A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest

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B.D.L. initiated and led the project, assembled and analysed the data and wrote the manuscript with input from all co-authors. J.K. and K.V. contributed to the acquisition of fungal community data and initiated the statistical modelling. K.E.C. contributed expertize on fungal ecology, bioinformatics and statistics. A.D. initiated the project and contributed expertize of fungal ecology and taxonomy. E.K. contributed expertize on physical geography and biogeochemistry. J.S. initiated the project, led the sampling campaign, assembled stand and soil data and contributed expertize on physical geography and biogeochemistry.

Data accessibility
All data is included as supplementary files, except sequence data, which are stored at NCBI SRA as Bioproject (data under submission).

Abstract

Boreal forests soils are important global carbon sinks, with significant storage in the organic topsoil. Decomposition of these stocks requires oxidative enzymes, uniquely produced by fungi, of which many live in ectomycorrhizal symbiosis with the trees. Here we show that presence of a group of closely related species of ectomycorrhizal fungi – *Cortinarius acutus* s.l. – decreased local carbon storage in the organic topsoil by 33% across Swedish forests. Our findings challenge the prevailing view that ectomycorrhizal fungi generally act to increase carbon storage in soils and show that certain ectomycorrhizal fungi can complement free-living decomposers, maintaining nutrient cycling and tree productivity under nutrient poor conditions. The finding that a narrow group of fungi exerts a major influence on carbon cycling refutes the prevailing dogma of functional redundancy among microbial decomposers. *Cortinarius acutus* s.l. responds negatively to forestry, and population declines are likely to increase soil carbon sequestration while impeding nutrient cycling.

Introduction

It is well recognized that impacts of biological species and their traits propagate through ecosystems, affecting processes and, ultimately, ecosystem services (Mace *et al.* 2012). Yet, the influence of individual microbial taxa at the ecosystem scale is often questioned, and the potential of microbial community composition to affect ecosystem functioning is one of the major unresolved questions in terrestrial ecology (Schimel 1995; Schimel & Schaeffer 2012; Graham *et al.* 2016). The pivotal role of microorganisms as drivers of ecosystem processes is indisputable, but functional redundancy among microorganisms has been thought to restrict influence of microbial diversity and community composition on overall process rates (Andrén & Balandreau 1999; Nannipieri *et al.* 2003). Holistic approaches based on measurements of pools and fluxes have prevailed (Nannipieri *et al.* 2003), and microbial communities have largely been treated as a "black box" (Schloter *et al.* 2018). However, recent evidence suggests that microbial keystone taxa may exert a major influence on community functions (Banerjee *et al.* 2018). Our capacity to understand variation in ecosystems and predict

responses to disturbance and global change is severely impeded by poor knowledge of the interplay between microbial community composition, traits and functions (Allison & Martiny 2008; Strickland *et al.* 2009; McGuire & Treseder 2010; Philippot *et al.* 2013; Clemmensen *et al.* 2015; Glassman *et al.* 2018).

In mineral soils, storage and stability of organic matter is largely regulated by mineral aggregation rather than by inherent biochemical recalcitrance (Schmidt *et al.* 2011). Boreal forest soils are an important exception, with the purely organic topsoils constituting a relatively unprotected carbon pool that is of global significance (Deluca & Boisvenue 2012). Decomposition of soil organic matter is usually considered a "broad" process, carried out by a diverse assembly of microorganisms with a high degree of functional redundancy (Schimel 1995; Nannipieri *et al.* 2003). However, Schimmel and Schaeffer (2012) hypothesised that "In physically unprotected organic detritus, chemical structures remain complex and … exoenzyme breakdown is necessary for microbes to metabolize them. Thus, their breakdown remains under biological control and sensitive to the specific identities of the decomposers present". As decomposition of more recalcitrant organic pools depends on a subset of more capable microbial decomposers, it may be a more "narrow" process in terms of redundancy (McGuire & Treseder 2010). Organic matter in boreal forest topsoil has its origin primarily in root litter and fungal mycelium (Clemmensen *et al.* 2013; Kyaschenko *et al.* 2019; Adamczyk *et al.* 2019), and there is evidence that pool sizes are regulated largely by the rate of degradation of biochemically demanding compounds, primarily by manganese-dependent peroxidase enzymes (Stendahl *et al.* 2017; Kyaschenko *et al.* 2017a). Manganese peroxidases have evolved uniquely within the fungal class Agaricomycetes (Floudas *et al.* 2012) (the "mushroom forming" fungi). This phylogenetic constraint of potent decomposer capacity to a limited group of microorganisms may reduce functional redundancy.

Microbial communities in boreal forest soils are characterized by a large contribution of ectomycorrhizal fungi, which live in symbiosis with tree roots (Högberg & Högberg 2002; Read &

Perez-Moreno 2003). Interactions between symbiotic ectomycorrhizal fungi and free-living saprotrophs play a central role in the regulation of carbon accumulation in forest ecosystems (Talbot *et al.* 2013; Averill *et al.* 2014; Kyaschenko *et al.* 2017a). As ectomycorrhizal fungi may both hamper and promote accumulation of organic matter below ground (Frey 2019), there is a need to assess the importance and direction of ectomycorrhizal effects across large spatial scales. Most ectomycorrhizal fungi have lost the capacity to decompose during evolution from saprotrophic ancestors (Kohler *et al.* 2015), but the genetic capacity to produce manganese peroxidase is retained in a limited number of ectomycorrhizal taxa, notably members of the genus *Cortinarius* (webcaps) (Bödeker *et al*. 2014). Exclusion of ectomycorrhizal fungi radically reduced manganese peroxidase activity in a Swedish boreal forest (Sterkenburg *et al.* 2018). Furthermore, spatial correlation between manganese peroxidase activity and *Cortinarius* DNA markers was observed in a pine chronosequence (Kyaschenko *et al.* 2017b), and high abundance of *Cortinarius* species coincided with more rapid organic matter turnover in forest on islands in a northern Swedish lake (Clemmensen *et al.* 2015).

These reports led us to hypothesise *that presence of ectomycorrhizal Cortinarius species would correlate with low stocks of organic matter in the topsoil of boreal forests*. This hypothesis challenges two widespread null hypotheses:

1) that microbial community composition has little influence on decomposition (Andrén & Balandreau, 1999; Nannipieri *et al.* 2003), or that such influence is related to abundance of broad functional guilds, rather than more narrow phylogenetic taxa (Schimmel & Schaeffer, 2012).

2) that ectomycorrhizal fungi generally increase organic matter storage by supressing saprotrophic decomposers (Gadgil & Gadgil 1975; Orwin *et al.* 2011; Averill *et al.* 2014; Averill & Hawkes 2016; Fernandez & Kennedy 2016; Kyaschenko *et al.* 2017a).

To test our hypothesis, we used high-throughput sequencing of DNA markers (Nilsson *et al.* 2019) to analyse Agaricomycete communities in the organic topsoil of boreal forest stands, systematically distributed across a wide geographical area, in conjunction with the National Forest Inventory of

Sweden. Due to the large size and longevity of Agaricomycete individuals in boreal forest soils (Dahlberg & Stenlid 1994) we expected that the presence of *Cortinarius* species would be reflected in low organic matter stocks, conditional on other important drivers. Although the study relied on correlative relations, it is important to point out that the data were used to test a clear *a priori* hypothesis, based on previous studies, rather than to draw inductive *post-hoc* conclusions (Prosser, 2020).

Materials and Methods

Sampling

Sampling was carried out in June-September 2014-2016 in connection with the Swedish Forest Soil Inventory (Fridman *et al.* 2014). The soil inventory collects data from locations that are distributed systematically in a grid across Sweden. We sampled the organic topsoil in 3 m^2 plots, geographically distributed across Swedish coniferous forest land. Our sampling campaign covered a 240 000 km^2 area spanning the entire latitudinal range of the boreal forest biome (Fig. S1). Two data sets were analysed: “All forests” encompassed 359 stands distributed in the boreal part of Sweden. Only forest stands with more than 70% of the tree basal area consisting of conifers and an organic topsoil (O horizon) that was distinct from the mineral soil beneath were included. Stands with *Sphagnum* peat were excluded. “Old forests” was distinguished as a subset of “All forests” that included 173 stands older than 60 years, to avoid disturbance by clear-cutting forestry. The study area extended from 60° to 68° N and from 12° to 24° E with the stands more or less evenly distributed across the area, albeit with a somewhat higher density in the southern parts.

At each stand, basal area, species composition and average age (weighted by basal area) of trees were assessed within a 10 m radius circle. For estimation of organic stocks, samples of the organic topsoil were collected with a 10 cm diameter corer in 1-9 locations along a 60 cm radius circle within the larger circular area. After removal of freshly fallen, structurally intact plant litter, cores were collected, spanning the entire organic topsoil down to the mineral soil transition (or to rock at stands

without mineral soil), until at least 1.5 l of organic matter was collected. The mass of the topsoil was calculated on an area basis by drying, sieving (2 mm mesh) and weighing the samples, and dividing the dry weight by the total area of the collected cores. Carbon and nitrogen concentrations were determined using an elemental analyser (TruMac CN; LECO, St. Joseph, MI, USA), and topsoil carbon stocks were determined by multiplying carbon concentrations with the topsoil mass. The nitrogen concentration of organic matter were expressed relative to the carbon content (i.e. as the N:C ratio). The pH in the topsoil was determined in a slurry consisting of 2 g soil and 25 ml deionised water, using an 855 Robotic Titrosampler with an Aquatrode Plus combined pH electrode (Metrohm, Herisau, Switzerland).

Separate samples of the organic topsoil were collected for analysis of the fungal community. For this purpose, material from the uppermost 10 cm was collected and pooled from five locations within a 1m radius circle (3 m^2) concentric with the 60 cm radius circle. Samples were, on average, frozen within 8 days after sampling, freeze-dried, and finely ground in a ball mill.

Sequencing of fungal ITS markers

DNA was extracted from 50-400 mg of organic soil, using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany), and the ITS2 region was amplified using the fungal-specific primers gITS7 and a 3:1 mix of the reverse primers ITS4 and ITS4arch, with both forward and reverse primers fitted with unique 8 bp sample identification tags (Ihrmark *et al.* 2012; Clemmensen *et al.* 2016). DNA extracts were diluted to 0.25 ng/µl in a 50-µl PCR reaction volume and amplified using the following cycling program: 5 min at 95°C; 20-35 cycles of 30 s at 95°C, 30 s at 56°C and 30 s at 72°C; 7 min at 72°C. The number of PCR cycles was optimized for each sample by re-running samples with too strong or too weak PCR products (according to gel electrophoresis) with cycle numbers adopted to obtain “weak but visible” bands on the gel (Castaño *et al.* 2020). PCR products were run in duplicates, which were pooled and cleaned using the AMPure kit (Beckman Coulter Inc., Brea, CA, USA). DNA concentrations were established using a Qubit fluorometer (Life Technologies, Carlsbad, CA, USA), and DNA from

each sample was pooled and again purified using the E.Z.N.A. Omega cycle pure kit (Omega Bio-tek, Norcross, GA, USA). Amplicon size distribution was pre-checked using the 2100 Bioanalyzer system (Agilent, Santa Clara, CA, USA) and composite samples were sequenced on the RSII or SEQUEL platforms (Pacific Biosciences, Menlo Park, CA, USA) by SciLifeLab NGI (Uppsala, Sweden) after addition of sequencing adaptors by ligation. The PacBio platform was chosen to minimize bias due to size variation in the amplicon pool, which is considerable for the fungal ITS2 region (Castaño *et al.* 2020).

Bioinformatics

Sequences were filtered and clustered using the SCATA pipeline (scata.mykopat.slu.se) (Ihrmark *et al.* 2012). Sequences were quality checked to remove sequences shorter than 100 bp, with mean quality scores lower than 20, with individual bases with a quality score of <3, or with a missing 3' or 5' tag. Sequences were screened for the gITS7 and ITS4 primers, requiring a minimum match of 90%, and reverse complemented if necessary. Global singletons (sequences that were unique in the entire data set) were removed to reduce the incidence of sequencing errors. Quality filtering removed 53% of the total sequences, and another 16% were removed as singletons. Remaining sequences were clustered into species hypotheses (SHs) (Kõljalg *et al.* 2013) through pairwise comparison with USEARCH (Edgar 2010) followed by single linkage clustering, with the minimum similarity to the closest neighbour required to enter a cluster set at 99%.

The most abundant sequence from each SH was selected as a representative and identified by BLAST comparisons with the UNITE) (Kõljalg *et al.* 2013) and NCBI databases. After removal of non-fungal sequences (15%), all SHs that accounted for more than 1% of the sequences in any sample were manually evaluated for taxonomic identity and capacity to form ectomycorrhizal symbiosis. The relative abundance of ectomycorrhizal fungi was calculated for each sample as the added number of sequences in SHs assigned to ectomycorrhizal taxa divided with the total number of sequences in all identified SHs. The relative abundance of saprotrophic agaricomycetes was calculated in a similar

way, based on all agaricomycetes SHs that were not assigned as ectomycorrhizal or belonged to the order Sebacinales (the group may also contain some parasitic taxa).

Clustering was repeated with 94-98% clustering thresholds, grouping sequencing into broader phylogenetic sequence clusters. Representative sequences from the 99% SHs were included in the data, to trace the finer SHs among the broader sequence clusters. For each sequence clustering level a “core agaricomycete community” was derived, containing all agaricomycete sequence clusters that were present at more than 10% of the “Old forests” stands (>17 stands), with a relative abundance higher than 1% of the total amplicon pool required for a sequence cluster to be recorded as present.

Statistics

The annual temperature sum, calculated as a function of latitude and altitude (Odin *et al.* 1983), was used as a climate index. Tree basal area was used to represent tree biomass. Tree species was expressed as % *Picea abies* of the total basal area of conifers (*Picea abies* and *Pinus sylvestris*). The topsoil carbon pool, pH and relative abundance of saprotrophic agaricomycetes were log-transformed. The nitrogen concentration of organic matter (N:C) and relative abundance of ectomycorrhizal fungi were square root-transformed.

The smaller set of 173 old forest stands was used to evaluate potential direct and indirect predictors of carbon stocks in the organic topsoil. The overall correlation between the composition of the core agaricomycete community, at different clustering thresholds, and the topsoil carbon pool was estimated by fitting logistic regression models to the occurrence (presence/absence) of agaricomycete sequence clusters with the carbon pool as explaining variable. Community-aggregated statistics were derived using mvabund (Wang *et al.* 2012), and the strongest correlation with the carbon pool was found with the community clustered at 96% single linkage similarity (Fig. S2). Individual sequence clusters (96% sequence similarity) of the core agaricomycete community were then evaluated as predictors of the carbon pool by including them one by one as binary variables (presence/absence with a relative abundance >1% required to be recorded as present) in a linear

model with soil nitrogen, soil pH, temperature, tree biomass, tree species, ectomycorrhizal fungi and saprotrophs as covariates. A sequence cluster containing the ectomycorrhizal species *Cortinarius acutus* and closely related taxa (*C. acutus* s.l.) was singled out as the only significant (P=0.0015) predictor of the carbon pool (Table S1), both with and without Bonferroni correction for multiple tests (α = 0.0045).

Raw Pearson correlations between parameters are presented in Fig. S3.

A piecewise structural equation model was fitted using the R-package piecewiseSEM (Lefcheck 2016) according to the relationships presented below. Presence of *C. acutus* s.l. was modelled with a binomial linking function (*i.e.* logistic regression) after backward selection of predictors based on AIC in the R-package MASS.

$$\sqrt{N\ conc.} = \alpha_1 + \beta_1 Temperature + \beta_2 \ln(pH) + \beta_3 Spruce + \beta_4 Tree\ biomass + \varepsilon_1$$

$$\sqrt{ECM} = \alpha_2 + \beta_5 \sqrt{N\ conc.} + \beta_6 Temperature + \beta_7 \ln(pH) + \beta_8 Spruce + \beta_9 Tree\ biomass + \varepsilon_2$$

$$\ln(SAP) = \alpha_3 + \beta_{10} \sqrt{N\ conc.} + \beta_{11} Temperature + \beta_{12} \ln(pH) + \beta_{13} Spruce + \beta_{14} Tree\ biomass + \varepsilon_3$$

$$Prob.(C.\ acutus) = \alpha_4 + \beta_{15} \sqrt{N\ conc.} + \beta_{16} Tree\ biomass + \beta_{17} \sqrt{ECM} + \varepsilon_4$$

$$\ln(C\ pool) = \alpha_5 + \beta_{18} \sqrt{N\ conc.} + \beta_{19} Temperature + \beta_{20} \ln(pH) + \beta_{21} Spruce + \beta_{22} Tree\ biomass + \beta_{23} \ln(SAP) + \beta_{24} \sqrt{$$

where α_{1-5} denote model intercepts, β_{1-25} denote correlation coefficients and ε_{1-5} denote error terms. “Soil N” denotes the nitrogen concentration of organic matter (N:C), “Temperature” denotes the annual temperature sum (Odin *et al.* 1983), “Spruce” denotes the share of the coniferous basal area that was *Picea abies* and “Tree biomass” denotes basal area of conifers. “SAP” and “ECM” denote the relative sequence abundances of saprotrophic agaricomycetes and ectomycorrhizal fungi, respectively, among all fungal DNA markers. “*C. acutus*” refers to presence of the ectomycorrhizal fungus *C. acutus* s.l., with relative sequence abundance >1% required to be recorded as present. “C pool” refers to the amounts of carbon in the organic top soil expressed on an area basis.

The larger data set, which included both younger stands that were planted after clear-cutting, as well as stands with long continuity, was used to evaluate responses of *C. acutus* s.l. to forestry, using piecewise structural equation modelling (Lefcheck 2016) according to relationships presented below. Presence of *C. acutus* s.l. was modelled with a binomial linking function. Stand age was initially included both non-transformed and square root-transformed, to test for second order relationships, and the least correlated variable was removed unless both were significant.

$$\sqrt{N\ conc.} = \alpha_6 + \beta_{26}\sqrt{Stand\ age} + \varepsilon_6$$

$$\sqrt{ECM} = \alpha_7 + \beta_{27}\sqrt{N\ conc.} + \beta_{28}\sqrt{Stand\ age} + \beta_{29} Stand\ age + \varepsilon_7$$

$$Prob.(C.acutus) = \alpha_8 + \beta_{30}\sqrt{N\ conc.} + \beta_{31}\sqrt{ECM} + \beta_{32}\sqrt{Stand\ age} + \varepsilon_8$$

where α_{6-8} denote model intercepts, β_{26-32} denote correlation coefficients and ε_{6-8} denote error terms. “Stand age” refers to the average age of trees weighted by basal area.

Results

Sequencing yielded on average 1247 (range: 217-7917) high quality PacBio reads per sample. Agaricomycetes accounted for 27% of the fungal sequences among the 173 stands that were older than 60 years (i.e. never clear-cut and planted). The remaining fungal community consisted of ascomycetes and zygomycetes, which lack manganese peroxidases and are generally weak decomposers. The ectomycorrhizal community was dominated by the genera *Cortinarius*, *Piloderma* and *Russula*, and *Mycena* was the most common genus among saprotrophic agaricomycetes (Fig. S4). The core agaricomycetes community that accounted for >1% of the sequences on more than 10% of the stands (with a 96% similarity single-linkage clustering threshold) encompassed 11 phylogenetic groups (Table S1).

The power of individual taxa of the core agaricomycete community to explain carbon stocks was tested by including them one by one as binary variables (presence/absence) in a statistical model. We found that presence of a single group of closely related ectomycorrhizal species - *Cortinarius*

acutus s.l. - correlated negatively (P = 0.0015) with the amount of organic matter in the topsoil, conditional on climate, soil pH and nitrogen concentration, tree species and biomass, saprotrophs and general abundance of ectomycorrizal fungi (Table S1). Presence of *C. acutus* s.l. decreased carbon stocks in the organic layer by 33% (Fig. 1), and inclusion of this fungal taxon clearly improved the capacity of a statistical model to predict carbon stocks, decreasing AIC from -201.5 to -210.2. The standardised effect size of *C. acutus* s.l. on carbon stocks was in the same range as effects of temperature, soil nitrogen and saprotroph abundance (Fig. 2, Table S2). None of the other taxa of the core agaricomycete community correlated significantly with carbon stocks (P > 0.1). Relaxation of phylogenetic resolution, using a lower sequence similarity threshold to cluster a wider range of *Cortinarius* species, led to loss of statistical power, as did separation of the *C. acutus* s.l. complex into several taxa by application of a higher sequence similarity threshold (Fig. S2).

Organic matter stocks also decreased significantly with increasing relative abundance of saprotrophic agaricomycetes in the fungal communities (Fig. 2, Table S2) but did not correlate significantly with the over-all relative abundance of ectomycorrhizal fungi. Organic matter stocks correlated positively with warmer temperatures and a higher contribution of spruce among tree species, but negatively with the nitrogen concentration in organic matter. Soil pH and nitrogen, spruce abundance and tree biomass were all positively related with each other, together indicating high ecosystem fertility (Fig. S3). The over-all contribution of agaricomycetes to the total fungal community tied in with these fertility indicators, as saprotroph abundance correlated positively with pH and soil nitrogen, whereas ectomycorrhizal fungi correlated positively with tree biomass. In contrast, *C. acutus* s.l. occurred most frequently in forests with low nitrogen concentration in the soil (Fig. 3). DNA of *C. acutus* s.l. was encountered in 13% of the stands, evenly distributed across the study area, indicating that mycelium of this species occurs ubiquitously in Swedish boreal forests.

When the sample set was expanded to 359 stands by including young forests planted after clear-cutting, *C. acutus* s.l. was not detected in stands younger than 29 years, and its incidence increased

progressively with stand age (Fig. 4). This correlation remained significant when the over-all abundance of ectomycorrhizal taxa in the fungal community was included as a co-variate (Fig. 5, Table S3). Soil nitrogen concentration correlated negatively with both stand age and *C. acutus* s.l., and a major part of the positive influence of stand age on *C. acutus* s.l. was estimated as indirect via decreasing nitrogen levels in older stands. However, the direct correlation between stand age and presence of *C. acutus* s.l. remained marginally significant.

Discussion

Our finding that a single fungal species-complex plays a key role in the regulation of organic matter accumulation at the ecosystem scale is a clear demonstration of a low degree of microbial redundancy, even for the supposedly "broad" process of decomposition. Our results disprove previous claims of a high degree of functional redundancy among communities of forest soil fungi in biogeochemical processes (Talbot *et al.* 2014) and challenge the idea that relevant functional guilds of microorganisms would only be defined at high phylogenetic levels (Schimel & Schaeffer 2012). Nutrient cycling and ecosystem production in boreal forests depend on turnover of the organic topsoil (Kyaschenko *et al.* 2019), and *C. acutus* s.l. could well be considered a keystone species (Banerjee *et al.* 2018) in this context. Our study demonstrates that ecosystem functionality may depend on a non-redundant interplay between fungal traits and community composition across large spatial scales, supporting previous theoretical proposals (Koide *et al.* 2014; Crowther *et al.* 2014). The results confirmed our *a priori* hypothesis that presence of certain *Cortinarius* species would restrict organic matter accumulation, which was based on previous literature (Bödeker *et al.* 2014; Clemmensen *et al.* 2015; Kyaschenko *et al.* 2017b; Sterkenburg *et al.* 2018). The opposite causality, i.e. that thin organic topsoils would selectively favour establishment of certain *Cortinarius* species, finds no support in literature.

We also found that organic matter stocks decreased with increasing relative abundance of saprotrophic agaricomycetes in the fungal communities, which in turn correlated positively with pH

and soil nitrogen, in agreement with previous observations (Sterkenburg *et al.* 2015). These links support the wider occurrence of a positive feedback between free-living fungal decomposers and soil fertility, as proposed based on local observations (Kyaschenko *et al*. 2017a). In contrast, *C. acutus* s.l. occurred most frequently in forests with low nitrogen concentration in the soil (Fig. 3), in line with descriptions of *Cortinarius* as a generally nitrophobic genus (Lilleskov *et al*. 2019). Thus, acidic and nutrient limited conditions constrained efficient decomposers (i.e. manganese peroxidase producers) to the extent that only species supported by symbiosis were able to persist. This correlative pattern indicates that environmental filtering among non-redundant decomposers plays a major role in regulating below-ground organic matter stocks in this system. Our results support the hypothesis that certain ectomycorrhizal fungi may complement, and even replace, saprotrophs as decomposers when strong nutrient limitation makes it favourable for trees to invest in symbionts that facilitate nutrient mobilisation from recalcitrant organic matter (Lindahl & Tunlid 2015; Baskaran *et al.* 2017). While both organic matter oxidation and ectomycorrhizal symbiosis may be considered as “broad” traits with high redundancy, the combination of these two traits in a single organism is a more “narrow” feature, to the extent that efficient decomposition depends on the population dynamics of a handful of fungal species. The negative correlation between *C. acutus* s.l. and soil nitrogen is probably based on bi-directional causalities. *C. acutus* s.l. is favoured by carbon from nutrient limited tree hosts, but its selective mining for nutrients to sustain tree growth, in turn, reduce soil nitrogen (Clemmensen *et al.* 2015; Kyaschenko *et al.* 2019) and intensify nutrient limitation outside the symbiosis. The fungi would thereby mediate a positive plant-soil feedback, which is characteristic of ectomycorrhizal systems (Bennett *et al.* 2017) and essential to counteract ecosystem retrogression under less fertile conditions (Clemmensen *et al.* 2013, 2015).

Previous local studies (Gadgil & Gadgil 1975; Fernandez & Kennedy 2016; Averill & Hawkes 2016; Kyaschenko *et al.* 2017a), global analyses (Averill *et al.* 2014) and theoretical models (Orwin *et al.* 2011) have suggested that ectomycorrhizal fungi generally increase soil carbon storage by competing with saprotrophic decomposers. Here, no positive correlation was observed between the over-all

relative abundance of ectomycorrhizal fungi and organic stocks. Rather, within the context of boreal forest, certain ectomycorrhizal species seem to restrict local carbon storage in the soil by taking active part in decomposition (Lindahl & Tunlid 2015; Baskaran *et al.* 2017). The clear difference in decomposer capacity that we observe among different ectomycorrhizal fungi highlights the drawbacks of categorizing organisms into fixed guilds with uniform functional properties (Zak *et al.* 2019).

The minuscule spatial scale of microbial habitats promotes high diversity and functional redundancy on larger scales (Nannipieri *et al.* 2003), and Schimel (1995) envisioned that "the challenge of relating microbial diversity to ecosystem function is fundamentally one of relating the scales of microbial life to the scale of the ecosystem". Agaricomycetes differ from other microorganisms by their mycelia that sometimes extend over several square meters, acting as co-ordinated individuals that forage for resources in the heterogeneous soil. The composition of ectomycorrhizal communities is commonly patchy at the meter scale (Pickles *et al.* 2010), and individual mycelia may occupy the same habitat for decades (Dahlberg & Stenlid 1994). Our snapshots of fungal communities suggest that a fungal species, most likely individual mycelia, can dominate a 3 m^2 habitat for sufficiently long periods to regulate organic matter storage in the topsoil. It may be questioned if these fungi really fit within the general concept of "microorganisms", as they seem to operate at a similar spatiotemporal scale as trees. Due to insufficient statistical power, we were only able to pinpoint one particularly frequent taxon, but the guild of "ectomycorrhizal decomposers" is likely to contain many taxa within and outside the genus *Cortinarius*. For example, "mat forming" mycorrhizal *Hysterangium* species have been connected to high rates of decomposition in North American Douglas fir forests (Entry *et al.* 1991).

Allison and Martiny (2008) stated that "only if community composition is sensitive to a disturbance, not resilient, and functionally dissimilar to the original community do changes in community composition matter for predicting ecosystem process rates". We assessed the sensitivity and

resilience of *C. acutus* s.l. to stand-replacing disturbance and did not detect *C. acutus* s.l. in clear-cut and replanted forests younger than 29 years. The decreasing incidence of *C. acutus* in younger stands diverged from the over-all abundance of ectomycorrhizal fungi, which recovered faster after clear-cutting (Fig. 4; Fig. 5, Table S3). Thus, *C. acutus* s.l. is less resilient to forestry than other ectomycorrhizal taxa, similar to many other *Cortinarius* species (Kyaschenko *et al.* 2017b; Varenius *et al.* 2017). High nutrient availability after disturbance seem to act as an environmental filter that suppresses reestablishment of the nitrophobic *C. acutus* s.l.. Yet, other mechanisms, such as constraints on dispersal and priority effects of more rapid mycorrhizal colonisers, may also contribute to the delayed re-establishment after clear-cutting. Our finding that a group of fungal species with a central role in organic matter turnover and nutrient cycling is sensitive to disturbance and have low resilience raises concerns about the long-term sustainability of stand-replacing forestry operations. On a local scale, delayed recolonization by mycorrhizal decomposers may increase carbon sequestration in the organic topsoil, but at the same time impede nutrient cycling and decrease productivity in maturing planted forest. On larger scales, declining populations of mycorrhizal keystone species may put central ecosystem functions at risk, with nitrogen poor forests expected to be particularly sensitive.

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Figure legends

Fig. 1 Carbon stocks in the organic topsoil of Swedish old boreal forest in stands with, or without, presence of the ectomycorrhizal fungus *Cortinarius acutus* s.l., which was recorded as present when the species accounted for >1% of fungal ITS2 sequences (N=23), otherwise as absent (N=150). Bars represent averages ± SE.

Fig. 2 Correlations between properties of stands, soils and fungal communities across old stands of Swedish boreal forest (N=173) with organic stocks in the topsoil as the ultimate dependent variable. Numbers represent standardized correlation coefficients, established in a structural equation model, and are reflected by arrow width with blue arrows indicating positive correlations and red arrows indicating negative correlations. “Soil N” refers to the square-root transformed nitrogen concentration of organic matter (N:C). “Temperature” refers to the annual temperature sum. “Soil pH” refers to the log transformed pH in the organic topsoil. “Tree biomass” refers to basal area. “% Spruce” refers to the proportion of *Picea abies* among conifer basal area (the rest being *Pinus sylvestris*), “Ectomycorrhizal fungi” and “Saprotrophs” refers to the relative sequence abundances of ectomycorrhizal fungi (square root transformed) and saprotrophic agaricomycetes (log transformed) among fungal ITS2 sequences. “*Cortinarius acutus*” refers to presence of *C. acutus* s.l. (>1% of fungal ITS2 sequences required to be recorded as present). “Organic stocks” refers to the log transformed amount of carbon in the organic top soil expressed on an area basis.

Fig. 3 Abundance of saprotrophic agaricomycetes and frequency of occurrence of the ectomycorrhizal fungus *Cortinarius acutus* s.l. in organic topsoils of old stands of Swedish boreal forests. Fungal abundances are related to soil nitrogen concentration with data separated in quintiles according to the nitrogen concentration in the topsoil (N=34-35). Relative abundances of saprotrophic agaricomycetes (red bars; right y-axes; error bars = SE) are based on sequencing of

amplified ITS2 markers. Frequency of occurrence of *C. acutus* s.l. among the stands (green bars; left y-axes) is based on presence/absence requiring that the species accounted for >1% of the fungal ITS2 sequences to be recorded as present.

Fig. 4 Total abundance of ectomycorrhizal fungi and frequency of occurrence of the ectomycorrhizal fungus *Cortinarius acutus* s.l. in organic topsoils of Swedish boreal forest in relation to stand age. Data is organised according to age classes ranging from recently clear-cut and planted stands to old forests. Relative abundances of ectomycorrhizal fungi (blue bars; right y-axes; error bars = SE) are based on sequencing of amplified ITS2 markers. Frequency of occurrence of *C. acutus* s.l. (green bars; left y-axes) among the stands is based on presence/absence requiring that the species accounted for >1% of the fungal ITS2 sequences to be recorded as present.

Fig. 5 Correlations between stand age, soil nitrogen and ectomycorrhizal fungi across Swedish boreal forest stands, including younger forests planted after clear-cutting (N=359) with occurrence of the ectomycorrhizal fungus *Cortinarius acutus* s.l. as the ultimate dependent variable. Numbers represent standardized correlation coefficients, established in a structural equation model, and are reflected by arrow width with blue arrows indicating positive correlations and red arrows indicating negative correlations. “Stand age” refers to the average age of trees weighted by basal area and was tested both with and without square-root transformation (to enable second order dependencies). “Soil N” refers to the square-root transformed nitrogen concentration of organic matter (N:C). “Ectomycorrhizal fungi” refers to the relative sequence abundances of ectomycorrhizal fungi (square root transformed) among total fungal ITS2 sequences. “*Cortinarius acutus*” refers to presence of *C. acutus* s.l. (>1% of total sequences required to be recorded as present).

Fig. S1 Locations of 359 sampled forest stands in boreal Sweden with 173 stands older than 60 years indicated by stars.

Fig. S2 Correlation between carbon stocks in the organic top soil of boreal forests and fungal community composition, assessed by sequencing of amplified ITS2 markers. Deviance[57] explained by

carbon stock is related to different similarity thresholds used during sequence clustering (single linkage), with increasing similarity indicating a finer scale of phylogenetic resolution.

Fig. S3 Pearson correlations between parameters measured in 173 old (>60 years) forest stands in boreal (>60˚N) Sweden. Blue symbols indicate positive correlation and red symbols indicate negative correlation with symbol size corresponding to the absolute size of the correlation coefficient. “Soil N” refers to the square-root transformed nitrogen concentartion of organic matter (N:C). “Temperature” refers to the annual temperature sum. “pH” refers to the log transformed pH in the organic topsoil. “Tree biomass” refers to basal area. “Spruce” refers to the proportion of *Picea abies* among conifer basal area (the others being *Pinus sylvestris*). “Stand age” refers to the average age of trees weighted by basal area. “Ectomycorrhizal fungi” and “Saprotrophs” refers to the relative sequence abundances of ectomycorrhizal fungi (square root transformed) and saprotrophic agaricomycetes (log transformed) among fungal ITS2 sequences. “*C. acutus*” refers to presence of the ectomycorrhizal fungus *Cortinarius acutus* s.l. (>1% of total sequences required to be recorded as present). “Organic stocks” refers to the log transformed amount of carbon in the organic top soil expressed on an area basis.

Fig. S4 Over-all community composition of fungi in the organic topsoil of boreal forests. The figure is based on sequencing of amplified ITS2 markers from 173 old (>60 years) stands in boreal (>60˚N) Sweden.

Fig. S5 Illustration of *Cortinarius acutus* fruitbodies, painted by Liza Johansson using *Cortinarius* pigments.

Supplemented data

Data S1. (Data_all_forests.csv) Geographical coordinates, year of sampling, soil N, stand age, abundance of ectomycorrhizal fungi and occurrence of *C. acutus* s.l. for 359 soil samples from Swedish boreal forest.

Data S2. (Data_old_forests.csv) Latitude, temperature sum, stand age, carbon stock, soil N, soil pH, tree basal area, % spruce among conifers, abundance of ectomycorrhizal fungi and saprotrophic agaricomycetes, and occurrences of 11 taxa of the core agaricomycete community for 173 soil samples from old Swedish boreal forest.

Data S3. (Data_taxonomy.csv) Species identifications, UNITE SH codes and representative ITS2 sequences for members of the core agaricomycete community of soil samples from old Swedish boreal forests.