Expanding forests in alpine regions: space-for-time indicates a corresponding shift in belowground fungal communities

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Abstract

Climate change causes upward shift of forest lines worldwide, with consequences on soil biota and carbon (C). Using a space-for-time approach, we 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 subalpine mountain birch forests to low-alpine vegetation. Soil fungi and micro-eukaryotes were surveyed using DNA metabarcoding of the 18S and ITS2 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, we observed relatively more root-associated ascomycetes, including Archaeorhizomycetes, ericoid mycorrhizal fungi and dark septate endophytes. Ergosterol and percentage C content in soil strongly and positively correlated with the abundance of root-associated ascomycetes. The predominance of ectomycorrhizal and saprotrophic fungi below the forest line likely 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 belowground biota linked to C sequestration processes.

Introduction

Ecosystems all over the world are affected by climate change (Parmesan, 2006; Pecl et al., 2017). High latitude and altitude ecosystems are especially 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 (Fig. 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 decades (Harsch, Hulme, McGlone, & Duncan, 2009). Although many mountain regions are influenced by land use, the uppermost alpine forest lines are mainly temperature driven (Körner, 2012). Hence, as a response to climate warming, changes in vegetation are predicted, including an upward shift of the forest line into mountain ecosystems (Beckage et al., 2008; Chen, Hill, Ohlemuller, Roy, & Thomas, 2011). Recent studies have documented positive climate feedbacks from expanding alpine forest lines (de Wit et al., 2014) and an increase in the elevation of alpine forest lines will probably increase the local temperature and therefore accelerate the ongoing expansion further (Rydsaa, Stordal, Bryn, & Tallaksen, 2017).

Large areas in Fennoscandia are covered by mountain birch (*Betula pubescens* ssp. *czerepanovii*) forest, that also forms the alpine forest lines in most areas in this region (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 low-alpine vegetation (Fig. 1b). In Norway,

an upward shift in forest lines has been observed, both due to climate warming and land use change (Bryn & Potthoff, 2018). The expansion of subalpine trees and shrubs leads to increased primary production, and thus aboveground 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, Subke, & Wookey, 2015; Sorensen et al., 2018). Parker *et al.* (Parker et al., 2015) showed that soil C turnover was faster and belowground C pools smaller in the forest and shrub vegetation compared to arctic heath. Similarly, Sørensen *et al.* (Sorensen et al., 2018) showed that C stocks were lowest beneath shrub vegetation and significantly higher beneath ericaceous plants in the alpine heath. Furthermore, it is documented that litter decomposes faster in the shrub and mountain birch forest vegetation than in the alpine heath, independent of the litters resistance to decomposition (Parker et al., 2015). These differences in soil C pools may largely be regulated by belowground microorganisms, including fungi, similar to in lowland systems (Clemmensen et al., 2013; Frey, 2019). Improved knowledge of these communities and their functional roles are important for a deeper understanding of the C pool dynamics across the forest line ecotone.

Belowground fungi contribute to soil C processes in various ways, and can roughly be grouped into parasites, mutualistic symbionts and saprotrophs, although there is a blurry transition between nutritional strategies (Selosse, Schneider-Maunoury, & Martos, 2018). Saprotrophic fungi decompose and recycle dead organic matter, thus releasing C to the atmosphere. Mycorrhizal fungi form mutualistic symbiosis 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, Allison, & Treseder, 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), respectively (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, Turner, & Finzi, 2014; Koide, Fernandez, & Malcolm, 2014; Orwin, Kirschbaum, St John, & Dickie, 2011). Root-associated dark septate endophytes (DSE) are also common in alpine and arctic vegetation and often found in the same environments as ErM fungi (Newsham, Upson, & Read, 2009; Olsrud, Michelsen, & Wallander, 2007). It has recently been shown that DSE can promote nutrient uptake in plants (Hill et al., 2019). Taxonomically, it can be hard to separate between fungi forming DSE and ErM since 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 belowground dominating functional groups is expected, potentially shifting current soil C dynamics. 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 belowground C processes is not well studied.

To improve our understanding of the belowground biota across the mountain birch forest line ectone, we conducted a survey designed to measure belowground 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 belowground biota. We aimed to couple these results with both abiotic (soil edaphic factors, climate and bedrock) and biotic factors (aboveground vegetation), to investigate the relationships between above- and belowground changes across the ecotone. More specifically, we wanted to investigate whether: (i) there is a strong compositional change in the soil biota, both in terms of taxonomic and functional groups, across the forest line ecotone; (ii) the changes in taxonomic and functional groups of soil fungi are strongly correlated with changes in aboveground vegetation, and (iii) more fungal biomass is found above the forest line, which may be due to the presence of

more ErM/DSE fungi with recalcitrant mycelium. To assess the generality of our observations, we analysed soil samples obtained along ecotones in nine replicated sites spread across southern Norway.

Materials and methods

Study design and sampling

We conducted sampling in September/October 2017 at nine different high-elevation forest line sites in south-central Norway (Fig. 1). Our selection of sites was based on a forest line model for Norway (Bryn & Potthoff, 2018). 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 vegetation and located at least 15 km from each other (Fig. 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; Fig. 1c). At each plot, five soil cores were collected in each orientation (i.e. North, East, South, West) and the centre within a circle with 1.5 m radius using a 3.8 cm in diameter soil corer, and pooled to one representative soil sample, excluding the mineral soil layer. Most often, there was no clear division between the litter and organic layers and we therefore analysed them together. 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 understory 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 latter making arbutoid mycorrhiza.

We obtained the following site-specific environmental variables from a published data (Horvath et al., 2019): aspect, annual precipitation, mean temperature, bedrock and slope. The three types of bedrock provided in this dataset ("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.

Soil analyses

Soil samples were thawed and handled further in a laminar flow hood, where plants and coarse (>2mm) pieces of wood and roots were removed. Approximately 70 g of soil sample was freeze-dried (Labconco corporation, Kansas City, MO, USA) in falcon tubes for 36 h and then pulverised using a FastPrep-24 beadbeater (M.P. Biomedicals, CA, USA) in two rounds of 20 sec 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 dH_2O for one hour, and measured pH using a LAQUA-TWIN-11 pH Meter (Horiba Scientific, Kyoto, Japan) following the manufacturers protocol. Soil C and N concentration was determined, using 0.5 g of freeze-dried soil, by a flash elemental analyzer (Thermo Finnigan Flash EA 1112, ThermoFisher Scientific, Waltham, USA). Soil P concentration was determined by a segmented flow analyzer (SEAL AA3 HR AutoAnalyse, SEAL Analytical Ltd, Southampton, UK). We used approximately 200 mg of freeze-dried soil for measuring free and total soil ergosterol concentrations (mg g^{-1}DW) using a similar protocol as in (Ransedokken, Asplund, Ohlson, & Nybakken, 2019).

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 manufacturers protocol (Omega Bio-tek, Norcross, USA). Technical replicates and extraction negatives (negative controls) were introduced during DNA extraction, while mock communities (positive controls) were introduced during the PCR step. We PCR amplified both the rDNA ITS2 and 18S regions. 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 (MID). Each PCR reaction consisted of 1 µl DNA template and 24 µl master mix: 15.7 µl dH_2O, 2.5 µl Gold Buffer, 2.5 µl Gold $MgCl_2$, 1 µl 20mg/ml BSA, 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 run PCR reactions for ITS2 with initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec 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: PCR reactions were run with initial denaturation at 98°C for 7 min, followed by 32 cycles of denaturation at 98°C for 30 sec, primer annealing at 53°C for 30 sec and elongation at 72°C for 45 sec. 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, California, USA). DNA concentrations for each sample were measured with the Qubit 2 fluorometer dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and pooled to equimolar concentration into four pools. Each pool was cleaned and concentrated with DNA Clean & Concentrator-5 (Zymo Research, California, USA). The four libraries (two for each targeted DNA region) were sequenced by Fasteris SA (Switzerland) 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.

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 tags and primers removal 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 truncate 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 datasets, 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 8 nucleotides when merging ITS2 and 18S reads, respectively. Chimeras were checked and removed with a denovo approach using the DADA2 bimera 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 non-fungal reads. Due to widespread intraspecific variation in ITS2, an additional clustering step with 97% similarity was performed using VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahe, 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 datasets.

For taxonomic annotation, the ITS2 dataset was query searched using VSEARCH global against UNITE v8.0 (Nilsson, Larsson, et al., 2019), whereas the 18S dataset 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 dataset, 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 most fungi's ecologies and functions.

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

Statistics

We performed all statistical analyses in R (R Core Team, 2014).*ggplot2* was used for graphical plotting (Wickham & Chang, 2016); the *vegan* package for community composition analyses, i.e., rarefaction, OTU count data transformation, ordinations, variation partitioning, environmental correlations with ordination analyses (Oksanen et al., 2007) 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 non-metric multidimensional scaling (GNMDS) with the "metaMDS" function on the rarefied OTU matrix, using settings as recommended by Liu *et al.* (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 Son and Halvorsen (van Son & 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. In order to investigate the proportion of variation explained by different environmental variables, variation partitioning of the ITS2 and 18S data was performed using 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 squared values extracted from the models using the "r.squaredGLMM" function.

Results

Distribution of taxonomic and functional groups across the ecotone

In the 18S dataset, 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 kingdom level. We observed a slight increase in the proportion of Metazoa from the forest to the alpine heath vegetation (Fig 2a). At phylum level, Nematoda (422 OTUs; 29,096 reads) and Filosa-Sarcomonadea (411 OTUs; 13,278 reads) included highest richness, while Annelida (52 OTUs; 38,922 reads) possessed the highest number of reads. We observed no clear trends for the distribution of Annelida and Nematoda at phylum level, but the amount of reads belonging to Arthropoda and Rotifera increased slightly towards the low-alpine vegetation (Fig. 2b). At both kingdom and phylum level, none of the protists, represented by Ciliophora, Cercozoa, Apicomplexa, Lobosa and Conosa, showed any clear distribution patterns across the ecotone (Fig. 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 (Fig. 2b and c). We observed that the relative abundance of Ascomycota increased from the mountain birch forest to the low-alpine vegetation, while we saw an opposite trend for Mucoromycota (Fig. 2b). The relative abundance of Archaeorhizomycetes, which belongs to Ascomycota, increased distinctly towards the low-alpine vegetation, with an opposite trend for Mucoromycetes (Fig. 2c). We observed no clear trend for the distribution of Agaricomycetes (Basidiomycota).

The ITS2 dataset (Fig. 2d) 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 abundance, compared to the 18S data. In the ITS2 data, the proportion of Basidiomycetes (especially Agaricomycetes) decreased along the ecotone towards the low alpine vegetation, while Ascomycetes (especially Leotiomycetes and Eurotiomycetes) showed an opposite trend (Fig. 2d). Leotiomycetes made up the highest proportion of Ascomycetes at class level. In contrast to the 18S data, Archaeorhizomycetes and Mortierellomycetes/Mucoromycetes only made up a small proportion of the ITS2 reads.

For the fungal ITS2 dataset, we assigned OTUs to functional guilds (Fig. 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 other were EcM fungi. The abundance of EcM and saprotrophs decreased towards the low-alpine vegetation, while root-associated ascomycetes showed an opposite trend of being far more abundant in the low-alpine vegetation compared to the subalpine mountain birch forest (Fig. 3a). Furthermore, we observed a strong correlation between the percent C content in the dry mass of the samples and ergosterol (R^2=0.73), and percent C content and read abundance of root-associated ascomycetes (R^2=0.45; Fig. 4) across the ecotone.

Drivers of community composition

GNMDS analyses on both datasets demonstrated a clear gradient across the ecotone in community composition of all micro-eukaryotes (18S dataset, Fig. 5a) and fungi alone (ITS2 dataset, Fig. 5b). In both diagrams, the first ordination axis (GNMDS1) identified the ecotone stretching from subalpine mountain birch forest to low-alpine vegetation 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 (Fig. 3b and c, Fig. 5a and b). Percent C in dry mass soil and ergosterol content increased from the birch forest to the low-alpine vegetation, while pH and P content decreased (Fig. 3b). We observed no systematic trend for % total N content in the soil samples. The amount of ErM forming plants increased above the forest line, while AM plants decreased (Fig. 3c). By definition, the EcM forming *Betula pubescens* was only present below the forest line, while there was a slight increase in other understory EcM plants towards the low-alpine vegetation (Fig. 3c). In both datasets, the second axes (GNMDS2) were structured by the site-specific environmental variables (Fig. 5a, b), in addition to soil N in the ITS2 diagram (Fig. 5b).

The CCA analyses demonstrated that site-specific factors, which account for regional (between-site) variability, explained 4.69% of the compositional variation in the 18S dataset, and 13.68% in the ITS2 dataset (Table 1). Plot-specific factors, which also account for variability within the individual gradients, explained 11.27% in the 18S dataset and 13.44% in the ITS2 dataset, respectively. Thus, site- and plot-specific variables were about equally important in explaining variation in community composition in the ITS2 dataset. Interaction effects, both explained at site and plot level, were 3.3 (18S) and 4.2% (ITS2). Total variation explained, as a fraction of total variation, was 25.96% in the 18S dataset, and 27.12% in the ITS2 dataset.

Species score GNMDS ordination of ITS2 OTUs (Fig. 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 low-alpine vegetation and relatively more EcM in the birch forest. The largest proportion of root-associated ascomycetes was associated with the heath part of the low-alpine vegetation.

Discussion

We found that the ecotone, stretching from subalpine mountain birch forest to treeless low-alpine vegetation, 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 latter affecting nutrient content.

We observed that the belowground community composition largely reflected the aboveground 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 dataset, and Chytridiomycota and Cryptomycota were also abundant across the entire ecotone. In contrast, these groups were poorly represented in the ITS2 dataset. On the contrary, the subphylum Pezizomycotina, represented mainly by Leotiomycetes, Eurotiomycetes, Lecanoromycetes and Dothideomycetes, was largely absent in the 18S dataset, but made up a large proportion of the ITS2 dataset. We suggest that these contrasting results can largely be explained by primer biases (Nilsson, Anslan, et al., 2019; Rosling et al., 2011; Tedersoo et al., 2015). The employed ITS primers have been shown to discriminate against e.g. 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, was expected to amplify all eukaryotes (Hadziavdic et al., 2014) and provide a more general overview of the fungal community composition compared to the ITS2 dataset. 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 numerous Pezizomycotina lineages. Irrespective of the biases, there was a general trend of more Ascomycota above the forest line, most notably Archaeorhizomycota (18S data), and Leotimycetes and Eurotiomycetes (ITS2 data).

Several groups of Ascomycota in the ITS2 data represented plant root-associated fungi. For example, the overall most common 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 low alpine vegetation (ITS2 data) is in line with the pattern showed in Tedersoo *et al.*(Tedersoo et al., 2014), where these groups had highest relative abundances in arctic tundra. ErM and DSE fungi living under stressful conditions typically have melanized hyphae. Melanin 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, in comparison with non-melanized fungi (Fernandez, Heckman, Kolka, & Kennedy, 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 melanised mycelia may explain the strong positive correlation between root-associated ascomycetes and percent C content in this study. We also observed an increase of Archaeorhizomycetes towards the alpine heath. Little is known about the ecology of the Archaeorhizomycetes (Rosling et al., 2011), but is has been hypothesised that some Archaeorhizomycetes may be root-associated mutualists (Menkis, Urbina, James, & Rosling, 2014). The high abundance of Archaeorhizomycetes in the low alpine vegetation may indicate that they are linked to ericaceous plants, along with ErM fungi and DSEs. Other studies confirm that Archaeorhizomycetes are relatively abundant in stressful environments (Sterkenburg, Bahr, Durling, Clemmensen, & Lindahl, 2015), e.g. in high altitude and latitude ecosystems (Pinto-Figueroa et al., 2019; Schadt, Martin, Lipson, & Schmidt, 2003).

In parallel to the root-associated ascomycetes, we observed that percent soil C and ergosterol content had a strong positive correlation towards the low-alpine vegetation, in line with our hypotheses 3. Clemmensen*et al.* (Clemmensen et al., 2015) showed a similar correlation between ergosterol and total soil C stock, 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; Clemmensen et al., 2015; Hagenbo et al., 2017; Kyaschenko, Clemmensen, Karltun, & Lindahl, 2017). These processes may also account for the high C and ergosterol content in the low-alpine vegetation. Percent C content correlated strongly with the community composition in the ordination diagrams, but with respect to decomposition and cycling processes, C content is not regarded as a driver, rather a consequence of the fungal community composition (Clemmensen et al., 2015). However, it must be emphasised that in this study we only have data on percent C content and not overall C stock measurements. Hence, to conclude about overall C stock dynamics, additional data must be collected.

In accordance with hypothesis 2, the distribution of EcM fungi across the ecotone reflected their host plants distributions, with higher abundances of EcM fungi below the mountain birch forest line. Our observations are in line with what observed by Vašutová *et al* . (Vasutova, Edwards-Jonasova, Baldrian, Cermak, & Cudlin, 2017), where EcM fungi declined with altitude. Many EcM fungi are associated with relatively fast soil organic matter turnover (Bodeker et al., 2014; Clemmensen et al., 2013; Lindahl & Tunlid, 2015) and may, together with the saprotrophic fungi, account for the lower amount of soil C observed below the forest line in our study.

In general, we observed a higher proportion of saprotrophic fungi in the alpine birch forests compared to the heath, which may account for the lower fraction of soil C observed here, due to faster decomposition.

Based on the 18S dataset, Mucoromycetes dominated the fungal community below the forest line. These fungi are foremost known as saprotrophs, and there is likely a higher abundance of available 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.

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 fungi Ascomycota might have been poorly amplified by the 18S marker, so it remains unclear whether the proportions reflect an unbiased picture of the belowground 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). This is in line with the result of this study, where annelids and nematodes are the dominant groups. 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, Ejsmont-Karabin, & Yakovenko, 2011; Fontaneto & Ricci, 2006), which corresponds well with their relatively higher abundance above the forest line. Studies have shown that soil moisture strongly influence protist communities (Bates et al., 2013; de Araujo et al., 2018; Stefana, Cornelia, Jorg, & Michael, 2014), while others regard soil pH as more important (Heger, Derungs, Theurillat, & Mitchell, 2016; Shen et al., 2014). Heger *et al.* (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 likely largely dependent on the presence of host species (Arthropodes) (Mahe et al., 2017; Seppey et al., 2020). However, at kingdom and phylum level, none of the protists showed any clear distribution pattern across the ecotone.

The ecotone, stretching from subalpine mountain birch forest to treeless low-alpine vegetation, represents a corresponding shift in belowground fungal communities, from soils dominated by EcM and saprotrophic fungi to soils dominated by root-associated ascomycetes, respectively. This study has shown there is a parallel shift in belowground ergosterol content across the ecotone, which is strongly associated with the abundance of root-associated ascomycetes in the low-alpine vegetation. Our findings corroborate the view that despite higher aboveground productivity, shrubification and raise in forest line in northern ecosystems may lead to soil C loss because of higher soil respiration and C cycling (Parker et al., 2015; Sjogersten & Wookey, 2009; Sorensen et al., 2018), through corresponding shifts in fungal communities. However, C stock data is 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 comprehensive picture of soil fungal communities.

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Data accessibility

All data that support the findings of this study are openly available in Dryad.org at http://doi.org/[doi], [reference number].

Author Contributions

This study represent a revised version of the MSc thesis of LRT. LRT, ET, LM, AB and HK designed the research and LRT, ET, LM and HK conducted fieldwork. LRT, LM, ET, SM and LN did lab work and LRT, LM, SM and SB analysed the data. LRT wrote the manuscript in collaboration with HK and ET and all other co-authors edited and commented.

Figure legends

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 represents the plots where soil samples were collected every 20 meters along the 200 m transect stretching from 100 meters below to 100 meters above the mountain birch forest line.

Figure 2. Barplots illustrating the relative abundances of taxonomic groups across the ecotone based on the number of reads. (a) Kingdom and (b) phylum level distribution of the 18S data. (c) Class level distribution of the fungal 18S data. (d) Distribution of fungal classes based on the ITS2 dataset. Plot number 01 is the lowermost plot in the boreal mountain birch forest, while plot number 11 is the uppermost in the low-alpine vegetation.

Figure 3. Boxplots illustrating how (a) the tree 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 part of the box represents 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 low-alpine vegetation.

Figure 4. Scatter plots showing the relationship between (a) ergosterol and soil carbon content and (b) proportional 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 squared values shown were extracted. The lines represent least squares regression lines.

Figure 5. GNMDS ordination plots of (a) the 18S dataset and (b) ITS2 dataset based on rarefied OTU matrices. Each point represents one soil sample. Plots belonging to the low-alpine vegetation are colored red/yellow, while plots belonging to the boreal mountain birch forest are green (see legend for details). Variables that significantly correlated with the community structure ($p<0.05$) are shown as arrows in the diagrams. Dark arrows represent edaphic factors, dark green arrows site-specific factors, while light green arrows illustrates the distribution of mycorrhizal plant types. (c) GNMDS ordination plot of the ITS2 dataset 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 (low-alpine vegetation) to 11 (boreal mountain birch forest). Colors indicate functional groups (see legend), and size 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* .

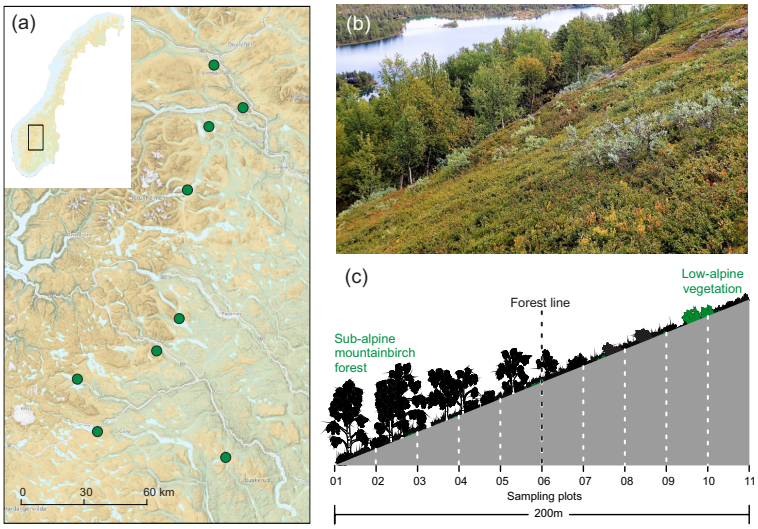

Figure 1

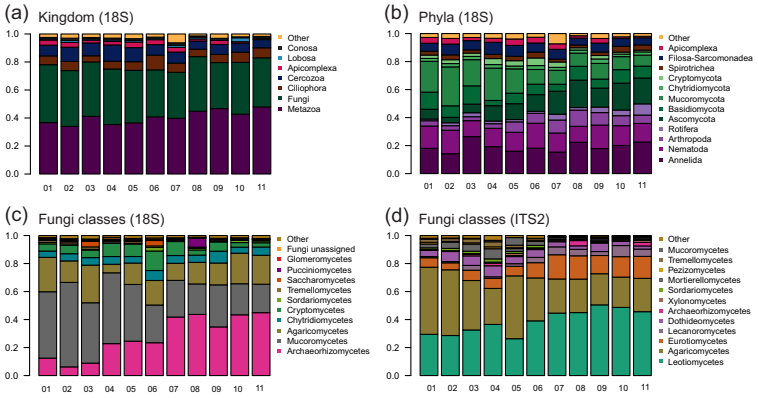

Figure 2

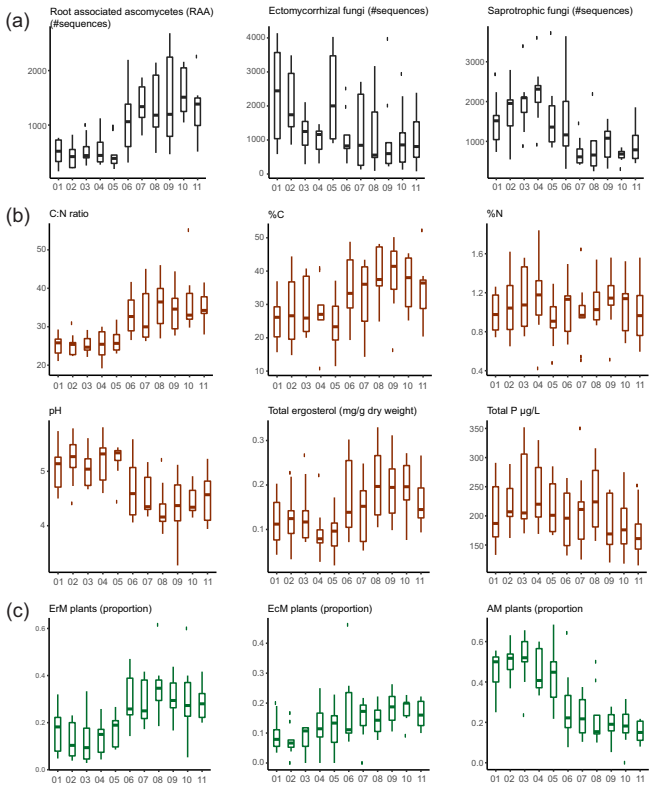

Figure 3

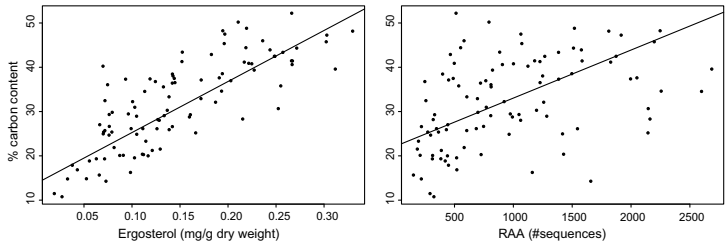

Figure 4

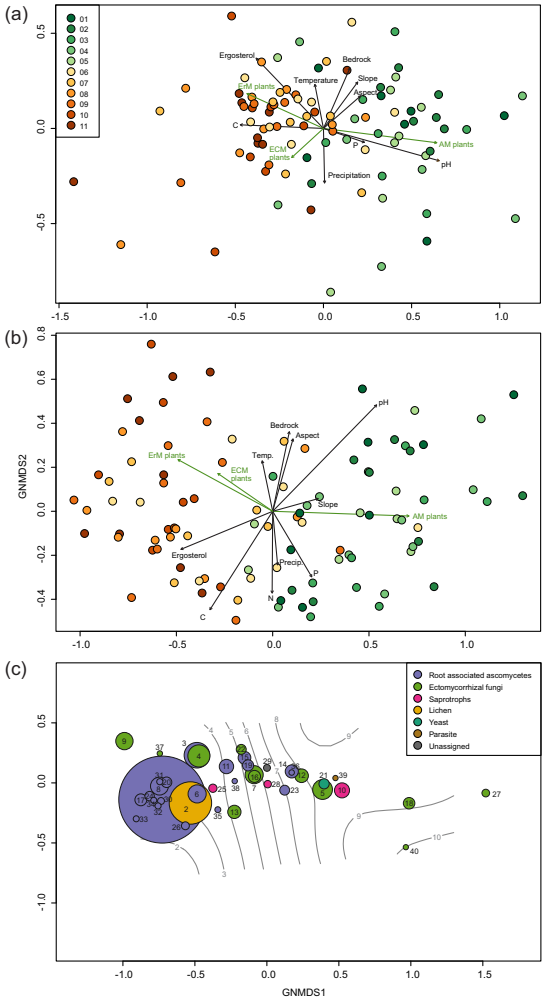

Figure 5