



Fungal community dynamics across a forest–alpine ecotone

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Abstract

Climate change is causing upward shift of forest lines worldwide, with consequences for soil biota and carbon (C) sequestration. We here analyse compositional changes in the soil biota across the forest line ecotone, an important transition zone between different ecosystems. We collected soil samples along transects stretching from sub-alpine mountain birch forests to alpine heath. Soil fungi and micro-eukaryotes were surveyed using DNA metabarcoding of the ITS2 and 18S markers, while ergosterol was used to quantify fungal biomass. We observed a strong shift in the soil biota across the forest line ecotone: Below the forest line, there were higher proportions of basidiomycetes and mucoromycetes, including ectomycorrhizal and saprotrophic fungi. Above it, we observed relatively more root-associated ascomycetes, including Archaeorhizomycetes, ericoid mycorrhizal fungi and dark septate endophytes. Ergosterol and percentage C content in soil correlated strongly and positively with the abundance of root-associated ascomycetes. The predominance of ectomycorrhizal and saprotrophic fungi below the forest line probably promote high C turnover, while root-associated ascomycetes above the forest line may enhance C sequestration. With further rise in forest lines, there will be a corresponding shift in the below-ground biota, probably leading to enhanced release of soil C.

KEYWORDS

climate change, forest line, mycorrhiza, saprotroph, soil carbon, soil fungi, treeline

1 | INTRODUCTION

Ecosystems all over the world are being affected by climate change (Parmesan, 2006; Pecl et al., 2017). High-latitude and high-altitude ecosystems are particularly exposed because of arctic amplification and elevation-dependent warming (Pepin et al., 2015; Serreze & Barry, 2011). In temperate and boreal regions, one of the most striking ecological transitions in high-elevation areas is the change from boreal lowland forests to alpine highlands without forests (Figure 1a). This marked ecotone, termed the forest line (here) or treeline,

varies in altitude and species composition across the globe, and has in many regions moved towards higher elevations during the last few decades due to climate warming (Beckage et al., 2008; Chen et al., 2011; Harsch et al., 2009). Although many mountain regions are influenced by land use, the uppermost alpine forest lines are mainly temperature-driven (Körner, 2012). Recent studies have also documented positive climate feedbacks from expanding alpine forest lines (de Wit et al., 2014); an increase in the elevation of alpine forest lines will probably increase the local temperature and therefore accelerate the ongoing expansion further (Rydsaa et al., 2017).

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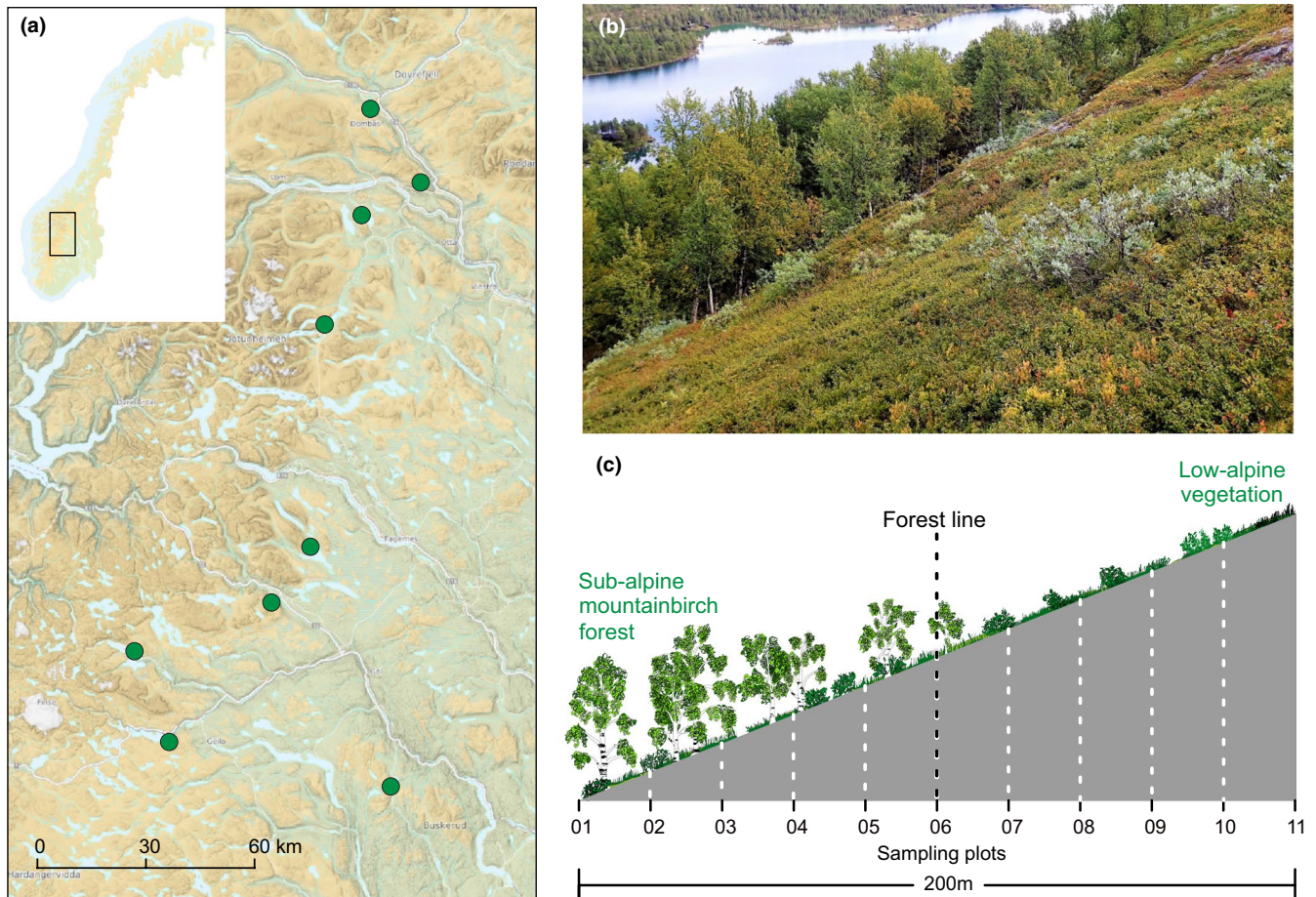


FIGURE 1 (a) The nine sampled sites in south-central Norway, from south to north: Haglebu, Ustevatn (Hardangervidda), Strandavatnet, Skyrvedalen (Hemsedal), Storlifjell (Vestre Slidre), Bessheim (Jotunheimen), Lemonsjøen (Vågå), Sel and Dombås. The map was drawn in qgis version 3.4.14, with map data from geonorge.no. (b) Photo of the abrupt vegetation change at the forest line at the Lemonsjøen site. (c) Schematic view of the studied transects stretching across the mountain birch forest line. Numbers 1 to 11 represent the plots where soil samples were collected every 20 m along the 200-m transect stretching from 100 m below to 100 m above the mountain birch forest line

In Norway, mountain birch (*Betula pubescens* subsp. *czerepanovii*) is the dominant species that forms the alpine forest lines (Bryn & Potthoff, 2018). At the forest–alpine ecotone, the vegetation is normally shrub-dominated in a transition zone, before shifting to vegetation dominated by ericaceous plant species, commonly referred to as alpine heath vegetation (Figure 1b). In Norway, an upward shift in forest lines has been observed, due to both climate warming and land use change (Bryn & Potthoff, 2018). The expansion of subalpine trees and shrubs leads to increased primary production, and thus above-ground carbon (C) fixation. However, studies from alpine and arctic regions have shown that soil organic C content is significantly higher under alpine heaths than under shrub and forest vegetation (Parker et al., 2018; Sorensen et al., 2018). Similarly, Sorensen et al. (2018) showed that C stocks were lowest beneath shrub vegetation and significantly higher beneath ericaceous plants in the alpine heath. It has been documented that litter decomposes faster in the shrub and mountain birch forest vegetation than in the alpine heath, independently of the litter decomposability, based on litter biochemistry (Parker et al., 2018).

Soil C pools are to a large extent regulated by below-ground microorganisms, including fungi (Clemmensen et al., 2013; Frey, 2019). Below-ground fungi contribute to soil C processes in various ways, and can roughly be grouped into parasites, mutualistic symbionts and saprotrophs, although there is no clear transition between nutritional strategies (Selosse et al., 2018). Saprotrophic fungi decompose and recycle dead organic matter, thus releasing C to the atmosphere. Mycorrhizal fungi form mutualistic symbioses with plant roots, where they receive freshly fixed C from their plant host in exchange for water and nutrients. Saprotrophic and mycorrhizal fungi are thus crucial components of the global C cycle, as well as the nitrogen (N) and phosphorus (P) cycles (Lindahl & Tunlid, 2015; Smith & Read, 2008; Talbot et al., 2008). Mycorrhizal fungi can be divided into functional groups depending on their plant hosts, structure and function (Smith & Read, 2008). The three main groups are arbuscular mycorrhizal (AM), ectomycorrhizal (EcM), and ericoid mycorrhizal (ErM) fungi, which establish symbiosis with mainly herbs and graminoids (AM), trees and shrubs (EcM), and ericoid plants (ErM) (Smith & Read, 2008). EcM fungi, which dominate in northern

forest ecosystems, can promote turnover of organic matter in some systems (Bodeker et al., 2014; Frey, 2019; Lindahl & Tunlid, 2015; Talbot et al., 2008) or C sequestration in others (Averill et al., 2014; Koide et al., 2014; Orwin et al., 2011). In a recent study, Clemmensen et al. (2021) suggested that certain EcM fungi were the main biological drivers for C turnover and release in a Swedish mountain birch forest. Root-associated dark septate endophytes (DSEs), which can promote plant nutrient uptake (Hill et al., 2019), are also common in alpine and arctic vegetation and often found in the same environments as ErM fungi (Newsham et al., 2009; Olsrud et al., 2007). Taxonomically, it can be hard to separate between DSE and ErM fungi because both groups are dominated by ascomycetes, especially Leotiomycetes. Both DSEs and ErM fungi have melanized hyphae resistant to decomposition, which has been proposed to play a central role in soil C sequestration (Clemmensen et al., 2015; Fernandez & Koide, 2013). Above the forest line, ericoid dwarf shrubs dominate the alpine heath. Thus, as the forest migrates upwards, a shift in below-ground-dominating functional groups is expected, potentially shifting current soil C dynamics (Clemmensen et al., 2021). Other micro-eukaryotes, including invertebrates and protists, are also essential members of the soil biota and the soil food web (Geisen et al., 2018; Phillips et al., 2019), although their contribution to below-ground C processes has not been well studied.

To improve our understanding of the below-ground biota across the mountain birch forest line ecotone, we analysed below-ground compositional changes across the ecotone in nine sites spread across southern Norway. Using DNA metabarcoding, we targeted the ITS2 (fungi only) and 18S (all eukaryotes) regions of ribosomal DNA from soil, and we measured ergosterol (a proxy for fungal biomass) to obtain both qualitative and quantitative (fungi only) information about the below-ground biota. We aimed to couple these results with both abiotic (soil edaphic factors, climate and bedrock) and biotic factors (above-ground vegetation), to investigate the relationships between above- and below-ground changes across the ecotone. More specifically, we wanted to investigate whether: (i) there is a strong compositional change in the soil biota, in terms of both taxonomic and functional groups, across the forest line ecotone; (ii) the changes in taxonomic and functional groups of soil fungi are correlated with changes in above-ground vegetation; and (iii) the amount of fungal biomass changes throughout the ecotone. To assess the generality of our observations, we analysed soil samples obtained along ecotones in nine replicated sites spread across southern Norway.

2 | MATERIALS AND METHODS

2.1 | Study design and sampling

We conducted sampling in September/October 2017 at nine different high-elevation forest line sites in south-central Norway (Figure 1). Based on a wider set of a priori candidate sites, we selected nine sites with a clear elevation gradient, a distinct shift from mountain birch forests to alpine heath and located at least 15 km

from each other (Figure 1a). At each site, we collected soil samples from plots located every 20 m along a 200-m transect, stretching from 100 m distance below the mountain birch forest line to 100 m distance above (i.e., 11 plots per transect and 99 plots all together; Figure 1c). At each plot, five soil cores were collected in each orientation (i.e., north, east, south, west) and the centre within a circle of 1.5 m radius using a soil corer (3.8 cm in diameter, 30 cm height), and pooled to one representative soil sample. We focused on the organic (O) horizon. Mineral soil was inconsistently present and not considered. Most often, there was no clear division between the litter and humus layers and we therefore analysed the entire organic layer together as composite samples. This also enabled us to better compare the relative proportions of different functional guilds. The depth of the organic horizon varied widely, from ~1 cm up to 10 cm. We removed living green plants and mosses. Soil samples were kept at -20°C during fieldwork and later stored at -80°C before further processing. Within each plot, we recorded the understorey vegetation to species level for lichens, mosses and higher plants and categorized the abundance of each species into three levels: rare (1), common (2) and dominant (3). Based on this information, we calculated the proportion of each plant species per plot, as well as the overall proportion of the following plant groups: ErM plants, EcM plants, AM plants, lichens, mosses and *Pyrola* species, the last comprising arbutoid mycorrhiza.

We obtained the following site-specific environmental variables from published data (Horvath et al., 2019): aspect (i.e., the compass direction that the terrain surface faces), annual precipitation, mean temperature, bedrock and slope. The three types of bedrock provided in this data set ("nutrient-poor bedrock," "nutrient-average bedrock," "nutrient-rich bedrock") were ranked on a 1–3 scale. All environmental and climatic variables were then zero skewness-transformed and standardized.

2.2 | Soil analyses

Soil samples were thawed and handled further in a laminar flow hood, where plants and coarse (>2 mm) pieces of wood and roots were removed. Approximately 70 g of soil sample was freeze-dried (Labconco) in falcon tubes for 36 h and then pulverized using a FastPrep-24 beadbeater (M.P. Biomedicals) in two rounds of 20 s at 25 MHz and subsampled for the different analyses. For soil pH measurements, we diluted 0.5 g of freeze-dried soil in 5 ml distilled H₂O for 1 h, and measured pH using a LAQUA-TWIN-11 pH Meter (Horiba Scientific) following the manufacturer's protocol. Soil C and N concentration was determined, using 0.5 g of freeze-dried soil, by a flash elemental analyser (Thermo Finnigan Flash EA 1112; ThermoFisher Scientific). Note that we do not possess data on soil C stocks, only concentration. Soil P concentration was determined by a segmented flow analyser (SEAL AA3 HR AutoAnalyse; SEAL Analytical). We used ~200 mg of freeze-dried soil for measuring free and total soil ergosterol concentrations (mg/g dry weight [DW]) using a similar protocol as in Ransedokken et al. (2019).

2.3 | Molecular methods

We extracted DNA using a CTAB-chloroform DNA extraction protocol followed by a column-based DNA purification using the E.Z.N.A. Soil DNA Kit following the manufacturer's protocol (Omega Bio-tek). Technical replicates and extraction negatives (negative controls) were introduced during DNA extraction, while mock communities (positive controls) were introduced during the PCR (polymerase chain reaction) step. We amplified both the rDNA ITS2 and 18S regions by PCR. The primers gITS7 (forward) and ITS4 (reverse) (Ihrmark et al., 2012) were used for amplifying the ITS2 region, and TAReuk454FWD1 (forward) and TAReukREV3 (reverse) (Stoeck et al., 2010) for 18S. All primers were tagged with unique molecular identifiers (MIDs). Each PCR consisted of 1 µl DNA template and 24 µl master mix: 15.7 µl dH₂O, 2.5 µl Gold Buffer, 2.5 µl Gold MgCl₂, 1 µl 20 mg ml⁻¹ bovine serum albumin, 0.2 µl dNTPs, 0.1 µl AmpliTaq Gold, 1.5 µl 10 µM forward primer and 1.5 µl 10 µM reverse primer. We ran PCR reactions for ITS2 with initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s and elongation at 72°C for 1 min. We added a final elongation step at 72°C for 7 min, before cooling down to 4°C. The protocol for 18S was slightly different: PCRs were run with initial denaturation at 98°C for 7 min, followed by 32 cycles of denaturation at 98°C for 30 s, primer annealing at 53°C for 30 s and elongation at 72°C for 45 s. A final elongation step was included at 72°C for 10 min, before cooling down to 4°C. We controlled each PCR product for positive amplification with gel electrophoresis using a 2% agarose gel, before individual clean-up and purification of the amplicons with ZR-96 DNA Clean & Concentrator-5 kit (Zymo Research). DNA concentrations for each sample were measured with the Qubit 2 fluorometer dsDNA BR Assay Kit (Thermo Fisher Scientific) and pooled to equimolar concentration into four pools. Each pool was cleaned and concentrated with DNA Clean & Concentrator-5 (Zymo Research). The four libraries (two for each targeted DNA region) were sequenced by Fasteris SA in two runs using Illumina MiSeq with a 250-bp paired-end (PE) with V3 chemistry. A ligation protocol, specifically designed to minimize tag-jumping, was used to ligate the amplicons with the Miseq flow-cell adapters.

2.4 | Bioinformatics

We performed all bioinformatics analyses on the Abel high-performance computer cluster at the University of Oslo. The raw PE reads were demultiplexed separately with simultaneous removal of tags and primers using CUTADAPT (Martin, 2011). No miss-match with primer and MID tags were allowed. Further processing of the data was performed with the DADA2 pipeline (Callahan et al., 2016) using the statistical environment R version 3.6.0 (R Core Team, 2014). We generated sequence quality profiles and used this information to decide parameters for filtering low-quality reads. A truncated length of 200 bp was set for the 18S sequence reads (hereafter called reads). Due to natural length variability of the ITS2 marker, no truncation

was imposed. In both data sets, maximum expected error was set to 2.5. We then independently corrected forward and reverse reads using the DADA2 machine-learning algorithm that estimates correction parameters from the data itself. The default settings were used for 18S, while for ITS2 the band size was set to 32 due to the higher indel rate of this marker. The corrected forward and reverse reads were merged. We used a minimum overlap of 50 and eight nucleotides when merging ITS2 and 18S reads, respectively. Chimeras were checked and removed with a de novo approach using the DADA2 bimer algorithm with default settings. We then constructed an amplicon sequence variant (ASV) table with the chimera-free reads. For the ITS2 data, we used ITSX (Bengtsson-Palme et al., 2013) for extracting the ITS2 region and filtering of nonfungal reads. Due to widespread intraspecific variation in ITS2, an additional clustering step with 97% similarity was performed using VSEARCH (Rognes et al., 2016) and singleton sequences were discarded. Finally, to adjust for over-splitting of operational taxonomic units (OTUs), we performed post-clustering curation using the LULU algorithm (Froslev et al., 2017) for both data sets.

For taxonomic annotation, the ITS2 data set was query searched using VSEARCH global against the UNITE version 8.0 database (Nilsson, Larsson, et al., 2019), whereas the 18S data set was annotated using the Eukref/PR2 database (del Campo et al., 2018). We used FUNGUILD (Nguyen et al., 2016) for functional annotation of the ITS2 data set, where we annotated the fungi into the following functional groups: saprotrophs, ectomycorrhizal fungi (EcM), lichens, yeasts, pathotrophs, root-associated ascomycetes (including DSEs and ErM fungi) and other ascomycetes. Notably, these groups should be regarded tentative (i.e., as hypotheses), since we still lack solid evidence for the ecologies and functions of most fungi.

In the ITS2 data set, we removed one sample due to low read number (286 reads). The number of reads per sample was rarefied to the lowest sample read number (6317 reads). The final ITS2 data set consisted of 98 samples and 3090 OTUs. In the 18S data set, we removed six samples due to low read number (<9). Plant OTUs were removed, and the number of reads per sample was then rarefied to the lowest sample read number (2486 reads). The final 18S data set consisted of 93 samples and 4595 OTUs.

2.5 | Statistics

We performed all statistical analyses in R (R Core Team, 2014). GG-PLOT2 was used for graphical plotting (Wickham & Chang, 2016), the VEGAN package (Oksanen et al., 2007) for community composition analyses (i.e., rarefaction, OTU count data transformation, ordinations, variation partitioning, environmental correlations with ordination analyses), and the NLME (Pinheiro et al., 2017) as well as MuMIn (Barton, 2019) packages for modelling correlations.

To investigate structure in community composition, we constructed ordination diagrams using global nonmetric multidimensional scaling (GNMDS) with the "metaMDS" function on the rarefied OTU matrix, using settings as recommended by Liu et al. (2008).

GNMDS and detrended correspondence analysis (DCA), using default settings, were run in parallel to assess the correlation between GNMDS and DCA axes. As shown by van Son and Halvorsen (2014), a good correspondence between these methods can be interpreted as robust ecological gradients. We fitted the environmental variables to the ordinations with the “envfit” function, and plot isolines were fitted with the “ordisurf” function. To investigate the proportion of variation explained by different environmental variables, variation partitioning of the ITS2 and 18S data was performed using the canonical correspondence analysis (CCA) function in the *vegan* package. We ran a forward model selection until no more significant variables could be added. Correlations between soil C and ergosterol and DSEs were modelled as linear mixed models with sites as a random factor using the “lme” function, and r^2 values were extracted from the models using the “r.squaredGLMM” function.

3 | RESULTS

3.1 | Distribution of taxonomic and functional groups across the ecotone

In the 18S data set, Fungi was the overall richest group (1083 OTUs; 85,101 reads), followed by Metazoa, which included the highest number of reads (1015 OTUs; 94,166 reads). These two groups dominated the soil with about 80% of all reads at the kingdom level. We observed a slight increase in the proportion of Metazoa from

the forest to the heath (Figure 2a). At the phylum level, Nematoda (422 OTUs; 29,096 reads) and Filosa-Sarcomonadea (411 OTUs; 13,278 reads) had the highest richness, while Annelida (52 OTUs; 38,922 reads) possessed the highest number of reads. We observed no clear trends in the proportions of Annelida and Nematoda at the phylum level across the gradient, but the number of reads belonging to Arthropoda and Rotifera increased slightly towards the heath (Figure 2b). At both kingdom and phylum levels, none of the protists, represented by Ciliophora, Cercozoa, Apicomplexa, Lobosa and Conosa, showed any clear distribution patterns across the ecotone (Figure 2a).

Only assessing fungi in the 18S data, the majority of OTUs belonged to the phylum Cryptomycota (318 OTUs; 5804 reads), followed by Basidiomycota (226 OTUs; 16,810 reads). However, the majority of reads belonged to Mucoromycota (47 OTUs; 30,137 reads) and Ascomycota (181 OTUs; 26,096 reads), together making up about 60% of the fungal reads (Figure 2b,c). We observed that the relative abundance of Ascomycota increased from the mountain birch forest to the heath, while we saw an opposite trend for Mucoromycota (Figure 2b). The relative abundance of Archaeorhizomycetes (Figure 2c), which belongs to Ascomycota, increased distinctly towards the heath, with an opposite trend for Mucoromycetes (Figure 2c). We observed no clear trend for the distribution of Agaricomycetes (Basidiomycota).

The ITS2 data set was dominated by the phyla Ascomycota (1522 OTUs, 380,072 reads) and Basidiomycota (903 OTUs, 200,356 reads) and showed a different pattern in relative abundances compared to

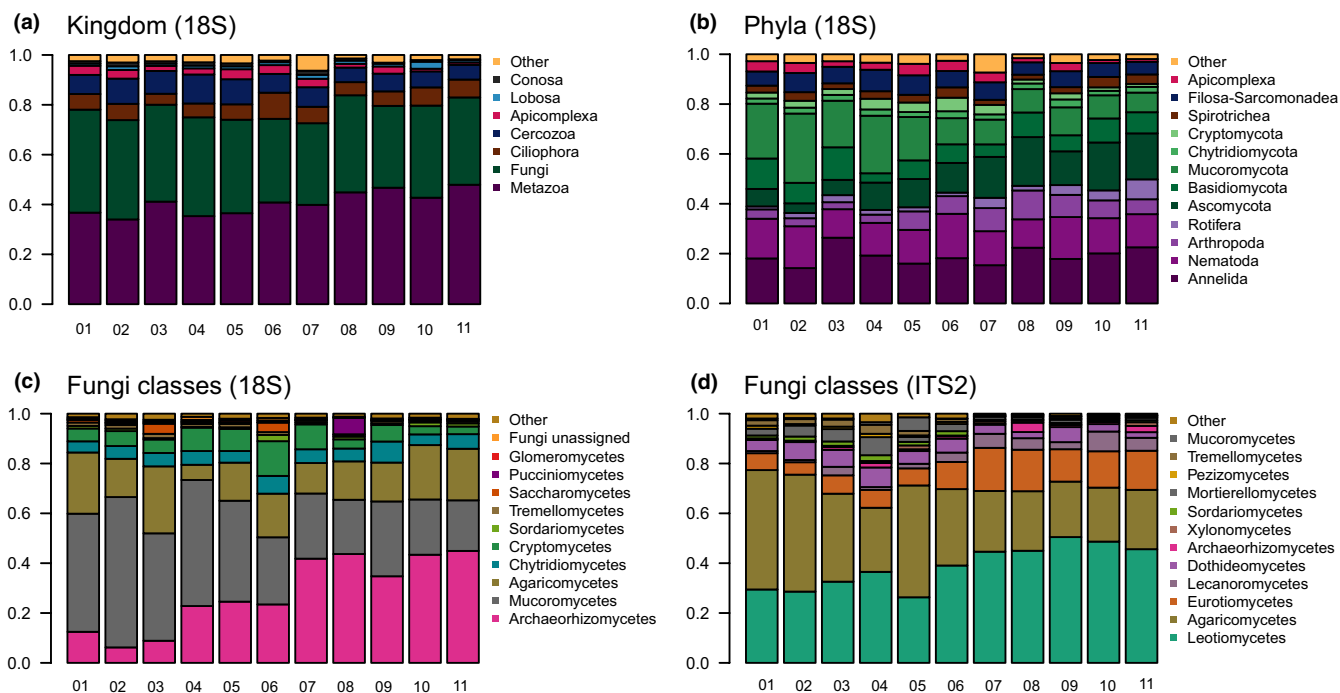


FIGURE 2 Bar plots illustrating the relative abundances of taxonomic groups across the ecotone based on the number of reads. (a) Kingdom/phylum-level and (b) phylum/subphylum-level distribution of the 18S data. (c) Class-level distribution of the fungal 18S data. (d) Distribution of fungal classes based on the ITS2 data set. Plot number 01 is the lowermost plot in the boreal mountain birch forest, while plot number 11 is the uppermost in the heath

the 18S data. In the ITS2 data, the proportion of Agaricomycetes decreased along the ecotone towards the heath, while the Ascomycota groups Leotiomycetes and Eurotiomycetes showed an opposite trend (Figure 2d). In contrast to the 18S data, Archaeorhizomycetes and Mortierellomycetes/Mucoromycetes only made up a small proportion of the ITS2 reads.

For the fungal ITS2 data set, we assigned OTUs to functional guilds (Figure 3a), which revealed that saprotrophs made up the largest group (815 OTUs, 130,986 reads), followed by unassigned ascomycetes (626 OTUs, 148,884 reads), EcM fungi (321 OTUs, 137,428 reads), yeasts (149 OTUs, 43,932 reads), pathotrophs (135 OTUs, 7612 reads), root-associated ascomycetes (115 OTUs, 94,454 reads) and lichens (108 OTUs, 20,586 reads). About half of the 40 most common OTUs were root-associated ascomycetes and most of the others were EcM fungi. The abundance of EcM and saprotrophs decreased towards the heath, while root-associated ascomycetes showed an opposite trend of being far more abundant in the heath compared to the subalpine mountain birch forest (Figure 3a). Furthermore, we observed a positive correlation between the percentage C content in the dry mass of the samples and ergosterol ($R^2 = .73$), and percentage C content and read abundance of root-associated ascomycetes ($R^2 = .45$; Figure 4) across the ecotone.

3.2 | Drivers of community composition

GNMDS analyses demonstrated a clear gradient across the ecotone in community composition of all micro-eukaryotes (18S data set, Figure 5a) and fungi alone (ITS2 data set, Figure 5b). In both diagrams, the first ordination axis (GNMDS1) identified the ecotone stretching from subalpine mountain birch forest to alpine heaths as the main gradient driving the compositional changes in soil communities. Soil edaphic factors, together with the plant groups, were largely structured along the same main gradient as the soil biota (Figures 3b,c and 5a,b). The percentage C in dry mass soil and ergosterol content increased from the forest to the heath, while pH and P content decreased (Figure 3b). We observed no systematic trend for percentage total N content in the soil samples. The amount of ErM-forming plants increased above the forest line, while AM plants decreased (Figure 3c). By definition, the EcM-forming *Betula pubescens* was only present below the forest line, while there was a slight increase in other understorey EcM plants towards the heath (Figure 3c). In both data sets, the second axes (GNMDS2) were structured by the site-specific environmental variables (Figure 5a,b), in addition to soil N in the ITS2 diagram (Figure 5b).

The CCAs demonstrated that site-specific factors, which account for regional (between-site) variability, explained 14.69% of the compositional variation in the 18S data set and 13.68% in the ITS2 data set (Table 1). Plot-specific factors, which also account for variability within the individual gradients, explained 11.27% in the 18S data set and 13.44% in the ITS2 data set. Thus, site- and plot-specific variables were about equally important in explaining variation in community composition in the ITS2 data set between

the soil samples. Interaction effects, both explained at site and plot level, were 3.3% (18S) and 4.2% (ITS2). Total variation explained, as a fraction of the total variation, was 25.96% in the 18S data set and 27.12% in the ITS2 data set.

Species score GNMDS ordination of ITS2 OTUs (Figure 5c) revealed the same trends as outlined above, where OTUs distributed along the ecotone with relatively more root-associated ascomycetes and lichen-forming fungi in the heath and relatively more EcM in the birch forest. The largest proportion of root-associated ascomycetes was associated with the part of the heath that stretched above the shrub-dominated vegetation.

4 | DISCUSSION

We found that the ecotone, stretching from subalpine mountain birch forest to treeless alpine heath, was the primary structuring gradient shaping the soil biota. Both the fungal communities, as well as the overall micro-eukaryotic communities, were structured primarily along the ecotone. Variation in community composition between sites was largely accounted for by regional variation in climate, as well as other site-specific factors, such as slope, aspect and bedrock, the last of these affecting nutrient content.

We observed that the below-ground community composition largely reflected the above-ground shift in vegetation. However, for fungal communities, the 18S and ITS2 markers showed substantial differences in abundance and distribution of fungal groups across the ecotone. Most noteworthy, Archaeorhizomycetes (Ascomycota) and Mucoromycota dominated in the 18S data set, and Chytridiomycota and Cryptomycota were also abundant across the entire ecotone. In contrast, these groups were poorly represented in the ITS2 data set. However, the subphylum Pezizomycotina, represented mainly by Leotiomycetes, Eurotiomycetes, Lecanoromycetes and Dothideomycetes, was largely absent in the 18S data set, but made up a large proportion of the ITS2 data set. We suggest that these contrasting results can be explained by primer biases (Nilsson, Anslan, et al., 2019; Rosling et al., 2011; Tedersoo et al., 2015). The ITS primers employed have been shown to discriminate against Archaeorhizomycetes (Ihrmark et al., 2012; Rosling et al., 2011; Tedersoo et al., 2015) and they also seemingly amplify Mucoromycota, Chytridiomycota and Cryptomycota poorly. The 18S primers we used in this study were expected to amplify all eukaryotes (Hadziavdic et al., 2014) and provide a more general overview of the fungal community composition compared to the ITS2 data set. However, the low amount of Pezizomycotina in the 18S data indicated that primer biases may be present. Indeed, after a closer inspection, a mismatch in the reverse primer was observed in several of the Pezizomycotina lineages. Irrespective of the biases, there was a general trend of more Ascomycota above the forest line, most notably Archaeorhizomycota (18S data), and Leotiomycetes and Eurotiomycetes (ITS2 data).

Several groups of Ascomycota in the ITS2 data represented plant root-associated fungi. For example, the overall most common

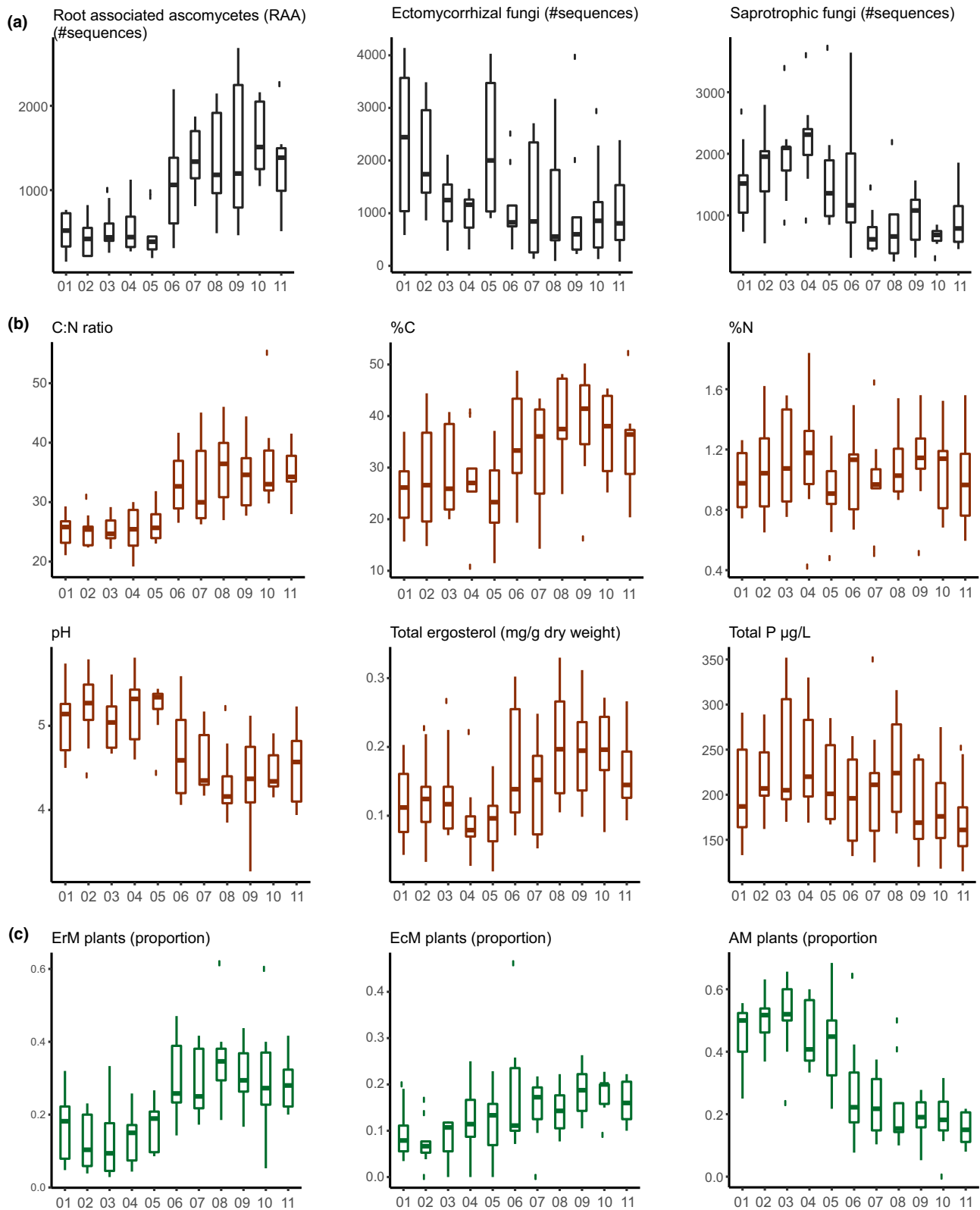


FIGURE 3 Boxplots illustrating how (a) the three dominant fungal guilds, (b) soil edaphic factors and ergosterol, and (c) plant groups based on mycorrhizal status vary across the ecotone. The bold lines in the boxes show the median, and the upper and lower parts of the box represent the 25th and 75th percentiles. Outliers are represented by black dots. Plot number 01 is the lowermost plot in the boreal mountain birch forest, while plot number 11 is the uppermost in the heath

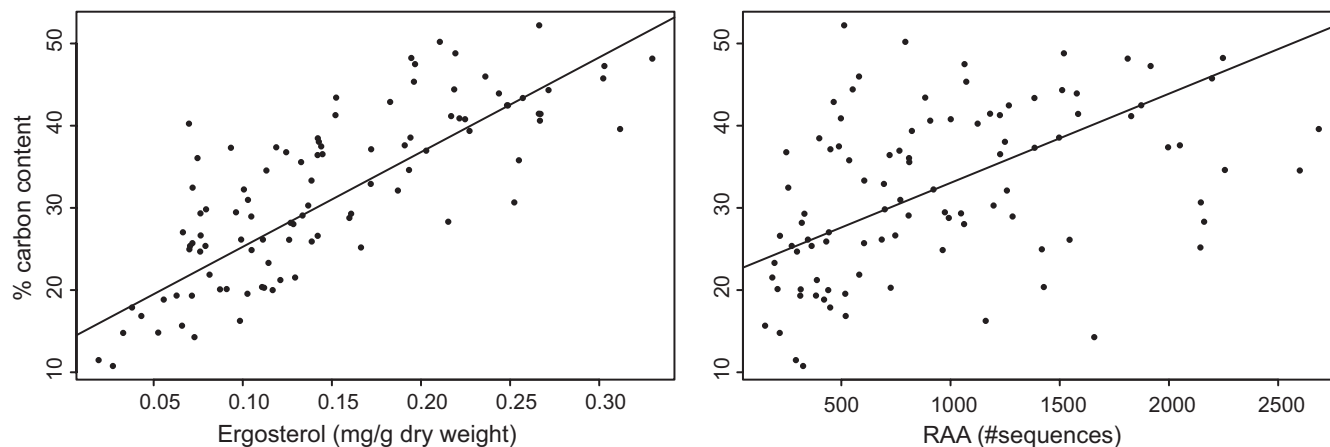


FIGURE 4 Scatter plots showing the relationship between (a) ergosterol and soil carbon content and (b) read abundance of dark septate endophytes (number of reads) and soil carbon content. The relationships were modelled using mixed models with sites as a random factor, from which the R^2 values shown were extracted. The lines represent least squares regression lines

OTU showed high sequence similarity to *Pezoloma ericae*, a common ErM fungus (Smith & Read, 2008). In the species ordination plot, most of the root-associated ascomycetes, representing ErM and DSE fungi, clustered in the alpine heath along the ecotone, where ericaceous plants, especially *Empetrum nigrum*, dominated. The gradual increase of Leotiomycetes and Eurotiomycetes towards the heath (ITS2 data) is in line with the pattern shown by Tedersoo et al. (2014), who revealed that these fungal groups dominate in the arctic tundra. We also observed an increase of Archaeorhizomycetes (Ascomycota) towards the heath. Little is known about the ecology of the Archaeorhizomycetes (Rosling et al., 2011), but it has been hypothesized that some Archaeorhizomycetes may be root-associated mutualists (Menkis et al., 2014). The high abundance of Archaeorhizomycetes in the heath may indicate that they are linked to ericaceous plants, along with ErM fungi and DSEs. Other studies have confirmed that Archaeorhizomycetes are relatively abundant in stressful environments (Sterkenburg et al., 2015), such as in high-altitude and high-latitude ecosystems (Pinto-Figueroa et al., 2019; Schadt et al., 2003). There is a strong need for additional functional studies of Archaeorhizomycetes.

In parallel with the root-associated ascomycetes, we observed that percentage C and ergosterol content had a positive correlation towards the heath, in line with our third hypothesis. Clemmensen et al. (2015) showed a correlation between ergosterol and total soil C stocks, and argued that this relationship is due to slower mycelial turnover rate leading to a long-term build up and increased soil C sequestration (Clemmensen et al., 2013, 2015; Hagenbo et al., 2017; Kvaschenko et al., 2017). These processes may also account for the high C and ergosterol content in the heath. ErM and DSE fungi living under stressful conditions typically have hyphae containing melanin, which is a recalcitrant polymer of the hyphal cell wall that may protect fungi against desiccation (Fernandez & Koide, 2013). Melanin induces slower decomposition, and the retention time of highly melanized fungi in the soil may thus be longer than

with nonmelanized fungi (Fernandez et al., 2019; Fernandez & Koide, 2014). Fungal necromass (i.e., dead biomass) is an important part of boreal soil carbon pools (Clemmensen et al., 2015), and the longer retention time of melanized mycelia may contribute to the positive correlation between root-associated ascomycetes and percentage C in this study.

In accordance with hypothesis 2, the distribution of EcM fungi across the ecotone reflected their host plant distributions, with higher abundances of EcM fungi below the mountain birch forest line. Our observations are in line with Vařutová et al. (2017), who observed that EcM fungi declined with increasing altitude. Many EcM fungi are associated with relatively fast soil organic matter turnover (Bodeker et al., 2014; Clemmensen et al., 2013, 2021; Lindahl & Tunlid, 2015) and may, together with the saprotrophic fungi, account for the lower soil C content observed below the forest line in our study. In a recent study, Clemmensen et al. (2021) found support for the proposal that EcM fungi in mountain birch forests contribute to decomposition when mining N from organic matter. In particular certain members of the genus *Cortinarius* have a high capacity for lignolytic activity (Clemmensen et al., 2021). In parallel with the EcM fungi, we observed a higher proportion of saprotrophic fungi in the subalpine birch forests compared to the heath, which also may contribute to the lower fraction of soil C observed here due to faster decomposition. Based on the 18S data set, Mucoromycetes dominated the fungal community below the forest line. These fungi are foremost known as saprotrophs, and there is probably a higher abundance of available above-ground plant litter in the forest compared to the alpine heath. However, it has also been observed that Mucoromycotina, as fine root endophytes, are important symbionts with cryptogams (Field et al., 2015; Hoysted et al., 2019), receiving plant C in exchange of soil N. Hence, Mucoromycetes may play different functional roles, which may explain the high relative abundance of Mucoromycota across the entire ecotone. It must be emphasized that in this study we only have data on percentage C content and not overall C stock measurements. Hence, additional data are needed to

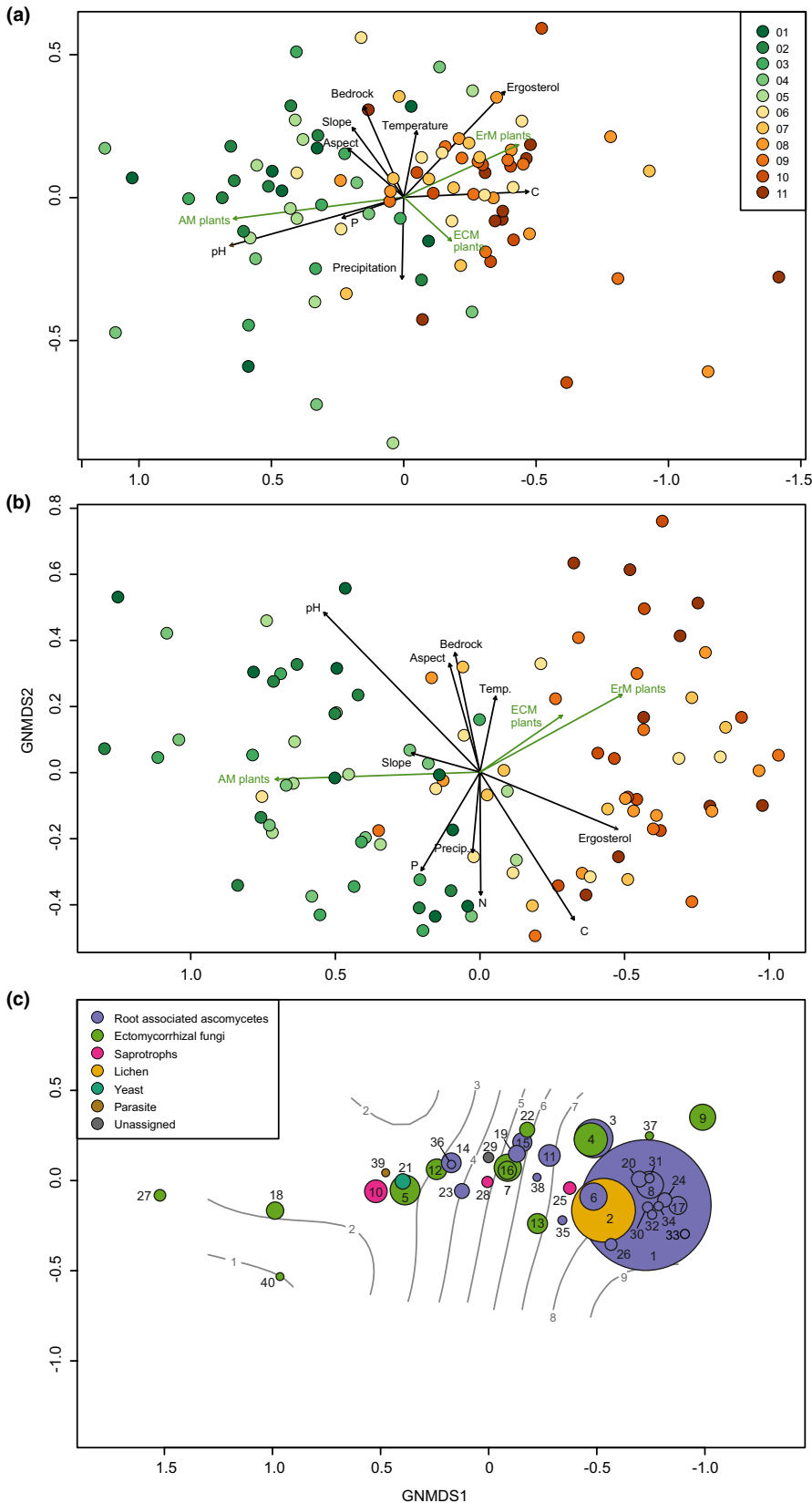


FIGURE 5 GNMDs ordination plots of (a) the 18S data set and (b) ITS2 data set based on rarefied OTU matrices. Each point represents one soil sample. Plots belonging to the heath are coloured red/yellow, while plots belonging to the boreal mountain birch forest are green (see key for details). Variables that significantly correlated with the community structure ($p < .05$) are shown as arrows in the diagrams. Dark arrows represent edaphic factors, dark green arrows site-specific factors, while light green arrows illustrate the distribution of mycorrhizal plant types. (c) GNMDs ordination plot of the ITS2 data set based on species scores. Each point represents one OTU. Only the 40 most common OTUs are visualized. Isolines are numbered according to the plot numbers, ranging from 01 (subalpine mountain birch forest) to 11 (alpine heath). Colours indicate functional groups (see key), and sizes of circles are proportional to the number of reads. (1) *Pezoloma ericae*, (2) *Lecanorales* sp., (3) *Helotiales* sp., (4) *Cortinarius caperatus*, (5) *Piloderma* sp., (6) *Chaetothyriales* sp., (7) *Cenococcum* sp., (8) *Chaetothyriales* sp., (9) *Cortinarius armillatus*, (10) *Mortierella humilis*, (11) *Herpotrichiellaceae* sp., (12) *Cortinarius pseudocandelaris*, (13) *Cortinarius alpinus*, (14) *Venturiales* sp., (15) *Herpotrichiellaceae* sp., (16) *Pseudotomentella tristis*, (17) *Meliniomyces* sp., (18) *Amanita* sp., (19) *Helotiales* sp., (20) *Herpotrichiellaceae* sp., (21) *Solicoccozyma terricola*, (22) *Piloderma bicolor*, (23) *Phialocephala* sp., (24) *Helotiales* sp., (25) *Luellia* sp., (26) *Chaetothyriales* sp., (27) *Hygrophorus albicastaneus*, (28) *Mucor abundans*, (29) *Agaricales* sp., (30) *Helotiales* sp., (31) *Meliniomyces* sp. 2, (32) *Meliniomyces* sp. 3, (33) *Herpotrichiellaceae* sp., (34) *Meliniomyces* sp. 4, (35) *Hyaloscypha* sp., (36) *Leohumicola minima*, (37) *Hygrophorus* sp., (38) *Meliniomyces bicolor*, (39) *Muriformistrickeria rosae*, (40) *Cortinarius talus*

conclude about overall C stock dynamics. Numerous other factors may also influence the C dynamics. For example, we did not analyse soil bacteria, which may be relatively more abundant in the subalpine birch forests soils, where pH is higher.

Although our study was mainly focused on fungi, we found that Metazoa was about equally abundant as fungi in the soil measured as sequence reads. As pinpointed earlier, some Ascomycota might have been poorly amplified by the 18S marker, so it remains unclear

TABLE 1 Results from variation partitioning (CCA) of 18S and ITS2 community data with respect to measured variables, where the numbers indicate the percentage of variation explained. A forward model selection of variables was conducted, where only variables that contributed significantly to the model were included

Variable	18S	ITS2
Site-specific variables		
Site	9.37	9.21
Slope	1.22	-
Bedrock	0.82	1.54
Aspect	1.20	1.08
Annual precipitation	0.92	0.84
Annual mean temperature	1.16	1.01
Plot-specific variables		
Plot	10.12	9.78
pH	1.15	1.42
%N	-	1.17
%C	-	1.06
Total variation explained	25.96	27.12
Interactions	4.17	3.30

whether the proportions reflect an unbiased picture of the below-ground biota. The two most abundant Metazoa groups, Annelida and Nematoda, were common across the entire ecotone, whereas Arthropoda and Rotifera were relatively more abundant above the forest line. Other studies have shown that annelids and nematodes typically are more abundant at high latitudes and altitudes (Phillips et al., 2019; Procter, 1990). Rotifera has also been shown to be abundant in soils at high latitudes and altitudes, and are often present in mosses and lichens (Bielańska-Grajner et al., 2011; Fontaneto & Ricci, 2006), which corresponds well with their relatively higher abundance above the forest line. Studies have shown that soil moisture strongly influences protist communities (Bates et al., 2013; de Araujo et al., 2018; Stefana et al., 2014), while others regard soil pH as a more important factor (Heger et al., 2016; Shen et al., 2014). Heger et al. (2016) also showed a correlation between protist communities and altitude. In general, Cercozoa is commonly observed in various soil types, whereas Ciliophora and Apicomplexa have been shown to be relatively more abundant in humid soils (Bates et al., 2013). Due to the parasitic lifestyle of Apicomplexa, their distribution is probably largely dependent on the presence of host species (Arthropods) (Mahe et al., 2017; Seppey et al., 2020). However, at the kingdom and phylum levels, none of the protists showed any clear distribution pattern across the ecotone.

The ecotone, stretching from subalpine mountain birch forest to treeless alpine heath, represents a corresponding shift in below-ground fungal communities, from soils dominated by EcM and saprotrophic fungi to soils dominated by root-associated ascomycetes, respectively. This study has shown that there is a parallel shift in below-ground ergosterol content across the ecotone, which is strongly associated with the abundance of root-associated ascomycetes in the heath. Our

findings corroborate the view that despite higher above-ground productivity, shrubification and a rise in the forest line in northern ecosystems may lead to soil C loss because of higher soil respiration and C cycling (Clemmensen et al., 2021; Parker et al., 2015; Sjøgersten & Wookey, 2009; Sorensen et al., 2018), through corresponding shifts in fungal communities. However, C stock data are needed to conclude on this matter. On a technical note, our study also underlines that in future community studies of fungi, a wider set of DNA markers and primers should be considered to obtain a more comprehensive picture of soil fungal communities.

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CONFLICT OF INTEREST

There are no conflict of interest related to this article.

AUTHOR CONTRIBUTIONS

This study represents a revised version of the MSc thesis of L.R.T. L.R.T., E.T., L.M., A.B. and H.K. designed the research and L.R.T., E.T., L.M. and H.K. conducted fieldwork. L.R.T., L.M., E.T., S.M. and L.N. did laboratory work and L.R.T., L.M., S.M. and S.B. analysed the data. L.R.T. wrote the manuscript in collaboration with H.K. and E.T. and all other co-authors edited and commented.

DATA AVAILABILITY STATEMENT

All data that support our findings, including raw sequence data, mapping files, the final data matrices and metadata, are openly available in Dryad.org at <https://doi.org/10.5061/dryad.9w0vt4bg6>.

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