Resistance of subarctic soil fungal and invertebrate communities to disruption of belowground carbon supply

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Abstract

1. The supply of recent photosynthate from plants to soils is thought to be a critical mechanism regulating the activity and diversity of soil biota. In the Arctic, large-scale vegetation transitions are underway in response to warming, and there is an urgent need to understand how these changes affect soil biodiversity and function.
2. We investigated how abundance and diversity of soil fungi and invertebrates responded to a reduction in fresh belowground photosynthate supply in treeline birch and willow, achieved using stem girdling. We hypothesised that birch forest would support greater abundance of ectomycorrhizal fungal species and fauna than willow shrubs, and that girdling would result in a rapid switch from ectomycorrhizal fungi to saprotrophs as canopy-supply of C was cut, with a concomitant decline in soil fauna.
3. Birch forest had greater fungal and faunal abundance with a large contribution of root-associated ascomycetes (ericoid mycorrhizal fungi and root endophytes) compared to willow shrub plots, which had a higher proportion of saprotrophs and, contrary to our expectations, ectomycorrhizal fungi. Broad-scale soil fungal and faunal functional group composition was not significantly changed by girdling, even in the third year of treatment. Within the ectomycorrhizal community, there were some changes, with genera that are believed to be particularly C-demanding declining in girdled plots. However, it was notable how most ectomycorrhizal fungi remained present after three years' isolation of the belowground compartment from contemporary photosynthate supply.
4. *Synthesis:* In a treeline/tundra ecosystem, distinct soil communities existed in contrasting vegetation patches within the landscape, but the structure of these communities was resistant to canopy disturbance and concomitant reduction of autotrophic C inputs.

Introduction

The climate is changing in the Arctic faster than anywhere else on earth (Mudryk *et al.*, 2019), causing complex changes and feedbacks within terrestrial ecosystems (Post *et al.*, 2009; Wookey *et al.*, 2009). One such prominent change in parts of the Arctic is the increase in productivity and associated compositional shifts in vegetation cover (Elmendorf *et al.*, 2012b). Deciduous shrubs are documented to have increased in cover and range in many areas, and treelines are shifting northward and upslope, in line with shifts in climate and other factors (Myers-Smith *et al.*, 2011; Rees *et al.*, 2020). Aboveground, shrub or treeline expansion is reflected in important changes in primary productivity, reflectance and microclimate (Myers-Smith *et al.*, 2011). Belowground, the implications of vegetation change are less well understood, but there is potential for major biogeochemical feedbacks (Parker *et al.*, 2021). Greening and productivity trends across the Arctic are being punctuated and even counteracted by increasingly frequent 'browning' events and trends in some places (Phoenix & Bjerke, 2016; Myers-Smith *et al.*, 2020). Parts of tundra and treeline forests face increased incidents of tundra fires (Bret-Harte *et al.*, 2013), caterpillar outbreaks (Jepsen *et al.*, 2008; Dahl *et al.*, 2017), extreme winter warming events (Treharne *et al.*, 2020) and range shifts of major canopy herbivores (Tape *et al.*, 2016), all of which can contribute to reductions in ecosystem productivity.

'Greening' and 'browning' of the Arctic could represent fundamental changes to how ecosystems function in this biome. Greening is characterised by a shift from plants that form ericoid mycorrhiza (Read *et al.*, 2004; Hobbie & Hobbie, 2006) to more productive trees and deciduous shrubs that associate with ectomycorrhizal (ECM) fungi, whereas browning events may reduce the C supply to fungal symbionts (Saravesi *et al.*, 2015; Parker *et al.*, 2017, 2020). The resulting change in belowground productivity (Sloan *et al.*, 2013) and mycorrhizal association (Hobbie *et al.*, 2009) will alter the supply of C to soil food webs (e.g. fungi and fauna), the community composition of which is vital for regulating the turnover of C in soils (Handa *et al.*, 2014). Mycorrhizal fungi are key components of food webs because they have a fundamental role in regulating soil organic matter (SOM) storage and turnover (Frey, 2019), and are phylogenetically and functionally diverse (Hibbett *et al.*, 2000). These fungi represent a critical physical and energetic link between plants and other components of soil food webs and soil processes, because they are recipients of plant-derived C and sometimes also decomposers of SOM. Some species are known "as potent decomposers, 'mining' organic N by producing oxidative enzymes (Bödeker *et al.*, 2014; Lindahl *et al.*, 2021), while others are better adapted for 'scavenging' of mineral N with a less direct effect on SOM (Zak *et al.*, 2019).

77 Hence it is important to determine the factors driving food web community assembly in order to
78 better understand the implications of plant community change for ecosystem functioning
79 (Clemmensen *et al.*, 2021).

80 Browning events could restructure ECM fungal communities by reducing the amount of C
81 that can be allocated belowground and thereby adjusting the competitive balance of 'C-demanding'
82 vs less demanding fungi (Saikkonen *et al.*, 1999). Trees at the relatively productive subarctic birch
83 treeline are associated with ECM fungi that specialise in rapid mobilisation, or 'mining', of organic N
84 from the soil (Bödeker *et al.*, 2014; Clemmensen *et al.*, 2021). In particular, the *Cortinarius* genus,
85 which consists of cord-forming Agaricomycetes, has been linked to oxidation of organic matter by
86 production of manganese peroxidases in low-fertility boreal forests (Bödeker *et al.*, 2014; Lindahl *et*
87 *al.*, 2021; Pérez-Izquierdo *et al.*, 2021). Conversely, other fungi that form 'short distance' or 'contact'
88 mycorrhizal morphotypes (with hyphae concentrated close to the root tip (Agerer, 2001)) may be
89 favoured under more restrictive C supply from their hosts, especially after a browning event
90 (Saikkonen *et al.*, 1999). Indeed, defoliation of subarctic canopies by geometrid moth caterpillars
91 ('autumnal' and 'winter' moths of the genera *Epirrita* and *Operophtera*, respectively) has been found
92 to shift mycorrhizal communities from medium and long distance exploration types to contact-types
93 (Saravesi *et al.*, 2015; Parker *et al.*, 2017). In addition, we have already demonstrated that stem
94 girdling (disconnection of the phloem) results in a large reduction in the production of extra-radical
95 mycelium in treeline mountain birch forest, but not in tall willow plots, which had lower baseline
96 rates of hyphal production (Parker *et al.*, 2020). Potentially, these results are related to a higher
97 allocation of C to ectomycorrhizal fungi in the birch forest. Therefore we predicted that girdling
98 would result in selective reduction of ectomycorrhizal species in the fungal community, in particular
99 of more C-demanding species, and most markedly in the birch forest.

100 The traditional view represented in food web models is that C enters soil fauna
101 predominantly from plant litter inputs, either via bacterial or fungal-based energy channels (Hunt *et*
102 *al.*, 1987). However, there is increasing evidence that C from recent photosynthate is a major source
103 of energy for soil fauna (Ruf *et al.*, 2006; Pollierer *et al.*, 2007; Eissfeller *et al.*, 2013; Gilbert *et al.*,
104 2014; Goncharov *et al.*, 2016; Chomel *et al.*, 2019). Mycorrhizal fungi are likely to be a key pathway
105 for C delivery to microbivore fauna, such as microarthropods (e.g. Collembola and oribatid mites),
106 because the substantial flux of recent photosynthate through extra-radical mycelium in forests
107 (Högberg *et al.*, 2001, 2008; Heinemeyer *et al.*, 2007). Indeed, experiments in laboratory model
108 systems have demonstrated transfer of recent photosynthate to Collembola (Kanters *et al.*, 2015)
109 and that the presence of ectomycorrhizal fungi may modify the composition of faunal communities
110 (Setälä *et al.*, 1999; Setälä, 2000). Despite these findings from laboratory experiments, it is unclear

111 whether changes in the belowground supply of recent photosynthate and associated alterations of
112 fungal communities lead to changes in microarthropod communities in the field.

113 Soil biodiversity under shrub willows (*Salix* spp.), which are particularly prevalent in wetter
114 areas of the tundra (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011; Elmendorf *et al.*, 2012a), is also
115 poorly characterised. The fungal and faunal communities associated with *Salix* shrubs may be
116 distinct from those of *Betula* shrubs or forest (Clemmensen *et al.*, 2021) because of a tendency of
117 *Salix* to grow in moister areas prone to seasonal flooding, potential anoxia, and a higher influx of
118 aeolian and snow-borne mineral nutrients (Nadelhoffer *et al.*, 1991; Sturm *et al.*, 2005). Relatively
119 high nutrient availability may reduce the need for *Salix* shrubs to invest in ectomycorrhizal fungi, or
120 at least in C-demanding ectomycorrhizal fungi that specialise in 'N-mining' (Clemmensen *et al.*,
121 2021). Reduced competition from ectomycorrhizal fungi may favour proliferation of saprotrophic
122 fungi when N is more available (Kyaschenko *et al.*, 2017). Lower ECM hyphal production was
123 measured in willow soils compared to drier mountain birch soils (Parker *et al.*, 2020), and we
124 therefore expect a smaller proportion of ECM fungal species in willow plots. Consequently,
125 microarthropods, who may depend on recent photosynthate delivery by ECM fungi and/or on their
126 hyphal (necro)mass as a food source (Setälä *et al.*, 1999; Setälä, 2000), are expected to be less
127 abundant under willow than in birch soils, particularly in deeper soils where mycorrhizal fungi
128 dominate in this system (Clemmensen *et al.*, 2021).

129 The objective of our study is to characterise soil fungal and microarthropod communities in
130 mountain birch forest (*Betula pubescens*) and tall willow stands (*Salix* spp.); two important subarctic
131 and arctic vegetation types that are documented to be expanding in range and cover at high
132 latitudes (Myers-Smith *et al.*, 2011; Rees *et al.*, 2020). We also documented the response of soil
133 communities to a stem girdling treatment, in order to test their dependence on the delivery of
134 recently-fixed autotrophic C. We hypothesised that H1) willow and birch would support distinct soil
135 communities, reflecting differences in host and soil conditions. Specifically, we predict greater
136 abundance of ECM fungi and microarthropods in birch plots, reflecting greater overall mycelium
137 production and drier conditions. In a preceding study on the same experiment (Parker *et al.*, 2020),
138 we observed a large reduction in soil CO_2 efflux in both treeline mountain birch forest and willow
139 shrub stands in response to girdling, therefore we here further hypothesised that H2) disruption of
140 the supply of recently fixed photosynthate to the rhizosphere would cause a shift in soil fungal
141 communities with saprotrophs replacing ECM fungi and a reduction in microarthropods in deeper
142 organic soil layers, where ECM fungi are dominant. Based on previous findings of a large decrease in
143 hyphal production in birch plots, but not in willow plots (Parker *et al.*, 2020), we further

144 hypothesised H3a) that girdling would result in a larger decline in ECM species H3b) particularly of
145 medium and long-distance exploration types in birch forest compared with willow shrubs.

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Materials and Methods

Site selection and experimental design

A girdling experiment, outlined in detail by Parker *et al.* (2020), was carried out in a permafrost-free forest-tundra ecotone 4-5 km south of the Abisko Scientific Research Station, Sweden (68°18 N 18°49 E, ~600 m asl). Briefly, six pairs of plots in mountain birch forest (*Betula pubescens* with a dense ericaceous understorey of primarily *Empetrum nigrum* and *Vaccinium* spp.) and five pairs of willow thickets (*Salix lapponum*) were located across a 0.88 km^2 area. Average soil and canopy characteristics did not vary significantly between control and girdled plots prior to girdling (Parker *et al.*, 2020), and one of each pair was girdled in June 2017. During girdling, the bark and phloem were removed around the circumference of all birch or willow stems, resulting in a disruption of the transport of photosynthate from canopy to roots. Birch plots had a circular area with a radius of 10 m and willow plots had a radius of 2 m (with a trenched perimeter to prevent root ingrowth from adjacent plants). Re-sprouting shoots from below the girdle-line were removed whenever observed during the experiment. Birch and willow plants retained leaves until natural senescence in 2017, and all birch trees produced leaves above the girdle-line in spring 2018. However, leaf production in girdled birch canopies mostly failed in 2019 and in girdled willow shrub canopies in both 2018 and 2019.

Soil sampling and DNA amplification for analyses of fungal communities

The organic soil horizon (O horizon) was sampled on 3rd August 2017, 1st August 2018 and 5th August 2019. For each plot (22 in total), nine 3.8 cm diameter soil cores were collected; in the birch plots, cores were taken in a grid across a 3 x 3 m central area (corresponding to the central area in which soil CO_2 efflux, root and mycelium production measurements were taken; (Parker *et al.*, 2020). In the willow plots, the nine cores were distributed evenly across the plot area within the trenched perimeter. The uppermost litter layer, as well as the mineral horizons underlying the organic horizon (clearly identifiable at these sites), were removed from cores immediately after coring. The nine cores from each plot were homogenised and pooled within 6 hours of sampling, coarse roots (> 2 mm diameter) were removed and samples were frozen at -20°C until further analysis. Some birch forest samples from 2019 were lost, resulting in fewer replicates for that year, and 59 samples (out of 66 plots) went forward for analysis. A further homogenisation of the pooled soil samples was carried out in the lab using a custom-built large grinder that breaks up soil cores within one second, using rotating blades, while maintaining them in a frozen state.

Soil sub-samples (approximately 10 g) were freeze-dried and ball milled to a fine powder. DNA was extracted from a 50 mg sub-sample using the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany). ITS2 markers were amplified using the fungal gITS7 forward (Ihrmark *et al.*, 2012) and reverse primer mix of 3/4 of ITS4 (for general eukaryotes (White *et al.*, 1990)) and 1/4 of ITS4arc (adapted for Archaeorhizomycetes (Sterkenburg et al. 2018)) with minimal cycle numbers (51 samples were amplified with 23 cycles, 5 samples at 21 cycles and 3 at 25 cycles) in order to minimise biases in the community data (Castaño *et al.*, 2020). PCR reactions were run in duplicates per sample, with 50 µl in each reaction containing the following reactants: Approximately 25 ng of DNA template, 0.2 mM dNTPs, 0.75 mM $MgCl_2$, 1.25 units of DreamTaq polymerase in its buffer (ThermoFisher, Waltham, MA, USA) and primer concentrations of 0.5 µM of gITS7, 0.3 µM of ITS4 and 0.1 µM of ITS4arc. The PCR cycling conditions were: 95 °C for 5 minutes, then 21-30 cycles (depending on the sample) of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, and 7 min at 72 °C. The duplicate PCR products from each sample were pooled and purified using AMPure magnetic beads (Beckman Coulter, Carlsbad, CA, USA), concentrations measured with Qubit (Invitrogen, Carlsbad, CA, USA) and equal DNA amounts mixed into a single pool. The pool was further cleaned using the E.Z.N.A CyclePure kit (Omega, Nocross, GA, USA) and sequenced with the Pacific Biosciences SMRT Sequel technology after adaptor ligation (Castaño *et al.*, 2020) by SciLifeLab NGI (Uppsala, Sweden).

Bioinformatic processing and fungal taxonomic identification

We used a community metabarcoding approach on the pooled DNA samples to identify distinct taxonomic groups at species level, which we now term 'species hypotheses' (SHs). Raw sequences were quality filtered and clustered into sequence clusters at an approximate species level, using the SCATA pipeline (scata.mykopat.slu.se; (Ihrmark *et al.*, 2012)). Sequences were first quality filtered (requiring a mean quality score of 20 or higher) and screened for primer sequences (90% similarity required) and identification tags, which were removed. Sequences were then pairwise compared using USEARCH (Edgar, 2013) and clustered into SHs using a single linkage algorithm with a 98.5% similarity cut-off (Lindahl *et al.*, 2013). Plant sequences were removed. Sequencing produced 181560 reads with 100282 passing quality control. Only SHs that contributed >1% of the fungal sequences in at least one sample were retained. In the final analysis, 38659 reads were analysed in a matrix of 521 SHs in 59 samples. Three reference datasets (Swedish Soil Inventory: (Lindahl *et al.*, 2021)), Swedish Boreal forest: (Clemmensen *et al.*, 2015) and nearby Abisko database: (Clemmensen *et al.*, 2021)) were included in the clustering process in order to aid taxonomic classifications. These were verified against the UNITE database using 98 % similarity for

species-level identification. SHs were assigned to functional guilds (ECM fungi, moulds, litter saprotrophs and root-associated ascomycetes) based on the FungalTraits database (Põlme *et al.*, 2020). The root-associated ascomycetesincluded ericoid mycorrhizal fungi and root endophytes but was deliberately left as an unspecified group due to the often broad or undefined ecology of many species (Kohout, 2017). Ectomycorrhizal SHs were assigned to one of five well-defined exploration types (ET) (Agerer, 2001) based on established datasets (Tedersoo & Smith, 2013). Fungal community composition and ITS copy numbers (see next section) data were stored at the NERC EIDC (Parker *et al.*, 2022a).

ITS region quantitative PCR

Copy numbers of the fungal ITS2 region were estimated by quantitative PCR (qPCR) using the IQ SYBR green supermix on an iQ5 real time PCR system (Bio-Rad, Hercules, California). The 20 µl reactions contained approximately 5 ng of DNA template, 0.1 % bovine serum albumin and the ITS4/ITS4arc and gITS7 primers (as above). The thermal cycling conditions were 95 °C for 5 minutes, then 40 cycles of 95 °C for 15 s, 56 °C for 30 s, 72 °C for 40 s and 78 °C for 5 s, with the fluorescent signal acquired at the last step of each cycle. PCR inhibition tests where known plasmid copy numbers were amplified with M13 primers (pGEM-T plasmid, Promega, WI, USA) showed no significant inhibition by the sample extracts. Standard curves for quantification were obtained by serial dilutions of linearized plasmids containing the ITS2 marker. The relative abundance of each SH and functional guild (which is well preserved during PCR cycles and PacBio sequencing (Castaño *et al.*, 2020)) was multiplied by total fungal ITS copy numbers (after correcting for amplification of non-fungal markers based on sequencing results) to estimate copy numbers for each SH and guild in each sample.

Soil mesofauna sampling and extraction

Soils were sampled across all plots for microarthropods (Collembola and mites) on July 29th 2019. Within each plot, three 4.5 cm diameter soil cores were taken to the depth of the soil horizon (until rock or last clasts) within the central 3 x 3 m area of the birch plots, and across the willow plots. The mineral horizon of each soil core was removed and discarded, while the litter horizon was retained and pooled separately for each plot. Cores were inserted into tight fitting plastic rings to maintain soil structure for optimal extraction efficiency. The soil and litter samples were extracted for soil fauna using a Tullgren funnel fauna extractor (Van Straalen & Rijninks, 1982). 22 pooled soil

and 22 pooled litter samples were placed at random under individual heat lamps (in order to avoid spatial biases in extraction efficiency) and left for 10 days to extract fauna into ethanol. At the end of the extraction the soil and litter samples were oven dried for 24 hours at 70 °C and weighed to determine soil dry weights. The fauna samples were preserved in 70 % ethanol prior to identification and quantification. Extracted fauna were counted and identified under a dissecting microscope to species level for Collembola using the key of Hopkin (2007) and to order level for Acari (Oribatid, Mesostigmatid, Prostigmatid). Other invertebrates were separated at higher taxonomic levels (e.g. Diptera, Coleoptera, Araneae etc.). Soil mesofauna data were stored at the NERC EIDC (Parker *et al.*, 2022b).

To facilitate trait analyses needed for testing H2, Collembola species were assigned trait values from 1-6 for soil vertical life form and moisture preference according to Kuznetsova (2003) and maximum body length according to Fjellberg (2007). Community-weighted mean (CWM) values were calculated for each trait according to

$$\mathrm{CWM}_j = \sum_{k=1}^{n_j} \mathrm{A}_{kj} \times \mathrm{FT}_{kj}$$

Where in the community in sample *j,* n_j is the number of species samples, A_{kj} is the relative abundance of species *k* and FT_{kj} is the functional trait of species *k* (Krab *et al.*, 2013b). CWMs were calculated for organic soil and litter horizons separately.

Statistical analyses

Total fungal ITS copy numbers were compared between birch and willow plots using a linear mixed effects model (the lme function from the nlme package in R (Pinheiro *et al.*, 2017)) with vegetation type, sample year and girdling treatment as fixed effects, and plot nested within plot pair as a random intercept term. Linear mixed effects models were used to test the effect of vegetation type, girdling treatment and soil horizon depth on mite and Collembola abundances and CWM vertical preference, moisture preference and body length of collembola. Plot nested within plot pair were used as random intercept terms for soil fauna models. Models were simplified by removing non-significant three-way interaction terms in order to increase statistical power to test fixed factors. The effect of each factor in the final model was assessed relative to the null model (intercept only) by likelihood ratio tests (Crawley, 2007).

Differences and trends in fauna and fungal community composition were visualised by non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix using the metaMDS function of the 'Vegan' package in R (Oksanen, 2013). The effect of vegetation type, girdling treatment and sampling year, and their interactions, on fungal community composition (functional guild or SH) was assessed by multivariate analysis of copy numbers assigned to each fungal group. The *manyglm* function of the "MVABUND" package in R V 4.0.0 (Wang *et al.*, 2012) was used to test the effect of the above factors on the multivariate copy number data of fungal SHs, guilds, ectomycorrhizal genera, and ectomycorrhizal exploration types using a Poisson distribution (which was found to fit the mean-variance assumption of the analysis). The role of different factors was compared using the ANOVA function, which applied Wald statistics following 999 PIT-trap resamplings (Warton *et al.*, 2017). Models were simplified by removing interaction terms if they were not significant ($P < 0.05$). Furthermore, to assess effects on individual functional groups within each test, additional univariate tests were carried out within the *manyglm* function.

287 *Results*

288 Soil in birch forest had a greater fungal abundance (ITS copies mg OM^{-1}) than willow plots
289 ($F_{1,9}$ = 5.7, *P* = 0.043), with no significant variation between years ($F_{1,9}$ = 0.9, *P* = 0.33). The differences
290 in fungal abundance between birch and willow plots were primarily accounted for by a greater
291 abundance of root-associated ascomycetes in the birch plots, particularly ericoid mycorrhizal genera
292 such as *Hyaloscypha*. Willow plots contained slightly more ECM fungi (Table S1, $Wald_{1,51}$ = 2.4, *P* =
293 0.02), but a similar abundance of litter saprotrophs and moulds. The differences in abundance of
294 root associated ascomycetes and ECM fungi between birch and willow plots were linked to a
295 statistically significant difference in fungal community composition at the functional guild level
296 (Table S1, $Wald_{1,51}$ = 8.3, *P* < 0.001). The fungal community of birch plots contained 60 % root
297 associated ascomycetes (Fig. 1) with litter saprotrophs as the second most abundant guild (15%),
298 followed by ECM fungi (9.5 %) and moulds (4.5 %). Willow plots had a more even abundance of the
299 four guilds (Fig. 1), with root-associated ascomycetes contributing, on average, 29% of the fungal
300 community, and litter saprotrophs, ECM fungi and moulds contributing 23, 22 and 6.8 %,
301 respectively (Fig. 1).

302 At the level of individual species, community composition of both fungi (Fig. 2a, Table S2,
303 $Wald_{1,51}$ = 48.5, *P* < 0.001) and soil fauna (Fig. 2b, Table S3, $Wald_{1,20}$ = 6.608, *P* < 0.001) contrasted
304 significantly between the drier and mycelium-rich birch soils and the wetter, more nitrogen (N) rich
305 willow soils. Fungal communities in birch forest soils were also less variable than fungal communities
306 in willow soils.

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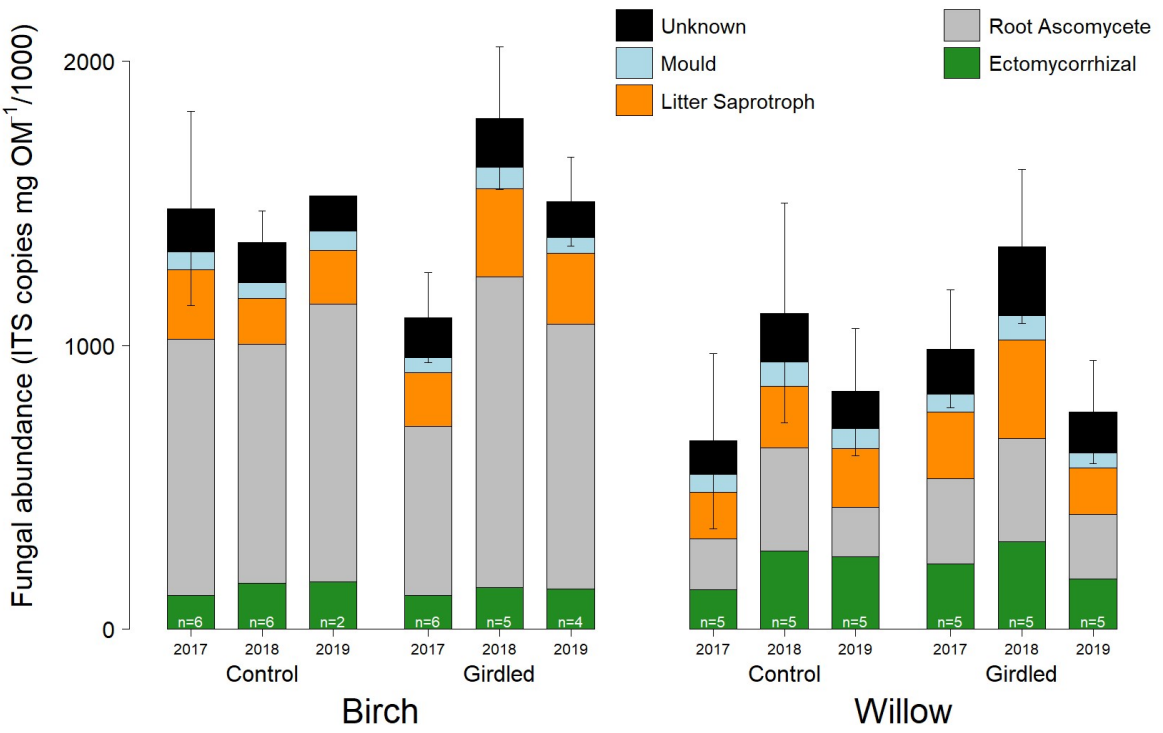


Figure 1: Fungal abundance partitioned into four functional guilds over the years 2017-2019 in soil from girdled and control plots of birch and willow at a subarctic treeline. Error bars represent ± 1 standard error of the mean total copy numbers. Birch control plots in 2019 do not have an error estimate because n = 2; replicate numbers for each bar are indicated at the base of the figure.

Across both birch and willow plots, girdling induced no significant change in total fungal abundance (Fig. 1, $F_{1,39} = 1.7$, $P = 0.21$), abundance of different fungal guilds (Fig.1, Table S1, $Wald_{1,46} = 2.3$, $P = 0.37$) or fungal community composition at the species level (Fig. 2a, Table S2, $Wald_{1,46} = 22.3$, $P = 0.31$). The abundance of litter saprotrophs and moulds did not increase relative to ECM fungi in any of the sampling years up to 26 months after girdling. Further, there was no significant interaction between vegetation type and girdling treatment on total fungal abundance ($F_{1,39} = 1.3$, $P = 0.27$), abundance of different fungal guilds (, $Wald_{1,47} = 1.6$, $P = 0.69$) or fungal community composition ($Wald_{1,47} = 16.0$, $P = 0.48$). Thus, the community composition and guild abundances did not change significantly more in birch plots than willow plots in response to girdling.

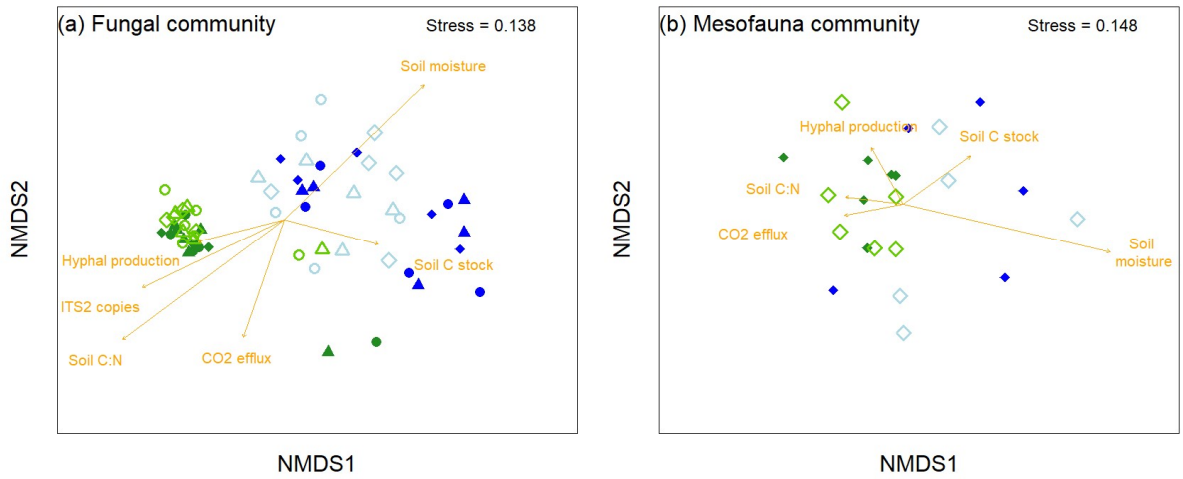
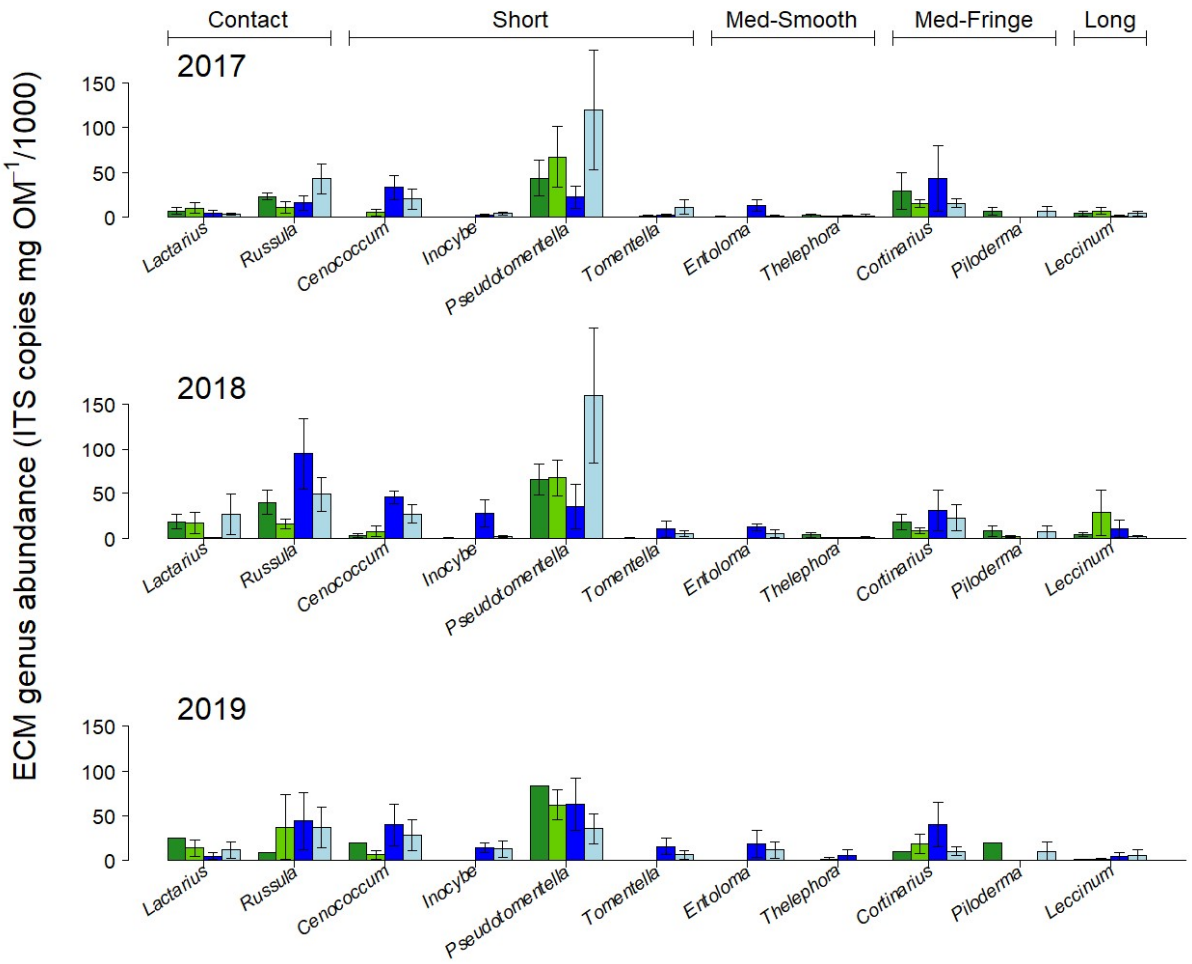


Figure 2: Nonmetric multidimensional scaling (NMDS) ordination of (a) fungal community composition in the 59 organic horizon samples across vegetation type, treatment and year and (b) soil fauna community composition in the organic horizon from 2019 at a subarctic treeline. Environmental vectors (orange) in (a) are based on data from Parker et al. (2020) and show the degree and direction of correlation of measured variables with fungal community composition; vectors in (b) are based on new data collected in 2019 using the same protocols as Parker et al. (2020).

Birch and willow plots had significantly different communities of ECM fungi at the genus level (Fig. 3, Table S4, $Wald_{1,51} = 11.9$, $P = 0.001$), with higher abundance of *Cenocccum, Entoloma, Inocybe, Russula and Tomentella* (Fig. 5, Table S5) as well as more medium distance -smooth exploration types (Fig. 6, Table S6) in the willow plots. Overall, there was a small but statistically significant effect of girdling on the genus composition among ECM fungi (Fig. 3, Table S4, $Wald_{1,50} = 6.2$, $P = 0.034$) with reductions in *Piloderma, Entoloma and Cortinarius* and an increase in *Pseudotomentella* ($P < 0.1$, Fig. 3, Table S4). Despite the reductions in *Piloderma* and *Cortinarius* in response to girdling, medium distance-fringe types showed a less clear reduction whereas medium distance-smooth types responded negatively ($P = 0.02$, Fig.4, Table S5). There were no detectable differences in ECM community composition between years and no significant interaction between girdling and vegetation type (Fig. 3, Fig. 4, Table S4, Table S5, $Wald_{1,47} = 3.7$, $P = 0.42$), meaning that the response of ECM communities to girdling was similar in birch and willow plots.



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349 **Figure 3: Abundance of ectomycorrhizal (ECM) genera in non-girdled (dark shade) and girdled**
350 **(light shade) plots of birch (green) and willow (blue) at a subarctic treeline. Different panels show**
351 **the three sampling years. Genera are grouped according to their exploration types, with short-to-**
352 **long ranging strategies ordered from left to right. Sample sizes for each group per year are**
353 **indicated in Figures 1 and 4. Error bars signify ± 1 SE of the mean; birch control plots in 2019 do**
354 **not have an error estimate because n = 2.**

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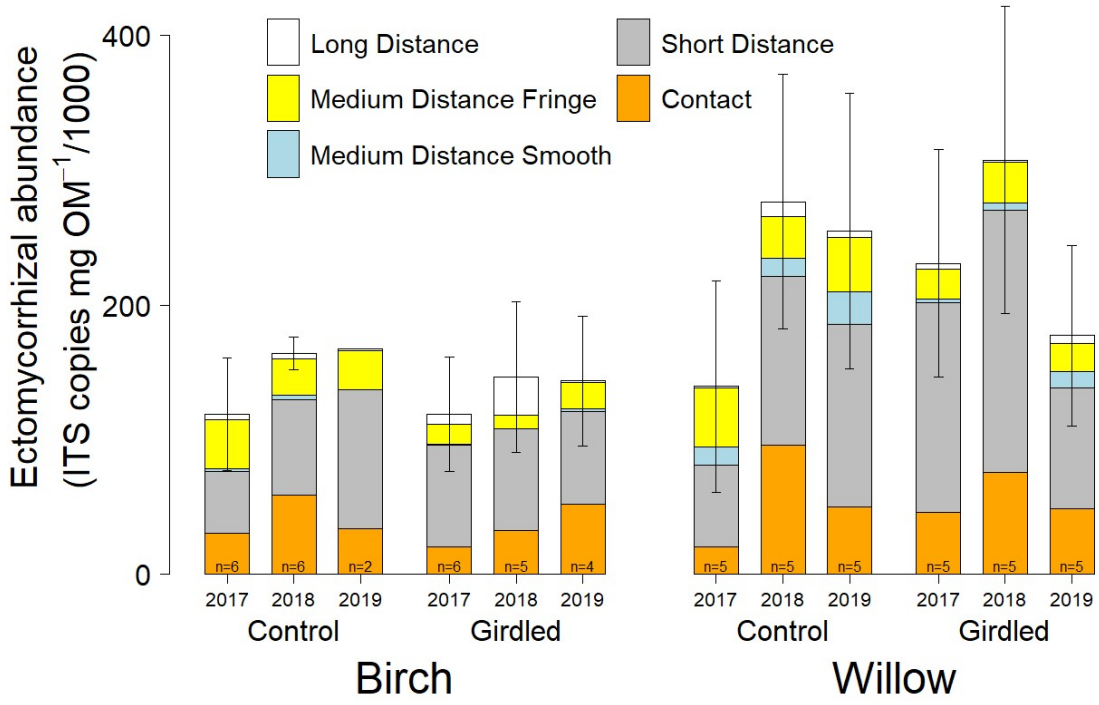
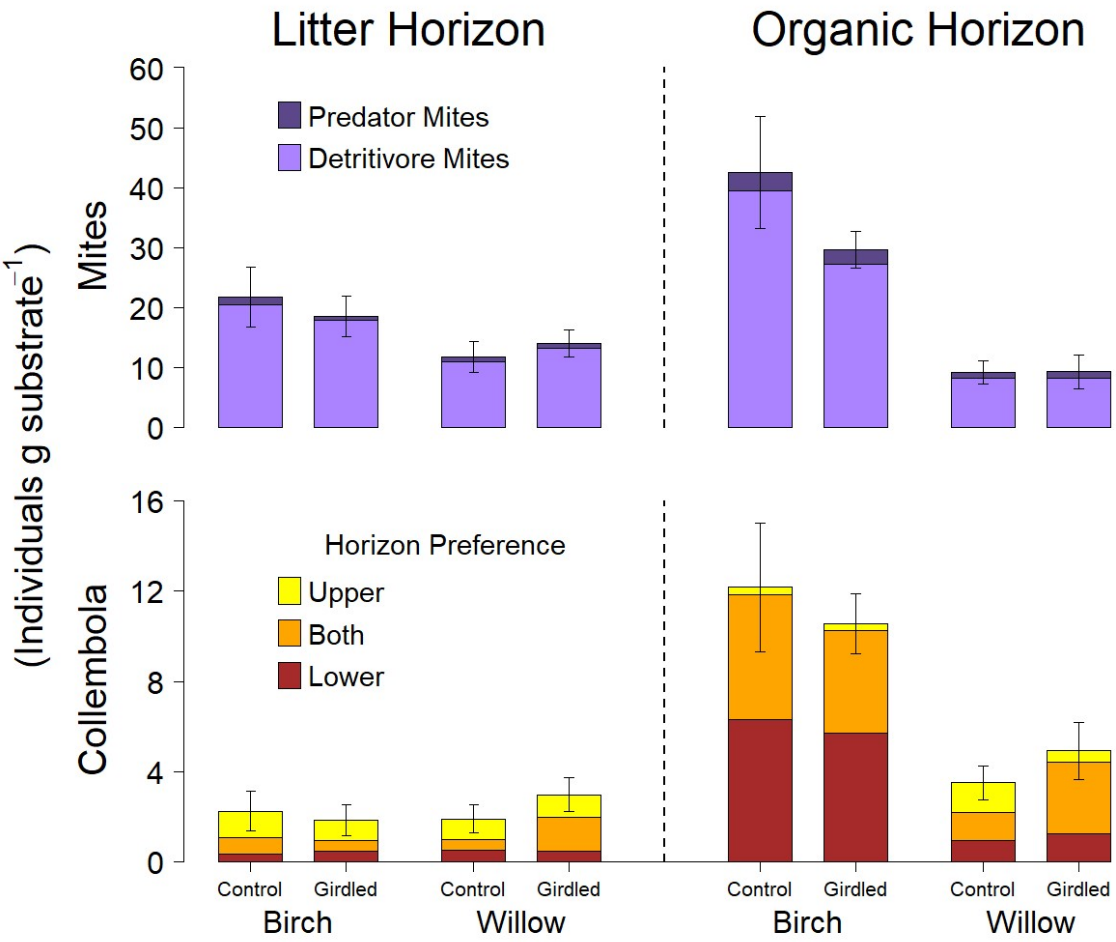


Figure 4: Abundance of ECM fungi partitioned into five exploration types over the years 2017-2019 in girdled and control plots of birch and willow at the subarctic treeline at a subarctic treeline. Error bars represent ± 1 standard error of the mean total copy numbers. Birch control plots in 2019 do not have an error estimate because n = 2.

There was greater abundance of mites and Collembola in birch plots compared with willow plots, which was primarily the result of a disproportionately higher density of these faunal groups in the organic horizon. Collembola in birch plots were more stratified according to vertical preference, with typically deeper dwelling species found in high abundances in the organic horizon and shallow dwelling species mainly found in the litter, whereas communities of the organic and litter horizons in willow were more similar. Collembola communities in birch and willow had similar moisture preference, but, as with vertical preference, there was greater stratification with depth in the birch plots, with drought sensitive species dwelling preferably in the organic horizon and drought tolerant species in the litter horizon. Willow plots typically contained collembola with larger body size in both the litter and organic horizons (Fig. 6, Table S6). There was no statistically significant girdling effect on the abundance of mites or Collembola, or on any collembolan traits (Fig. 5, Fig. 6, Table S6).



374

375 **Figure 5: Soil mite and Collembola abundances in the litter horizon and organic soil horizons of**
376 **control and girdled plots of birch and willow-dominated plant communities at a subarctic treeline.**
377 **Bars represent six samples per treatment in birch plots and five samples in willow plots. Error bars**
378 **represent ± 1 standard error of the mean of total mites or Collembola.**

379

380

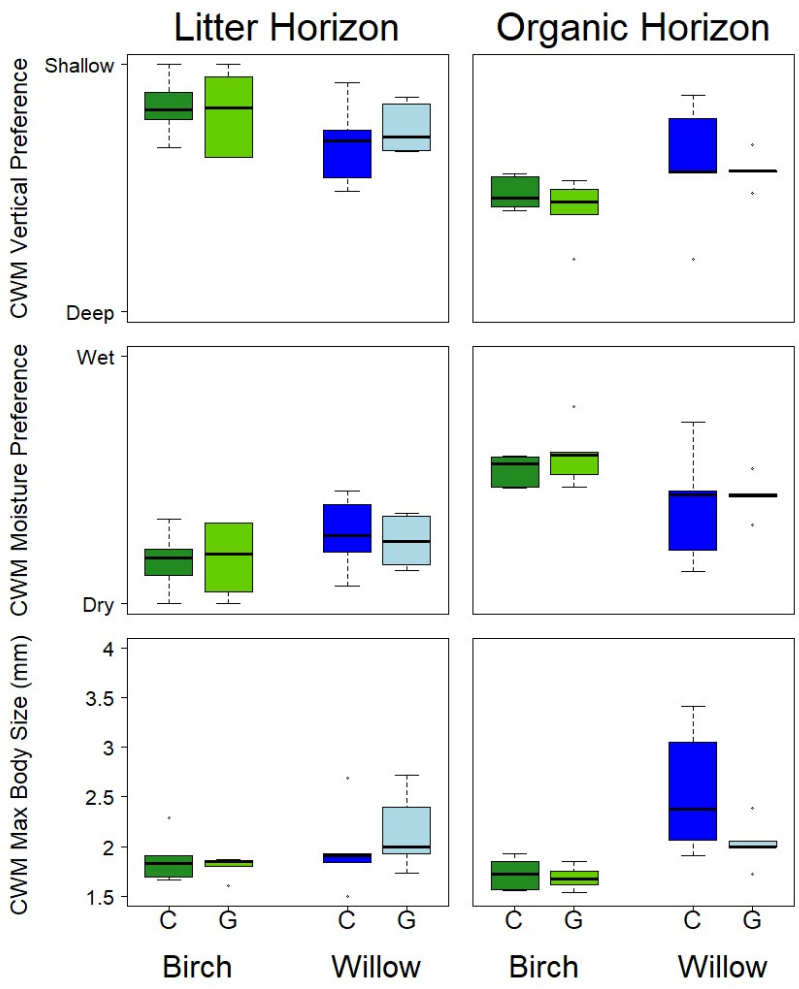


Figure 6: Community weighted mean (CWM) values of three traits (soil vertical preference, moisture preference and maximum body size) for abundant Collembola species in the litter horizon and organic soil horizon of control (C) and girdled (G) birch and willow plots at the subarctic treeline. Boxplots show the median, interquartile range and extreme values of each treatment.

Discussion

The abundance and diversity of soil fungi and fauna in treeline forest and shrubs are resistant to declines in belowground allocation of photosynthates

We found that fungal and faunal soil communities in two distinct treeline and tundra ecosystems were resistant to cessation of below ground transfer of C from the phloem. Girdling completely severed photosynthate transport between canopy and roots, but even after two years, there was no significant restructuring of the soil communities, neither among fungi or collembolan species, nor among fungal or microarthropod functional groups. Informed by a nearby trenching and root ingrowth experiment (Clemmensen *et al.*, 2021), as well as trenching and clearcutting experiments from further south (Kohout *et al.*, 2018; Sterkenburg *et al.*, 2018, 2019), we hypothesised (H2) that there would be a replacement of ECM fungi by saprotrophic fungi and a reduction in soil fauna abundance in the organic soil. However, we observed that the proportion of ECM and free-living saprotrophic fungi in the community was maintained after girdling. Furthermore, faunal communities did not restructure or decline in abundance. Our results are even more surprising given that we previously observed large reductions in response to girdling both in soil CO_2 efflux in the same experiment and in hyphal production in the birch plots (Parker *et al.*, 2020).

Resistance of fungal and faunal community structure to girdling

The apparent stability of the soil communities in treeline and tundra shrub ecosystems after a disruption to supply of C belowground contrasts with results from other experiments, which observed significant community shifts over similar time frames, particularly for fungi. Here we discuss the potential mechanisms and ecological drivers for our observed results in fungal and faunal communities.

With regards to soil fungal community composition, our result contrasts with trenching and clear-cutting experiments, but these differences may provide explanations as to why we observed no change in fungal community composition. In long-lived plants, a proportion of recently fixed C is allocated to storage as non-structural carbohydrates that can be retained for a number of years in parenchyma of various organs, including in the hardwood and coarse roots of trees (Hartmann & Trumbore, 2016). In fact, a large proportion of contemporary root activity in a boreal forest was found to be driven by metabolism of years-old non-structural carbohydrates (Schuur & Trumbore, 2006). From a methodological perspective, we conducted a girdling experiment with phloem

disruption located at the bottom of the stem, potentially leaving a number of metres of roots (Friggens *et al.*, 2019) between the point of girdling and the root tips where mycorrhizal symbiosis is formed and activity may be highest. In trenching experiments, where root-shoot connection is severed less than 50 cm from root tips (typically in a 1 x 1 m plot (Sterkenburg *et al.*, 2018)), the root tips are disconnected, not only from aboveground photosynthesis, but also from a significant amount non-structural carbohydrates stored elsewhere in the plant. A lack of non-structural carbohydrate supply might explain why the fungal community response is rapid in trenching experiments (within a year (Lindahl *et al.*, 2010; Sterkenburg *et al.*, 2018)). It is possible that ECMs were kept alive in the birch plots by roots from outside the plot (less likely in willow plots which where the perimeter was trenched). However this is unlikely given that lateral spread of boreal forest trees was found to be at maximum 5 m (Göttlicher *et al.*, 2008) and the minimum distance between the sampling points and the perimeter of our plots was 8.5 m. We speculate that, even though the large flux of photosynthesis product from the shoot was eliminated by the girdling treatment, and hyphal production declined (Parker *et al.*, 2020), root tips were able to survive and maintain their fungal partners through the mobilisation and allocation of a small supply of C from non-structural carbohydrates.

Clearcutting of temperate spruce forest (Kohout *et al.*, 2018) and subarctic pine forest (Sterkenburg *et al.*, 2019), leads to a substantial shift away from ECM fungi within twelve months. Clearcutting differs from girdling as the total removal of the xylem connection to the canopy results in a complete loss of the tree water and nutrient sink, and light conditions are also altered. In the present study, birch trees and willow shrubs maintained leaves for up to two seasons after girdling (Parker *et al.*, 2020). This could mean that the continued demand for nutrients may have maintained a reliance on, and continued C delivery to, mycorrhizal symbionts, for longer than if connections to the canopy had been completely severed. Of course, the present experimental system contrasts from previous similar experiments in many other ways, not least in the environmental stress that forest and tundra are adapted to, with extreme cold and disturbance being a regular feature of the landscape (Bjerke *et al.*, 2014).

Abundance and community composition of soil fauna were also resistant to stem girdling. Mites and collembola were extracted two years after girdling treatment in birch and willow plots but their community structure remained relatively unchanged, suggesting that they are not sensitive to a change in C resource availability. Hyphal production decreased in the birch plots in this experiment (Parker *et al.*, 2020), but there was no proportional decline in the fauna (although there were some reductions in mite abundance). Fungal hyphae form a significant part of mite and Collembola diets (Renker *et al.*, 2005; Anslan *et al.*, 2018), but in the treeline systems studied here, root associated

454 ascomycetes and saprotrophs were the dominant functional guilds and represent a viable food
455 source (Anslan *et al.*, 2018). In boreal forests, girdling negatively affected specialised fungivores,
456 such as fungivorous nematodes (Kudrin *et al.*, 2021) or proturans (Malmström & Persson, 2011), and
457 specific species like the Oribatid mite *Oppiella nova* (Remén *et al.*, 2008), but had no effects on
458 Collembola communities (Malmström & Persson, 2011) or other Oribatid species (Remén *et al.*,
459 2008). Mites and Collembola are flexible heterotrophs that are able to feed on a variety of food
460 resources, including fungi, algae and detritus (Schneider *et al.*, 2004; Chahartaghi *et al.*, 2005; Krab
461 *et al.*, 2013a; Ferlian *et al.*, 2015; Anslan *et al.*, 2018). Although it has been shown that Collembola
462 can feed on ECM fungi (Schneider *et al.*, 2005; Pollierer *et al.*, 2007), they could probably easily shift
463 to a heavier reliance on saprotrophic fungi if ECM hyphae become rare (Pollierer & Scheu, 2021).
464 Furthermore, girdling could induce an important input of dead roots and fungal hyphae that could
465 compensate for the decrease in fresh ECM-derived carbon. It seems important to further investigate
466 the complex ecological controls over soil fauna communities in the Arctic, as climate and vegetation
467 cover are changing around them.

468 The fungal community, as assessed by our DNA-based approach, was largely resistant to the
469 dramatic disturbance aboveground. An immediate explanation for this observation could be that
470 DNA in dead fungal mycelium was still present in the soil, indeed, across biomes, it is known that
471 extracellular 'relic DNA' makes up, on average, 40 % of extracted and amplified DNA in soils (Carini *et*
472 *al.*, 2016). Our results are likely to be influenced by a larger fraction of relic DNA in girdled plots.
473 However, several experiments, in which the C delivery to the rhizosphere was disrupted, found very
474 different results from our own. Using DNA amplification of Swedish boreal forest soils, Lindahl et al.
475 (2010) found that ECM species decreased in abundance two weeks after root trenching, and
476 Sterkenburg et al. (2018) observed a large reduction in amplifiable DNA from ECM fungi after a year.
477 Low temperatures at our subarctic site may have preserved DNA from dead fungi (Strickler *et al.*,
478 2015) for a longer time compared to these warmer boreal sites, however, clear-cutting at a cold
479 subarctic *Pinus sylvestris* forest (67 °N) resulted in a 70 % reduction in ECM fungal abundance in the
480 O horizon (Sterkenburg *et al.*, 2019). Furthermore, canopy defoliation by caterpillars in subarctic
481 birch forests resulted in large changes in fungal community structure as measured using DNA
482 techniques (Saravesi *et al.*, 2015). Therefore, despite an important, yet unquantified contribution of
483 relic DNA to assessed fungal communities, we do not believe that the apparent resistance of the
484 fungal community to girdling was only an artefact of the method.

485 An ecological factor that may be related to the observed resistance of treeline soil
486 communities to girdling is that both mountain birches and willow shrubs are well adapted to regular,
487 intense canopy disturbance. Mountain birch forests across Northern Fennoscandia endure cyclical

outbreaks of geometrid moth caterpillars, which cause wide-spread and often complete, defoliation of the canopy (Jepsen *et al.*, 2008). However, mountain birch trees often survive these outbreaks by resprouting stems from their base (Karlsson & Weih, 2003; Tenow *et al.*, 2005), again pointing to a significant storage of non-structural carbohydrates. Arctic willows are equally subject to intense disturbance by herbivores (te Beest *et al.*, 2016; Tape *et al.*, 2016; Dahl *et al.*, 2017) and change their morphology in response to herbivory, e.g. by resprouting shoots (Christie *et al.*, 2014). We speculate that there is a belowground facet to birch and willow survival after disturbance: the ability to maintain the mycorrhizosphere until aboveground productivity can rebound to normal levels, which can take a number of years (Karlsson & Weih, 2003; Vindstad *et al.*, 2019). As much as some areas of the tundra are increasing in productivity and undergoing shrub expansion, others are experiencing more 'browning events' in the form of disturbance (Phoenix & Bjerke, 2016; Myers-Smith *et al.*, 2020). Therefore it is important to know how short and long-term cessation in C-fixation influence root-driven processes (Parker *et al.*, 2021). Our data suggest that maintenance of a soil fungal community in the face of disturbance may aid willow survival through intermittent 'browning events' as they continue on their overall 'greening' trend (Myers-Smith *et al.*, 2020).

Ectomycorrhizal community dynamics in response to changes to photosynthate delivery

We hypothesised (H3) that girdling would result in a large compositional change in the ECM fungal community within the treeline birch forest (more so than in the willow plots) with a reduction in supposed C-demanding genera. Although we did observe some systematic shifts within the ECM community after girdling, changes were as large in willow plots as in birch plots. In support of our hypothesis, species of 'medium distance-fringe' genera, like *Piloderma* and *Cortinarius*, declined in abundance across both plant communities in response to girdling, probably because the reduction in photosynthate delivery reduced the competitiveness of these supposedly C-demanding species (Saikkonen *et al.*, 1999) that turn over biomass quickly (Clemmensen *et al.*, 2021) and/or express costly extracellular enzymes at high levels (Bödeker *et al.*, 2014). Saikkonen et al. (1999) argued that, as plant C delivery to ECM root tips is reduced by disturbance in subarctic ecosystems, less 'C-demanding' species hold an advantage. This hypothesis has empirical support from studies on defoliation (Saravesi *et al.*, 2015; Parker *et al.*, 2017) and now also in the context of this girdling experiment. It is now important to understand whether the large-scale changes in productivity occurring in the Arctic and boreal biomes will influence ECM community composition and function. More specifically, will major disruptions in C delivery to ECM fungi alter the rate of mycorrhizal-driven turnover of organic matter (Clemmensen *et al.*, 2021)?

Soil community composition in important tundra vegetation types

Characterising the soil community in willow soils is important, as willows play a key role in Arctic 'greening' and 'shrubification' (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011). We hypothesised that ECM fungi would be less prolific under willow hosts as a result of high soil moisture and relatively high nutrient availability (Nadelhoffer *et al.*, 1991; Chen *et al.*, 2020), as suggested by the observation that overall hyphal production was less in willow plots (Parker *et al.*, 2020). Soil in willow plots was wetter and had lower C:N ratio but the concentration of ECM fungal gene copies in the organic horizon was greater than in the birch plots. Conversely, there were significantly fewer mites and Collembola in willow plots (as we hypothesised), which may be linked to lower overall fungal biomass (Fig. 1) and soil respiration (Parker *et al.*, 2020). Willows in the tundra tend to grow in depressions in the landscape where snow accumulates and water saturation is a regular feature over the annual cycle (Tape *et al.*, 2006; Parker *et al.*, 2020). The regularly high water table may restrict proliferation of soil fauna as the 'habitable' volume of soil is small. This hypothesis is supported by our observation that the Collembola community in willow plots tended to include more species with a preference for surface habitats. The ECM fungal community in willow plots differed from that of birch plots, with more short distance and medium distance-smooth morphotypes. The higher abundance of *Tomentella* and *Cenococcum*, which tend to respond positively to N deposition (van der Linde *et al.*, 2018), accords with a more nutrient-rich environment, whereas *Piloderma,* which typically responds negatively to N deposition (van der Linde *et al.*, 2018), was less abundant. The molecular data presented here show that there is an important ECM community in willow plots but this assemblage may produce either fewer hyphae, or hyphae that are less likely to grow into sandbags (the standard method for measuring hyphal production (Wallander *et al.*, 2013)).

In birch plots, ECM fungal species had a relative abundance of only 9.5 % of the total fungal community, but considering that fast mycelial growth rates have been consistently observed in mountain birch forests (Parker *et al.*, 2015, 2020; Friggens *et al.*, 2019; Clemmensen *et al.*, 2021), it is likely that this small constituent of the fungal community has a disproportionately large effect on turnover of C. Indeed, Clemmensen *et al.* (2021) linked ECM fungi in treeline birch forests to a high potential for SOM degradation and belowground C turnover. As with our study, Clemmensen *et al.* (2021) showed that root-associated ascomycetes are the dominant fungal guild in mountain birch forest; their DNA is present in abundance but they may have slower metabolism and turnover rate than ECM fungi (Clemmensen *et al.*, 2015). A high proportion of a collembolan fungal diet comprises

554 ascomycetes (Anslan *et al.*, 2018), and the large stock of ascomycete biomass could be a primary
555 driver of high soil fauna abundance which, in turn, controls ascomycete biomass turnover. As
556 Clemmensen *et al.* (2021) suggested, the mycelium of mycorrhizal fungi associated with ericaceous
557 plants may contribute to SOM formation, while the less abundant, but more active, ECM fungi may
558 act to reduce SOM stocks. The high numbers of soil fauna in birch forests could therefore be driving
559 the turnover of mycelial biomass that would otherwise be difficult to access (Clemmensen *et al.*,
560 2015). Due to the large-scale vegetation change occurring at Arctic treelines and in the tundra,
561 further characterisation of soil and fungal communities in relation to plant communities from
562 around the Arctic should be a research priority, if we are to understand the C cycle feedbacks
563 associated with this change (Parker *et al.*, 2021).

564

565 *Conclusions*

566 We show that fungal and microarthropod communities of mountain birch forest and willow shrub
567 are distinct, but once established, they are resistant to complete cessation of below ground C supply
568 from the canopy phloem. This finding has implications for how Arctic systems may respond to
569 disturbance events that have similar impacts on below ground C supply, such as canopy disturbance
570 events. Our results contrast similar experiments from more southerly biomes, raising questions
571 about the mechanisms driving the ecology and adaptations of Arctic ecosystems to stress and
572 disturbance. Given the progressive greening trend around the Arctic and the growing importance of
573 intermittent browning events, unpicking the belowground-mediated feedbacks will be key to
574 understanding Arctic biome-wide responses to climate change.

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Conflict of Interest

We declare no conflicts of interest.

Author Contributions

TCP, IK, PAW, IPH, DJ, BDL, KEC, NLF, LES and J-AS designed, implemented and collected plot-level data from the experiment. MC processed and identified fauna extractions. TCP analysed data and wrote the manuscript. All authors significantly contributed to multiple drafts of the paper.

Data Availability Statement

All sequence data is stored at NCBI-SRA at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA846260/. ITS copy numbers and species hypothesis relative abundance data are available at https://doi.org/10.5285/d6c787ec-146c-461b-b8a2-e0251259036c. Flux, hyphae and root data are available at https://doi.org/10.5285/4418c631-c39c-467c-b3b8-c75142fcae0a, soil fauna data are available at https://doi.org/10.5285/d3c98f24-7a4d-40b8-989a-6cc340e91cac.

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