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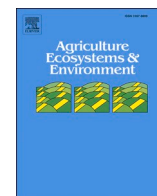
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Grassland litter decomposition is accelerated by herbivore dung deposition via changes in bacterial communities

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ABSTRACT

The decomposition of herbivore dung and plant litter are two crucial ecological processes that maintain the biogeochemical cycles and functions of grazing grassland ecosystems. Herbivore dung deposition causes heterogeneous microenvironmental conditions (e.g., temperature, moisture, nutrients, and microbial activities), yet its influence on litter decomposition and the involved biotic and abiotic processes remains underexplored. This study conducted a three-year dung-litter decomposition experiment in a yak-grazed alpine pasture on the Tibetan Plateau to examine how dung deposition influences soil microclimate, litter chemical quality, extracellular enzyme activities, and microbial communities, and how these factors collectively regulate the decomposition of *Elymus nutans* litter, a dominant plant species in the region. Results showed that dung deposition accelerated litter mass loss by 22 %, primarily by enhancing the degradation of cellulose and hemicellulose. Dung deposition raised soil temperature by 1.5 °C and increased moisture content by 19 % on average, and enriched litter chemical quality by reducing the carbon to nitrogen (C: N) ratio and increasing N, dissolved organic C, and dissolved organic N content. It also boosted the activities of lignocellulose-degrading enzymes, including β -1,4-glucosidase (BG), β -1,4-xylosidase (BX), β -D-cellobiosidase (CBH), xylanase (XYL), peroxidase, and polyphenol oxidase. Changes in microbial communities were distinct: bacterial communities responded strongly in the first two years, driven by improved litter chemistry and the introduction of dung-derived bacteria, while fungal community shifts occurred mainly in years 2–3, influenced by C and N dynamics (e.g., C: N ratio). Dung deposition increased the abundances of bacterial phyla such as *Bacteroidota* and *Proteobacteria*, which correlated positively with cellulase activity (BG, BX, CBH, and XYL) and the degradation of cellulose and hemicellulose in litter. Structural equation modeling revealed that the acceleration of litter decomposition by dung deposition was predominantly mediated by bacterial communities and their associated enzymatic activities. Overall, this study underscores the pivotal role of herbivore dung deposition in accelerating litter decomposition via regulating bacterial-driven processes. The findings offer valuable insights for grassland management and enhance predictions of herbivore dung deposition impacts on ecosystem functions under global herbivore expansion.

1. Introduction

Grasslands cover approximately 40 % of Earth's terrestrial area, with litter decomposition playing a central role in driving carbon (C) and nutrient cycling, essential for regulating biogeochemical cycles and maintaining grassland ecosystem functions (Guo et al., 2023; Zhao et al., 2024). This process is influenced by a combination of factors, including soil microclimate conditions (e.g., temperature and moisture), litter chemical quality (e.g., nitrogen (N) content and C: N ratio), and

decomposers such as bacteria, fungi, and their extracellular enzymes (Handa et al., 2014; Zhao et al., 2024). Changes in any of these factors can significantly affect litter decomposition rates and, consequently, the functionality of grassland ecosystems.

Herbivore grazing is a dominant grassland management approach worldwide, converting a portion of grassland productivity into livestock products for human use, while recycling the remainder back into the ecosystem as excreta, such as dung (Bardgett et al., 2021). Dung deposited on litter may cause changes in soil temperature and moisture

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content, litter chemical quality, and microbial communities and related extracellular enzyme activities (EEAs) (Cai et al., 2014; Liang et al., 2018). Consequently, herbivore dung deposition may impact litter decomposition in grasslands (Bloor, 2015; Liang et al., 2018; Wang et al., 2022). However, this significant ecological process and its biotic and abiotic mechanisms remain insufficiently understood. This is particularly relevant as the total deposited amount of herbivore dung is projected to continue to increase with the continuous expansion of global herbivore populations (Castonguay et al., 2023). Therefore, unraveling these processes is essential for advancing a mechanistic understanding and accurately predicting the impacts of herbivore dung deposition on grassland ecosystem functions, particularly under scenarios of global livestock expansion.

Soil temperature, moisture, and litter chemical quality are key abiotic factors influencing litter decomposition by shaping microbial communities and their enzyme activity (Joly et al., 2017; Wahdan et al., 2023). For example, warmer and moister soils provide optimal conditions for the metabolic activities of decomposers, thereby enhancing decomposition (Curiel Yuste et al., 2007). Litter with high chemical quality typically supports high decomposition rate due to they create a more suitable habitat and a complementary food source for decomposer microbes (Wang et al., 2021). Microorganisms secrete extracellular enzymes to break down lignocellulose, the tough structural component of plant litter, is a fundamental process in litter decomposition (Wang et al., 2023; Chakrawal et al., 2024). Generally, bacteria and fungi play distinct roles in lignocellulose decomposition, with bacteria thriving in nutrient-rich conditions due to their rapid growth rates, while fungi are better suited for nutrient-poor environments due to their higher enzymatic efficiency and resilience (Wilhelm et al., 2019; Christiansen et al., 2017). Consequently, the continuous inputs of high-quality exogenous substrates are expected to promote a bacterial-dominated decomposer community, thus facilitating the lignocellulose decomposition. The deposition of herbivore dung can alter soil conditions by increasing moisture and lowering surface temperature through shading effects, and enrich the litter quality by seeping the liable carbon and nutrients into the litter layer (Cai et al., 2014; Liang et al., 2018). Additionally, herbivore dung contains diverse microbial communities and active enzymes, which may transfer to the litter layer and influence its decomposition. For example, yak dung has been found to support a higher bacterial diversity and activity than fungi (Zhang et al., 2025). Despite these observations, the effects of herbivore dung on soil temperature, moisture, litter quality, microbial communities, and enzyme activity as well as how these factors regulate litter decomposition remain poorly understood, which is essential to fully comprehend the role of herbivore dung in grassland ecosystems.

The Tibetan Plateau, spanning approximately 2.5 million km² on the Eurasian continent, represents one of the world's most significant grassland ecosystems (Mi et al., 2021). Yak grazing dominates the region, with around 14 million yaks contributing over 10.2 billion dung patches annually (Bodin and Chen, 2023; Zhang et al., 2024). *Elymus nutans* (*E. nutans*), a prevalent perennial grass species, accounts for about 12 % of the surface vegetation in this ecosystem (Feng et al., 2010). To investigate how herbivore dung deposition may affect litter decomposition, a three-year dung-litter decomposition experiment was conducted in a yak-grazing alpine pasture on the Plateau. The study examined the effects of yak dung deposition on soil temperature, moisture, litter chemical quality, EEAs, and bacterial and fungal communities (composition and diversity), as well as how these factors collectively regulate litter decomposition. It was hypothesized that yak dung deposition would significantly accelerate the decomposition of litter materials in the Tibetan grassland ecosystem and that bacteria, often facilitated by the high-quality carbon and nutrient content of yak dung and its rich bacterial diversity, would play a more dominant role than fungi in the decomposition processes.

2. Materials and methods

2.1. Research site

This dung-litter decomposition experiment was established in the Gansu Gannan Grassland Ecosystem National Observation and Research Station (34°57'N, 102°53'E, 2960 m a.s.l.), located in the northeastern Tibetan Plateau. The site with a mean annual precipitation of 570 mm, over 80 % of which falls from July to September. The mean annual temperature is ~ 2.0 °C ranging from a monthly maximum of 22.5 °C in July to a minimum of -8.9 °C in January. The site is an alpine meadow ecosystem dominated by *E. nutans* (~ 72 %), *Agropyron cristatum* (~ 10 %), *Potentilla anserina* (~ 8 %), and *Astragalus membranaceus* (~ 5 %). The soil is alpine meadow soil, with surface layer (0–20 cm) has a pH of 7.2 and contains 50.2 g kg⁻¹ organic C and 5.0 g kg⁻¹ N. The site has been subjected to open yak-grazing (*Bos grunniens*) from early May to late October for three decades, with an estimated stocking density of 3–5 yaks per hectare.

2.2. Sample preparation

Yak dung was collected from randomly selected subgroups of yaks within five corrals, each housing eight mature yaks. Fresh dung samples were combined, thoroughly mixed, and divided into uniform dung patches weighing 2 kg each, with dimensions of 20 cm in diameter and 5 cm in height. These measurements were based on field observations indicating that a mature yak typically deposits three dung patches daily, each weighing an average of 2 kg and covering 0.03 m² of ground. To prevent material loss while allowing macroinvertebrates such as dung beetles and earthworms to access the dung, the patches were enclosed in cylindrical polyethylene nylon bags (D × H: 22 cm × 6 cm, with a 1 cm mesh size).

Freshly senescent *E. nutans* leaf litter was collected during mid-October 2018, when the majority of leaf pools had accumulated on the soil surface at the study site. The litter was thoroughly mixed, air-dried, and cut into ~ 15 cm sections before being stored in a desiccator for later application in the field. Initial physicochemical properties, extracellular enzyme activities (EEAs), and bacterial and fungal communities of the fresh yak dung and litter were determined from four fresh samples of each.

2.3. Experimental setup

Litter decomposition was assessed with litterbags (20 g of air-dried litter per bag) made from circular polyethene nylon nets, featuring an 18 cm diameter to ensure it is fully covered by dung patches and a 2 mm mesh size to reduce litter loss while permitting access by soil fauna. Based on previous studies, fresh yak dung deposited during the cold season is estimated to take approximately three years to fully decompose (Yu et al., 2013). Thus, this study was designed as a three-year-long decomposition experiment.

Four 6 m × 6 m replicate blocks within the research area were established with a distance of at least 2 m between each block. The litter layer and dung residues were removed from each plot, and vegetation was periodically trimmed to maintain a height of approximately 1 cm throughout the experiment. Ten litterbags were placed within each block, with an edge distance of 1.5 m to prevent mutual interference. Litterbags were placed on the soil surface and secured with five 10 cm nails to prevent movement. In each block, five dung patches were placed over individual litterbags to simulate natural dung deposition, while the remaining five litterbags served as controls without dung patches. RT-1 temperature sensors and EC-5 moisture sensors were installed beneath one dung-applied litterbag and one control litterbag in each block to monitor soil microclimate conditions. These sensors were connected to an EM50 digital datalogger (Decagon Devices, Pullman, WA, USA) for data collection at 2-hour intervals.

2.4. Sampling of litter, dung, and soil

Sampling was carried out at 0.5, 1, 1.5, 2, and 3 years after the experiment began to capture different stages of decomposition. On each sampling date, one control litterbag and one dung-treated litterbag were randomly selected from each of the four blocks. Remaining decomposed litter and dung samples were collected intact, along with surface soil cores (4 cm in diameter and 20 cm in depth, $n = 4$) from beneath each litterbag. Samples were placed in sterilized polyethylene bags and transported to the laboratory in liquid nitrogen to preserve their integrity. In the laboratory, soil samples were cleaned of plant debris and stones, then sieved to 2 mm. Litter and dung samples were weighed, cut (approximately 0.2 cm²), and crushed with mortar, respectively.

The processed samples were divided into three portions: one was dried at 65 °C and ground using a vibration ball mill for analyzing total carbon (TC), total nitrogen (TN), and lignocellulosic fractions; another was stored at 4 °C for measuring dissolved organic compounds and EEAs; and the third was frozen at -80 °C for DNA extraction. All analyses were completed within one week of sample collection.

2.5. Physicochemical characteristics analysis

Gravimetric moisture content was determined by oven-drying fresh subsamples at 65 °C until a constant weight was achieved. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted using 100 mL of 0.5 M K₂SO₄ solution (1 g for litter and dung and 10 g for soil) and centrifuged at 10,000 rpm for 15 minutes. The supernatant was filtered through 0.45 µm nitrocellulose membrane filters, and the carbon and nitrogen concentrations in the extracts were analyzed using a TOC analyzer (Multi N/C 2100S, Analytik Jena, Germany). TC and TN were measured with an elemental analyzer (Elementar vario MACRO cube, Elementar Analysensysteme, Hanau, Germany). Lignocellulosic fractions in litter and dung, including lignin, cellulose, and hemicellulose, were quantified using a Fiberte™ 8000 Fiber Analyzer (Foss Technology, Hillerød, Denmark).

2.6. Extracellular enzyme activities analysis

Six EEAs including hydrolases β-1,4-glucosidase (BG), β-1,4-xylosidase (BX), β-D-cellobiosidase (CBH), xylanase (XYL) and oxidases peroxidase (POD) and polyphenol oxidase (PPO) were quantified. The litter, dung, and soil sample suspensions were prepared by adding 0.5 g of fresh sample into 125 mL of 50 mM Tris buffer and homogenizing for 3 minutes.

Hydrolase activities were assessed using standard high-throughput fluorometric techniques following the method described by Saiya-Cork et al. (2002). Microplates were incubated in the dark at 25 °C for 4 h, then centrifuged. The supernatant was transferred into black 96-well plates, and 10 µL of 1.0 M NaOH was added to each well. Fluorescence was measured within 1 minute using a microplate fluorometer (Thermo Scientific, Varioskan Flash, Finland) at an excitation wavelength of 365 nm and emission wavelength of 450 nm.

Oxidase activities were quantified using the method proposed by DeForest (2009). Microplates were incubated in the dark at 25 °C for 4 hours for PPO and 24 hours for POD. Enzyme activity was measured using a microplate spectrophotometer at an absorbance of 450 nm, corrected using an extinction coefficient of 7.9 for DOPA. Enzyme activities were expressed as µmol g⁻¹ dry weight (gdw) per hour (h⁻¹).

2.7. DNA extraction, gene amplification, and high-throughput sequencing

Total genomic DNA was extracted from approximately 200 mg of litter, dung, and soil subsamples using the MoBio PowerSoil DNA Isolation Kit (MO BIO, USA). Libraries were prepared following the protocols provided in the HiSeq Reagent Kit Guide (Illumina, USA). The bacterial 16S rRNA gene (V3–V4 region) and fungal rRNA gene (ITS1

region) were amplified in triplicate using primer pairs 338 F/806 R and ITS1F/ITS2R, respectively. The triplicate amplicons were combined, and successful amplification was confirmed via electrophoresis on a 2 % agarose gel.

Amplicons were purified using the GeneJET Gel Extraction Kit (Thermo Scientific, USA), and their concentrations were quantified with a Qubit 2.0 fluorometer (Invitrogen, USA). Samples were then pooled in equal proportions to create the sequencing library, which was sequenced on an Illumina HiSeq 2500 platform (Illumina, USA).

High-quality sequences were clustered into Operational Taxonomic Units (OTUs) at a 97 % similarity threshold using the UPARSE pipeline. Chimeric sequences were identified and removed with the UCHIME algorithm during clustering. Representative OTUs sequences were classified using the Ribosomal Database Project (RDP) classifier against the SILVA 138 database for bacteria and the UNITE 8.0 database for fungi, with a confidence threshold of 80 %. To ensure consistency, all samples were rarefied to the smallest sequence count (22,447 for bacteria and 32,237 for fungi).

2.8. Statistical analyses

Statistical analyses were conducted using R software (version 4.1.1) (R Core Team, 2021). Soil temperature and moisture content data were averaged over each sampling interval for analysis. Linear mixed models (LMMs) analyses were used to evaluate the effects of dung deposition on litter mass loss, physicochemical properties, EEAs, and bacterial and fungal community properties (*nlme* package). In these models, dung deposition and sampling time (years) were treated as fixed factors, while individual samples nested within blocks were included as random factors.

Nonmetric multidimensional scaling (NMDS) was applied to examine dissimilarities in bacterial and fungal communities among treatments, based on weighted UniFrac distances from OTU similarity matrices (*vegan* package). Permutational multivariate analysis of variance (PERMANOVA) assessed the effects of dung application and decomposition time on bacterial and fungal communities (*vegan* package). NMDS results showed that decomposition time primarily distinguished samples along the first axis, while the second axis differentiated treatments (control vs. dung deposition) (Fig. 5). Redundancy analysis (RDA) was used to explore relationships between litter chemical properties, lignocellulose content, EEAs, and bacterial and fungal communities at the phylum and class levels (*vegan* package). The individual contributions of explanatory variables in RDA were quantified using hierarchical partitioning method (*rdacca.hp* package) (Lai et al., 2022).

A piecewise structural equation model (SEM) (*piecewiseSEM* package) was constructed to elucidate how dung deposition influences litter decomposition through changes in microclimate, chemical quality, EEAs, and microbial communities (the priori conceptual model see Fig. S1). The model is constructed using linear mixed effect models that allows to consider the block as error term (Lefcheck, 2016). Variables were standardized and grouped into six categories: (1) microclimate (soil temperature and moisture content), (2) chemical quality (C, N, C: N ratio, DOC, and DON), (3) EEAs (BG, BX, CBH, XYL, POD, and PPO), (4) bacterial community (OTU richness, Shannon index, and NMDS second-axis scores), (5) fungal community (OTU richness, Shannon index, and NMDS second-axis scores), and (6) litter decomposition (mass loss). The dimensionality of the multiple parameters within each category was reduced to one primary principal component (PC1) by the PCA (*stats* package), which explained 66–87 % of the total variance for each category (Table S1), and then fed into the SEM analysis. In the SEM, dung deposition (coded as 0 = control and 1 = dung application) was treated as an exogenous variable influencing all response variables. Model fit was evaluated using Fisher's C statistic, with $P > 0.05$ indicating acceptable fit. Standardized direct, indirect, and total effect sizes of the factors on litter decomposition were calculated.

3. Results

3.1. Effects of dung deposition on litter mass loss, chemical properties, and soil microclimate conditions

After three years of decomposition, dung deposition accelerated the litter mass loss of *E. nutans* by 22 %, with the majority of the loss occurring within the first two years (Fig. 1a, $P < 0.001$). Lignocellulose, which constituted 82 % of the initial litter mass, included 39 % cellulose, 39 % hemicellulose, and 4 % lignin (Table S2). The decomposition of cellulose and hemicellulose predominantly occurred within the first 1.5–2 years in both control and dung deposition treatments (Fig. 1b–d), with dung application enhancing their mass loss by 12 % and 25 %, respectively, compared to the control after three years (Table S2). Lignin content initially increased during the first two years of decomposition in both treatments, followed by a decline (Fig. 1d). By the end of the experiment, lignin mass loss was 7 % higher in the dung-applied treatment compared to the control (Table S2).

On average, dung deposition significantly enhanced litter N content by 19 % and increased the concentrations of DOC and DON by 63 % and 105 %, respectively, while reducing the C: N ratio by 10.4 % (Fig. 1f–i, $P < 0.001$). Additionally, dung application raised soil temperature and moisture content by an average of 1.5 °C and 19 %, respectively, over the course of the experiment, with these effects being more pronounced during cold and dry seasons (Fig. 2, $P < 0.001$).

3.2. Effects of dung deposition on litter EEAs

Dung deposition significantly enhanced the activities of hydrolases (BG, BX, CBH, and XYL) during the first 1.5 years of decomposition and

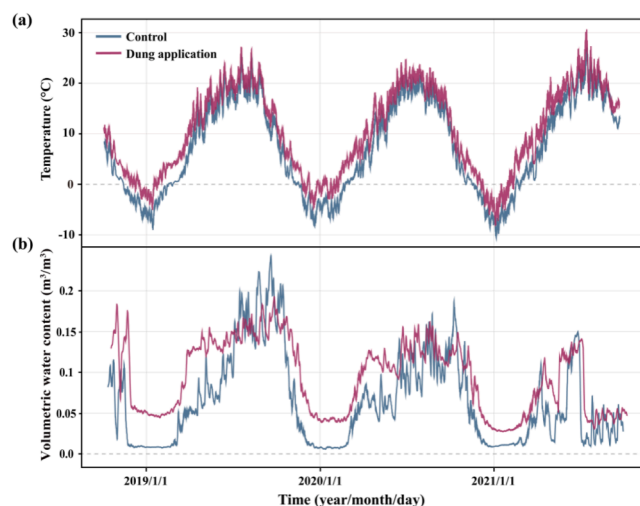


Fig. 2. Temperature (a) and volumetric water content (b) in the litter-soil interface in control and dung application plots throughout the experimental period. Values are daily means ($n = 4$). The effect of dung application was significant at $P < 0.001$ for all variables.

increased the activities of oxidases (POD and PPO) throughout the entire decomposition period (Fig. 3, $P < 0.01$). The activities of hydrolases showed a declining trend over the three-year decomposition in both the control and dung deposition treatments (Fig. 3a–d), whereas the activities of oxidases progressively increased (Fig. 3e and f).

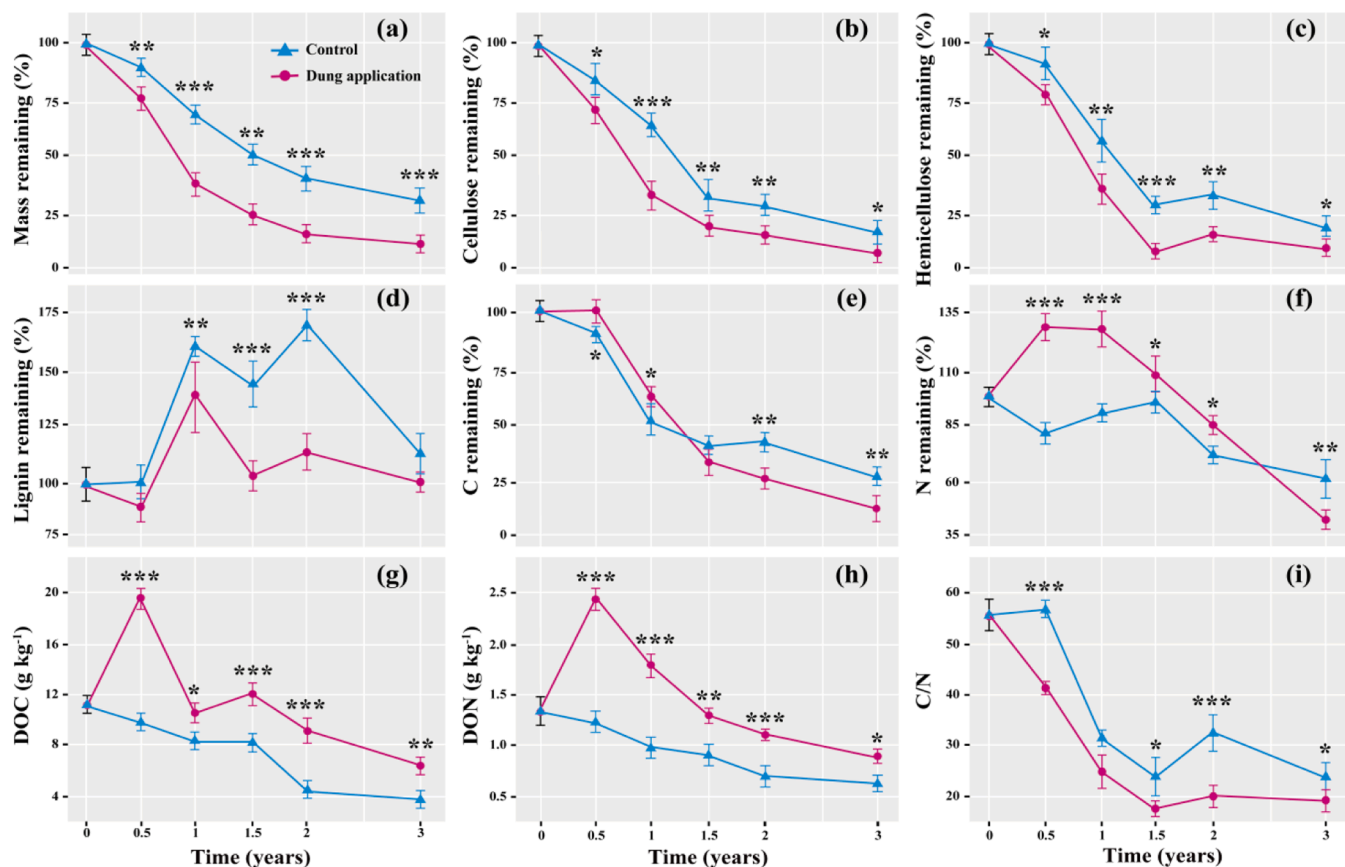


Fig. 1. Litter mass loss and chemical properties. Values are mean $\pm$ standard error ($n = 4$). The effect of dung application was significant at $P < 0.001$ for all variables. Asterisks indicate significant differences between control and dung application in each sampling time (linear mixed model results: with * = $P < 0.05$ and *** = $P < 0.001$). Abbreviations: C, total carbon; N, total nitrogen; DOC, dissolved organic C; DON, dissolved organic N; and C: N, the ratio of TC to TN.

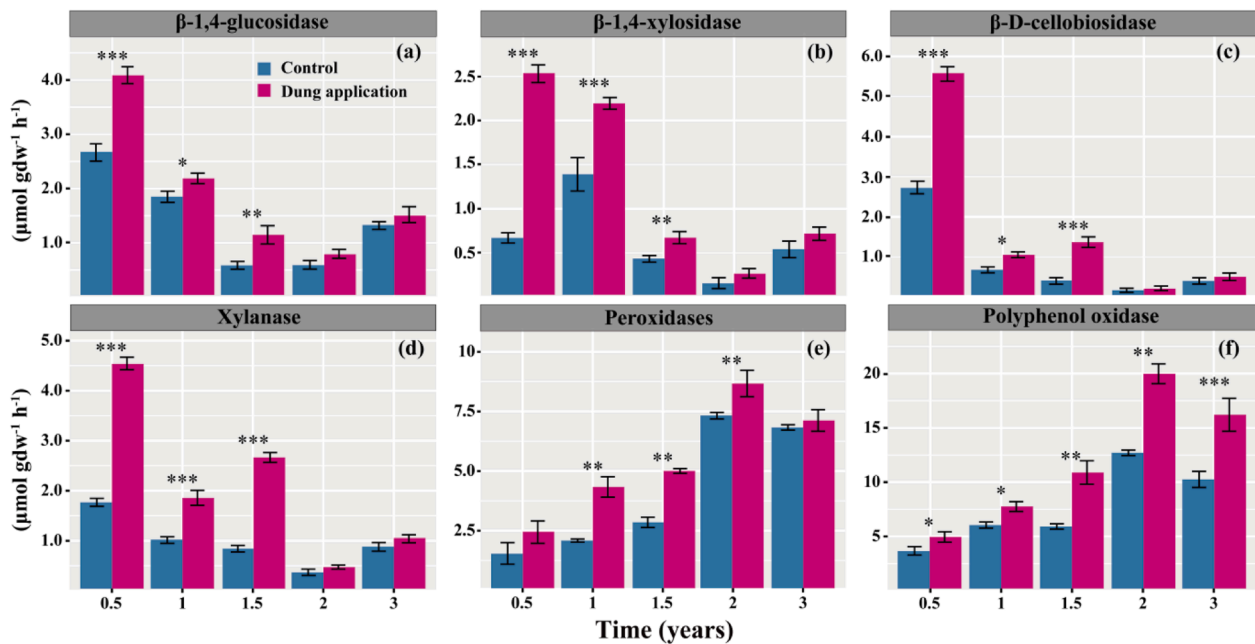


Fig. 3. Activities of litter extracellular enzymes. Values are mean $\pm$ standard error ($n = 4$). The effect of dung application was significant at $P < 0.01$ for all variables. Asterisks indicate significant differences between control and dung application in each sampling time (linear mixed model results: with * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$).

3.3. Effects of dung deposition on litter bacterial and fungal communities

Litter bacterial communities were dominated by phyla of *Proteobacteria* (22–78 %), *Actinobacteriota* (5–51 %), *Bacteroidota* (4–27 %), *Acidobacteriota* (0.4–14 %), and *Chloroflexi* (0.7–13 %) (Fig. 4a), and by the classes of *Alphaproteobacteria* (23–44 %), *Bacteroidia* (6–26 %), *Gammaproteobacteria* (3–27 %), and *Actinobacteria* (5–44 %) (Fig. S2). Dung deposition increased the abundances of *Proteobacteria* and *Bacteroidota* in the first 1.5–2 years of decomposition, and *Acidobacteriota* and *Chloroflexi* in the 1.5–3 years of decomposition (Fig. S3a, $P < 0.05$), while decreased the abundances of *Actinobacteriota* and *Cyanobacteria* (Fig. S3a, $P < 0.05$). At the class level, dung deposition increased the abundances of *Alphaproteobacteria* and *Gammaproteobacteria* (Phylum *Proteobacteria*) and *Bacteroidia* (Phylum *Bacteroidota*) in the first 1.5–2 years of decomposition ($P < 0.05$), and decreased the abundance of *Actinobacteria* (Phylum *Actinobacteriota*) in the first 2 years of decomposition (Fig. S4, $P < 0.05$). In addition, dung deposition significantly altered the bacterial community (PERMANOVA result: $R^2 = 0.76$, $P < 0.001$) and increased the OTUs richness and Shannon diversity mainly during the first 2 years of decomposition (Fig. 5a, Fig. S5 a and c, Table S4).

Litter fungal communities were dominated by phyla of *Ascomycota* (62–94 %) and *Basidiomycota* (1–35 %) (Fig. 4b), and by the classes of *Sordariomycetes* (47–65 %), *Leotiomycetes* (2–26 %), *Dothideomycetes* (7–24 %), and *Agaricomycetes* (1–7 %) (Fig. S2b). Dung deposition increased the abundances of minor (relative abundance < 0.1 %) phyla *Mortierellomycota*, *Rozellomycota*, and *Glomeromycota* in the third year of decomposition ($P < 0.05$), but no significant differences were detected for dominant phyla *Ascomycota* and *Basidiomycota* (Fig. S3b). Dung deposition significantly altered the fungal community (PERMANOVA result: $R^2 = 0.19$, $P < 0.01$) and increased the OTUs richness and Shannon diversity mainly within the third year of decomposition (Fig. 5b, Fig. S5 b and d, Table S4).

3.4. Relationships among litter chemical properties, EEAs, and microbial communities

Changes in litter chemical quality explained 69 % of the variations in the litter bacterial community ($P < 0.001$), with DOC, DON, and TN having the strongest effects (accounting for ~ 82 %). These factors were positively correlated with the abundances of the bacterial phyla *Bacteroidota* and *Proteobacteria* (Fig. 6a). In contrast, changes in litter

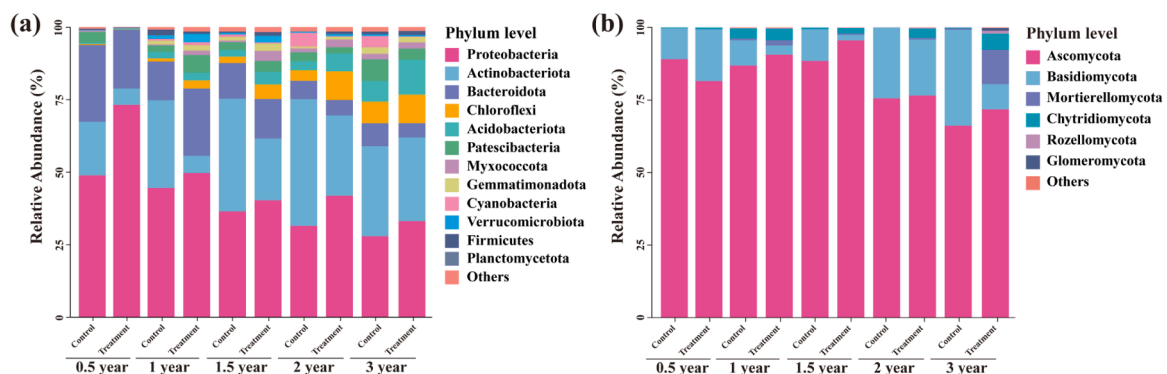


Fig. 4. Relative abundances of dominant (relative abundance > 1 %) litter bacterial (a) and fungal (b) phyla in control and dung application. The results presented are means ($n = 4$).

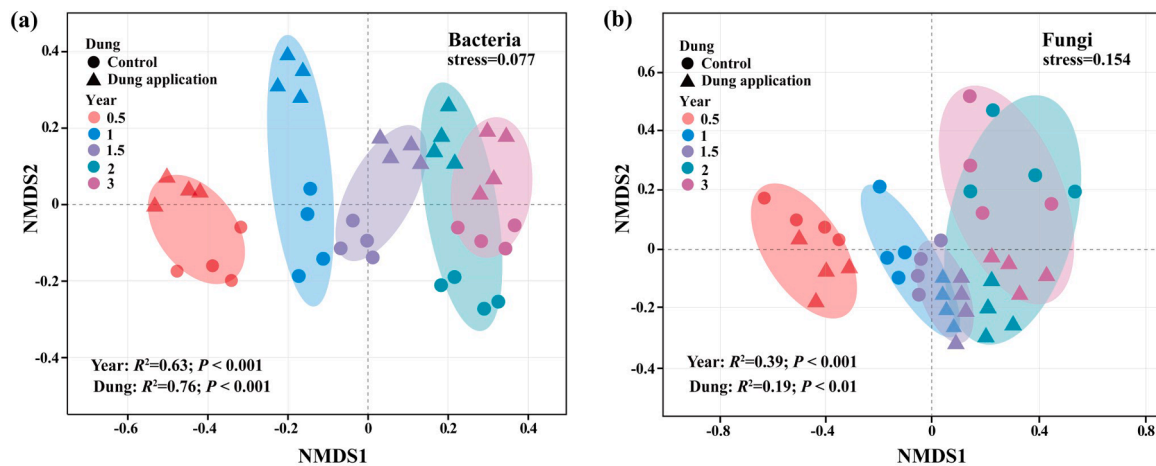


Fig. 5. Unconstrained nonmetric multidimensional scaling (NMDS) showing the differences in litter bacterial (a) and fungal (b) communities under dung treatment and different decomposition stages. Shaded 95 % confidence ellipses are shown around sample groups. Similarity values between dung treatment and among different decomposition stages were examined using the PERMANOVA test, which are shown in each panel.

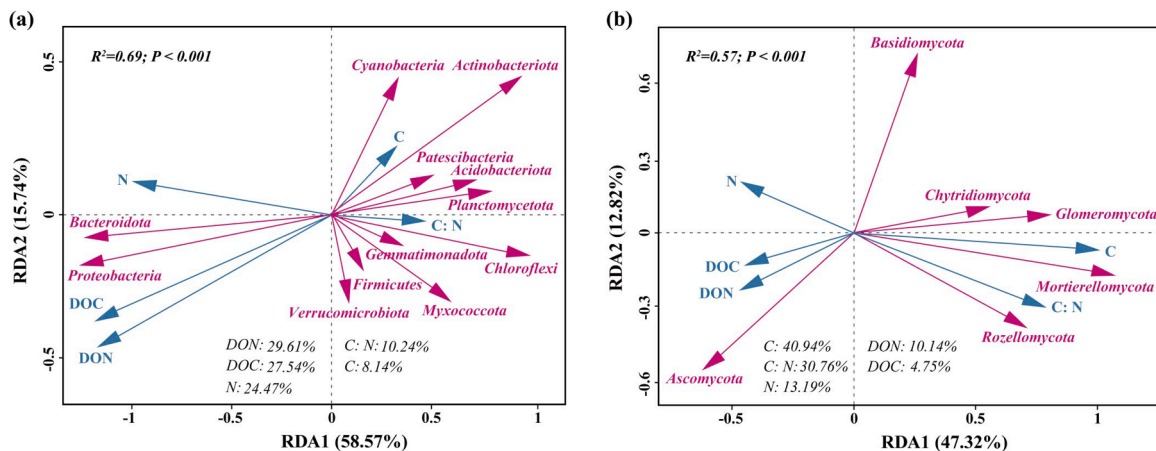


Fig. 6. Redundancy analysis (RDA) showing the relationships between bacterial (a) and fungal (b) communities with litter chemical quality. Blue arrows represent explanatory variables, while red arrows denote response variables. Only phyla with relative abundances > 0.1 % were displayed. The statistical significance of models (adjusted R^2) and RDA axes were obtained by a Monte Carlo permutation test with a permutation number of 999. Percentages in the panels represent the individual explained proportion of each explanatory variable on the response variable. The values were obtained by a hierarchical partitioning method. Abbreviations: C, total carbon; N, total nitrogen; C:N, the ratio of TC to TN; DOC, dissolved organic C; and DON, dissolved organic N.

chemical quality explained 57 % of the variations in the litter fungal community ($P < 0.001$), where TC, TN, and the C:N ratio had the most substantial impacts (accounting for ~ 85 %). These factors were positively correlated with the abundances of the fungal phyla *Glomeromycota*, *Mortierellomycota*, and *Rozellomycota* (Fig. 6b).

The bacterial phyla *Bacteroidota* and *Proteobacteria* were positively correlated with the activities of hydrolase enzymes, including BG, BX, CBH, and XYL (Fig. 7a). Similarly, the abundances of these two bacterial phyla and the activities of the associated hydrolases were positively correlated with the cellulose and hemicellulose content in litter (Fig. 7b and c). In contrast, the abundances of *Actinobacteriota* and *Cyanobacteria* and the activities of the lignin-degrading enzymes POD and PPO were positively correlated with lignin content (Fig. 7b and c). At the class level, the relative abundances of *Alphaproteobacteria*, *Bacteroidia*, and *Gammaproteobacteria* were positively correlated with cellulose and hemicellulose content, while *Actinobacteria* and *Cyanobacteriia* showed positive correlations with lignin content (Fig. 7d).

3.5. Pathways of effects of dung application on litter decomposition

Dung deposition-induced changes in soil microclimate conditions

and litter chemical quality explained 83 % and 76 % of the variance in these variables, respectively (Fig. 8a). Direct effects of dung deposition, along with its influence on soil microclimate and litter chemical quality, accounted for 90 % of the variance in litter bacterial communities. In contrast, changes in litter chemical quality alone explained 78 % of the variance in litter fungal communities.

The combined direct effects of dung deposition and indirect effects through altered soil microclimate, litter chemical quality, and bacterial and fungal communities explained 92 % of the variance in litter EEAs. EEAs showed a stronger positive correlation with bacterial communities. Furthermore, dung-induced changes in bacterial communities and EEAs were positively correlated with litter decomposition, together explaining 89 % of its variance.

Standardized total effects derived from the SEM showed that bacterial communities (1.46), litter chemical quality (1.41), and EEAs (0.88) played more substantial roles in litter decomposition compared to soil microclimate conditions (0.42) and fungal communities (0.10) (Fig. 8b). Overall, the accelerated litter decomposition following dung deposition was primarily driven by improved litter chemical quality, which subsequently enhanced bacterial communities and EEAs (Fig. 8a).

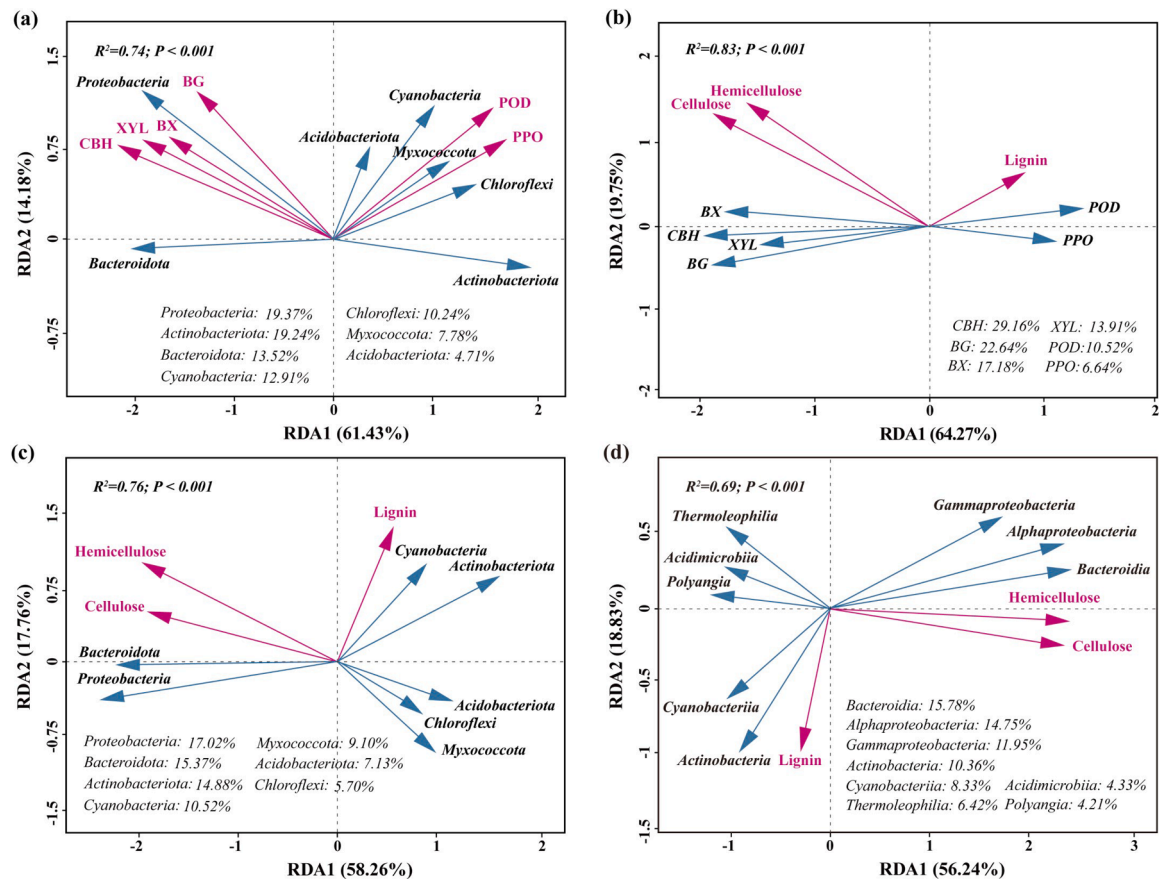


Fig. 7. Redundancy analysis (RDA) showing the relationships between bacterial and fungal phyla with extracellular enzyme activities (a), and extracellular enzyme activities (b), bacterial and fungal phyla (c) and classes (d) with litter lignocellulose content. Blue arrows represent explanatory variables, while red arrows denote response variables. Only those significant explanatory variables were displayed in each panel. Statistical information is described see Fig. 6. Abbreviations: BG, β -1,4-glucosidase; BX, β -1,4-xylosidase; CBH, β -D-cellobiosidase; XYL, xylanase; POD, peroxidases; and PPO, polyphenol oxidase.

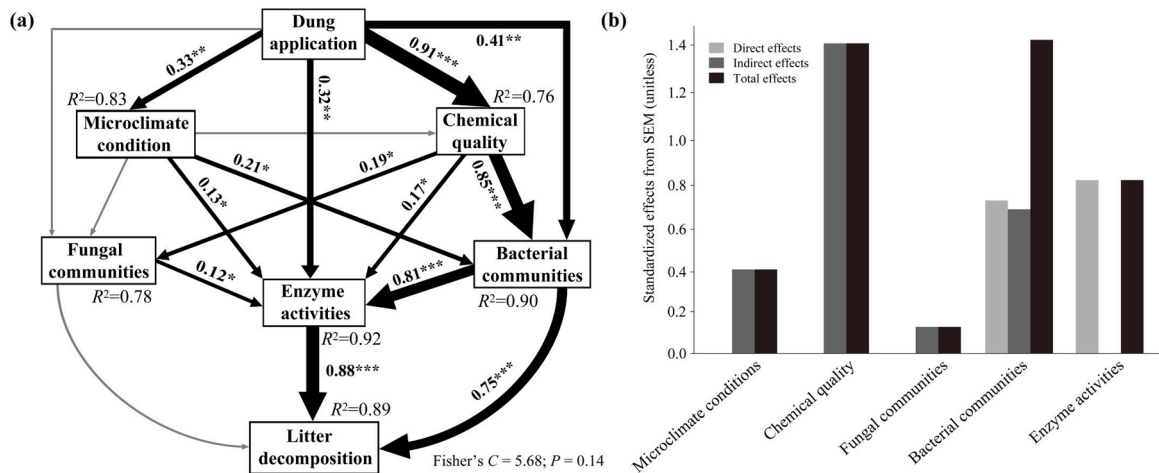


Fig. 8. The piecewise structural equation modeling (SEM) reveals the direct and indirect effects of yak dung application on litter decomposition (a). The model fitness was: Fisher's $C = 5.68$ ($P = 0.14$). The paths with non-significant ($P > 0.05$) and positive significant (with $*$ = $P < 0.05$, $**$ = $P < 0.01$, and $***$ = $P < 0.001$) effects are shown with grey and black arrows, respectively. The width of the significance paths is scaled based on the magnitude of the standardized regression coefficient. Conditional R^2 values of the fitted linear mixed-effect models are displayed in composite variables. Panel (b) presents the standardized direct, indirect, and total effect sizes of soil microclimate, litter chemical quality, enzyme activities, and bacterial and fungal communities on litter decomposition.

4. Discussion

4.1. Effects of yak dung deposition on soil microclimate conditions and litter chemical quality

Yak dung deposition can significantly alter soil microclimate conditions, as demonstrated in this study, with soil temperature and moisture content increasing by an average of 1.5 °C and 19 %, respectively, over the entire experiment. The increases were primarily observed during cold and dry seasons, where the large patches of yak dung likely elevated soil temperature by reducing heat loss and increased soil moisture by minimizing water evaporation, while minimal or negligible effects during warm and rainy seasons, which were likely due to the shading effect of yak dung and its obstruction of rainfall infiltration.

Yak dung contains high exogenous carbon and nutrients, which can be enriched into the litter layer through the leaching of mineral nutrients and the decomposition of lignocellulose. High litter chemical quality is often characterized by high contents of N and dissolved organic compounds and a low C: N ratio (Uselman et al., 2012; Li et al., 2019). In this study, yak dung deposition significantly increased litter N, DOC, and DON contents while reducing the C: N ratio. Similarly, Liang et al. (2018) reported a decrease in the C: N ratio in four common plant litters after yak dung deposition, further demonstrating that yak dung improves litter chemical quality.

4.2. Effects of yak dung deposition on litter EEAs and microbial communities

Yak dung deposition enhanced the activities of litter enzymes, including BG, BX, CBH, XYL, POD, and PPO (Fig. 3). This may be attributed to two primary factors. First, bacterial communities showed a stronger positive correlation than fungal communities with EEAs (Fig. 8a), indicating that bacteria play a more dominant role in regulating the production of litter EEAs after yak dung deposition. Consequently, yak dung deposition stimulated microbial activity in the litter, particularly bacterial communities, which led to increased the production of extracellular enzymes. Second, the elevated EEAs present in yak dung itself (Liu et al., 2017; Zhang et al., 2024, 2025; Table S3) contributed to the transfer of dung-derived enzymes into the litter layer (Fig. 8a), further amplifying the observed EEAs.

Yak dung deposition also altered the microbial community structure by increasing the alpha diversity and modifying the composition of litter bacterial communities earlier in the decomposition process, while changes in fungal communities were more apparent later. This suggests that bacterial communities are more responsive than fungal communities in litter to dung deposition. Similar patterns have been reported by Hatch et al. (2000) and Lazcano et al. (2013), where bacterial communities exhibited rapid responses, while fungal communities demonstrated slower changes following organic manure application in permanent alpine pastures.

The changes in litter bacterial communities were primarily driven by increases in DOC, DON, and TN levels. Yak dung provides abundant liable nutrients, creating favorable conditions for bacteria that thrive in nutrient-rich environments (Liu et al., 2017; Wilhelm et al., 2019). Additionally, bacteria present in the dung itself likely contributed to changes in the bacterial community when introduced into the litter layer (Table S3). As a result, bacterial communities responded quickly to dung deposition but showed no significant effects after two years, as the availability of dung-derived nutrients declined. In contrast, litter fungal communities were influenced mainly by total carbon content and the C: N ratio. Over time, as liable nutrients were exhausted, recalcitrant carbon became richer in the remaining substrates with a higher C: N ratio, which likely encouraged fungal growth and led to changes in the fungal community composition during the later stages of decomposition.

4.3. Effects of yak dung deposition on litter decomposition and underlying mechanisms

Yak dung deposition significantly accelerated the decomposition of *E. nutans* litter and showed a strong correlation with bacterial communities, including their diversity, composition, and enzyme activities. This supports our initial hypothesis that bacterial processes play a more dominant role than fungal processes in litter decomposition following yak dung deposition. Herbivore dung is rich in high-quality carbon, nutrients, and microbial diversity, particularly bacteria, which exhibit high activity levels (Zhang et al., 2025). The introduction of these dung-derived substrates and microbes creates favorable conditions for bacterial growth and metabolism in the litter layer, ultimately leading to an accelerated litter decomposition rate.

In this study, *Bacteroidota* and *Proteobacteria* were the most abundant bacterial phyla and were closely associated with the decomposition of cellulose and hemicellulose. These bacteria, categorized as copiotrophs, are enriched with genes encoding cellulases (Trivedi et al., 2016), enabling them to use the breakdown products of cellulose and hemicellulose as carbon and energy sources during litter decomposition (Fierer et al., 2007; Gharechahi et al., 2021). At the class level, *Bacteroidia*, *Alphaproteobacteria*, and *Gammaproteobacteria* were identified as the key bacterial groups involved in cellulose and hemicellulose decomposition. Similar findings by Schneider et al. (2012) and Wilhelm et al. (2019) have highlighted these classes as major contributors to litter decomposition. *Bacteroidia* and *Alphaproteobacteria*, in particular, are efficient decomposers with a diverse array of lignocellulose-degrading genes (Pandit et al., 2016). The increased abundances of *Bacteroidota*, *Proteobacteria*, and their key subtaxa were mainly in the first 1.5–2 years of yak dung decomposition, largely driven by the introduction of exogenous substrates and bacteria from the dung (Fig. 8a, Fig. S6), which explained the elevated cellulase activities and accelerated breakdown of cellulose and hemicellulose during this period.

The results showed that the abundance of *Actinobacteriota* was strongly associated with lignin content, indicating that *Actinobacteriota* was the primary agent in litter lignin decomposition in this study. *Actinobacteriota* are oligotrophs equipped with genes encoding a comprehensive set of carbohydrate-active enzymes, enabling them to break down recalcitrant biomaterials such as lignin (Lewin et al., 2016). The process of decomposition led to extensive lignification, resulting in increased lignin content in both treatments. Consequently, the abundance of *Actinobacteriota* and the activities of oxidase enzymes POD and PPO increased during decomposition. Additionally, a positive correlation was observed between the abundances of *Actinobacteriota* and *Cyanobacteria* in this study (Fig. 7c). *Cyanobacteria*, which often host heterotrophic microbes as epiphytes (Vidal et al., 2021; Papazachariou et al., 2024), can provide fixed inorganic nitrogen, potentially enhancing the decomposition capacity of associated microbes (Kuehn et al., 2014). Fungi can also play a vital role in lignin decomposition due to their capacity to break down recalcitrant compounds (Schneider et al., 2012). In this study, the fungal phyla *Mortierellomycota*, *Rozellomycota*, and *Glomeromycota* were closely associated with lignin decomposition, and their abundances increased in response to dung deposition during the later stages of decomposition (2–3 years). Together with the bacterial phylum *Actinobacteriota*, these fungal groups were also the primary contributors to the decomposition of lignin in the litter (Fig. S7).

5. Conclusions and outlooks

This study demonstrates that yak dung deposition significantly accelerates *E. nutans* litter decomposition by improving soil microclimate conditions, enhancing litter chemical quality, and increasing extracellular enzyme activities. Yak dung deposition also alters both bacterial and fungal communities within the litter, with bacterial communities showing a stronger and more immediate response than fungal

communities. The acceleration of litter decomposition is primarily driven by the enhanced chemical quality of the litter, which promotes bacterial activity, as well as the direct contribution of dung-derived bacteria. Particularly, copiotrophic bacteria taxa (e.g., *Bacteroidota* and *Proteobacteria*) and cellulases (e.g., BG, BX, CBH, and XYL) are the primary agents of litter decomposition upon yak dung deposition. Future research should incorporate litter from a variety of plant species with differing qualities and extend investigations to broader grassland ecosystems, which will help validate the generality of the findings and provide a more comprehensive understanding of herbivore dung's role in grassland ecosystems.

Given the continuous expansion of global herbivore populations and the projected increase in dung deposition in grasslands (Castonguay et al., 2023), which would lead to adverse environmental and ecological effects by accelerating litter decomposition, such as nutrient imbalances and increased greenhouse gas emissions. To mitigate these potential impacts, strategies such as adopting intensive husbandry practices and removing excess dung from grasslands could be effective in maintaining ecosystem health and agricultural sustainability. Overall, this study enhances our understanding of how herbivore dung deposition influences litter decomposition processes and provides valuable insights for grassland management and conservation, which are critical for developing sustainable practices in grassland ecosystems under the pressures of global herbivore population growth.

CRedit authorship contribution statement

Zhiyang Zhang: Writing - original draft, Methodology, Investigation, Formal analysis, Visualization, Data curation. **Xiaogang Dong:** Investigation, Writing - review & editing. **Jing Tang:** Resources, Writing - review & editing. **Shiting Zhang:** Funding acquisition, Writing - review & editing. **Riikka Rinnan:** Resources, Funding acquisition, Writing - review & editing. **Yi Jiao:** Writing - original draft, Writing - review & editing, Formal analysis, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

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

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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.2025.109557.

Data availability

Data will be made available on request.

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