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RESEARCH ARTICLE

# Ectomycorrhizal decomposers and their niche(s) in boreal forests

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## Abstract

1. Ectomycorrhizal fungi that produce oxidative enzymes—ectomycorrhizal decomposers—may limit soil carbon stocks while maintaining forest productivity in nutrient-poor forest soils by mobilising nitrogen from organic matter. Yet, these fungi are difficult to study in laboratory experiments. Here, we used a correlation-based analysis of field-measured properties to study traits of these unculturable fungi.
2. Two datasets were used to test hypotheses on the effect and response traits of ectomycorrhizal decomposers. Based on samples at the centimetre scale, correlations between fungal abundances and manganese peroxidase activity were tested, enabling assignment of potential important taxa. In a national scale inventory, the niche(s) of the assigned ectomycorrhizal decomposers, concerning mean stand age and soil fertility, were investigated.
3. We found 10 ectomycorrhizal taxa that were significantly co-localised with manganese peroxidase hotspots. Collectively, in pine-dominated forests these taxa were most frequent in relatively young stands with more fertile soils, whereas in spruce-dominated forests, they were most frequent in stands with more nutrient-poor soils. However, individual taxa varied in their responses.
4. There was evidence for niche variation related to stand age and soil fertility among the 10 investigated taxa, suggesting that they do not share one common niche and that ectomycorrhizal decomposers may contribute to oxidative decomposition across a variety of forests with different management histories.

**KEYWORDS**
ectomycorrhizal fungi, effect traits, forest management, fungal communities, manganese peroxidases, response traits, soil inventory, soil nutrients

[Correction added on 17 June 2025, after first online publication: Section headings are renumbered.]

## 1 | INTRODUCTION

Decomposition of organic matter by oxidative enzymes, such as manganese peroxidases (Mn-peroxidases), is a crucial step in carbon and nutrient cycling in organic forest soils (Jones et al., 2020; Kellner et al., 2014; Kranabetter, 2019; Stendahl et al., 2017; Talbot et al., 2013). In the presence of $Mn^{2+}$ and $H_2O_2$, Mn-peroxidases oxidise organic compounds that are resistant to hydrolysis, such as lignins, melanins and tannin complexes (Kirk & Farrell, 1987). Previous observations of negative correlations between available manganese and carbon stocks have been attributed to oxidation by Mn-peroxidases (Kranabetter, 2019; Stendahl et al., 2017; Zhang et al., 2024). Therefore, oxidative decomposition is of interest when modelling ecosystem and global carbon (C) cycling (Baskaran et al., 2017; Chen et al., 2018; McGuire & Treseder, 2010; Moorhead & Sinsabaugh, 2006). The capacity to produce Mn-peroxidases is phylogenetically dispersed across the fungal class Agaricomycetes, including lineages that evolved ectomycorrhizal symbiosis (Floudas et al., 2012; Ruiz-Dueñas et al., 2021). Current research on soil C stabilisation focuses on interactions of microbially processed C with minerals and physical protection of C in aggregates (Lehmann & Kleber, 2015; Schmidt et al., 2011). However, the uppermost soil horizon in boreal forests consists of organic matter with insignificant mineral content (Lindahl et al., 2007). Thus, intrinsic biochemical properties of the litter, chemical stabilisation and the occurrence of fungi with enzymes capable of decomposing persistent organic matter are important factors in regulating C and nutrient dynamics in the organic layer. However, the key fungal species that are relevant for oxidative decomposition and regulation of these enzymes remain largely unknown.

Evidence of oxidative decomposition in the presence of ectomycorrhizal *Hydnellum* (Hintikka & Näykki, 1967) and *Hysterangium* (Cromack et al., 1988; Entry et al., 1991) mats was observed as early as the late 1960s, but recent research on ectomycorrhizal fungi with oxidative capacity (i.e. ectomycorrhizal decomposers) has focused on the genus *Cortinarius* (Bödeker et al., 2014; Defrenne et al., 2023; Lindahl et al., 2021; Pellitier & Zak, 2021). Peroxidase encoding genes have also been detected in some species from the ectomycorrhizal genera *Russula* and *Lactarius* (Russulales), *Tylospora* and *Piloderma* (Atheliales), *Hebeloma* and *Hygrophorus* (Agaricales), and *Gomphus* and *Gautieria* (Gomphales), but are apparently absent in species from the orders Boletales, Cantharellales, Sebacinales and Thelephorales (Bödeker et al., 2009, 2014; Chambers et al., 1999; Miyauchi et al., 2020). However, it cannot be inferred that peroxidases produced by ectomycorrhizal fungi are relevant for decomposition based on gene presence alone (Barbi et al., 2020). Yet, *Cortinarius* Mn-peroxidase genes were found to be actively transcribed in forest soils (Bödeker et al., 2014; Hasby, 2022), and large reductions (over 80% decrease) in Mn-peroxidase activity were observed following the removal of ectomycorrhizal fungi by root trenching (Sterkenburg et al., 2018) or fire-induced tree mortality (Pérez-Izquierdo et al., 2021), suggesting that active enzymes are produced and quantitatively relevant by at least some species within the genus. Both Lindahl et al. (2021) and Bödeker et al. (2014) observed that there may not be strong phylogenetic conservation of Mn-peroxidase production within genera and that taxonomic affiliation alone is also not sufficient to infer relevance of ectomycorrhizal species for decomposition. Thus, the key species of 'ectomycorrhizal decomposers' still remain to be pinpointed. Ectomycorrhizal fungi display a high degree of patchiness at the meter scale (Pickles et al., 2010), but expression of Mn-peroxidases is not likely to be evenly distributed throughout mycelia. Hotspots of Mn-peroxidase activity occur not only at the micrometre scale between fungal hyphae and their substrate (Keiluweit et al., 2015) but also at the centimetre-to-metre scale within single fungal mycelia (Šnajdr et al., 2008). The patchiness of both ectomycorrhizal communities and enzyme activities opens up possibilities to assign likely decomposers through correlative inference. Such correlative studies linking enzyme activity with the presence of specific fungi on the spatial scale of individual mycelia are one of the few possible approaches to assign ectomycorrhizal decomposers (Bödeker et al., 2014), since most ectomycorrhizal fungi cannot be cultivated.

The mobilisation of persistent organic nitrogen by ectomycorrhizal fungi through oxidative enzyme activity has a major influence on ecosystems (an effect trait) but also simultaneously modulates the ectomycorrhizal fungal niche (a response trait; Koide et al., 2014). Response traits refer to the properties of organisms that define how they respond to their environment, i.e. their ecological niches, while effect traits refer to traits that influence abiotic conditions and other species in their surrounding environment (Lavorel & Garnier, 2002). Effect traits that have major relevance for ecosystem processes and a low degree of redundancy, i.e. are linked/synonymous with a narrow set of response traits in certain species, are particularly important to understand fungal influences on ecosystem processes, and how these will be altered by environmental change (Allison & Martiny, 2008; Crowther et al., 2014; Koide et al., 2014). In Sweden, most forests are intensely managed through clear-cutting and replanting resulting in even-age stands. Mean stand age has a marked effect on the soil nutrient status and fungal communities (Kyaschenko et al., 2017; Twieg et al., 2007; Wallander et al., 2010). Ectomycorrhizal fungal community composition may shift in response to changes in the below-ground input of symbiotic C, and there is evidence of variation among ectomycorrhizal fungi in their C requirements and growth rate (Jörgensen et al., 2023; Saikkonen et al., 1999). Presumably, decomposition by Mn-peroxidases has a high energy requirement due to concurrent production of $H_2O_2$ (Hagenbo et al., 2019; Kirk & Farrell, 1987; Lindahl & Tunlid, 2015; Shimizu et al., 2005). In contrast, strong constraints on nutrient availability in older forest stands may increase the competitive advantage that ectomycorrhizal decomposers have compared with ectomycorrhizal fungi that lack this capability, in spite of larger C costs (Hagenbo et al., 2018; Kyaschenko et al., 2017). During periods of elevated mineralisation (in younger stands following tree harvest or wildfires) and N deposition (fertilisation and atmospheric N deposition), plant below-ground C allocation decreases (Högberg et al., 2003), which as proposed by van der Linde et al. (2018), may hamper ectomycorrhizal decomposers that grow slowly and have high C requirements (Agerer, 2001; Jörgensen et al., 2024; Wasyliw & Karst, 2020). Furthermore, variation in inherent soil fertility affects peroxidase activity (Jörgensen et al., 2024). Therefore, in comparison with other

ectomycorrhizal fungi, ectomycorrhizal decomposers should be particularly sensitive to declines in below-ground C supply and physical disturbances to the soil (Argiroff et al., 2022; Defrenne et al., 2019; Lilleskov et al., 2002; Lindahl et al., 2021).

In line with the reasoning above, Lilleskov et al. (2011) proposed that ectomycorrhizal fungi that tend to form hydrophobic mycelial cords (e.g. *Cortinarius* species) or dense mycelial mats (e.g. many members of the family Bankeraceae and subclass Phallomycetidae) are associated with enhanced organic matter decomposition and are preferably found in nutrient-poor soils. This framework has been commonly applied to suggest that ectomycorrhizal decomposers may have their main niche in older, nutrient-poor forests, leading to the perpetuation of organic matter turnover, even as N-limitation intensifies during ecosystem retrogression (Clemmensen et al., 2015; Dynarski & Houlton, 2020). Thus, there is a risk that stand-replacing forestry, through host-tree removal and increased soil nutrient availability, may interfere with the functionality of this group of fungi (Kyaschenko et al., 2017; Lindahl et al., 2021).

The objective of this study was to pinpoint the most important ectomycorrhizal taxa related to local Mn-peroxidase activity (as an effect trait) and to identify the niche of these taxa (their response traits) with respect to forest stand age and soil fertility. Our *a priori* hypotheses were tested in two connected steps (Figure 1). A regional dataset with fine-scaled sampling was used to study the Mn-peroxidase activity as an effect trait of ectomycorrhizal decomposers, based on our first hypothesis:

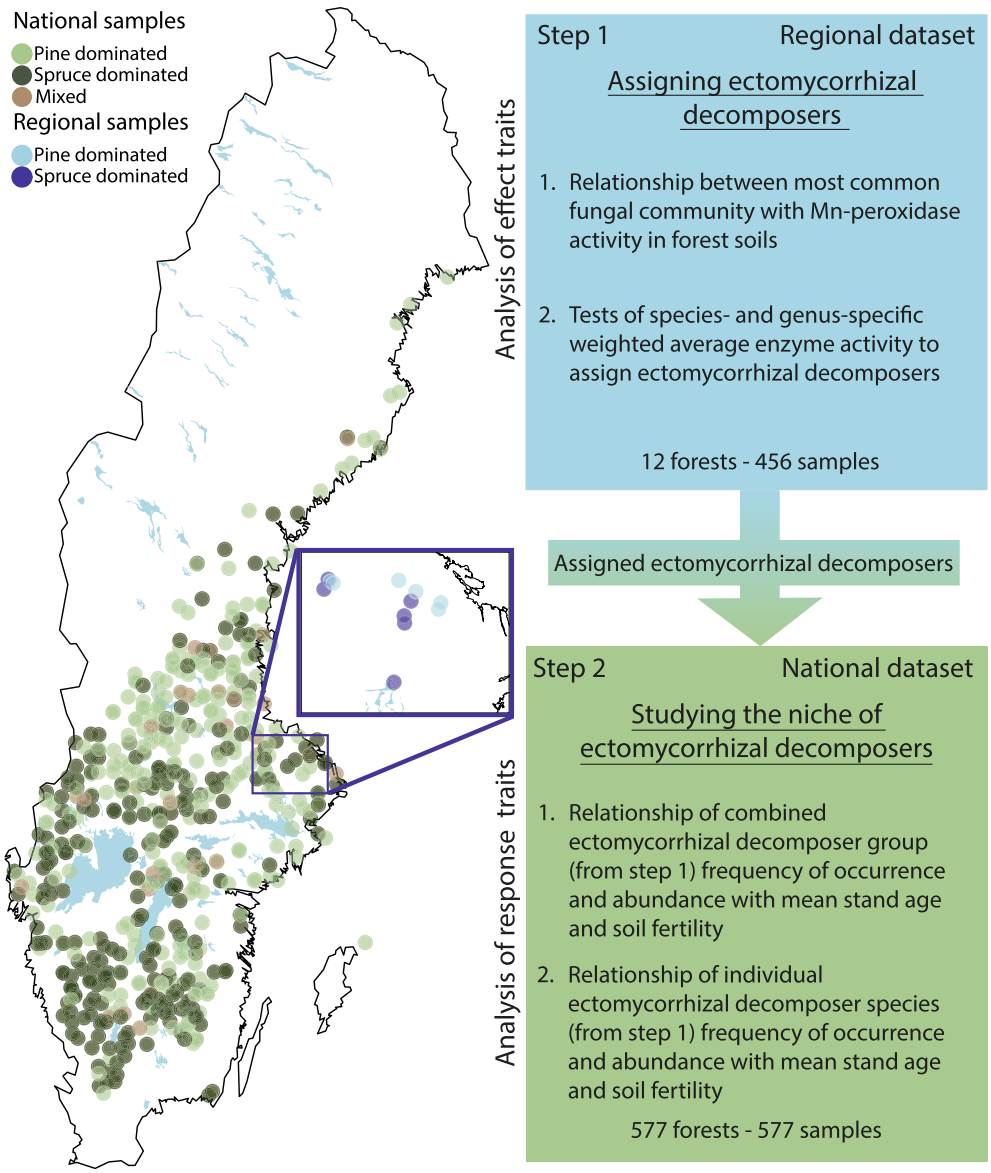


**FIGURE 1** Distribution of 12 regional and 577 national sampling sites within Sweden and structure of data analysis. The regional samples were collected for a study focussed on local spatial heterogeneity and are used in Step 1 (Figure S1). The national samples were collected as a part of the Swedish Forest Soil Inventory and are used in Step 2. The national sites have >90% basal area of pine and spruce, and were categorised as pine-dominated or spruce-dominated (>60% basal area either pine or spruce). Stand age ranged from 15 to >80 years for the regional sites and from 10 to 215 years for the national sites (Figure S2).

1. That fungal community composition would be predictive of Mn-peroxidase activity on the mycelial scale (cm to m), and that some ectomycorrhizal species from genera with documented Mn-peroxidase genes would correlate positively with community-level Mn-peroxidase activity.

Subsequently, a national dataset was used to study the realised niche of these fungi, based on our second hypothesis:

2. That ectomycorrhizal Mn-peroxidase-producing fungi, as defined in our first analysis, would correlate positively with forest stand age and negatively with soil fertility.

## 2 | MATERIALS AND METHODS

### 2.1 | Soil sampling and analyses

#### 2.1.1 | Regional dataset

Soil samples were collected from 12 boreal forests differing in dominant tree species and stand age (Figure 1; Table S1). No permits were required for fieldwork. In early October 2019, we collected 38 soil cores (2.5 cm in diameter) at different spatial distances from each other (0.1–10 m) following a hierarchical nested sampling design implemented in each of the 12 forests (456 samples total; Figure S1). This design was intended for a separate study focused on assessing spatial heterogeneity of various soil parameters on the meter scale (Lindahl et al., 2023), but here we focused on links between Mn-peroxidase activity and fungal communities. Upon collection, all green plant parts and any mineral soil were removed, retaining only the organic mor layer (the O-horizon, including litter). The soils were stored at −20°C from the time of sampling till further analyses. Samples were freeze-dried, weighed and milled. Total C and N were determined with an Isotope ratio mass spectrometer (DeltaV; Thermo Fisher Scientific, Bremen, Germany) coupled to an elemental analyser (Flash EA 2000; Thermo Fisher Scientific). Soil pH was measured in a 1:5 (v:v) ratio of dry soil to deionised water slurry with a 744 pH meter (Metrohm, Herisau, Switzerland).

Potential enzyme activity of Mn-peroxidase was measured in freeze-dried soils (Valášková & Baldrian, 2006) from the regional dataset using 3-Methyl-2-benzothiazolinone hydrazine hydrochloride monohydrate (MBTH) and 3-(dimethylamino)benzoic acid (DMAB) in a coupled colorimetric assay (Supporting Information). In brief, activity was measured in 200 µL reactions containing 50 µL of soil extracted enzymes in 50 mM sodium acetate (pH 5) and 150 µL of reaction solution containing 0.05 mM MBTH, 2.5 mM DMAB, 25 mM sodium lactate (pH 4.5), 25 mM sodium succinate (pH 4.5), 0.25 mM $H_2O_2$ and either 0.1 mM $MnSO_4$ or 0.2 mM EDTA (i.e. no reactive Mn present). The changes in absorbance were measured at 590 nm in a SpectraMax Plus 384 Microplate Reader (Molecular Devices, Sunnyvale, USA) over 45 min to determine the rate of activity. To determine Mn-dependent activity, reactions with EDTA were subtracted from reactions with $MnSO_4$ (Baldrian et al., 2000; Ngo & Lenhoff, 1980). Total peroxidase activity was determined as the difference in the rate of activity between reactions with and without $H_2O_2$.

#### 2.1.2 | National dataset

As a part of the Swedish Forest Soil Inventory, soil samples across all of Sweden were collected from sites chosen based on a grid design (Fridman et al., 2014). We used a subset of 577 sites in similar climatic conditions to the regional sites that were sampled between 2014 and 2021 (Temperature sum between 900 and 1400; Odin et al., 1983; Figure 1). We further restricted to only productive forests (annual growth >1 $m^3 ha^{-1}$) with a mean stand age greater than 10 years (a different subset of the samples was previously used in Lindahl et al., 2021). This age threshold was made to avoid confounding effects of recent clear-cutting forestry on the ectomycorrhizal community (Wallander et al., 2010). Stand age was assessed as the average tree age weighted by their basal area (Nilsson et al., 2013). Furthermore, we selected only coniferous sites (90% of the basal area as *Pinus sylvestris* L. and/or *Picea abies* L.) with either pine or spruce dominance. No permits were required for the use of data from these field sites.

Soil was collected separately for analyses of soil parameters and fungal communities as described in Lindahl et al. (2021). In brief, tree parameters were measured within a 10 m radius circle, and within that, two concentric circles were sampled for fungal communities (1 m radius) and soil properties (0.6 m radius). For the fungal community, samples from the uppermost 10 cm of the soil were pooled from five points, and for the soil properties, a 10 cm diameter corer was used to sample the organic layer until at least 1.5 L was collected (1–9 cores). The soil was frozen at −20°C within 6 days of sampling (on average) and then freeze-dried and finely ground with a ball mill (Bertin Technologies; Montigny-le-Bretonneux; France). C and N concentrations were measured from dry sieved soil using an elemental analyser (TruMac CN; LECO, St. Joseph, USA), and soil pH was determined with an Aquatrode Plus Pt1000 pH meter (Metrohm) in a 1:2.5 (dry weight: volume) ratio with deionised water.

### 2.2 | Fungal community analysis

DNA was extracted from 100 mg of homogenised soil from both sample sets using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. ITS2 markers were PCR amplified using fungal specific primers gITS7 (Ihrmark et al., 2012) and a 3:1 mix of the two reverse primers ITS4 (White et al., 1990) and ITS4arch (Kyaschenko et al., 2017), fitted with unique 8-bp sample identification tags (Clemmensen et al., 2023). PCR reactions (50 µL volume) were run: 5 min at 94 (regional samples) or 95°C (national samples) for denaturation, 20–35 cycles of 30 s at

94 or 95°C, 30 s at 56°C and 30 s at 72°C, and a final elongation period at 72°C for 7 min. To reduce fragment length bias, the number of PCR cycles was minimised to obtain 'weak but visible' bands on electrophoresis gels (Castaño et al., 2020). For samples from the national dataset, all PCR products were run in duplicates that were pooled before cleaning with AMPure (Beckman Coulter, Indianapolis, USA). PCR products from each sample of the regional dataset were purified with Sera-Mag magnetic carboxylate modified particles (Hydrophobic; GE Healthcare, Chicago, USA), measured with Qubit fluorometer (Thermo Fisher Scientific) and mixed in equal concentrations into six composite pools before purifying pools with E.Z.N.A cycle pure kit (Omega bio-tek; Nocross, USA). BioAnalyzer was used to check the amplicon size distribution (Agilent Technologies, Santa Clara, USA). Adaptor ligation and PacBio (Pacific Biosciences, Menlo Park, USA) sequencing were performed by SciLifeLab NGI (Uppsala, Sweden) using one SMRT cell per pool. National samples collected between 2014 and 2016 were sequenced on the RSII platform and samples collected between 2017 and 2021, as well as all regional samples, were sequenced with Sequel 1 (both PacBio technologies).

Sequence quality control and clustering into species hypothesis (hereafter species) (Kõljalg et al., 2013) was performed in the SCATA pipeline (Ihrmark et al., 2012). Only sequences with tag matching at both ends, and >90% match with both primers were accepted. After removal of primer sequences and global singletons, pairwise comparisons were made with USEARCH (Edgar, 2010) followed by single-linkage clustering, with 98.5% similarity required for sequences to enter clusters. An internal annotated reference database was included during clustering to assign sequences to species, with additional manual identification performed with UNITE (Abarenkov et al., 2024).

## 2.3 | Statistics

Data analysis was performed in R v4.4.1 (R Core Team, 2025). The significance level used was $\alpha = 0.05$ for two-sided tests and $\alpha = 0.1$ for one-sided tests. We used dplyr (Wickham et al., 2023), ggplot2 (Wickham, 2016), factoextra (Kassambara & Mundt, 2020), patchwork (Pedersen, 2022), tmap (Tennekes, 2018) and vegan (Oksanen et al., 2022) for data processing and visualisations. Scripts for analysis are available on Zenodo.

### 2.3.1 | Regional dataset: Relationships between Mn-peroxidase activity and fungal community composition

To test for spatial autocorrelation in Mn-peroxidase activity between cores, the standard deviation of the three points in each triangle (Figure S1) was calculated. We used a linear model to test the relationship between variation in Mn-peroxidase activity (scaled and centred by site) within triangles and the size of triangles (0.1, 0.3, 1.0, 3.0 and 10.0 m), dominant host-tree species (spruce or pine) and stand age (young or old) as categorical explanatory factors, including the interactions between triangle size and tree species or stand age. Variation in Mn-peroxidase activity within triangles was not significantly related to triangle size, that is the distance between cores (ANOVA; df = 4; $F = 0.91$; $p = 0.460$). Spatial autocorrelation was not observed at any scale, so patchiness of enzyme activity should occur at scales <0.1 m. Therefore, the distance between cores was not considered, and each core was treated as an independent sample with respect to Mn-peroxidase activity in subsequent analyses.

We aimed to test whether the most frequent species in the regional samples (1212 species from the total fungal community that were present in ≥10 of the 456 samples and ≥3 of the 12 sites) explained variation in Mn-peroxidase activity (Figure S3). The square root-transformed relative abundances (Hellinger-transformation) of the fungal communities were analysed in a canonical correspondence analysis (CCA) with log-transformed Mn-peroxidase activity as a predictor, which was evaluated with PERMANOVA with 1000 permutations constrained to within sites to focus on within-site variation.

### 2.3.2 | Regional dataset: Exploring for ectomycorrhizal decomposers

To assign species as potential ectomycorrhizal decomposers, we determined the species-specific weighted average enzyme activity ($WAE_{sp}$; Equation 1; Bödeker et al., 2014) of the most frequent Agaricomycetes species (214 species of any ecology, i.e. ectomycorrhizal and saprotrophic). A higher $WAE_{sp}$ indicates species with higher relative abundance in samples with high peroxidase activity. For this, Mn-peroxidase and total peroxidase activity (untransformed) were first centred by site (value minus the arithmetic mean) to reduce inter-site variation but maintain intra-site variation. To assess whether the trait was phylogenetically clustered at the genus level, we taxonomically aggregated species by genera and repeated the assessments of weighted average enzyme activity ($WAE_{gen}$; Equation 2).

$$WAE_{sp} = \frac{\sum_{i=1}^{n} E_i P_{si}}{\sum_{i=1}^{n} P_{si}}. \quad (1)$$

$$WAE_{gen} = \frac{\sum_{i=1}^{n} E_i P_{gi}}{\sum_{i=1}^{n} P_{gi}}. \quad (2)$$

($E_i$ is peroxidase activity in sample $i$; $P_{si}$ is the relative abundance of the species in sample $i$; $P_{gi}$ is the relative abundance of the genus in the sample; $n$ is the total number of samples (456)).

Each species and/or genus was evaluated with a one-sided permutation test for their relationship with both Mn-peroxidase and total peroxidase activity. A simulated random distribution of each *WAE* was formed by 10,000 re-samplings of the enzyme activity ($E_i$) within Equation (1) or (2), and each observed WAE was evaluated against its simulated distribution. *p*-values were determined based

on the sum of observations in the simulated distributions that were ≥ the test statistic ($WAE_{sp}$ or $WAE_{gen}$) divided by the total number of permutations. $WAE_{sp}$ and $WAE_{gen}$ were also tested to see which taxa had a negative relationship with peroxidase hotspots (i.e. sum of observations in the simulated distributions that were ≤ the test statistic).

Ectomycorrhizal species with an uncorrected *p*-value <0.1, for either Mn-peroxidase and/or total peroxidase activity, were assigned as ectomycorrhizal decomposers for subsequent analysis of their niche at the national scale. In addition, we chose a more exclusive approach for assigning ectomycorrhizal decomposers that also excluded ectomycorrhizal species lacking Class-II peroxidase genes (at the genus level) based on currently available genomic data and fungi of unknown or saprotrophic ecologies.

For all sequenced species within the Agaricomycetes genera observed in this study, the number of genes coding for Class-II peroxidases was extracted from Miyauchi et al. (2020) and the Joint Genome Institute (mycocosm.jgi.doe.gov; Balasundaram et al., 2018; Branco et al., 2015; Eastwood et al., 2011; Gaskell et al., 2017; Grigoriev et al., 2014; Harder et al., 2024; Kohler et al., 2015; Lebreton et al., 2022; Lofgren et al., 2021; Looney et al., 2022; Martinez et al., 2009; Miyauchi et al., 2020; Nagy et al., 2016). We determined the mean number of Class-II peroxidase genes per genus. In cases where no genomes were sequenced within a genus, data from species within the same family were used. Kendall's Rank correlation test was used to test whether $WAE_{sp}$ correlated with the mean number of Class-II peroxidase genes across species within the genera.

#### 2.3.3 | National dataset: Niche(s) of ectomycorrhizal decomposers

Soil pH and N/C were strongly co-linear, so these variables were incorporated into a principal component analysis (after centring and scaling), where the first PC axis was interpreted as a soil fertility index (Figure S4). Fisher's exact test was used to test whether the ectomycorrhizal decomposer group and the individual assigned species occurred more frequently in pine- or spruce-dominated forests. A linear model with (log-transformed) relative abundance of the ectomycorrhizal decomposer group, when present, and dominant tree species as a fixed factor was used to test whether their abundance differed between forest cover types. For subsequent analyses, the data were analysed both across all forest cover types (577 sites) and split into spruce-dominated (253 sites) and pine-dominated (295 sites) datasets (sites with 50/50 host species dominance were omitted).

Mean stand age and the soil fertility index were used as independent predictor variables to model the niche of the combined ectomycorrhizal decomposer group assigned in the regional dataset. Separate models were made with stand age either as a continuous log-transformed variable or a categorical variable (young [<70 years] or old [>70 years]). Forests with a stand age less than 70 years have likely been clear-cut, while forests with a stand age more than 70 years likely have a longer forest continuity (Antonson & Jansson, 2011; Lundmark et al., 2013). By testing stand age in two ways, we aimed to assess whether the history of clear-cutting or mean stand age was more relevant to the niche of ectomycorrhizal decomposers. In both presence/absence (frequency of occurrence) and relative abundance models, the sequencing platform was included as a random factor. Frequency of occurrence of the combined assigned ectomycorrhizal decomposer group was tested with generalised linear mixed models (*glmer* from lme4; Bates et al., 2015) with binomial linking functions, and relative abundance (log-transformed) was tested with linear mixed models (*lmer* from lme4). The relative abundance of the ectomycorrhizal decomposer group, when present, was tested either as a proportion of the total fungal community or of the ectomycorrhizal community. In cases where data appeared unimodal, we tested polynomial terms for both stand age and the soil fertility index. Evaluation of multicollinearity between predictor variables (soil fertility and stand age) was evaluated (*vif* function from car; Fox & Weisberg, 2019) and was unproblematic, as variance inflation factors were less than three in all models tested. Adjusted $R^2$ were determined with the *r.squaredGLMM* function from the MuMIn package (Bartoń, 2023).

Correlations between stand age or soil fertility with the presence/absence of individuals of ectomycorrhizal decomposer species were evaluated with generalised linear models with a binomial linking function. *p*-values were adjusted for multiple testing using the false discovery rate method (Benjamini & Hochberg, 1995).

## 3 | RESULTS

### 3.1 | Regional dataset: Mn-peroxidase activity as a trait of ectomycorrhizal decomposers

Fungal community composition was correlated with Mn-peroxidase (PERMANOVA; df = 1; proportion explained of CCA = 0.003; $F = 1.28$; $p \leq 0.001$) and total peroxidase activity (PERMANOVA; df = 1; proportion explained of CCA = 0.003; $F = 1.27$; $p = 0.004$) at the scale of individual soil cores. Twenty-nine fungal taxa had significantly higher $WAE_{sp}$ than expected by random variation, of which 16 were ectomycorrhizal (Table 1). Ten of the 29 taxa lacked Class-II peroxidase genes: *Coniophora puteana*, *Postia ptychogaster*, *Serpula himantioides*, *Sistotrema* sp., *Suillus luteus*, *Tomentellopsis submollis*, *Thelephora terrestris*, *Thelephora longisterigmata*, *Thelephora eucoerulea* and *Tylospora asterophora* (Shah et al., 2016). Nine of the 29 taxa were saprotrophic species of known ligninolytic capacity: two *Trechispora* taxa (Trechisporales); *Galerina* sp., *Galerina calyptrata*, *Gymnopilus penetrans*, *Mycena rubromarginata* and *Mycena sanguinolenta* (Agaricales); *Ganoderma lucidum* (Polyporales); and *Rescinicium bicolor* (Hymenochaetales) (Floudas, 2021). *Coniophora puteana*, *Postia ptychogaster* and *Serpula himantioides* are considered 'brown-rot' saprotrophs, while *Sistotrema* sp. has uncertain ecology. The assigned ectomycorrhizal decomposers consisted of 10 taxa:

**TABLE 1** Fungal taxa with significantly higher estimated specific Mn-peroxidase and/or total peroxidase activity than expected by random variation tested each with one-sided permutation tests.

| Species | UNITE species hypothesis | Mean Class-II peroxidase genes ± SE (number of species in genus with published genomes) | MnP | | | Total peroxidase | | |
|---|---|---|---|---|---|---|---|---|
| | | | *p*-value | WAEsp rank order | WAEgen rank order | *p*-value | WAEsp rank order | WAEgen rank order |
| ***Lactarius necator*** | **SH0961100.10FU** | **2.8 ± 0.2 (10)** | **<0.001** | **1** | **15[a]** | **0.029** | **4** | **25** |
| ***Cortinarius anomalus*** | **SH1017270.10FU** | **9 (1)** | **0.022** | **2** | **58** | | **16** | **56** |
| *Trechispora* sp.2 | SH0841098.10FU | 12 (1)[b] | 0.023 | 3 | 21 | 0.057 | 6 | 38 |
| *Mycena rubromarginata* | SH0748954.10FU | 17.2 ± 0.4 (25) | 0.03 | 4 | 36 | 0.099 | 17 | 36 |
| ***Gautieria* cf. *otthii*** | **SH0932000.10FU** | **25 (1)** | **0.029** | **5** | **1[a]** | **0.067** | **11** | **3[a]** |
| ***Suillus luteus*** | **SH0946483.10FU** | **0 (22)** | **0.019** | **6** | **20** | **0.026** | **7** | **15** |
| ***Russula aquosa*** | **SH0944250.10FU** | **1.63 ± 0.2 (8)** | **0.026** | **7** | **23** | | **27** | **28** |
| *Rescinicium bicolor* | SH0860289.10FU | 15 (0) | 0.064 | 8 | 2[a] | | 24 | 6 |
| *Coniophora puteana* | SH0883820.10FU | 0 (2) | 0.069 | 10 | 6[a] | 0.068 | 15 | 4[a] |
| ***Cortinarius fulvescens* coll.** | **SH1017507.10FU** | **9 (1)** | **0.080** | **11** | **58** | **0.072** | **14** | **56** |
| *Serpula himantioides* | SH0992263.10FU | 0 (3) | 0.092 | 12 | 4[a] | | 28 | 7 |
| ***Cortinarius comptulus* coll.** | **SH0986335.10FU** | **9 (1)** | **0.059** | **13** | **58** | **0.007** | **2** | **56** |
| *Postia ptychogaster* | SH0755398.10FU | 0 (2) | 0.094 | 14 | 7 | | 35 | 12 |
| *Trechispora* sp.1 | SH0888744.10FU | 12 (1)[b] | 0.076 | 15 | 21 | | 32 | 38 |
| ***Tomentellopsis submollis*** | **SH0936881.10FU** | **0 (2)[b]** | **0.099** | **16** | **33** | | **44** | **46** |
| *Ganoderma lucidum* | SH0762718.10FU | 10.2 ± 0.3 (5) | 0.062 | 21 | 26 | | 42 | 16 |
| ***Thelephora terrestris*** | **SH0919166.10FU** | **0 (2)** | **0.045** | **23** | **30** | | **43** | **23** |
| ***Thelephora eucoerulea*** | **SH0920796.10FU** | **0 (2)** | | **26** | **30** | **0.060** | **12** | **23** |
| *Galerina calyptrata* | SH0994936.10FU | 22 (1) | | 27 | 12 | 0.028 | 8 | 8 |
| ***Tylospora asterophora*** | **SH0826300.10FU** | **0 (5)[b]** | | **29** | **16** | **0.084** | **26** | **13** |
| ***Lactarius tabidus*** | **SH0961123.10FU** | **2.8 ± 0.2 (10)** | **0.084** | **30** | **15[a]** | | **55** | **25** |
| *Mycena sanguinolenta* | SH0748984.10FU | 17.2 ± 0.4 (25) | | 37 | 36 | 0.021 | 18 | 36 |
| *Sistotrema* sp. | SH1030361.10FU | 0 (1) | | 49 | 74 | 0.055 | 13 | 57 |
| ***Russula rhodopus*** | **SH0956387.10FU** | **1.63 ± 0.2 (8)** | | **73** | **23** | **0.003** | **1** | **28** |
| ***Cortinarius mucosus* coll.** | **SH1017686.10FU** | **9 (1)** | | **78** | **58** | **0.031** | **10** | **56** |
| *Galerina* sp. | SH0886132.10FU | 22 (1) | | 99 | 12 | 0.026 | 9 | 8 |
| *Gymnopilus penetrans* | SH0886174.10FU | 15 (1) | | 117 | 46 | 0.010 | 5 | 2[a] |
| ***Thelephora longisterigmata*** | **SH0918425.10FU** | **0 (2)** | | **125** | **51** | **0.007** | **3** | **26** |
| ***Cortinarius spilomeus*** | **SH1017671.10FU** | **9 (1)** | | **196** | **58** | **0.093** | **20** | **56** |

*Note*: All species hypotheses with an unadjusted *p*-value <0.1 are shown. Ectomycorrhizal taxa are in bold (guild assignment in accordance with FungalTraits database; Põlme et al., 2020). Taxa considered as potential ectomycorrhizal decomposers are highlighted in grey. $WAE_{sp}$ and $WAE_{gen}$ are ranked from low (stronger correlation) to high (weaker correlation). Mean number of Class-II peroxidase genes per species within genera is extracted from Miyauchi et al. (2020) and the Joint Genome Institute Mycocosm portal.

[a]Significant at the genus level.

[b]Gene counts from within family.

*Cortinarius anomalus*, *C. comptulus* coll., *C. fulvescens* coll., *C. mucosus* coll., *C. spilomeus*, *Gautieria* cf. *otthii*, *Lactarius necator*, *L. tabidus*, *Russula aquosa* and *R. rhodopus*.

Mean Class-II peroxidase gene counts per genus (or per family in certain cases) and $WAE_{sp}$ did not correlate across the 214 studied Agaricomycete species (Kendall's rank; $z = -0.155$; $p = 0.877$) or 76 genera (Kendall's rank; $z = -0.153$; $p = 0.878$). The share of genera that had a significant relationship with estimated Mn-peroxidase activity ($WAE_{gen}$) was smaller (9 of 75 genera) than that of species ($WAE_{sp}$; 29 of 214 species). The nine genera significantly related to Mn-peroxidase and/or total peroxidase activity ($WAE_{gen}$ with unadjusted *p*-values <0.1) were *Gautieria*, *Coniophora*, *Lactarius*, *Postia*, *Naucoria*, *Rescinicium*, *Gymnopilus*, *Clavulina* and *Serpula* (Table S2). *Gautieria*, *Rescinicium* and *Serpula* were represented by only one species each (thus $WAE_{gen}$ and $WAE_{sp}$ did not differ). Other genera containing taxa with significant $WAE_{sp}$, *Cortinarius*, *Galerina*, *Ganoderma*, *Mycena*, *Russula*, *Sistotrema*, *Suillus*, *Thelephora*, *Tomentellopsis*, *Trechispora* and *Tylospora*, were not significant at the genus level ($WAE_{gen}$).

There were 44 taxa that were negatively co-localised with Mn-peroxidase activity hotspots (Table S3). Genera that contained both positively and negatively correlated species included *Cortinarius*, *Lactarius*, *Mycena*, *Russula*, *Sistotrema*, *Thelephora* and *Trechispora*.

### 3.2 | National dataset: Niche(s) of ectomycorrhizal decomposers

As a group, the assigned ectomycorrhizal decomposers were present in 47.6% of the samples of the national inventory (individual taxa average 7.3 ± 4.6%; range between 1% and 15%), and when present, they accounted for on average 1.2% of the total fungal sequences and 4.5% of the ectomycorrhizal sequences. There were 473 ectomycorrhizal species hypotheses identified in the national inventory.

The assigned ectomycorrhizal decomposer group was collectively more frequent (Fisher's exact; Figure S6a; odds ratio = 2.90; 95% CI = 2.02–4.18; $p \leq 0.001$) and had a marginally higher relative abundance (ANOVA; Figure S6b; df = 1; $F = 3.48$; $p = 0.063$) in spruce-dominated forests. However, *C. mucosus* coll. was 2.2 times more frequent in pine-dominated forests (Figure S7; Table S4).

Across all the included forests, the group of assigned ectomycorrhizal decomposers tended to have a peak in frequency of occurrence in mature secondary forests (Figure 2a). The decline in the oldest forest stands was supported by a negative and significant polynomial term. However, the increase at early succession (~10–45 years; linear term) had weak statistical support (Table 2). This was also the case in pine-dominated stands but not in spruce-dominated stands (Figure 2b,c). There were no significant differences in the frequency

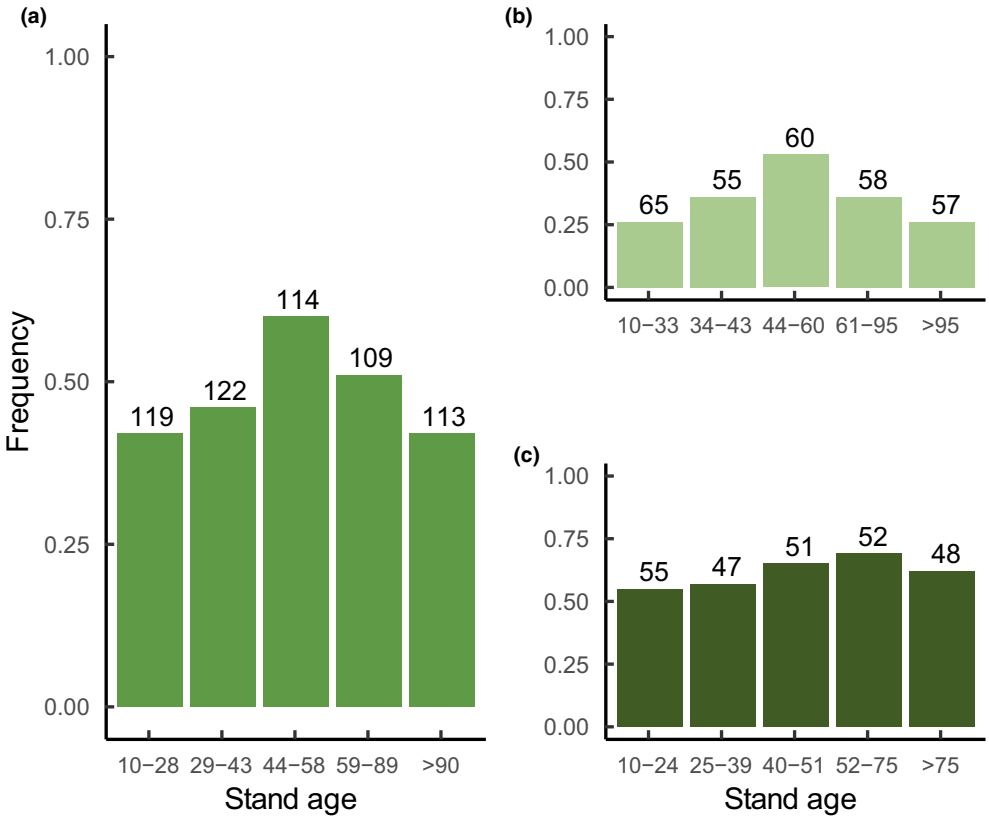


FIGURE 2 Frequency of occurrence of species assigned as ectomycorrhizal decomposers across forest stand ages in (a) all forests, (b) pine-dominated and (c) spruce-dominated forests. Stand age is sectioned into five evenly distributed bins (for graphical representation) with their inclusive age ranges shown on the *x*-axis. Numbers on top of bars indicate the total number of forests in each age range. Statistics are presented in Table 2.

**TABLE 2** Correlations between combined frequency of occurrence of species assigned as ectomycorrhizal decomposers and stand age and soil fertility with sequencing platform as a random effect analysed by generalised linear mixed models.

| | | Stand age (log-transformed years) | | | Stand age (old vs. young) | | |
|---|---|---|---|---|---|---|---|
| | | Estimate (SE) | *p*-value | Model Pseudo delta $R^2$ | Estimate (SE) | *p*-value | Model Pseudo delta $R^2$ |
| All forests (577 sites) | Intercept | (+) 0.341 (0.28) | | 0.10 | (−) 0.150 (0.24) | | 0.03 |
| | Age | (+) 0.110 (0.09) | 0.240 | | (−) 0.067 (0.19) | 0.728 | |
| | Age$^2$ | **(−) 0.279 (0.08)** | **<0.001** | | n.a | | |
| | Soil fertility index | **(+) 0.329 (0.09)** | **0.001** | | (+) 0.070 (0.7) | 0.298 | |
| | Soil fertility index$^2$ | **(−) 0.157 (0.04)** | **<0.001** | | n.a | | |
| Pine-dominated (295 sites) | Intercept | (−) 0.257 (0.26) | | 0.14 | (−) 0.642 (0.24) | | 0.05 |
| | Age | (+) 0.199 (0.16) | 0.210 | | (−) 0.132 (0.28) | 0.633 | |
| | Age$^2$ | **(−) 0.531 (0.14)** | **<0.001** | | n.a | | |
| | Soil fertility index | **(+) 0.308 (0.12)** | **0.007** | | **(+) 0.345 (0.11)** | **0.002** | |
| Spruce-dominated (253 sites) | Intercept | (+) 0.424 (0.30) | | 0.09 | (+) 0.401 (0.31) | | 0.08 |
| | Age | (+) 0.150 (0.14) | 0.284 | | (−) 0.032 (0.33) | 0.924 | |
| | Soil fertility index | **(−) 0.337 (0.10)** | **<0.001** | | **(−) 0.356 (0.10)** | **<0.001** | |

*Note*: Polynomial terms of stand age (age$^2$) and/or soil fertility (soil fertility index$^2$) were tested when data appeared unimodal. Soil fertility index represents the first axis of a principal component analysis (PCA) with soil pH and N/C from all sites. *p*-values <0.05 are in bold.

of occurrence of the group between young or old forest stands (Table 2). While no individual species had a significant response to stand age in pine-dominated forests, in spruce-dominated forests some species responded, but in contrasting directions. Frequency of occurrence of *Cortinarius fulvescens* coll. was higher in older stands and *C. spilomeus* was lower (Figure S5; Table S5). *Cortinarius comptulus* coll., *Lactarius necator* and *Russula aquosa* were also marginally more frequent in older forests, yet *Russula rhodopus* tended to be less frequent ($p < 0.1$; Table S5).

The frequency of occurrence of the group had a unimodal response to soil fertility (Figure 3a). In spruce-dominated forests, they collectively declined with increasing soil fertility; yet, they had the opposite relationship to soil fertility in pine-dominated forests (Figure 3b,c; Table 2). As in the response to stand age, individual species also responded in varying directions to soil fertility. The individual taxa most strongly driving the overall pattern were *C. comptulus* and *C. fulvescens* in spruce-dominated forests (Figure 4b,d; Table S5) and *G.* cf. *otthii* and *R. aquosa* in pine-dominated forests (Figure 4a,c; Table S5).

There was no relationship between the relative abundance of the group of ectomycorrhizal decomposers, when present, when assessed either as a proportion of the whole fungal community or relative to other ectomycorrhizal fungi (Tables S6 and S7).

## 4 | DISCUSSION

### 4.1 | Mn-peroxidase activity as a trait of ectomycorrhizal decomposers

Manganese peroxidase activity was highly variable (Figure S3) but did not display spatial autocorrelation at scales >10 cm across the 12 forests studied here. Nevertheless, enzyme activities correlated with fungal community composition, and we explore this covariation to assign potential key ectomycorrhizal decomposers. The 10 taxa highlighted here should be relevant for oxidative decomposition of organic matter in Swedish forest soils. We note that our assignment of taxa is limited by the local context of the regional study; thus, we cannot extrapolate with certainty across all boreal forests. In line with previous observations (Bödeker et al., 2014; Lindahl et al., 2021; Pellitier & Zak, 2021), species from the genus *Cortinarius* made up half of the highlighted ectomycorrhizal decomposers in this study. Furthermore, *Gautieria* stands out as having a particularly high number of Class-II peroxidase genes (Miyauchi et al., 2020) and can be grown in pure culture with insoluble protein–tannin complexes as the sole nitrogen source, suggesting a capacity to mobilise recalcitrant nitrogen (Griffiths & Caldwell, 1992), which is consistent with a high $WAE_{sp}$. The ecology of the truffle-like fungus *Gautieria* is understudied, but production of peroxidases in combination with a tendency to produce dense mycelial mats would suggest that they could have a strong localised influence on the decomposition of soil organic matter. *Russula* and *Lactarius* species tend to have a relatively modest number of Class-II peroxidase genes (Table 1). Their mycorrhizal structures are morphologically classified as contact exploration types (Agerer, 2001), yet they tend to proliferate in organic ingrowth bags (Jörgensen et al., 2023), which together with a high $WAE_{sp}$ of some members of these genera goes against the idea that organic nutrient mobilisation is primarily a trait of cord and/or mat-forming fungi. Among Agaricomycetes (ectomycorrhizal and saprotrophic), the trait of Mn-dependant oxidation did not appear to be phylogenetically clustered at the genus level: aggregation of species within genera weakened statistical patterns, particularly for more species-rich genera. Furthermore, several genera contain species with both high and low $WAE_{sp}$ (e.g. *Cortinarius* and *Mycena*). It seems possible that not all species within a genus have active

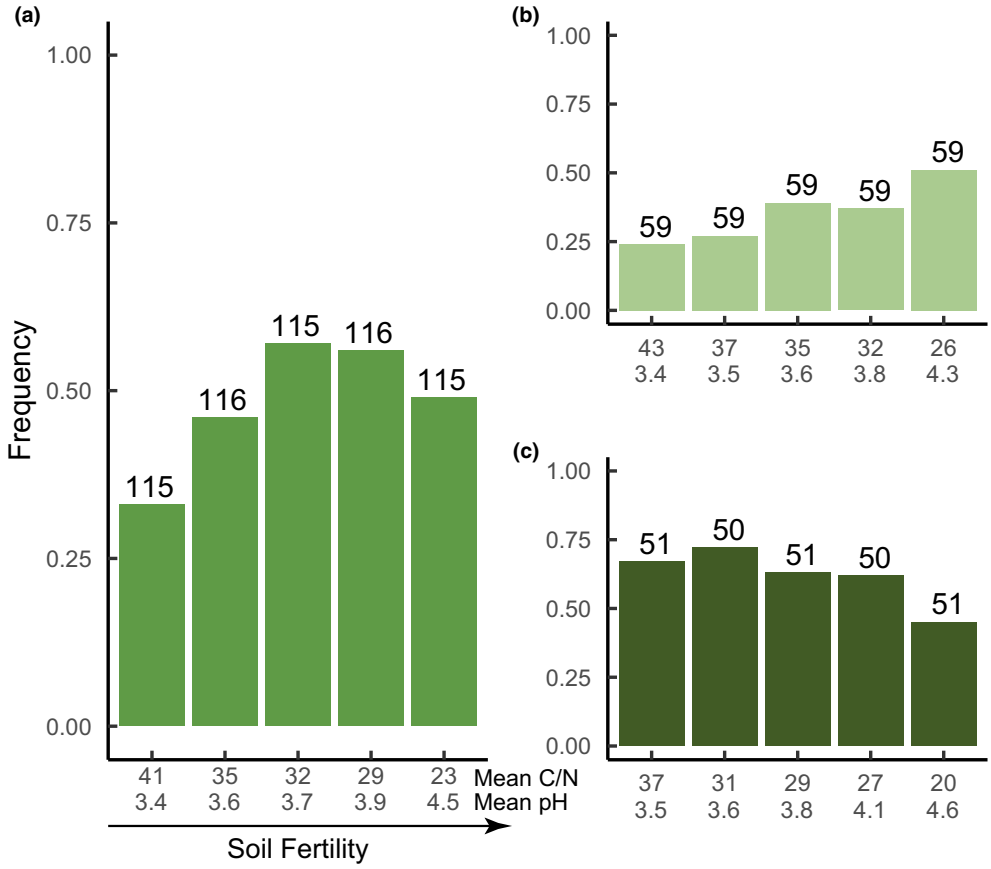


FIGURE 3 Frequency of occurrence of species assigned as ectomycorrhizal decomposers across soil fertility in (a) all forests, (b) pine-dominated and (c) spruce-dominated forests. Soil fertility is sectioned into five evenly distributed bins (for graphical representation) with their mean C/N and pH shown on the *x*-axis. Numbers on top of bars indicate the total number of forests in each soil fertility bin. Note that the range of mean C/N and pH is different between pine-dominated and spruce-dominated forests. Statistics are presented in Table 2.

Mn-peroxidase genes or expressed them under the observed conditions. This is not unexpected given that gene loss and duplication occurs at a slower pace than trait differentiation (Hess et al., 2021; Kohler et al., 2015). In particular, substantial production of Mn-peroxidases is not likely to be a general effect trait for all *Cortinarius* species, but has to be assessed at the species level, in concordance with Lindahl et al. (2021).

Fungal traits may be studied more directly and experimentally in pure culture (albeit under unrealistic conditions), but for fungi that are not amendable to isolation, correlations with field-measured properties, as used here, remain one of the few options. Although the fungal community composition explained a minor proportion of the variation in Mn-peroxidase activity, there was a link to the local composition of fungal species, as posed by our first hypothesis. The longevity of peroxidases in soil is largely unknown (Allison, 2006; Sinsabaugh, 2010), and the precision of our method depends on temporal and spatial synchronisation between Mn-peroxidase activity and the fungi that produce them. Localisation of extracellular enzymes to hotspots rather than an even distribution throughout mycelia may also contribute unexplained variation (Lindahl & Finlay, 2006), as supported by the observed lack of Mn-peroxidase spatial autocorrelation across the different spatial scales. Further, most species are infrequent, leading to low predictability. Presence of ITS sequences, especially at low relative abundances, could also be attributed to inactive fungal propagules. With this in mind, we selected a more lenient α value for our one-sided permutation tests, aiming to strike a balance between type-I and type-II error. Species from genera with a generally high number of Class-II peroxidase genes in genome-sequenced representative species did not necessarily have a high $WAE_{sp}$ (except *Gautieria* cf. *otthii*). Thus, assigning decomposition traits to unculturable fungi is possible via statistical inference, but there are certainly many less frequent ectomycorrhizal decomposers that were not detected, even with this large sampling effort. Despite challenges in assigning traits based on community-level enzyme activity and ITS relative abundances, we found some signal, especially for the more frequent taxa, which may anyway be the most important for understanding ecosystem processes.

The assignment of traits based on correlative data can be susceptible to false positives. However, the probability of correctly assigning a trait seems reasonably high, given that 10 out of 16 ectomycorrhizal species assigned in our permutation-based analysis of $WAE_{sp}$ also have evidence of genetic capacity within the genus, despite that most ectomycorrhizal fungi do not (Miyauchi et al., 2020).

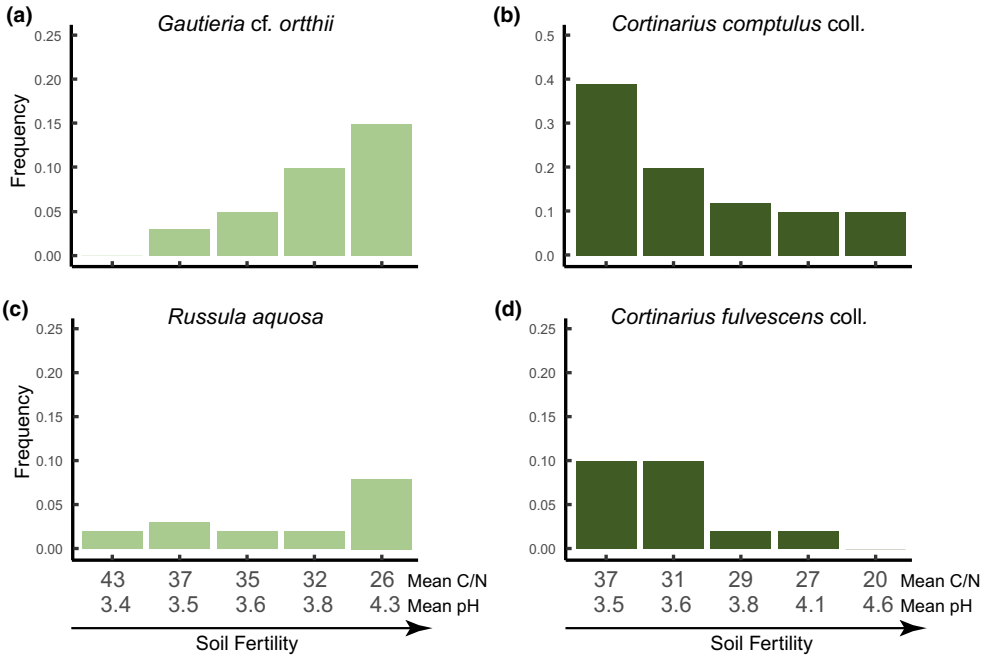


FIGURE 4 Frequency of occurrence (a) *Gautieria* cf. *otthii*, (b) *C. comptulus* coll., (c) *Russula aquosa* and (d) *C. fulvescens* coll. in pine-dominated (a, c; light-green) and spruce-dominated (b, d; dark-green) forests with varying soil fertility. Soil fertility is sectioned into five evenly distributed (59 pine-dominated forests or 50/51 spruce-dominated forests each) bins with the mean C/N and soil pH shown on the *x*-axis. Note that range of C/N and soil pH is different between pine-dominated and spruce-dominated forests. All show a significant relationship between frequency and soil fertility ($p$-value <0.05). Statistics are presented in Table S5.

Notably, *Suillus luteus* and several *Thelephorales* spp. were co-localised with Mn-peroxidase hotspots, yet do not have evidence of Class-II peroxidase genes. They may co-occur with peroxidase-producing fungi through several means of facilitation. Presumably, niche modification occurs during soil organic matter decomposition, as it does during wood decomposition (Hiscox et al., 2015). Furthermore, fungi lacking Mn-peroxidases may scavenge for degradation products of oxidative decomposition that they otherwise would not be able to access. In addition, *Suillus luteus*, which probably has decomposition mechanisms similar to Fenton reactions of 'brown-rot' fungi (Shah et al., 2016), could take advantage of $H_2O_2$ produced by Mn-peroxidase producers for their own non-enzymatic decay mechanisms (or vice versa, i.e. provide $H_2O_2$ that is hijacked by ectomycorrhizal decomposers).

## 4.2 | Niche(s) of ectomycorrhizal decomposers

We found no support for our hypothesis that previous clear-cutting would have long-lasting negative impacts on the presence of ectomycorrhizal decomposers. Actually, they occurred more frequently in mature secondary forests established after clear-cutting than in forests with mostly no history of clear-cutting. Despite that no consistent response to stand age was observed for all of the ectomycorrhizal decomposers in spruce-dominated forests, it was apparent that individual species tended to have their niches in either older or younger forests. Further, this varied between species in the same genus, highlighting that there is not only wide variation in response traits within genera but also that fungi with predicted similar effect traits (i.e. oxidative capacity) vary widely in their response to their environment (Koide et al., 2014). While some ectomycorrhizal decomposers, such as *Cortinarius acutus* s.l., are found increasingly present in older nutrient-poor forest stands (Lindahl et al., 2021), it is evident that this is not the rule for all. Splitting of stand age into categories of forests older or younger than 70 years explained less variation in the frequency of ectomycorrhizal decomposers than stand age as a continuous variable. Although both models had low explanatory power, this may be an important distinction in terms of forest management, as this suggests that, at least for the fungi investigated, whether a forest has been clear-cut or not may not be more important for fungal assemblages than the stand age per se.

While we expected, based on the prevalent view (Argiroff et al., 2022; Lilleskov et al., 2019; Lindahl et al., 2021), that ectomycorrhizal decomposers would be restricted to the least fertile soils, this was not the case. There was a unimodal response to soil fertility across all forests included in our study with a maximum at intermediate fertility. The pattern across all forests reflects that pine-dominated forest soils tend to be less fertile than spruce-dominated forest soils (pine mean C:N ratio = 34 ± 8, $n$ = 295; spruce mean C:N ratio = 29 ± 6, $n$ = 253), such that the upward slope in Figure 3a corresponds to increasing frequency in more fertile pine-dominated forest soils (Figure 3b) and the downward slope corresponds to decreasing frequency in more fertile spruce-dominated forest soils (Figure 3c). The decline of ectomycorrhizal decomposers in fertile spruce forest soils is in line with the idea that they are sensitive to higher available nitrogen, as in temperate forests (Argiroff

et al., 2022). Counter to our expectations, all individual ectomycorrhizal decomposer taxa, except *Cortinarius mucosus* coll., were more common in spruce-dominated forests, and many taxa tended to be more frequent in fertile soils, some significantly so. In the most acidic and least fertile soils, ectomycorrhizal decomposers may be replaced by ericoid mycorrhizal fungi or other more stress-tolerant fungi (Fanin et al., 2022; Sterkenburg et al., 2015). Pine forests also tend to have coarser soil textures, lending to drier and drought-prone soils, and ectomycorrhizal communities are influenced by these microclimatic conditions (Castaño et al., 2018). Yet, all these forests are within the organic nutrient cycling state (*sensu* Phillips et al., 2013), and it is possible that ectomycorrhizal decomposers, even in more nutrient-rich boreal forests, play an important role in maintaining soil fertility (Clemmensen et al., 2015; Jörgensen et al., 2024).

When present, the abundance of the ectomycorrhizal decomposers relative to the total fungal or ectomycorrhizal community did not significantly relate to stand age or forest soil fertility. This also points to limitations in modelling relative abundances of a small group of individual species. While relative abundance data may give an indication of whether these ectomycorrhizal fungi are present as just a few spores or as dense mycelium, our objective to assess the niche(s) of these fungi is similarly achieved through a frequency-based analysis, which indicates whether or not they are present in a given environment. Although some species may respond negatively to tree removal and high nutrient availability (as we hypothesised), there may be broad enough niche variation among ectomycorrhizal decomposers to sustain nutrient and carbon dynamics, even in previously clear-cut and/or nutrient-rich forests, as is also found to be the case with ectomycorrhizal fungi producing hydrolytic enzymes (Jones et al., 2010; Walker et al., 2016).

In conclusion, correlation-based analyses of community-level peroxidase activity and fungal community composition may expand our knowledge of key decomposer taxa and their niches, but several challenges remain, some of which may be addressed by metatranscriptomics (Auer et al., 2024). The effect trait of oxidative decomposition with Mn-peroxidases is likely not conserved within genera, which makes trait assignment through extrapolation from closely related species problematic, and a way forward may be to focus on the most frequent species. The group of 10 ectomycorrhizal decomposers assigned here may have the potential to influence C stocks and N cycling in boreal forests, as they were represented in nearly half of the samples. We found little support for our hypothesis that ectomycorrhizal decomposers, as a group, would have their niche in older forest stands with less fertile soils (at least not in the context of this study). Rather, our findings suggest that there is large niche variation among ectomycorrhizal fungi with oxidative decomposer capacity in boreal forests. Ectomycorrhizal decomposers are likely also constrained by the most acidic and nutrient-poor forest soils. Additionally, certain ectomycorrhizal decomposers (e.g. *Gautieria* species) may not only be adapted to higher fertility conditions but also maintain ecosystem fertility by contributing to the decomposition of persistent soil organic matter (Jörgensen et al., 2024). Furthermore, the trait of ectomycorrhizal oxidative decomposition has a degree of redundancy in relation to stand age and soil fertility, with different species maintaining this function in different forest types. Thus, stand-replacing forestry may not pose as much of a threat for ectomycorrhizal decomposition over the long term, as previously proposed (Kyaschenko et al., 2017; Lindahl et al., 2021).

## AUTHOR CONTRIBUTIONS

Björn D. Lindahl, Anders Dahlberg, Johan Stendahl and Erica E. Packard conceptualised the study. Leticia Pérez-Izquierdo performed field sampling and laboratory analyses of the regional dataset. Erica E. Packard compiled the data, performed all statistical analyses and wrote the first draft of the manuscript. Karina E. Clemmensen, Anders Dahlberg, Björn D. Lindahl, Erica E. Packard, Leticia Pérez-Izquierdo, Marie Spohn and Johan Stendahl contributed to the interpretation of results and revisions of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

## DATA AVAILABILITY STATEMENT

The raw sequence data analysed during this study are available on NCBI SRA under the accessions numbers: PRJNA693127 and PRJNA1088460, for national and regional datasets, respectively. The R code for this analysis is available at Zenodo: https://doi.org/10.5281/zenodo.15343882 (Packard, 2025).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Data S1:** Manganese peroxidase (MnP) and total peroxidase (Perox) activity per gram organic matter in soil samples collected for regional study.

**Table S1:** Descriptions of the 12 regional sites located in South-Central Sweden.

**Table S2:** List of genera with significantly higher estimated specific Mn-peroxidase and/or total peroxidase activity than expected by random variation tested each with a one-sided permutation test.

**Table S3:** List of taxa with significantly lower estimated specific Mn-peroxidase and/or total peroxidase activity than expected by random variation tested each with a one-sided permutation test.

**Table S4:** Results of Fisher's exact test of differences in frequency of individual ectomycorrhizal decomposers in pine- or spruce-dominated forest.

**Table S5:** Correlations between frequency of occurrence of species assigned as ectomycorrhizal decomposers and stand age and soil fertility analysed by generalized linear models.

**Table S6:** Correlations between combined relative abundance of the assigned ectomycorrhizal decomposers, when present, and stand age and soil fertility with sequencing platform as a random effect analysed by linear mixed model.

**Table S7:** Correlations between combined relative abundance of the assigned ectomycorrhizal decomposers among ectomycorrhizal fungi, when present, and stand age and soil fertility with sequencing platform as a random effect analysed by linear mixed model.

**Figure S1:** Sampling design at each of the twelve sites from the small-scale dataset.

**Figure S2:** Distribution of 12 small-scale (inset) and 577 national sampling sites within Sweden.

**Figure S3:** Manganese peroxidase activity (change in absorbance per minute per gram of organic matter across the 12 regional plots).

**Figure S4:** Principal Components Analysis of N/C ratio, soil pH, spruce percent basal area, and stand age of 548 large-scale samples.

**Figure S5:** Frequency of occurrence of select individual ectomycorrhizal decomposers across forest stand age in spruce-dominated forests.

**Figure S6:** Differences in (a) frequency of occurrence of the assigned ectomycorrhizal decomposer species group and (b) relative abundance of the assigned ectomycorrhizal decomposers as a proportion of the total fungal community, when present between dominant tree species.

**Figure S7:** Frequency of occurrence of individual assigned ectomycorrhizal decomposer species in pine- versus spruce-dominated forests.

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