

Reindeer grazing alter soil fungal community structure and litter decomposition related enzyme activities in boreal coniferous forests in Finnish Lapland

Minna Santalahti^{a,b,c,*}, Hui Sun^d, Outi-Maaria Sietiö^{a,b}, Kajar Köster^{b,e}, Frank Berninger^{b,e}, Tuomas Laurila^f, Jukka Pumpanen^g, Jussi Heinonsalo^{a,b,e}

^a Department of Microbiology, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland

^b Institute for Atmospheric and Earth System Research/Forest Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland

^c Environmental Soil Sciences, Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland

^d Collaborative Innovation Center of Sustainable Forestry in Southern China, College of Forestry, Nanjing Forestry University, Nanjing 210037, China

^e Department of Forest Sciences, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, 00014 Helsinki, Finland

^f Finnish Meteorological Institute, Climate System Research, P.O. Box 506, FI-00101 Helsinki, Finland

^g Department of Environmental and Biological Science, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

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ABSTRACT

Reindeer grazing in northern boreal zone affects forest floor vegetation heavily and alters the vegetation structure. However, the effect of grazing on soil fungal communities, which are intimately linked to plants, is not currently known. Therefore, our objectives were to investigate changes caused by reindeer grazing on soil fungal communities, litter decomposition rate and litter degrading extracellular enzyme activities. The study was conducted in four areas divided into grazed and non-grazed sites (all together 38 sample plots) in northern boreal forests in Finnish Lapland. Fungal communities were analyzed from humus with high-throughput sequencing technology (454-pyrosequencing), and litter mass loss and extracellular enzyme activities were analyzed after a one-year litterbag experiment. The results showed that grazing significantly affected the fungal community structure and the abundance of certain fungal genera and species. Grazing also decreased laccase and enhanced cellobiohydrolase I activities from the litterbags. Our study is one of the first to describe detailed fungal community composition in sites with long-term history of reindeer grazing and exclusion. Our results indicate that reindeer grazing alter fungal community structure and litter degradation related enzyme activities in the northern boreal forest soils.

1. Introduction

Semi-domesticated reindeer (*Rangifer tarandus* L.) are the most numerous large mammalian herbivores in the northern boreal zone, especially in areas where their husbandry is practiced. Currently, there are on average 200 000 reindeer (number in winter after culling) (Suominen and Olofsson 2000) grazing freely in Finnish Lapland, on an area covering one third of Finland. The reindeer density is on average 1.5 individuals per km² (Susiluoto et al., 2008; Turi, 2002), although the density varies between reindeer herding districts and are above the ecosystem's carrying capacity in many areas in Finnish Lapland (Suominen and Olofsson 2000, Kumpula et al. 2014).

Reindeer are strongly affecting the forest floor vegetation in northern latitudes and their direct and indirect consequences on

ecosystems are important to understand. Compared to many other large mammalian grazers, reindeer feed on only a minor part of net primary production (NPP) but alter the vegetation structure by selective grazing and trampling. The main impact of grazing on forest floor vegetation is the reduction of lichen biomass (den Herder et al., 2003; Köster et al., 2015b; Stark et al., 2000; Susiluoto et al., 2008). A reduction of tree regeneration, total ground vegetation biomass, mosses and vascular plants in forested ecosystems has also been observed (Köster et al., 2013, 2015b; Olofsson et al., 2010). Furthermore, reindeer affect plant diversity and coverage, change soil microclimate, moisture conditions, nutrient cycling and organic matter decomposition (Akujärvi et al., 2014; Köster et al., 2013, 2017, 2015b; Olofsson et al., 2004; Stark et al., 2010; Vowles et al., 2017; Väre et al., 1996).

The impact of grazing on ground vegetation, soil processes and

* Corresponding author at: Department of Microbiology, Faculty of Agriculture and Forestry, P.O. Box 56 (Viikinkaari 9), FI-00014, University of Helsinki, Finland.
E-mail address: minna.santalahti@helsinki.fi (M. Santalahti).

microbial communities have mainly been studied in open tundra, and less is known about the potential effects of reindeer grazing in the boreal forest ecosystem. Grazing reduced lichen mat cover resulting in faster soil warming in spring and higher soil temperatures during growing season, which can enhance Scots pine (*Pinus sylvestris*) growth (Fauria et al., 2008). On the other hand, reduced lichen mat can increase soil moisture fluctuation and decrease plant growth (Olofsson et al., 2010). Grazing can also decrease soil microbial respiration and activity in boreal forests humus, which was suggested to result in decreased decomposition rate (Väre et al. 1996, Stark et al. 2003). In Alaskan taiga, mammalian browsers (moose and snowshoe hares) were observed to significantly reduce the annual production, decomposition and survival of fine roots (Ruess et al., 1998), and to reduce ECM infections of fine roots of willow and balsam poplar compared to exclosures protected from browsing (Rossow et al., 1997). However, in Finnish northern boreal forest, reindeer grazing was not observed to affect tree root biomass (Köster et al., 2013, 2015b) and field layer respiration was found to be higher on grazed sites compared to non-grazed sites due to higher soil temperatures (Köster et al., 2017, Köster et al., 2018).

Reindeer lichens (*Cladonia* spp.) have been reported to produce allelopathic extracts that can inhibit the growth of many mycorrhizal fungi, including mycorrhiza of Scots pine and dwarf shrubs in pure and axenic synthesis cultures (Brown and Mikola, 1974). Since mycorrhiza formation is essential for nutrient uptake of woody plants in boreal ecosystems (Smith and Read, 2008), growth of pine seedlings may be positively affected by reindeer grazing due to reduction of lichen cover. Lichen extracts were found to reduce seed germination and mycorrhizal colonization percentages of roots on pine seedlings in a greenhouse experiment (Sedia and Ehrenfeld, 2003). In contrast, Kytöviita and Stark (2009) demonstrated that in microcosms pure usnic acid, which is the most abundant secondary metabolite of the lichen *Cladonia stellaris*, or lichen fragments did not retard the growth of pine seedlings or negatively affect the plant nutrient uptake. In addition, usnic and perlatolic acids produced by *C. stellaris* lichens showed no evidence of antimicrobial activities, as these substances did not leach out from the lichen thallus to the rainwater, inhibit microbial respiration or enrich in soil underneath the lichen mat (Stark et al., 2007). However, to our knowledge, no field studies under natural conditions have been carried out to confirm or contest these findings in lichen rich boreal forests.

Only a few published studies have investigated the effects of reindeer grazing on soil microbial communities in northern boreal ecosystems. Generally, these studies have used phospholipid fatty-acid analysis (PLFA) and concluded that grazing had no clear effect on microbial community structure or biomass (Bardgett et al., 1996; Francini et al., 2014; Rinnan et al., 2009; Stark et al., 2010, 2008). As PLFA is not a highly sensitive method to describe microbial, and especially fungal community changes, current methods based on new sequencing technologies may therefore better reveal these patterns. Vowles et al. (2018) studied mycorrhizal communities in Scandies Mountain Range with ingrowth bags and found that although grazing did not affect the diversity of mycorrhizal species, it decreased extramatrical mycelial biomass and the abundance of genus *Cortinarius* in mountain birch forest site. Thorough, sequencing-based analysis on the effects of reindeer grazing on soil fungal communities are still lacking, especially from the northern boreal forests.

With variety of extracellular enzymes, fungi are the predominant decomposers of plant residues and soil organic matter (SOM) (Lundell et al., 2014; Rytioja et al., 2014). In mesotrophic tundra heat in northernmost Norway, microbial respiration, β -glucosidase, acid-phosphatase and leucine-aminopeptidase activities were higher and N-acetylglucosaminidase activity was lower in heavily grazed areas compared to the lightly grazed areas (Stark and Väisänen, 2014). Sedia and Ehrenfeld (2006) tested litter decomposition rates and soil enzyme activities under lichen and moss covers and found that the decomposition rate tended to be slower underneath lichen than moss cover.

In the present study, our objectives were to describe the fungal community structure and to estimate the overall, long-term impact of reindeer grazing on soil fungal communities in grazed and non-grazed sites in northern boreal forest. Furthermore, our objectives were to show how grazing affects microbial communities' functionality by comparing the litter decomposition rates and extracellular enzyme activities between the grazed and non-grazed sites. To study these aims, we had four unique natural forest areas separated by 20, 55 or 100 years old fences, creating sites with and without reindeer grazing side by side. This allowed us to test whether in long-term reindeer grazing as a whole is a determining factor that shape the soil fungal communities in natural environment. We hypothesized that vegetation changes caused by reindeer grazing alter fungal community structure and accelerates the decomposition rate of litter. We also hypothesized that ECM fungi would be more abundant in the grazed than non-grazed sites, due to the allelopathic substances secreted by *Cladonia* lichens (Brown and Mikola, 1974). To our knowledge, this is the first study to document long-term changes in fungal communities caused by reindeer grazing in northern boreal forest by using high-throughput sequencing.

2. Materials and methods

2.1. Sites and sampling

Our study areas were located in northern boreal coniferous forests at close vicinity of ICOS/SMEAR I station in Värriö (67°46'N, 29°35'E) and the Arctic Research Centre of the Finnish Meteorological Institute in Sodankylä (67°21'N, 26°38'E). We had four sampling areas: Nuortti 1, Nuortti 2 and Kotovaara in Värriö and one area in Sodankylä. All areas were non-managed, nature reserve forests with the average age of the dominant trees in Nuortti 1 and Nuortti 2 approx. 100 years, Kotovaara approx. 65 years and Sodankylä approx. 70–80 years. All areas had a fence that excluded all reindeer, dividing the areas to the grazed and non-grazed sites. Nuortti 1 and 2 were located close to the border of Finland and Russia and the reindeer fence along the border-line has prevented Finnish reindeer from going to Russian side for approx. 100 years. Kotovaara and Sodankylä areas were located close to the research stations and have been fenced for around 20 and 55 years, respectively. The size of the exclosures were 90 × 110 m in Kotovaara and 40 × 50 m in Sodankylä (Supplementary Fig. S1). The distance between Nuortti 1 and Nuortti 2 areas was approx. 1 km, Nuortti areas and Kotovaara approx. 7 km, and Kotovaara and Sodankylä approx. 150 km.

The soils in the Värriö study areas were classified as haplic podzol (FAO, 1990) on sand tills, where the bulk of the mineral soil consisted of sand (Köster et al., 2017). The study sites belonged to the Pohjois-Salla reindeer herding district, with approx. 2.2 reindeer per km² (Turunen et al., 2016). In Sodankylä, the soil was haplic podzol on glaciofluvial sandy deposit. The soil properties of each area are presented more detail in Supplementary Table S1. The main tree species in the study areas were Scots pine (*Pinus sylvestris* L.) and the ground vegetation consisted mainly of shrubs (mostly *Vaccinium vitis-idaea* L., *Empetrum nigrum* L. and *Calluna vulgaris* L.) and various mosses. *Cladonia* lichens were abundant on the sites where reindeer grazing was excluded (Köster et al., 2013, 2015b). The vegetation biomass and tree distribution of each area are presented more detail in Supplementary Table S2. There were no underlying permafrost in the areas.

The sampling followed a split plot experiment with four different whole plots (Sodankylä, Nuortti 1, Nuortti 2 and Kotovaara) described in Köster et al. (2013, 2015b, 2017), referred here as study areas. The Sodankylä, Nuortti 1, Nuortti 2 and Kotovaara constituted of 5, 4, 6 and 4 subplots, respectively (Supplementary Fig. S1). These subplots were divided into grazed and non-grazed sites with pre-established fences. The subplots were assigned in a systematic manner into even areas with no visible differences in stand structure, soil or topography within each whole plot. The distance between each subplot was approx. 50 m and

each replicate approx. 2 m.

Soil samples were collected in June 2013 from the humus horizon, from a 0.25 m by 0.25 m quadrat area after removing the litter. For each sample, two adjacent humus samples were taken and pooled. The samples were transferred into 1.5-ml Eppendorf vials, frozen at -180°C in liquid nitrogen within a few hours after collection and transported to the laboratory on dry ice. In total, we had 38 pooled soil samples from four sampling areas, with 19 grazed and 19 non-grazed samples. The number of replicate DNA samples from Nuortti 1, Nuortti 2, Kotovaara and Sodankylä were 5, 4, 6 and 4, respectively.

2.2. Litterbag experiment and enzyme activity measurements

For the litter bag experiment, Scots pine needle litter from a pine stand from Hyytiälä ($61^{\circ}51'\text{N}$, $24^{\circ}17'\text{E}$) Finland were collected in year 2012 using litter traps. The collected litter was air dried at room temperature until a constant mass was reached. The litter bags ($7\text{ cm} \times 10\text{ cm}$) were made of 0.2 mm nylon mesh that prevented plant roots but allowed fungal hyphae to penetrate in the litter bags (Köster et al., 2015a). Approx. 5 g of dried litter were placed in the bags and the bags were buried into the organic horizon in year 2013 to the same grazed and non-grazed study areas in Värriö. Litter bags were harvested one year later and frozen after collection. Before the analysis, samples were homogenized, weighed and a subsample was taken for the enzyme activity measurements. The rest of the litter was dried in an oven at 50°C until a constant mass was reached to measure the litter dry weight and moisture content. Mass loss from the litter was determined as the difference between the initial (in 2013) and final (in 2014) dry weights of the litter.

The activities of extracellular enzymes related to organic matter degradation were measured as described in Heinonsalo et al. (2012). Shortly, approx. 0.2 g of needle litter was placed in triplicates into 1.5 ml microcentrifuge tube (0.22 μm filter) (Corning INC., NY, USA), 100 μl of sterile distilled water was added and incubated in $+4^{\circ}\text{C}$ for 1 h. The samples were centrifuged at $16\,000 \times g$ in $+4^{\circ}\text{C}$ for 30 min. Supernatant was collected and pooled from the three subsamples and diluted to 3 ml volume using distilled water. The activities of leucine amino peptidase (EC 3.4.11.1), β -xylosidase (EC 3.2.1.37), β -glucuronidase (EC 3.2.1.31), cellobiohydrolase I (CBH I) (EC 3.2.1.91), N-acetylglucosaminidase (EC 3.2.1.14), β -glucosidase (EC 3.2.1.21), and acid phosphatase (EC 3.1.3.2) were detected with fluorescent based assay. The reactions were carried out at $+22^{\circ}\text{C}$, at pH 4.5, or pH 6.5 for leucine aminopeptidase (Courty et al., 2005), with incubation times described in Pritsch et al. (2011). The fluorescence was measured with Victor³ plate reader (Perkin Elmer) and the obtained fluorescence reads were compared against standard curves prepared from 4-methylumbelliferone, or aminomethylcoumarin in case of leucine aminopeptidase. Four technical replicates were measured from each sample. Background fluorescence of the individual samples were determined with the method described in Adamczyk et al. (2016) and the activities were calculated to $\text{pmol min}^{-1} \text{g}^{-1}$. Laccase (EC 1.10.3.2) activities were measured with colorimetric assay in $+22^{\circ}\text{C}$, pH 4.5 using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as a substrate (Pritsch et al., 2011) with Infinite M200 Plate Reader (Tecan, Switzerland), and the activities were calculated to $\text{pmol min}^{-1} \text{g}^{-1}$. All the substrates and standards were purchased from Sigma-Aldrich (St. Louis, USA).

2.3. Fungal community analysis

DNA was extracted from 0.1 g (fresh weight) of humus soil with Mobio PowerSoil DNA extraction kit (Mo Bio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. Bead beating was conducted with FastPrep instrument (MP Biomedicals, LLC, France) with speed 4 m s^{-1} for 30 s. DNA was further purified using GeneClean Turbo -kit (MP Biomedicals, LLC, France). DNA was quantified using

NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the concentrations were adjusted to $10\text{ ng } \mu\text{l}^{-1}$.

Fungal ITS2 region was amplified with gITS7 and ITS4 primers (Ihrmark et al., 2012), containing the appropriate 454 pyrosequencing A- and B-adapters and a unique 6-bp barcode sequences (Supplementary Table S3). The PCR was conducted in 25- μl volume, using Phusion high-fidelity DNA polymerase (Thermo Scientific, Vantaa, Finland) with 30 ng of template DNA. All samples were amplified as triplicates and the amplification was conducted as in Santalahti et al. (2016). The triplicate PCR products were pooled and purified using Agencourt AMPure XP beads (Beckman Coulter, Bernried, Germany). DNA concentrations were determined and 100 ng of each amplicon sample were combined into the sequencing library. The sequencing was conducted in the Institute of Biotechnology at the University of Helsinki using 454 GS-FLX Titanium protocol (454 Life Sciences/Roche Diagnostic, CT, USA). The sequence data are available in the European Nucleotide Archive at the European Bioinformatics Institute under study PRJEB21587 with accession numbers ERS1810502-ERS1810541.

The sequencing data were analyzed using mothur (v. 1.33.3) pipeline (Schloss et al., 2009), and the modified protocol for fungi was used as in Santalahti et al. (2016). Shortly, the sequences were trimmed and quality checked. Sequences containing any ambiguous (N) bases, homopolymers longer than eight nucleotides, or the average Phred quality score lower than 25 were discarded. All sequences were truncated to 200 bp after removing the primers and barcode sequences. Sequences were pre-clustered with 1 bp distance using a pseudo-single linkage algorithm in mothur (Huse et al., 2010) to remove sequences resulting from pyrosequencing errors. Chimeric sequences were removed using mothur-embedded UCHIME (Edgar et al., 2011). Unique sequences were pairwise aligned using the Needleman method (Needleman and Wunsch, 1970) and the obtained distance matrices were clustered into operational taxonomic units (OTUs) using the average neighbor algorithm with 97% similarity. All global singletons were omitted due to their uncertain origin (Tedersoo et al., 2010). The sequence counts were normalized as relative abundance data within each sample by dividing the read count of each OTU by the library size obtained from the sample.

For each OTU, the representative sequences were assigned to taxa with an 80% confidence threshold using the naïve Bayesian classifier (Wang et al., 2007) and the mothur-formatted UNITE taxonomy reference database (UNITE + INSD, version 6, released 2015-03-02) in mothur. Non-fungal OTUs were removed from further analysis. For calculating the diversity estimates, the sequence data were randomly subsampled to 2500 reads per sample to ensure comparable diversity estimators across the samples (Gihring et al., 2012) using the rarefy_even_depth function in phyloseq package (McMurdie and Holmes 2013) in R software version 3.3.3 (R Core Team 2017). The diversity estimates of observed species richness (Sobs), estimated species richness (Chao1) (Chao, 1984) and inverse Simpson index (InvSimpson, $1/D$) were further calculated using estimate_richness function.

To calculate the number of shared and unique OTUs, the OTU-data was presence/absence transformed. The percentages of the shared and unique OTUs were calculated between the grazing treatments or sampling areas, and the data were visualized using venn diagram. Fungal OTUs were further categorized into ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), saprotrophic (SAP) and 'other' groups based on their functional role, by annotating the OTUs against the FUNGuild database (Nguyen et al. 2016). The group 'other' contained all other functional groups than ECM, ERM and SAP, such as endophytic, pathogenic, parasitic and lichenized fungi. Those OTUs that could not be assigned to any groups were named 'unassigned'.

2.4. Statistical analysis

Two-way ANOVA was used to analyze the statistical differences between the grazing treatments and the sampling areas for each diversity estimates (Sobs, Chao1 and Inverse Simpson index), and mass loss percentage from the needle litter. The percentage data was arcsine transformed to meet the criteria for two-way ANOVA. In the analysis, grazing was used as fixed and area as random factor. Two-way ANOVA was performed using software IBM SPSS Statistic 22 and $P \leq 0.05$ was set as the limit for statistical significance.

The effect of area and grazing for the fungal communities and each fungal phylum were tested using multivariate analysis of variance (MANOVA) from the normalized sequence counts with adonis-function in vegan package (Oksanen et al., 2017) in R software (R Core Team 2017). Statistical significance of the grazing effect for the relative abundances of the most abundant genera and species, ecological groups, and extracellular enzyme activities were tested with non-parametric Kruskal-Wallis test using IBM SPSS Statistic 22.

Canonical correspondence analysis (CCA) was used to explore the relationship between the fungal community structure and driving environmental variables (all numerical variables from Supplementary Tables S1 and S2) measured in earlier studies from the same reindeer grazing sites (Köster et al. 2013, 2015b) using cca-function from vegan package (Oksanen et al., 2017) in R (R Core Team 2017). For the analysis, both relative abundance sequence data and environmental data were logarithmic normalized with the decostand-function and the 300 most abundant OTUs were used in the analysis. The environmental variables were chosen for the CCA by model building using ordistep-function in vegan package. The cross-correlation between the selected variables in the final model were further tested with the vif.cca-function in vegan (variance inflation factor, $VIF < 4$). The statistical significance of each environmental variable and CCA-axis were tested with anova.cca function with 999 permutations. The species scores of each OTU were selected from the model using ordiselect function in goevg-package, and those OTUs which were 80% of the most abundant and 15% of the best fit, were visualized in the CCA.

CCA was also used to explore the relationship between the extracellular enzyme activities and explanatory variables from the needle litter using vegan package (Oksanen et al., 2017). Moisture content and mass loss measured from the litter bags were used as explanatory variables. Before the analysis, the enzyme activity data were normalized by dividing each value by the total sum of the enzyme activities across all the studied samples with the decostand-function and the total-method. CCA was conducted similarly as for the sequencing data. The species scores of individual enzymes were shown and the visualized CCA was scaled according to the site scores (scaling = 1). The grouping of the variables was further visualized with ellipses representing the 75% confidence intervals using ordiellipse function from vegan package (Oksanen et al., 2017).

3. Results

3.1. General information on the pyrosequencing data, species richness and diversity

The 454 pyrosequencing of the 38 samples resulted in total of 213 905 high quality fungal sequences (without singletons) that were assigned to 1 186 OTUs with 97% similarity. The grazed and non-grazed sites shared 52.8% of all OTUs, and 21.1% and 26.1% of all OTUs were unique to the sites, respectively (Supplementary Fig. S2a). The four areas Sodankylä, Nuortti 1, Nuortti 2 and Kotovaara shared 9.7% of all OTUs, and 14.8%, 7.6%, 17.5% and 6.9% OTUs were unique for the areas, respectively (Supplementary Fig. S2b). The sequencing effort of all samples are presented as rarefaction curves in Supplementary Fig. S3. Overall, reindeer grazing increased the estimated species richness (Chao1) ($P \leq 0.01$, with two-way ANOVA), but

Table 1

Diversity estimates for each study area and grazing treatment. All diversity estimates, observed (Sobs) and estimated (Chao1) species richness and diversity with the inverse Simpson index (InvSimpson) were calculated from the sub-sampled data (2500 sequences for each). Chao1 was statistically higher in the non-grazed compared to the grazed sites ($P \leq 0.01$ with two-way ANOVA). There were no statistical differences between the grazed and non-grazed sites for Sobs and InvSimpson. SD indicates for standard deviation.

Area	Grazing	n	Sobs \pm SD	Chao1 \pm SD	InvSimpson \pm SD
Sodankylä	grazed	5	100 \pm 10	136 \pm 11	8.8 \pm 4.5
Sodankylä	non-grazed	5	113 \pm 12	150 \pm 16	13.6 \pm 4.8
Nuortti 1	grazed	4	82 \pm 8	99 \pm 15	7.7 \pm 3.8
Nuortti 1	non-grazed	4	77 \pm 8	113 \pm 18	4.9 \pm 2.1
Nuortti 2	grazed	6	95 \pm 24	128 \pm 30	8.9 \pm 5.2
Nuortti 2	non-grazed	6	100 \pm 15	135 \pm 18	6.5 \pm 4.6
Kotovaara	grazed	4	88 \pm 14	121 \pm 18	5.2 \pm 2.6
Kotovaara	non-grazed	4	100 \pm 8	141 \pm 34	7.9 \pm 0.8

had no effect on observed OTUs (Sobs) or fungal diversity (Inverse Simpson's index $1/D$) (Table 1).

3.2. Litter mass loss and enzyme activity

In the litter bag experiment, the needle litter had lost 25–30% of the mass after being buried underneath the humus horizon for one year. The mass loss varied between each study area, but did not differ significantly between the grazed and non-grazed sites.

Grazing significantly affected the extracellular enzyme activities measured from the needle litter, and was the most important individual parameter explaining the variation in the data ($P \leq 0.001$, $F = 7.9676$, anova.cca) in the CCA (Fig. 1). Grazing increased CBH I activities ($P \leq 0.05$, with Kruskal-Wallis) and decreased laccase activities ($P \leq 0.05$) (Supplementary Table S4). The activities of β -xylosidase, β -glucuronidase, N-acetylglucosaminidase, β -glucosidase and acid phosphatase did not differ significantly between the grazed and non-grazed

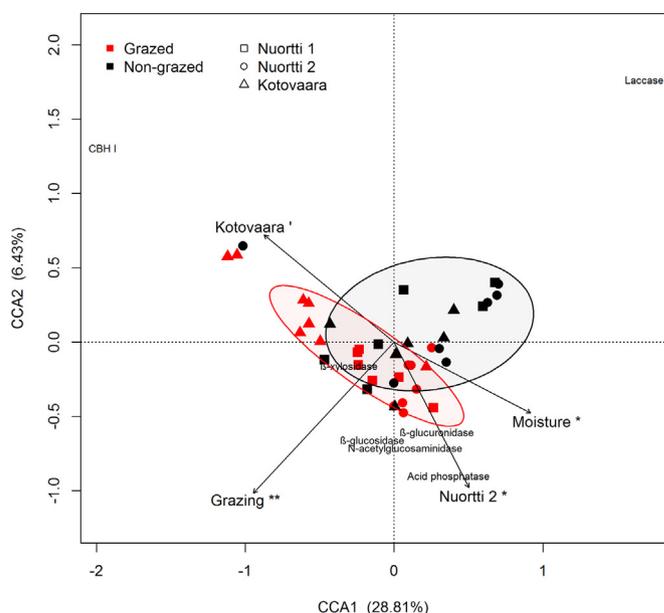


Fig. 1. CCA showing the differences in the enzyme activities between each sampling area and grazing treatment. Normalized enzyme activities were used. CCA 1 represents the constrained ordination of the data with 28.81% ($P \leq 0.001$, $F = 15.4715$) and CCA 2 with 6.43% ($P \leq 0.05$, $F = 3.4535$) of the total variation. The grazing effect is further visualized with ellipses representing the 75% confidence intervals. The species scores of individual enzymes are presented and the significance of each explanatory variable are calculated with anova.cca and marked with asterisk (** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.1$).

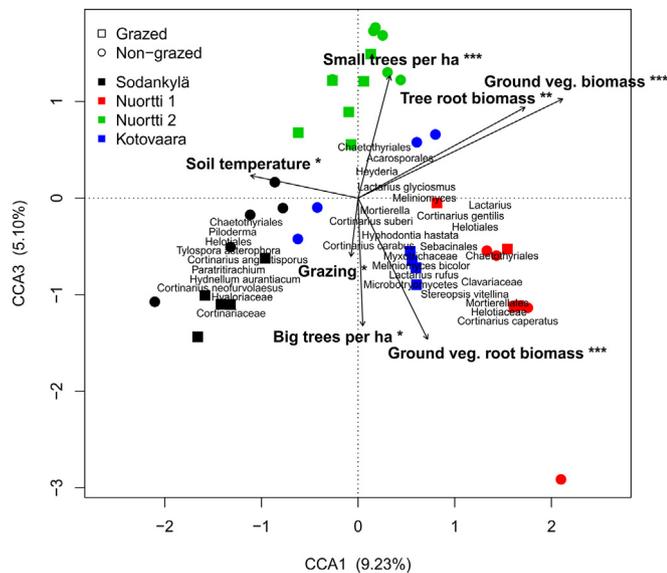


Fig. 2. CCA showing the differences in the fungal community structure between each sampling area and grazing treatment with environmental parameters as explanatory variables. Logarithmic normalized data from the 300 most abundant OTUs were used. CCA 1 represents the constrained ordination of the data with 9.23% ($P \leq 0.001$, $F = 4.0281$) and CCA 3 with 5.10% ($P \leq 0.001$, $F = 2.2232$) of the total variation of each axis. The species scores of those OTUs which were 80% of the most abundant (from the top 300 OTUs) and 15% of the best fit are presented and the significance of each environmental parameter are calculated with *anova.cca* and marked with asterisk (***) $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$). The significance of each environmental parameter are presented in Table 2.

sites (Supplementary Table S4), but the overall pattern of these enzymes were associated to the grazed sites in the CCA. Within the Nuortti 2 area, the activities of β -xylosidase, N-acetylglucosaminidase ($P \leq 0.05$, for both) and β -glucuronidase ($P \leq 0.1$) were higher in the grazed compared to the non-grazed side (Supplementary Table S4). Moisture content of the needle litter was a significant environmental parameter explaining the variation in the enzyme activity data ($P \leq 0.05$, $F = 4.5236$, with *anova.cca*), (Fig. 1). Mass loss was not a significant parameter and was discarded from the analysis.

3.3. Fungal community structure

Each study area harbored unique fungal community, as the CCA showed that the fungal communities within each area clustered together (Fig. 2). Grazing was significant parameter explaining the variation in the sequence data ($P \leq 0.05$, $F = 1.3072$, *anova.cca*), and the fungal communities of the grazed and non-grazed sites mainly clustered separately within each area.

In the model building for the environmental variables, ground vegetation biomass, number of small trees (lower than 1.3 m) per hectare, ground vegetation root biomass, tree root biomass, number of big trees (higher than 1.3 m) per hectare and soil temperature were significant variables explaining the data and were selected in the final model. Further analysis showed that the selected variables significantly explained the variation in the fungal community structure (Fig. 2, Table 2). Ground vegetation biomass, number of small trees per hectare and ground vegetation root biomass were the most significant variables explaining the variation in the fungal community structure ($P \leq 0.001$, *anova.cca*), followed by tree root biomass ($P \leq 0.01$), and number of big trees, soil temperature and grazing ($P \leq 0.05$), (Table 2).

Table 2

The significance of the environmental parameters explaining the variation in the fungal community structure (Fig. 2). The significance of each parameter are marked with asterisk (***) $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

	Df	ChiSquare	F	Pr(> F)	Sig.
Grazing	1	0.0868	1.3072	0.027	*
Ground vegetation biomass	1	0.22768	3.4288	0.001	***
Number of small trees per ha	1	0.14785	2.2265	0.001	***
Tree root biomass	1	0.10083	1.5184	0.009	**
Ground vegetation root biomass	1	0.15974	2.4056	0.001	***
Number of big trees per ha	1	0.09088	1.3686	0.019	*
Soil temperature	1	0.09121	1.3736	0.025	*
Residual	30	1.99208			

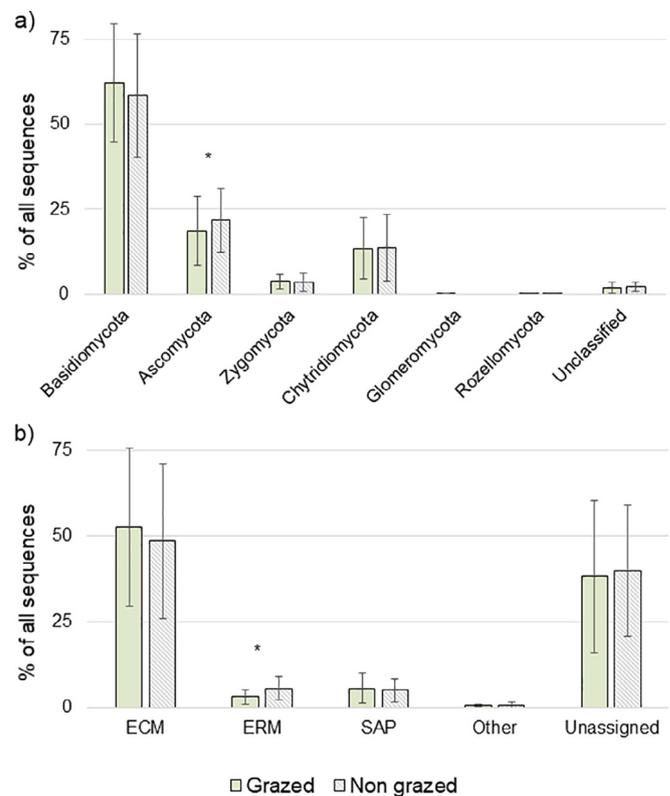


Fig. 3. Relative abundance of fungal phyla and ecological groups in the grazed and non-grazed sites. a) Combined picture of fungal phyla from all sites included b) Combined picture of fungal functional groups from all sites included. 'ECM' indicates ectomycorrhiza, 'ERM' ericoid mycorrhiza, 'SAP' saprotroph and 'other' mainly parasitic, pathogenic or endophytic ecology. OTUs belonging to 'unassigned' could not be assigned to any functional group. Statistical significance of the grazing effect for each phylum and ecological group with Kruskal-Wallis. Statistically significant differences are marked with asterisk (* $P \leq 0.05$). The error bars indicates for standard deviation, $n = 19$.

3.4. Fungal community structure on phylum, genus and species level

Fungal OTUs were classified into six fungal phyla. Basidiomycota were the most abundant phylum in all grazed and non-grazed areas, with on average 60.3% of all sequences and 37% of all OTUs (Fig. 3a, Supplementary Table S5). Ascomycota were the second most abundant phylum with 20.2% of all sequences and 30.8% of all OTUs (Fig. 3a). Ascomycota was the only phylum which had statistically significant difference for grazing ($P \leq 0.05$, $F = 1.9644$ with MANOVA) and area ($P \leq 0.001$, $F = 3.7727$ with MANOVA). Chytridiomycota covered 13.5% of all sequences and 2.7% of all OTUs (Fig. 3a). Former phylum of Zygomycota covered 3.7% of all sequences and 3.5% of all OTUs

Table 3

The relative abundance of the most abundant genera and species in the grazed and non-grazed sites. Statistical significance of the grazing effect are calculated with non-parametric Kruskal-Wallis test and marked with asterisk ($^*P \leq 0.05$, $^*P \leq 0.1$). SD indicates for standard deviation, n = 19.

Genus	Abundance of OTU (%)	
	Grazed \pm SD	Non-grazed \pm SD
<i>Cortinarius</i>	24.34 \pm 26.74	25.22 \pm 26.52
<i>C. semisanguineus</i>	2.48 \pm 5.60	1.74 \pm 3.61
<i>C. caperatus</i>	0.02 \pm 0.06	3.47 \pm 14.70
<i>C. coleoptera</i>	1.18 \pm 2.65 [*]	0.15 \pm 0.58
<i>C. pseudoduracinus</i>	0.04 \pm 0.15 [*]	0.09 \pm 0.17
<i>C. brunneus</i> var. <i>glandicolor</i>	0.11 \pm 0.27 [*]	0.01 \pm 0.04
<i>Piloderma</i>	10.00 \pm 10.23	10.31 \pm 9.30
<i>P. sphaerosporum</i>	8.20 \pm 9.74	9.80 \pm 9.02
<i>Suillus</i>	3.60 \pm 7.44	8.46 \pm 13.90
<i>S. variegates</i>	3.58 \pm 7.41	8.43 \pm 13.85
<i>Lactarius</i>	7.50 \pm 17.48	2.58 \pm 8.69
<i>L. rufus</i>	5.93 \pm 17.47	2.13 \pm 8.69
<i>L. musteus</i>	1.37 \pm 2.85 [*]	0.42 \pm 1.29
<i>Rhizoscyphus</i>	2.34 \pm 1.87 [*]	4.13 \pm 2.86
<i>R. ericace</i>	2.29 \pm 1.82 [*]	4.01 \pm 2.80
<i>Mortierella</i>	2.90 \pm 1.77	3.09 \pm 2.36
<i>Hydnellum</i>	3.37 \pm 7.77	0.23 \pm 0.96
<i>Hygrophorus</i>	1.41 \pm 4.56	0.26 \pm 0.95
<i>Meliniomyces</i>	0.53 \pm 0.38	0.94 \pm 0.82
<i>Russula</i>	0.03 \pm 0.11	1.28 \pm 3.80
<i>Oidiodendron</i>	0.32 \pm 0.23 [*]	0.56 \pm 0.38
<i>Mycena</i>	0.31 \pm 0.87	0.44 \pm 0.99

(Supplementary Table S5). Glomeromycota and Rozellomycota were found in minority (0.02% and 0.03% of all sequences, respectively). Sequences that could not be classified into phylum level covered 2.2% of all sequences and 25.3% of all OTUs.

ECM fungi dominated the humus communities in both grazed and non-grazed sites with approx. 50% of all sequences, as ERM, SAP and ‘other’ fungi were less abundant (4.3%, 5.3% and 0.6%, respectively) groups (Fig. 3b). Approx. 39% of all sequences could not be assigned to any functional group. The abundance of ERM fungi were higher in the non-grazed sites ($P \leq 0.05$, with Kruskal-Wallis), and the abundance of other functional groups were similar in the grazed and non-grazed sites.

At genus level, *Cortinarius* was the most abundant genus with 24.8% of all sequences, followed by *Piloderma* (10.2% of all sequences), *Suillus* (6%), *Lactarius* (5%), *Rhizoscyphus* (3.2%), *Mortierella* (3%) and *Hydnellum* (1.8%). Grazing affected the abundance of certain fungal genera, but the differences were more obvious in the youngest enclosure areas of Kotovaara and Sodankylä (Table 3, Supplementary Table S5). *Rhizoscyphus* and *Oidiodendron* were more abundant in the non-grazed compared to the grazed sites ($P \leq 0.05$ and $P \leq 0.1$, respectively with Kruskal-Wallis). Within the youngest exclusion area of Kotovaara, the abundance of *Lactarius* was higher and the abundance of *Rhizoscyphus*, *Meliniomyces* and *Umbellopsis* were lower in the grazed side compared to the non-grazed side ($P \leq 0.05$, for each) (Supplementary Table S5).

Up to 24 *Cortinarius* species were identified, and their abundances varied between the grazed and non-grazed sites and the study areas (Supplementary Table S5). *C. semisanguineus* was the most abundant *Cortinarius* species and was present in both grazed and non-grazed sites and all study areas (Table 3, Supplementary Table S5). The second most abundant species *C. caperatus* was only found in the Nuortti 1 area and mainly in the non-grazed side. *C. coleoptera* and *C. brunneus* were more abundant in the grazed sites and *C. pseudoduracinus* was more abundant in the non-grazed sites ($P \leq 0.05$, for all). *C. traganus*, *C. testaceofolius* and *C. carabus* were only found in the grazed sites. In the CCA, *C. angustinusporus* and *C. neofurvolaeus* associated with Sodankylä area, *C. caperatus* and *C. gentilis* associated with Nuortti 1 area, and *C. suberi* and *C. carabus* associated with Kotovaara area (Fig. 2).

The abundance of other fungal species also varied between the grazed and non-grazed sites (Supplementary Table S5). *Rhizoscyphus ericace* (with 97% of *Rhizoscyphus* sequences) was more abundant in the non-grazed sites ($P \leq 0.05$). *Lactarius rufus* and *L. musteus* (with 82% and 15% of all *Lactarius* sequences, respectively) were generally more abundant in the grazed sites ($P \leq 0.1$, for *L. musteus*). *Russula decolorans*, *R. altorubens* and *R. vinosa* were mainly found in the Nuortti 1 and Kotovaara non-grazed sites. In the CCA, *Hydnellum aurantiacum* and *Tylospora asterophora* associated with the Sodankylä area in the CCA (Fig. 2). *L. rufus* and *Meliniomyces bicolor* associated with the Kotovaara grazed site, and *Lactarius glyciosmus* associated with the non-grazed site. OTUs belonging to Mortierellales, Helotiales and Chaetohyriales associated with the Nuortti 1 area.

4. Discussion

To our best knowledge, our field study is the first to describe detailed fungal community structure in sites with long-term reindeer grazing and exclusion in northern boreal coniferous forests using high-throughput sequencing. Overall, reindeer grazing had significant impact on soil fungal community structure and activity of enzymes related to litter degradation, even though the decomposition rate of litter was not affected. Grazing increased the estimated species richness but had no significant effect on soil fungal diversity. Vegetation changes caused by reindeer grazing altered the fungal community structure, as multiple grazing related environmental variables significantly explained the variation of the fungal communities. On a phylum level, grazing decreased the abundance of Ascomycota and ERM fungi. Contrarily to our hypothesis, the abundance of ECM fungi was similar in the grazed and non-grazed sites and was not affected by the presence of lichens. Grazing also affected the abundance of certain fungal genera and species, but the effect was more visible in the youngest exclusion areas of Kotovaara and Sodankylä. Our study sites were located in four areas within 150 km from each other, thus our data provides a representative insight into the reindeer grazing effects in northern boreal forests in Finland.

4.1. Impact of reindeer grazing on litter decomposition and enzyme activities

Fungi produce wide range of extracellular enzymes, and are the most efficient organic matter decomposers in boreal areas. Mass loss of the needle litter was approx. 25–30%, and did not differ significantly between the grazed and non-grazed sites, after being buried in organic horizon for one year. Our results are consistent with the previous studies by Stark et al. (2010, 2002) showing that no clear grazing-related differences in litter decomposition rate were found in northern boreal forest. Contradicting result have also been obtained as reindeer grazing retarded the decomposition rate of *V. myrtillus* leaves in Scots pine forest in Finland (Stark et al., 2000) and fine roots in Alaskan taiga (Ruess et al., 1998), and accelerated litter decomposition rate and nutrient availability in tundra heat in northern Norway (Olofsson et al., 2004).

In our study, reindeer grazing generally enhanced the extracellular enzyme activities related to cellulose and hemicelluloses degradation and nutrient acquisition from the needle litter. Grazing significantly enhanced cellulose chain hydrolyzing CBH I activity. In addition, the activities of β -xylosidase, β -glucosidase, β -glucuronidase and N-acetylglucosaminidase, as well as phosphate group cleaving acid phosphatase activities were associated to the grazed sites in the CCA. Previously, the activities of β -glucosidase, acid phosphatase and leucine-aminopeptidase activities were higher and N-acetylglucosaminidase was lower in heavily than lightly grazed areas in a mesotrophic tundra heat (Stark and Väisänen 2014). They further suggested that grazing altered the enzyme activities through substrate availability for soil microorganisms (Stark and Väisänen 2014). In our

study, moisture content of the needle litter was also significant variable explaining the variation in the enzyme activities, which was in line with previous studies where soil moisture content was found to correlate positively with enzyme activities (Baldrian et al. 2010a,b).

Contrarily to other measured enzyme activities, grazing significantly decreased the activity of lignin modifying laccase. Previously, grazing was found to decrease soil microbial respiration and activity, which was suggested to result in decreased decomposition rate (Väre et al. 1996, Stark et al. 2003). Increased N availability from reindeer feces in heavily grazed areas have also been found to reduce the decomposition rate of recalcitrant organic matter (Craine et al. 2007), microbial biomass and activity of ligninolytic enzymes (Sinsabaugh 2010; Männistö et al. 2016). Although we did not measure N availability, microbial biomass was generally lower in the grazed areas (Köster et al., 2015b). Further, as several lichens (including some *Cladonia* species) have shown to possess laccase activities (Beckett et al. 2014; Zavarzina and Zavarzin 2006), we cannot rule out the possibility that the higher abundance of lichens in the non-grazed sites could have affected to the higher laccase activities on these sites, although the laccase activities from non-*Peltigeralean* lichens have generally been low (Beckett et al. 2013).

There are numerous contradictory results concerning the effects or reindeer grazing on litter and SOM decomposition rate, activity of various extracellular enzymes and soil microbial activity. It is evident that several factors are simultaneously affecting soil processes and therefore, the grazing effect should be investigated more thoroughly in a multidisciplinary manner. It is also noted that our one-year litter bag experiment might not had enough time to induce clear differences in the litter decomposition rate, even though there were clear differences in the enzyme activities between the grazed and non-grazed sites. As grazing decreases the number of young tree seedlings, it may affect the litter input, and consequently alter litter and SOM quality. Nevertheless, these processes take time as the tree growth and SOM decomposition processes in northern boreal forests are extremely slow.

4.2. Impact of reindeer grazing on soil fungal community structure

Reindeer grazing altered the fungal community structure, as grazing was significant parameter explaining the variation in the CCA. However, area was more important parameter, as the communities clustered together based on the study areas in the CCA, and all study areas harbored a unique species composition. These findings are in line with earlier studies showing that spatial heterogeneity is an important factor affecting microbial communities more than e.g. grazing (Stark et al., 2008; Sørensen et al., 2009).

The relationship between fungal community structure and driving environmental variables showed that many of the studied variables were significant and explained the variation in the fungal community structure. Previously, reindeer grazing was found to significantly reduce lichen and total ground vegetation biomasses (mainly due to the decrease in lichen biomass) (Köster et al., 2013, 2015b). Contrarily to our hypothesis, the abundance of ECM fungi in general varied inconsistently between the grazed and non-grazed sites, and the presence of lichens did not suppress most ECM fungi. The vegetation changes due to reindeer grazing were not only limited to the reduction of lichen biomass, but also to the reduction of small tree seedlings (Köster et al., 2013, 2015b). The inconsistency in the number of big trees between the treatments may have caused differences in the abundance of root and fine root biomass, and thus may also reflect to the abundance of ECM fungi. Even though the fine roots were not quantified in this study, the tree root biomass significantly explained the variation in the fungal community structure. Therefore, further studies are needed to validate this via quantifying the fine root biomass.

4.3. Genus and species level changes

Cortinarius was the most abundant genus by the number of sequences and OTUs, and the abundance of *Cortinarius* species varied between the grazed and non-grazed sites. Genus *Cortinarius* are abundant and species rich ECM fungi in boreal forest soils (Bödeker et al., 2014; Lindahl et al., 2010, 2007; Santalahti et al., 2016; Sun et al., 2015), in Arctic tundra (Deslippe et al., 2011) and in eutrophic heaths and meadows in Fennoscandia (Lye, 1975). *Cortinarius* are strongly proteolytic fungi that are adapted to the typical N-limited conditions of boreal forests and derive C from their host for biomass and extracellular enzyme production (Deslippe et al., 2011). In the Scandies Mountain Range, *Cortinarius* was found to be more abundant in non-grazed enclosures than grazed control plots (Vowles et al., 2018).

According to our previous study from Värriö, genus *Cortinarius* was clearly associated with the functional gene profile observed at the old growth forest site (Sun et al., 2015), suggesting that *Cortinarius* also are highly important genus for the functioning of the old growth forest soils in the area, close to our study sites. Furthermore, ECM *Cortinarius* species have been detected to possess oxidative enzymes, such as laccase and manganese peroxidase (MnP) (Bödeker et al., 2014; Courty et al., 2006), and it has been suggested to have significant role in the decomposition of complex SOM. Several *Cortinarius* species have been demonstrated to co-localize with high MnP activity (Bödeker et al., 2014). In our study, some species e.g. *C. caperatus*, *C. biformis* and *C. suberi* were in general more abundant in the non-grazed sites with higher laccase activity. In addition to genus *Cortinarius*, several other ECM fungi have also been demonstrated to produce various oxidative enzymes, including genera *Piloderma*, *Lactarius* and *Suillus* (Bödeker et al., 2014; Heinonsalo et al., 2012; Kohler et al., 2015; Shah et al., 2015), which all were abundant in our study sites. Since fungi possess large pool of various lignin modifying enzymes, further study to detect grazing induced differences in the patterns to modify lignin to its subunits would be needed.

Other highly abundant genera in our study were *Piloderma*, *Suillus*, *Lactarius*, and *Rhizoscyphus*, which all are typical mycorrhizal fungi of boreal forest soils (Markkola et al., 2002; Santalahti et al., 2016; Sun et al., 2015). Genus *Rhizoscyphus* and species *R. ericae*, which forms ERM with roots of *Ericaceae* plants (Smith and Read, 2008), were statistically more abundant in the non-grazed sites, even though grazing did not affect shrub biomass (Köster et al., 2013, 2015b). It is also possible that the roots and especially the fine roots of *Ericaceae* and other shrubs are more susceptible for the trampling of reindeer than e.g. tree roots that mainly are located deeper in the soil (Makkonen and Helmisaari, 1998; Helmisaari et al., 2007).

Suillus variegatus, which was the most abundant species among genus *Suillus* in our study areas, and *Piloderma croceum* has previously been reported to suffer from lichen extracts (Brown and Mikola, 1974; Markkola et al., 2002), but also stimulated by reindeer lichen *Cladonia rangiferina* (Brown and Mikola, 1974). In our study, genus *Suillus* and *Piloderma* varied inconsistently between the grazed and non-grazed sites and did not seem to suffer from lichens. In contrast, the ECM genus *Lactarius* and the most abundant species *L. rufus* and *L. musteus* were on average more abundant on the grazed sites and might have suffered from the lichens.

5. Conclusions

Our study is the first to describe detailed fungal community structure using a 454-pyrosequencing analysis in reindeer grazed and non-grazed sites in northern boreal coniferous forest soils. Reindeer grazing altered significantly the soil fungal community structures and the abundance of Ascomycota, as well as certain fungal genera and species. Furthermore, the activities of extracellular enzymes related to litter degradation were also affected by grazing, even though the measured litter mass loss did not differ between the grazing treatments after one

year. Our data indicates that with longer time scales, grazing may affect litter decomposition through changes in fungal community structure and enzyme activities in the northern boreal forest soils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.apsoil.2018.08.013>.

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