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Ericoid shrubs shape fungal communities and suppress organic matter decomposition in boreal forests

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Summary

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- Mycorrhizal fungi associated with boreal trees and ericaceous shrubs are central actors in organic matter (OM) accumulation through their belowground carbon allocation, their potential capacity to mine organic matter for nitrogen (N) and their ability to suppress saprotrophs. Yet, interactions between co-occurring ectomycorrhizal fungi (EMF), ericoid mycorrhizal fungi (ERI), and saprotrophs are poorly understood.
- We used a long-term (19 yr) plant functional group manipulation experiment with removals of tree roots, ericaceous shrubs and mosses and analysed the responses of different fungal guilds (assessed by metabarcoding) and their interactions in relation to OM quality (assessed by mid-infrared spectroscopy and nuclear magnetic resonance) and decomposition (litter mesh-bags) across a 5000-yr post-fire boreal forest chronosequence.
- We found that the removal of ericaceous shrubs and associated ERI changed the composition of EMF communities, with larger effects occurring at earlier stages of the chronosequence. Removal of shrubs was associated with enhanced N availability, litter decomposition and enrichment of the recalcitrant OM fraction.
- We conclude that increasing abundance of slow-growing ericaceous shrubs and the associated fungi contributes to increasing nutrient limitation, impaired decomposition and progressive OM accumulation in boreal forests, particularly towards later successional stages. These results are indicative of the contrasting roles of EMF and ERI in regulating belowground OM storage.

Introduction

Plant–fungi interactions are key regulators of carbon (C) storage in boreal forests (Clemmensen *et al.*, 2013; Baskaran *et al.*, 2017), and plant species vary in the extent to which they can stimulate or inhibit the activity of specific fungal groups that drive soil organic matter (OM) decomposition and stabilisation (Kyaschenko *et al.*, 2017; Adamczyk *et al.*, 2018). Furthermore, fungal groups also interact to drive these processes; a long-standing theory about the effects of interaction among fungal guilds on soil C cycling is called the ‘Gadgil hypothesis’ (Gadgil & Gadgil, 1971, 1975). This hypothesis stipulates that competition between ectomycorrhizal fungi (EMF) and free-living saprotrophs for organic nitrogen (N) may slow down litter decomposition rates (Orwin *et al.*, 2011; Fernandez & Kennedy, 2016), in turn promoting C accumulation in the long term (Averill *et al.*, 2014; Averill & Hawkes, 2016). Although the ‘Gadgil

effect’ has been experimentally tested by several studies over the past half century, the results among those studies are largely inconsistent (Fernandez & Kennedy, 2016). The lack of consistency across studies and systems may be driven by variation in environmental factors, such as soil fertility and soil moisture (Koide & Wu, 2003; Smith & Wan, 2019), substrate quality (Sietiö *et al.*, 2019; Fernandez *et al.*, 2020) or the position within the soil profile (Bödeker *et al.*, 2016; Clemmensen *et al.*, 2021; Lindahl *et al.*, 2021). For instance, Sterkenburg *et al.* (2018) demonstrated that the ‘Gadgil effect’ was restricted to the surface litter, mainly because saprotrophs were unable to exploit substrates with a low proportion of hydrolysable compounds in deeper horizons, irrespective of EMF presence. Furthermore, in boreal forests the understorey vegetation is dominated by ericaceous dwarf shrubs, which occur in association with ericoid mycorrhizal fungi (ERI), as well as by feather mosses (Wardle *et al.*, 2012, 2020). Although these two components are major

contributors to OM accumulation (Clemmensen *et al.*, 2015; Fanin *et al.*, 2019a), they have never been considered within the context of interactions between fungal guilds, and therefore the ‘Gadgil effect’.

Ericaceous shrubs and their root-associated ERI contribute significantly to the build-up of OM in boreal forest soils through the production of poor quality litter and highly melanised necromass, respectively (Nilsson & Wardle, 2005; Clemmensen *et al.*, 2015, 2021). Furthermore, ERI have saprotrophic abilities through their extracellular enzyme production and promotion of some oxidative mechanisms (Wurzburger *et al.*, 2012; Martino *et al.*, 2018), which enables them to access N in organic soil layers (Read & Perez-Moreno, 2003). The functional abilities of EMF to effectively mine the OM for nutrients have been proposed to be the primary mechanism driving the ‘Gadgil effect’ (Fernandez & Kennedy, 2016), but it is possible that the ERI associated with ericaceous dwarf shrubs have similar impacts on saprotrophic fungi, but also on EMF. This would in turn have consequences for soil processes such as litter decomposition and nutrient fluxes, as well as for belowground C inputs and OM quality (Ward *et al.*, 2021). For instance, if ERI compete directly with EMF and free-living saprotrophs for the same soil resources (Mielke *et al.*, 2022), then the overlapping niches may slow the breakdown of OM due to increasing nutrient limitation, a phenomenon that could be further amplified by a greater incorporation of highly recalcitrant dwarf shrub litter and ERI necromass in the soil OM (Clemmensen *et al.*, 2015). The presence of feather mosses, which are a major component of the ground layer vegetation in many boreal forests, may further contribute to the impairment of OM recycling, because feather mosses typically produce highly recalcitrant litter (Jonsson *et al.*, 2015; Fanin *et al.*, 2019a). However, whether and how the presence of ericaceous dwarf shrubs and mosses may contribute to explaining the context dependency of the ‘Gadgil effect’ is still unknown, and the consequences of this for soil biogeochemical cycling have never been evaluated, despite these plant groups being a prominent feature of most boreal forests worldwide.

In this study, we sought to assess the impact of ericaceous shrubs and their ERI on competition between EMF and saprotrophs over a long-term chronosequence of boreal forest stands and in the presence vs absence of feather mosses, and how this interplay in turn affects litter decomposition rates (including the ‘Gadgil effect’), OM quality and N availability. To achieve this, we used a biodiversity manipulation experiment that had been running for 19 yr, which includes full factorial removals of tree roots, ericaceous dwarf shrubs and mosses on each of 30 forested islands in northern Sweden, which collectively represent a lengthy post-fire chronosequence (Wardle & Zackrisson, 2005; Gundale *et al.*, 2010; Fanin *et al.*, 2018). With increasing time since fire on these islands, soil fertility and plant productivity have declined (Wardle *et al.*, 2012). Furthermore, different mycorrhizal types and fungal species characterised by different morphologies and exploration strategies dominate at different positions along this type of gradient (Agerer, 2006; Dickie *et al.*, 2013; Anthony *et al.*, 2022; Argiroff *et al.*, 2022). For instance, EMF species with mycelia that extend far from the roots by creating hydrophobic

hyphal cords (cord-formers) dominate in the organic topsoil at earlier stages along our island chronosequence (Clemmensen *et al.*, 2015), whereas those EMF species with hydrophilic mycelia that explore the soil close to the roots (simple mycelia) make up a rather constant proportion of the EMF communities across the gradient (Clemmensen *et al.*, 2015). By contrast, a high relative abundance of ERI, which is often associated with slow breakdown of OM and inorganic N cycling, dominates in the later stages of the gradient (Clemmensen *et al.*, 2013). Given that cord-forming EMF and ERI play contrasting roles in C accumulation in boreal ecosystems (Clemmensen *et al.*, 2015), this experiment allowed us to explore the ecological effects of the removal of tree roots (and therefore EMF) and ericaceous shrubs (and therefore ERI) on soil biogeochemical cycling across an environmental gradient, in which EMF and ERI change strongly in relative abundance.

First, we asked the question: (1) do EMF and ERI compete with each other? We hypothesised that removal of one of the guilds through removal of the host plants would lead to proliferation of the other (H_{1a}), mainly because they may compete for the same soil nutrient resources. Furthermore, we expected that the removal of tree roots (and associated EMF) should have a greater effect on ERI earlier in the chronosequence (in which EMF dominate the fungal community), and removal of shrubs should have a greater effect on EMF later in the chronosequence (in which ERI dominates) (H_{1b}) (Wardle *et al.*, 2012; Clemmensen *et al.*, 2015). We also explored if removing shrubs and ERI would have a greater effect on EMF fungi with simple mycelia than on cord-forming EMF, given that simple mycelia make up a larger proportion of the EMF community in the ecosystems in which ERI are more abundant (i.e. small islands) (H_{1c}) (Clemmensen *et al.*, 2015).

Second, we asked the question: (2) how does the relative importance of EMF vs ERI within the mycorrhizal community influence fungal saprotrophs and decomposition? The EMF are thought to be better exploiters of N than ERI (Clemmensen *et al.*, 2021), and especially certain cord-forming EMF that are particularly efficient at competing against saprotrophic decomposers by mobilising organic N from complex organic polymers (Hobbie *et al.*, 2013). For this reason, we tested the hypothesis that removal of EMF would have a stronger stimulatory effect on saprotrophs and litter decomposition than would removal of ERI (H_{2a}). We also hypothesised that removal of tree roots would have a stronger stimulatory effect on saprotrophs earlier in the chronosequence, in which trees and their cord-forming EMF are a larger component of total biomass relative to shrubs and ERI (H_{2b}). This stimulatory effect on saprotrophs should be accentuated by moss removals as they are important contributors of highly recalcitrant litter (Jonsson *et al.*, 2015; Fanin *et al.*, 2019a), especially in late successional stages in which mosses are most abundant (H_{2c}) (Wardle *et al.*, 2012).

Finally, we asked the question: (3) how does the relative importance of EMF vs ERI and their interactions with saprotrophs impact OM quality? We hypothesised that effects of plant functional group removal on decomposition dynamics across the chronosequence would be matched by changes in OM quality as

revealed by changes in stable isotopes and chemical shifts in the OM (Högberg *et al.*, 1999; Clemmensen *et al.*, 2013; Kyaschenko *et al.*, 2019). Specifically, we tested the hypothesis that plant removals should increase soil ^{13}C and ^{15}N (as indicators of OM age and processing) ($\text{H}_{3\text{a}}$) (Clemmensen *et al.*, 2013; Kyaschenko *et al.*, 2019), whereas the proportion of carbohydrate-like C forms should decrease as a consequence of reduced plant C inputs and increased decomposition rates (due to greater action of saprotrophs) ($\text{H}_{3\text{b}}$) (Fanin *et al.*, 2018). Consequently, the effects of removing tree roots and EMF on OM quality should be higher for large islands where they play a larger role on decomposition and N recycling (Clemmensen *et al.*, 2015), whereas removing shrubs and ERI should have a greater impact on OM quality in small islands where they exert a stronger impact on OM dynamics ($\text{H}_{3\text{c}}$) (Clemmensen *et al.*, 2021).

Materials and Methods

Study site and experimental design

We used a long-term and ongoing biodiversity manipulation experiment across a chronosequence consisting of 30 forested islands in two adjacent lakes (Lake Hornavan and Lake Uddjaure) in northern Sweden ($65^{\circ}55'$ to $66^{\circ}09'\text{N}$; $17^{\circ}43'$ to $17^{\circ}55'\text{E}$). In this study system, the frequency of fires is the main abiotic factor that varies across islands; larger islands are struck by lightning more often and have therefore burned more recently than have smaller islands, and the time since the most recent fire across the 30 islands ranged from 60 to 5000 yr (Wardle *et al.*, 1997, 2003). Therefore, these islands collectively represent a chronosequence of increasing time since the most recent fire; all extrinsic drivers other than fire history including climatic conditions across islands were relatively consistent (Wardle *et al.*, 2012). With increasing time since fire, soil fertility, plant productivity and rates of nutrient fluxes and decomposition decline, whereas OM accumulates. Consistent with previous work in this system (Wardle *et al.*, 2003; Kardol *et al.*, 2018; Fanin *et al.*, 2019a), we categorised these 30 islands into 10 'large' islands representing young successional stages (>1.0 ha; mean time since fire 585 yr), 10 medium islands representing intermediate successional stages (0.1 to 1.0 ha, 2180 yr), and 10 small islands representing late successional stages (<0.1 ha, 3250 yr) (please refer to Supporting Information Table S1 for more details).

To understand the importance of effects of loss of plant functional groups on fungal communities and their importance for decomposition across contrasting ecosystems, we used an ongoing removal experiment (Wardle & Zackrisson, 2005; Gundale *et al.*, 2010; Fanin *et al.*, 2018). In this experiment, eight plots were established on each of the 30 islands (240 plots in total) in August 1996, comprising a full factorial combination of removals of each of the three main plant functional groups present on these islands, that is trees (performed annually by root trenching to a depth of 40 cm with a hand saw, or less when the saw hits underlying bedrock), ericaceous dwarf shrubs (manual pulling out of

rhizomes directly from the soil), and feather mosses (performed manually by removing all the moss from the top of the humus layer). All plots were 55×55 cm, but only the inner 45×45 cm area was used for measurement and sampling. Removal treatments have been maintained annually each year since 1996. This study builds further on previous work in the experiment, showing that the identities of plant functional groups impacted soil microbial communities (as assessed by both phospholipid fatty acid analysis and metabarcoding), plant litter decomposition, OM quality and soil nutrients (Fanin *et al.*, 2018, 2019a,b; Wardle *et al.*, 2020). However, whether and how removing shrubs and trees may affect interactions among fungal guilds with links to altered decomposition rates and N availability has not been evaluated.

Between 3 and 15 August 2015, 19 yr after the experiment started, five soil cores per plot were sampled to a depth of 10 cm using a stainless steel cylinder (diameter of 3 cm) (following the protocol of Fanin *et al.*, 2018). The green-to-brown transition of the moss layer was set as the soil surface (0 cm depth) when mosses were present in the plot. The 0–10 cm depth layer included most of the plant roots and mycorrhizal fungi in our study system (Clemmensen *et al.*, 2013; Fanin *et al.*, 2018); it consisted of an uppermost litter layer with recognisable plant parts in which saprotrophic fungi dominate, and a fragmented litter and humus layer in which EMF and ERI dominate (Clemmensen *et al.*, 2015). When the organic layer was <10 cm (which occurred in 1.3% of plots on large islands), we sampled only to the depth of the organic layer and excluded any mineral soil.

Of the five soil cores taken from each plot, one was taken in the centre of the plot. Living roots and rhizomes with a diameter of >2 mm, coniferous cones, stones and mineral parts were removed in the laboratory, and the remaining soil was homogenised, freeze dried and milled to a fine powder before subsampling for analysis of fungal community composition (PacBio sequencing of amplified ITS2 markers, please refer to Methods S1) and characterisation of OM chemistry. Mid-infrared spectroscopy was used to characterise OM chemistry and to proportionally assign the signal to eight defined nuclear magnetic resonance (NMR) chemical regions (Table S2) as predicted by partial least squares regression against a training set of 60 samples which were analysed directly by ^{13}C CP/MAS NMR (Forouzangohar *et al.*, 2015; Fanin *et al.*, 2018; Baldock *et al.*, 2021). The other four soil cores were collected 20 cm from the plot centre and each corner, and bulked, for measurements of other soil physicochemical properties (mineral N, soil C : N ratio, ^{15}N , ^{13}C , soil moisture) and microbial phospholipid fatty acids (PLFAs) to estimate fungal biomass (following the protocol of Fanin *et al.*, 2018). An increasing soil C : N ratio is interpreted as enhanced N foraging by EMF and ERI, because mycorrhizal fungi use plant-derived C to acquire soil-derived N (Lindahl *et al.*, 2007; Clemmensen *et al.*, 2013). Stable isotopes also provide information about the role of mycorrhizal communities in controlling C and N cycling in boreal soils (Clemmensen *et al.*, 2013; Kyaschenko *et al.*, 2019). In particular, because ectomycorrhizal mycelium is characterised by high ^{13}C values

(Högberg *et al.*, 1999; Mikusinska *et al.*, 2013), it has been proposed that higher soil ^{13}C values indicate larger contributions of mycorrhizal fungal necromass to soil OM (Ehleringer *et al.*, 2000; Boström *et al.*, 2007), but higher ^{13}C content may also indicate older OM (Kyaschenko *et al.*, 2019). Regarding ^{15}N signatures, it has been proposed that high soil ^{15}N values are indicative of an important role of mycorrhizas in N cycling (Lindahl *et al.*, 2007; Clemmensen *et al.*, 2013) because mycorrhizal fungi transfer mostly ^{15}N depleted to their plant hosts (Högberg *et al.*, 1996, 1999). All soil cores were either refrigerated (4°C) for soil analyses or frozen (-20°C) for microbial community analyses within a few hours after sampling. Furthermore, the rate of plant litter decomposition was assessed in each plot by measuring litter mass loss using litter bags (mesh size 1.0×0.1 mm) incubated in the organic horizon at 5 cm depth with a standardised substrate (senesced leaves from the shrub *Vaccinium myrtillus*) over a full year (following the protocol of Fanin *et al.*, 2018). All methodological details for these measurements can be found in the [Supporting Information](#).

Analyses of fungal communities

Fungal community structure was assessed by high-throughput sequencing of amplified ITS2 markers, as described for this experiment by Fanin *et al.* (2018) (please refer to Methods S1 for a detailed description). After sequencing, fungi identified to species (i.e. species hypotheses or SHs) or genera with a well known life-form (i.e. based on published literature) were categorised into the functional groups (please refer to Figs S1–S4 for the assignment of SHs to fungal guilds): ‘Litter-associated saprotrophs’ (SAP), ‘Moulds and yeasts’, ‘Pathogens’, ‘Ectomycorrhizal fungi’ (EMF), ‘Ericoid mycorrhizal fungi’ (ERI) and ‘Root-associated fungi’. This last category included species matching reference sequences isolated from roots or rhizoids (excluding known ERI or EMF taxa) as well as fungi of uncertain mycorrhizal type. Fungi included in supported clades representing either a higher phylogenetic level with supposed common ecology (i.e. Archaeorhizomycetes; Fig. S1), or matching with database references derived from a particular substrate (i.e. surface-sterilised roots of ectomycorrhizal or ericoid hosts or fresh plant litter) were categorised into putative functional groups: ‘Putative ectomycorrhizal fungi’, ‘Putative ericoid mycorrhizal fungi’, ‘Putative root-associated fungi’ or ‘Putative litter-associated saprotrophs’. SHs of low abundance or with insufficient information to be classified into a putative functional group were left in the group of ‘Fungi with unknown ecology’.

Overall, 601 968 reads passed quality control, representing 38% of the total sequencing output. Removal of nonfungal sequences left 520 450 sequences (86% of the high quality sequences), which clustered into 2974 local SHs. Among them, 514 SHs (each with > 50 sequences) were assigned taxonomic affiliation, and 398 SHs (445 426 sequences; 86% of fungal sequences) were assigned to putative functional guilds (Figs S1–S4). On average, fungal communities were represented by 1257 reads per sample, with a range from 44 to 3480.

Statistical analyses

Using a regularised-logarithm transformation that stabilises the variance across the mean for ‘count data’ (Love *et al.*, 2014), we calculated the overall similarity in fungal communities among removal treatments across the different islands using Bray–Curtis distance. We then used permutational multivariate analysis of variance (PERMANOVA, $n=999$ permutations) to assess the effect of removal treatments (i.e. tree roots, shrubs, and mosses), island size class and their interactions on the composition of total fungal communities (i.e. all identified SHs), fungal guilds (i.e. SHs summed by guild) and EMF communities (i.e. identified EMF SHs). We also performed a second analysis for EMF communities only using Hellinger-transformed data (i.e. using relative abundance by dividing counts for individual EMF species by the total EMF counts). Island identity was considered as a random factor to account for the subplot design. The diversity and overlap of EMF taxa across the three island size classes and the treatments with or without shrubs were visualised by assembling a bipartite network (Dormann *et al.*, 2009). Linear mixed models (LMM) were used to assess the effects of removal treatments, island size class and their interaction on concentrations of fungal PLFAs (18:2 ω 6 and 18:1 ω 9), relative abundances of fungal guilds, soil properties (including soil abiotic parameters and litter mass loss) and relative predicted NMR signals of spectral regions following the same model design as detailed for the PERMANOVA above. Normality of residuals was checked using the Shapiro–Wilk test. We then used redundancy analysis (RDA) to elucidate how much of the total variation (inertia) in fungal guild composition was related to the environmental variables, that is measures of components of OM quality and soil properties (Ramette, 2007). All statistical analyses were carried out in R v.4.0.0 (R Core Team, 2013).

Results

Fungal community and guild composition

The fungal community composition at the level of species (i.e. species hypotheses; from this point forwards SHs) was significantly affected by island size, shrub removal, moss removal and the moss \times shrub removal interaction (Table S3a). Effects of tree root removal on fungal SHs composition were marginally non-significant at $P=0.05$. The effects of plant functional group removals on fungal community structure were invariant along the chronosequence, as there were no interactive effects between island size class and plant functional group removals (please refer to Fig. S5 for details about higher taxonomic levels).

The fungal community at the level of guilds revealed similar patterns, with overall significant effects of island size, shrub removal, moss removal and the moss \times shrub removal interaction, as well as a significant effect of tree root removal (Table S3b). The relative abundances of saprotrophs, pathogens, moulds and yeasts were higher on medium than on small islands, whereas abundance of other fungal guilds did not vary among island size classes (Table 1; Fig. S6). Manipulation of plant

Table 1 Results from fully factorial mixed linear models to test for the effects of functional group removals on fungal guilds, soil properties and organic matter (OM) quality classified in three categories: 'Main effects' (i.e. interactive effects among removal treatments) and 'Context dependency' (i.e. interactive effects between removal treatments and island size class). *F*-values ($df_{den} \sim 165$) were indicated for each variable when statistically significant (***) $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$; † $P \leq 0.10$ and the signs (+, blue box) or (-, orange box) are shown when the effects of plant functional group removals are positive or negative, respectively.

Variable	Main effects			Interactions			Context dependency							
	Island size (IS)	Tree root removal (-T)	Moss removal (-M)	Shrub removal (-S)	-T x -M	-T x -S	-M x -S	IS x -T	IS x -M	IS x -S	IS x -T x -M	IS x -T x -S	IS x -M x -S	IS x -T x -M x -S
Fungal biomass (PLFA, nmol g OM ⁻¹)		28.8***(-)		113.4***(-)	3.2 [†]									
Fungal guilds (% ITS markers)														
Ericoid fungi	2.7 [†]		4.6*(-)	19.9***(-)										
Saprotrophs	4.6*			9.6**(-)		4.0*								
Root-associated fungi				9.0**(-)										
Ectomycorrhizal fungi		4.2*(-)		15.6***(+)										
Pathogens	5.8*													
Moulds and yeasts	3.4*		3.3 [†] (-)											
Soil properties														
Mineral N (mg N m ⁻²)	6.8**			32.1***(+)		5.0*		4.9**		6.4**				
Soil C : N ratio (unitless)	17.9***			58.6***(+)		3.4 [†]		5.5**		3.9*				3.8*
¹⁵ N δ _{am} (‰)	23.6***			10.1**(+)		3.8 [†]		3.9*						
¹³ C δ _{org} (‰)	13.0***			7.1**(+)		9.5**								
Decomposition rate (% mass loss)				14.8***(+)						2.5 [†]				
Organic matter quality (% NMR signal)														
Alkyl	9.2***	3.2 [†] (+)	16.5***(+)	7.2**(+)	4.4*					2.5 [†]				2.9 [†]
N-alkyl		11.4***(+)	23.0***(+)	12.0***(+)										
O-alkyl	4.6*	5.4*(-)	20.3***(-)	4.6*(-)			6.1*							3.0 [†]
di-O-alkyl	6.3**	9.6**(-)	22.7***(-)	10.9**(-)	3.9*		7.3**							2.3 [†]
Aryl	3.3 [†]													
di-O-aryl	5.7**			15.1***(-)			3.6 [†]							
Carbonyl	7.1**	15.8***(+)	14.9***(+)	13.9***(+)			3.2*							3.1*
Ketone	7.7**	3.6 [†] (+)		20.2***(+)			4.4*							6.3**

Significant effects of island size and interactive effects are highlighted in green. Dark and light shading in the boxes indicate significant ($P < 0.05$) and marginally nonsignificant effects ($0.05 < P < 0.10$), respectively.

functional groups had distinct effects on the relative abundance of different fungal guilds: tree root removals reduced the relative abundance of ectomycorrhizal fungi (EMF), moss removals reduced the relative abundance of ericoid mycorrhizal fungi (ERI), and shrub removals decreased the relative abundance of saprotrophs, root-associated fungi and ERI but increased the relative abundance of EMF (Table 1; Fig. 1). The effects of plant functional group manipulations on the different fungal guilds were invariant along the chronosequence, as there were no significant interactive effects between any removal treatment and island size (Table 1).

When we considered the effects of shrub removals on fungal species, we found that removals caused declines in the relative abundance of several species of ERI (e.g. *Rhizoscyphus ericae* also referred to as *Hyaloscypha hepaticicola*, and species belonging to the order Chaetothyriales) and other root-associated fungi (e.g. Archaeorhizomycetes) (Fig. S7). Shrub removals also decreased the relative abundance of some dominant saprotrophic basidiomycetes (e.g. *Mycena clavicularis* and *Mycena monticola*) but favoured some ascomycetes (e.g. *Leptodontidium*) (Fig. S7). Furthermore, shrub removal significantly affected the composition of the EMF community, regardless of whether abundance was calculated relative to all fungal sequences or to EMF sequences only (Table S4). When considering only EMF sequences, the effect of shrub removals on the EMF community structure varied with island size (Table S4), with smaller effects on large islands (PERMANOVA, $n = 999$ permutations, $df = 1$, $P = 0.421$) than on medium islands ($df = 1$, $P = 0.009$) and small islands ($df = 1$,

$P = 0.003$) (data not shown). As such, we found that 20 out of 37 EMF species present increased as a proportion of the total EMF sequences after shrub removals (Fig. 2a), notably the abundant, simple-mycelial species *Russula decolorans* on large and medium islands (i.e. in Fig. 2b), and *Lactarius rufus* on medium and small islands (Fig. 2b). We also found a significant increase in the simple-mycelial species *R. paludosa* on large islands, *Pseudotomentella tristis* on medium islands, and *P. humicola* and *Cenococcum geophilum* on small islands (Fig. 2b). Shrub removal also increased the cord-forming fungi *Cortinarius acutus* and *C. semisanguineus* on large- and medium-sized islands and *C. flexipes* on small islands (Fig. 2b). Shrub removal decreased the relative abundance of *Amphinema byssoides* across all three island size classes, and abundances of *Hyaloscypha finlandica* and *Cortinarius collinitus* for medium and small islands (Fig. 2b).

Decomposition, N availability and OM quality

We found that the decomposition rates of *Vaccinium* litter were highest on the large islands, whereas soil mineral N was highest in medium islands and soil OM ^{13}C signatures (with higher values indicating older OM and/or a larger contribution of mycorrhizal mycelium to OM) increased gradually from large to small islands (Fig. 3; Table 1). Tree root removals decreased the soil C : N ratio, moss removals increased the values of soil ^{13}C and ^{15}N (Fig. S8), and shrub removals increased the values of ^{13}C and ^{15}N (with higher values indicating a greater contribution of mycorrhizas to N cycling), as well as mineral N and

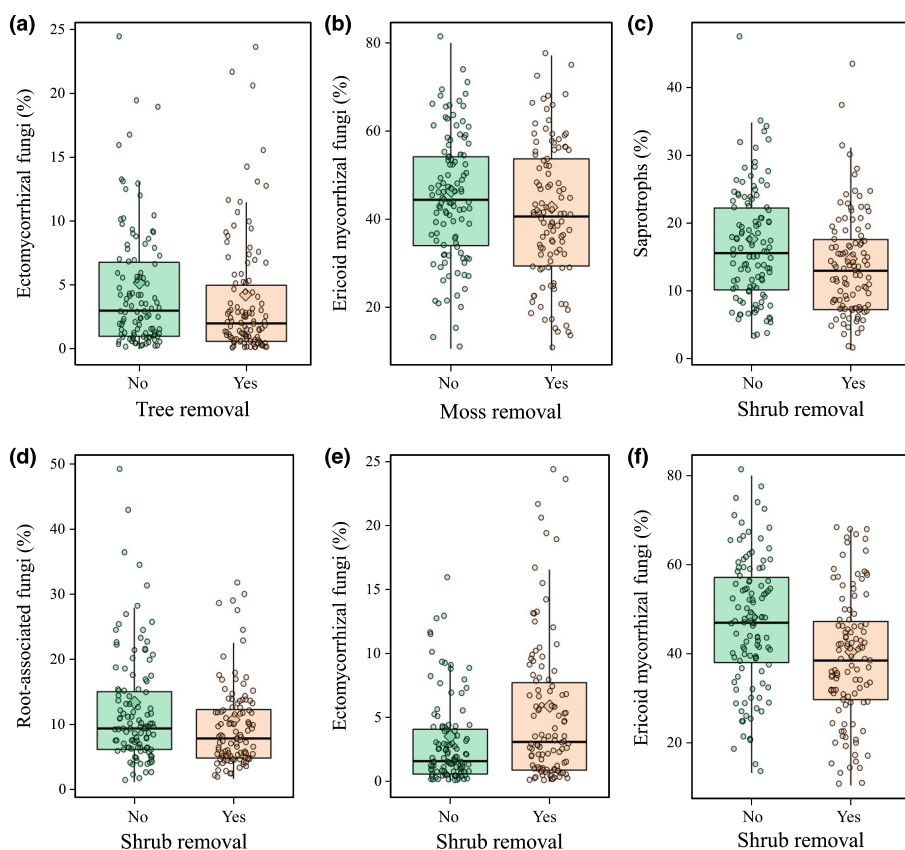


Fig. 1 Boxplots of statistically significant ($P < 0.05$) effects of plant functional group removals on relative abundances of fungal guilds. Effect of (a) tree root removals on ectomycorrhizal fungi, (b) moss removals on ericoid mycorrhizal fungi, and shrub removals on (c) saprotrophs, (d) root-associated fungi, (e) ectomycorrhizal fungi, and (f) ericoid mycorrhizal fungi. The boxes and whiskers represent the central 50% and 95% of the data, respectively. Circular symbols represent individual plots, and the diamond and solid line within each box represents the mean and median, respectively.

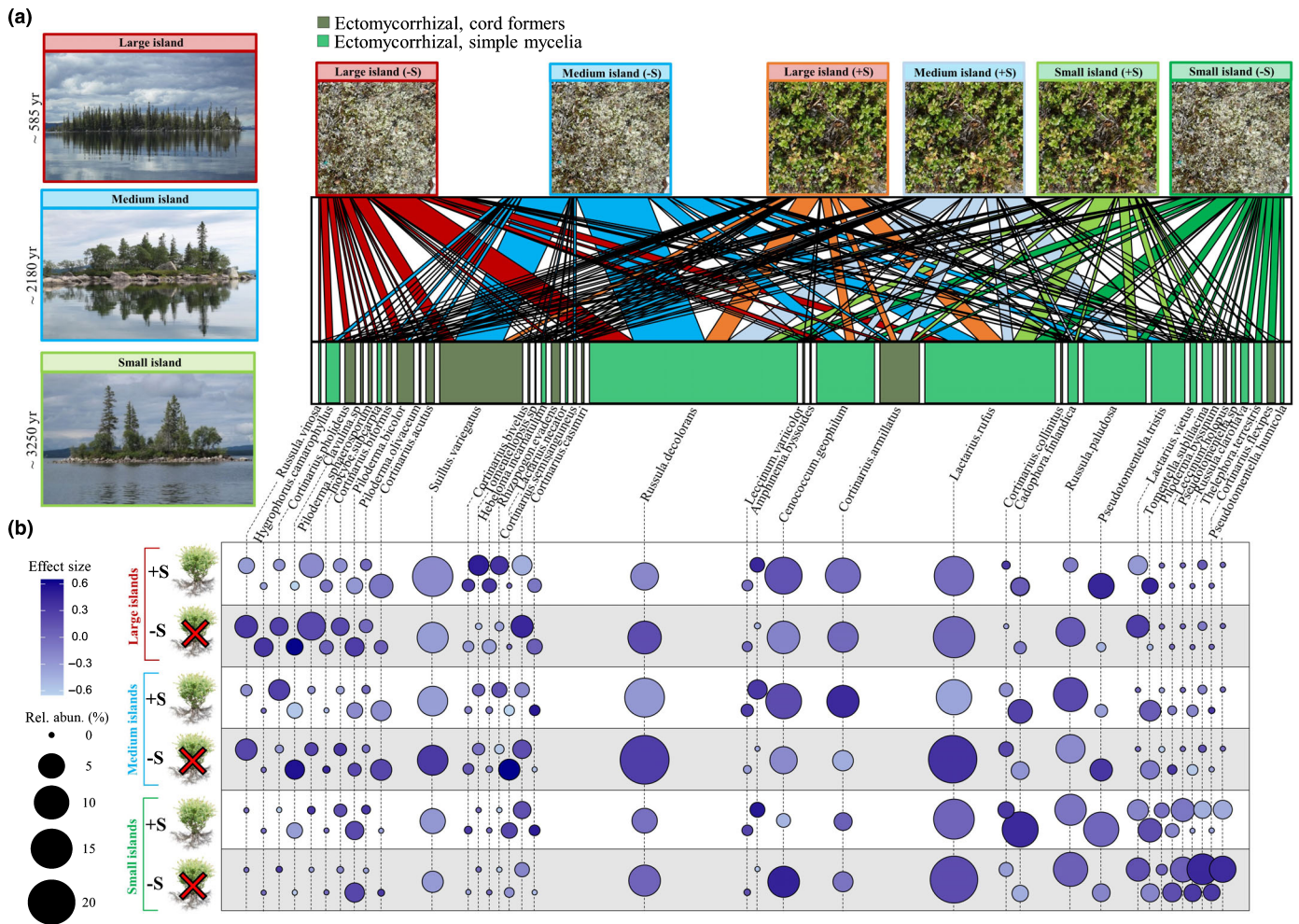


Fig. 2 Effects of shrub removals on ectomycorrhizal fungi (EMF) communities. (a) Bipartite network representing the EMF species richness and (b) relative abundance of ectomycorrhizal fungi across the three island size classes (large (red), medium (blue) and small (green)) when the shrubs are present (+s) or absent (–s). For (a), the association between treatments and EMF species was evaluated using a bipartite network. Each rectangle in the bottom level represents a different EMF species. The width of each link represents the interaction intensity for each combination of island size and shrub presence vs absence. For (b), the effect size comparing the presence/absence of shrubs is represented by the shade of purple (with dark/light purple corresponding to positive/negative effects of shrubs on EMF species) and the size of circles represents the relative abundance of that species within the whole EMF community. EMF communities were subsampled to equal sequence number for this analysis. The corresponding PERMANOVA results can be found in Supporting Information Table S4. Photography credit: N. Fanin.

decomposition rates (Fig. 3). There were significant and positive interactive effects between shrub and moss removals on decomposition rates and the soil C : N ratio (Table 1; Fig. S9). We also found that removal of shrubs or tree roots significantly reduced fungal biomass (fungal PLFAs; Table 1; Fig. 3).

Furthermore, we found that some effects of plant functional group removals on soil properties depended on island size class (Table 1). The effects of shrub removals on soil C : N ratios was positive, neutral and negative for large, medium and small islands, respectively (Fig. 3). The effects of tree root and shrub removals on mineral N were highest on medium islands, whereas effects on ^{13}C and ^{15}N were highest in small islands (Figs 3, S8). We also found a three-way interaction between island size \times trees \times shrubs on the soil C : N ratio (Table 1), with the greatest negative effects occurring when trees and shrubs were removed from small islands (Fig. S9).

The chemical composition of OM varied significantly with island size class (Table 1): the alkyl : O-alkyl C ratio (i.e. higher ratios indicating a greater extent of decomposition) decreased from large to small islands as a result of increase in alkyl C on large islands, whereas O-alkyl and di-O-alkyl C were highest on small islands (Fig. S10). Carbonyl and di-O-aryl C (i.e. chemically recalcitrant C; Table S2) were both highest on medium and small islands (Fig. S10). The composition of OM was also strongly affected by plant functional group removals (Table 1): tree root, moss and shrub removals affected the relative proportion of O-alkyl and di-O-alkyl C negatively but N-alkyl and carbonyl C positively (Fig. S11). Alkyl C was positively affected by moss and shrub removals, while ketone and di-O-aryl C were positively and negatively affected by shrub removals, respectively (Fig. S11). The effects of plant functional group removals on OM quality also varied with island size class for some NMR

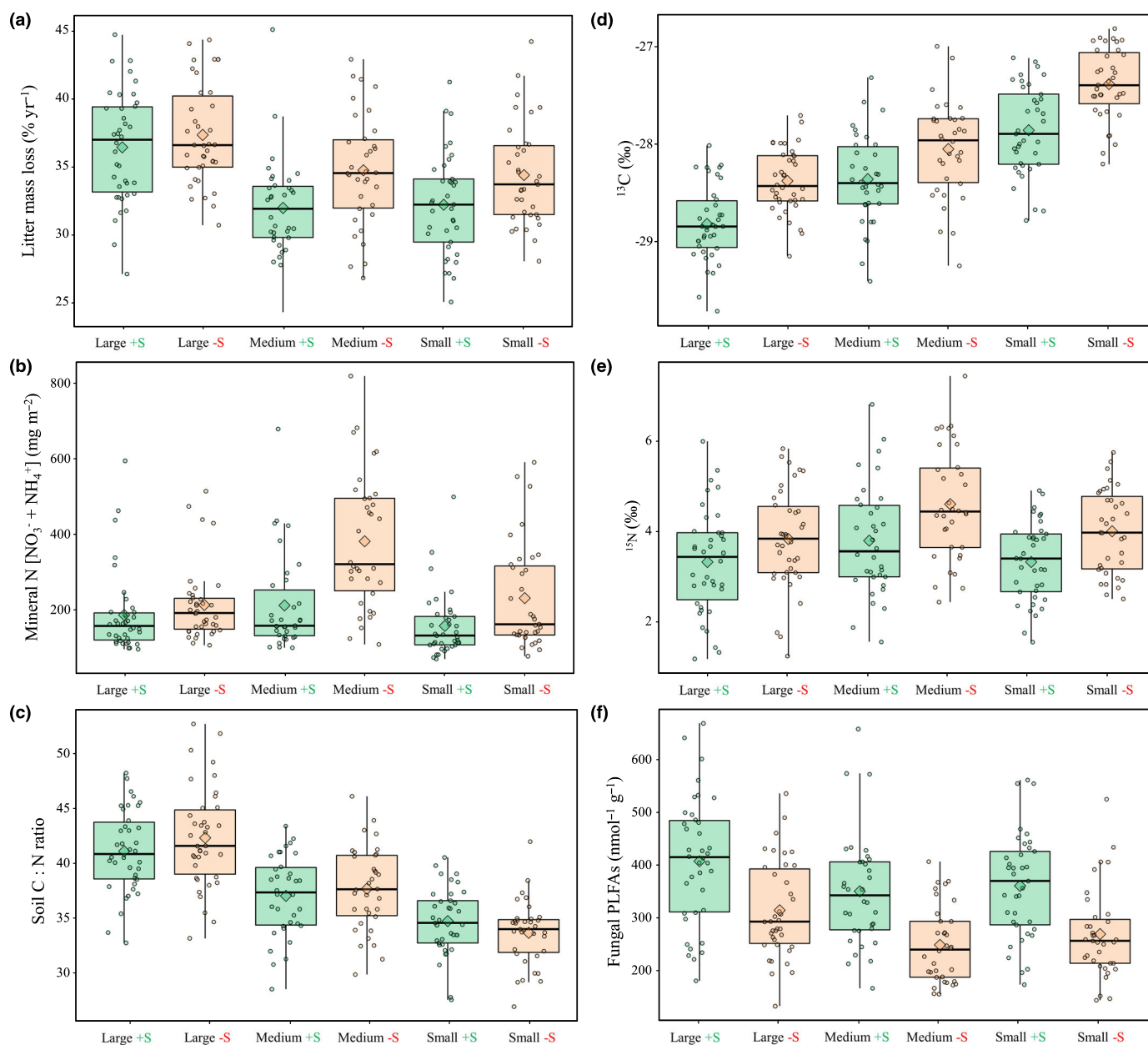


Fig. 3 Boxplots of the effects of shrub removals (Shrubs present (+S) and Shrubs absent (–S)) on (a) decomposition rates, (b) mineral N, (c) soil C : N ratio, (d) ^{13}C , (e) ^{15}N and (f) fungal phospholipid fatty acids (PLFAs) across island size classes (large, medium and small). The boxes and whiskers represent the central 50% and 95% of the data, respectively. Different points represent individual plots, and the diamond and solid line within each box represent the mean and median, respectively.

regions (Table 1): the effect of moss and shrub removals on ketone C was higher for small islands, whereas the effect of shrub removals on di-*O*-aryl C was higher for large islands (Fig. S12).

Linking fungal guilds to OM quality and nutrient availability

The relative decrease in ERI abundance and corresponding increase in EMF resulting from shrub removal were associated with higher soil N availability and ^{15}N values (with higher values indicating a greater contribution of mycorrhizas to N cycling), and to a lesser extent with greater decomposition rates and ^{13}C

values (with higher values indicating older OM and/or a larger share of OM derived from mycorrhizal mycelium) (Fig. 4). Decreasing the relative abundance of ERI was also associated with lower values in the humus of *O*-alkyl and di-*O*-alkyl (representing carbohydrates and aliphatics) but higher values of alkyl, *N*-alkyl, carbonyl and ketone (representing amino acids and more complex and chemically recalcitrant C compounds). The shift from recently formed to more processed C compounds, as represented by an increase in the alkyl to *O*-alkyl ratio, was associated with a lower fungal biomass, but a higher proportion of EMF (Fig. 4).

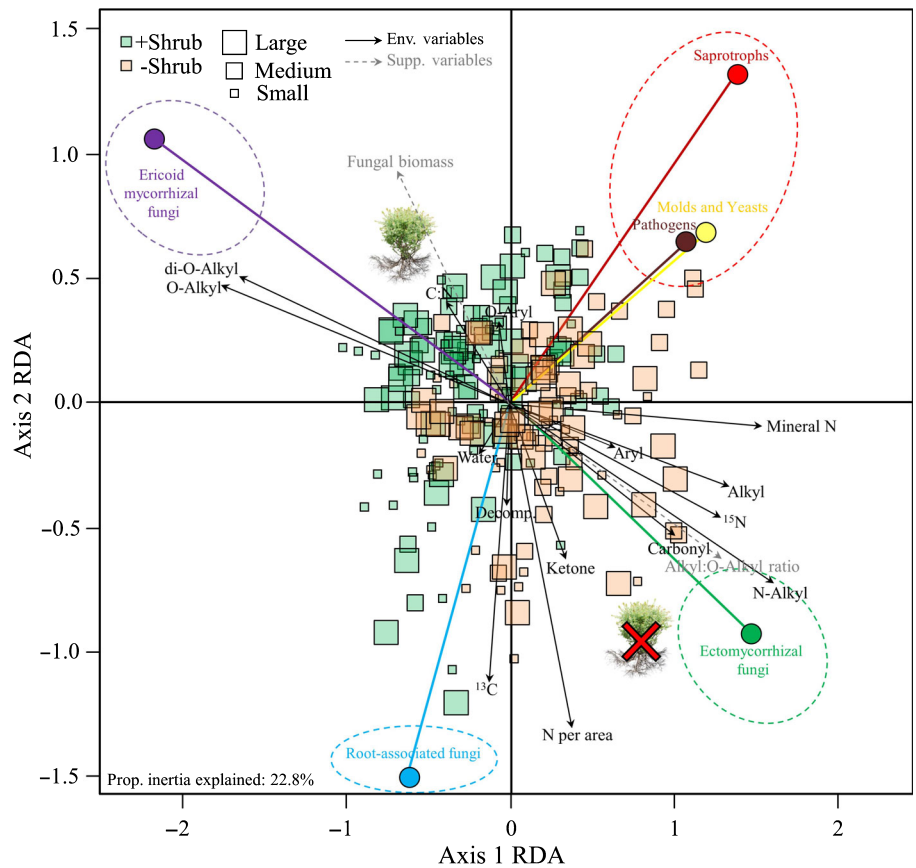


Fig. 4 Redundancy analysis (RDA) ordination biplots of the relative abundance of fungal guilds, ecosystem properties and mid-infrared (MIR)-derived nuclear magnetic resonance (NMR) regions of soil organic matter. We depicted shrub removals in the RDA, because this was the main factor affecting the structure of fungal communities along the island gradient. Island size class is indicated by square size and shrub presence vs absence by colour. The vectors representing ecosystem properties or MIR-derived NMR regions of soil organic matter point in the direction of greater values.

Discussion

Interactions between fungal guilds after plant functional group removals

Our study enabled us to assess the consequences of plant functional group removal on mycorrhizal and saprotrophic fungal guilds in the upper part of the organic soil layer, and on SOM quality and litter decomposition after almost two decades. The effect of plant removal on fungal biomass was relatively consistent along the island chronosequence (i.e. tree and shrub removals decreased fungal biomass similarly for all island size classes). In line with our expectations, tree root removal significantly decreased the relative abundance of EMF, whereas shrub removal significantly decreased the relative abundance of ERI. The large decrease in total fungal biomass that we found following the loss of EMF or ERI (due to the removal of tree roots or ericaceous shrubs, respectively) means that other fungi were not able to colonise the niche left empty by EMF or ERI removal, even after 19 yr. This was contrary to our hypothesis H_{1a}, and suggested that EMF and ERI occupy complementary, rather than overlapping, niches. One explanation could be that these two guilds did not compete directly for the same resources, suggesting that they could be utilising different N sources in the humus (Michelsen *et al.*, 1996; Näsholm *et al.*, 1998; Read & Perez-Moreno, 2003). For instance, it is generally accepted that some EMF are efficient at exploring and exploiting resources in patchy environments

through the production of extensive mycelia, using oxidative decomposition mechanisms (Hobbie *et al.*, 2013; Bödeker *et al.*, 2014; Shah *et al.*, 2016; Sterkenburg *et al.*, 2018), whereas ERI are more efficient at detoxifying phenolic compounds for accessing organic N sequestered in tannin–protein complexes in their immediate surroundings (Bending & Read, 1996; Wurzbarger & Hendrick, 2009). The apparent lack of colonisation of open soil niches by fungi following ERI or EMF loss resulting from plant removals could have resulted from overall low C fixation rates (and therefore low belowground C inputs) associated with low ecosystem productivity across this island chronosequence and which may restrain root and mycorrhizal recolonisation (Wardle *et al.*, 2003, 2012).

Interactions between fungal guilds along the island chronosequence

Our results revealed that shrub removals significantly influenced EMF community composition (i.e. when only EMF sequences were considered), and that these effects were larger in late successional forests (i.e. on medium and small islands) than in early successional forests (i.e. on large islands). In line with our hypothesis H_{1b}, these results indicated that ericaceous shrubs and associated ERI played a more important role in structuring EMF communities in ecosystems in which they occupy a higher relative biomass (Clemmensen *et al.*, 2015), such as the small islands (Wardle *et al.*, 2012). This finding aligns with several previous

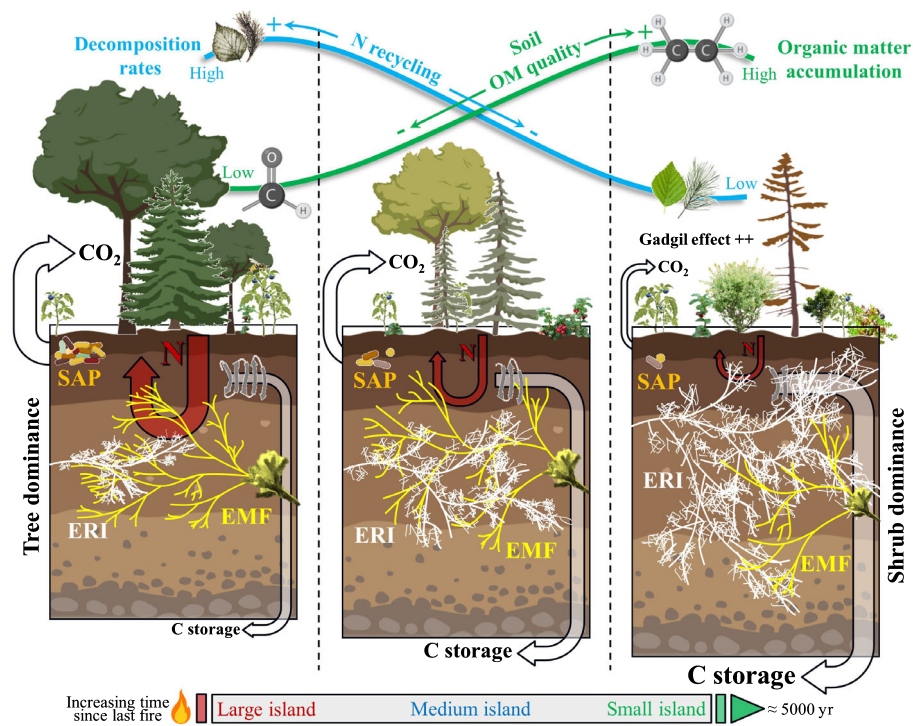


Fig. 5 Schematic diagram representing the changes that occur in plant–fungi interactions between tree-dominated and shrub-dominated ecosystems, and their consequences for C and N cycling, as the chronosequence progresses (i.e. as island size declines with increasing time since fire). Contrary to the common belief that organic matter (OM) accumulation is mainly due to decreasing quality of litter originating from plants as chronosequences proceed, our results supported the idea that the greater abundance of dwarf shrubs and associated ericoid mycorrhizal fungi slows down C and nutrient cycling mainly through decreasing the contribution of ectomycorrhizal fungi (EMF) and saprotrophs to promoting decomposition. This means that, although the quality of OM inputs derived from plants is higher earlier in the chronosequence, the faster decomposition rates (i.e. the increase in OM recycling and CO₂ release to atmosphere; white arrow) in large islands leads to poorer quality OM remaining in the soil compared with later stages in the chronosequence. Therefore, we propose that the increasing abundance of slow-growing ericaceous shrubs and associated fungi contributes to progressive soil OM accumulation (i.e. increase in soil C stocks; grey arrows) and increasing nutrient limitation (i.e. decrease in soil N availability; red arrows) in boreal forest, mainly because of an impairment of decomposition and nutrient recycling towards later successional stages. ERI, ericoid mycorrhizal fungi; SAP, saprotrophs. This figure was created using BioRENDER and MS POWERPOINT.

field studies showing that abundant occurrence of ericaceous shrubs can inhibit EMF colonisation and reduce EMF diversity due to antagonistic effects and/or changes in environmental conditions (Nilsson *et al.*, 1993; Walker *et al.*, 1999; St Martin & Mallik, 2016). In our study, the negative effects of the presence of shrubs and ERI in medium and small islands were found for a variety of EMF species, including *Lactarius rufus*, *Pseudotomentella tristis*, *P. humicola*, *Cenococcum geophilum*, *Piloderma sphaerosporum*, *Cortinarius acutus*, *C. semisanguineus* and *C. flexipes*, although a smaller number of EMF species were positively affected by the presence of shrubs, such as *Amphinema byssoides*, *Cadophora finlandica* and *C. collinitus*. In contrast with our hypothesis H_{1c}, these results highlighted the finding that the removal of shrubs and their ERI have relatively similar effects on simple mycelia EMF (e.g. *Pseudotomentella* species) and cord-forming EMF (e.g. *Cortinarius* species), and suggest that, although EMF and ERI can co-exist at the guild level, species turnover within guilds is necessary for this inter-guild complementarity to occur. Furthermore, and while it seems unlikely that these two fungal guilds compete directly for the same resources, our experiment showed that some fungal guilds may influence the community composition of other fungal guilds, with the

strength of these effects being enhanced in ecosystems that are unproductive and nutrient poor.

Gadgil effect of shrub removals

In contrast with our hypotheses H_{2a} and H_{2b}, we did not find that the removal of tree roots significantly increased the activity of saprotrophs (and therefore plant litter decomposition rates), nor that the effects were higher in the more productive larger islands. Similarly, and in contrast with H_{2c}, we did not find that removal of mosses, which contribute highly recalcitrant litter, had a positive effect on the activity of saprotrophs. Instead, our results showed that shrubs and ERI exerted a direct control on processes regulating C cycling and N availability across all island size classes, as indicated by the large increase in mineral N and accelerated litter mass loss after shrub removals (Fig. 3). One possible explanation was that shrubs and ERI lock up nutrients through the production of highly recalcitrant (e.g. tannin-rich and nutrient poor) litter and fungal necromass (Nilsson & Wardle, 2005; Clemmensen *et al.*, 2015), and thereby reduce OM loss from the soil by saprotrophic decomposition (Sterkenburg *et al.*, 2018). In line with this, root-associated ERI

ascomycetes belonging to groups such as Chaetothyriales and Helotiales, which were dominant in our study, commonly formed dark mycelia with thick and melanised cell walls that were highly resistant to degradation (Koide *et al.*, 2014; Finlay & Clemmensen, 2017; Fehrer *et al.*, 2019; Fernandez *et al.*, 2019).

In line with our hypotheses H_{3a} and H_{3b}, we showed that the reduction in plant C inputs and the positive effects of removing shrubs on decomposition were reflected in OM quality and soil properties (Fig. 4). Our data on SOC chemistry revealed that the removal of shrubs decreased the proportion of carbohydrate-like C forms (i.e. those that are represented mainly by O-alkyl and di-O-alkyl) in the soil, and is likely to be due to the lack of new shrub-derived OM inputs and therefore an increase in average OM age. This loss of shrub contributions to OM resulting from their removal was also supported by an increase in soil ¹⁵N, because ericaceous shrubs are depleted in ¹⁵N relative to other plant functional types (Nadelhoffer *et al.*, 1996; Craine *et al.*, 2018; Wardle *et al.*, 2020). Furthermore, the higher soil ¹³C that occurred due to shrub removal was consistent with the sequestered OM becoming older (Clemmensen *et al.*, 2013). In addition, shrub removals stimulated saprotrophic decomposition and release of mineral N from fresh litter. This finding was consistent with a loss of total soil OM stocks in the shrub removal plots across the island gradient (Grau-Andrés *et al.*, 2020). Altogether, our findings of increased N availability and stimulated decomposition by saprotrophs after shrub removals suggested that the ‘Gadgil effect’ was not a feature of only EMF, but was also applicable to ERI. These results are consistent with shrubs acting as a dominant N sink in boreal forests through their large contribution to OM inputs and negative effects on decomposition rates (Clemmensen *et al.*, 2015; Friggens *et al.*, 2020). However, and in contrast with our hypothesis H_{3c}, these effects were relatively consistent across our island chronosequence, despite significant variation in the relative contribution of ericaceous shrubs and ERI.

Mechanisms of plant–soil interactions across the gradient

The results of our long-term removal experiment contributed to our understanding of the mechanisms of plant–soil interactions during long-term succession and ecosystem development (Fig. 5). In addition to previously identified mechanisms revealing why soil OM accumulation accelerates later in succession in our study system through an accumulation of poor quality plant litter and associated fungal necromass (Clemmensen *et al.*, 2015; Wardle *et al.*, 2020), our results indicated an additional mechanism in which shrubs can promote OM accumulation by hampering litter decomposition (i.e. through a ‘Gadgil effect’ caused by shrubs and their ERI). Although the quality of plant litter input is higher for large islands (i.e. early successional stages) (Wardle *et al.*, 2012), the faster decomposition rates have led to poorer soil OM quality relative to small islands (i.e. late successional stages) (Fig. 5). These results corroborate the recent findings of Clemmensen *et al.* (2021) in suggesting that the negative effects of shrubs and ERI on decomposition rates (through reducing decomposition and loss of labile compounds) contribute to long-

term OM accumulation. Increasing moss biomass along the island chronosequence may further contribute to OM formation as mosses produce heavily recalcitrant litter (Fanin *et al.*, 2019a). Therefore, we propose that an increasing abundance of mosses, slow-growing ericaceous shrubs and ERI over time contributes to progressive OM accumulation, not only through necromass accumulation, but also through impairment of decomposition processes due to increasing nutrient limitation (Fig. 5), a pathway that can only be reset by major disturbances such as fire or loss of ericaceous shrubs (Lindahl & Clemmensen, 2016).

Conclusions

Our study suggests that, across the island chronosequence, the inhibition of leaf litter decomposition by mycorrhizas was stronger in the presence of ERI-shrub roots than in the presence of EMF-tree roots. These results are in contrast with our hypothesis predicting enhanced decomposition primarily after removals of tree roots and their associated EMF, and have several implications. First, they highlight that ericaceous shrubs have a strong and direct control on OM accumulation, mainly because shrubs and ERI impair decomposition by saprotrophs, through a ‘Gadgil effect’ of shrub removals. Second, they indicate that the presence of ericaceous shrubs contributes to the context dependency of ‘Gadgil effects’ among different studies and ecosystems. This is notable because most studies to date have not considered the role of ERI in inter-guild interactions (Ward *et al.*, 2021), and the occurrence of ericaceous shrubs could help explain the inconsistent results found among those studies. Third, our data demonstrate that although the quality of OM inputs derived from plants and their associated fungi is higher in early successional stages, the higher decomposition rates of labile substrates leads to a lower quality of OM remaining in the soil, compared with later stages. Finally, our data suggest that ericaceous shrubs and their associated ERI can contribute to the important role that boreal forest soils play as C and N sinks, in a large part through their role in promoting belowground OM accumulation. For this reason, changes in the balance of trees vs ericaceous shrubs and their associated fungal communities that may result from global changes, such as climate warming, more frequent fires or N deposition, will have important future consequences for OM accumulation and nutrient limitation in boreal forest ecosystems.

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Author contributions


DAW acquired the necessary funding and designed and implemented the experiment. NF, KEC, BDL, DAW, PK, MJG, M-


CN and MF contributed to the collection of data. NF analysed data and wrote the first draft of the manuscript in close consultation with DAW, KEC and BDL who contributed critically to data interpretation and ideas. All authors contributed to manuscript completion and revision.


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Data availability

The data that support the findings of this study are available on request from the corresponding author. DNA sequence data are accessible at the NCBI-SRA under the accession no. SRP125544.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Neighbour-joining tree of representative sequences from basal fungal lineages and basal clades of Ascomycota.

Fig. S2 Neighbour-joining tree of representative sequences from Pezizomycotina.

Fig. S3 Neighbour-joining tree of representative sequences from Leotiomycetes.

Fig. S4 Neighbour-joining tree of representative sequences from Basidiomycota.

Fig. S5 Clustering analysis on fungal species hypotheses and fungal guilds.

Fig. S6 Boxplots of different fungal guilds per island size classes.

Fig. S7 Detrended correspondence analysis of fungal communities when shrubs are present or removed.

Fig. S8 Boxplots of the effects of plant functional group removals on soil physicochemical properties.

Fig. S9 Boxplots of the effects of plant functional group removals on litter mass loss and C : N ratio.

Fig. S10 Boxplots of the effects of island size classes on different NMR regions.

Fig. S11 Boxplots of the effects of plant functional group removals on different NMR regions.

Fig. S12 Boxplots of the effects of plant functional group removals on *O*-aryl and Ketone.

Methods S1 Supplementary methods.

Table S1 Ecosystem properties across island size classes.

Table S2 ¹³C-NMR spectral assignment of carbon functional groups.

Table S3 PERMANOVA results on fungal community composition and fungal guild composition.

Table S4 PERMANOVA results on the structure of ectomycorrhizal communities.

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