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The balance between accumulation and loss of soil organic matter in subarctic forest is related to ratios of saprotrophic, ecto- and ericoid mycorrhizal fungal guilds

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ABSTRACT

Free-living saprotrophic fungi and symbiotic mycorrhizal fungi affect organic matter dynamics differently because of contrasting ecological adaptations. We investigated how mass-loss, C:N-ratio and stable isotope dynamics of leaf litter and humus substrates depended on presence of living tree roots and associated fungal communities in a forest-to-tundra ecotone over three years. Litter mass-loss was stimulated by tree roots, contrary to a Gadgil effect. Increases in the litter nitrogen pool and $\delta^{15}N$ suggested import of nitrogen from deeper soil by the dominating saprotrophic fungi. Over time, humus first lost, then gained, mass, and corresponding shifts in $\delta^{15}N$ and $\delta^{13}C$ suggested fluctuating pools of fine roots and fungal mycelium. Ectomycorrhizal tree roots consistently reduced longer-term humus mass-gain, counteracting positive effects of ericoid roots and associated fungi. Across all substrates, mass dynamics correlated with the balance between ectomycorrhizal and litter-saprotrophic fungi, both linked to mass-loss, and ericaceous shrubs and associated fungi, linked to mass-gain.

1. Introduction

Most northern forest and tundra ecosystems have accumulated soil organic matter since the last glaciation, and they continue to be globally important belowground carbon sinks (Post et al., 1982; Pan et al., 2011). This accumulation reflects a positive net balance between organic matter inputs, ultimately derived from plant photosynthesis, and losses, mainly driven by decomposer organisms. Free-living saprotrophic fungi dominate early stages of plant litter decomposition, driving overall mass-loss when the organic matter still contains ample amounts of high-energy resources, such as cellulose (Lindahl et al., 2007; Baldrian et al., 2012). At later decomposition stages in deeper soil layers, different types of mycorrhizal fungi affect both organic matter inputs and losses. The traits of dominant mycorrhizal fungi have been proposed to determine the net balance between inputs and losses, and thus the magnitude of the belowground sink (Clemmensen et al., 2013; Frey, 2019; Kyaschenko et al., 2019; Lindahl et al., 2021; Ward et al., 2021; Argiroff et al., 2022; Mayer et al., 2023). Mycorrhizal fungi receive sugars directly from their plant hosts through specialized root structures and transport carbon further into the soil matrix to support mycelial growth, metabolism and production of exoenzymes (Bödeker et al., 2014; Shah et al., 2016; Martino et al., 2018; Frey, 2019; Nicolas et al., 2019). They thus provide a direct pathway for recent photosynthate from the plants into the soil, but some mycorrhizal fungi may also drive organic matter turnover directly by enzyme production, or indirectly, by supplying other decomposer organisms with easily available organic molecules (Phillips et al., 2013; Lindahl and Tunlid, 2015; Mayer et al., 2023). Furthermore, in some cases, mycorrhizal fungi have been found to restrict decomposition by competing for nutrients with free-living saprotrophs (Gadgil and Gadgil, 1971; Fernandez and Kennedy, 2016), the so-called “Gadgil effect”.

Boreal and sub-arctic forests are dominated by trees, such as pine, spruce and birch, which grow in ectomycorrhizal symbiosis with fungi primarily belonging to the Basidiomycota, and often have a dense understory of ericaceous shrubs with hair roots hosting a diversity of other fungi. These fungi, which include both well-characterized ericoid

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mycorrhizal fungi and other root associated ascomycetes, have been proposed to be ecologically versatile, potentially mixing biotrophic and saprotrophic lifestyles (Martino et al., 2018; Perotto et al., 2018). Both ectomycorrhizal and ericoid mycorrhizal fungi have been found to suppress early-stage decomposition of plant litter, although this effect is not consistent and usually rather small, around a 10% reduction in mass-loss after 1–2 years (Gadgil and Gadgil, 1971; Sterkenburg et al., 2018; Fanin et al., 2022). Other studies have suggested that trees may accelerate decomposition through association with ectomycorrhizal fungi that mine organic nitrogen and consequently release carbon from more decomposed organic matter (Clemmensen et al., 2021; Lindahl et al., 2021; Parker et al., 2021). Ericoid mycorrhizal plants and fungi, in contrast, have been associated with greater soil organic matter accumulation (Ward et al., 2021), potentially linked to production of recalcitrant above- and belowground litters (Cornelissen et al., 2007; Fernandez et al., 2019) and chemical stabilization (Adamczyk et al., 2019). Although ericoid mycorrhizal fungi have a broad repertoire of enzymes to degrade polysaccharides and other easily hydrolysable litter components, as well as some capacity to oxidize polyphenolic compounds (Cairney and Burke, 1998; Martino et al., 2018), they generally lack the most potent oxidative enzymes – the class II peroxidases (Floudas et al., 2012; but see: Ferrari et al., 2021), which basidiomycete litter degraders and some ectomycorrhizal fungi use to attack recalcitrant organic polymers, such as lignin, tannins, melanin and other non-hydrolysable compounds (Lindahl and Tunlid, 2015; Shah et al., 2016). In the face of ongoing climate warming, with an overall vegetation shift towards increasing abundance of ectomycorrhizal trees and shrubs at the forest-to-tundra ecotone, it is important to evaluate effects of saprotrophic, ectomycorrhizal and ericoid mycorrhizal fungal guilds on decomposition of different organic substrates and how the balance between these groups, and potentially within-group species composition and traits, may regulate net accumulation of belowground organic matter.

Decomposition of organic substrates can be accurately monitored by following the mass-loss of discrete organic resources, such as wood blocks or plant litters, incubated for different time spans in the laboratory or in field experiments (Moore et al., 2017; Fukasawa et al., 2020). Input of organic matter through biomass production by mycorrhizal fungal mycelia and roots, on the other hand, can be assessed by quantifying ingrowth into inert substrates, such as sand, in mesh bags or cores (Wallander et al., 2013). However, in naturally decomposing organic substrates these two processes occur simultaneously, and it is their net balance that determines long-term belowground carbon accumulation (Clemmensen et al., 2013; Kyaschenko et al., 2019). Changes in stable carbon isotope ratios, *i.e.*, ^{13}C:^{12}C (notated as $\delta^{13}C$), of organic substrates can help to identify the carbon pools that are lost and gained over time, even without a net change in substrate mass, and may complement measurements of mass-loss for pinpointing the dominant processes underlying organic matter dynamics (Ehleringer et al., 2000; Heath et al., 2005; Godbold et al., 2006). However, an isotopic mass balancing approach requires good knowledge of $\delta^{13}C$ of various relevant input sources in an ecosystem, for instance, roots and mycorrhizal mycelium, which are commonly ^{13}C-enriched relative to aboveground litters (Ehleringer et al., 2000). Similarly, stable nitrogen isotope ratios, *i.e.*, ^{15}N:^{14}N ($\delta^{15}N$), of decomposing substrates can indicate which nitrogen sources have been lost or gained during different decomposition phases. In early stages of decomposition, nitrogen may be imported into litter substrates by saprotrophic fungi (Melillo et al., 1989). Release from competition by ectomycorrhizal fungi may manifest as higher $\delta^{15}N$-levels in the decomposing litter, because the saprotrophs can access nitrogen from deeper soil layers (Melillo et al., 1989; Sterkenburg et al., 2018), potentially stimulating litter decomposition (Gadgil and Gadgil, 1971; Boberg et al., 2014; Bödeker et al., 2016). At later decomposition stages, differences in mycorrhizal nitrogen uptake and translocation to the host also affect soil $\delta^{15}N$ signatures, as there is strong discrimination against ^{15}N during transfer to the host roots, increasing the $\delta^{15}N$ signature of the residual nitrogen pool (Högberg et al., 1999a; Clemmensen et al., 2021).

Previously we examined mass-loss, respiration, fungal biomass and guild composition in organic substrates incubated in a mountain birch forest close to the subarctic-alpine treeline and found that tree roots and associated ectomycorrhizal fungi, notably from the genera *Cortinarius* and *Leccinum,* accelerated decomposition, potentially explaining the smaller soil organic matter stocks in forest compared to heath (Clemmensen et al., 2021). In the present study we compared these data with mass-loss of the same substrates during incubation in a nearby dwarf shrub heath above the forest. We incubated both fresh litter from aboveground plants (hereafter "litter") and more decomposed material from deeper in the organic horizon (hereafter "humus"). In the forest, the substrates were exposed to both tree roots and understory dwarf shrubs, or to only dwarf shrubs in trenched plots. For reference, trenched plots were included also on the heath sites, although no trees were affected there. Fungal community composition was assessed by ITS2 amplicon sequencing. By monitoring changes in carbon:nitrogen (C:N) ratios and $\delta^{13}C$ and $\delta^{15}N$ signatures of substrates incubated in the forests we were able to disentangle effects of ectomycorrhizal tree roots and fungi on carbon and nitrogen dynamics from those of ericoid plants and their associated fungi.

We hypothesized that:

(**H1**), all organic substrates would decompose more rapidly when exposed to the tree roots, microorganisms and less harsh environment of the forest compared to the heath, although this effect would only slightly offset larger differences among substrate types (Parker et al., 2018).

(**H2**), in forest, the presence of ectomycorrhizal fungi associated with living tree roots would inhibit decomposition of fresh litter by free-living saprotrophic fungi (a "Gadgil effect"). Tree root exclusion would result in increased decomposition and $\delta^{15}N$ in the litter due to enhanced access by saprotrophic fungi to nitrogen in deeper layers (Sterkenburg et al., 2018).

(H3), in forest, the presence of living tree roots would accelerate mass-loss of more decomposed humus, linked to nitrogen "mining" by ectomycorrhizal fungi, particularly the genera *Cortinarius* and *Leccinum* (Clemmensen et al., 2021), which would increase the C:N ratio and decrease the residual nitrogen pool, relative to plots from which tree roots were excluded.

(H4), in the longer-term, humus would increase in mass linked to ingrowth of ericoid mycorrhizal roots and fungi ("accumulation"), but only in the absence of simultaneous mining of organic matter by ectomycorrhizal fungi associated with forest trees. The $\delta^{15}N$ and $\delta^{13}C$ of accumulated carbon and nitrogen in humus would thus match isotope abundances in roots and mycorrhizal fungi.

2. Material and methods

2.1. Site description

The work was conducted at two sites situated 300 m apart, 5 km south-east of Abisko in Northern Sweden; an arctic downy birch (*Betula pubescens* ssp. *czerepanovii*) forest (68°19′49.6″N, 18°50′53.1″E; 500 m above sea level) and a dwarf shrub heath above the forest edge (68°19′33.8″N, 18°51′16.1″E; 540 m above sea level). In the forest, the birch trees are the only ectomycorrhizal plants, and the dense understory is dominated by ericaceous dwarf shrubs (*Empetrum nigrum, Vaccinium vitis-idaea, V. myrtillus* and *V. uliginosum*) and a grass (*Deschampsia flexuosa*). The heath vegetation is dominated by a small-scale mosaic of ericaceous (*Cassiope tetragona*, *V. uliginosum*, *E. nigrum*) and ectomycorrhizal (*Betula nana*, *Salix* spp., *Dryas octopetala*) dwarf shrubs, and some herbs (*Carex* spp.) and mosses (Clemmensen et al., 2021). At both sites, a purely organic soil layer overlies a well-drained mineral soil without permafrost. The soil is podzolized with a 10 cm and 15–25 cm

deep organic layer in the forest and heath, respectively. The climate in the area is subarctic, with mean summer and winter temperatures of 10 °C and −9 °C, respectively, an annual precipitation of ~300 mm, and a snow-free season from late May to early October (Abisko Scientific Research Station).

2.2. Decomposition experiment

Mesh-bag samples from Clemmensen et al. (2021) were analysed for elemental (C and N) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition. Data on mass-loss and respiration from the forest site were obtained from Clemmensen et al. (2021) and compared with new data from bags incubated in a parallel experiment in the heath. Within each site, six pairs of 60 cm-diameter sampling plots were established 10 m apart from each other. In one of each of the paired plots, horizontal root ingrowth was eliminated by permanent barriers (trenched), with the aim of disrupting ectomycorrhizal connections, while the other plot was kept intact (control). Trenched plots were constructed by excavating an intact turf, well into the mineral soil, with a spade, and insulating the margin of the resulting hole with a double, sealed plastic sheet before replacing the turf (Fig. S1). The plastic liner allowed drainage through the bottom and the shrub vegetation remained unmanipulated throughout the experiment. The trenching treatment was thus expected to have largest effects in the forest plots, which were dominated by extensive, ectomycorrhizal tree root systems, but trenching with permanent barriers was also implemented in the heath to address long-term, non-target effects on shrubs. Long-term side-effects caused by severing the understory shrubs in the forest would likely be less well captured in disturbance-controls (with initial trenching but no permanent barrier), which typically converge towards the control state within the first year due to recolonization from the surroundings (Mielke et al., 2022).

Mesh-bags (4 × 8 cm) were constructed of 45 μm nylon mesh (Sintab Produkt AB, Oxie, Sweden) and filled with five different organic substrates: (1) forest litter, (2) heath litter, (3) forest humus, (4) upper heath humus and (5) lower heath humus. The litters were mixtures of vascular plant leaf litters collected at the soil surface. The humus was the amorphous organic horizon below the layer of intact and fragmented leaf litter. Forest humus substrates represented the entire humus depth (2–10 cm depth), while heath humus was split into the upper (2–10 cm) and lower (10–20 cm) fractions of the humus depth, due to the deeper and more stratified humus (Fig. S1). Substrates were collected from 6 subplots per site, mixed, cleaned from living stems and roots of >1 mm diameter and dried at 40 °C until attaining constant weight. The bags were filled with 2 g litter or 4 g humus and sealed. Bags were incubated at 2–10 cm depth in the six paired plots at each site. Two sets of substrates were incubated from late July 2009 until either August 2010 or August 2012. Three bags of each substrate type were not incubated but used for measurements of initial substrate characteristics. Upon recovery in 2010, bags were directly frozen at −20 °C and later freeze-dried and weighed to assess mass change. In 2012, bags were carefully transported to the laboratory and respiration measured, block-wise, within 10 h. The intact bags were placed in a closed chamber (173 cm^3) connected to an infrared gas analyser (EGM-4 Environmental Gas Monitor; PP Systems, Amesbury, MA, USA) and linear increase in CO_2 concentration was monitored over 4 min at 18 ± 1 °C and field moisture conditions (average water content of 71%, range 57–80%, of fresh weight). The bags were then frozen at −20 °C and later freeze-dried, weighed, and milled to a fine powder for further analyses. The full, retrieved sample set consisted of 5 substrates x 2 treatments x 6 replicates x 2 sites x 2 harvests, minus four bags lost during incubation = 236 samples in total.

2.3. Carbon, nitrogen and stable isotope analyses and mass balance

To test the more specific hypotheses related to effects of tree root exclusion on carbon and nitrogen dynamics in the forest (H2 and H3), freeze-dried subsamples from forest incubations and initial substrates were analysed for total carbon and nitrogen as well as ^{13}C:^{12}C and ^{15}N:^{14}N ratios on an Isoprime isotope ratio mass spectrometer (Elementar, Cheadle Hulme, UK) coupled to a Eurovector CN elemental analyser (Pavia, Italy) using continuous flow. The isotopic composition is expressed as the isotopic ratios relative to the international standards Pee Dee Belemnite ($CaCO_3$) for carbon (notation $\delta^{13}C$) and atmospheric N_2 for nitrogen ($\delta^{15}N$). We used isotopic mixing models (Godbold et al., 2006) to predict the isotopic signatures of the carbon and nitrogen pools that were gained or lost during each incubation phase:

$$\delta_{initial}M_{initial} = \delta_{final}M_{final} + \delta_{source}M_{source}, \text{ with } M_{initial} = M_{final} + M_{source},$$

where $M_{initial}$ is the pool of carbon or nitrogen in the original sample (g bag^{-1}) and $\delta_{initial}$ is its isotopic composition, M_{final} is the amount of carbon or nitrogen after the field incubation and δ_{final} its isotopic composition, M_{source} is the amount of carbon or nitrogen that was lost or gained during the incubation, *i.e.,* $M_{source} = M_{initial} - M_{final}$, and δ_{source} is the isotopic composition of this pool. Replacing M_{source} in the equation by $M_{initial} - M_{final}$ and rearrangement will give the isotopic composition of the source, *i.e.,* of the lost or accrued pool:

$$\delta_{source} = \left(\delta_{initial}M_{initial} - \delta_{final}M_{final}\right) / \left(M_{initial} - M_{final}\right).$$

To evaluate dynamics of the absolute carbon and nitrogen pool per bag over time, we calculated the pool changes per incubation period, *i.e.,* for year 0–1 and for year 1–3, as:

$$M_{change} = \left(M_{final} - M_{initial}\right) / M_{initial} \text{ x } 100\%,$$

which gives positive values when pools increase and negative values when pools decrease. Our five substrates varied in initial organic matter content (litters had c. 40% carbon, and forest and upper heath humus had c. 36% and lower heath humus 32% carbon), therefore calculations based on carbon pools were more exact and comparable, although total mass dynamics were similar.

2.4. Fungal community analysis

Total DNA was extracted from 50 mg freeze-dried and milled material from the second forest harvest using the CTAB protocol (Clemmensen et al., 2016). Extracts were cleaned using the Wizard DNA clean-up system (Promega, Madison, WI, USA). Fungal community amplicons for sequencing were generated by PCR amplification of ITS2 markers using the forward primer gITS7 (Ihrmark et al., 2012) and the reverse primer ITS4 (White et al., 1990) elongated with 8 bp long sample-identification tags. PCRs were run in technical triplicates with template concentration and amplification cycles optimized to obtain quantitative amplification (Castaño et al., 2020). The PCR mix consisted of 0.2 mM dNTPs, 0.75 mM $MgCl_2$, 0.5 μM of gITS7 primer, 0.3 μM of ITS4 primer and 0.5 U of DNA polymerase (Dream Taq, Fermentas, Sweden) in 50 μl reactions. Thermal cycling conditions were 5 min at 95 °C, followed by 27–35 cycles of 30 s at 95 °C, 30 s at 56 °C and 30 s at 72 °C, and a final 7 min at 72 °C. Amplicons were purified using the Agencourt AMPure kit (Beckman Coulter, Beverly, MA, USA) and concentrations measured fluorometrically (Qubit high sensitivity kit, Invitrogen, Carlsbad, CA, USA). Amplicons were mixed in equal amounts into one sequencing pool that was further cleaned with the Cycle Pure kit (EZNA, Omega Bio-Tek, Nocross, GA, USA). Adaptor ligation and 454-sequencing were performed by LGC Genomics GmbH (Berlin, Germany) on a GS-FLX Titanium system (Roche, Basel, Switzerland). Initial substrates had 1/10 as much DNA as incubated samples and were not sequenced.

Sequences were quality filtered and clustered using the bioinformatics pipeline SCATA (http://scata.mykopat.slu.se/; Ihrmark et al., 2012). High quality sequences (>20 in average score and >3 of individual bases) with 100% match with the sample-identification tags and

at least 90% match with the primer sequences passed quality control and were clustered into species level clusters by single-linkage clustering using a 98.5% sequence similarity criterion for pairwise comparisons (Usearch; Edgar, 2010). All fungal reference sequences in the UNITE database (https://unite.ut.ee/; Abarenkov et al., 2010) were included passively in the clustering to verify species delimitation and provide identification. Thus, our clusters simulate global species hypotheses as defined in UNITE (Koljalg et al., 2020), and we hereafter refer to fungal clusters as "species" although this is an approximation. In total, 110,610 high quality sequences were assembled into 1128 global clusters. After removing singletons (6355 reads), non-fungal clusters (10 clusters; 3062 reads), and clusters with less than 5 reads (536 clusters; 1354 reads), the final data set consisted of 582 fungal species and 99,839 reads. The 232 species with at least 30 reads (93% of total reads) were further assessed for taxonomic identities and functional guilds, by comparing reference sequences to annotated UNITE and INSD reference databases using the massBLASTer in the UNITE PlutoF module. At least 98% similarity was required for species identification, otherwise species were identified to higher taxonomic levels or left unidentified. Species that could not be assigned to functional guilds based on taxonomic information were assigned based on sequence similarity to reference sequences obtained from well-defined substrates, such as cleaned roots or plant litter. For each main phylum (Basidiomycota, Ascomycota, Zygomycota) the following fungal guilds were classified: ectomycorrhizal fungi, other root associated fungi (including ericoid mycorrhizal species) and saprotrophic fungi with subgroups of moulds and yeasts. Ectomycorrhizal fungi were sub-divided into "long" and "short" distance exploration types, where the "long" type included genera that form abundant extramatrical mycelium (*i.e.*, long and medium-fringe species of *Cortinarius*, *Piloderma*, *Leccinum*, *Suillus*, *Rhizopogon*, *Chalciporus*) and the "short" type included genera with less differentiated mycelium (*i.e.*, contact, short and medium-smooth species of *Polyozellus*, *Tomentella*, *Russula*, *Lactarius*, *Inocybe*, *Hebeloma*, *Laccaria*) (Agerer, 2006). Representative sequences, classifications and data matrices are available at DRYAD (https://doi.org/10.5061/dryad.79cnp5htw). Sequence raw data are archived at the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under accession number PRJNA662784. Ergosterol concentration was used as a proxy for fungal biomass (data from Clemmensen et al., 2021), and proportion of summed ITS2 markers belonging to *Empetrum nigrum, Vaccinium vitis-idaea*, *V. uliginosum* and *V. myrtillus* (together representing 95% of non-fungal reads) out of total quality filtered ITS2 amplicons was used as a proxy for biomass of ericaceous dwarf shrub roots.

2.5. Statistical analyses

Univariate data were analysed with SAS 9.4 (Statistical Analysis System Institute, Cary, NC, USA) using linear mixed models (PROC MIXED). Fixed factors were substrate type (df = 4), trenching (df = 1) and their interaction for data analysed separately per time point (source carbon and nitrogen pools and isotopic signatures, ergosterol concentration, fungal guild and ericoid root abundances), while incubation time (df = 1) and all interactions were included in models of substrate nitrogen pool, C:N and stable isotope signatures. Models for mass-loss (obtained from both forest and heath incubations) also included site (df = 1) and all interactions. All models included treatment block as a random factor to account for potential spatial dependencies. Degrees of freedom (df) for *F* values were estimated using the Kenward-Roger adjustment, and results were evaluated using the Tukey-Kramer adjustment for multiple comparisons with $\alpha = 0.05$. Proportional data (relative abundances of fungal guilds and ericoid roots) were arcsine transformed and mass-loss was square root transformed to remove heteroscedasticity and improve residual distribution (visual inspection).

Multiple linear regression models of the carbon pool change across all five organic substrates during the second incubation period (year 1–3) against relative abundances of major fungal guilds as potential predictors (log10 transformed) were ranked based on Akaike's information criterion (AIC), using the PROC REG statement. All models were run with intercept fixed at zero so that guilds included with a positive sign were associated with net carbon gain and guilds with a negative sign were associated with net carbon loss.

Fungal community composition at species level was evaluated using correspondence analysis (CA) and canonical correspondence analysis (CCA) in CANOCO 5 (Microcomputer Power, Ithaca, NY, USA). For initial visual inspection, a CA was performed across all samples, and due to large differences among substrates, separate CAs were run for humus and litter substrates to visualize trenching effects. To inform interpretation of the axes, the net mass loss over the three years and the relative abundances of the four main fungal guilds (litter saprotrophs, moulds and yeasts, ectomycorrhizal fungi, and other root-associated fungi) were correlated to the axes (without affecting the ordination). We judged CA outputs to be robust and not affected by artificial arch effects based on parallel NMDS analyses yielding similar patterns. A CCA was then used to specifically test how shifts in fungal community composition related to the net change in the carbon pools in the humus substrates during the last two years of incubation. This analysis thus included one constraining factor resulting in one constrained axis related to net carbon pool change. Sequencing depth and substrate type were included as covariates. To further inform interpretation of axes, the same variables as for the CAs were correlated with the axes post-ordination. Community data was Hellinger transformed for these analyses.

3. Results

3.1. Substrate mass dynamics in heath versus forest

The overall strongest determinant of mass-loss was substrate type ($F = 380$; $p < 0.0001$; Fig. 1). Mass-loss during the first incubation year was higher for forest litter than heath litter, while the upper heath humus had the largest mass-loss and forest humus the smallest among the humus substrates. There was no main effect of incubation site, but the mass-loss pattern across substrates depended on site, particularly after three years (significant interactions). Thus, as expected, litter substrates progressively lost mass with longer incubation. For humus substrates, in contrast, after initial mass-loss during the first incubation year, mass was either constant or increased during the subsequent two years of incubation (that is, their net mass-loss, relative to initial mass, declined from year 1 to year 3). The increase in humus mass during the second incubation period was particularly evident for samples incubated in the forest (Fig. 1). After three years, trenching had decreased mass-loss across all substrates and at both sites (significant trench × year interaction), although heath substrates seemed less affected by trenching at the heath. Accordingly, during the second period, root disruption in the forest led to a larger carbon pool gain in humus, and a smaller carbon pool loss in litter, relative to substrates incubated in control plots with intact root systems (Table 1).

Respiration from the substrates measured directly after the three-year harvest correlated positively with mass-loss over the preceding three years (Fig. S2). Substrates incubated in the forest site, however, had overall higher respiration rates than substrates in the heath, for comparable mass-loss levels.

3.2. Dynamics of nitrogen and carbon sources in the forest incubations

The overall pattern in C:N ratio across substrates reflected C:N ratio before incubation but did not align with patterns of mass-loss. Heath litter had a higher C:N ratio than forest litter, but forest humus had higher C:N ratio than the heath humus substrates; the heath humus collected from deeper layers had the lowest C:N ratio (Fig. 2A). Litter C:N ratio declined more with time than humus C:N, which stayed close to initial values throughout the experiment. Litter substrates maintained a stable total nitrogen pool in the bags during the first year, while humus

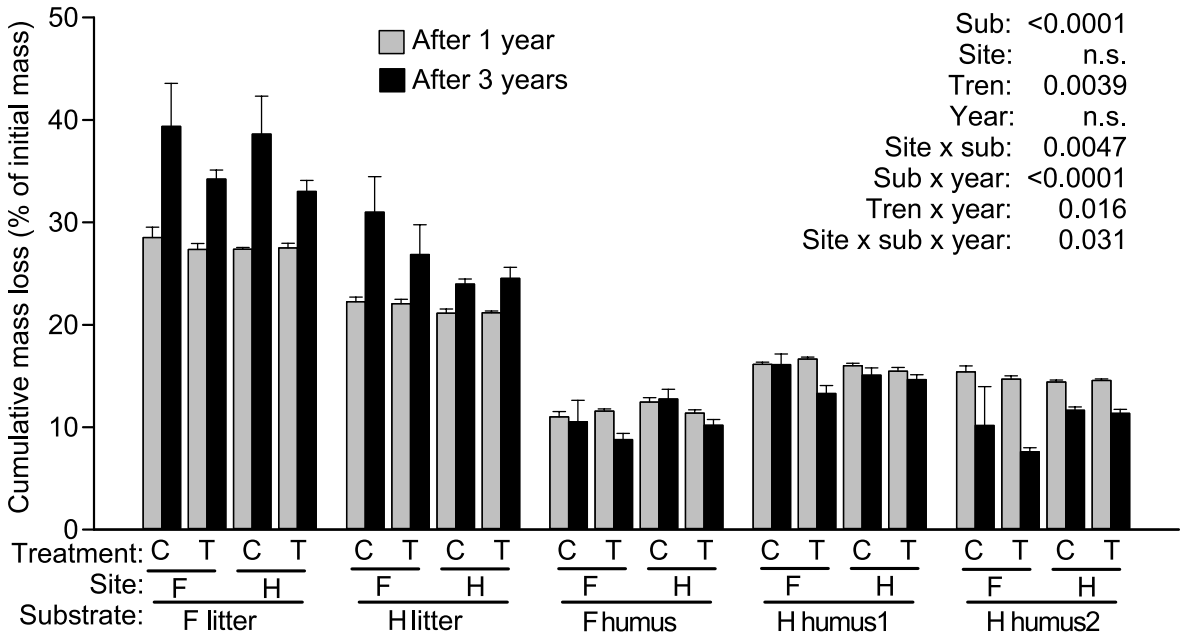

Fig. 1. Cumulative net mass loss of five decomposing substrates incubated for one (grey bars) or three (black bars) years in either control plots (C) or in plots from which living tree roots were excluded by trenching (T). The experiment was conducted at two sites, a subarctic forest (F) and a close-by alpine heath (H). The substrates were litter and humus collected at the same forest and heath; two humus depth layers were collected from the heath. All bars represent averages (n = 6) with the error bar representing one standard error of the mean. Results (p-values) from linear mixed models testing effects of substrate type (Sub), site of incubation (Site), trenching treatment (Tren) and incubation duration (Year) and their interactions are shown; interaction terms with $p > 0.20$ were excluded from the model. The data from the forest site are from Clemmensen et al. (2021).

Table 1
Estimates of stable isotope signatures of the lost or gained C and N pools (sources) during two incubation phases (year 0–1 or year 1–3) of five organic substrates in either control (C) plots or in plots from which living tree roots were excluded by trenching (T). The ericaceous understory vegetation was intact throughout the experiment. When C and N pools changed by less than ±5% of initial mass for an incubation period, estimated source isotopic signatures were uncertain and therefore excluded (average shown if n = 3–6), while all replicates were used for calculating C and N pool changes (n = 6). Results (P-values) of linear mixed models testing effects of substrate type (Sub) and trenching (Tren) and their interaction on each variable are shown.

Substrate		Year 0–1				Year 1–3			
		C change (%)	Source $\delta^{13}C$ (‰)	N change (%)	Source $\delta^{15}N$ (‰)	C change (%)	Source $\delta^{13}C$ (‰)	N change (%)	Source $\delta^{15}N$ (‰)
Forest litter	**C**	−28 ± 1.2	−30.9 ± 0.1	1 ± 3		−17 ± 5	−32.1 ± 0.3	7 ± 4	1.3 ± 4.2
	T	−28 ± 0.5	−30.7 ± 0.1	0 ± 2		−10 ± 1	−31.3 ± 0.9	7 ± 3	−5.0 ± 6.8
Heath litter	**C**	−23 ± 0.8	−28.8 ± 0.1	0 ± 1		−11 ± 5	−30.1 ± 2.6	9 ± 3	8.1 ± 7.9
	T	−22 ± 0.7	−28.7 ± 0.1	−1 ± 2		−7 ± 4	−31.7 ± 1.0	16 ± 4	2.2 ± 6.2
Forest humus	**C**	−10 ± 0.7	−19.4 ± 0.7	−15 ± 2	−1.2 ± 0.2	1 ± 2		1 ± 3	
	T	−12 ± 0.5	−21.1 ± 0.3	−17 ± 1	−0.4 ± 0.5	7 ± 1	−18.3 ± 1.5	13 ± 2	−6.1 ± 1.7
Heath upper humus	**C**	−16 ± 0.6	−22.6 ± 0.3	−17 ± 1	−4.1 ± 0.5	1 ± 2		13 ± 3	−4.4 ± 2.8
	T	−17 ± 0.6	−23.0 ± 0.2	−19 ± 2	−3.0 ± 0.3	8 ± 2	−20.4 ± 1.7	16 ± 3	−4.3 ± 2.2
Heath deep humus	**C**	−15 ± 0.7	−22.0 ± 0.3	−19 ± 1	−2.3 ± 0.2	8 ± 3	−19.5 ± 1.3	12 ± 5	−1.0 ± 1.4
	T	−15 ± 0.9	−21.9 ± 0.3	−21 ± 1	−1.6 ± 0.1	10 ± 1	−18.9 ± 0.8	19 ± 2	−4.1 ± 2.1
P-values									
	Sub	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.045**	0.20
	Tren	0.50	0.062	0.10	**0.0021**	**0.013**	0.21	**0.009**	0.11
	Sub x Tren	0.12	**0.019**	0.97	0.79	0.88	0.57	0.51	0.91

substrates lost 15–20% of the initial nitrogen pool (Fig. 2B). During the second incubation period, the total nitrogen pool increased in all substrates. There was no significant main effect of tree root trenching on C:N ratio or nitrogen pools in any of the substrates. However, after three years, the nitrogen pool had increased more (relative to after one year) in substrates incubated in trenched than in un-trenched plots (Fig. 2B; Table 1).

Overall, substrates differed in natural abundance of ^{15}N and ^{13}C (Fig. 3). Both $\delta^{15}N$ and $\delta^{13}C$ were lowest in litter substrates and increased towards deeper humus, but substrates collected from the forest had lower $\delta^{13}C$ and higher $\delta^{15}N$ than heath substrates. For litter, $\delta^{13}C$ remained rather stable over time, apart from a shift towards higher values during the second period heath litter. Correspondingly, the estimated $\delta^{13}C$ of the lost carbon pools reflected the $\delta^{13}C$ values of the original forest and heath litters (−31 and −29‰, respectively), during the first incubation period, while the carbon pool lost during the second period had $\delta^{13}C$ of −30 to −32‰ for both substrates (Table 1). For all humus substrates, $\delta^{13}C$ decreased by about 1‰ during the first incubation year and then increased towards pre-incubation values during the second period (Fig. 3A). The lost (year 0–1) and gained (year 1–3) humus carbon pools were isotopically similar and highly enriched in ^{13}C, with $\delta^{13}C$ ranging from −19 to −23‰ and −18 to −21‰, respectively (Table 1). Trenching led to loss of a carbon pool of slightly lower $\delta^{13}C$ from forest humus during the first period, but overall trenching

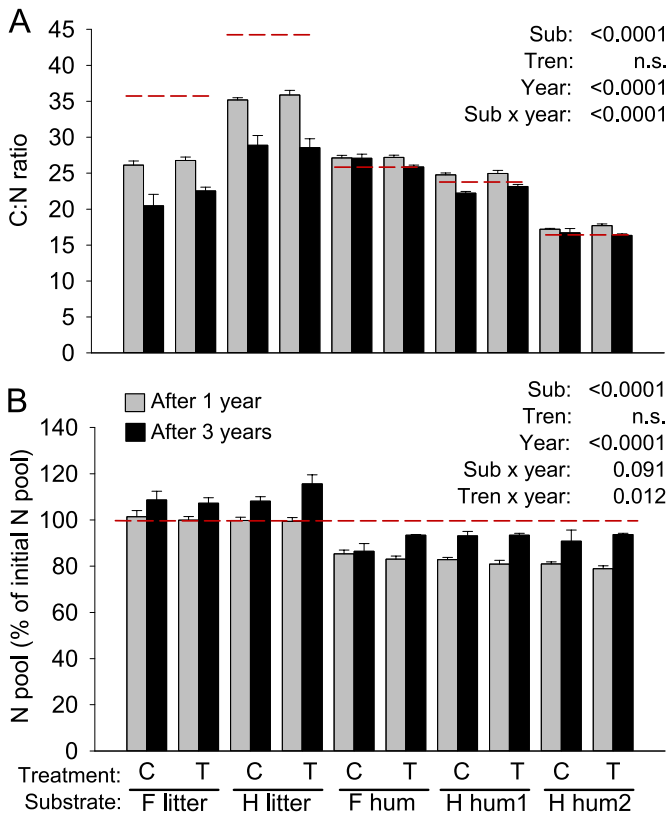

Fig. 2. **A)** Carbon-to-nitrogen (C:N) ratio and **B)** nitrogen (N) pool (% of initial nitrogen pool) of five organic substrates incubated for one (grey bars) or three (black bars) years in control plots (C) or in plots from which living tree roots were excluded by trenching (T) in a subarctic birch forest. The substrates were: leaf litter and humus (hum) collected at the forest (F) and heath (H) sites; two humus depth layers were collected from the heath. The horizontal broken, red lines indicate initial levels in substrates before incubations. All bars are averages (n = 6) with the error bar showing one standard error of the mean. Results (p-values) from linear mixed models testing effects of substrate type (Sub), trenching treatment (Tren) and incubation duration (Year) and their interactions are shown; interaction terms with p > 0.20 were excluded from the models.

effects on $\delta^{13}C$ were minor.

For forest litter, $\delta^{15}N$ did not change significantly during the first period, when the total nitrogen pool also remained stable (Figs. 2 and 3). The $\delta^{15}N$ of the nitrogen pool gained during the second period, however, seemed to be higher in control than in trenched plots, but these estimates were variable (Table 1). In heath litter, $\delta^{15}N$ increased by more than 2‰ during the full 3 year incubation (Fig. 3B), and the nitrogen pool gained in the second period was clearly ^{15}N-enriched, $\delta^{15}N$ of +2–8‰, compared to the initial pool (Table 1). All humus substrates maintained rather stable $\delta^{15}N$ signatures, despite the large initial nitrogen losses followed by varying extents of nitrogen regain during the second period (Figs. 2 and 3). The $\delta^{15}N$ of the nitrogen pools that were lost from humus during the first period ranged between −0.4 and −4.1‰, reflecting the range in the substrates themselves. During the second period, the $\delta^{15}N$ of the humus substrates generally decreased by about 0.5‰ (Fig. 3), and the gained nitrogen pools had $\delta^{15}N$ of −1 to −6‰, although these estimates were variable, and no significant differences were detected among substrates (Table 1). Across all humus substrates, trenching led to loss of the nitrogen pool with significantly higher $\delta^{15}N$ during the first period.

3.3. Fungal communities in mesh bags incubated in the forest

After the full three years of incubation, mesh bags containing heath humus had higher ingrowth of ericaceous fine roots than bags containing litters, as indicated by the summed relative abundance of the four dwarf shrub species in the system (Fig. S3). Most of the plant sequences (85%; ranging 69–98% across replicates) belonged to *Empetrum nigrum*, which was the dominant ericaceous dwarf shrub in the forest site. Trenching did not affect the abundance of Ericaceae in the bags (Table S1). Fungal biomass was lower in heath humus (both depths) than in forest humus and both litters and, overall, decreased significantly (by 7% on average, ranging 0–17%) after trenching (Fig. S3, Table S1; data from Clemmensen et al. 2021).

The relative abundance of different fungal guilds varied significantly among substrates (Fig. 4A, Table S1). Overall, the relative abundance of litter associated saprotrophs (particularly Basidiomycota within Agaricales, Trechisporales, Atheliales and Auriculariales, and Ascomycota within Venturiales, Rhytismatales and Helotiales) was higher in litters than in humus. Root associated guilds (Helotiales, Chaetothyriales and Archaeorhizomycetes, Sebacinales and ectomycorrhizal fungi) and moulds (mainly Mortierellales, Mucorales and Eurotiales) dominated in humus substrates. Yeasts (Tremellomycetes and Microbotryomycetes) had low abundance in all substrates. Tree root trenching had a large negative effect on the relative abundance of ectomycorrhizal Basidiomycota and other root associated Basidiomycota (Sebacinales), as well as on Basidiomycota yeasts. In contrast, trenching increased the relative abundance of root-associated Ascomycota (in heath humus) and litter-associated Basidiomycota (in both litter types and forest humus), although this effect is likely to be smaller in absolute terms considering the simultaneous decrease in fungal biomass.

The combination of fungal guilds that produced the best model explaining changes in the substrate carbon pools during the second incubation period (across all substrates) included litter saprotrophs and ectomycorrhizal fungi, both with negative coefficients, and other root-associated fungi (all groups summed; mainly Ascomycota) with a positive coefficient, indicating that the former were associated with a decreasing carbon pool, while the latter were associated with an increasing carbon pool (Table 2A). When the ectomycorrhizal guild was divided into long- and short-distance exploration types, the best model included long-distance-exploration types only (still with a negative coefficient), with a slightly better fit than when the ectomycorrhizal guild was not subdivided (Table 2B). The fungal guild ratio "(saprotrophic fungi + long-distance-exploration ectomycorrhizal fungi)/(root associated fungi)" was thus the best predictor of net change in the carbon pool across all substrate types during the second incubation period (Fig. 4B).

Correspondence analyses of fungal communities at the species-level across all substrates confirmed that different substrates hosted different communities, although the two heath humus substrates were relatively similar (Fig. 5A). Across all substrates, the relative abundance of litter saprotrophs correlated positively with mass loss (year 0–3) towards the litter substrates, while ectomycorrhizal fungi and yeasts and moulds increased towards forest humus, and other root-associated fungi were more abundant in heath humus substrates. In analyses of humus and litter communities separately (Fig. 5B–E), the first CA axis still described differences among substrates, while the second CA axis separated samples from trenched and control plots and correlated positively with mass loss and abundance of the ectomycorrhizal guild. In both litter and humus, certain ectomycorrhizal species associated with higher mass loss in some replicates of the bags incubated with access by roots.

To enable more precise identification of fungal taxa linked to net mass loss or gain, a CCA of fungal communities constrained by the net carbon pool change in the three humus substrates over the second period was conducted. Bags with net carbon gain had higher dominance of the root-associated fungal guild (*i.e.*, excl. ectomycorrhizal fungi), particularly ascomycetes, including the ericoid mycorrhizal *Hyaloscypha hepaticicola* and many other species in Helotiales as well as certain species from Sebacinales and Trechisporales (Figs. S4 and S5). Humus bags with net carbon loss, in contrast, had higher dominance of the ectomycorrhizal guild driven by higher abundance of several ectomycorrhizal

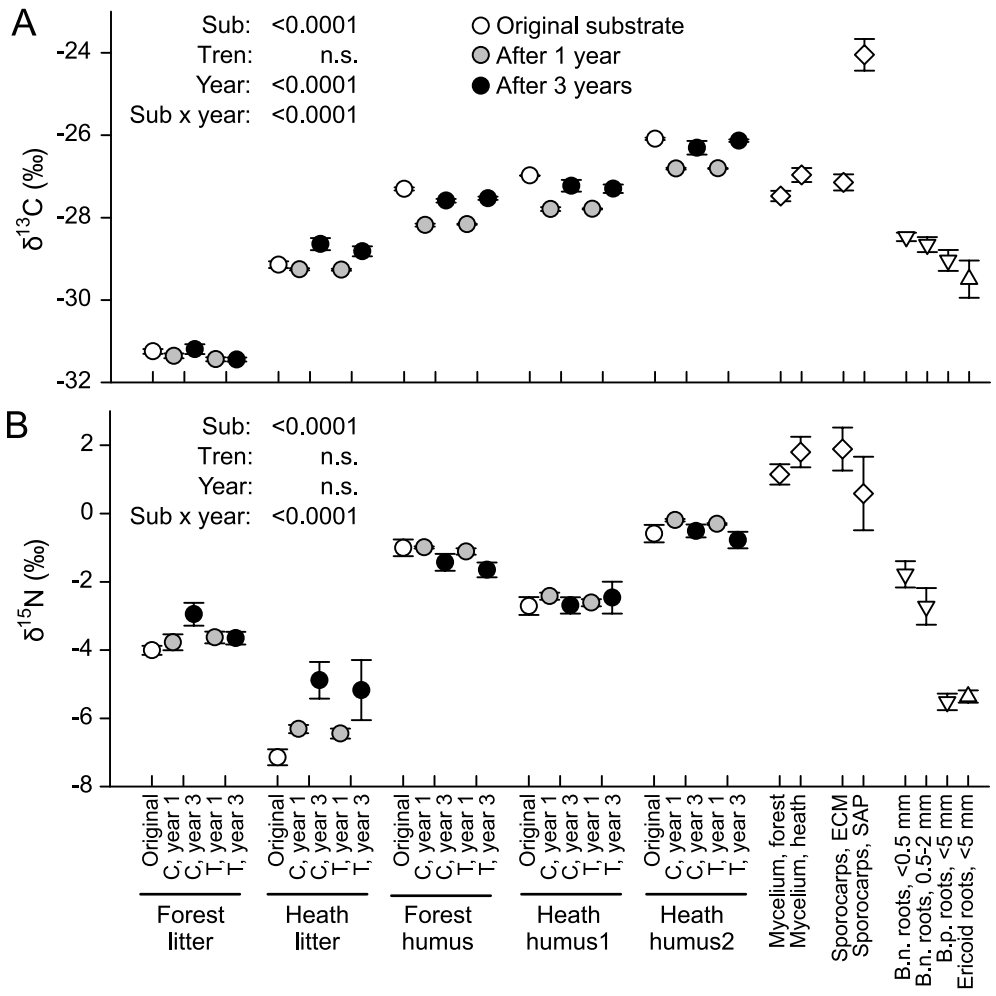

Fig. 3. Isotopic signatures of five decomposing organic substrates incubated in 45 μm mesh bags for one or three years in forest plots with (C, control) or without (T, trench) access by tree roots. Isotopic signatures of original substrates (open circles) and reference material (potential organic matter sources) were derived from earlier studies (Clemmensen et al., 2006, 2021; A. Michelsen, unpublished): fungal sporocarps of saprotrophic (SAP, n = 6 species) and ectomycorrhizal (ECM, n = 10 species) fungi collected at the same heath; mycorrhizal mycelium collected in the forest (n = 24 samples) and at the heath (n = 20 samples); *Betula nana* roots from the heath (B.n., <0.5 mm and 0.5–2 mm diameter, n = 5 and 10, respectively); *B. pubescens* roots (B.p., <5 mm, n = 5) and ericaceous roots (upward triangle; <5 mm, n = 22 samples of *Vaccinium myrtillus*, *V. vitis-idaea*, *V. uliginosum* and *Empetrum nigrum*) from a nearby forest. The results from linear models testing effects of substrate, trenching, year and their interactions on the isotopic signatures of the incubated bags are shown (n = 6 of averages ± SE).

Basidiomycota, particularly *Polyozellus tristis*, *Lactarius vietus*, *L. trivialis*, *Russula versicolor*, *R. nana, Leccinum scabrum*, *L. holopus* and *Hebeloma velutipes* (Figs. S4 and S5). A few ectomycorrhizal fungal species (*Russula* sp., *Tomentella* sp., *Inocybe* sp., *Suillus granulatus*, *Rhizpogon mohelnensis*, *Chalciporus piperatus*, *Lactarius sanguifluus*) were present in forest humus at low abundance, irrespective of trenching. These ectomycorrhizal species, as well as *Cenococcum geophilum, Cortinarius bivelus, C. casimiri, Piloderma* sp. and *Laccaria* sp., were associated with humus without large gains or losses of carbon (Figs. S4 and S5). A group of moulds (particularly *Leptodontidium*, *Mortierella* and *Umbellopsis* spp.) was associated with net carbon loss in some of the humus substrates, but the total mould and yeast guilds were unrelated to carbon loss. The total relative abundance of litter saprotrophs tended to be higher in humus substrates with higher gains of carbon, but many of the most abundant litter saprotrophs and root associated fungi were found across all humus substrates and were unrelated to carbon pool changes.

4. Discussion

In this field experiment we followed the decomposition dynamics of five organic substrates incubated in fine-meshed bags in a mountain birch forest and a nearby heath tundra over three years. The substrates were natural mixtures of leaf litters or organic material from different humus layers collected at the same two sites. The mesh-bags allowed ingrowth of fungi and ericaceous fine roots, but not mountain birch tree roots. As expected, (H1), the five substrates differed markedly in decomposition dynamics. The variation in mass-loss after three years correlated closely with respiration from the substrates directly after bag retrieval, suggesting that contemporary communities and activities reflected the longer-term decomposition dynamics well. Forest litter decomposed most rapidly and lost about 28% of mass during the first year, and an additional 5–10% during the following two years, while heath litter lost about 23% and 3–8% of mass during these two periods. These results are similar to mass-loss recorded for single plant-species litters in an experiment across similar heath and forest habitats, and likely reflect higher chemical quality of forest litter than of heath litter (Parker et al., 2018). The carbohydrate content was higher in mountain birch (*Betula pubescens*) litter than in litter of the ericaceous dwarf shrub *Empetrum nigrum*, while the lipid content was much higher in *Empetrum*, and lignin content did not differ (Parker et al., 2018). In the same study, 5–8% greater litter mass-loss was observed when these litters were incubated in forest, and this was mainly attributed to greater loss of more easily degradable carbohydrates and lipids. We also observed slightly larger mass-loss of the heath litter when incubated in the forest

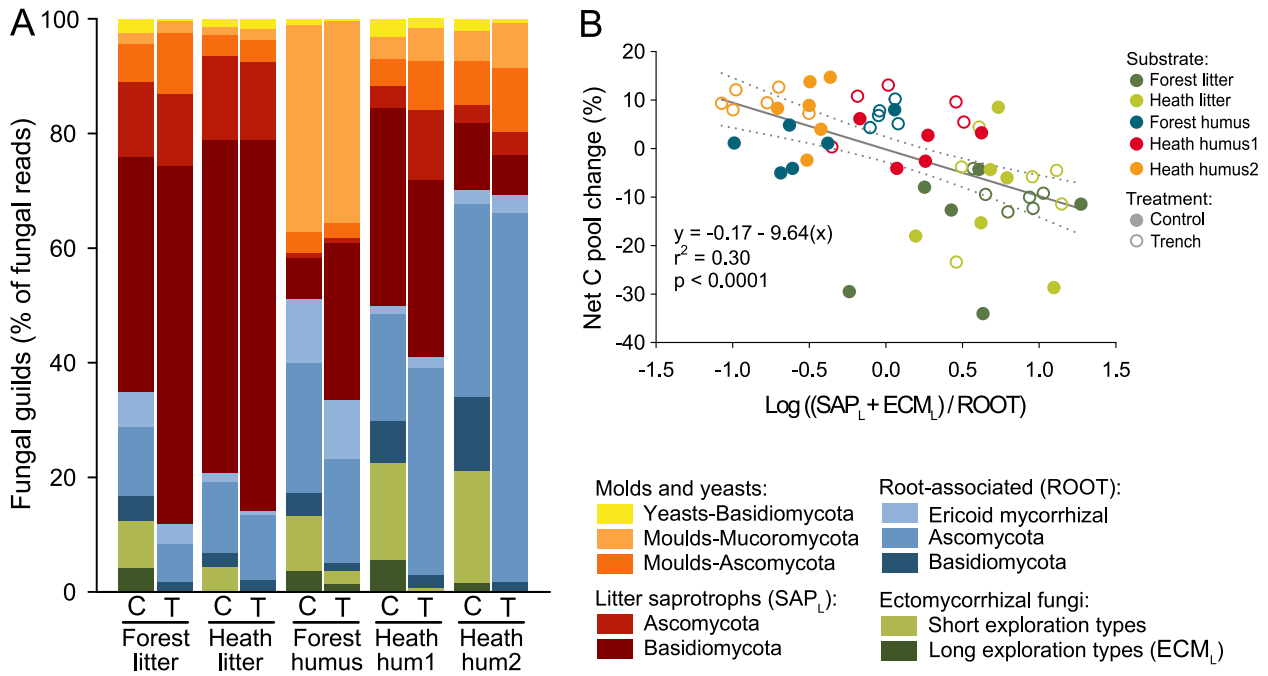

Fig. 4. **A)** Relative abundance of fungal guilds as assessed by sequenced fungal ITS2 markers in five organic substrates after three years of incubation in control (C) plots or in forest plots from which living tree roots were excluded by permanent barriers (T). **B)** The change in substrate carbon pools from year 1 to year 3 linearly regressed against the fungal guild ratio of highest predictive power across all substrates (see Table 2), with 95% confidence intervals shown. Symbol colours indicate sample types. The guild ratio is the summed litter saprotrophic (SAP_L) and ectomycorrhizal long exploration type (ECM_L) fungi versus other root-associated fungi (ROOT). The ericaceous dwarf shrub understory was intact in all plots throughout the experiment. The substrates were: litter and humus (hum) collected at the forest and heath sites; two humus depth layers were collected from the heath. All bars represent averages (n = 6).

habitat (H1), potentially due to faster loss of energy-rich organic matter fractions. The $\delta^{13}C$ signature of both litter substrates was rather stable during decomposition, and the $\delta^{13}C$ of the lost carbon pools had a narrow range of −29 to −31‰ during the first incubation period and −30 to −32‰ during the second period. These signatures are consistent with cellulose as the main decomposed pool during the first year, and lipids, which are often ^{13}C-depleted relative to sugars, as an increasing component being decomposed during the later stages (Brüggemann et al., 2011), leading to a higher $\delta^{13}C$ of the remaining heath litter carbon pool after three years.

The faster decomposition of forest litter may also relate to its lower C:N ratio as compared to heath litter (H1), suggesting that decomposition of heath litter was more strongly constrained by low nitrogen content (Berg, 2000). However, during the second incubation period, we observed an import of ^{15}N-enriched nitrogen into both litters – although more clearly so in heath litter, indicating import of nitrogen from deeper organic soil layers where $\delta^{15}N$ is higher (Högberg et al., 1996; Sterkenburg et al., 2018). The fungal communities in both litter types were dominated by saprotrophic fungi, particularly species of *Mycena* and *Luellia* (Basidiomycota), that can bridge heterogenous resource patches by expansive mycelia and possess both hydrolytic and oxidative enzymatic capacities (Frey et al., 2003; Boberg et al., 2014). Thus, both carbon and nitrogen dynamics were consistent with decomposition driven by saprotrophic decomposer communities. Nitrogen immobilization during early-stage decomposition of nutrient-poor plant litter is frequently observed (Melillo et al., 1989), but in our study this was only apparent during the later incubation period, potentially because the nitrogen import was driven by *Mycena* and other Basidiomycota which only established after an initial phase dominated by litter saprotrophic ascomycetes and moulds (Bödeker et al., 2016). It is noteworthy, and consistent with fungal immobilization of nitrogen in the litters, that the $\delta^{15}N$ signatures of the imported nitrogen – although variable – mostly ranged above zero, and that sporocarps and mycelium were the only potential organic matter sources within our reference material with positive values (Fig. 3B).

In line with a "Gadgil effect" (Gadgil and Gadgil, 1971; Fernandez and Kennedy, 2016), we hypothesized (H2) that the presence of intact tree roots and associated ectomycorrhizal fungi would inhibit litter decomposition by free-living saprotrophic fungi in the forest, and that root exclusion would increase decomposition and $\delta^{15}N$ in the litter relative to controls, due to enhanced access to nitrogen in deeper layers. We did not observe such effects of root trenching. In contrast, we observed a decreased mass-loss with trenching after three years, suggesting that roots and associated fungi stimulated, rather than inhibited, mass-loss at least in the later stages of litter decomposition, as also observed in a tree girdling experiment (Subke et al., 2011). Nitrogen dynamics and isotopic signatures of litters were also largely unaffected by trenching, apart from the increased nitrogen pool in heath litter after three years. Without a simultaneously stimulated mass-loss, this may suggest increased carbon use efficiency and nitrogen immobilization by the saprotrophic fungi in the absence of competition from roots and associated fungi (Boberg et al., 2014; Sterkenburg et al., 2018). Indeed, in the forest, ectomycorrhizal fungi only colonized the litters in control plots, and litter mass-loss correlated positively with the relative abundance of the ectomycorrhizal guild, suggesting that they stimulated carbon loss, at least at late stages of litter decomposition. While it is unlikely that ectomycorrhizal basidiomycetes contributed directly the decomposition of cellulose (Lindahl and Tunlid, 2015), their potential stimulation of saprotrophic decomposition should be further investigated.

As expected, the humus substrates containing older organic matter decomposed more slowly than the litter substrates, but their mass dynamics over time were also distinct from those of litter. During the first incubation year, forest humus lost the smallest mass fraction (c. 10%) while both humus layers from the heath displayed greater, and rather similar, mass-loss (c. 15%), despite their different initial C:N ratios. Thus, the mass-loss differences among substrates during the first year appeared unrelated to initial C:N ratios, and neither the incubation site

Table 2
Multiple linear regression models of net C pool change (% loss or gain relative to initial C mass) of five organic substrates during the last two years of incubation in plots with or without access by tree roots (n = 57). The four best models were selected based on Akaike's information criteria (AIC) for analyses including either **A**) the four major guilds (four parameters) or **B**) the four guilds but with the ectomycorrhizal guild sub-divided into short and long exploration types (five parameters) as potential independent predictors (all log10 transformed). All models were run with intercept fixed at zero so that guilds included with a positive sign associate with net C gain, while guilds with negative sign associate with net C loss. The model fit statistics, the root mean square error (RMSE) and R^2, should be minimized and maximized, respectively. The coefficients of factors included in the best four models were selected out of a) 16 and b) 32 possible models.

A	Fungal guild	Model 1	Model 2	Model 3	Model 4
Parameter estimates:	Litter saprotrophs	−41.43	−42.16	−41.48	−41.27
	Moulds and yeasts			−14.10	−3.66
	Ectomycorrhizal	−38.70			−37.68
	Other root-associated	51.29	44.78	51.39	52.84
Model fit statistics:	RMSE	9.52	9.63	9.69	9.61
	R^2	0.33	0.31	0.31	0.33
	AIC	259.85	260.12	261.82	261.83
B	Fungal guild	Model 1	Model 2	Model 3	Model 4
Parameter estimates:	Litter saprotrophs	−41.87	−42.16	−41.82	−41.76
	Moulds and yeasts				−2.33
	Ectomycorrhizal-long	−123.10		−119.40	−121.70
	Ectomycorrhizal-short			−3.81	
	Other root-associated	49.06	44.78	49.48	50.10
Model fit statistics:	RMSE	9.40	9.63	9.48	9.48
	R^2	0.35	0.31	0.35	0.35
	AIC	258.33	260.12	260.31	260.32

nor trenching treatment affected humus mass-loss much. During the following two years, all humus substrates, in contrast to litters, either regained mass or stabilized their mass, and variation among replicates increased. The mass gain appeared particularly pronounced and consistent for humus incubated in trenched plots in the forest. Thus, in agreement with our hypothesis (H3), presence of intact tree roots (control plots) in the forest overall increased cumulative net mass-loss after three years. However, while the mass dynamics of the indigenous heath substrates appeared rather unaffected by trenching in the heath, forest substrates were more similarly affected by trenching at the two sites. This may indicate that the trenching treatment also affected some of the smaller root systems in the heath, which hampered the decomposer communities to some degree.

The carbon pools that were lost from the humus substrates during the first year displayed high $\delta^{13}C$ signatures across all three humus types (−19 to −23‰), with narrow variation among replicates of each substrate type. Furthermore, for the substrates with a significantly increased carbon pool over the following two years, the gained carbon pool had an even higher $\delta^{13}C$ signature (−18 to −20‰). The observed shifts in substrate $\delta^{13}C$ signatures, first away from, and then back to, the $\delta^{13}C$ of the original humus substrates are thus consistent with fluctuation of a strongly ^{13}C-enriched organic pool. However, none of the known soil organic matter sources, such as fungal or root biomass fractions (Fig. 3), matched these high $\delta^{13}C$ signatures, and the fluctuating pool was likely a more distinct chemical fraction. Plant organs that are sinks for carbon, such as stems and roots, are typically enriched in ^{13}C relative to photosynthetic organs (Brüggemann et al., 2011), as also reflected across our reference materials. This is because various post-photosynthetic metabolic pathways (*i.e.*, after the initial RuBisCO-mediated carboxylation) and the export of specific compounds out of the photosynthetic organ involve a range of isotopic fractionation processes (Hobbie and Werner, 2004). The main transport form of carbon in plants, sucrose, is ^{13}C-enriched relative to other photosynthesis products and becomes increasingly ^{13}C-enriched during phloem transport further away from the photosynthetic tissues in woody plants (Brandes et al., 2006; Bowling et al., 2008). Sucrose is then utilized for various purposes in the sink tissues, and although the fine roots from our system had an overall $\delta^{13}C$ of −28 to −30‰, it is possible that a more labile chemical fraction with higher $\delta^{13}C$ was first lost due to decomposition of dead roots in the homogenized substrate, and then regained as new roots colonized the substrates. For instance, it is known that transitory leaf starch reserves may become several ‰ more ^{13}C-enriched than total leaf carbon, displaying $\delta^{13}C$ of up to −21‰, and transported sucrose may become particularly ^{13}C-enriched when carbon is re-mobilized from leaf starch reserves during periods of limited photosynthesis (Gleixner et al., 1993; Jäggi et al., 2002; Brandes et al., 2006). Isolated studies indicate that belowground starch storage may be important in cold-climate herbs (Körner, 1999) and in Ericaceae species in the Cape region (Verdaguer and Ojeda, 2002). Ericaceous fine roots did indeed colonize all our decomposing substrates, and addition of root-derived organic compounds such as starch potentially contributed to the increased mass and $\delta^{13}C$ signature during the later incubation period.

Another relatively labile carbon pool in the initial humus substrates is newly-produced fungal mycelial necromass, and a potential pathway of carbon back into the humus during incubation could be via re-established mycelial growth and biomass turnover. Saprotrophic wood-decomposers (Basidiomycota) may become 4‰ enriched in ^{13}C relative to their substrate source, and specifically, fungal chitin is 1.5–2‰ enriched ($\delta^{13}C$ of −22 to −23‰) relative to cellulose in the wood (Gleixner et al., 1993). This ^{13}C-enrichment of chitin is a consequence of a preference for the light hexose units (with ^{12}C at position 3 and 4) in catabolic reactions and the heavy molecules preferentially repolymerized into chitin (Gleixner et al., 1993). Indeed, saprotrophic sporocarps sampled in our sites had 2–3‰ higher $\delta^{13}C$ (c. −24‰) than the ectomycorrhizal mycelia and sporocarps ($\delta^{13}C$ of −27 to −28‰), as previously observed across systems (e.g. Högberg et al., 1999b; Trudell et al., 2003). Mycorrhizal fungi receive ^{13}C-enriched sugars from their plant partners, and likewise enrich their biomass carbon relative to plants, due to preferential use of ^{13}C in chitin biosynthesis (Hobbie et al., 1999). In an old-growth, lowly-productive boreal forest with a similar ergosterol content of the organic soil layer as in our humus substrates (c. 100 μg gOM^{-1}), chitin constituted only about 1% of the humus mass (Clemmensen et al., 2013). Thus, although ^{13}C-enriched chitin in fungal cell walls likely accounted for some of the highly ^{13}C-enriched carbon pool that fluctuated over time in the humus substrates, other pools were also important. After tree girdling in a boreal pine forest, a transient increase in $\delta^{13}C$ of soil respiration was proposed to be caused by metabolism of ^{13}C-enriched starch and sugars in roots and ectomycorrhizal mycelium (Bhupinderpal-Singh et al., 2003), suggesting

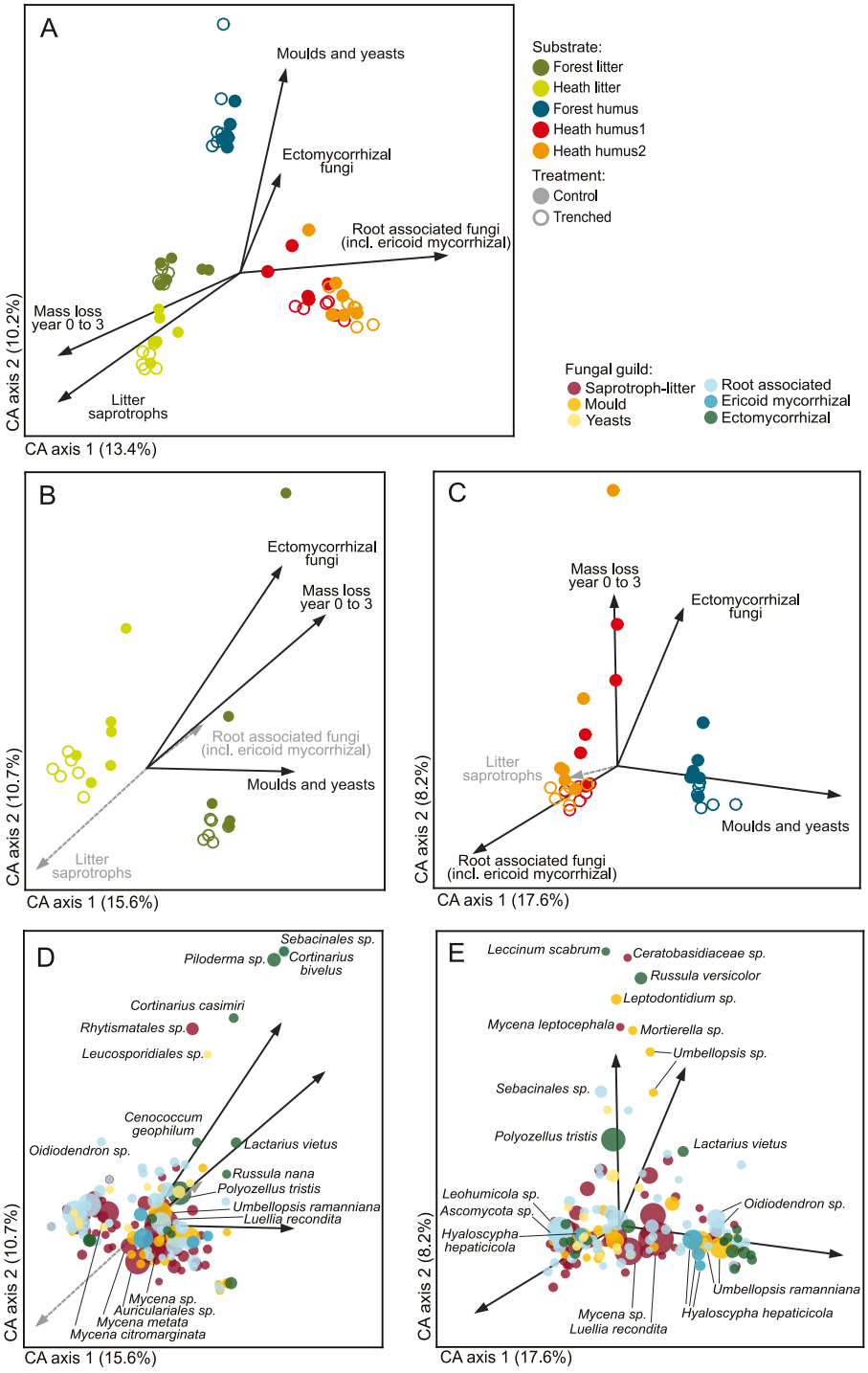

Fig. 5. Correspondence analyses (CAs) of fungal communities across all samples (**A**, n = 57), litter substrates (**B** and **D**, n = 24) and humus substrates (**C** and **E**, n = 33) after three years of incubation in forest plots with or without access by tree roots. Analyses were based on relative abundance of 232 (A), 199 (B) or 203 (C) fungal species, and total inertia was 3.98 (A), 2.23 (B) and 2.85 (C) with percentage variation explained by the two first CA axes indicated on each graph. The vectors point in the direction of the steepest increase in total relative abundance of each guild or net mass loss (year 0–3), and degree of correlation with axes is indicated by vector length (black vectors $p < 0.05$ for correlation with at least one axis; hatched grey vectors $p > 0.05$, n = 33). Vectors in D and E are the same as in B and C.

ecosystem-scale quantitative importance of a relatively mobile, ^{13}C-enriched carbon pool. We suggest that the decomposition of the pool of recently produced fine root- and mycorrhizal and saprotrophic fungal-necromass during the first incubation period, and then recolonization by ericaceous fine roots and associated mycorrhizal fungi, as well as by saprotrophic fungi, in the following two years, caused the observed fluctuations of substrate $\delta^{13}C$, at least partly linked to turnover and reestablishment of starch reserves in roots and chitin in fungal cell walls.

All humus substrates first lost 15–20% of their nitrogen pools and then regained nitrogen during the following two years, and as for carbon, $\delta^{15}N$ signatures of the lost and gained pools spanned a similar range (about 0 to −6‰). This $\delta^{15}N$ range overlapped with the fine root and fungal reference materials, and thus the patterns in both nitrogen pools and isotopes were consistent with fine roots and fungal mycelium being the main sources of organic matter losses and gains.

We hypothesized, (H3), that tree-root-mediated acceleration of humus decomposition would be linked to nitrogen "mining" by ectomycorrhizal fungi, particularly by the genera *Cortinarius* and *Leccinum* that were earlier found to be dominant in the forest soil (Clemmensen et al., 2021). This would in turn increase the C:N ratio and decrease the residual nitrogen pool of humus, relative to when tree roots were excluded, due to decomposition and preferential export of nitrogen out of residual substrate by the ectomycorrhizal fungi. However, although tree root exclusion did indeed efficiently exclude ectomycorrhizal fungi, it had little effect on overall nitrogen pool dynamics, C:N ratios and isotopic patterns in humus substrates. Thus, we did not find clear evidence for preferential fungal nitrogen "mining" (in a strict sense) as a mechanism underlying increased humus mass-loss in the presence of tree roots and ectomycorrhizal fungi. However, root exclusion did lead to a larger increase in the nitrogen pool, paralleling the larger humus mass-gain, during the last two years of incubation. Our data thus demonstrated that the extent to which nitrogen (and carbon) pools increased in the later incubation period was likely to be linked to increased decomposition when ectomycorrhizal roots and associated fungi were present.

To further test our hypothesis, (H4), that humus substrates would increase in mass in the longer-term due to ingrowth of ericoid mycorrhizal roots and fungi ("accumulation"), but more so in the absence of simultaneous decomposition activity by ectomycorrhizal fungi, we selected the best model of the carbon pool change in the forest during the second incubation period, testing combinations of fungal guild abundances as predictors. Carbon pools largely reflected total mass of our humus substrates but accounted for the different mineral contents of the substrates, which made this response variable more exact and comparable across substrates than dry mass. The best model suggested that both litter saprotrophs and ectomycorrhizal fungi were associated with decreases in the carbon pool, while other root-associated fungi (mainly Ascomycota, including ericoid mycorrhizal fungi) overall were associated with increases in the carbon pool. Furthermore, a model substituting the entire ectomycorrhizal fungal guild with the subset of long-distance exploration types, including the putative decomposing genera *Cortinarius* and *Leccinum*, produced a slightly better fit, and an ordination pinpointed eight ectomycorrhizal fungal species that were clearly associated with carbon loss (Figs. S4 and S5). Among these were two *Leccinum* species, putatively with brown-rot-based capacity for oxidative decomposition of organic matter, similar to other members (*e.g.*, *Suillus* and *Paxillus*) of the order of Boletales (Rineau et al., 2012; Shah et al., 2016), lending some support for the importance of ectomycorrhizal fungi with decomposer capacity for restricting carbon (and linked nitrogen) gain in the substrates. *Polyozellus tristis* (earlier: *Pseudotomentella tristis*) was the most abundant ectomycorrhizal fungus colonizing the humus substrates, and it was also linked to lower carbon gain. This fungus was resistant to disturbance through tree girdling in a similar mountain birch forest site (Parker et al., 2022), but it has no known capacity to decompose recalcitrant organic matter. Potentially, uptake and transport of inorganic nitrogen out of the substrate by this and other ectomycorrhizal species may have restricted colonization by saprotrophic and ericoid mycorrhizal fungi that are more prone to transport nitrogen into the bags. Against our expectations, we did not find any *Cortinarius* species associated with lower carbon gains across the humus substrates. *Cortinarius* species have earlier been associated with production of Mn-peroxidase – one of the most potent oxidative exoenzymes, in soils of similar mountain birch forest (Bödeker et al., 2014) and the presence of certain *Cortinarius* species has been linked to particularly small carbon stocks across Swedish boreal forest (Lindahl et al., 2021). In the same mountain birch forest as studied here, both *Leccinum* and *Cortinarius* species were associated with smaller soil carbon stocks and increasing C:N ratio of the deeper humus layers, as tree density increased along a gradient from alpine heath to forest, implying their involvement in organic matter decomposition and nitrogen mobilization (Clemmensen et al., 2021). Although categorized as one of the long-distance exploration types (medium-distance fringe), *Cortinarius* species are generally sensitive to disturbance (Lindahl et al., 2021) and were among the slowest colonizers of ingrowth-bags containing various inorganic and organic substrates (Jörgensen et al., 2023). Longer incubation times may therefore be needed for all members of the ectomycorrhizal community to colonize, and the nitrogen and carbon dynamics to fully mimic, the soil (Hagenbo et al., 2018). Furthermore, a partly different ectomycorrhizal fungal community, including two *Cortinarius* species, correlated with higher mass loss across the litter substrates, which points to substrate specificity and differential ecological niches within the ectomycorrhizal fungal guild (Dickie and Reich, 2005).

Irrespective of tree root trenching, fungal communities in the humus bags included a large proportion of the root-associated fungal guild (mainly ascomycetes), and the positive relationship between this guild and increases in the carbon pool may partly be linked to the elimination of ectomycorrhizal fungi, *per se*, in the trenched plots, increasing the relative abundance of remaining guilds. However, the dense ericaceous dwarf shrub understory was maintained in all plots, and these plants and their associated root fungi probably dominated soil processes upon removal of the tree roots and associated fungi, which could have led to the observed net increases in organic matter, carbon and nitrogen pools (Clemmensen et al., 2021; Ward et al., 2021; Fanin et al., 2022). When humus substrates simultaneously were colonized by tree-root associated ectomycorrhizal fungi, mass gain and nitrogen immobilization was counteracted by increased decomposition. Based on this study of relationships between fungal guilds and organic matter dynamics on small spatial scales in a single treeline ecotone, we propose that the net balance between processes leading to longer-term organic matter accumulation *versus* loss likely depends on the relative dominance of different vegetation components and their associated fungal communities across various spatial scales. In the broader context of the treeline ecotone, which is currently expanding due to a milder climate in many regions (Körner and Hoch, 2023), it is particularly important to understand the factors that restrict or promote encroachment of trees into previous dwarf shrub tundra. This is because this tundra type often holds large belowground organic stocks (Post et al., 1982; Parker et al., 2021; Castaño et al., 2023), and increased dominance of trees and associated ectomycorrhizal fungi may tip the accumulation-decomposition balance towards increased organic matter losses, leading to an amplifying feedback to global warming.

In conclusion, our study suggests that:

- Free-living saprotrophic fungi mediated mass-loss of fresh litter substrates. Tree roots and associated ectomycorrhizal fungi stimulated, rather than inhibited, mass-loss, at least during later stages of litter decomposition, and similar $\delta^{15}N$ patterns with and without root exclusion did not support a Gadgil effect.
- Ericoid dwarf shrubs and associated fungi promoted organic matter and nitrogen accumulation through root and fungal colonization of humus substrates, irrespective of tree root presence. However, trait diversity in the species-rich, and often dominant, fungal communities associated with ericoid dwarf shrubs needs further study.
- In the forest, tree roots and associated ectomycorrhizal fungi restricted organic matter and nitrogen accumulation in humus substrates during the later incubation phase, likely due to ectomycorrhizal decomposition (*sensu* Lindahl and Tunlid, 2015). We observed no direct evidence of preferential ectomycorrhizal nitrogen mining, as carbon and nitrogen pools remained coupled (*i.e.*, no increase in C:N ratio of the remaining substrate). Longer incubation times would likely be needed for ectomycorrhizal fungal communities and related element dynamics in incubated humus substrates to fully reflect those in undisturbed soil.
- The best predictor of net carbon balance during year two and three across all substrate types was the ratio (litter saprotrophs + long-distance ectomycorrhizal fungi)/(other root-associated fungi), but

the ratio (litter saprotrophs + all ectomycorrhizal fungi)/(other root-associated fungi) was almost equally good.

CRediT authorship contribution statement

K.E. Clemmensen: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **A. Michelsen:** Data curation, Formal analysis, Funding acquisition, Writing – review & editing. **R.D. Finlay:** Conceptualization, Resources, Writing – review & editing. **B.D. Lindahl:** Conceptualization, Methodology, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: KEC reports financial support was provided by European Commission Seventh Framework Programme for Research and Technological Development. AM reports financial support was provided by Independent Research Fund Denmark, DFF Nature and Universe. Two of the authors, KEC and BDL, of this submission serves in the editorial board of the journal Fungal Ecology. KEC serves as one of the editors of the Special Issue to which the manuscript is submitted. KEC has not been involved in the review or editorial process of this submission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2024.101359.

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