI database under the accession no.: PRJNA1163065.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Layout of the experimental design and aerial picture of the field experiment.

Fig. S2 Difference in bulk density among different trampling levels.

Fig. S3 Effects of simulated moderate and intense ungulate herbivores on β-diversity and β-deviation.

Fig. S4 Relative effects of multiple predictors on soil biota β-deviation under moderate-density and high-density ungulate herbivores using the residuals from linear models.

Fig. S5 Relative abundance of plant functional groups in different defoliation levels.

Fig. S6 Specific root length of 10 common species in control, selective defoliation, and non-selective defoliation treatments.

Fig. S7 Effects of different defoliation types on arbuscular mycorrhizal fungi and saprotroph fungi abundance.

Fig. S8 Relative abundance of nematode functional groups and nematode maturity index under different defoliation levels.

Fig. S9 Effects of simulated moderate and intense ungulate herbivores on alpha diversity of soil bacteria, fungi, and nematodes.

Methods S1 Root traits of common species and statistical analyses.

Table S1 All nematode genera in the experiment.