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Reindeer grazing history determines the responses of subarctic soil fungal communities to warming and fertilization

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Summary

- Composition and functioning of arctic soil fungal communities may alter rapidly due to the ongoing trends of warmer temperatures, shifts in nutrient availability, and shrub encroachment. In addition, the communities may also be intrinsically shaped by heavy grazing, which may locally induce an ecosystem change that couples with increased soil temperature and nutrients and where shrub encroachment is less likely to occur than in lightly grazed conditions.
- We tested how 4 yr of experimental warming and fertilization affected organic soil fungal communities in sites with decadal history of either heavy or light reindeer grazing using high-throughput sequencing of the internal transcribed spacer 2 ribosomal DNA region.
- Grazing history largely overrode the impacts of short-term warming and fertilization in determining the composition of fungal communities. The less diverse fungal communities under light grazing showed more pronounced responses to experimental treatments when compared with the communities under heavy grazing. Yet, ordination approaches revealed distinct treatment responses under both grazing intensities.
- If grazing shifts the fungal communities in Arctic ecosystems to a different and more diverse state, this shift may dictate ecosystem responses to further abiotic changes. This indicates that the intensity of grazing cannot be left out when predicting future changes in fungi-driven processes in the tundra.

Introduction

Ongoing global change is altering the Arctic (IPCC, 2014) and increasing the abundance of shrubs and dwarf shrubs, a phenomenon commonly known as Arctic 'greening' (Myers-Smith *et al.*, 2015). Increasing shrub abundance in tundra is likely to exert major impacts on soil fungal communities (Deslippe *et al.*, 2011; Morgado *et al.*, 2015), and thereby alter carbon (C) and nutrient cycling (Weintraub & Schimel, 2005; Phillips *et al.*, 2019). Of the different fungal guilds, saprotrophs break down plant litter and complex accumulated organic matter for their energy, whereas symbiotrophic mycorrhizal fungi largely rely on recently fixed labile photosynthates from their host plants (Lindahl & Tunlid, 2015). In addition, ericoid mycorrhizal (ErM) and some ectomycorrhizal (EcM) fungi have advanced abilities to break down complex organic compounds to fill their own and their host plants' demand for nutrients (Read *et al.*, 2004; Lindahl & Tunlid, 2015; Clemmensen *et al.*, 2021). The activity and presence of saprotrophic and mycorrhizal fungi depend, first, on the abiotic environment; consequently, fungal biomass, functioning, and community structure may shift in response to increased temperatures and nutrient availability (Frey *et al.*, 2008; Deslippe *et al.*, 2011; Geml *et al.*, 2015; Morgado *et al.*, 2015; Semenova *et al.*, 2015). Second, soil fungi also respond to changes in vegetation community composition, with fungal activity further impacting resources available for vegetation (Gavazov *et al.*, 2016). In the warming tundra, fungi-driven changes in ecosystem functions have been particularly linked with the expansion of deciduous shrubs (*Salix* spp., *Betula nana*) and their symbiotic EcM fungal partners that access nutrients through organic matter degradation (Deslippe *et al.*, 2012; Parker *et al.*, 2021).

Although climate affects ecosystems directly and indirectly, biotic interactions can sometimes override trends driven by climate change (Callaghan *et al.*, 2013). In the tundra, annually occurring reindeer (*Rangifer tarandus* L.) migrations are an example of such a driver. A heavy pulse of grazing increases soil nutrient availability through the deposition of urine and feces (Barthelemy *et al.*, 2015) and promotes plant species that are tolerant to defoliation and trampling (Bardgett & Wardle, 2003; van der Wal, 2006). Through these mechanisms, heavy grazing

may locally transform tundra dominated by dwarf shrubs and moss into a graminoid and herb-dominated tundra with substantially warmer soil temperatures (Olofsson *et al.*, 2004; Ylänne *et al.*, 2018, 2020; Ylänne & Stark, 2019), enhanced nitrogen (N) availability (Stark & Väisänen, 2014) and higher ecosystem productivity (van der Wal, 2006). Altered abiotic conditions and higher litter quality impact soil microbial communities, resulting in a more bacterial-dominated soil microbial community (Männistö *et al.*, 2016; Ylänne *et al.*, 2020). Given the change in abiotic conditions and the shift from predominantly EcM and ErM-associated woody species into nonmycorrhizal and arbuscular mycorrhizal herbs and grasses, grazing is also likely to cause a major shift in the soil fungal community composition. For example, along with the changes in mycorrhizal fungi (Barthelemy *et al.*, 2017), the vegetation shift may be also accompanied by increases in saprotrophic fungi degrading the graminoid litter.

Long-term grazing-driven ecosystem change in the tundra could also dictate fungal responses to further compounding environmental changes, such as elevated temperatures and enhanced soil nutrient availability (Callaghan *et al.*, 2013; Väisänen *et al.*, 2014; Ylänne *et al.*, 2020). First, in the heavily grazed areas, soil fungi may be already adapted to higher soil temperature and nutrient levels, making fungal communities less responsive to further increases in temperature and nutrient availability. Second, grazing may mitigate the warming-induced expansion of shrubs (Post & Pedersen, 2008; Olofsson *et al.*, 2009; Vowles *et al.*, 2017; Ylänne & Stark, 2019) and, thereby, slow down the associated soil fungal community composition changes driven by increasing shrub abundance (Deslippe *et al.*, 2012). However, to our knowledge, the role of grazing intensity in shaping fungal community responses to warming and nutrient availability has never been investigated.

In this study, we analyzed tundra soil fungal communities under contrasting long-term (50 yr) grazing intensities and assessed their responses to growing seasonal warming and enhanced nutrient availability. We used a unique study design along a reindeer migration route, where the annually occurring pulse of heavy grazing during reindeer migration has shifted the subarctic tundra ecosystem towards increased graminoid dominance, higher soil temperature and nutrient availability (Väisänen *et al.*, 2014; Ylänne *et al.*, 2020; Fig. 1). We compared the heavily grazed area to an adjacent area with long-term light grazing intensity and used open-top-chambers and N fertilization to simulate climate change over four consecutive summers under both grazing intensities. We characterized soil fungal communities and assigned the taxonomies to their known ecological functions to find out how long-term difference in grazing has shaped fungal community composition and how fungal communities respond to warming and higher nutrient availability under contrasting grazing intensities. First, we hypothesized soil fungal communities to differ between the grazing intensities and, more specifically, EcM and ErM fungi to be more abundant under light grazing and saprotrophic and pathotrophic fungi to be more abundant under heavy grazing. Second, given that fungal communities under heavy grazing can be assumed to be already adapted to higher soil temperature and nutrient availability, we also hypothesized that warming and fertilization will induce grazing-dependent responses in fungal communities, which will be more pronounced under light grazing (Fig. 1). Furthermore, we examined linkages between fungal community composition and soil organic C (SOC) stocks (Ylänne *et al.*, 2020).

Materials and Methods

Study site, experimental design, and soil sampling

The study site was a subarctic-alpine tundra heath on a northern slope of Ráisduoddar fell in northern Norway (69°31′29″N, 21°19′16″E; altitude 490–520 m above sea level (asl)). At the study site, the annual precipitation is 935 mm and the annual mean temperature is −0.6°C (2006–2015, Norwegian Water Resources and Energy Directorate, www.senorge.no). A pasture rotation fence built in the 1960s divides the area into a migration range with light grazing (LG) intensity, which is influenced by reindeer only transiently during the spring and autumn migrations, and a summer range with heavy grazing (HG) intensity experiencing intensive grazing annually for 2–3 wk in August (Olofsson *et al.*, 2004). Vegetation under LG is dominated by evergreen and deciduous dwarf shrubs like *Empetrum nigrum* L. ssp. *hermaphroditum* (Hagerup), *B. nana* L., and *Vaccinium* L. species (abundance as hits per pin is 0.8, 0.2, and 0.6, respectively). On the contrary, HG is dominated by grasses and sedges with patches of evergreen dwarf shrubs, such as *E. hermaphroditum* (abundances as hits per pin 1.1, 1, and 0.2, respectively). Throughout the growing season, HG soil has higher N concentrations than LG soil does due to reindeer excrement, which increase soil N, especially during the migration (Stark & Väisänen, 2014). In July 2013, the mineral N content (i.e. sum of ammonium-N (NH_4N) and nitrate-N (NO_3N)) in the organic soil was $0.138 \pm 0.048\ g\ m^{-2}$ (mean ± SE) during LG and $0.537 \pm 0.144\ g\ m^{-2}$ during HG (analyzed in 0.5 M potassium sulfate extractions: ISO standard SFS 3032, UV-1700 spectrometer (Shimadzu, Kyoto, Japan) for NH_4N; SFS-EN ISO 133395CFA, AA3 analyzer (Seal Analytical, Mequon, WI, USA) for NO_3N). Organic soil pH range during LG was 4.15–5.96, the average being 5.1, whereas soil pH range during HG was 4.44–6.06, the average being 5.3 (analyzed in 3 : 5 v/v soil : water suspensions, Model 220 pH conductivity meter; Denver Instrument, Bohemia, NY, USA).

In 2010, eight spatial blocks were established, extending to both sides of the pasture rotation fence that separated the LG and HG sides. Within each block, four study plots with similar exposure and hydrological status were selected from both the LG and HG sides and assigned to the following treatments: control (Ctrl), fertilization (F), warming (W), and warming and fertilization (WF). In total, there were 64 study plots. Warming was applied using hexagonal open-top chambers (OTCs) made of polycarbonate of 1.5 mm thickness (Marion *et al.*, 1997). The plots containing OTCs (height 0.4 m and basal diameter 1.04 m) had an area of $0.935\ m^2$ (W and WF), whereas plots without OTCs (Ctrl and F) were $1\ m^2$. The OTCs were constructed right after snowmelt (16 June 2010, 31 May 2011, 5 June 2012, 4 June 2013) and removed just before reindeer arrival to the

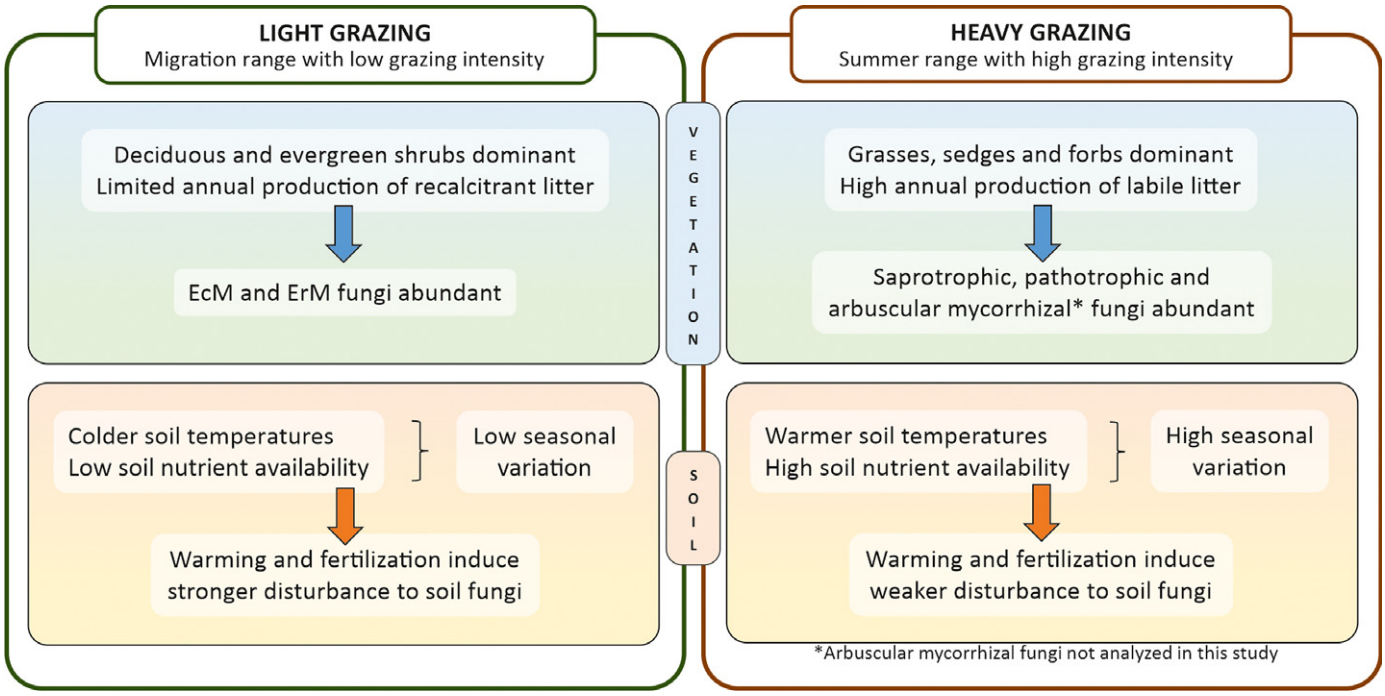

Fig. 1 Vegetation and soil characteristics and hypothetical fungal responses to warming and fertilization under light and heavy grazing. Soil temperatures and seasonal variation imply the conditions shown during the growing season.

study site in early August (7 August 2010, 4 August 2011, 8 August 2012, 4 August 2013). OTCs generally increase air temperature by 1.5–3°C and soil temperature by 0.6–1.1°C (Molau & Mølgaard, 1996; Marion *et al.*, 1997). These increased temperatures are known to be accompanied by other microclimatic changes, such as decreased wind speed and soil moisture and changes in humidity (Bjorkman *et al.*, 2020). In our experiment, soil temperatures at 3 cm depth ($n = 3$, EasyLog EL-USB-1; Lascar Electronics, Whiteparish, UK) did not differ depending on the warming treatment in 2013 (Ylänne *et al.*, 2020), but the surface temperature in 2012 was *c.* 1.4°C higher in the warmed plots under both grazing intensities (Väisänen *et al.*, 2013). Fertilization was implemented by dissolving ammonium nitrate equivalent to 10 g N m^{-2} to 1 l of local water and applying it on the plots at the time of bud burst (16 June 2010, 9 June 2011, 28 June 2012, 4 June 2013). The fertilizer addition dose was selected to mimic previous manipulations in the Arctic (e.g. Mack *et al.*, 2004). In 2013, fertilization increased soil mineral N content over seven-fold under LG and four-fold under HG (Ylänne *et al.*, 2020).

Previous studies using the same experimental set-up have shown substantial ecosystem-level impact of experimental treatments that depend on each other and grazing intensity (Ylänne *et al.*, 2020). Under both grazing intensities, warming generally decreased soil moisture and soil mineral N availability next to increasing vegetation biomass, and both warming and fertilization increased the activities of several extracellular enzyme activities responsible for catalyzing organic matter degradation (Ylänne *et al.*, 2020). In addition, in 2014, 1 yr after the present investigation, warming had decreased soil C by 22% under both grazing intensities in unfertilized plots but had no effect on soil C in fertilized plots (Ylänne *et al.*, 2020). Additionally, soil C stocks decreased as soil fungal : bacterial ratio increased under both grazing intensities (Ylänne *et al.*, 2020).

In July 2013, composite soil samples were collected from the organic horizon (LG: 3–8.5 cm, mean 4.5 cm; HG: 2.2–8.5 cm, mean 4.3 cm) by taking three to four soil cores (2.8 cm diameter) from each plot and pooling these together. All cores extended through the whole organic soil layer, and the litter and mineral soil layers were removed before pooling. Soil samples were immediately homogenized in the field (2 mm mesh size) following aseptic procedures, and duplicate samples of 2 ml from each plot were snap-frozen in cryo vials in liquid N_2. These were preserved at −80°C for later DNA analysis.

Molecular analyses and bioinformatics

The preserved duplicate samples (0.138–0.566 g) were extracted for total DNA using a modified phenol–chloroform–isoamylalcohol method (Griffiths *et al.*, 2000; Männistö *et al.*, 2016) with 600 µl of CTAB buffer and a mixture of beads (0.7 g ceramic beads (1.0 mm), 0.3 g glass beads (0.1 mm), and two large glass beads (3.5 mm); BioSpec, Bartlesville, OK, USA). DNA extracts were diluted to 1 : 50 for PCR amplification, since in this dilution the concentration of coextracted humic substances no longer inhibited PCR. PCR of the fungal internal transcribed spacer 2 (ITS2) ribosomal DNA region were run in duplicate for each subsample (1.5 µl of 1 : 50 dilution) in 15 µl reaction volume using Phusion High-Fidelity DNA Polymerase (ThermoScientific, Waltham, MA, USA), 0.2 µM of fungal-specific primer pair fITS7 (Ihrmark *et al.*, 2012) and barcoded ITS4 (White *et al.*, 1990) (primers included sequencing adapters trP1 and A,

respectively), and 0.2 mM of each deoxynucleoside triphosphate. The cycling regime was initial denaturation of 98°C for 1 min, followed by 27 cycles of 98°C for 10 s, 57°C for 20 s, and 72°C for 30 s, and final extension of 72°C for 7 min. 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 an Ion 314™ chip and Ion Torrent PGM (Life Technologies, Guilford, CT, USA) at the Biocenter Oulu Sequencing Center (Finland).

Raw data with 326 388 sequences were processed using QIIME 1.8.0 (Caporaso *et al.*, 2010). Sequences were demultiplexed and quality filtered (i.e. one mismatch per barcode was accepted, sequences with mean quality score < 20 with quality score window size of 50 bp and sequences shorter than 150 bp were removed along with primers and adapters). We employed a double *de novo* operational taxonomic unit (OTU) picking (Nguyen *et al.*, 2015), first removing chimeras and clustering all sequences using USEARCH 6.1 (Edgar, 2010) followed by second clustering with UCLUST (Edgar, 2010) (at 97% sequence similarity level in QIIME). Taxonomy for each representative sequence was assigned using BLAST (Altschul *et al.*, 1990) against dynamic release of UNITE v.7.0 (Kõljalg *et al.*, 2013) with 90% similarity, and for the most common OTUs (over 1000 sequence reads) we also performed a second BLAST against National Center for Biotechnology Information GenBank with 95% similarity. All nonfungal OTUs were discarded and singletons were removed, leaving the final sequence count to 133 047 reads (forming 5223 OTUs) with the sequence count per sample varying from 827 to 2985. Finally, ecological function for each fungal OTU was assigned using the online tool FUNGUILD (Nguyen *et al.*, 2016) or based on published ecological information of the taxon in question. The acquired functions were further combined into seven functional groups: ErM (including all fungi with ErM function), EcM (all ectomycorrhizas except the ones already grouped into ErM), endophytes (undefined non-ErM/EcM root endophytes and arbuscular mycorrhizal fungi), lichenized, saprotrophs (all saprotrophs except the ones with symbiotrophic functions), pathotrophs (all pathotrophs excluding the ones with symbiotrophic or saprotrophic functions), and undefined function. The ecological function could not be determined for most of the fungal community (mean relative abundance of fungi with undefined function 55.6%). The sequences with associated metadata for this study have been deposited in the European Nucleotide Archive (ENA) at the European Bioinformatics Institute (EMBL-EBI) under accession no. PRJEB38257 (https://www.ebi.ac.uk/ena/browser/view/PRJEB38257).

Statistical analyses

To test the main effects of grazing (hypothesis 1) and its interaction with warming and fertilization (hypothesis 2) on the relative abundance of taxonomic groups (phyla, classes, orders, families and genera), functional groups of the ecological function and function within the phyla, multivariate generalized linear models (mGLMs; MVABUND package; Wang *et al.*, 2012) were built with block within grazing intensity set as strata. Non-rarefied data was used for calculating relative abundances, these were rounded up to closest 0.01% and only subgroups with a relative abundance of > 0.1% were included in the tests (including 99.8% of the abundance in the phyla level, 98.7% in the order level, 94.8% in the genera level, and 100% within the functional groups). Models were fitted with a negative binomial distribution using 999 bootstrap iterations and reported with log-likelihood ratio statistics. To find out which fungal groups drove the overall community shifts, the initial multivariate tests were accompanied by univariate tests. In the univariate tests, we report the adjusted *P*-values, where multiple tests are accounted for using a step-down resampling algorithm as in Westfall & Young (1993). Since grazing had significant interactions with warming and/or fertilization (Supporting Information Table S1), the multivariate generalized linear model and the univariate tests were ran separately for the different grazing intensities to explore the divergent effects of warming and fertilization in LG and HG.

We further explored the main effect of grazing (hypothesis 1) and the three-way interactions between grazing, warming, and fertilization (hypothesis 2) on the fungal community by three additional means: by testing these with permutational multivariate ANOVA (PERMANOVA), by running partial constrained correspondence analysis (CCA), and by assessing diversity differences among the treatments. For the PERMANOVA we used Hellinger-transformed data (square root of relative abundance data), for the CCA we used relative abundances and applying the same prevalence criteria as in the mGLM (i.e. only testing OTUs with a relative abundance > 0.1%), and for the alpha diversity metrics we used data rarefied to minimum sequence read number (827). We tested the main and interactive effects of grazing, warming, and fertilization on the fungal community with the function adonis (VEGAN package; Oksanen *et al.*, 2020), setting block as a random factor and using Bray–Curtis distance and 999 permutations. We then assessed multivariate homogeneity of group dispersions with the function betadisper – testing separately the impacts of grazing, warming, and fertilization and their interactions. The number of unique OTUs within both grazing intensities and across treatments was further visualized by Venn diagrams. The CCA was performed with the PHYLOSEQ package (McMurdie & Holmes, 2013), accounting for the variation induced by blocks by setting block as a random factor, and the plots were drawn to show the impacts of grazing, warming, and fertilization. CCA1 and CCA2 scores of each plot were tested for the main and interactive effects of grazing, warming, and fertilization with a linear mixed effect (LME) model (NLME package; Pinheiro *et al.*, 2020) with block within grazing intensity as a random factor. Since grazing had significant interactions with warming and fertilization on both CCA1 and CCA2 scores (Table S2), separate CCAs were performed for LG and HG and the CCA axis scores were tested within these. We used the envfit command in VEGAN to correlate the shown community compositions to fungal phyla, orders, genera and functional groups, diversity indices, and explanatory environmental variables (including vegetation and soil parameters; see Table S3 for more information). We

calculated diversity indices – richness (number of OTUs), Pielou evenness, and Shannon, Simpson, and inverse Simpson diversities – using the VEGAN package and tested these with the LME using suitable transformations to achieve normal distribution of variables (Table S4). To study more closely the correlation of different fungal taxa and functional groups with SOC stocks in the same plots (Ylänne *et al.*, 2020), we used Pearson correlation and Akaike information criterion (AIC) tests (AICCMODAVG package; Mazerolle, 2020). All statistical analyses were carried out with R software for statistical computing, v.3.5.0–v.4.0.0 (R Core Team, 2020).

Results

Grazing intensity affected soil fungal communities

Grazing intensity was the most determinant factor shaping soil fungal community composition across functional groups and taxonomic levels (mGLM: $P < 0.001$; Table S1). Of the most abundant fungal orders (relative abundance > 0.5%), 59% responded to grazing (Table 1), and grazing impact was evident for five out of the seven functional groups (Table 2). Also on the overall community composition, grazing exerted the strongest impact (PERMANOVA: $F_{1,63} = 6.807$, $P < 0.001$; Table S5) and the effect of grazing paralleled with the CCA1 axis (LME: $F_{1,42} = 102.187$, $P < 0.001$; Fig. 2; Table S2). A few dominant OTUs were characteristic to LG, and communities under LG associated with higher abundance of shrubs and lichens and higher organic soil C : N ratio (Fig. 2; Table S3). On the other hand, fungal community under HG was characterized by numerous OTUs with low abundance forming a more even and diverse community (higher Pielou evenness and Shannon, Simpson, and inverse Simpson diversity indices; Figs 2, S1; Table S4), as also indicated by the higher heterogeneity of group dispersion under HG (betadisper: $F_{1,62} = 13.619$, $P < 0.001$; Table S5). Fungal communities under HG associated with higher graminoid abundance and higher soil moisture, soil N availability, and soil C stocks (Fig. 2; Table S3). Regardless of these differences, both grazing intensities hosted a similar number of unique OTUs (Fig. S2).

At phylum level, soils for both grazing intensities were dominated by Ascomycota (mean relative abundance 62%), followed by Basidiomycota (31%) (Fig. 3a; Table 1). Ascomycota was more abundant under LG than under HG and especially the dominating orders Chaetothyriales and Helotiales (including genus *Rhizoscyphus*) occurred in higher abundance in LG (Fig. 3a; Table 1). *Rhizoscyphus* was the most abundant OTU in the whole data set, contributing 4% of all sequence reads with 4802 reads under LG vs 567 reads under HG. Some less abundant ascomycetous taxa, such as orders Saccharomycetales (including genus *Basidioascus*) and Hypocreales, and genus *Meliniomyces*, were more abundant under HG (Fig. 3a; Table 1). Also, Basidiomycota and its orders Auriculariales, Geminibasidiales (all from genus *Geminibasidium*), Trechisporales, and Tremellales (including genus *Cryptococcus*) were more abundant under HG (Fig. 3a; Table 1). From other phyla, Mortierellomycota (3.2%; including order Mortierellales and genus *Mortierella*) was more abundant under HG, while the proportion of unidentified phyla (3.5%) was higher under LG (Fig. 3a; Table 1).

Communities in both grazing intensities were dominated by fungi with currently undefined ecological functions (55.6%). Most of the fungi with undefined function were ascomycetes, and the abundance of these was higher under LG than under HG (Fig. 3b; Table 2). ErM and lichenized fungi were more abundant under LG, whereas saprotrophic and pathotrophic fungi occurred more abundantly under HG (Fig. 3b; Table 2). Although abundances of EcM fungi did not differ significantly between grazing intensities, except for EcM ascomycetes, which were slightly more abundant under HG (Table 2), EcM seemed to constitute a bigger proportion of the fungal community under HG than under LG (Fig. 3b), as indicated by the CCA (Fig. 2; Table S3).

Effects of warming and fertilization treatments on fungal communities under light grazing and heavy grazing intensities

W and F treatments induced distinct responses in soil fungal communities under both grazing intensities (Tables S1, S2). Whereas F had an evident main effect at the genus level, fungal responses to W and WF differed depending on the grazing intensity (mGLM: $P < 0.050$; Table S1). For the overall community, there was no clear effect of W or F treatments (PERMANOVA; Table S5). In the CCA, the treatments diverged from each other along CCA2 axis under HG but were lumped under LG (Fig. 2). Under HG, communities in the Ctrl and W treatments were associated with positive CCA2 scores, whereas the community in the F treatment was located in the middle of the CCA2 axis, and the WF treatment associated with negative CCA2 scores. Communities in F treatment under LG and HG did not differ along the CCA2 axis and approached each other along the CCA1 axis (Fig. 2). W and F treatments had no effect on fungal diversity indices (Fig. S1; Table S4), but they altered the homogeneity of group dispersions depending on the grazing intensity (betadisper: $P < 0.050$; Table S5).

Almost all taxonomic levels of the fungal community showed three-way interactions among grazing intensity, W, and F when analyzed with mGLM, indicating that the impacts of W and F depended on each other and the grazing intensity (Table S1). When the effects of W and F were tested separately for the two grazing intensities, the W and F treatments exerted significant ($P < 0.050$) effects on the most abundant fungal taxonomical levels and ecological functions only under LG (Table S1), and also the PERMANOVA test on communities found significant treatment effects only under LG (Table S5). These patterns deviated from the CCA ordination, where W and F treatments affected both CCA1 and CCA2 scores under both grazing intensities (Fig. 4; Table S2). Under LG, communities in W and WF treatments were grouped together and differed from communities in Ctrl and F treatments, which in turn diverged from each other (Fig. 4a). Under HG, all treatments differed along both CCA axes (Fig. 4b). Even though the univariate tests found only a few

Table 1 Relative abundances of the most abundant fungal taxa on phyla, order, and genus levels and the responses of these to grazing intensity (G), warming (W), fertilization (F) and their interactions as indicated by the multivariate generalized linear model (deviance-values and significance).

		Response						
Taxon	Abundance (%)	$G_{62,1}$	$W_{61,1}$	$F_{60,1}$	$W \times F_{42,1}$	$G \times W_{44,1}$	$G \times F_{43,1}$	$G \times W \times F_{42,1}$
Ascomycota	62	**24.393*****↓	0.167	1.677	0.262	**9.936****a	1.288	1.32
Chaetothyriales	27.7	**10.748**†↓	0.618	0.851	0.06	4.57	0.638	2.935
Capronia (endo)	4.3	4.288	3.199	1.808	0.321	0.872	0.49	5.1
Helotiales	15.4	**21.17*****↓	0.563	1.354	0.208	0.809	0.12	0.08
Rhizoscyphus (ErM)	3.6	**34.156*****↓	0.043	0.536	0.734	0.565	0.262	0.38
Hypocreales	0.9	**27.224*****↑	0.242	6.79	1.519	0.383	0.007	0.118
Hysteriales	0.9	7.22	0.319	0.037	1.052	0.071	1.537	2.494
Cenococcum (EcM)	0.9	7.321	0.309	0.031	1.163	0.088	1.597	2.647
Leotiomycetes i.s.	2.1	2.664	0.021	3.197	4.284	0.607	0.23	5.427
Meliniomyces (ErM)	0.8	**17.054***↑	1.402	0.002	1.527	0.394	0.174	2.326
Saccharomycetales	0.9	**33.291*****↑	0.045	0.357	1.118	1.798	0.058	0.63
Basidioascus (sapro)	0.8	**32.948****↑	0.566	0.421	5.989	3.002	0.468	**14.153***ns
Basidiomycota	31	**24.055*****↑	0.409	2.979	1.434	**14.53****b	0.054	3.868
Agaricales	15.1	2.048	2.043	6.938	0.138	4.529	0.265	1.342
Clavaria (sapro)	3.6	3.893	0.858	7.615	1.031	0.828	0.468	0.15
Cortinarius (EcM)	1.3	10.657	0.214	4.956	2.906	0.828	0.014	2.888
Mycena (sapro)	2.7	5.999	3.7	0.174	0.118	0.749	0.031	3.005
Auriculariales	0.7	**12.583***↑	0.426	4.326	0.386	0.047	0.019	0.006
Geminibasidiales	0.9	**25.704*****↑	2.824	1.868	0.132	**14***ns	**12.994***ns	0
Geminibasidium (sapro)	0.9	**25.704****↑	2.824	1.868	0.132	**14***ns	**12.994***ns	0
Russulales	2.8	0.152	0.02	0.053	2.834	0.827	0.006	0.18
Russula (EcM)	2.1	0.454	0.153	0.181	2.202	0.337	0.009	0.002
Sebacinales	3.4	0.087	0.215	1.655	0.833	1.362	1.212	0.523
Thelephorales	2.9	7.29	0.068	0.323	3.25	0.57	5.4	0.01
Tomentella (EcM)	1.1	4.475	4.069	0.098	6.774	4.26	0.575	0
Trechisporales	1.3	**30.877*****↑	3.886	1.804	0.138	1.85	1.959	1.43
Tremellales	0.8	**32.805*****↑	2.164	7.309	3.485	2.269	0.945	**13.143***ns
Cryptococcus (sapro)	0.7	**29.116****↑	2.459	7	2.933	2.515	0.774	**13.235**†ns
Mortierellomycota[1]	3.2	**24.402*****↓	1.103	0.738	0.11	1.149	1.243	0.477
Mortierellales	3.1	**26.107*****↑	1.143	0.535	0.119	1.566	1.118	0.561
Mortierella (sapro)	2.6	**26.994****↑	0.961	0.473	0.146	1.5	1.019	0.46
Unidentified fungi	3.5	**6.092***↓	0.098	1.858	0.223	0.133	1.738	0.079

For genus level, the ecological function is listed in parentheses: EcM, ectomycorrhizal; endo, root endophyte; ErM, ericoid mycorrhizal; patho, pathotroph; sapro, saprotroph; i.s., *incertae sedis*.
Residuals and difference in degrees of freedom (Res.Df and Df.diff, respectively) are presented as subscripts in the title row. Defiance values with $P < 0.1$ are in bold, and the significance is coded as follows: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$. In the case of significant grazing effect, the direction of change towards heavily grazed site is indicated by an arrow. In the case of significant interactions with grazing, the test was repeated within the grazing intensities, the results of which are presented with the following letters: a, Ascomycota increased by W in LG ($W_{30,1}$: Dev = 6679); b, Basidiomycota decreased by W in LG ($W_{30,1}$: Dev = 7106); ns, the group did not respond significantly to warming or fertilization, when tested in the grazing regimes separately. Complete results of all fungal taxa tested are found in Supporting Information Table S6.
[1]Mortierellomycota refers to the prior phylum Zygomycota and also contains 0.1% Mucoromycota.

significant responses of individual fungal groups to W and F (Tables 1, 2, S6), the CCA ordinations separately for LG and HG revealed several taxa and functional groups that correlated with different treatments under each grazing intensity (Fig. 4; Table S3).

Under LG, the community in F treatment was associated with a higher number of unique OTUs (Fig. S2). These were particularly EcM fungi (103 unique EcM OTUs in LG-F vs 27 in LG-Ctrl, 37 in LG-W, and 32 in LG-WF); and in line with this, EcM abundance increased towards F treatment along with higher graminoid abundance and soil N availability (Fig. 4a; Table S3). Furthermore, F treatment was linked with higher abundances of saprotrophic ascomycetes and mortierellomycetes and genus *Cryptococcus* (Fig. 4a; Table S3). Communities in W and WF treatments had a higher abundance of Ascomycota and a lower abundance of Basidiomycota (Table 1) and were associated with a higher shrub abundance and lower soil moisture (Fig. 4a; Table S3). Endophytes (e.g. genus *Capronia*) and saprophytic basidiomycetes were characteristic to Ctrl treatment under LG, along with a higher abundance of bryophytes and lower litter cover (Fig. 4a; Table S3).

Under HG, the communities in F and WF treatments were associated with a higher abundance of pathotrophs, mostly consisting of plant pathogens, and these treatments were also characterized by higher soil N availability (Fig. 4b; Table S3). In addition, WF treatment was linked with a higher abundance of Trechisporales and a lower abundance of Pezizales (Tables S3, S6), whereas W treatment seemed to have a decreasing effect on

Table 2 Relative abundances of the most abundant fungal ecological functions and the responses of these to grazing intensity (G), warming (W), fertilization (F), and their interactions as indicated by the multivariate generalized linear model (deviance-values and significance).

		Response						
Ecological function	Abundance (%)	$G_{62,1}$	$W_{61,1}$	$F_{60,1}$	$W \times F_{42,1}$	$G \times W_{44,1}$	$G \times F_{43,1}$	$G \times W \times F_{42,1}$
Ectomycorrhizal	9.3	2.756	0.25	0.051	4.423	0.32	3.264	1.531
EcM asco	1	**7.37**$^{\dagger}\uparrow$	1.122	0.001	0.865	0.001	1.376	2.871
EcM basidio	8.3	1.815	0.114	0.079	4.95	0.454	3.013	1.051
Ericoid mycorrhizal	4.8	**26.211**$^{***}\downarrow$	0.781	0.559	0.404	0.655	0.328	0.195
Endophytes	5.8	1.443	0.451	2.849	4.549	0.61	3.676	3.333
Endo asco	4.9	4.768	3.556	1.903	0.75	1.013	0.662	7.679
Endo basidio	0.8	0.397	0.004	0.004	7.672	5.691	2.601	1.035
Lichenized	1.8	**6.551**$^{\dagger}\downarrow$	2.327	1.099	0.076	0.35	0.941	0.799
Saprotrophs	21.7	**29.024**$^{***}\uparrow$	0.77	0.954	0.264	4.964	1.667	3.118
Sapro asco	5.2	**23.332**$^{***}\uparrow$	0.157	7.105	0.016	0.128	2.14	4.405
Sapro basidio	13.2	**15.802**$^{***}\uparrow$	0.355	6.474	0.161	**8.756**†ns	7.27	3.44
Sapro mortierello	3.2	**24.402**$^{***}\uparrow$	1.103	0.738	0.11	1.149	1.243	0.477
Pathotrophs	1.1	**20.874**$^{***}\uparrow$	0.406	5.995	1.506	1.937	0.629	0.817
Undefined function	55.6	**12.864**$^{**}\downarrow$	0	0.053	0.664	2.751	0.062	1.245
Undef.func. asco	43.5	**21.354**$^{***}\downarrow$	0.022	0.552	0.19	6.272	0.502	0.83
Undef.func. basidio	8.5	5.174	0.035	2.073	0.683	1.537	1.149	0.379

Abbreviations for functions: asco, ascomycetes; basidio, basidiomycetes; EcM, ectomycorrhizal; endo, endophyte; mortierello, mortierellomycetes; sapro, saprotroph; undef.func., undefined function.
Residuals and difference in degrees of freedom (Res.Df and Df.diff, respectively) are presented as subscripts in the title row. Defiance values with $P < 0.1$ are in bold, and the significance is coded as follows: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$; In the case of significant grazing effect, the direction of change towards heavily grazed site is indicated by an arrow; ns, the group did not respond significantly to warming or fertilization, when tested in the grazing regimes separately. Complete results of ecological functions are found in Supporting Information Table S6.

genus *Hebeloma* and saprophytic ascomycetes (e.g. order Sordariales) along with SOC (Fig. 4b; Table S3). None of the treatments had significant effects on diversity indices, even though Ctrl treatment had slightly more unique OTUs than other treatments under HG did (Fig. S2; Table S3).

Fungal taxa correlating with soil organic carbon stock

Under LG, low SOC stocks occurred in combination with a high relative abundance of ErM genus *Rhizoscyphus* ($r = 0.506$, $P = 0.003$; Fig. S3). In addition, AIC analysis indicated that aside from *Rhizoscyphus*, the abundances of *Meliniomyces* and unidentified Leotiomycetes orders also explained SOC stocks. Under HG, no correlation was found between SOC stocks and any fungal taxonomical or functional group.

Discussion

In support of our first hypothesis, the fungal community composition differed considerably depending on the 50 yr difference in grazing intensity. This effect of grazing consequently drove the responses of fungal communities to compounding environmental changes, warming and fertilization. More precisely, in line with our second hypothesis, the multivariate generalized model showed fungal communities to respond more profoundly to the experimental treatments under light grazing. However, the CCA of both grazing intensities revealed treatment-induced differences, particularly under heavy grazing. These together demonstrate a determinant role of long-term difference in grazing intensity in shaping fungal communities and their responses to abiotic changes but also reveal their interaction to be ambiguous.

Soil fungal community composition in response to grazing history

Grazing intensity affected all taxonomical levels and most of the identified functional groups. More precisely and in accordance with our hypothesis, the abundances of ErM and lichenized fungi were higher under light grazing, while the abundances of saprotrophs and pathotrophs were higher under heavy grazing. However, in contrast to our hypothesis, the abundances of EcM fungi did not differ between grazing intensities except for a small group of EcM ascomycetes that were more abundant under heavy grazing.

Under light grazing, the higher abundance of ErM and lichenized fungi aligns with the higher abundances of ErM-associated ericaceous dwarf shrubs and lichens (Väisänen *et al.*, 2014; Ylänne *et al.*, 2020). In particular, the ErM genus *Rhizoscyphus*, the most abundant fungal OTU in our study, was associated with light grazing, following the cover differences in *E. hermaphroditum*, but also potentially affected by a decrease in ErM colonization of *E. hermaphroditum* under heavy grazing (Barthelemy *et al.*, 2017). In line with the lower abundance of ErM and lichenized fungi under heavy grazing – both dominated by ascomycetous taxa – grazing decreased the abundance of Ascomycota, as also found in northern boreal forests (Santalahti *et al.*, 2018).

In contrast to light grazing, the heavy grazing site was characterized by a higher abundance of saprotrophs (e.g. Auriculariales,

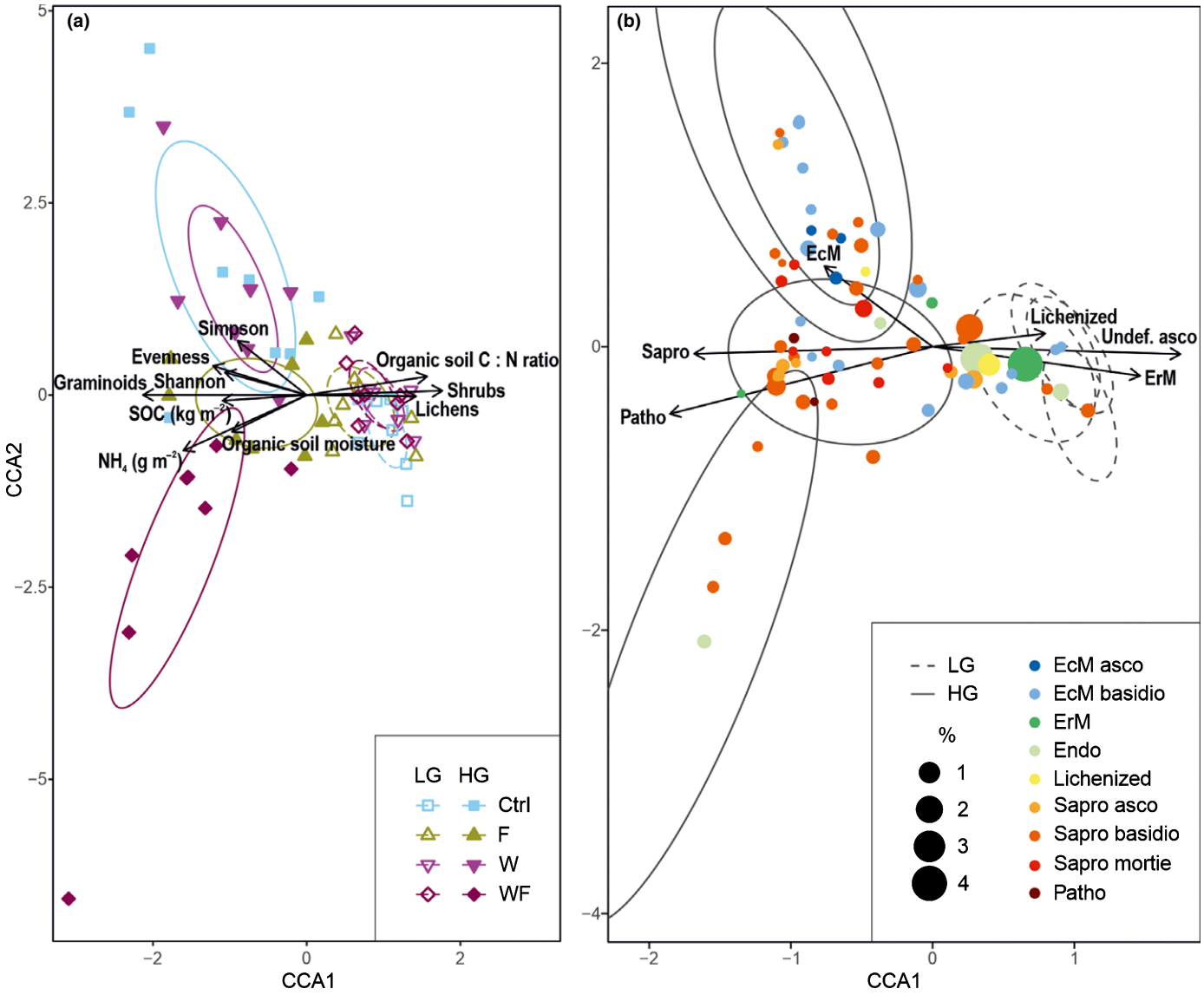

Fig. 2 A canonical correspondence analysis showing the interacting effects of different grazing intensities, experimental warming, and enhanced nitrogen availability on soil fungal communities. (a) Samples within treatments and (b) operational taxonomic units within the functional groups of the ecological function. Ellipses encompass the factorial treatments – that is, control (Ctrl), fertilization (F), warming (W), and combined fertilization and warming (WF) – within the grazing intensities with a 95% confidence interval ($n = 8$). Environmental variables that explain the constrained correspondence analysis (CCA) division significantly ($P < 0.05$) are shown as vectors. asco, ascomycetes; basidio, basidiomycetes; C, carbon; EcM, ectomycorrhizal; endo, endophytic; ErM, ericoid mycorrhizal; mortie, mortierellomycetes; N, nitrogen; NH_4, ammonium; patho, pathotrophic; sapro, saprotrophic; SOC, soil organic carbon; undef., undefined function.

Geminibasidiales, Hypocreales, Mortierellales, Saccharomycetales, Trechisporales, and Tremellales). These molds and yeasts have a limited capacity to decompose complex organic matter (Lindahl *et al.*, 2010; Fernandez & Kennedy, 2018), and thus they depend on easily available C derived from leaf, root, and mycelial litter (Parker *et al.*, 2018; Ylänne *et al.*, 2021). The higher abundances of these taxa under heavy grazing are thus likely linked to the graminoid and herb dominance resulting in a higher input of more labile litter (Olofsson & Oksanen, 2002) and higher soil N availability (Stark & Väisänen, 2014). Interestingly, increases in the abundance of opportunistic saprophytic fungi have also been reported in subarctic mountain birch forests during moth outbreaks, where massive insect herbivory led to a higher dissolved N in soil (Kaukonen *et al.*, 2013; Saravesi *et al.*, 2015) and to an increase in labile C input in larval frass (Kristensen *et al.*, 2018). Ultimately, the abundance of saprophytic basidiomycetes could explain the higher abundance of Basidiomycota under heavy grazing.

Regardless of the higher abundance of EcM host *B. nana* under light grazing (Ylänne *et al.*, 2020), EcM abundance did not differ between grazing intensities, contrasting with the known association of deciduous shrubs with EcM Basidiomycota (Wallenstein *et al.*, 2007; Clemmensen *et al.*, 2015). The lack of grazing effect on EcM abundance could be explained by several mechanisms. EcM fungi could be inhibited by ErM fungi (Kohout *et al.*, 2011) and/or by the allelopathic compounds produced by

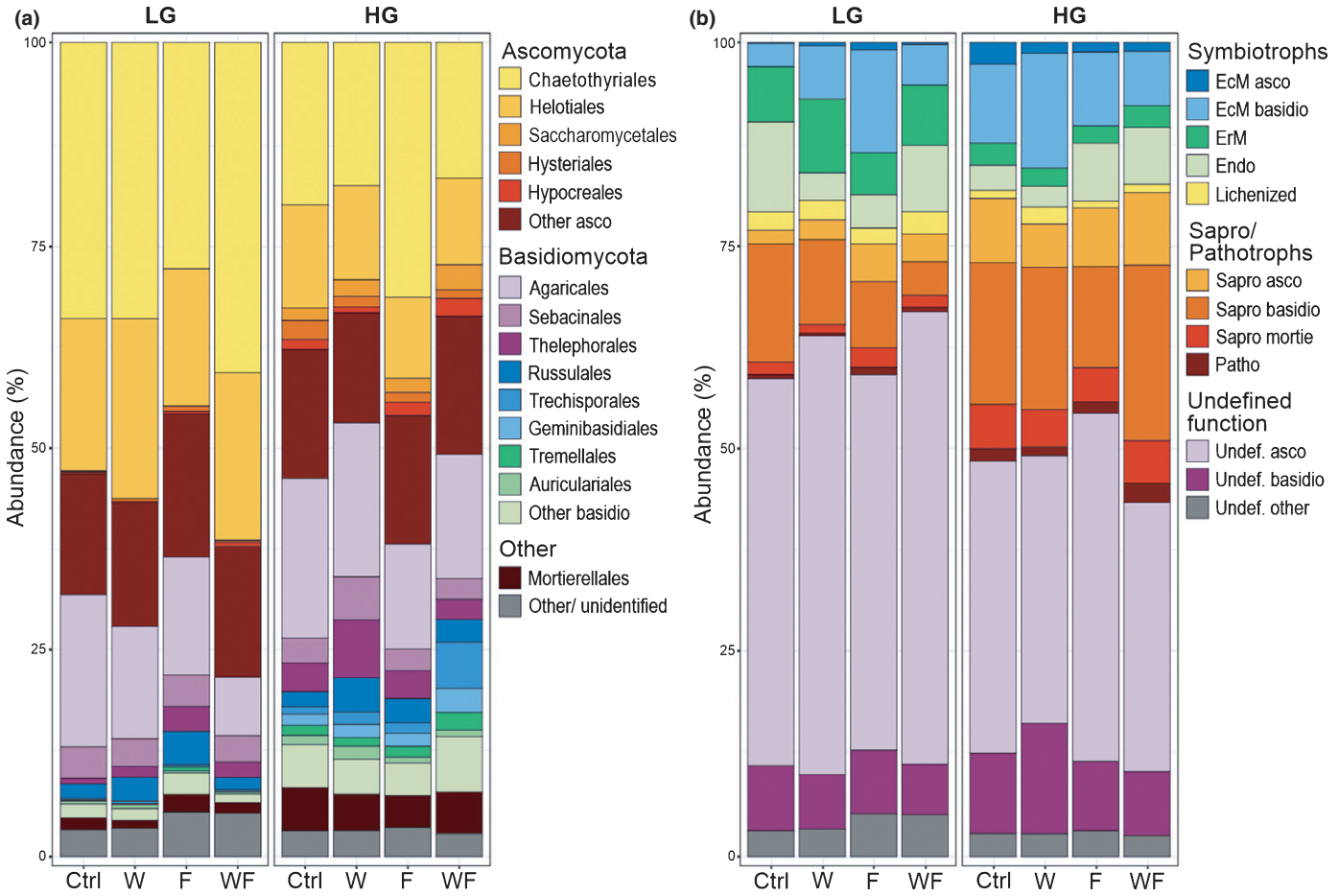

Fig. 3 Mean relative abundances of fungal orders (a) and ecological functions (b) in control (Ctrl), warmed (W), fertilized (F) and combined warmed and fertilized (WF) plots under light (LG) and heavy grazing (HG). asco, ascomycetes; basidio, basidiomycetes; EcM, ectomycorrhizal; endo, endophytic; ErM, ericoid mycorrhizal; mortie, mortierellomycetes; patho, pathotrophic; sapro, saprotrophic; undef., undefined function.

E. hermaphroditum (Nilsson *et al.*, 1993; Tedersoo *et al.*, 2020) under light grazing. Some EcM are likely also hidden behind the classification bias, as several fungi classified here as ErM may also form EcM symbioses (e.g. *Rhizoscyphus* and *Meliniomyces*; Nguyen *et al.*, 2016). Furthermore, some typical tundra herbs, such as *Bistorta vivipara*, which was more abundant under heavy grazing (E. Kaarlejärvi *et al.*, unpublished data), form EcM with several fungal taxa (Mundra *et al.*, 2015), including *Cenococcum* and *Tomentella*, which are abundant in this study. Yet, these fungal groups could have also responded to the abiotic changes brought by grazing, as fungi differ by their abilities to compete in different environments. For example, *Cenococcum* is well known for its wide host and habitat range (LoBuglio, 1999), and some *Tomentella* species have been mentioned as nitrophilic (Defrenne *et al.*, 2019). Thus, the effects of grazing on fungal communities might not be driven by the vegetation change alone.

Responses of fungal community composition to warming and increased nitrogen

Experimental warming and fertilization exerted very different responses across the grazing intensities, thus supporting our assumption that a long-term difference in reindeer grazing can control the consequences of compounding environmental changes on fungal communities. In line with our hypothesis, fungal community was generally more responsive to warming and fertilization under light grazing than under heavy grazing. However, depending on the modelling approach, we could also detect strong fungal community responses under heavy grazing. These controversies suggest that, in addition to being already adapted to warmer and nutrient-rich soil *per se* (Olofsson *et al.*, 2004; Stark & Väisänen, 2014), the observed high diversity of fungal community under heavy grazing could also weaken detection of clear patterns in the multivariate model, as we discuss later herein.

We found that the fungal community responses to warming were evident under light grazing. At the phylum level, warming under light grazing increased the dominance of Ascomycota at the expense of Basidiomycota. This shift was counterintuitive in relation to the increased abundance of deciduous shrubs under light grazing (Ylänne *et al.*, 2020) that generally associate with basidiomycete EcM. Yet, the pattern aligns with the negative responses of EcM basidiomycetes to warming at a moist tundra site in Alaska (Geml *et al.*, 2015) and with the contrasting responses of different EcM genera to warming (Morgado *et al.*, 2015). Ultimately, the indirect changes brought by vegetation shifts (Semenova *et al.*, 2015; Hagedorn *et al.*, 2019) could occur

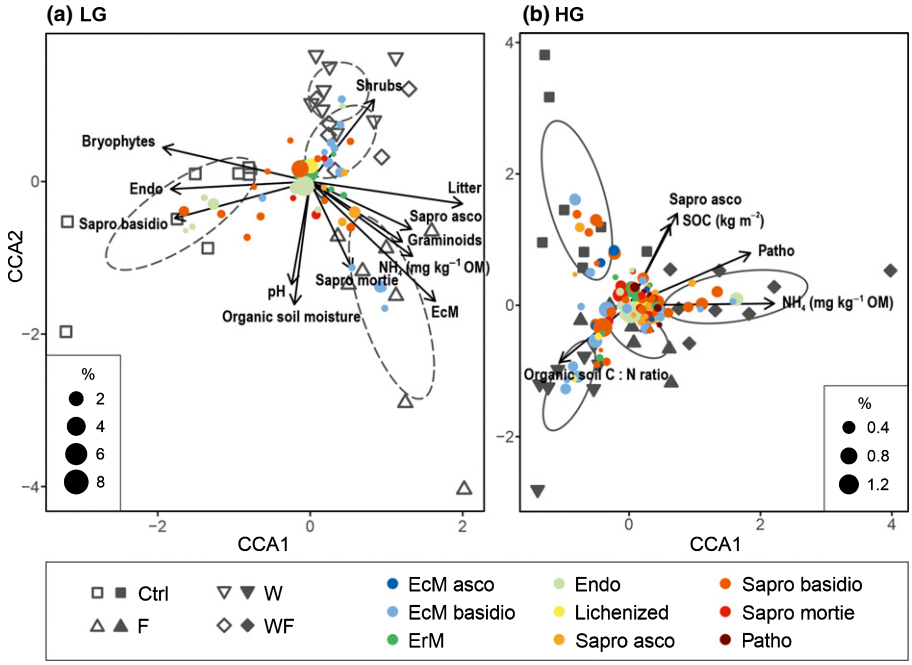

Fig. 4 A canonical correspondence analysis showing the effects of experimental warming and enhanced nitrogen availability on soil fungal community separately under (a) light grazing (LG) and (b) heavy grazing (HG). Ellipses encompass the factorial treatments – that is, control (Ctrl), fertilization (F), warming (W) and combined fertilization and warming (WF) – within the grazing intensities with a 95% confidence interval ($n = 8$). Environmental variables that explain the constrained correspondence analysis (CCA) division significantly ($P < 0.05$) are shown as vectors. asco, ascomycetes; basidio, basidiomycetes; C, carbon; EcM, ectomycorrhizal; endo, endophytic; ErM, ericoid mycorrhizal; mortie, mortierellomycetes; N, nitrogen; NH_4, ammonium; OM, organic matter; patho, pathotrophic; sapro, saprotrophic; SOC, soil organic carbon.

more slowly than captured in this study (Timling & Taylor, 2012).

Fungal communities also changed in response to fertilization, which partly interacted with warming and grazing. Under light grazing, fertilization was associated with a higher number of unique OTUs, many of those EcM fungi, supporting previous findings from tundra where fertilization increases EcM growth (Clemmensen *et al.*, 2006). This adds to the evidence that EcM abundance is not necessarily linked with deciduous shrub abundance but, as discussed earlier, may reflect nitrophilic taxa. Under heavy grazing, by contrast, the combined warming and fertilization treatment was associated with a high abundance of plant pathogens. This agrees with previous reports of pathogens benefiting from increased plant biomass, higher temperatures (Geml *et al.*, 2015; Semenova *et al.*, 2015), and N addition (Nordin *et al.*, 2006). Further, the responses of saprotrophs to the increase in graminoid litter could be negated by treatment impacts on soil moisture (Christiansen *et al.*, 2017). Under both grazing intensities, fertilization increased soil moisture. Yet, when fertilization was combined with warming under LG it led to drier soils (Ylänne *et al.*, 2020), potentially inhibiting saprotrophic activity.

Depending on the modelling approach, we detected conflicting results in the responses of fungal community to warming and fertilization under heavy grazing. We suggest this to be related to the dynamic and heterogeneous fungal communities under heavy grazing, which limit the detection of significant responses using linear regressions. CCA, on the other hand, can tackle the heterogeneity in the community. As discussed before, heavy grazing was associated with saprotrophic fungi that generally possess a capacity to respond to changes in resource availability (Lindahl *et al.*, 2010). In our study area, the reindeer migration induces large seasonal variation in soil mineral N concentrations only under heavy grazing (Stark & Väisänen, 2014), and the dominant vegetation, herbs and graminoids, produces more labile litter. These regular pulses of fast resources may favor saprotrophs and, in combination with the higher fluctuation in soil temperature (Stark *et al.*, 2015), favor a more diverse fungal community – as indicated by the higher alpha and beta diversity under heavy grazing. Therefore, the more diverse fungal communities under heavy grazing could be adapted to higher variation in resource availability and have a stronger capacity for rapid responses to resource shifts. Yet, such responses could remain hidden in the multivariate models, where the heterogeneous communities were tested at the levels of taxonomical and functional groups.

Linkages between fungal community composition and soil carbon

Future changes in vegetation and fungal communities have the potential to critically impact global C cycling, including shifts in SOC stocks (e.g. Parker *et al.*, 2021). The deciduous shrubs *B. nana* and *Salix* spp., which host EcM fungi, have often been associated with lower SOC stocks (Hartley *et al.*, 2012; Parker *et al.*, 2015), but we found no association between EcM fungi and SOC in this study. EcM fungal abundance in our study area was rather low, with no particular EcM genus being dominant.

The most common EcM fungal genera at our site were characterized with contact-type (e.g. *Russula*, *Tomentella*) or short-distance (e.g. *Cenococcum*) exploration strategies (Wei & Agerer, 2011; Tedersoo *et al.*, 2012), whereas EcM fungi with long-distance exploration strategies (e.g. Boletaceae, *Cortinarius*) were rare, potentially explaining why EcM fungi were not linked to SOC (Clemmensen *et al.*, 2021). Instead, under light grazing, lower SOC was associated with higher abundance of the ErM genus *Rhizoscyphus*. Taxa in the genus *Rhizoscyphus* produce a rich repertoire of degradative enzymes and have the ability to break down recalcitrant organic matter (Martino *et al.*, 2018). The negative association between *Rhizoscyphus* and SOC contrasts with previous research suggesting that ErM species are associated with slow rates of soil organic matter breakdown and higher SOC accumulation (Clemmensen *et al.*, 2013; Fernandez & Kennedy, 2018) and rather indicates an important role of this group in soil C degradation in the tundra. Our study may provide the first evidence from field conditions (*sensu* Parker *et al.*, 2021) that *Rhizoscyphus* could be an example of a specialized ErM fungal genus controlling SOC loss analogous to the role of long-distance exploration types among EcM fungi (Clemmensen *et al.*, 2021). Even though the warming treatment was found to decrease SOC under both grazing intensities (Ylänne *et al.*, 2020), fungal groups could not be linked to decreased SOC under heavy grazing. Thus, interestingly, the more diverse microbial communities under heavy grazing could lead to a similar outcome on SOC.

Conclusions and implications

As expected by a strong shift in the vegetation from shrubs to graminoids, an annually occurring pulse of heavy grazing for the past 50 yr had led to distinct shifts in soil fungal communities when compared with a nearby area with light grazing. The long-term grazing difference largely overrode the effects of short-term warming and fertilization, which were not unidirectional under different grazing intensities. This agrees with previous reports showing locally varying fungal responses to changes in abiotic environment (Morgado *et al.*, 2015; Semenova *et al.*, 2015), revealing for the first time that biotic interactions may dictate the consequences of compounding environmental changes on fungal communities.

Our evidence derives from a study set-up where grazing has induced the vegetation shift from a shrub-dominated tundra heath towards graminoid dominance. Earlier studies have demonstrated that, after such a vegetation shift takes place, soil microbial responses to grazing become independent of the actual grazing event (Stark & Väisänen, 2014). Interestingly, these long-term consequences of grazing may also dictate the responses to short-term exclusion of grazers – the most common approach to test the impacts of herbivory (e.g. Andriuzzi & Wall, 2017). In our study area, we have found that the short-term responses to grazer exclusion did not resemble long-term consequences of heavy grazing, but rather amplified these (Väisänen *et al.*, 2014; Ylänne *et al.*, 2020). The contrasting responses warn against making far-reaching conclusions based on exclusion studies and indicate that long-term grazing differences should be more commonly utilized when assessing the interactive effects of grazers and climatic changes.

It is noteworthy that the observed ecosystem responses to grazing were highly analogous to the reported consequences of several stochastic events that the Arctic is facing, such as insect and pathogen outbreaks (Olofsson *et al.*, 2011; Kaukonen *et al.*, 2013) and extreme winter warming and N deposition events (Choudhary *et al.*, 2016; Phoenix & Bjerke, 2016). Similar to grazing, these climate-change-driven effects may hinder the greening of the Arctic (Phoenix & Bjerke, 2016) and promote the abundance of graminoids and herbs (Kaukonen *et al.*, 2013) while increasing the abundance of opportunistic saprotrophs (Saravesi *et al.*, 2015). If these stochastic events lead the Arctic fungal communities to shift to different and more diverse states, they could also alter how fungal communities respond to further abiotic changes. This highlights the need to foresee stochastic events, especially those that enhance N availability, when predicting future changes in fungal communities and fungi-driven processes, such as soil C sequestration. We thus recommend that future work should test the effects of these cumulative changes.

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Author contributions

SS and MV designed the research. SS, MV and HY performed the field manipulations. MKM designed and organized the microbial community analyses. SHKA performed the fungal community composition analyses, and AM and ALR helped to interpret the results. SHKA wrote the manuscript with contributions from all authors. All authors read and approved the final version of the manuscript for publication.

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Data availability

The data that support the findings of this study are openly available in the ENA at EMBL-EBI (www.ebi.ac.uk), accession no. PRJEB38257.

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Supporting Information

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

Fig. S1 Richness, Pielou's evenness, Shannon's, Simpson's and Inverse Simpson's diversity indices in control, warmed, fertilized and combined warmed and fertilized plots under light and heavy grazing.

Fig. S2 Unique, shared and total number of operational taxonomic units in control, warmed, fertilized and combined warmed and fertilized plots under light and heavy grazing.

Fig. S3 Pearson's correlation between organic soil carbon stock and the relative abundance of fungal genus Rhizoscyphus in control, warmed, fertilized and combined warmed and fertilized plots under light grazing.

Table S1 Deviance and significance values of the multivariate generalized linear model showing the own and interacting effects of grazing intensity, warming and fertilization on the taxonomic and functional composition of soil fungal community.

Table S2 *F*-values and significance of the linear model showing the own and interacting effects of grazing intensity, warming and fertilization on the CCA1 and CCA2 axis values when tested on all data and separately under light and heavy grazing.

Table S3 Results of vector analyses for CCA-ordinations from environmental variables and fungal taxonomical and functional group abundances.

Table S4 *F*-values and significance of the linear model showing the own and interacting effects of grazing intensity, warming and fertilization on the diversity indices.

Table S5 *F*-values and significance of permutational multivariate ANOVA and multivariate homogeneity of group dispersion showing the own and interacting effects of grazing intensity, warming and fertilization on the soil fungal communities when tested on all data and separately under light and heavy grazing.

Table S6 Relative abundances of the most abundant fungal taxa on phyla, order and genus levels, and fungal ecological functions, and the responses of these to grazing intensity, warming, fertilization and their interactions as indicated by the multivariate generalized linear model.

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