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Does long-term grazing cause cascading impacts on the soil microbiome in mountain birch forests?

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ABSTRACT

In subarctic mountain birch forests, reindeer grazing and moth outbreaks act as important biotic drivers of ecosystem functioning. We investigated how a long-term contrast in reindeer grazing regimes and short-term ungulate exclusion affected soil fungal and bacterial communities in mountain birch forests recovering from a recent moth outbreak. We separately described the impacts on microbial communities for organic and mineral soil layers. Differences in fungal communities were mainly explained by variations between grazing regimes, whereas the four-year exclusion of ungulates had little effect. Soil microbial communities showed a high level of specificity between organic and mineral layers. Our results suggest that long-term grazing may have cascading impacts, especially on ectomycorrhizal fungal communities. In contrast, ericoid mycorrhizal and saprotrophic fungal communities and soil bacterial communities were less affected by grazing and appeared to be more resilient to aboveground herbivory in mountain birch forests recovering from a moth outbreak.

1. Introduction

In Northern Fennoscandia, mountain birch (*Betula pubescens* ssp. *czerepanovii)* forests form the main ecotone between boreal coniferous forest and treeless tundra (Wielgolaski, 2005). The structure and functioning of these forests are strongly shaped by biotic disturbance agents, most prominently grazing by semi-domestic reindeer (*Rangifer tarandus*) and large-scale defoliation events caused by cyclic outbreaks by geometrid moths (autumnal moth *Epirrita autumnata* and winter moth *Operophtera brumata*) (Calderón-Sanou et al., 2021; Kumpula et al., 2011). The linkages between aboveground herbivory and belowground soil processes (Bardgett and Wardle, 2003) are likely to result in altered soil microbial communities in mountain birch forests.

Reindeer influence the subarctic forest ecosystem in multiple ways e.g., via selective feeding, trampling, and urine and feces deposition which can lead to an increased abundance of graminoids in relation to evergreen and deciduous shrubs (Lempa et al., 2005). Intensive reindeer grazing on basal sprouts and seedlings, especially during summer, can reduce birch regeneration (Kumpula et al., 2011). It has been suggested that the realized tree line of subarctic mountain birch forests is determined by reindeer via grazing and trampling effects on sprouts and seedlings (Cairns and Moen, 2004). Reindeer grazing can increase nutrient availability in soils via urine and feces deposition (Barthelemy et al., 2015; Stark et al., 2002), by changing the amount and quality of litter (Olofsson et al., 2004) along with alterations in plant community structure (Mitchell et al., 2012; Wardle et al., 2004), and by increasing litter decomposition rates (Väisänen et al., 2014). In addition to reindeer grazing, moth outbreaks, typically occurring at approximately ten-year intervals (Jepsen et al., 2008; Neuvonen et al., 2001), can cause similar effects on vegetation and soil. Mountain birch can partly compensate for moth-induced foliage loss through intensive sprouting. However, when massive moth outbreaks coincide with intensive reindeer grazing during the growing season (Biuw et al., 2014), entire forest stands can suffer diebacks (Lehtonen and Heikkinen, 1995), especially when outbreaks

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occur in successive years (Kaukonen et al., 2013) or are accompanied by other simultaneous disturbances (Bjerke et al., 2014). Similar to reindeer grazing, severe moth outbreaks can significantly alter ground vegetation, favoring graminoids over evergreen and deciduous shrubs (Karlsen et al., 2013). Furthermore, high larval densities in tree canopies lead to the deposition of moth carcasses and feces, increasing soil nitrogen and carbon levels (Frost and Hunter, 2004; Kaukonen et al., 2013).

Fungi are the main decomposers in subarctic acidic soils, thanks to their diverse extracellular enzymes that efficiently break down recalcitrant plant material (Van der Wal et al., 2013). Mycorrhizal fungi are also vital for carbon, nitrogen, and phosphorus dynamics in soil (van der Heijden et al., 2015). In addition to fungi, bacteria are essential players in soil functions. They play a crucial role in nitrogen fixation and they assist in decomposing dead plant material and fungal mycelia and interact with plant roots and mycorrhizal fungi in the rhizosphere, either as mutualistic partners or by supporting mycorrhizal processes (Lladó et al., 2017). Reindeer grazing has been shown to affect soil microbes in various ways. Grazing has been demonstrated to lower the fungal:bacterial ratio in tundra heath (Männistö et al., 2016; Ylänne et al., 2020) and in mountain birch forest (Biuw et al., 2014). Grazing decreased ericoid mycorrhizal (ErM) fungi, while no effect was found on ectomycorrhizal (EcM) fungi in the boreal forest (Santalahti et al., 2018) or in tundra (Ahonen et al., 2021). In contrast, 16 years of ungulate exclosures had a positive effect on EcM biomass in shrub heaths and mountain birch forests (Vowles et al., 2018). In tundra heaths, grazing has been found to lead to a saprotroph-dominated fungal community due to grazing-induced vegetation change from shrubs to graminoids, resulting in increased labile litter quality and higher N availability (Ahonen et al., 2021). Conversely, in mountain birch forests, differences in long-term grazing history decreased free-living saprotrophs (molds and yeasts) in soil, which was related to the reduction of evergreen litter (Ylänne et al., 2021). Moth outbreaks have been found to lead to effects similar to reindeer grazing, including higher microbial biomass, increased microbial activity, and enhanced soil respiration (Meyer et al., 2022), a lower fungal:bacterial ratio (Kaukonen et al., 2013), and a decline in EcM fungi seen right after the outbreak event (Parker et al., 2017) as well as later in recovering stands (Calderón-Sanou et al., 2021). The shifts in fungal communities induced by both reindeer grazing and moth outbreaks have also been linked to reduced decomposition rates in mountain birch forests (Sandén et al., 2020; Ylänne et al., 2021). Ultimately the amount and quality of litter deposited on the ground directly affect heterotrophic soil microbiota, as well as subsequent decomposition, carbon sequestration, and nutrient mobilization and transformation processes in subarctic soils (Wardle et al., 2004; Ylänne et al., 2021). Grazing effects on soil bacterial communities have been studied mainly in the tundra (Männistö et al., 2016; Stark et al., 2015) and in grasslands (e.g., Fernández-Guisuraga et al., 2022; Xun et al., 2018) while, to our knowledge, no studies on bacterial communities have been conducted in the mountain birch forests.

Here, we studied the impacts of reindeer grazing on soil microbiota in subarctic mountain birch stands recovering after severe moth herbivory. We contrasted long-term (from decades to century) and short-term (years) grazing in the experimental setup. To investigate the effects of long-term grazing regimes we focused on a spatial contrast between year-round-grazing (hereafter YRG) and winter grazing (hereafter WG) in an area where this contrast has persisted for over a century due to specific national management restrictions placed on reindeer movement. To further explore the short-term effects of grazing we employed a control/exclosure design where ungulates were excluded from half of the plots over four consecutive years, encompassing both long-term grazing regimes. Earlier study from the same site reported that after the massive moth outbreak over 90% of birch tree trunks in the area were dead. However, the regeneration of mountain birch appears to be more successful in the WG regime (Biuw et al., 2014). Therefore, we assumed to find a higher relative abundance of birch associated EcM fungi in the WG regime. We further hypothesized that short-term exclusion of grazing would increase the abundance of EcM fungi in the YRG regime while no such increase would be seen under WG. As the environment of soil microbiome, soil physicochemical properties, i.e., organic matter, pH, and nutrient availability, are known to contrast in different podzol soil layers, we also describe and present results of the microbial communities in the organic and (upper) mineral soil layers in more detail.

2. Material and methods

2.1. Study site, design, and soil sampling

The study site is located in the northern-boreal/subarctic mountain birch forest near Buolbmát on the Finnish-Norwegian border area in northern Fennoscandia (70°1′N, 28°58′E; altitude 110–150 m a.s.l.). The area is characterized by relatively mild winters and cool summers with average temperatures around −12 °C in January and 12 °C in July, and annual precipitation of approximately 445 mm (Utsjoki Nuorgam observation station, https://en.ilmatieteenlaitos.fi). The vegetation in the area consists of discontinuous areas of short-statured, shrub-like polycormic growth forms of mountain birch and an understory layer dominated by dwarf shrubs (*Empetrum nigrum* ssp. *Hermaphroditum*, *Vaccinium myrtillus*, *V. vitis-idaea*). The area lies within two distinct reindeer districts, Kaldoaivi on the Finnish side, and Rákkonjárga on the Norwegian side. The national border has been closed to migrating reindeer since 1852 by a fence (Horstkotte et al., 2022). On the Finnish side of the border, the maximum allowable reindeer herd size is ~2.3 reindeer/km^2, and on the Norwegian side ~1.7 reindeer/km^2 (Biuw et al., 2014; https://www.reinbase.no). On the Finnish side, reindeer have year-round access to the site, thus the area is grazed more intensively (the YRG regime), especially during the spring and summer months. On the Norwegian side, the grazing mainly occurs during the winter or early spring months, when they don't feed on birch (the WG regime), thus the overall grazing intensity is lighter compared to the Finnish side. The entire study area was severely defoliated during outbreaks of geometrid moth in 2006–2008 (Jepsen et al., 2009) which caused high stem mortality on both sides of the border fence (Biuw et al., 2014). The same area was subjected to a severe moth outbreak in the mid-1960s (Lehtonen and Heikkinen, 1995). More detailed information on the study site can be found in Biuw et al. (2014).

In 2011, an experimental herbivore exclosure/control setup was established on both sides of the fenced border to investigate the impacts of mammalian herbivores on forest regeneration and succession under the two contrasting grazing regimes. The setup consists of six fenced 30 × 30 m ungulate exclosures on either side of the border, each matched by an open 30 × 30 m control plot randomly allocated in the immediate vicinity to either the southern or northern side of exclosure (altogether 24 plots).

On 2015 August 15th, composite soil samples were collected from all study plots using a soil corer of a diameter of 3 cm to a depth of ~15 cm. Organic and mineral layers were separated in the field and the samples were kept at ambient temperature during the sampling and then stored at −20 °C. Molecular and soil analyses were performed separately for organic and mineral soil layer fractions, and five organic/mineral soil cores from each plot were pooled together for each analysis (N = 24 plots).

2.2. Molecular analyses and bioinformatics

For the DNA analyses, we used freeze-dried soil samples from which larger pieces of roots and plant debris had been removed. Organic soil samples were further homogenized in mortar and pestle using 2.5 g of mixed organic soil sample with liquid nitrogen. DNA was extracted from 50 to 90 mg DW of organic soil and 300–600 mg DW of mineral soil using a PowerSoil DNA isolation kit (QIAGEN, Hilden, Germany)

following the manufacturer's instructions with an extra incubation of 30 min in 60 °C after adding solution C1. All templates were quantified with NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and adjusted to a final 5 ng μl^{-1} for PCR amplification. The Internal Transcribed Spacer 2 (ITS2) region was amplified for sequencing using fungal-specific primer set fITS7 (Ihrmark et al., 2012) and barcoded ITS4 (White et al., 1990), and 16S V4–V5 region using bacteria-targeted primer set barcoded U519F and U926R (Baker et al., 2003). Fungal and bacterial amplifications were done in triplicate 20-µl reactions using 0.2 mM of dNTPs, 0.2 µM of both primers, 0.02 U of Phusion High-Fidelity DNA polymerase with Phusion HF buffer (Thermo Fisher Scientific), and 10 ng of template DNA in fungal PCR and 5 ng of template in bacterial PCR. PCR cycling conditions for fungi were an initial denaturation for 1 min at 98 °C; followed by 27 cycles of 10 s at 98 °C, 20 s at 57 °C, 30 s at 72 °C"; and a final extension for 7 min at 72 °C. PCR cycling conditions for bacteria were an initial denaturation for 1 min at 98 °C; 26 cycles of 10 s at 98 °C, 30 s at 64 °C, 30 s at 72 °C; and a final extension for 7 min at 72 °C. The triplicate samples were pooled, and the sizes of PCR products were visualized using a MultiNA microchip electrophoresis system (Shimadzu Scientific Instruments, Columbia, MD, USA). Amplicons were magnetically cleaned with AMPure XP Kit (Beckman Coulter, Brea, CA, USA), quantified with PicoGreen (Invitrogen, Carlsbad, CA, USA) according to manufacturers' instructions and pooled with equimolar volume. The pooled amplicon library was sequenced using Ion Torrent PGM (Thermo Fisher Scientific) with Ion 316™ v2 chip and PGM Template OT2 400 kit, at the Biocenter Oulu Sequencing Center (Finland).

All sequences under 100 bp were removed from the raw data using Cutadapt (Martin, 2011), and QIIME2 (versions 2019.10–2021.2; Bolyen et al., 2019) was used for the following bioinformatic steps. Pre-trimmed sequences were demultiplexed followed by primer removal with q2-cutadapt plugin (Martin, 2011). Sequence data was denoised using a *denoise-pyro* command with DADA2 (Callahan et al., 2016). Taxonomy was assigned to fungal and bacterial amplicon sequence variants (ASVs) using pre-trained naïve Bayes q2-feature-classifiers (Bokulich et al., 2018). The fungal classifier was trained against the full-length UNITE database (version 8.2 QIIME release for Fungi 2; dynamic data (Abarenkov et al., 2020)) and the bacterial classifier was trained against trimmed SILVA database (version 138; SSU data (Quast et al., 2013) modified compatible for QIIME2 using a python script (https://github.com/mikerobeson/make_SILVA_db). Bacterial database trimming was done using a *feature-classifier extract-reads* command with the modified full-length database and primers from bacterial PCR. All non-fungal/bacterial reads, mitochondria, and chloroplast hits were removed from the data, and only ASVs with at least a phylum level match were kept for further analyses. Both ASV data sets were collapsed to species level and then calculated to relative abundances per sample. Fungal taxa were further classified into the following functional groups using FUNGuild (Nguyen et al., 2016): ericoid mycorrhizal (ErM, all fungi with ErM function), ectomycorrhizal (EcM, all fungi with EcM but no ErM function), arbuscular mycorrhizal (AM, all fungi with AM, but no EcM/ErM function), lichenized, endophytic (all other fungi with symbiotrophic function), saprotrophic (all fungi with saprotrophic but no symbiotrophic function) and pathotrophic (pathotrophic function only) fungi. The sequences with associated metadata for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB62634 (https://www.ebi.ac.uk/ena/browser/view/PRJEB62634).

2.3. Soil and vegetation analyses

We analyzed soil physicochemical properties, pH, electrical conductivity, and amount of soluble nutrients, Ca, K, Mg, as well as phosphorus (PO^{4-}) from thawed fresh soil samples, and total C and N from lyophilized samples (first air-dried in 40 °C and then pulverized with a ball mill). Total C and N were measured using an automatic elemental analyzer (EA 1110, CE Instruments, Wigan, UK). Electrical conductivity and pH were measured following van Reeuwijk (2002). Soluble nutrients, Ca, K, and Mg were analyzed with AAS (atomic absorption spectrophotometer; SpectrAA 220FS, Varian, Veriton, Australia) using an ammonium acetate extraction. PO^{4-} was analyzed colorimetrically (John, 1970) from the same ammonium acetate extracts using a UV–Vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan). Soil moisture (gravimetric water content) was determined by drying fresh samples at 105 °C overnight and total organic matter (OM) content with ignition loss after burning the dried samples at 550 °C for 5 h.

The Point Intercept technique was employed to analyze the vegetation community by counting the number of hits for each plant species across ten vegetation plots within each site. We used a 40 × 40 cm aluminum frame with nine pins, which were lowered into the vegetation. Additionally, we counted the number of birch stems taller than 1.3 m, noting the number of live trees and those with basal shoots.

2.4. Statistical analyses

We calculated diversity indices (richness, Pielou's evenness, and Shannon, Simpson's, and inverse Simpson's diversities) for sequence read data rarified to minimum sample size using the vegan package (Oksanen et al., 2022) and tested with a linear model (aov function; Chambers et al., 2017) how these measures were affected by the long-term grazing regimes (YRG/WG) and their interaction with short-term reindeer exclusion (exclosure/control) and soil layer (organic/mineral). A linear model was further used for testing differences in soil physicochemical properties. Suitable transformations were employed to achieve a normal distribution of variables. We fitted multivariate generalized linear models (mGLM; mvabund-package; Wang et al., 2012) to test the effects of the grazing regime, exclosures, and soil layer as well as their interactions on the relative abundance of fungal and bacterial taxonomic groups and fungal ecological functions. Only subgroups with relative abundance over 0.05% were included. Models were fitted with a negative binomial distribution using 999 bootstrap iterations and reported with log-likelihood ratio statistics. Multivariate tests were followed by univariate tests to find out which microbial groups were linked to the differences in the communities, reported as adjusted P-values (Westfall and Young, 1993). We also performed a non-metric multidimensional scaling (NMDS) and permutational multivariate ANOVA (metaMDS and adonis functions in vegan) using Bray-Curtis distance on species composition data standardized by Hellinger transformation (square root of relative abundance data) and 999 permutations. This was followed by a multivariate homogeneity of group dispersions test (betadisper function in vegan). Since the soil layer showed significant interactions with grazing regime and exclosure in mGLM and linear model analyses (Table 1; Supplementary Table S1), all tests were run also separately for the different soil layers to explore the possible divergent effects of grazing regime and exclosures in organic and mineral layers. All statistical analyses were carried out with R software for statistical computing, versions 4.1.3–4.2.0 (R Core Team, 2020).

3. Results

3.1. Microbial community

The molecular analysis showed that Ascomycota (mean relative abundance 75.2% of all reads, with orders Chaetothyriales 37.8%, Helotiales 21.5%) was the most dominant fungal phylum in our data, followed by Basidiomycota (22.4%, with order Agaricales 13.6%) and Mortierellomycota (1.8%) (Fig. 1a). Saprotrophic fungi (44.8%) were the most abundant fungal ecological group, followed by ErM (13.3%), EcM (3.9%), lichenized (2.5%) and pathotrophic (0.6%) fungi (Fig. 1b). The mean relative abundance of AM and endophytic fungi was very low (<0.1%). The ecological function of 34.8% of fungal taxa could not be

Table 1
Deviance values from the multivariate generalized linear model (mGLM) indicating the own and interacting effects of grazing regime (R), treatment (E) and soil layer (S) on the taxonomic composition of fungi and bacteria, as well as the functional composition of the fungal community.

	Organic & Mineral							Organic			Mineral		
	$R_{46,1}$	$E_{45,1}$	$S_{44,1}$	$R \times E_{43,1}$	$R \times S_{42,1}$	$E \times S_{41,1}$	$R \times E \times S_{40,1}$	$R_{22,1}$	$E_{21,1}$	$R \times E_{20,1}$	$R_{22,1}$	$E_{21,1}$	$R \times E_{20,1}$
FUNGI													
Phylum	5.125	8.857	**40.839**	3.333	1.801	3.348	0.915	5.155	**21.420**	**4.256**	4.354	10.585	0.761
Class	20.304	21.039	**153.950**	22.168	**36.768**	12.050	27.347	16.839	13.182	27.914	**37.457**	25.185	18.496
Order	**66.891**	27.716	**349.666**	**66.699**	**63.541**	32.602	48.384	52.894	34.917	54.848	**54.700**	28.549	38.229
Family	**92.427**	44.224	**502.087**	**103.991**	71.160	62.147	54.159	84.855	51.642	83.312	58.971	31.485	45.981
Genus	**115.048**	56.008	**645.768**	**109.892**	80.883	74.345	66.607	**110.181**	69.116	81.458	65.866	42.243	64.742
Function	**19.684**	9.254	**109.229**	8.505	3.088	6.991	7.103	14.012	6.287	3.490	9.046	12.894	9.779
Func-Phy[a]	28.758	13.317	**175.621**	15.904	6.726	15.585	12.195	24.629	10.472	7.965	13.289	19.688	13.137
BACTERIA													
Phylum	14.156	7.500	**262.199**	**28.526**	**67.613**	**47.955**	5.081	5.662	**49.091**	14.019	**142.385**	16.736	16.104
Class	20.056	10.694	**366.362**	**34.629**	**80.356**	**54.198**	**34.236**	11.763	**61.673**	**28.771**	**147.340**	20.360	25.549
Order	31.391	16.290	**664.113**	56.979	37.680	**54.788**	**82.135**	40.456	35.520	45.969	77.457	39.677	86.697
Family	45.458	21.681	**876.888**	90.132	62.269	87.969	79.587	39.990	30.596	39.551	90.975	38.818	109.929
Genus	47.811	26.049	**1021.185**	109.894	81.533	107.944	81.205	57.581	36.338	47.651	103.447	55.125	112.042

Columns to the left show the combined effects on organic and mineral data, while columns to the right show the effects separately under organic and mineral soil layers.
Subscript numbers represent residuals and difference in degrees of freedom (Res.Df and Df.diff, respectively). Defiance values with $P < 0.05$ are indicated in bold.
[a] Fungal phyla within the functional groups.

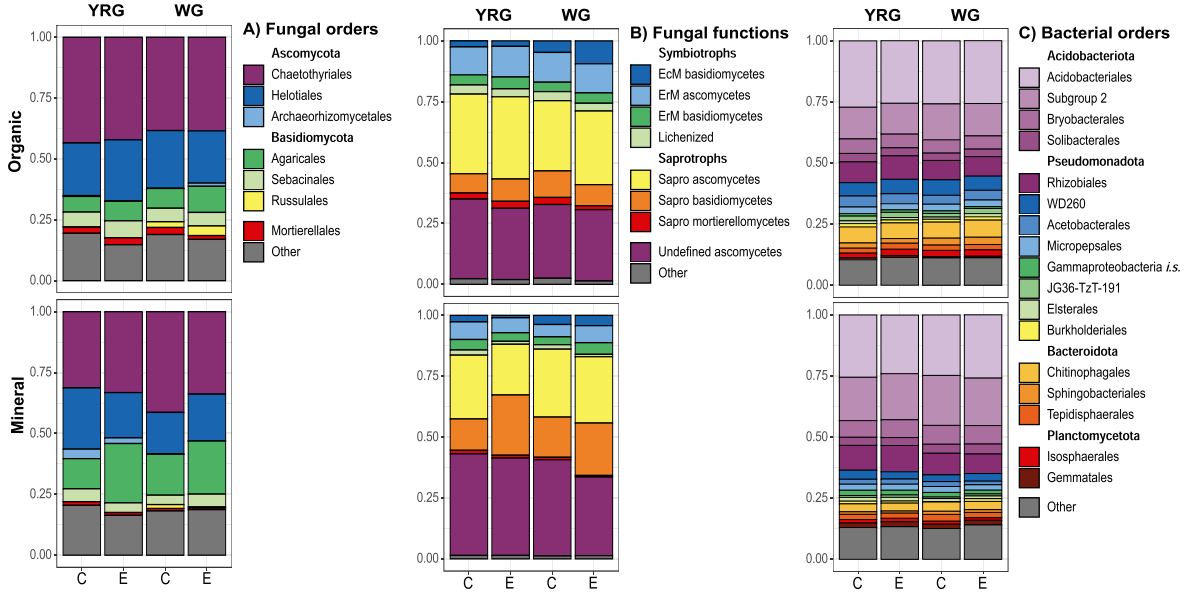

Fig. 1. Mean relative abundances of A) fungal orders, B) fungal ecological functions, and C) bacterial orders in different treatments (control, C and exclosures, E) under year-round grazing (YRG) and winter grazing (WG) regimes in organic and mineral soil layers. Other = undefined and <1% mean relative abundance orders/fungal functions, EcM = ectomycorrhizal, ErM = ericoid mycorrhizal, Sapro = saprotrophic.

identified. Acidobacteriota (mean relative abundance 52.4% of all reads, with orders Acidobacteriales 25.6%, and Subgroup 2 16.2%) was the most dominant bacterial phylum (Fig. 1c). Other abundant bacterial phyla were Pseudomonadota (27.1%), Bacteroidota (7.3%), Planctomycetota (6.1%), Actinomycetota (1.7%), and Chloroflexota (1.1%).

Fungal community structure differed between grazing regimes (Adonis: $P < 0.05$; Table S2) at genus, family, and order levels and by their ecological functions (Table 1). EcM basidiomycetes and fungal family Russulaceae (Russulales) were more abundant in the WG regime than in the YRG regime, especially in the organic layer (Table 2). Bacterial community structure showed no significant difference between grazing regimes (Table 1; Table S2). However, the bacterial family Xanthobacteraceae (Rhizobiales) was more common in the YRG regime ($P < 0.05$; Table S3). Fungal and bacterial alpha diversity (Fig. S1; Table S4) and heterogeneity of group dispersion did not differ between grazing regimes (Table S2). Furthermore, the grazing regime had divergent impacts on microbial communities in the organic and mineral soil layers (Adonis: $P < 0.05$; Table 1; Table S2; Fig. S2). Fungal class Archaeorhizomycetes, represented by a single *Archaeorhizomyces*, was more abundant in the mineral layer of the YRG regime and bacterial phylum Acidobacteriota (and its class Acidobacteriae) was more abundant in the mineral layer of the WG regime (Table 2; Table S3).

Short-term exclosures showed a statistically significant effect on fungal and bacterial community structure only in the organic layer (Table 1). Fungal communities in the organic layer differed inside and outside of exclosures at the phylum level and bacterial communities at phylum and class levels (Table 1). Ascomycota and Acidobacteriae were more abundant in the organic layer of the grazed plots ($P < 0.05$; Table 2; Table S3). Furthermore, within the organic layer, Archaeorhizomycetes were more abundant inside the exclosures in the WG regime, while in the YRG regime, they were more abundant in the plots outside of the exclosures (Table 2). This was also seen at the fungal

Table 2
Relative abundances (mean % ± SE) of the most abundant bacterial and fungal taxa (with mean relative abundance <0.1%) at phylum, order, and genus level, along with fungal ecological functions in organic and mineral soil layers. The responses of these groups to grazing regime (R), treatment (E), soil layer (S) and their interactions as indicated by deviance values from multivariate generalized linear model (mGLM) univariate tests.

	Organic	Mineral	$R_{46,1}$	$E_{45,1}$	$S_{44,1}$	$R \times E_{43,1}$	$R \times S_{42,1}$	$E \times S_{41,1}$	$R \times E \times S_{40,1}$
FUNGAL TAXA									
Ascomycota[a]	78.3 ± 1.7	72.2 ± 2.1	1.366	2.699	5.653	0.085	0.285	1.743	0.025
Archaeorhizomycetales[b]	0.5 ± 0.2	1.6 ± 0.4	7.972	1.082	0.915	8.761	**13.641**	1.700	5.420
Archaeorhizomyces[b]									
Chaetothyriales	40.6 ± 1.7	34.9 ± 1.8	0.064	0.347	5.703	0.789	4.066	0.299	1.685
Helotiales	23 ± 0.9	20 ± 1.3	1.725	0.129	4.233	0.475	1.160	0.774	5.825
Meliniomyces	4.1 ± 0.3	1.2 ± 0.1	0.744	0.000	**52.710**	3.327	1.524	0.005	7.456
Pezoloma	6.7 ± 0.5	4.6 ± 0.5	0.039	0.581	8.689	0.432	0.747	0.036	0.941
Mycosymbioces	1.1 ± 0.2	2 ± 0.4	0.005	1.284	6.171	0.029	0.234	0.742	0.186
Basidiomycota	18.8 ± 1.6	26 ± 2.2	1.911	4.011	8.943	0.052	0.708	1.004	0.120
Agaricales	8.3 ± 1.1	18.8 ± 2.3	0.259	3.690	**19.134**	0.310	0.246	0.412	0.516
Clavaria	4.5 ± 0.8	16.5 ± 2.2	0.254	2.975	**29.708**	0.243	0.460	0.447	0.016
Russulales	1.6 ± 0.6	0.5 ± 0.3	**14.846**	1.402	2.480	3.271	0.009	1.883	0.453
Sebacinales	6.1 ± 0.7	4.6 ± 0.5	0.403	0.068	2.660	0.415	0.262	0.008	1.416
Serendipita	4.1 ± 0.5	3.9 ± 0.4	0.126	0.159	0.118	0.651	0.340	0.010	1.715
Mortierellomycota	2.4 ± 0.3	1.1 ± 0.2	1.404	2.215	**22.069**	2.045	0.755	0.068	0.581
Mortierellales									
Mortierella									
FUNCTIONAL GROUPS									
Ectomycorrhizal	4.8 ± 1.1	3 ± 0.7	**15.171**	0.000	3.451	4.826	0.136	2.737	0.223
EcM basidiomycetes[c]	4.7 ± 1	2.9 ± 0.7	**16.338**	0.035	3.799	5.385	0.178	3.209	0.124
Ericoid mycorrhizal	16.4 ± 0.8	10.3 ± 0.7	0.123	0.435	**26.023**	1.830	0.079	0.031	4.365
ErM ascomycetes	12.1 ± 0.7	6.4 ± 0.5	0.005	0.251	**32.240**	1.020	0.434	0.038	2.350
ErM basidiomycetes	4.3 ± 0.5	4 ± 0.5	0.106	0.497	0.189	0.610	0.186	0.002	1.246
Lichenized	3.5 ± 0.3	1.5 ± 0.3	0.048	1.505	**19.669**	0.000	0.233	1.620	0.007
Lichenized ascomycetes									
Saprotroph	43.4 ± 1.3	46.2 ± 2.4	0.038	0.876	0.981	0.515	1.484	0.880	0.056
Sapro ascomycetes	31.4 ± 1.4	25.5 ± 1.8	0.051	0.054	7.472	0.542	3.152	1.545	0.301
Sapro basidiomycetes	9.2 ± 1	18.9 ± 2.2	0.026	2.126	**18.333**	1.915	0.029	3.100	0.002
Sapro mortierellomycetes	2.4 ± 0.3	1.1 ± 0.2	1.669	1.849	**22.235**	2.262	0.598	0.132	0.741
Pathotroph	1 ± 0.1	0.2 ± 0	0.305	2.047	**29.608**	0.919	0.189	0.051	0.027
Undefined function	30.9 ± 1	38.6 ± 2.2	1.765	1.958	**11.110**	0.079	0.502	0.146	0.901
Undefined ascomycetes	30.4 ± 1	38.4 ± 2.2	2.058	2.450	**12.841**	0.240	0.340	0.047	0.575
BACTERIAL TAXA									
Acidobacteriota[d]	48.5 ± 0.9	56.3 ± 0.8	1.059	0.182	0.000	7.608	51.016	36.115	0.057
Acidobacteriales	26.1 ± 0.5	25 ± 0.7	0.002	0.436	1.789	1.844	0.000	4.033	5.857
Acidipila	3.2 ± 0.2	1.6 ± 0.2	0.023	0.074	**18.753**	1.146	0.038	1.350	2.712
Granulicella	5.1 ± 0.3	1.4 ± 0.1	0.012	0.172	**73.935**	0.339	0.037	0.127	0.000
Occallatibacter	2.4 ± 0.2	1.1 ± 0.1	0.694	0.406	**11.372**	0.831	3.369	0.586	0.916
Acidobacteria SG2	13.4 ± 0.6	19.1 ± 0.5	1.524	0.114	36.389	0.975	0.001	0.525	0.039
Bryobacterales	5.6 ± 0.1	7.3 ± 0.1	0.158	0.003	**44.885**	0.116	0.000	6.303	9.179
Bryobacter									
Solibacterales	3.2 ± 0.1	3.6 ± 0.1	0.931	0.090	7.785	1.968	0.000	2.965	0.471
Candidatus Solibacter									
Actinomycetota	2.4 ± 0.2	0.9 ± 0.1	0.764	0.220	28.744	7.525	0.034	1.291	0.352
Bacteroidota	9.6 ± 0.4	5.1 ± 0.2	1.780	0.016	67.994	0.493	0.025	0.741	0.318
Chitinophagales	6.6 ± 0.3	3.4 ± 0.1	0.376	0.002	**75.273**	0.077	0.139	0.617	0.897
Puia	4.2 ± 0.2	2.3 ± 0.1	0.756	0.071	**45.986**	0.774	0.555	0.109	0.325
Sphingobacteriales	2.4 ± 0.2	1.1 ± 0.1	4.503	0.198	46.138	0.286	0.582	0.486	0.087
Mucilaginibacter	2.1 ± 0.2	0.7 ± 0	2.242	0.493	**62.331**	0.026	0.133	0.390	0.297
Chloroflexota	0.4 ± 0	1.9 ± 0.2	0.444	0.243	**66.706**	0.085	0.298	0.107	0.094
Planctomycetota	5.8 ± 0.3	6.3 ± 0.3	0.001	0.192	1.350	0.617	0.003	1.015	0.000
Gemmatales	0.6 ± 0.1	1.9 ± 0.1	0.390	0.161	**47.581**	0.409	0.622	0.045	0.787
Isosphaerales	2.4 ± 0.2	1.3 ± 0.2	0.001	0.048	3.904	0.094	0.308	0.157	0.009
Aquisphaera	1.5 ± 0.2	0.9 ± 0.2	0.160	0.020	0.668	0.003	0.051	0.462	0.053
Tepidisphaerales	2.2 ± 0.1	2.3 ± 0.1	0.425	0.009	0.266	1.392	1.291	2.615	0.038
Pseudomonadota	29.3 ± 0.6	25 ± 0.7	4.274	0.137	19.632	0.020	2.318	0.209	0.211
Acetobacterales	4 ± 0.2	1.9 ± 0.1	0.639	0.078	**26.172**	0.108	0.049	0.287	1.231
Burkholderiales	1.3 ± 0.2	0.8 ± 0.2	0.212	0.340	1.986	2.200	1.063	1.344	1.832
Caulobacterales	1.2 ± 0.1	0.8 ± 0.1	0.119	0.093	3.625	1.295	0.626	1.044	1.200
Elsterales	1.1 ± 0.1	1.3 ± 0.1	1.104	0.029	2.031	0.781	1.876	0.492	1.415
Gammaproteobacteria *i.s.*	1 ± 0.1	1.9 ± 0.1	0.346	0.375	7.193	0.229	0.009	0.009	0.063
Acidibacter	0.9 ± 0.1	1.8 ± 0.1	0.462	0.294	6.158	0.261	0.004	0.041	0.137
JG36-TzT-191	1.9 ± 0.1	0.8 ± 0.1	0.242	0.524	8.514	0.082	0.004	0.000	0.045
Micropepsales	2.7 ± 0.1	2.4 ± 0.1	0.027	0.421	3.866	0.022	1.193	0.028	0.575
Rhizobiales	8.5 ± 0.3	9.4 ± 0.4	8.658	0.129	3.206	1.497	0.630	0.643	0.047
Roseiarcus	2.1 ± 0.1	1.8 ± 0.1	0.818	0.125	1.388	2.649	0.428	0.385	0.406
Bradyrhizobium	1.7 ± 0.2	1.4 ± 0.3	0.184	0.259	0.234	1.344	1.012	0.631	0.727
WD260	5.9 ± 0.3	3.1 ± 0.2	0.002	0.064	45.667	0.374	1.823	0.062	3.419

Abbreviations: EcM = ectomycorrhizal, ErM = ericoid mycorrhizal, sapro = saprophytic, SG2 = subgroup 2, *i.s.* = *insertae sedis*. WG = winter grazing, YRG = year-round grazing. Significant univariate test results for organic and mineral layer analyses separately.

Bacterial phylum-level nomenclature follows Oren and Garrity (2021). Subscript numbers represent Residuals and Difference in Degrees of Freedom (Res.Df and Df. diff, respectively). Defiance values with $P < 0.05$ are indicated in bold.

[a] Ascomycota in organic soil more abundant in control plots ($E_{21,1}$: dev = 18.626).

[b] Archaeorhizomycetales/*Archaeorhizomyces* in organic soil more abundant in WG exclosures ($R \times E_{20,1}$: dev = 13.054), and in mineral soil more abundant in YRG ($R_{22,1}$: dev = 14.858).

[c] EcM basidiomycetes in organic soil more abundant in WG ($R_{22,1}$: dev = 11.063).

[d] Acidobacteriota in mineral soil more abundant in WG ($R_{22,1}$: dev = 118.184).

phylum level indicated by a significant interaction between the grazing regime and treatment (Table 1). Bacterial Pielou's evenness was higher inside the exclosures, especially in the organic layer ($P < 0.05$; Table S4; Fig. S1). Fungal Pielou's evenness in the mineral layer was significantly higher in the grazed plots of the YGR regime compared to the exclosures of the YRG regime, while in the WG regime, the effect was opposite (region × exclosure interaction: $P < 0.05$; Table S4; Fig. S1).

Fungal and bacterial communities in the organic and mineral soil layers showed significant distinctions between them (Adonis: $P < 0.001$; Table S2; Fig. 2a and b) across all taxonomical and functional levels (Table 1). Fungal genera *Meliniomyces* (Helotiales) and *Mortierella* (Mortierellales), family Leotiaceae (Helotiales), class Lecanoromycetes, and fungal ecological groups ErM, lichenized, saprotrophic and pathotrophic ascomycetes were relatively more abundant in the organic layer, while class Agaricomycetes, especially saprotrophic basidiomycetes (including genus *Clavaria*), and ascomycetes with unknown ecological function were relatively more abundant in the mineral soil layer (Table 2). Bacterial genera *Acidipila*, *Granulicella*, *Occallatibacter* (all Acidobacteriales), *Puia* (Chitinophagales) and *Mucilaginibacter* (Sphingobacteriales), and family Acetobacteraceae (Acetobacterales) were relatively more abundant in the organic layer, and genus *Bryobacter* (Bryobacterales) and family Gemmataceae (Gemmatales) were relatively more abundant in the mineral soil layer (Table 2). Fungal species richness ($P < 0.001$) and fungal Shannon diversity ($P < 0.001$) were higher in the organic layer along with bacterial Pielou's evenness ($P < 0.01$) and bacterial inverse Simpson ($P < 0.05$) (Fig. S1; Table S4). The heterogeneity of group dispersions in fungal and bacterial communities did not differ between soil layers (Table S2).

3.2. Vegetation and soil physicochemical properties

The vegetation composition did not show differences between grazing regimes or inside and outside short-term exclosures in 2015 (Fig. S3), but there was a higher proportion of live mountain birch trees and higher numbers of birch basal shoots in the WG regime (Fig. S4).

Soil physiochemical properties were strongly affected by the soil layer. pH was lower in the organic layer ($P < 0.001$) while all other measured soil variables were higher in the organic than in the mineral layer (moisture, conductivity, PO4-, Ca, K, Mg, N, C, and OM; $P < 0.001$). The grazing regime and exclosures had little effect on soil properties. However, N content was significantly higher in YRG ($P < 0.001$), especially in the organic layer ($P < 0.001$). Furthermore, in the organic layer, OM and PO4- contents were highest in YRG exclosures ($P < 0.05$), while WG exclosures had the lowest organic layer PO4- content ($P < 0.05$). In mineral soil, Mg was higher inside exclosures ($P < 0.05$) and pH highest inside WG exclosures ($P < 0.05$). The thickness of the organic layer varied between 1 and 5.5 cm and did not differ significantly between the study plots. In addition, pH, Ca, and Mg showed no significant differences between the grazing regimes. More detailed results of soil properties are found in Fig. S5 and Table S1.

4. Discussion

Our results suggest that in mountain birch forests subject to recent and severe moth herbivory, the reindeer grazing regime affected the EcM fungal community. Lower grazing pressure in the form of winter grazing seemed to allow both mountain birch and the associated soil EcM community to recover better from the moth outbreak compared to the year-round grazing. Saprotrophic and ErM fungi were less impacted by grazing. Additionally, we observed that reindeer grazing had a greater effect on the soil fungal community compared to the bacterial community, with divergent impacts on microbial groups between the organic and mineral soil layers.

4.1. Fungal community responses to grazing

Differences in long-term grazing pressure and short-term exclusion had less impact on soil fungal communities than we expected. Nevertheless, our hypothesis that the relative abundance of EcM fungi in soil would mirror that of their mountain birch hosts held true. Birch trees

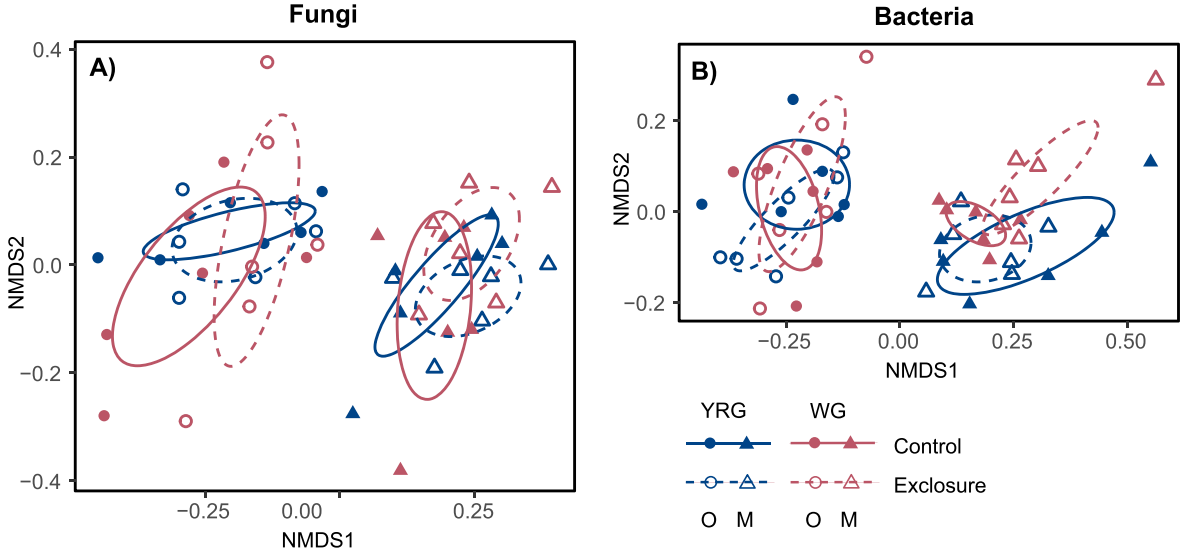

Fig. 2. A non-metric multidimensional scaling (NMDS) of A) fungal and B) bacterial communities in different treatments (control and exclosure), under year-round grazing (YRG) and winter grazing (WG) in organic (O) and mineral (M) soil layers. The ellipses show a 95% confidence interval.

subject only to winter grazing with moderate grazing pressure recovered more rapidly from the moth outbreak which was reflected in the higher abundance of EcM fungi, primarily Russulales, in the WG regime. However, the overall relative abundance of EcM in soil was lower than expected in both grazing regimes. The abundance of EcM mycelia in the soil is primarily influenced by EcM abundance in the roots. Severe and repeated moth herbivory has been shown to reduce EcM fungal abundance in the mountain birch roots (Saravesi et al., 2015), which could suggest that the EcM community at our site may have been significantly affected by the earlier moth outbreak. Observed high abundance of soil saprotrophic fungi, showing no clear difference between grazing regimes in our study, might also be a result of this. However, prior studies have found a similar low relative abundance of EcM fungi and a high relative abundance of ErM fungi from mountain birch forest soils with shrub-dominated understory (Clemmensen et al., 2021; Ylänne et al., 2021). In both grazing regimes at our site, *E. hermaphroditum* dominated the understory. Live and dead hair roots of *Empetrum* and other dwarf shrubs are intensively colonized by ErM fungi (Read, 1996). The low relative abundance of EcM fungi may be due to competition with ErM fungi (Kohout et al., 2011) and the inhibitory effects of allelopathic compounds produced by *E. hermaphroditum* (Nilsson et al., 1993). The abundance of EcM mycelia in soil depends also on the EcM community composition, specifically whether the fungal symbionts present in the roots have abundant external mycelia for nutrient uptake or not. Fungi with extensive mycelia, such as *Cortinarius,* are typically abundant in intact mountain birch forests, while fungi with limited mycelial network are usually dominated in heath and shrub vegetation (Clemmensen et al., 2021; Parker et al., 2015). From these so-called EcM exploration types (*sensu* Agerer, 2001), short-distance EcM fungi are often found in soils with relatively high inorganic N levels (Lilleskov et al., 2002; Sterkenburg et al., 2015) and they are suggested to be more resilient in response to disturbance (Tedersoo and Smith, 2013), while host benefits related to nutrient uptake of the long-distance EcM are lost in disturbed systems (Lilleskov et al., 2011). The most abundant EcM family at our site was Russulaceae, typically lacking mycelia extending over long distances. Interestingly, Ylänne et al. (2021) observed the same dominant family in another mountain birch forest area experiencing similar winter and year-round grazing conditions. They proposed that even the minor disturbances associated with winter grazing appear to favor short-distance EcM types, which do not rely on the extensive nutrient transport mechanism used by long-distance EcM fungi.

Our results align with the perspective that soil communities are relatively stable in mountain birch forests (Stark et al., 2008). Apart from the mountain birch trees, the composition of the field vegetation did not differ inside and outside of the short-term exclosures, nor did it vary between the grazing regimes. This lack of differentiation was somewhat surprising, considering the long-standing differences in grazing regimes. Notably, this consistency extended to the fungal community. In addition to reindeer grazing and moth outbreaks, cyclic outbreaks of rodents can cause major disturbance to understory vegetation and soil in the mountain birch forests (Olofsson et al., 2012). At the start of the experiment in 2011, the understory vegetation at our study site was in a strongly disturbed state due to an ongoing lemming and vole outbreak (Ehrich et al., 2020). Both the impact of the moth outbreak itself and the massive rodent outbreak could have contributed to eliminating any more subtle effects of the grazing regimes. Intense fertilization, extensive rodent grazing on understory vascular plants and bryophytes, and mechanical disturbance of the soil organic layer may have masked minor changes in the soil microbial community. It is worth highlighting, however, that the rodent outbreak was not able to mask the differences in soil nutrients between the grazing regimes. It is possible that changes in this environment, particularly those induced by the exclosures, occur at a rate too slow to be detected within the four years of our experiment, consistent with findings from previous studies from tundra (Ahonen et al., 2021) and mountain birch forest (Parker et al., 2022). Time lag could be considerably longer before any responses to increased nutrient supply may occur in microbial biomass and community composition of subarctic soils (Rinnan et al., 2007; Stark et al., 2008). The high relative abundance of saprotrophs and ErM fungi could indicate a long legacy of grazing, cyclic moth, and rodent outbreaks in the study area, which have shaped the soil community holistically possibly making it less susceptible to this kind of disturbance. Particularly, the dominance of ErM understory may play a role here, as it has been suggested that the resilience of ErM fungi could be linked to their ability to function as free-living saprotrophs (Ward et al., 2022).

4.2. Bacterial community responses to grazing

While fungal communities may be generally more affected by vegetation due to the dependency of mycorrhizal fungi on their plant hosts, soil bacteria are more affected by soil properties, such as pH, organic matter, nutrient content, and soil moisture (Lladó et al., 2017). Surprisingly, the bacterial communities showed only minimal response to grazing regimes, despite notable differences such as higher total N and P levels and a lower C:N ratio in the soil organic layer of the YRG as compared to the WG regime. Increased nutrient availability in soil follows the intensity of grazing (Stark et al., 2008) and, for example, nitrogen fertilization has been shown to affect nitrogen-fixing bacteria (Berthrong et al., 2014). However, contrary to conventional expectations, we found no evidence that the abundance of nitrogen-fixing bacteria decreased with increasing herbivory at our sites. The reason for not finding clear differences between the grazing regimes in nitrogen-fixing bacteria and bacterial communities, in general, might be partly due to the enormous functional diversity within each bacterial taxon (Cohan and Perry, 2007). For example, Xanthobacteraceae has been formerly associated with nitrogen-fixation (Oren, 2014), but the most abundant genus from that family in our study, *Bradyrhizobium*, is ecologically diverse and widely distributed in soil (VanInsberghe et al., 2015). Furthermore, it has been shown that soil microbial activity may not always increase with higher soil nutrient availability; instead, it might be influenced by substrate availability (Stark and Väisänen, 2014). Short growing seasons and overall low temperatures results in slow decomposition of low-quality shoot and root litter produced by evergreen dwarf shrubs, ultimately leading to the accumulation of highly resistant and inert organic matter (Stark et al., 2023). Consequently, it influences how bacterial communities respond to grazing in acidic soil already dominated by fungi.

4.3. Microbiome in the organic and mineral soil layers

In the organic layer, the fungal community had a higher relative abundance of ericoid mycorrhizal ascomycetes, pathotrophs, and Mortierellomycota. In contrast, the mineral layer had a higher relative abundance of saprotrophic basidiomycetes, predominantly belonging to *Clavaria*. Previous results on the vertical distribution of fungi indicate that free-living saprophytes, like as Mortierellomycota, along with pathotrophs are typically found in the litter layer of organic soil (Clemmensen et al., 2021). The organic soil layer in our study area is rich in hair roots of *Empetrum* which explains the higher relative abundance of ericoid mycorrhizal fungi in this layer. Interestingly, ErM basidiomycete Serendipitaceae (including *Serendipita*) was equally abundant in both soil layers. ErM fungi are important for organic N uptake for ericoid plants (Walker et al., 2010), and they have also been proposed to contribute to belowground organic matter accumulation through the production of recalcitrant necromass, progressively locking up nutrients (Clemmensen et al., 2013, 2015; Fanin et al., 2022; Fernandez and Kennedy, 2018; Ward et al., 2021). Although *Clavaria* was classified as saprotrophic in this study, *C. argillacea*, a common species in boreal and subarctic sandy soils, is suggested to potentially form mycorrhizal associations with *Empetrum* (Birkebak et al., 2013). Notably, grazing had varying effects on a cryptic root-associated fungus, Archaeorhizomycetes, in the organic and mineral soil layers. In the

mineral soil layer, it was most abundant in the YGR, whereas in the organic layer, it was more abundant in the WG exclosures. Their ecological function is unclear as they are neither ectomycorrhizal nor pathotrophic (Rosling et al., 2011). However, they are commonly found in mountain birch forest soils (Ylänne et al., 2021), and in deeper mineral soil layers, possibly participating in mineral weathering (Pinto-Figueroa et al., 2019). Some species of this group have also been associated with a high turnover of nutrients (Urbina et al., 2018), which could explain their higher relative abundance in the N-richer YRG regime.

The bacterial communities in the organic and mineral soil layers in our study site followed the common pattern observed in the forest soils overall: Acidobacteriota, Pseudomonadota, and Bacteroidetes are typically associated with organic layers, while Chloroflexota and Planctomycetota are commonly found in mineral layers (Lladó et al., 2017). Actinomycetota, while globally abundant in soils (Delgado-Baquerizo et al., 2018; Lladó et al., 2017), constituted only one to two percent of our bacterial data. This discrepancy may be due to the DNA extraction method, which has been known to impact the relative abundance of Actinomycetota (Pérez-Brocal et al., 2020; Zielińska et al., 2017), possibly due to insufficient cell lysis for their thick cell walls. Many bacterial genera found from the organic layer in our study, for example, *Granulicella*, *Acidipila*, and *Occallatibacter* from Acidobacteriaceae subgroup 1, have been associated with low pH and organic matter decomposition in previous studies. In contrast, *Bryobacter*, another Acidobacteriota genus, was more abundant in the mineral layer, possibly due to its preference for mildly acidic conditions (Dedysh et al., 2017). Other typical bacterial taxa associated with an organic layer in our study were Chitinophagales (including the genus *Puia*) and Sphingobacteriaceae (including the genus *Mucilaginibacter*). *Mucilaginibacter*, known for its chitinolytic abilities (Kim et al., 2022), likely benefits from the fungal mycelia-rich environment of the organic layer. However, *Puia*, despite belonging to Chitinophagales, does not possess chitin-degrading capabilities (Lv et al., 2017). While it has been linked to the organic soil layer in several other studies (Lv et al., 2017; Prada-Salcedo et al., 2022; Singavarapu et al., 2022) its function in the organic layer remains uncertain.

Fungal diversity and richness were significantly higher in the organic soil layer, while bacterial species richness did not decrease in the mineral layer but was slightly higher. This is consistent with previous findings that bacterial richness declines more gradually than fungal richness in the upper horizons of mineral soil (Mundra et al., 2021). However, there appears to be a knowledge gap regarding fungal and bacterial taxa in the mineral soil layer. We found a higher relative abundance of ascomycetes with undefined ecological function in the mineral layer, which could be the result of extensive fungal research primarily focusing on root-associated (basidiomycetous) fungi, leaving the fungal reference libraries lacking information about other common ascomycetes, especially the ones inhabiting lower soil horizons. Furthermore, limited information exists on Gemmatales and Chloroflexota and their ecological role in the mineral layer, despite their presence there reported also in other studies from birch forests (Mundra et al., 2021) and tundra environments (Ricketts et al., 2020; Viitamäki et al., 2022). They may be associated with mineral weathering, and Gemmatales has even been proposed as a possible phosphorus miner (Mason et al., 2021). Diverse bacterial taxonomic groups play various understudied roles in close association with fungi in soil. Exploring mineral soil layer biodiversity is a potentially crucial yet often overlooked component of soil research.

4.4. Conclusions

In a subarctic mountain birch forest recovering from extensive moth herbivory, our study revealed differences in soil microbial communities under long-term grazing regimes, while short-term ungulate exclusions had limited effects. A lower relative abundance of EcM basidiomycetes in the YRG regime compared to WG suggests that long-term ungulate grazing can lead to cascading impacts on mycorrhizal fungal communities. These varying grazing impacts may be indirect and largely due to the lower availability of the host trees for the EcM fungi in the YRG regime. Soil fungal saprophyte and bacterial communities showed little variation between grazing regimes, possibly indicating slow and inert nutrient and carbon cycling processes in acidic, dwarf shrub-dominated mountain birch forest soil as suggested by Stark et al. (2008). Our finding supports the perspective that in Fennoscandian subarctic mountain birch forests, soil microbial communities may have adapted to withstand elevated disturbance levels caused by aboveground herbivory and challenging climate conditions (Ylänne et al., 2021). This is seen as resilient dwarf shrub-associated ErM community and EcM community modified by dominating long-term grazing regime. Grazing impacts on soil microbial communities also vary between the organic and mineral soil layers, highlighting the need for a more comprehensive understanding of mineral soil-dwelling microbes in subarctic mountain birch ecosystems. This underscores the importance of investigating microbial communities at deeper soil levels in future research.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly and ChatGPT to check grammatic errors and improve the language of the original text. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2024.101332.

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