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RESEARCH ARTICLE

Ectomycorrhizal necromass turnover is one-third of biomass turnover in hemiboreal *Pinus sylvestris* forests

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Societal Impact Statement

Efficient mitigation of climate change requires predictive models of forest ecosystems as sinks for atmospheric carbon. Mycorrhizal fungi are drivers of soil carbon storage in boreal forests, yet they are typically excluded from ecosystem models, because of a lack of information about their growth and turnover. Closing this knowledge gap could help us better predict future responses to climate change and guide policy decisions for sustainable management of forest ecosystems. This study provides new estimates of the production and turnover of mycorrhizal mycelial biomass and necromass. This information can facilitate the integration of mycorrhizal fungi into new predictive models of boreal forest soils.

Summary

- In boreal forests, turnover of biomass and necromass of ectomycorrhizal extraradical mycelia (ERM) are important for mediating long-term carbon storage. However, ectomycorrhizal fungi are usually not considered in ecosystem models, because data for parameterization of ERM dynamics is lacking.
- Here, we estimated the production and turnover of ERM biomass and necromass across a hemiboreal *Pinus sylvestris* chronosequence aged 12 to 100 years. Biomass and necromass were quantified in sequentially harvested in-growth bags, and incubated in the soil for 1–24 month, and Bayesian calibration of mathematical models was applied to arrive at parametric estimates of ERM production and turnover rates of biomass and necromass.
- Steady states were predicted to be nearly reached after 160 and 390 growing season days, respectively, for biomass and necromass. The related turnover rates varied with 95% credible intervals of 1.7–6.5 and 0.3–2.5 times yr^{-1}, with mode values of 2.9 and 0.9 times yr^{-1}, corresponding to mean residence times of 62 and 205 growing season days.
- Our results highlight that turnover of necromass is one-third of biomass. This together with the variability in the estimates can be used to parameterize ecosystem models, to explicitly include ERM dynamics and its impact on mycorrhizal-derived soil carbon accumulation in boreal forests.

KEYWORDS
chitin, degradation, ergosterol, extramatrical mycelium, extraradical mycelium, fungal biomass, fungal necromass, mycorrhiza

1 | INTRODUCTION

Boreal forests store approximately half of the global forest ecosystem carbon (C) stock (Malhi et al., 1999), of which 60% is stored in the soil (Pan et al., 2011). The soil C stock is determined by the balance between input and loss of C from the soil, and because of their potential to sequester atmospheric C, boreal forest soils are regarded as a critical component for developing forest-based climate mitigation strategies (Ameray et al., 2021). Primary production drives the input of organic matter into the soil which is sourced from above- and below-ground litter depositions. Boreal trees allocate photoassimilated C to aboveground parts to promote the competition for light through growth. However, the majority, approximately 60% of the C is, allocated to belowground to support roots and mycorrhizal associations (Gill & Finzi, 2016; Litton et al., 2007). This allocation provides mycorrhizal fungi with direct access to carbohydrates, enabling them to form extensive extraradical mycelia (ERM) that explore the soil matrix to enhance tree's access to soil nutrients and water (Smith & Read, 2008). Ectomycorrhiza is the dominant mycorrhizal type in boreal forests and plays a central role in nutrient cycling, organic matter accumulation, and storage (Clemmensen et al., 2013; Kyaschenko et al., 2019; Smith & Read, 2008).

Whereas some groups of ectomycorrhizal fungi produce exoenzymes that degrade stable soil organic matter to mobilize organic nitrogen (N) (Bödeker et al., 2014; Lindahl et al., 2021), ectomycorrhizal fungi also contribute to soil organic matter accumulation through two interacting mechanisms. Firstly, ectomycorrhizal fungi produce ERM that may resist decomposition (Fernandez et al., 2016; Fernandez & Koide, 2014). Secondly, they immobilize N in their biomass which decreases soil N availability and suppresses the activity of free-living saprotrophic decomposers (Fernandez & Kennedy, 2016; Näsholm et al., 2013). The contribution of recalcitrant ERM to soil and the restricted N availability together drive the accumulation of fungal litter, and in boreal forest soils, the majority of the accumulated soil organic matter (humus) is derived from roots- and mycorrhizal fungal residues (Clemmensen et al., 2013; Godbold et al., 2006; Kyaschenko et al., 2019).

While production of ERM biomass by ectomycorrhizal fungi in boreal forests often exceeds several hundred kilograms per hectare per year (Ekblad et al., 2013) and represents a large C input into the soil (Cairney, 2012; Wallander et al., 2013), the turnover rate of ERM biomass coupled with the decomposition of ERM necromass (i.e. turnover of dead biomass) determines the persistence of fungal residues in soil (Fernandez & Kennedy, 2015; Langley & Hungate, 2003). Limited data is available on ERM necromass turnover across ecological gradients (Ekblad et al., 2016). ERM biomass, however, is typically reported to have a turnover of about 10 times per year, but measures range from 20 times per year to about one time per year (Cheeke et al., 2021; Ekblad et al., 2016; Hagenbo et al., 2017, 2018, 2021; Hendricks et al., 2016), with a relatively faster turnover in warm-temperate conditions compared to boreal conditions.

The apparent importance of ERM for the accumulation of soil organic matter and the information of a generally rapid ERM biomass turnover, together imply that restricted necromass decomposition (i.e. slow necromass turnover) may be a main contributing factor behind ectomycorrhiza-mediated soil organic matter accumulation. However, the quantitative relationship between turnover of biomass- and necromass is uncertain, as their combined estimation has only been obtained from a single *Pinus taeda* plantation in which turnover of biomass and necromass, respectively, were 13.1 and 1.5 times year^{-1} (28 to 243 days residence time; Ekblad et al., 2016).

While current soil C models can describe present soil C dynamics, their ability to predict future ecosystem responses to climate change and forest management practices is constrained by the lack of explicit and mechanistically accurate consideration of microbial processes in soil. For example, the lack of basic data on ERM-related soil C fluxes (particularly ERM growth and turnover) restricts quantitative analyses of mycorrhizal impacts on ecosystem function and soil C storage (Deckmyn et al., 2014; Orwin et al., 2011). This limits our ability to provide information for making well-informed policy decisions concerning the sustainable management of forest ecosystems and developing effective strategies for forest-based climate change mitigation. Therefore, further information about ERM biomass- and necromass dynamics is required to facilitate the incorporation of mycorrhizal-mediated processes into ecosystem models and improve quantitative modeling of microbial impacts on soil C dynamics.

Here, we simultaneously assessed ERM biomass and necromass dynamics to compare their quantitative contributions to belowground soil C pools and fluxes. We hypothesized that the turnover rate of ERM biomass would be greater than the turnover rate of ERM necromass. This hypothesis was inferred from two basic principles: 1) ectomycorrhizal ERM biomass has a short mean residence time (often 1–2 months) due to a relatively fast biomass turnover (Cheeke et al., 2021; Ekblad et al., 2016; Hagenbo et al., 2017, 2021; Hendricks et al., 2016) and 2) ectomycorrhizal fungi contribute to soil C accumulation through residual litter input (Clemmensen et al., 2013; Godbold et al., 2006; Kyaschenko et al., 2019), thus the contribution of ERM to soil C pools should be primarily driven by a slower rate of necromass turnover rather than the process of biomass turnover. We further hypothesized that necromass turnover would correlate with biomass turnover, resulting in a forest age-related decrease in necromass turnover and thus an increased contribution of necromass C to the accumulation of soil organic C. This hypothesis was drawn from previous work reporting declining ERM biomass turnover with increasing forest age (Hagenbo et al., 2017, 2018). It relies on the

assumption of an increased prevalence of recalcitrant soil organic matter, as suggested by the shifts in fungal community composition toward increased abundance of ectomycorrhizal taxa with oxidative enzymes (Kyaschenko et al., 2017), capable of mobilizing nutrients from biochemically demanding matter such as humus (Lindahl & Tunlid, 2015). To test these hypotheses, we used a hemiboreal chronosequence of seven even-aged *P. sylvestris* forest stands, ranging in age from 12 to 100 years. Estimates of ERM biomass and fungal mass were obtained from sequentially harvested ingrowth mesh bags, capturing mycelial growth and accumulation. The biomass was based on ergosterol measurements sourced from Hagenbo et al. (2018) and combined with chitin-derived estimates of fungal mass to estimate the stock of ERM necromass according to Ekblad et al. (2016). We performed Bayesian modeling to simultaneously determine biomass production, biomass turnover, and necromass turnover while considering potential variation in chemical conversion factors from chitin to fungal mass, and from ergosterol to fungal biomass. The parametric estimates obtained from the model fitting were interpreted as production and turnover estimates. The ERM biomass and mass estimates were supplemented with measurements of the C content of the mesh bags to allow the quantification of the contribution of fungal-derived C to the accumulation of total C in the mesh bags. Finally, the fraction of non-hydrolyzable organic C in ERM was assessed to test for recalcitrant ERM traits across the chronosequence.

2 | MATERIALS AND METHODS

2.1 | Site descriptions

The study was conducted along a hemiboreal chronosequence consisting of eight managed *Pinus sylvestris* L. stands, ranging in age from 12 to 100 years, situated in Uppsala County, central Sweden (Hagenbo et al., 2017; Kyaschenko et al., 2017). The stands were unfertilized, evenly aged, and shared similar water availability and site/fertility index (Hägglund, 1973). Soils at all sites were classified as Umbric podzols and composed of sandy loamy till with a high occurrence of boulders. The length of the growing season (mean temperature above 5°C) is approximately 185 days and spans from mid-April to late October. We refer to Hagenbo et al. (2017) for further site information.

2.2 | Mesh bags and experimental design

To assess ERM necromass dynamics we used a mesh bag setup for which we previously published data on mycelial biomass production and turnover (Hagenbo et al., 2017). Mycorrhizal fungal ERM was sampled from soil by field incubation of cylindrical nylon mesh bags (100 × 20 mm). The nylon mesh (50 µm) allows ingrowth of fungal hyphae but not of roots (Wallander et al., 2001). As mesh bags were filled with 40 g silica sand (0.36–2.0 mm, 99.6% SiO2, Silversand, Sibelco Nordic AB) and thus lacked organic C, they selected for mycorrhizal fungal ingrowth (Hagenbo et al., 2018; Parrent & Vilgalys, 2007; Wallander et al., 2001, 2010), and allowed standardized measurements across the chronosequence (Wallander et al., 2013). The bags were inserted at a 45° angle and to a depth of approximately 7 cm in the top humus layer beneath the organic fermentation layer, which is dominated by mycorrhizal fungi in boreal forest soils (Lindahl et al., 2007). Given the selection of a C-free growth substrate (sand) and the placement of mesh bags in the soil, the mesh bags discriminated against saprotrophic fungal ingrowth while favoring the ingrowth by ecto- and ericoid mycorrhizal fungi (Hagenbo et al., 2018). Furthermore, the previous analysis of the fungal community composition of the mesh bags (Hagenbo et al., 2018), indicated that the bags did not select for any mycorrhizal exploration types (Agerer, 2001). The bags were incubated over different overlapping incubation periods to enable the capturing of ERM biomass and necromass dynamics (Ekblad et al., 2016). Between 2012 and 2014, seven sets of mesh bags, each representing different periods, were incubated at each site (Figure 1), in five subplots (2 × 2 m; 10–50 m apart) to account for within-site variation in environmental conditions. After harvest, the collected bags were immediately replaced by new

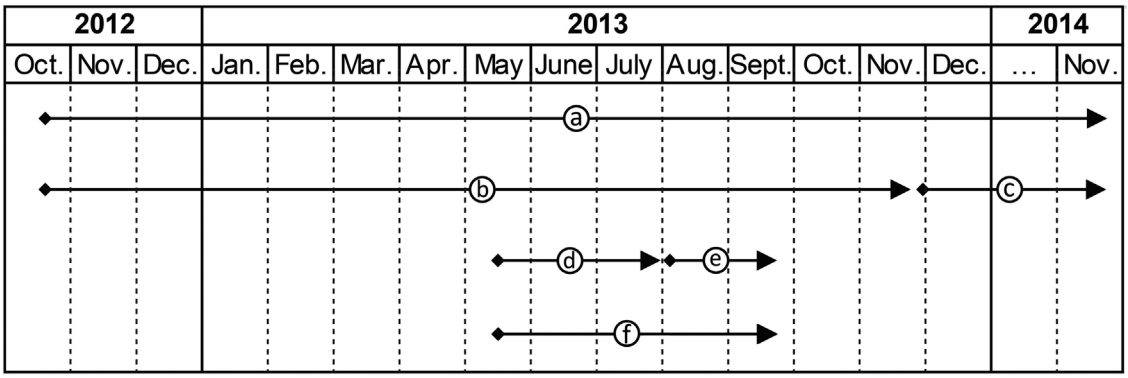

FIGURE 1 Incubation scheme for ingrowth mesh bags harvested between October 2012 and November 2014 in a *Pinus sylvestris* forest stand age gradient, used for capturing ectomycorrhizal extraradical mycelial biomass and necromass dynamics. Beginning of arrows indicates mesh bags installation and the end of arrows indicates time points of harvest. Letters annotate mesh bags with different incubation periods. Five bags were incubated during each period to cover spatial heterogeneity within stands.

bags which were inserted into the same holes to minimize soil disturbance. All bags were immediately stored in the dark and transferred to −20°C storage within a few hours after collection. The bags were then freeze-dried and contents representing the same site and incubation period were pooled and thoroughly ground, using a pestle and mortar, and stored at −20°C until further analyses.

2.3 | Chemical analyses and estimation of fungal biomass, total mass, and necromass

Ergosterol, a cell-membrane lipid found in living fungal cells, was used as a proxy for fungal biomass and was extracted according to Nylund and Wallander (1992), and chromatographically analyzed as described by Hagenbo et al. (2017). Chitin-derived glucosamine was used as a proxy for total fungal mass (Lezica & Quesada-Allué, 1990). As a component of the cell wall, chitin has been shown to correlate with fungal biomass (Wallander et al., 2013). However, rather than representing biomass alone, chitin may instead be viewed as a proxy for fungal mass (biomass + necromass) prior to its transformation into humus (Ekblad et al., 1998, 2016; Wallander et al., 2013). While decomposition studies have shown that chitin may degrade more rapidly relative to other fungal cell components (Drigo et al., 2012; Fernandez & Koide, 2012; Trofymow et al., 1983), other studies have reported chitin to be recalcitrant and may result in a build-up of fungal necromass in soil (Joergensen & Wichern, 2008; Langley & Hungate, 2003). This inconsistency between studies may stem from fungal compounds, particularly chitin, forming stable complexes with condensed tannins from plants, rather than the compound per se being recalcitrant (Adamczyk et al., 2019; Kraus et al., 2003).

Extraction of chitin-derived glucosamine was done by placing a 16 g subsample in 20 ml of 6 N hydrochloric (HCl) acid for 6 h at 100°C in sealed sample tubes (Ekblad & Näsholm, 1996). Sand and extract were then separated using a 1,003 grade filter paper (Munktell, Ahlstom, Helsinki, Finland), with a particle retention of 12 to 15 µm. The remaining sand was rinsed for remaining hydrolyzable residues with an additional 15 ml 6 N hydrochloric acid which was combined with the first extract. The filter paper and the remaining sand, containing unhydrolyzable residues, were separated after drying whereupon the sand content was transferred to a glass vial and stored at −20°C, until further analyses. The extract was filtered through a 0.45 µl polyethersulfone syringe filter (Filtropur S 0.45, Sarsted, Nümbrecht, Germany) and dried using a centrifugal vacuum evaporator (SpeedVac SPD111V, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and transferred to −20°C storage.

Glucosamine content was quantified using electrospray ionization-tandem mass spectrometry, as described by Olofsson and Bylund (2016). The instrumental setup consisted of an isocratic pump (Shimadzu LC-10 AD, Kyoto, Japan), mobile phase degasser (Uniflows, Tokyo, Japan), autoinjector (Agilent 1,100, Santa Clara, California, USA), ZIC-HILIC analytical column (150 × 2.1 mm, 5 µm, SeQuant, Umeå, Sweden), and a mass spectrometer (API3000, AB Sciex, Concord, Canada).

Ergosterol and chitin-derived glucosamine contents were converted to fungal biomass and total fungal mass, respectively, using conversion factors of 3.0 and 44.0 µg mg^{-1} (Ekblad et al., 1998, 2016; Salmanowicz & Nylund, 1988). The difference in efficiency between ergosterol and chitin extractions was compensated for by multiplying the extracted ergosterol and chitin amounts by 1.61 and 1.41, respectively (Montgomery et al., 2000; Alf Ekblad, pers. comm). The ERM necromass in samples was estimated as the difference between estimated fungal mass and biomass (Ekblad et al., 2016).

In sand samples subjected to acid hydrolysis, and in control samples (not subjected to acid hydrolysis) content of C was determined using a Trumac CN (Leco Corporation, St. Joseph, Michigan, USA). The C contents in hydrolyzed samples were interpreted as stable C and the ratio between the content of non-hydrolyzable C and total C was interpreted as the stable C fraction, hereafter referred to as the non-hydrolyzable C fraction. All C in the mesh bags was interpreted as organic C, as all forest stands had acidic soils (Kyaschenko et al., 2017), and were therefore assumed to not contain inorganic C (carbonates).

2.4 | Bayesian modeling and estimation of production and turnover of biomass and necromass

Production of biomass and turnover of biomass and necromass were estimated using Bayesian model fitting according to Ekblad et al. (2016), where two models describing biomass and necromass dynamics are fitted to data sets of biomass and necromass in mesh bags over time. The two models assume stable production and turnover rates and we refer to Ekblad et al. (2016) for further information. Bayesian inference was selected to allow simultaneous model fitting while considering uncertainties related to the ergosterol and chitin conversion factors. The models (Equation 1 and 2) describe the temporal change in biomass ($B[t]$) and necromass ($N[t]$) as a function of biomass production (P), biomass turnover rate (μ), necromass turnover rate (k), and incubation time of mesh bags (t), according to:

$$B(t) = \frac{P}{\mu}\left(1 - e^{-\mu t}\right) \quad \text{(Eqn1)}$$

$$N(t) = \frac{P}{k}\left(1 - e^{-kt}\right) - \frac{P}{k-\mu}\left(e^{-\mu t} - e^{-kt}\right) \quad \text{(Eqn2)}$$

The two models were simultaneously fitted to estimate all the parameters. Model fitting was performed in R v3.5.1 (R Core Team, 2017), within a Bayesian framework written in JAGS (Plummer, 2003), adopting a Metropolis-Hastings sampler of four separate Markov chains Monte Carlo. Each model was calibrated based on 100,000 runs for each of the four chains. In the calibrations, P, μ and k were assumed to vary according to uniform a priori probability distributions, ranging between 0 and 1,000 kg ha^{-1}, 0.001–25 times yr^{-1} and 0.0001–5.0 times yr^{-1}, respectively, based on previous estimates of ERM production and turnover (Ekblad et al., 2016; Hagenbo

et al., 2018, 2021; Hendricks et al., 2016). Uniform distribution was selected as it does not rely on assumptions of the distributions of *P*, μ, and *k*, thus allowing more conservative estimation of their parametric values, and the wide ranges were selected to avoid excessive model constraints. Since ergosterol and chitin per unit biomass have been shown to vary with mycelial age and between species (Hagerberg et al., 2003; Montgomery et al., 2000; Wallander et al., 2013), we also assumed a priori probability distribution for the conversion factors of ergosterol to fungal biomass and chitin to fungal mass. This was done to reduce model constraints and to compensate for potential variability in conversion factors across the study area and over the season. The conversion factors were assumed to be normally distributed and therefore represented by a Gaussian distribution with a mean of 3 μg mg^{-1} and 44 μg mg^{-1} and standard deviations of 0.367 and 0.104 for ergosterol and chitin, respectively (Ekblad et al., 1998, 2016). We assumed a larger variability in ergosterol per unit fungal biomass based on variability in available estimates (Djajakirana et al., 1996; Montgomery et al., 2000), whereas chitin appears to be more stable across conditions (Ekblad et al., 1998). Following the Bayesian modeling procedure, new conversion factors were obtained for ergosterol and chitin per unit biomass and mass, respectively. These conversion factors were applied to yield Bayesian estimates of biomass and necromass, which were implemented in the Bayesian fitting of Equation 1 and Equation 2 to describe biomass and necromass dynamics.

To test the effect of forest age on ERM necromass dynamics, the Bayesian calibration was performed for data subsets from young forests (12 and 19 years), intermediately aged forests (34, 46, and 59 years), and mature forests (80 and 100 years). The Bayesian model was also run over the whole dataset to assess overall necromass dynamics over the full chronosequence. After the Bayesian calibration, biomass production and turnover of biomass and necromass were estimated according to the mode of the Markov chains (i.e. the density function of the parametric estimates). Mode is the value that appears most often in a dataset and offers a more robust estimate than the mean for data with skewed distributions. Variability of the parametric estimates (i.e. biomass production, biomass turnover, and necromass turnover) was assessed by calculating the 95% credibility intervals of the Markov chains. Forest age-related differences in biomass and necromass in mesh bags were tested using multiple linear regression, where the differently aged forest stands were included as categorical factors to account for non-linear variation between stands.

2.5 | Modeling of fungal C dynamics

As the degradation of necromass progresses, it eventually transforms into a more stable form through humification. To evaluate the contribution of necromass to the accumulation of stable soil organic matter, the temporal development of C derived from fungal ERM (hereafter referred to as fungal-derived C) was assessed according to Equation 3, based on the measured total C content (C_{total}) and the estimated residual C content ($C_{residual}$):

$$C_{fungal-derived} = C_{total} - C_{residual} \quad \text{(Eqn3)}$$

Fungal-derived C represents the total amount of C derived from biomass, necromass, and decayed necromass, for which chitin may be degraded, thus not detectable from combined ergosterol and chitin measurements. The residual C content is the content of C that could not be assigned to be derived from biomass or necromass, e.g. C from dissolved organic C including exudates from roots and ERM. The residual C content was calculated based on the difference between the total C content and the combined content of C derived from biomass and necromass, from mesh bags with the shortest duration of incubation (43 days), according to Equation 4.

$$C_{residual} = C_{total\,(43\,d)} - \left(C_{bio\,(43\,d)} + C_{necro\,(43\,d)}\right) \quad \text{(Eqn4)}$$

Negligible turnover of necromass was assumed for mesh bags with 43 days incubation, and therefore the differences between the total C and the combination of biomass C (C_{bio}) and necromass C (C_{necro}) were assumed to represent C leached into the bag from surrounding soil matrix and/or exudates from ERM. For the calculations, we assumed a biomass C content of 44% (Zhang & Elser, 2017) and a necromass C content of 50%. Residual C was assumed to vary across sites but to be similar for mesh bags with different incubation duration, i.e. the stocks of C leachates in the bag were presumed to reach a steady-state equilibrium after one month in the field. This assumption was based on the fact that the mesh bags were incubated in the topsoil layer, thus limiting the flow of dissolved organic C with water percolation from above the soil layers. The different C forms referred to in this study are summarized below, with Figure 2 providing a visual representation.

1. Total C: content of C in mesh bags.
2. Biomass C: ergosterol-derived estimates of ERM biomass multiplied by 0.44.
3. Necromass C: ergosterol- and chitin-derived estimates of ERM necromass multiplied by 0.5
4. Residual C: content of C that could not be assigned to be derived from biomass or necromass in mesh bags with 43 days of incubation.

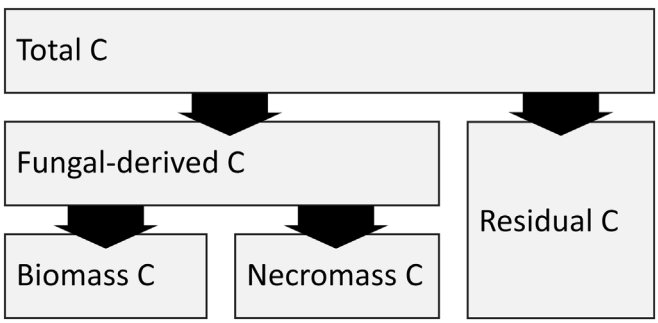

FIGURE 2 Graphical representation of the various carbon (C) forms associated with the total C content in mycelial ingrowth mesh bags. Each C form is represented by a non-hydrolyzable and acid-hydrolyzable C fraction, which has been omitted from the figure to enhance readability.

5. Fungal-derived C: The difference between total C and residual C.
6. Hydrolyzable C: Fraction of total C dissolvable in 6 N HCl.

Estimates of production and turnover of fungal-derived C and total C were obtained by fitting Equation 1 to two the respective sets of data using the R-package MINPACK-LM for nonlinear least squares fitting (Elzhov et al., 2016). These estimates, along with parametric estimates from the Bayesian model fitting of biomass C and necromass C production and turnover, are all included in Table 1 for comparison.

3 | RESULTS

3.1 | Biomass and necromass increased with incubation time and varied with forest age

Fungal biomass in mesh bags significantly increased with the incubation time of the mesh bags across all sites (adjusted $R^2 = 59.9\%$; $P < 0.001$) and was significantly higher in 34- and 80-years-old *P. sylvestris* forest ($P = 0.026$ and 0.041; Table 2) relative to the 12-year-old forest. Furthermore, necromass also increased with incubation time across all stands (adjusted $R^2 = 36.5\%$; $P < 0.001$) and reached a significant maximum ($P = 0.029$) in the 80-year-old forest (Table 2).

3.2 | Turnover of necromass was three times slower than the turnover rate of biomass

Turnover rate of necromass was approximately three times slower than the turnover rate of biomass, based on overall biomass and necromass dynamics over the whole chronosequence (12–100 years; Figure 3; Table 1). Specifically, the mode of the production estimate was 269 kg ha^{-1} (1.5 kg ha^{-1} day^{-1}) over the growing season (185 days), and the turnover rates for biomass and necromass were 2.9 and 0.9 times yr^{-1} (Figure 3a-c; Table 1), corresponding to a mean residence time of 63 and 210 growing season days, respectively. The biomass- and necromass functions (equation 1 and equation 2, respectively) parameterized by the production and turnover estimates, resulted in models that explained 50%, 58%, and 43% of the temporal changes in biomass, total mass (necromass + biomass) and necromass (Figure 4a-c). Based on the parameterization, our models suggest that ERM biomass and necromass would be close to a steady state (defined as when the increase in biomass or necromass is <1% wk^{-1}) at 160 and 390 growing season days, respectively. Corresponding to an ERM biomass of 85 kg ha^{-1} and ERM necromass of 238 kg ha^{-1}.

According to the calibration of the biomass and necromass functions, the conversion factor of 3 µg ergosterol mg^{-1} fungal biomass varied with 95% credibility interval from 1.9–3.4, corresponding to ± 30% of the mode of the density function of 2.6 µg ergosterol mg^{-1} fungal biomass (Figure 3d). Conversely, the conversion factor of 44.0 µg glucosamine mg^{-1} fungal mass varied with 95% credibility interval between 43.8 and 44.2 and the mode was 44.0 µg mg^{-1} fungal mass (Figure 3e).

Forest age-related variation in biomass and necromass dynamics was not possible to analyze since the models (Equations 1 and 2) failed to find a stable solution (i.e. lack of convergence) when applied to subsets of data from young forests (12–19 years), intermediate-aged forests (34–59 years), or mature forests (80–100 years).

3.3 | Total C and fungal-derived C in mesh bags increased with incubation time

The total carbon (C) content and fungal-derived C content in mesh bags increased with the incubation time of the mesh bags (Table 2). Fitting Equation 1 to the measured total C content and estimated fungal-derived C content in the mesh bags (Figure 5) resulted in parametric production estimates (Table 1). For fungal-derived C, the estimated production rate was 0.8 kg C ha^{-1} day^{-1} (144 kg C ha^{-1} yr^{-1}), and for total C, the estimate was 2.8 kg C ha^{-1} day^{-1} (504 kg C ha^{-1} yr^{-1}). Based on this model fit, the turnover estimates were 0.4 and 0.7 times yr^{-1} for fungal-derived C and total C, respectively, corresponding to a mean residence time of 1847 and 129 days (Table 1). In mesh bags with 43 days of incubation, an average C content corresponding to 97.8 kg ha^{-1} could not be assigned to biomass or necromass. This residual C content varied between 71.3 and 120 kg ha^{-1}. The hydrolyzable organic C fraction in mesh bags remained relatively stable with incubation time and forest age (Figure 6).

	Production rate (kg day^{-1})*		Turnover (times yr^{-1})*		
	Estimate	SE (±)	Estimate	SE (±)	Mean residence time (days)
ERM biomass C	0.64	0.31	2.92	1.25	63
ERM Necromass C			0.88	0.57	210
Fungal C†	0.78	0.38	0.10	0.41	1847
Total C	2.76	1.51	1.43	0.70	129

TABLE 1 Overview of production and turnover estimates of ectomycorrhizal extraradical mycelial (ERM) biomass carbon (C), necromass C, fungal-derived C, and total C. The data are from ERM ingrowth mesh bags that were incubated in *Pinus sylvestris* forests.

*The production and turnover values are scaled to represent the period of the growing season, April–October (185 days).
†Fungal C represents the difference between total C and residual C (i.e. content of C derived from sources other than biomass and necromass).

TABLE 2 Multiple linear regressions of mesh bag's contents of ectomycorrhizal extraradical mycelial (ERM) biomass, necromass, total C and fungal-derived C and their relationships with mesh bags' incubation duration (growing season days) and forest age (expressed as categorical variables). The data are from ERM ingrowth mesh bags that were incubated in *Pinus sylvestris* forests.

Term	Estimate	Std. error	T-value	P-value
ERM biomass (kg ha^{-1})				
Intercept	11.8	11.6	1.02	0.317
Incubation duration (days)	0.24	0.0326	7.24	**<0.001**
Forest age				
19	−13.0	14.3	−0.91	0.371
34	35.3	15.1	2.33	**0.026**
46	23.6	14.3	1.65	0.109
59	14.3	14.3	1.00	0.325
80	30.5	14.3	2.13	**0.041**
100	22.5	14.3	1.57	0.126
ERM Necromass (kg ha^{-1})				
Intercept	21.8	34.5	0.63	0.532
Incubation duration (days)	0.44	0.0966	4.59	**<0.001**
Forest age				
19	57.0	42.4	1.35	0.188
34	19.5	44.7	0.44	0.665
46	−0.6	42.4	−0.01	0.988
59	34.9	42.4	0.82	0.416
80	96.6	42.4	2.28	**0.029**
100	7.1	42.4	0.17	0.868
Total C (kg ha^{-1})				
Intercept	146.7	31.4	4.67	**<0.001**
Incubation duration (days)	0.6202	0.0880	7.05	**<0.001**
Forest age				
19	−51.5	38.6	−1.33	0.191
34	3.7	40.7	0.09	0.929
46	−23.5	38.6	−0.61	0.546
59	−46.4	38.6	−1.20	0.238
80	0.2	38.6	0.00	0.996
100	−19.5	38.6	−0.51	0.617
Fungal C (kg ha^{-1})				
Intercept	0.3302	0.0631	5.23	**<0.001**
Incubation duration (days)	0.0010	0.0002	5.88	**<0.001**
Forest age				
19	−0.0738	0.078	−0.95	0.348
34	0.0172	0.082	0.21	0.834
46	−0.0985	0.078	−1.27	0.213
59	0.0217	0.078	0.28	0.781
80	0.0273	0.078	0.35	0.727
100	0.1437	0.078	1.85	0.073

Note: Significant values (P < 0.05) are highlighted in bold. Adjusted R^2 = 59.9, 36.5% 53.7%, and 50.5% respectively for the biomass, necromass, total C, and fungal C regressions, n = 41.

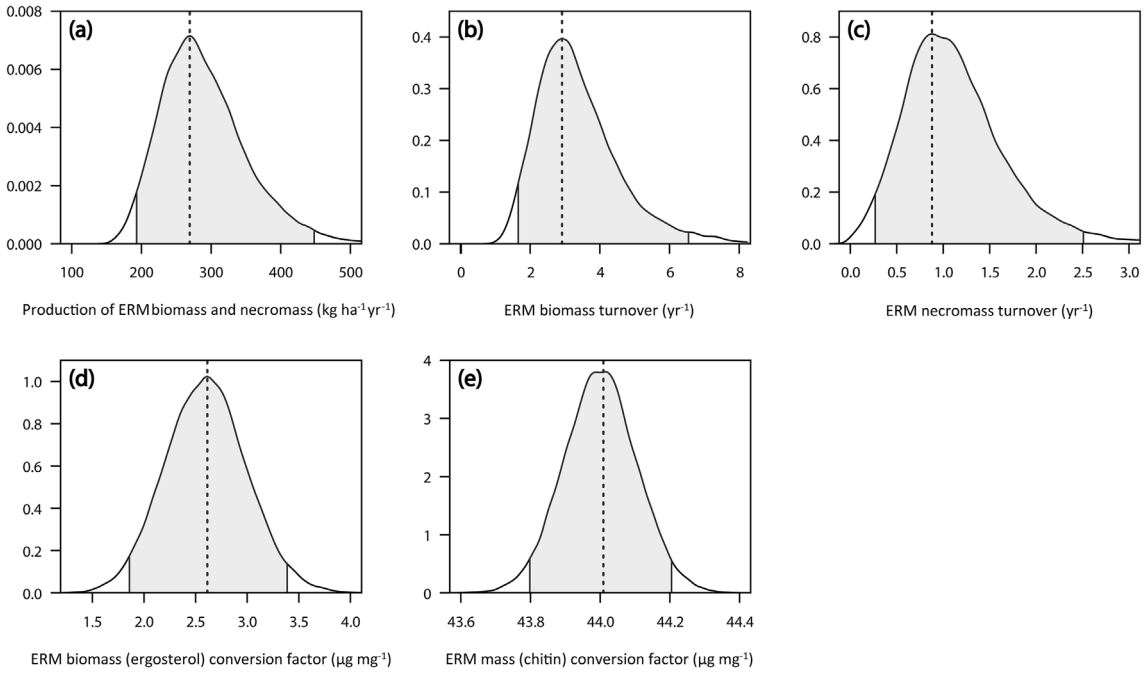

FIGURE 3 Probability density functions of parametric estimates from Bayesian modeling for (a) production of extraradical mycelium (ERM) biomass and necromass, (b) turnover of ERM biomass, (c) turnover of ERM necromass, (d) biomass (ergosterol) conversion factors, and (e) fungal mass (chitin) conversion factors. Dashed lines represent the modes of the functions, and shaded areas indicate limits of the 5th and 95th percentile of the credibility intervals.

4 | DISCUSSION

4.1 | Turnover of necromass is three times slower than turnover of biomass

Here we simultaneously quantified production and turnover of ERM biomass and necromass across a hemiboreal chronosequence of *P. sylvestris* forest stands. In agreement with our first hypothesis, the turnover of ERM biomass was three times more rapid compared to the turnover of ERM necromass. Consequently, the C allocated to ERM has a three-fold longer residence time in the necromass than in the biomass, and variation in necromass turnover (i.e. decomposition rate) has the potential to represent a stronger control on ERM-mediated soil C pools in boreal forest. The fitting of our models suggests that ERM biomass in the upper humus layer would be close to a steady state (i.e. when the increment of biomass is <1% wk^{-1}) at 85 kg ha^{-1}, after approximately 160 growing season days (<1 full year), while ERM necromass would reach a steady-state at 238 kg ha^{-1}, around 390 growing season days (i.e. > 2 full years). However, due to root-derived tannins potentially complexing with fungal proteins and chitin to stabilize the ERM necromass (Adamczyk et al., 2019), the point of steady-state may be delayed due increased degree of complexation over time, leading to a prolonged accumulation of necromass. Although this process is not considered in our models, the results presented here provide clear support that biomass and necromass dynamics of ectomycorrhizal ERM operate at different temporal scales, which have implications for the contribution of fungal-derived C in boreal forest soils.

Overall, our results further underline that mycorrhizal fungi are key players in mediating soil C fluxes with implications for soil C accumulation. This understanding is vital for achieving a more sustainable forest management approach that prioritizes the maintenance and preservation of forest soils as a sink for atmospheric C. Strategies for land use should consider the contribution of mycorrhizal-derived C to boreal forest soil, fostering a more mechanistic consideration of ecosystem processes in shaping and adapting climate policy frameworks.

4.2 | Contribution of ERM turnover to soil C fluxes and pools

The implementation of Bayesian calibration to simultaneously assess biomass and necromass dynamics yielded turnover estimates of 2.9 and 0.9 times yr^{-1} for biomass and necromass, respectively. This biomass turnover is similar to values of 1–7 times yr^{-1} (average: 3 times yr^{-1}) estimated earlier across the chronosequence (Hagenbo et al., 2017, 2018), despite deploying different modeling approaches. These earlier estimates involved the separate estimation of biomass dynamics for each site (Hagenbo et al., 2017, 2018), whereas the present study involved the simultaneous estimation of biomass and necromass dynamics across all sites, to allow a more robust and general estimation of biomass and necromass turnover. Furthermore, the estimated necromass turnover rate (0.9 times yr^{-1}) is similar to reported values in Ekblad et al. (2016), where necromass turnover was estimated to be 1.3–1.5 times yr^{-1} in control plots of a warm-temperate *P. taeda* forest.

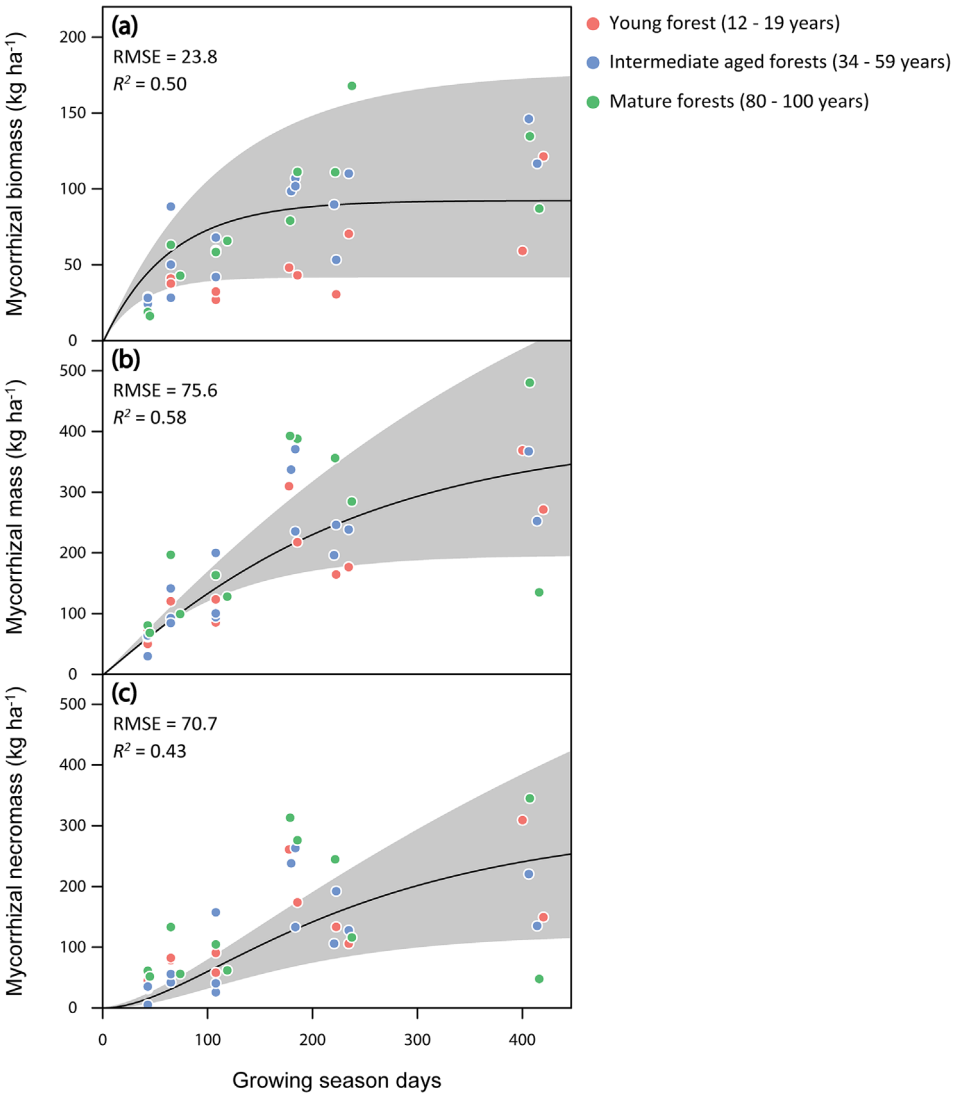

FIGURE 4 Relationships between growing season days and (a) ergosterol-derived biomass of ectomycorrhizal extraradical mycelium (ERM), (b) chitin-derived ERM mass, and (c) ergosterol and chitin-derived ERM necromass in young (12–19 years), intermediate (34–59 years), and mature (80–100 years) *Pinus sylvestris* forest stands. The lines represent predictions based on production and turnover parameters presented in Figure 2a-c and according to equations 1 and 2 (see material and methods section). The grey-shaded areas represent the limits of the 5th and 95th percentile of the Bayesian simulations. Length of a growing season is approximately 185 days in the study area. Thus, mesh bags incubated over 200 and 400 growing season days have been out in the field for approx. one and two years, respectively.

Fitting of Equation 1 to content of fungal-derived C and content of total C from ERM ingrowth bags resulted in turnover estimates of 0.1 and 1.4 times yr^{-1}, respectively, corresponding to a mean residence time ranging from >1,000 to 129 days. The fungal-derived C was based on the difference between total C and residual C, which were estimated as the content of C derived from other sources than ERM ingrowth (e.g. infiltration of dissolved organic C). While the differentiation between fungal-derived C and residual C is only approximate, and based on the assumption that the influx and efflux of residual C is rapid and close to steady-state at 43 days of incubation, the similarity in production estimates between biomass C and fungal-derived C (0.64 and 0.78 kg ha^{-1} day^{-1}, respectively), implies that our approximation of fungal-derived C and residual C is sensible. Furthermore, the content of C in the mesh bags increased at a greater rate than ERM biomass C (2.76 and 0.64 kg ha^{-1} day^{-1}, respectively), which is indicative of hydrological transport of dissolved organic C through the bags from the forest floor and surrounding soil. Typical concentrations of dissolved organic C leaving the forest floor of northern latitude forests are in the range of 20–90 mg l^{-1} (Fröberg et al., 2006; Michalzik et al., 2001). This amount corresponds to a net removal of 120–540 kg C ha^{-1} yr^{-1}, when assuming an annual precipitation of 600 mm (average over 2013–2014; Hagenbo et al., 2017), which is in a similar order of magnitude as the average residual C content (98 kg C ha^{-1}) found in mesh bags with 43 days of incubation. Moreover, dissolved organic C may also enter the bags via the direct release of exudates from ERM. These exudates typically consist of low-molecular-weight organic acids, amino acids, and sugars, with rapid turnover rates (van Hees et al., 2005). The concentration of these compounds appears to be relatively stable in soil (van Hees et al., 2000), which implies that they have a rapid turnover and are replenished at the scale of days (van Hees et al., 2005). However, the quantity of ERM exudates is complex and varies with factors such as plant species, plant age, soil type, and nutrient availability (Vives-Peris et al., 2020). Consequently, the precise quantitative contribution of exudates to the C content within the mesh bags remains uncertain. Nevertheless, it is reasonable to consider ERM exudates as another potential source of residual C within the bags.

Collectively our turnover rate estimates demonstrate that the mean residence time of C within the ERM pools extends from months to years. The C within the biomass has the overall shortest residence time, of about two months, and C within the necromass, (prior to its transformation to humus), has a residence time of about seven months. Finally, C within the degraded necromass (fungal-derived C) appears to have a residence time that extends over years. This basic information, together with the reported uncertainties, should offer a new direction for parametrizing and constraining models of boreal forest soil C dynamics that explicitly incorporate fluxes of C related to ERM dynamics.

We foresee that models incorporating mycorrhizal-mediated processes can contribute toward describing soil C dynamics in a more mechanistically correct way, which may facilitate our ability to predict the effects of forest management and ecosystem responses to climate change. Such models may facilitate and inform policy decision-making processes targeting forest-based climate mitigation, by offering a more comprehensive view of boreal soil C dynamics. Furthermore, integrating mycorrhizal-mediated processes into modeling frameworks may broaden the scope for exploring new hypotheses related to boreal soil C cycling. As such these models may contribute to achieving both predictive- and exploratory objectives in research and policy development for boreal forest ecosystems.

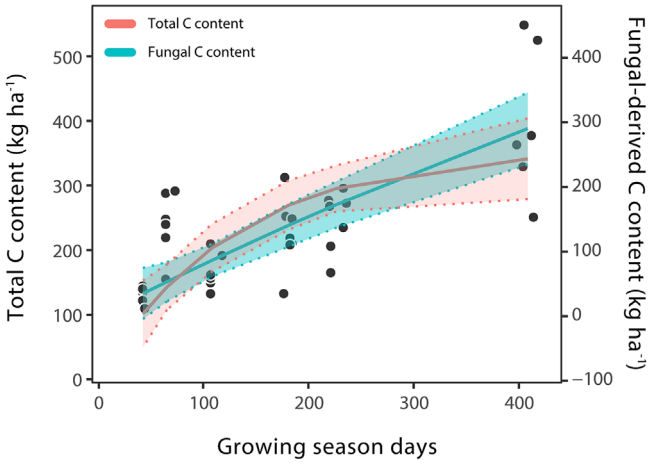

FIGURE 5 Relationship between growing season days and content of total carbon (C) (left-hand axis) and content of fungal-derived C (right-hand axis) in mycelial ingrowth mesh bags incubated in *Pinus sylvestris* forest stands aged 12–100 years. Blue and red colored lines represent equation 1 fitted to the data of total C and fungal-derived C, respectively. The shaded areas represent the limits of the 5th and 95th percentile of the fits. Length of a growing season is approximately 185 days in the study area, thus mesh bags incubated over 200 and 400 growing season days have been out in the field for approx. one and two years, respectively. Fungal-derived C represents the difference between total C and residual C. Residual C represents the content C that could not be assigned to be derived from biomass nor necromass, e.g. C sourced from dissolved organic C.

4.3 | Absence of forest age-related variation in necromass turnover

Previous studies reported declining ERM biomass turnover with increasing forest age over the present chronosequence (Hagenbo et al., 2017, 2018). However, despite the previously reported variability in ERM biomass dynamics and mycorrhizal-mediated soil C fluxes (Hagenbo et al., 2019) and shifts in community composition (Hagenbo et al., 2018; Kyaschenko et al., 2017), we did not observe any forest age-related shift in ERM necromass turnover, in disagreement to our second hypothesis. Fungal necromass decomposition depends on

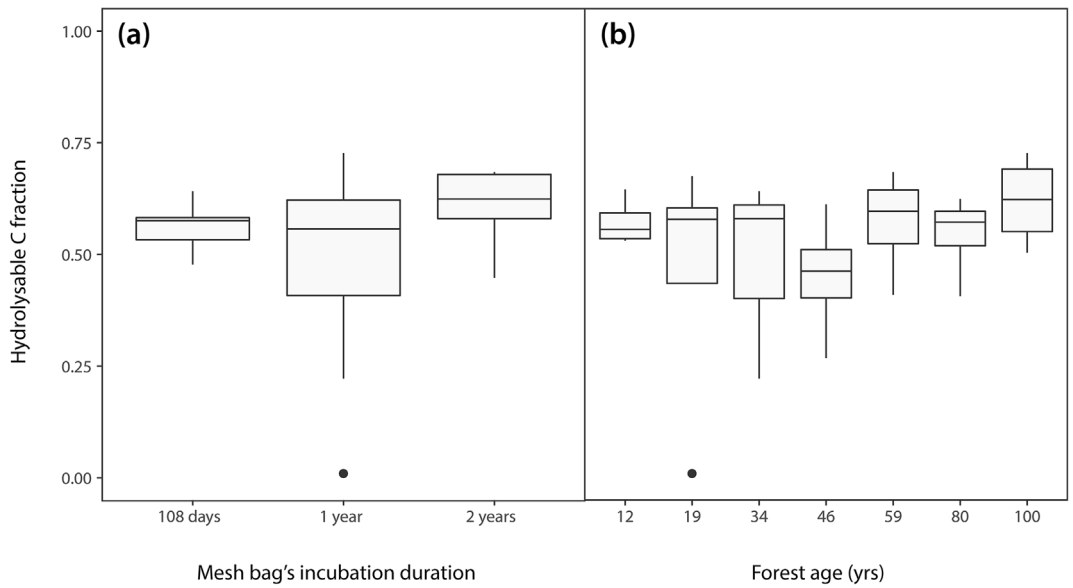

FIGURE 6 Hydrolyzable carbon (C) fractions in mycelial ingrowth mesh bags incubated in *Pinus sylvestris* forests. Average hydrolyzable C fraction relative to (a) their incubation duration, and b) forest age. Data is based on mesh bag sets (a-d) (Figure 1).

both the quality of the necromass, as well as the microbial community driving the degradation (Brabcová et al., 2016; Fernandez & Kennedy, 2018). Hence, we hypothesized that we would observe forest age-related variation in necromass turnover. However, when the data were subset into young forests (12–19 years), intermediately aged forests (34–46 years), and mature forests (80–100 years) the necromass model failed to yield stable parametric estimates due to a lack of convergence, thus were unable to statistically analyze forest age-related variation in necromass turnover.

The models used here are based on the assumption of stable production and turnover rates (Ekblad et al., 2016), and the variability in conditions introduced from incubating mesh bags over two full years likely contributed to uncertainty in the biomass and necromass estimates. Potentially, seasonality in conditions obscured the detection of forest age-related changes in necromass turnover, and thus the extent to which ERM necromass turnover varies with forest age is inconclusive from our results. However, the fact that the estimated ERM necromass reached a peak in the 80-year-old forest suggests that ERM necromass pools and dynamics were not constant across the forest ages. Potentially, the peak in ERM necromass may relate to the high abundance of ectomycorrhizal genus *Cenococcum* (Hagenbo et al., 2018), known for its high cell-wall melanin concentration, reducing the decomposability of the ERM necromass and promoting the accumulation of fungal-derived C in soil (Fernandez et al., 2016; Fernandez & Koide, 2014). In support of this, content of total C was also significantly higher in mesh bags incubated for 2 years in the 80-year-old forest. On the other hand, the fraction of non-hydrolyzable C in ERM remained relatively constant across the sites of the chronosequence, indicating similar stability of ERM necromass. However, it is worth noting that the non-hydrolyzable C fraction was determined based on HCl hydrolysis rather than the Klason lignin method, which relies on concentrated sulfuric acid hydrolysis. Hence, we estimated that the non-hydrolyzable C fraction of ERM was about 50% of the total C content, which is higher compared to other mycelial decomposability analyses (Huang et al., 2022). Therefore, it is possible that a stronger hydrolysis agent could have revealed clearer variations in the non-hydrolyzable C fraction across the sites of the chronosequence.

4.4 | Possible increase in biomass production rate with incubation duration

In the upper 7 cm of the humus layer, we estimated a daily biomass production of 1.5 kg ha^{-1} day^{-1} over the growing season, which is higher than the previously estimated average ERM biomass production of 0.7 kg ha^{-1} day^{-1} over the growing season (Hagenbo et al., 2017, 2018, 2019). This discrepancy likely stems from three primary reasons.

Firstly, in our Bayesian calibration, we used the mesh bags' incubation duration in growing season days instead of the total incubation duration. This was done because the biomass and necromass models assume stable conditions (Ekblad et al., 2016), and to account for some mesh bags being incubated over winter (i.e. mesh bags with one and two years incubation), while the remaining solely being incubated over the growing season. If calibrated based on the total incubation duration, rather than the growing season days, the total annual production would be 219 kg ha^{-1}, corresponding to daily production of 0.6 kg ha^{-1} over the full year.

Secondly, the concentration of ergosterol in ERM extracted from mesh bags seems lower compared to ERM from pure cultures (Hagerberg et al., 2003; Wallander et al., 2013). In this study, we loosened the constraints regarding the ergosterol and chitin conversion factors to account for this uncertainty in the calibrations. This enabled a lower ergosterol-to-biomass conversion factor (2.6 μg ergosterol mg^{-1} biomass, as opposed to 3.0 μg mg^{-1} found in culture studies), thus yielding 15% larger ERM biomass estimates and consequently higher production estimates.

Thirdly, the parameterized biomass model (Equation 1) tended to overestimate biomass in mesh bags with the shortest incubation time (43 days), suggesting that growth accelerated after the initial colonization of the bags, potentially linked to non-linear mycelial branching of maturing ERM. A lag phase before ERM enters the mesh bags could have contributed to similar results (Wallander et al., 2013). However, as no clear lag phase was observed in mesh bags incubated for only 2 weeks (Hagenbo et al., 2018), it seems more likely that proportionally slower-growing taxa colonized the bag at later stages (after 1 year; Hagenbo et al., 2018), or that the ERM colonization rate increased with mesh bags' necromass content. In the present study, our production estimates were derived from mesh bags with longer incubation durations than previous studies (only using up to 108 days of incubation; Hagenbo et al., 2017, 2018). Thus, if ERM colonization accelerates with the development of necromass over time, it could contribute to the higher ERM biomass production observed in this study.

4.5 | Methodological considerations

The results presented here are solely derived from semi-poor hemiboreal *P. sylvestris* forests and the extent to which the results apply to other forest ecosystems (e.g. boreal *Picea abies* forests) is not known. Furthermore, it is important to acknowledge that the mesh bags only covered the upper 7 cm of the humus layer, and at least half of the production of ERM is probably found below this depth (Ekblad et al., 2013). Another potential limitation is related to the application of biomarkers to estimate fungal biomass and mass, respectively from ergosterol and chitin measurements. Here, we assumed a priori distribution for ergosterol centered around 3.0 μg mg^{-1} biomass and for chitin centered around 44.0 μg mg^{-1} fungal mass. Both conversion factors have been applied many times before (Ekblad et al., 2016; Hagenbo et al., 2017; Salmanowicz & Nylund, 1988; Wallander et al., 2001), but because of variation between species and growth conditions, some degree of inaccuracy may persist in the estimates.

4.6 | Conclusions

This study stands as one of the few attempts to assess the relationship between the production and turnover of ectomycorrhizal ERM biomass and necromass under field settings. Our results demonstrate that biomass and necromass dynamics of ERM in hemiboreal *P. sylvestris* forests contribute to the cycling of soil C at different temporal scales, which has consequences for the contribution of mycorrhizal-derived C in the soil. We found that the turnover of biomass (2.9 times yr^{-1}) is three times greater than the turnover of necromass (0.9 times yr^{-1}), highlighting the importance of necromass dynamics in driving fungal-mediated soil organic C accumulation in boreal forest soils. Together with the reported variability in ERM estimates, these estimates can be used to further develop and parameterize ecosystem models aiming to explicitly include ERM dynamics and assess the impact of ectomycorrhizal ERM inputs on soil C accumulation in boreal forests. Such models may enhance our ability to predict the effects of forest management and ecosystem responses to climate change to inform policy development for climate mitigation in boreal forests.

AUTHORS CONTRIBUTIONS

AH, PF, KEC, and AE designed the study. AH conducted the majority of the fieldwork. AH performed the ergosterol analysis and MAO performed the chitin analysis. AH and LM applied models and AH performed statistical analysis. AH wrote the first draft and all authors contributed to data interpretation and revisions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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