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Understanding the impact of main cell wall polysaccharides on the decomposition of ectomycorrhizal fungal necromass

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Abstract

The extramatrical mycelium of ectomycorrhizal fungi (EMF) is an important source of soil carbon and nitrogen. While the importance of recalcitrant compounds in the fungal cell wall has been explored earlier, the contribution of highly abundant but labile components, like glucans, and the role of their temporal dynamics during decomposition remains unknown. For the first time, we examined how the concentration of three main fungal cell wall components (chitin, melanin, glucans) in EMF necromass are related to necromass decomposition, over a period of 6 weeks. Although the initial concentrations of the three components were not good predictors of necromass loss, we found species–specific trends of chitin and glucans loss over time. The chitin concentration during decomposition was tightly linked to the weekly necromass degradation, with trends of chitin loss being dissimilar across fungal species. Chitin concentration was positively correlated with the mass loss in the first week, but in the remaining 5 weeks, it was found to be weakly negatively correlated with mass loss. The similarity in susceptibility to the decomposition of glucans and chitin likely compensates for the impacts of interspecific differences in their initial concentration, leading to overall similar decomposition patterns. Alternatively, other, non-measured, components (e.g., glycoproteins, N content) may contribute to explaining similar decomposition patterns. Our results indicate that ectomycorrhizal necromass decomposition processes differ from those of plant litter decomposition with, unlike in plants, differences in initial concentrations of major structural carbohydrates (e.g., glucans) being unrelated to differences in decomposition rates. These findings indicate that the decomposition of fungal material cannot be inferred from assumptions based on data provided by studies of plant decomposition.

KEYWORDS
chitin, ectomycorrhizal extramatrical mycelium, fungal cell wall, fungal litter, glucans

Highlights

- Necromass of different ectomycorrhizal species decomposes similarly
- Main cell wall components show different decomposition patterns
- The relationship between cell wall compounds and mass loss changes over time
- Mycorrhizal necromass decomposition differs substantially from that of plant litter.

1 | INTRODUCTION

Soil organic matter (SOM) is known to be the largest carbon (C) pool in terrestrial ecosystems, ranging from 1500 to 2000 Pg in the top meter layer of soil. This is equivalent to three to four times the amount of C stored in the vegetation, which is approximately 500 Pg (Amundson, 2001; Butler & Day, 1998; Dixon et al., 1994). Considering that mycorrhizal fungi constitute a large proportion of the total microbial biomass in the soil of different ecosystems (e.g., temperate and boreal forests), the turnover of mycorrhizal fungal tissues is thought to be one of the main factors in the formation and stabilization of SOM (Cotrufo et al., 2013; Ekblad et al., 2013; Frey, 2019; Schmidt et al., 2011). This implies that environmental and ecological factors need to be considered in a complex ecosystem, as fungal necromass turnover frequently differs from the bacterial one. Estimates of ectomycorrhizal (EM) biomass, which constitutes the major fraction of soil fungal biomass, typically ranges from 100 to 600 kg ha^{-1} (Hendricks et al., 2016; Wallander et al., 2004), and, together with roots, produces more than 50% of the dissolved organic carbon in forest soil (Högberg & Högberg, 2002). In some coniferous forests, the dry fungal biomass per g of litter has been found to be three- to 10-fold the dry mass found in the soil (Baldrian et al., 2013). Moreover, in forest soil and litter, which are often nitrogen (N)-limited, fungal mycelia are more attractive than plant residues as a target for decomposers, given their high content of N, which can be up to 30 times greater than in plant litter (Koide & Malcolm, 2009; Mouginot et al., 2014). Due to the large fluxes of C and nutrients into the soil through fungal necromass, being dead fungal biomass, a better understanding of its decomposition dynamics will improve our understanding of SOM dynamics, and how these two are related.

A major driving factor of litter decomposition is the initial litter 'quality', that is, litter chemical composition (Berg, 1984). In general, decomposition is favoured by relatively low litter C:N ratios (Cleveland & Liptzin, 2007), which might help to maintain the low C:N ratio of microbial cells (Manzoni et al., 2010). Also the melanin:N ratio in the fungal necromass has been suggested to be a key factor in its decomposition (Fernandez & Koide, 2014; Koide & Malcolm, 2009; See et al., 2021). On one hand, fungal melanin, known to be one of the most recalcitrant cell wall compounds that is synthesized especially under stressful environmental conditions, such as extreme temperatures, oxidant-mediated and UV-light damages, heavy metal toxicity, hydrolytic enzymes and antimicrobial compounds (Butler & Day, 1998; Fogarty & Tobin, 1996), significantly slows down the necromass degradation (Fernandez et al., 2019; Fernandez & Koide, 2014; Ryan et al., 2020). On the other hand, similar to plant litter, fungal decomposition rates have been found to be positively correlated with tissue N concentration, especially in the early stage (Berg, 2000; Koide & Malcolm, 2009).

It is possible to distinguish a gradient of fungal necromass quality which ranges from a high-quality substrate, with relatively low melanin and comparatively high N content, and a low-quality substrate with relatively high melanin and comparatively low N content (Beidler et al., 2020). Hereto, based on stoichiometry, Beidler et al. (2020) have proposed a low litter quality to have N and melanin values around 3.5% and 4.3%, respectively, and litter of high quality to have around 7.0% N and 17.5% melanin. These two opposite necromass qualities likely have different impacts on the rates at which the C and N are released from the necromass into the soil. Among these two types, the high-quality necromass, rich in labile nitrogen and poor in melanin, deserves special attention, as the majority of studies conducted thus far addressed the decomposition process of mycorrhizal and saprotrophic fungi featuring low-quality necromass. Yet, the high-quality fungal necromass is an important hotspot of several biopolymer-degrading enzymes, high bacterial biomass (Brabcová et al., 2016), and driving the community composition of litter-inhabiting fungi (Algora Gallardo et al., 2021).

In addition, EM fungal chemical composition shapes the structure of the associated decomposer communities (Fernandez & Kennedy, 2018). Improving the knowledge of the temporal changes of the EM composition would therefore improve our ability to predict the temporal dynamics of necromass decomposition (Kennedy &

Maillard, 2022). This effect on decomposers community structure has been found to be true both for high-quality substrate as well as for low-quality substrate (Beidler et al., 2020).

While the cytoplasmic fraction does not likely play a significant role in the decomposition of EM necromass due to its high lability (Drigo et al., 2012), it seems that the chemical composition of the cell-wall drives the long-term decomposition of EM extramatrical necromass. This composition varies with age, genotype, taxon, and environment (Bowman & Free, 2006; Feofilova, 2010). EM fungal cell walls are composed mainly of polysaccharides such as glucans and chitin (70%–90% of dry biomass), proteins, which contain from 40% to 60% of cell wall total N, and melanin. The latter, despite being highly variable in concentration across different taxa (Butler & Day, 1998), is linked to the tolerance of various environmental stressors (Fernandez & Koide, 2013; Rosas & Casadevall, 1997; Wang & Casadevall, 1994).

Despite several studies suggesting that differences in initial concentrations of the main classes of chemical fungal cell-wall components (proteins, polysaccharides, melanin) underpin necromass degradation (Fernandez & Koide, 2012, 2014), there still are uncertainties concerning the mechanisms behind this relationship and we may not assume that the initial concentration of cell-wall compounds (Berg, 2000; Melillo et al., 1982) is a dominant predictor of the decomposition rate and dynamics. These uncertainties are caused by the dynamics of main components' contents during the decomposition process and by the interaction between the components themselves, considering the extensive covalent cross-linking of glucans and chitin (Cairney, 2012; Kang et al., 2018; Smolander et al., 2012). The fungal wall structure is highly variable. In contrast to plant litter, the fungal cell wall retains considerable plasticity despite the presence of a relatively rigid scaffold formed by chitin fibrils and α-1,3-glucans. This plasticity allows to easily reshape the molecular architecture of mycelia to survive through external stress. These dissimilarities with decomposition processes of plants suggest that we might need to consider different drivers to predict fungal decomposition compared to plants. Previous studies did not consider the impacts of changes in multiple cell wall components during decomposition. Since all these components are highly interconnected and each one has a specific role in maintaining the cell wall structure and function, an extensive temporal analysis of the dynamics of their composition through the fungal litter degradation process will improve our understanding of the mechanisms behind fungal necromass decomposition.

In this paper we examine the dynamics in the most abundant cell-wall compounds (chitin, glucans and melanin) from EMF mycelia necromass across six EM fungi species featuring necromass of high chemical quality, seeking an answer to the question of whether the dynamics in composition and concentration of these compounds is linked to mycelium decomposition dynamics. Hereto, we seek an answer to three research questions: (1) Do the initial concentrations of the cell-components alone predict fungal litter decomposition?; (2) Can the concentration dynamics of chitin, glucans and melanin during the decomposition process predict the dynamics of fungal litter mass loss?; and (3) Do distinct EM fungal species exhibit a similar temporal dynamics of losing cell wall components during the decomposition process?

2 | MATERIALS AND METHODS

2.1 | Fungal material and substrate

In this study, six EM fungal species (Table 1) were selected based on their representativeness and availability. The fungal strains were supplied by Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands) and by the Belgian Coordinated Collections of Microorganisms/Mycothèque de L'Université catholique de Louvain (BCCM/MUCL, Louvain-la-Nueve, Belgium). All the isolates were cultivated in specific agar media (Table 1) for approximately 40 days until the mycelium covered the entire surface of the petri dish. An average of eight plates for each species was harvested. Upon harvesting, mycelium was divided into three concentric rings, allowing comparison across different ages of the same mycelium. Age was approximated as the average of the growth between the inner edge and the outer edge. The rings were removed from the plate, and the mycelium was then rinsed three times with hot water (85°C) to wash out the remaining agar without damaging the mycelium. The mycelium was then dried overnight at 70°C. This provided approximately 120 mg of dried mycelium for each ring. The mycelium was put into a nylon mesh bag with 25 μm opening (Van Borselen Filters B.V., Zoetermeer, The Netherlands) and heat-sealed before incubation.

Five replicates were prepared for each ring, resulting in 90 bags in total. One of these replicates was used to analyse the concentrations of cell wall components of the initial necromass (Week 0). For *Lactarius deliciosus* only 65 mg of dry mycelium per bag could be prepared due to the scarcity of biomass. For the decomposition assay, soil from a pine stand (calcareous dune soils; fine sand) located in the Meijendel Dunes area (Wassenaar, The Netherlands, 52°08′57.0″ N 4°22′21.6″ E) was collected between 10 and 20 cm

TABLE 1 Isolates of ectomycorrhizal fungi with corresponding species names, collection codes, culture medium, isolate supplier, pre-decomposition values of chitin, glucans and melanin concentrations (mg/g), and decay rate constant (*k*).

Name	CBS/MUL No.	Medium	Chitin	Glucans*	Melanin	*k*
Paxillus involutus	CBS 100140	MOD	32.9 (18.9)	199.9 (30.6)^{BC}	56.84 (27.9)	0.05
Laccaria bicolor	CBS 445.79	CHA	15.2 (8.5)	230.8 (13.7)^{C}	51.82 (12.2)	0.07
Inocybe rimosa	CBS 210.55	X agar	21.3 (3.1)	155.1 (6.1)^{AB}	26.67 (7.1)	0.08
Hebeloma hiemale	CBS 376.89	MEA	55.6 (4.5)	102.3 (4.6)^{A}	21.19 (10.0)	0.10
Lactarius deliciosus	CBS 582.63	BAF	10.4 (5.4)	242.8 (9.4)^{C}	40.10 (14.1)	0.03
Scleroderma verrucosum	MUCL 34674	MA2	41.3 (19.0)	132.8 (0.8)^{AB}	6.14 (5.79)	0.08

Note: Mean values across all samples and standard error are shown. "A,B,C,*" superscripts correspond to p-value < 0.05. MUCL-Belgian Coordinated Collections of Microorganisms/Mycotheque de L'Université catholique de Louvain, Louvain-la-Neuve, Belgium. CBS-Filamentous fungi and Yeast Collection, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherands.

depth and sieved with a 2 mm mesh sieve. Each bag was incubated in a pot with approximately 400 g of soil for a period of 6 weeks in the dark, in a climate chamber at 15°C, 65% RH. Once a week the pots were watered to field capacity.

2.2 | Mass loss evaluation

Three mesh bags were harvested from the soil after one, two, four and six weeks of decomposition. The bags were rinsed in deionized water to wash off the remaining soil and dried overnight at 65°C. Mass loss was calculated by weighing the dried bags and dividing their weight with the dry weight before incubation. We further calculated the weekly mass loss (WML) by dividing the mass difference between two consecutive mass measurements by the number of weeks between the two measurements. The dried mycelium was collected and ground with a microcentrifuge tube pestle in a 1.5 mL tube for chemical analysis.

2.3 | Chemical analysis

Chitin was extracted according to the protocol of Fernandez and Koide (2012), with minor adjustments, which were mainly related to the lower amount of sample used and the respective volume of solvents; the concentration in the samples was determined with the spectrophotometer at 653 nm. Known amounts of pure chitin (Merck KGaA, Darmstadt, Germany) were assayed to obtain a standard curve which was used to obtain the concentrations in the samples. Melanin concentrations were measured according to Fernandez et al. (2014). The determination of melanin concentration was done by placing 10 mg of sample in 2 mL of Azure A dye solution and incubating for 24 h. The solution was filtered through a 0.45 mm nitrocellulose membrane, and the absorbance of the filtrate was measured using the spectrophotometer at 610 nm. A standard curve was constructed using known amounts of melanin previously isolated from the same material used in the decomposition assay. The change in absorbance was used to calculate the melanin content of the sample based on the standard curve.

Glucans were extracted with the Mushroom and yeast beta-glucans assay kit (K-YBGL 08/18, Megazyme). The protocol was followed with a few adjustments; a known amount of glucans was used to calculate the standard curve, which was used to calculate the glucans concentration after measuring the absorbance at 510 nm.

3 | STATISTICAL ANALYSES

The mycelium age, that is, ring, did not have any effect on the decomposition or chemical composition (Table 3). Therefore, rings of mycelium of different ages were treated as replicates, allowing to use $n = 3$ replicates for each week in subsequent statistical analyses. A two-way ANOVA model with species, time and their interactions were used to determine significant effects on the decomposition of necromass (% mass remaining). This was followed by Tukey's post-hoc test to evaluate significant differences between species. The effect of the concentration of the single compounds over 6 weeks on the decomposition rate k was tested through an ANCOVA analysis, using species as the covariate. In order to test the correlation between the three components, we used Pearson's correlation coefficient.

Differences in initial chitin, glucans and melanin contents among the necromass of the six species were assessed with one-way ANOVAs. Differences in the concentration of chitin, glucans and melanin between species over time and their interactions were assessed through an

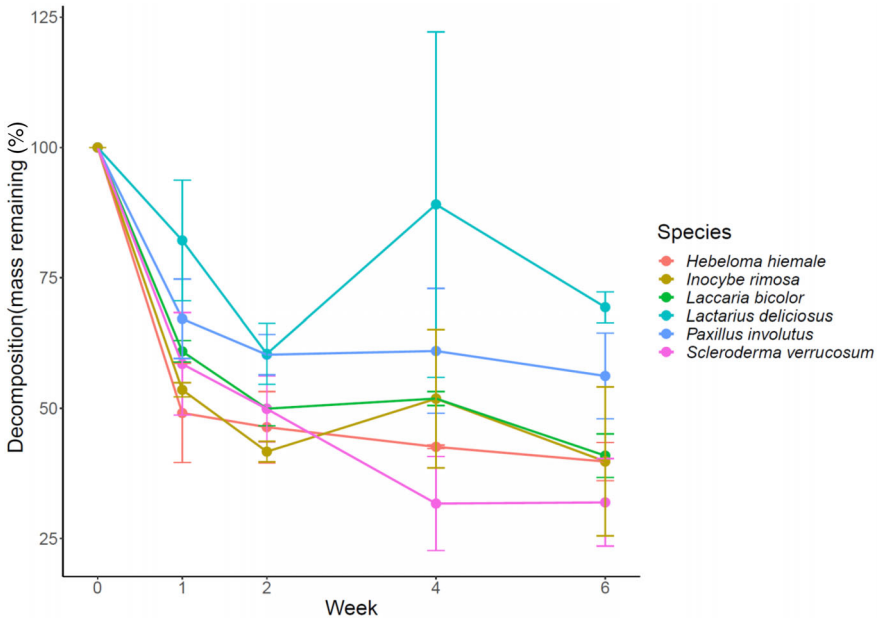

FIGURE 1 Mean per cent mass remaining of necromass from the six ectomycorrhizal fungal species. Individual points show the average of ectomycorrhizal fungi mass remaining in each week, error bars show the standard error.

TABLE 2 Outcomes of the ANCOVA on cell wall component concentration during 6 weeks decomposition of ectomycorrhizal fungal species.

Response	SSq	df	*F*	*p*
Chitin				
Week	12,720	1	24.16	5.38 e-06***
Species	63,093	5	23.97	4.11 e-14***
Week:Species	8689	5	3.30	0.00969**
Melanin				
Week	8962	1	8.24	0.00530**
Species	10,542	5	1.93	0.0976
Week:Species	2896	5	0.533	0.751
Glucans				
Week	16	1	0.014	0.905
Species	201,584	5	35.51	6.52 e-16***
Week:Species	61,296	5	2.66	0.0319*

Abbreviations: df, degrees of freedom; F, F-statistic; *p*, *p*-value; SSq, sum of squares.
Note: "*"<0.05; "**"<0.01; "***"<0.001.

ANCOVA. The relationship between the weekly mass remaining and the concentration of melanin, chitin and glucans were determined through linear model analyses. All statistical analyses were conducted using R studio (R Foundation for Statistical Computing, Vienna, Austria). In order to calculate the decay rate k, we fitted both single ($y = e^{-kX}$) and double ($y = a * e^{-k1X} + b * e^{-k2X}$) exponential decay curves by minimizing the sum of squares. The single exponential decay model was selected after comparing the fits based on the Akaike information criterion (AIC) of the two models.

TABLE 3 Results from the two way ANOVA model with species, week and age as predictors of decomposition (percent mass remaining) of the ectomycorrhizal necromass.

Response	df	SSq	*F*	*p*
Species	5	0.4650	6.402	<0.01***
Week	1	2.4661	92.3529	<0.01***
Age	1	0.0021	0.0648	0.79970
Species:Week	5	0.1207	0.9046	0.48266
Residuals	76	2.02942		

Note: One sample of *Lactarius deliciosus* harvested at the end of the 4th week was removed due to a suspicious negative value in the mass loss, likely being a measurement error.
Abbreviations: df, degrees of freedom; F, F-statistic; *p*, *p*-values; SSq, sum of squares.
Note: "*"<0.05; "**"<0.01; "***"<0.001.

TABLE 4 Pearson's correlation coefficients between the three cell wall components.

	Chitin	Glucan	Melanin
Chitin		−0.421***	0.209
Glucan	−0.421***		−0.053
Melanin	0.209	−0.053	

Note: "*"<0.05; "**"<0.01; "***"<0.001.

4 | RESULTS

Within the 6 weeks of the decomposition experiment, the EM fungal species lost on average 53.7% of the necromass, ranging from a minimum of 30.6% (*L. deliciosus*) to a maximum of 68.1% (*Scleroderma verrucosum*). The differences in decomposition were highly significant both

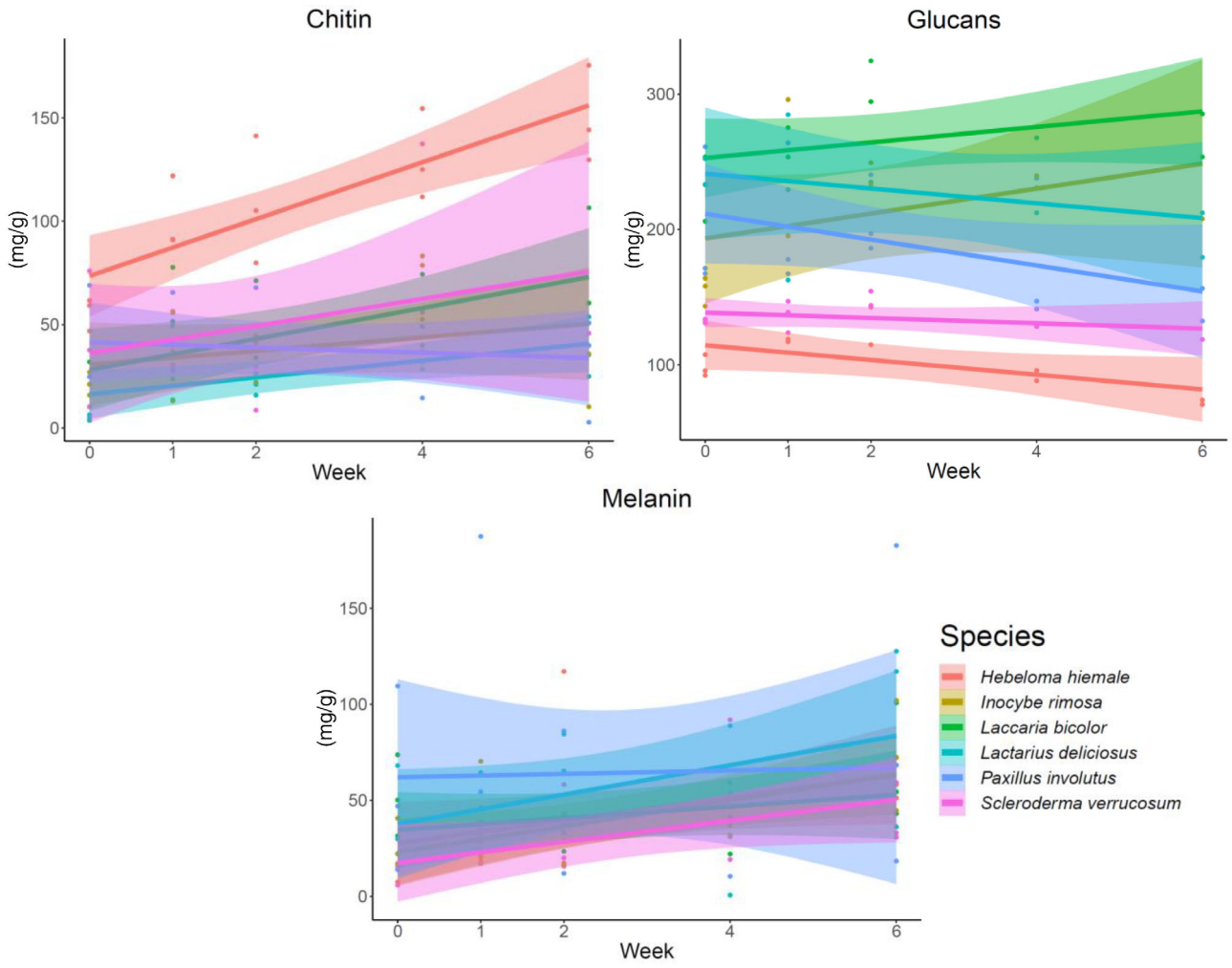

FIGURE 2 Concentration (mg component/g total mass) of the cell wall components (*y*-axis) from the necromass of ECM fungal species during 6 weeks of decomposition (*x*-axis). Significances are shown in the ANCOVA table (Table. 2). Chitin R2: *H. hiemale* = 0.67; *I. rimosa* = 0.09; *L. bicolor* = 0.37; *L. deliciosus* = 0.36; *P. involutus* = 0.02; *S. verrucosum* = 0.12. Glucans R2: *H. hiemale* = 0.35; *I. rimosa* = 0.16; *L. bicolor* = 0.17; *L. deliciosus* = 0.11; *P. involutus* = 0.20; *S. verrucosum* = 0.12. Melanin R2: *H. hiemale* = 0.24; *I. rimosa* = 0.38; *L. bicolor* = 0.10; *L. deliciosus* = 0.23; *P. involutus* <0.01; *S. verrucosum* = 0.26. Parameters of the relationship of each species is reported in Table S2.

between species and between weeks, with *L. deliciosus* decomposing significantly slower than the other species (Species: F value = 6.55, p value <0.001; Week: F value = 60.08, p value <0.001). There was no significant effect of the Species * Week interaction (F value = 1.29, p value = 0.22), indicating that all species underwent a similar pathway of total mass loss, with an average loss of around 38% of the initial mass during the first week, followed by a slower degradation during the last 5 weeks (Figure 1). Analysis of the effect of the concentration of each compound measured along with the assay on the decomposition rate k, generated by a negative exponential curve, did not reveal any significant result (Table S1). Between the three components, we found a significant correlation between the two polysaccharides, chitin and glucans (Table 4).

The six species showed different initial concentrations of glucans in their necromass. *Laccaria bicolor*, *L. deliciosus* and *Paxillus involutus* had significantly higher concentrations compared to *Hebeloma hiemale*, *S. verrucosum* and *Inocybe rimosa*. (F value = 13.432, p value <0.001). On the other hand, melanin and chitin initial concentrations did not show any significant difference among species (Figure 2).

After 6 weeks, the concentrations of glucans and melanin did not differ between species anymore, while the chitin concentration of *H. hiemale* was higher than in the other species, suggesting—in combination with the significant initial concentrations—that the loss of glucans and chitin differed among species (Figure 2). The analysis of covariance (Table 2) confirmed this supposition and provided more detailed insights. The dynamics of

compounds loss differed among fungal species: glucans concentration increased over time in *I. rimosa* and *L. bicolor*, while it decreased for the other species; chitin concentration increased over time in every species besides *P. involutus* (Figure 2; Table 2).

The initial concentrations of the main components were not a good predictor of decomposition (melanin: $R^2 = 0.015$, $p = 0.281$; chitin: $R^2 = 0.007$, $p = 0.542$; glucans: $R^2 = 0.003$, $p = 0.32$), therefore could not explain the difference in decomposition between *L. deliciosus* and the other species. Instead, when looking at the relationship between chitin concentrations during decomposition (chitin concentration measured at each week) and the WML, we found a significant negative correlation (F value = 6.34, df = 53, p value = 0.0149). This was not the case for melanin and glucans concentrations (melanin: F value = 0.398, df = 52, p value = 0.531; glucans: F value = 2.75, df = 42, p value = 0.104).

Interestingly, we found that the relationship between chitin concentration in the samples and the WML changed over time (Figure 3): in the first week, the effect of chitin concentration on WML was positive, while for the rest of the decomposition period, it was negative.

5 | DISCUSSION

Our study assesses how the concentration of multiple cell wall components of low melanin ECM necromass affects the decomposition of EM fungi (EMF). By monitoring the concentrations of three main cell wall components over time, we could assess how their changes affect the decomposition process. In this way, we showed how, despite the different decomposition trends of these components between species, the majority of these strains had a comparable mass loss, after a period of 6 weeks. Considering that all fungal species were cultivated *in vitro* in absence of external stressors, we can be sure that our results reflect the actual effects of fungal chemical composition.

Overall, the magnitude and rate of decomposition of the EM fungal species were consistent with previous findings, especially so concerning the rapid mass decline in the first 7–10 days (Drigo et al., 2012; Ryan et al., 2020). The absence of any significant differences in decay rate k might be due to the absence of ecosystem-specific effects (Throckmorton et al., 2012). Yet in contrast to these earlier studies, the necromass decomposition of the analysed species did not differ significantly, with the exception of *L. deliciosus*. Previous research suggested that the initial concentrations of main cell wall components, and especially of melanin (Fernandez et al., 2019; Fernandez & Koide, 2014) and chitin (Fernandez & Koide, 2012) underpin fungal decomposition rate. In these studies, while higher concentrations of melanin in fungal litter decreased the decomposition of EM fungal necromass, a higher concentration of chitin was shown to be generally positively related to the decomposition rate. The fungal species examined here did not show a significant difference in the initial concentrations of these two components, which likely explains the similar mass loss among species. Yet, initial concentrations of glucans differed between species. This difference did not result in differences in mass loss during the decomposition, which suggests a more complex interaction than just a simple correlation between mass loss and the concentration of a single cell wall component. Following the benchmark values proposed in previous studies (Beidler et al., 2020; Certano et al., 2018), the necromass of our fungal species could be considered to be of high quality (i.e., melanin concentration is relatively low (<5%), following the stoichiometry relationship proposed by Beidler et al. (2020)). The high quality is likely due to the combination of species identity and the lack of environmental stressors, which enhance the production of melanin (Certano et al., 2018). With this type of fungal material, our results give us new insights related to those conditions where the fungal necromass is poor in recalcitrant compounds (e.g., melanin).

Remarkably, *L. deliciosus*, the slowest decomposing species in our species set, did not stand out in terms of initial cell-wall biochemistry. This suggests, in contrast to previous studies, which did not evaluate multiple components during decomposition, that differences in their initial concentrations do not drive differences in decomposition rates. A higher C:N, which has not been measured, might also explain the slower decay of this strain, as well as lower amounts of other N-rich compound (e.g., proteins) not analysed in this study.

On the other hand, the dynamics of loss of chitin was related to fungal species biomass loss. This is in line with recent findings of Ryan et al. (2020), who also found fungal species decomposition to be strongly underpinned by the loss of carbohydrates from fungal biomass. However, our results indicate that this finding could not be generalized for all structural carbohydrates, as there was no significant relationship between necromass loss and the concentration of glucans. As a matter of fact, *H. hiemale* had the lowest glucans concentration but the highest concentration of chitin. This might suggest that as long as there is a compound which ensures sufficiently high N availability to the decomposers (e.g., chitin), these microorganisms can sustain the enzymatic attack of all types of available structural carbohydrates (e.g., glucans). Given that glucans are more abundant than chitin in necromass, glucans have a higher chance to be degraded first. This combination might have led to an increased concentration

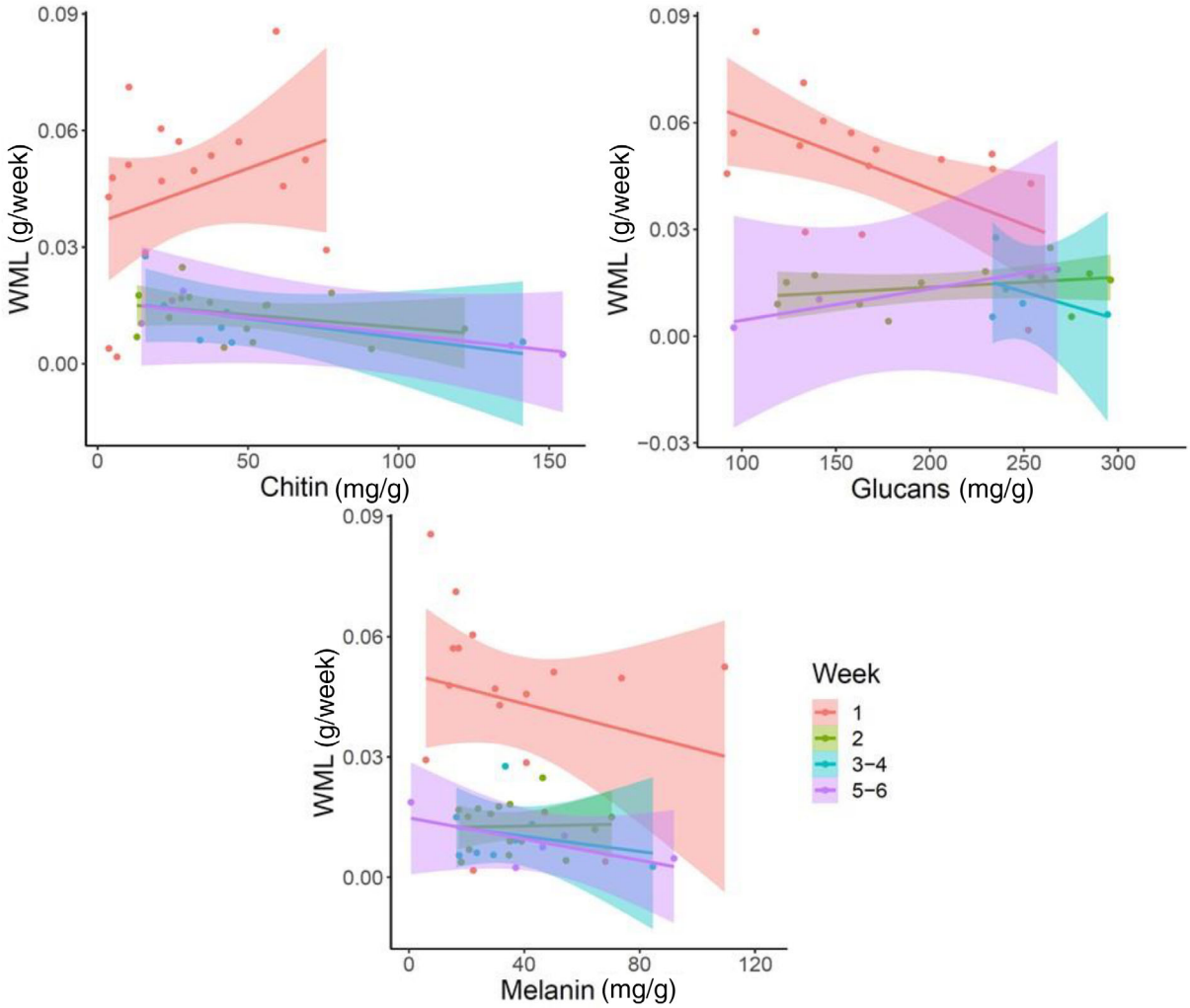

FIGURE 3 The relationship between concentration of the three analysed litter components and weekly mass loss (WML) of ectomycorrhizal fungal necromass. The data points include all the species in the study. The shades indicate the standard error. R^2 and p values of each week are reported in Table S3.

of chitin towards the later stage of the decomposition as well as a faster degradation of the isolate.

Interestingly, we observed that the impact of chitin on decomposition varies over time. We observed a positive relation between chitin and necromass loss during the first week of decomposition, while during the remaining weeks, the necromass loss had a weak negative relationship to chitin concentration. This might be the result of the increased nitrogen availability in the first stage of decomposition, which might be sufficient for decomposers to support the production of extracellular enzymes devoted to break down the organic matter (Schimel & Weintraub, 2003) later on.

The role of melanin in the decomposition process of fungi has been demonstrated previously (Fernandez et al., 2019), and specifically to a slow-degrading necromass pool (Ryan et al., 2020). In our study, the loss of melanin was not related to the necromass loss at all. This might be a consequence of the small quantity of melanin in our samples. These consistently low concentrations of melanin among the different species might be caused by the absence of external stressors during the growth and growing conditions were optimal in terms of temperature, light and nutrients.

The pattern of loss of glucans, the most abundant cell-wall compounds examined in this study, was highly species–specific (Figure 3). Yet, these differences between species in the pathways of the loss of cell-wall compounds were not reflected in the inter-specific differences in decomposition patterns. We hypothesize that the differences across fungal species in the loss of individual types of polysaccharides such as chitin and glucans, which constitute around 80-% of the fungal cell wall (Bartnicki-Garcia, 1968), may compensate for each other. This interpretation is consistent with the negative correlation between the two polysaccharides, which suggests that

where chitin is used as the main carbon source, glucan has not been depleted yet, and vice versa. Indeed, hydrolytic enzymes (e.g., endo-chitinase, *N*-acetylglucosaminidase, β-glucosidase; the same enzymes that degrade plant litter compounds such as cellulose) are produced by the majority of bacteria and saprotrophic fungi (Conn & Dighton, 2000). Many fungal decomposers (e.g., white-rot fungi) have the ability of degrading more than just one type of their components, a useful trait that enables them to attack the fungal cell wall in a similar manner as they degrade hemicellulosic matrix in plant biomass, known to be highly variable and featuring different links between numerous polysaccharides (Bentil et al., 2018). Although the decomposition of chitin is a common trait in most fungi because the chitinase activity is involved in the morphogenesis of their own mycelia, the production of endochitinases that efficiently cleave chitin is much less common (Baldrian et al., 2013). When it comes to bacteria, there is more involvement of distinct, substrate-specific guilds in the exploitation of biopolymers in the forest topsoil (Algora et al., 2022). On the other hand, the oxidative enzymes used mainly for degrading lignin and melanin, are less abundant because they are almost exclusively secreted by lignin decomposer fungi (Butler & Day, 1998). Specific communities of bacteria and fungi were previously found to be associated with decomposing mycelium, which differed between the initial and late phases of decomposition. This shift may explain the inversion of the relationship between chitin concentration and the weekly mass, where the species producing high levels of chitinase in the rapid decay stage might exploit a major part of the N released (Brabcová et al., 2016). However, while these mechanisms may explain the interactions among cell-wall compounds in affecting fungal litter decomposition and the consequent absence of significant relationships for individual cell-wall compounds, they do not fully explain the species–specific decomposition patterns.

To further understand this pattern, not only chitin lability deserves more attention, but especially glucans, as the most abundant carbohydrate fraction of the cell wall, should be further investigated. For instance, distinguishing between alpha-and beta-glucans could be an interesting line of research, as these compounds serve different functions in the cell wall compartment. Alpha-glucans contribute to the cell wall matrix (Sietsma & Wessels, 1994), while beta-glucans play a structural role, crosslinking the chitin fibres (Wessels et al., 1990). Once we understand the magnitude and the rate at which these components are lost from necromass and later stored in the SOM, we can more precisely predict what would be the C:N ratio of the SOM, which in many cases is essential to assess its chemical 'lability' (Cui et al., 2020). Further insights on this C input from EMF might provide a more comprehensive view of the contributions of these groups of microorganisms to SOM. While most of the research to date has focused on their capacity to mobilize organic C by decomposing organic matter (Leake et al., 2014; Read et al., 2005; Read & Perez-Moreno, 2003), we emphasize the importance of the decomposition of their necromass as a contribution to SOM dynamics.

Considering that glucans and chitin are both attacked by hydrolytic enzymes, it is also worth to further investigate the temporal activity of these enzymes under the influence of external factors. Such analysis of hydrolytic enzymes could provide more insights into the mechanisms that drive the EM decomposition, especially concerning environmental conditions. The activity of many enzymes depends on soil abiotic parameters (pH, temperature, moisture), which drastically change between soil layers and seasonality (Wittmann et al., 2004). This can have a major effect on the EM necromass decomposition, which can be modelled more easily once we can fine-tune them based on environmental conditions.

With the findings presented in this study, we encourage to follow two main paths to further understand the contribution of EM necromass to SOM. First, there is a need to dig deeper into the nature of interactions between the two most abundant polymers in the cell wall (glucans and chitin), to see if those interactions may have a significant effect on the decomposition of both single components and the overall necromass. In particular, whether these interactions are prone to change under the influence of environmental conditions or that they are highly species–specific. Understanding these interactions may take a long time, in analogy to the complications that arose to understand the interaction between lignin and cellulose on plant litter decomposition (Evans et al., 1994; Talbot & Treseder, 2012). Those interactions are particularly difficult to study in natural conditions due to the external biotic factors that are involved (Cuchietti et al., 2014). Secondly, when it comes to developing models which aim to predict SOM dynamic, without knowing the nature of the interactions within mycorrhizal necromass—as highlighted above, we can still use the decomposition estimates from our study and others to develop descriptive models. This would demand considering mycorrhizal necromass as a separate pool next to that of plant roots, instead of assuming that mycorrhizas could be considered as part of a root mass pool. Either way, our results suggest that necromass decomposition does not behave similarly to plant litter, while it is a critical component to consider in estimating SOM dynamics.

6 | CONCLUSIONS

Our results show that the rates and magnitudes of low-melanin necromass decomposition of EM fungal species studied here are mostly similar. However, there are significant differences in the pathway of chemical component loss from their cell walls, especially polysaccharides. As evidence of this, the concentration changes of the cell wall compounds over time do not correspond to the final biomass loss. For this reason, looking at the concentration of single compounds cannot ensure an accurate prediction of necromass loss in EMF. Together, this brings evidence that distinct EM fungal species affect SOM through distinct pathways of cell-wall compound loss, even if these fungi show a similar overall mass loss. These pathways differ remarkably from those of plant litter and need to be further understood to be able to better predict the contribution of fungal necromass on SOM dynamics.

AUTHOR CONTRIBUTIONS

R. Mancinelli: Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; formal analysis; data curation; visualization. **P. M. van Bodegom:** Writing – review and editing; conceptualization; supervision. **J. A. Lankhorst:** Investigation; methodology; writing – original draft; data curation. **N. A. Soudsilovskaia:** Conceptualization; funding acquisition; writing – review and editing; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest has been declared by the author(s).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in DRYAD at https://datadryad.org/.

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