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Research Article

RESEARCH ARTICLE

Establishment of spruce plantations in native birch forests reduces soil fungal diversity

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One sentence summary: Establishing spruce plantations in native birch forest areas causes dramatic changes in belowground fungal communities.

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ABSTRACT

Plantations of Norway spruce have been established well beyond its natural range in many parts of the world, potentially impacting native microbial ecosystems and the processes they mediate. In this study, we investigate how the establishment of spruce plantations in a landscape dominated by native birch forests in western Norway impacts soil properties and belowground fungal communities. Soil cores were collected from neighboring stands of planted spruce and native birch forests. We used DNA metabarcoding of the rDNA internal transcribed spacer 2 region and ergosterol measurements to survey the fungal community composition and its biomass, respectively. In the two investigated soil layers (litter and humus), fungal community composition, diversity and biomass were strongly affected by the tree species shift. Native birch stands hosted markedly richer fungal communities, including numerous fungi not present in planted spruce stands. In contrast, the spruce stands included higher relative abundance of ectomycorrhizal fungi as well as higher fungal biomass. Hence, establishing plantations of Norway spruce in native birch forests leads to significant losses in diversity, but increase in biomass of ectomycorrhizal fungi, which could potentially impact carbon sequestration processes and ecosystem functioning.

Keywords: forest plantations; Norway spruce; soil fungi; ectomycorrhiza; fungal communities; DNA metabarcoding

INTRODUCTION

In many parts of the world, plantations of non-native tree species have been established to provide humans with a variety of beneficial ecosystem services, such as timber and food production. Trees used for timber production, e.g. Norway spruce [*Picea abies* (L.) Karst.], display traits such as fast growth and frost hardiness, which make them desirable for commercial forestry.

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At the same time, these traits can facilitate naturalization and invasion of the species beyond their native range, where it may outcompete native species. Since the above- and belowground biota are strongly connected, a shift in the dominant tree species may result in corresponding changes in the belowground communities of microorganisms (Prescott and Grayston 2013; Uroz *et al.* 2016) and local biogeochemical processes (Mueller *et al.* 2012). This makes non-native tree invasions a subject of global environmental concern (Richardson and Rejmánek 2011).

Norway spruce's natural range spans from central and eastern Norway to Fennoscandia, the Baltic states, Belarus and Russia, as well as an unconnected southern range in Central and Southeastern Europe (Aarrestad *et al.* 2014). In the temperate zones in western Norway, the forests consist to a large extent of deciduous trees, such as birch (*Betula* spp.). However, intensive planting of Central European Norway spruce has been conducted here during the last 50 years. These trees are now spreading to new areas in western Norway and will likely become far more widespread in the future (Tveite 2006). Introducing coniferous species into deciduous forests changes the environment in many ways, including a reduction of solar radiation through the canopy, higher accumulation of litter and changes in soil moisture and nutrient availability (Smolander *et al.* 2005; Aarrestad *et al.* 2014). The tree species shift leads to a much sparser ground vegetation in spruce plantations, compared with the original birch forests. It is well established that aboveground vegetation is tightly linked to the belowground communities of various microorganisms, including fungi (Martínez-García *et al.* 2015; Bahnmann *et al.* 2018). Considering the strong effect of trees on soil microbial community structure (Bach *et al.* 2010), establishing plantations of Norway spruce in non-native habitats is expected to affect the diversity of the belowground fungal communities and the ecosystem processes they mediate.

Fungi play major roles in forest ecosystems as pathogens, nutrient cyclers and crucial symbionts. In temperate forest soils, they are mainly represented by two functional groups: saprotrophic and mycorrhizal fungi, both playing important roles in carbon (C) sequestration processes (Lindahl and Tunlid 2015). Saprotrophic fungi acquire energy by degrading dead organic matter, and are the principal decomposers of plant litter and wood. They thrive in the above energy-rich litter, but as the available energy in substrate decreases with soil depth, they are less competitive, and will be replaced by mycorrhizal fungi, which do not rely on litter-derived energy (Rosling *et al.* 2003; Lindahl *et al.* 2007; Baldrian *et al.* 2012). Mycorrhizal fungi, forming symbiosis with plant roots, obtain C derived from photosynthesis in exchange for soil minerals and water (Smith and Read 2008). Much of the atmospheric C fixed through photosynthesis is allocated belowground to the roots and the associated mycorrhizal symbionts (Clemmensen *et al.* 2013). Recent studies of ectomycorrhizal fungi have demonstrated that they to some extent have the potential to decompose organic matter when mining for nutrients, although not as efficiently as saprotrophs (Lindahl and Tunlid 2015). Competition for soil nitrogen (N) between mycorrhizal fungi and saprotrophs, i.e. the 'Gadgil effect' (Gadgil and Gadgil 1975), may slow down the C cycle, reducing soil respiration and increasing net soil C sink (Averill, Turner and Finzi 2014; Averill and Hawkes 2016). Thus, changes in the composition of fungal communities in soil may have consequences for the overall C budget, as C sequestration helps offset the release of greenhouse gases to the atmosphere.

In this study, we investigate how soil properties and belowground soil fungal communities are affected by the shift in aboveground tree species from native birch to Norway spruce in western Norway. We analyze whether the effects are strongest in the above litter or the below humus layers. First, we assess how different edaphic factors, such as pH and content of C, N and P, are affected by the tree species shift. Next, we analyze how the soil fungal communities are changing in terms of overall composition, diversity and biomass, and which edaphic factors correlate with the observed changes. Lastly, we assess how the composition of fungal functional guilds varies across forest types and the soil layers.

MATERIALS AND METHODS

Study site and sampling design

The two sampling sites are located in the Voss municipality, western Norway, around two neighboring lakes, Myrkdalsvatnet and Oppheimsvatnet, with an altitude of ∼230 and 330 m, respectively (Fig. S1, Supporting Information). The vegetation of the area is a mosaic of native birch (*Betula pubescens*) forests with a diverse understory of grass and herbaceous plants, and 40–50-year-old patches of planted, non-native Norway spruce stands, with an understory dominated by *Sphagnum* and *Polytrichum*. The natural birch forests were late successional phases of unknown age. They represented typical climax stage vegetation with an open canopy compared with the spruce plantations. We selected these two sites due to the alternating pattern of spruce and birch forests along the lakeshores (Fig. S1, Supporting Information), which make it possible to sample soil from the different forest types while minimizing variation in climatic and presumed local edaphic conditions prior to plantation establishment.

We collected soil cores during October 2016 from 40 plots, 20 along each lake, every other from birch and spruce stands. Within each stand, eight soil cores (3 cm in diameter) were collected from points in an outer and an inner circle with a radius of 4 and 2 m, respectively, and stored in field freezers. After thawing the soil cores, we removed all green plant parts and the cores were split according to soil layer: litter (topmost layer of freshly fallen litter) and humus (underlying layer of decomposed litter). The remaining lower layers containing mineral soil were discarded. We then pooled both layers to plot (resulting in 80 samples), freeze dried and then pulverized with ceramic beads for complete homogenization (3 times at 30 sec with 4 m/s speed).

Soil and fungal biomass analyses

Pulverized litter and humus were aliquoted for chemical analyses. C and N content were measured using a flash elemental analyzer (Thermo Finnigan Flash EA 1112, ThermoFisher Scientific, Waltham, MA, USA), while P was determined with a segmented flow analyzer (SEAL AA3 HR AutoAnalyzer, SEAL Analytical Ltd, Southampton, UK). We measured pH from a solution of 10 mL deionized water and 1 g litter/humus using LAQUA Twin pH Meter (Spectrum Technologies Inc, Aurora, IL, USA) following the manufacturer's protocol.

We measured total soil ergosterol concentrations (mg/g DW; fungal biomass proxy) following the protocol of Davey *et al.* (2009). In brief, ∼200 mg of each finely grounded soil sample was mixed with 7 mL 3 M KOH in MeOH, vortexed and sonicated in a 70°C ultrasonic water bath in darkness for 90 min. The samples were then centrifuged (∼16 400 × *g*, 15 min) and the supernatants mixed with 2 mL purified water in new tubes. Then, 5 mL hexane was added, vortexed and the hexane phase was collected after the two phases divided. This step was repeated twice. Both extracts were collected in the same vial,

evaporated to dryness and then redissolved in 500 μL MeOH. We then analyzed the extracts for total ergosterol content using HPLC. Ergosterol was separated using a reversed phase ODS ultrasphere column (250 mm × 4.6 mm; particle size 5 μm), with MeOH as the mobile phase (flow rate 1.5 mL/min; total analysis time 12 min). We measured ergosterol absorption at 280 nm and identified by cochromatography of a commercial standard (Sigma-Aldrich, St Louis, MO, USA).

Molecular analyses

We extracted DNA from the finely grounded litter and humus samples following a chloroform and CTAB DNA extraction protocol as described by Davey *et al.* (2009). Briefly, 1 g of sample was mixed with 10 mL CTAB to form a slurry. Then, 600 μL of the slurry was further homogenized in a Retsch ball mill (Retsch GmbH, Haan, Germany) using two tungsten carbide beads two times for 1 min at 24 Mhz. After extracting with 600 μL chloroform, we precipitated the DNA using 400 μL cold isopropanol. Following centrifugation, the liquid was carefully drained and the DNA pellet was resuspended in elution buffer, which then was cleaned up using the E.Z.N.A. soil kit (Omega Bio-tek, Norcross, GA, USA), following the manufacturer's protocol.

We amplified the internal transcribed spacer 2 (ITS2) with primers gITS7 and ITS4 (Ihrmark *et al.* 2012). Unique 6-bp (base pair) multiple identifier (MID) tags were attached to both primers. Altogether 1 μL DNA template was used for the 25 μL PCR reaction containing 14.6 μL dH_2O, 2.5 μL Gold buffer, 2.5 μL Gold $MgCl_2$, 0.2 μL dNTPs (25 mM), 0.2 μL AmpliTaq Gold (5U/μL), 1 μL 20 mg/mL BSA and 1.5 μL 10 μM of each primer (gITS7 and ITS4). We ran PCR reactions with an initial step of 5 min at 95°C, followed by 32 cycles of denaturation at 95°C for 30 s, annealing primers at 55°C for 30 s and elongation at 72°C for 1 min. A final step of elongation was performed at 72°C for 7 min. The DNA concentration was measured with Qubit, purified with Agencourt AMPure beads (Agencourt Bioscience, Beverly, United States) and pooled in equimolar amounts in two libraries, before paired-end (300 × 2) sequencing with Illumina MiSeq platform.

Bioinformatics

All bioinformatics were performed on a high-performance computer (Abel Cluster) at the University of Oslo. We corrected 10 108 861 forward and reverse sequences with *BayesHammer* (Nikolenko, Korobeynikov and Alekseyev 2012). Sequences were merged with *PEAR* (Zhang *et al.* 2014) with a minimum of 30-bp overlap, a maximum and minimum length of 550 and 200 bp, respectively, and a mean phred quality threshold of >30. We then quality filtered the merged reads using *FASTX-Toolkit*/0.0.14 (Gordon and Hannon 2010) keeping reads that had >35 quality score in at least 90% of the merged read. This was followed by *VSEARCH*/v2.4.3 (Rognes *et al.* 2016) to only keep sequences with maximum 0.5 expected errors. We demultiplexed the remaining high-quality sequences to individual samples based on the unique MIDs using simple demultiplexing (https://github.com/hildebra/sdm). Read direction was identified with *FQGREP*/v0.4.4 (https://github.com/indraniel/fqgrep/), and the reverse sequences were reverse complemented and merged with the forward sequences using the *FASTX-Toolkit*/0.0.14. *MOTHUR*/v.1.38.1 (Schloss *et al.* 2009) was used to dereplicate the reads (i.e. gather the unique sequences appending read abundance) before extracting the ITS2 region using *ITSx*/1.0.11 (Bengtsson-Palme *et al.* 2013). We discarded reads shorter than 100 bp. *VSEARCH* was used to dereplicate the ITSx extracted sequences. A total of 7 428 761 sequences, passing all above quality criteria, were grouped at 97% similarity threshold into operational taxonomic units (OTUs) and 6160 OTUs were generated. We then performed de novo-based chimera detection and removed global singletons, as well as OTUs with <10 reads. The OTU table was further analyzed using LULU (Frøslev *et al.* 2017), an algorithm designed to automatically correct for oversplitting of OTUs, resulting in 5092 OTUs. We considered the most abundant sequence of each cluster as the representative sequence and blasted these against the curated UNITE database (Abarenkov *et al.* 2010) for taxonomic annotations. After removing OTUs with no blast hits as well as <80% identity and <80% coverage to a UNITE reference fungal sequence, the final OTU table included 3626 OTUs representing 5 872 173 high-quality fungal sequences, with an average of 80 441 (±20 643) reads and 1181 (±180) OTUs per sample. We categorized OTUs into fungal functional guilds using the FUNGuild database (Nguyen *et al.* 2016).

Statistical analyses

We conducted all statistical analyses in R (R Core Team 2017). An initial ordination analysis identified four outlier samples (Fig. S2, Supporting Information). One of these samples included very few reads and the three others were interpreted to be mislabeled during laboratory analyses. The final OTU table with the four outliers excluded was rarefied to the minimum number of sequences obtained from a single sample (18 734 reads). We transformed the count data using Hellinger transformation. The community composition was inferred from a Bray–Curtis distance dissimilarity matrix of samples using the metaMDS function in the R package vegan (Oksanen *et al.* 2017). We determined linear correlations between the environmental variables and the ordination space using the envfit function in the vegan package. Significant correlated variables (alpha = 0.05) were fitted into the ordinations as vectors pointing toward increased abundance.

The mean relative abundance (per sample) of the most abundant fungal guilds (i.e. saprotrophs, pathotrophs, and ectomycorrhizal, arbuscular mycorrhizal and ericoid mycorrhizal fungi) were fitted into the ordination space using the envfit function. To test for differences between forest types and soil horizons in fungal community composition, we used multiple response permutation procedure with 999 permutations of the Bray–Curtis distance dissimilarity matrix. There was a strong effect of forest type on community composition, so we further investigated soil horizon and abiotic variable effects on community composition subsets of the data representing (i) natural birch forests and (ii) spruce plantations. To test the individual contributions of forest, soil horizon and soil chemical properties in explaining the variability of the community composition, we used PERMANOVA analysis of the Bray–Curtis distance dissimilarity with 999 permutations, as implemented in the Adonis function in the Vegan package. Potential spatial autocorrelation was investigated as in Mundra *et al.* (2015).

Fungal richness, Shannon diversity index and Pielou's evenness were calculated. ANOVA and Tukey HSD tests were used to evaluate whether there were significant differences between forest types and layers for the various measurements in the dataset. To address the effects of forest type and soil horizon in each fungal guild (sample-wise rarefied abundance), we also used ANOVA and Tukey HSD tests. Further, correlations between

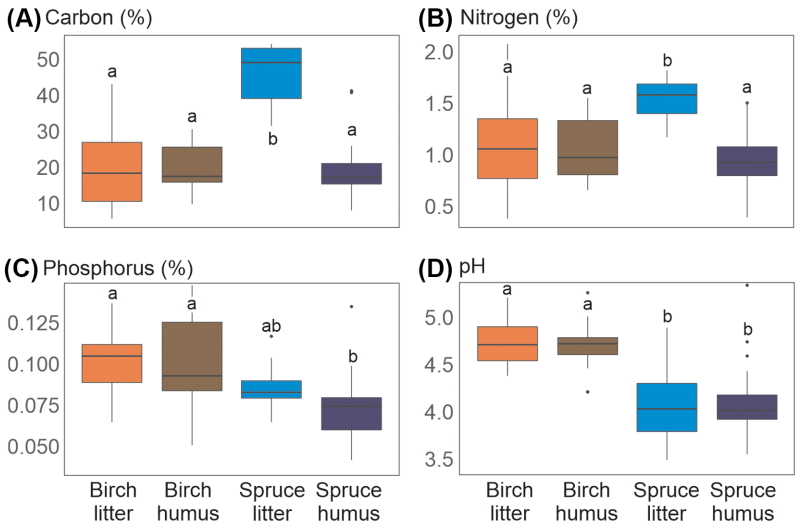

Figure 1. Box plots displaying the content of C, N and P in the freeze-dried soil samples **(A–C)** and pH **(D)**. The different letters above boxes indicate statistically significant differences at a significance level of $P < 0.05$ (Tukey post hoc tests). The box spans the interquartile range (IQR; first quartile to the third) with the median indicated by a dark horizontal line; the whiskers show the 1.5 × IQR, while outliers (>3 × IQR) are indicated by dark dots.

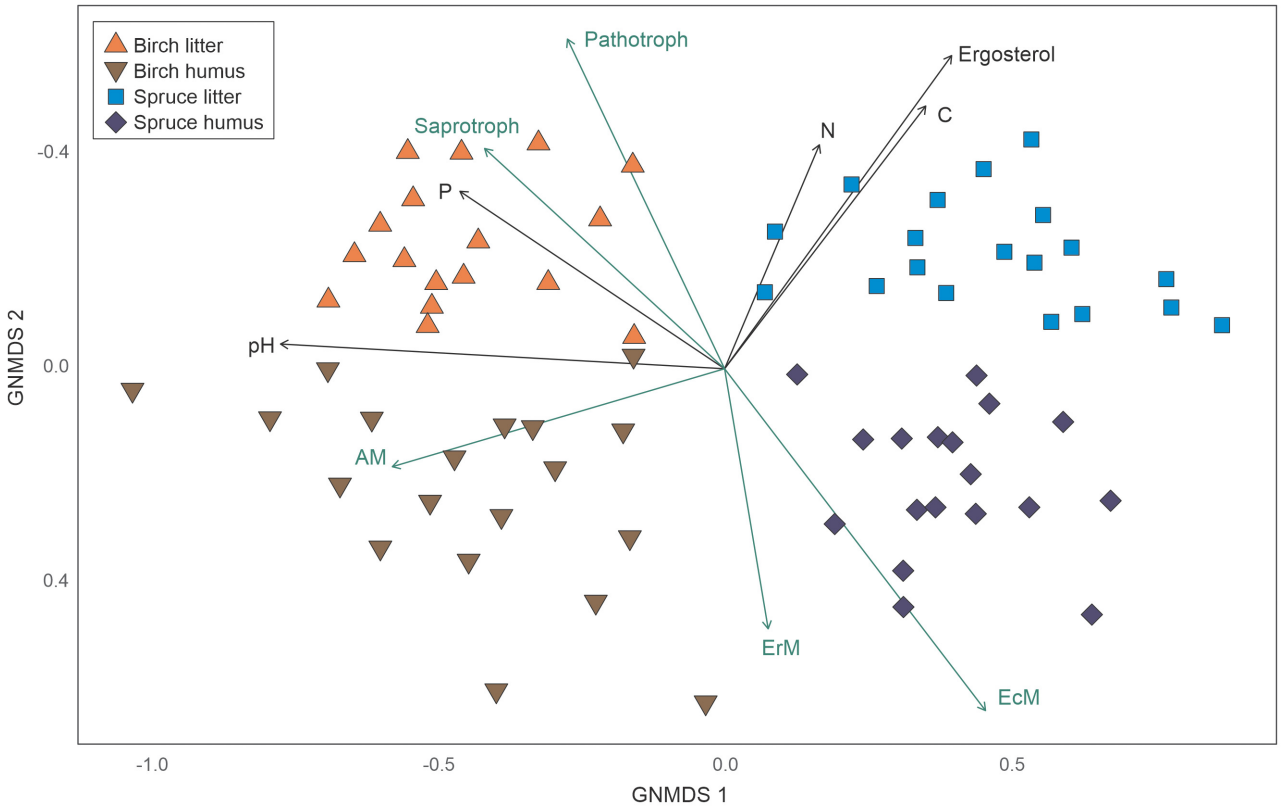

Figure 2. Global multidimensional scaling ordination biplot, displaying variation in fungal community composition across forest types (reflected by the first axis) and soil horizon (reflected by the second axis). Dark-colored vectors represent edaphic factors and ergosterol content, significantly correlated to the ordination plot, where vector sizes represent correlation strength. Green vectors indicate the distribution of various fungal functional guilds, where AM = arbuscular mycorrhizal fungi, EcM = ectomycorrhizal fungi and ErM = ericoid mycorrhizal fungi.

environmental variables and each fungal guild were addressed with linear models. Finally, individual OTU affinity with forest and soil horizon was determined using indicator species analyses using the multipatt function in the indicspecies R package (Cáceres and Legendre 2009). We used ggplot2 (Wickham 2009) for graphical representations of the results.

RESULTS

We observed that the tree species shift was associated with marked changes in edaphic factors: significantly higher content (%) of C and N were observed in spruce litter samples compared with the three other sample types, while spruce humus samples

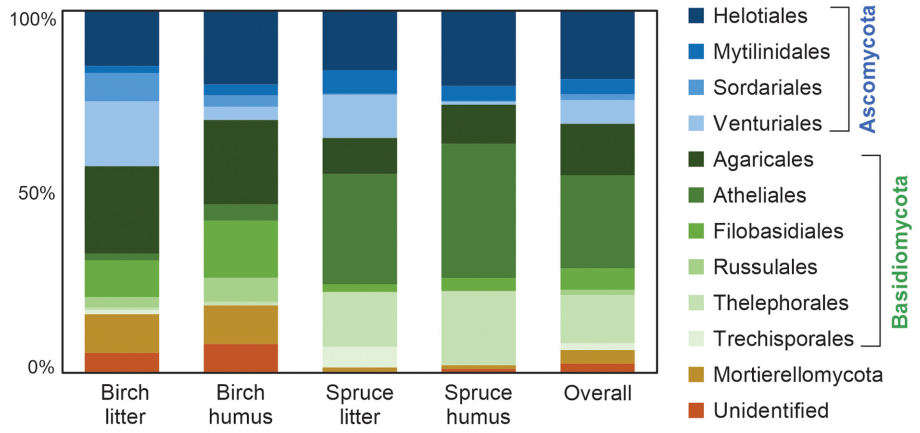

Figure 3. Proportion of taxonomic groups across the forest types and soil horizons, mainly given at order level. The proportions reflect relative sequence abundances.

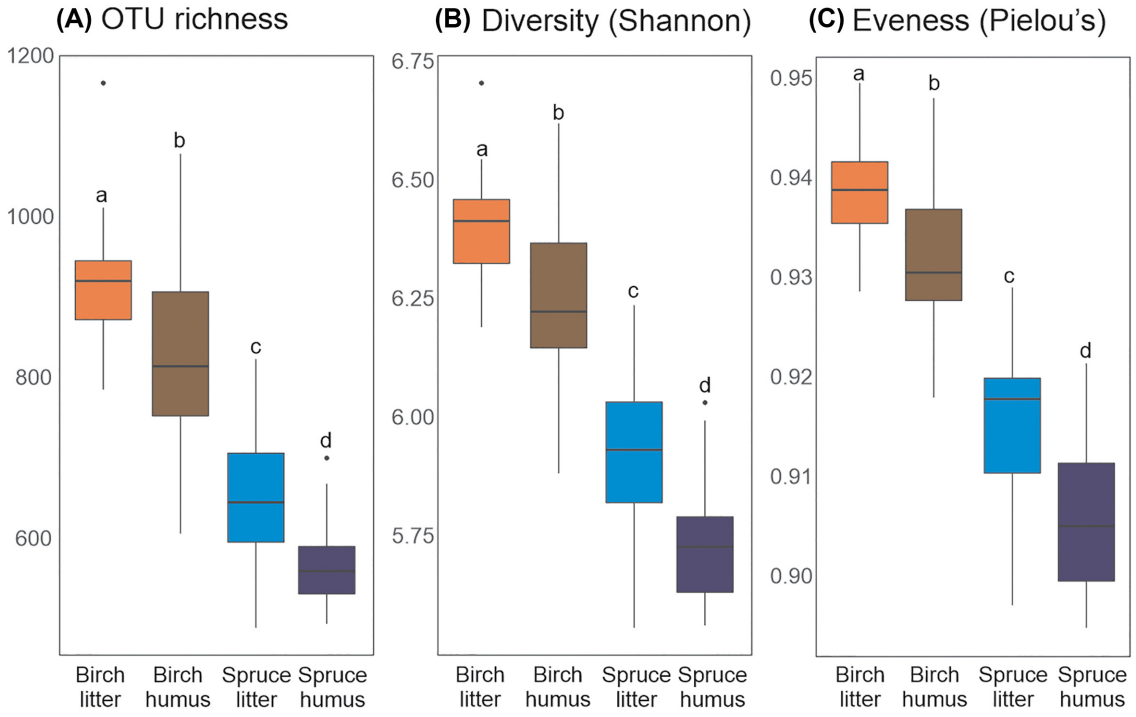

Figure 4. Box plots of OTU richness **(A)**, Shannon diversity index **(B)** and Pielou's evenness **(C)** in the two forest types and soil layers. The different letters above boxes indicate statistically significant differences at a significance level of $P < 0.05$ (Tukey post hoc tests). The box spans the IQR (first quartile to the third) with the median indicated by a dark horizontal line; the whiskers show the 1.5 × IQR, while outliers (>3 × IQR) are indicated by dark dots.

had a significantly lower P content compared with the birch forest samples (ANOVA and Tukey's PHT, $P < 0.05$; Fig. 1A–C). Further, soil pH was consistently higher in the birch samples, while there was no difference in pH between soil layers within forest types (ANOVA and Tukey's PHT, $P < 0.05$; Fig. 1D).

Mirroring the changes in edaphic factors, we observed a marked shift in fungal community composition from birch to spruce stands in both soil layers. As revealed by the NMDS analysis (Fig. 2), all samples were clearly separated by forest type, which accounted for 20% of the variation in community composition (PERMANOVA; Tables S1 and S2, Supporting Information). Soil horizon (i.e. litter versus humus samples) accounted for 6% of the variation in community composition (Table S1, Supporting Information), and segregated the samples along the second axis in the NMDS plot (Fig. 2). All measured edaphic factors correlated significantly to the compositional variation in the NMDS analysis (Table S3, Supporting Information). Above very small scales (>10 m), we did not observe any pronounced spatial effects on the community composition, as revealed by semi-variogram analyses (Fig. S2, Supporting Information), likely reflecting that the spatial variability in fungal communities was not associated with any unmeasured environmental factor influencing the fungal communities. Overall, Basidiomycota was the most abundant Phylum in the soil samples, with 62.4% of all sequences, followed by Ascomycota (31.3%) and Morteriellomycota (3.8%) (Fig. 3). The spruce stands had disproportionally higher amounts of Atheliales and Thelephorales, while the birch forests included more Agaricales, Filobasidiales and Mortierellomycota (Fig. 3). Fungal OTU richness, diversity (Shannon) and evenness (Pielou's) were all significantly higher

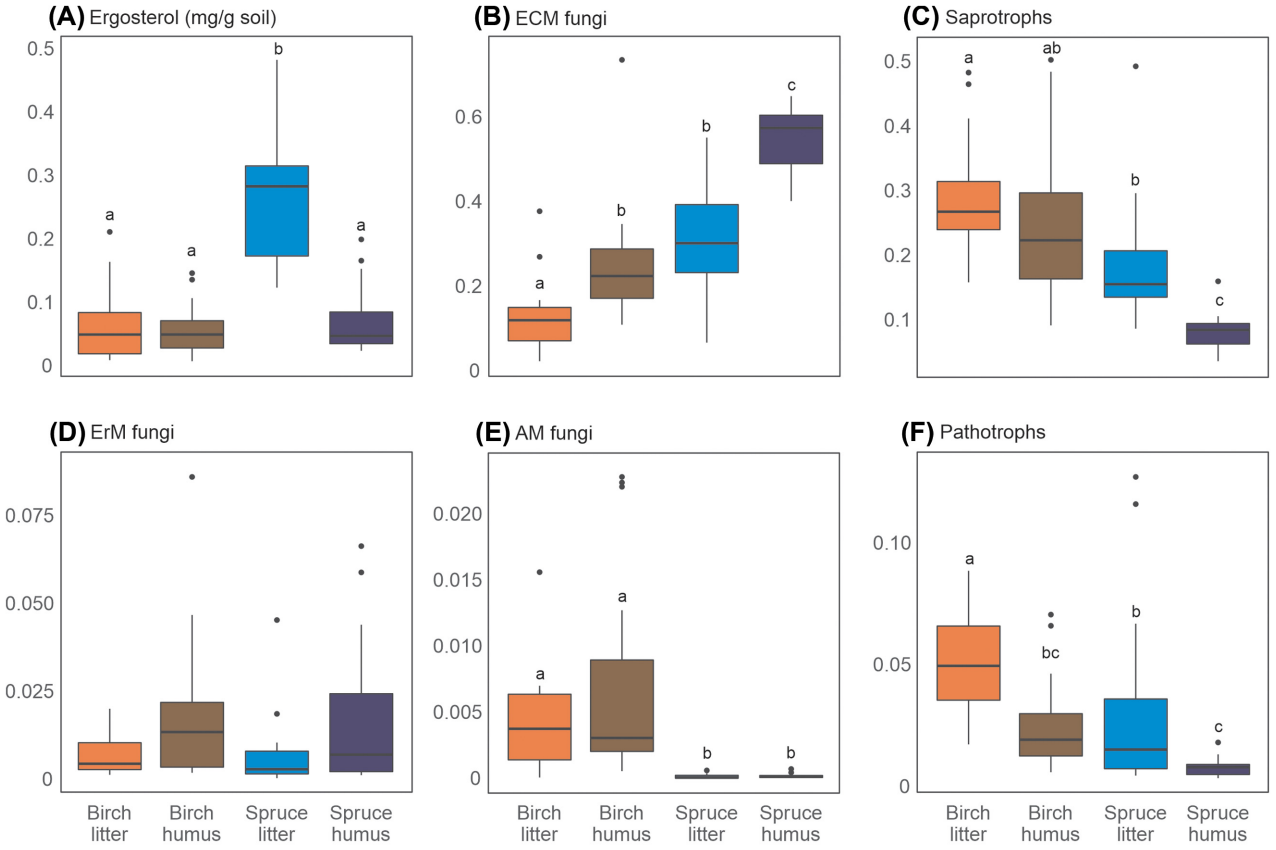

Figure 5. Box plots displaying **(A)** the relative abundance of ergosterol (mg/g soil) and main fungal guilds **(B–F)** across forest type and soil horizon, reported as mean relative sequence abundance per soil core. The different letters above boxes indicate statistically significant differences at a significance level of $P < 0.05$ (Tukey post hoc tests). The box spans the IQR (first quartile to the third) with the median indicated by a dark horizontal line; the whiskers show the 1.5 × IQR, while outliers (>3 × IQR) are indicated by dark dots.

in the birch forests in both soil layers, as well as in litter compared with the humus soil horizon (ANOVA and Tukey's PHT, $P < 0.05$; Fig. 4; Table S4, Supporting Information). Indicator species analyses revealed that the birch forests had, by far, the highest number of associated OTUs (779), as compared with spruce (320) (Table S5, Supporting Information). Mirroring the C and N differences, the amount of total ergosterol, which we used as a proxy for fungal biomass, was significantly higher in the spruce litter samples compared with the other sample types (Fig. 4A).

The three most abundant OTUs were EcM fungi, where the most abundant had 100% identity to the EcM fungus *Tylospora fibrillosa*, accounting for 4.5% of all sequences (Table S6, Supporting Information). EcM and saprotrophic fungi were the most abundant functional guilds in both the birch and spruce forest soils, but they were otherwise significantly differently distributed in the two forest types as well as soil horizons (ANOVA and Tukey's PHT, $P < 0.05$; Table S7, Supporting Information; Fig. 5). While the spruce stands were dominated by EcM fungi, the birch forests showed a more even distribution of the two guilds (Fig. 5; Fig. S3, Supporting Information). EcM fungi were particularly dominant in the humus horizon of the spruce forests, with on average >50% of the reads (indicated by the vector in Fig. 2). Saprotrophic fungi dominated the litter horizon in the birch forests, while it was a codominance of saprotrophic and EcM fungi in the humus horizon (Figs 2 and 5). AM fungi were significantly more abundant in the birch forest, ErM differed across the soil horizons, while pathotrophic fungi were associated with birch litter (ANOVA and Tukey's PHT, $P < 0.05$; Figs 2 and 5). The richness of AM fungi, pathotrophs and saprotrophs was significantly higher in the birch forest soil layers, and that of EcM fungi was significantly higher in the birch litter (ANOVA and Tukey's PHT, $P < 0.05$; Fig. S4, Supporting Information). As revealed by the indicator species analyses, numerous saprotrophs were significantly associated with the birch forests (156 in birch versus 6 in spruce). Even though the relative abundance of EcM sequences was significantly higher in the spruce forests, there was a higher number of EcM fungi preferentially associated with the birch forests (46 OTUs) compared with spruce (33) (Table S5, Supporting Information). AM fungi, which appeared in low abundances (19 OTUs), were associated with the birch forests only.

DISCUSSION

We observed distinct shifts in fungal community composition between the native birch forests (climax vegetation) and the planted spruce stands that mirrored corresponding changes in edaphic factors, in our study represented by C, N and P content and pH. The latter, pH, often found to be a strong predictor of fungal community turnover and biomass (Rousk, Brookes and Bååth 2009; Glassman, Wang and Bruns 2017), was significantly reduced in spruce forest soils, corresponding with what Smolander *et al.* (2005) observed in an earlier study also comparing spruce and birch humus. Our observation of a distinct turnover in belowground fungal communities with tree species shift cor-

responds well with previous studies also showing a strong tree species effect (Martínez-García *et al.* 2015; Urbnová *et al.* 2015; Uroz *et al.* 2016; Asplund *et al.* 2018; Bahnmann *et al.* 2018).

We observed a pronounced amplification in relative abundance of various EcM fungi, such as Atheliales and Theleophorales, in the spruce plantations and a corresponding decrease in Agaricales and Filobasidiales, as well as Mortierellomycota and AM fungi. This corresponds with results from a previous study (Urbanová, Šnajdr and Baldrian 2015), where higher abundance of EcM fungi also was observed under coniferous trees. In temperate forest systems, EcM fungi play an important role in C sequestration processes (Clemmensen *et al.* 2013, 2014), where in general more soil C is observed in EcM-dominated soils compared with AM-dominated soils (Averill and Hawkes 2016). This corresponds well with C stock measurements in spruce and birch forest soils in western Norway, where an increase in soil C stocks has been observed in spruce forest soils compared with the native birch forest soils (Kjønaas *et al.* submitted for publication). While the ECM fungi almost doubled in relative sequence abundance in the spruce forests, the abundance of saprotrophic fungi approximately halved. Although speculative, the relatively smaller community of saprotrophic fungi in the spruce litter compared with birch litter may be coupled to a tentatively slower turnover time of spruce litter. The amount of total ergosterol, used as a proxy for fungal biomass, was also markedly higher in spruce litter compared with the other soil types, paralleled by the higher amounts of C and N content. The accumulating spruce litter, with associated fungal mycelia, may play an important role in immobilization of N through a slower turnover of organic matter compared with the birch litter. A higher relative abundance of EcM fungi in spruce forests could possibly also lead to higher C sequestration because of a stronger 'Gadgil effect' in which EcM fungi compete with saprotrophs for limited N, suppressing decomposition rates (Gadgil and Gadgil 1975). However, whether the effect is present in this system needs to be confirmed experimentally.

In various studies, soil depth has earlier been found to be a strong driver of fungal community composition and the observed vertical distribution of fungal communities in our study was in accordance with earlier findings (Rosling *et al.* 2003; Lindahl *et al.* 2007; Baldrian *et al.* 2012). In the spruce plantations, saprotrophic fungi were, as expected, most abundant in the litter layer, while ectomycorrhizal fungi were more abundant in the humus layer. This niche partitioning was not so clear in the birch forest, where saprotrophic fungi were more evenly distributed in both horizons. This is probably due to a higher degree of mixing of soil, leading to a gradual transition between soil layers in the birch forest. In a recent study by Asplund *et al.* (2018), where fungal communities in adjacent spruce and beech forests were compared, soil depth was the primary factor structuring the fungal communities. In contrast, forest type came out as the strongest driver in the ordination analysis in our study, accounting for most of the compositional variation in fungal communities. This difference may indicate that a shift from birch to spruce forests has a stronger effect on belowground communities, compared with a shift from spruce to beech. Bahnmann *et al.* (2018) also observed that fungal communities in deeper soil horizons correlated strongly with pH, while fungal communities in the litter layer were more affected by dominating tree species. We were not able to separate these effects, since pH was highly correlated with forest type in both soil horizons. The studied spruce plantations were established some 60–70 years ago, which also suggests that spruce plantations established outside their native ranges can have a fast effect on reshaping belowground communities.

The shift to spruce plantations led to a significant reduction in the overall fungal richness and the indicator species analyses demonstrated that numerous fungi were preferentially associated with the birch forests. Hence, many species present in the soils prior to the tree species shift were not able to adapt to the new abiotic and biotic conditions. In general, the birch forest soils, with higher pH and amount of P, may provide more favorable conditions for many fungi. Further, the higher fungal richness in the birch forests may also be linked to the more diverse understory vegetation, including grasses and herbaceous species, compared with the *Sphagnum*-dominated spruce understory. The more diverse understory vegetation in birch forests likely facilitates a more varied community of both decomposers and root-associated fungi. We observed a markedly higher diversity of saprotrophs in the birch forests, which corresponds with the findings of Otsing *et al.* (2018), who demonstrated that richness in foliar litter types was the best predictor of fungal richness. The same trend appeared for the root-associated fungi. For example, AM fungi were in our study almost absent from the spruce forests, while numerous OTUs were significantly associated with the birch forests, likely growing on roots of grasses and herbs.

Our results demonstrate that the replacement of native birch forests with spruce plantations has major effects on belowground fungal communities, as well as different soil properties. The observed changes in the composition of functional guilds also indicate that ecosystem processes, mediated by the belowground fungi, also change, with tentative effects on C sequestration processes. In order to better understand the underlying mechanisms causing the observed changes, we call for more experimental studies in this study system, using ditching to study tentative Gadgil effects and ingrowth mesh bags and litter bags to study the production of belowground mycelia and C turnover processes.

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SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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DATA AVAILABILITY

The data underlying this article are available in Zenodo at https://zenodo.org/record/4936775.

AUTHOR CONTRIBUTIONS

This study is based on the M.Sc. thesis of JSD. JSD, LM, SM and HK designed the study and conducted field work. JSD, LM and SM conducted lab work and JSD, LM, SM and MD did bioinformatics

and statistics. JSD and HK wrote the manuscript with edits and comments from all coauthors.

Conflict of interest. None declared.

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