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LETTER

Melanin mitigates the accelerated decay of mycorrhizal necromass with peatland warming

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Abstract

Despite being a significant input into soil carbon pools of many high-latitude ecosystems, little is known about the effects of climate change on the turnover of mycorrhizal fungal necromass. Here, we present results from the first experiment examining the effects of climate change on the long-term decomposition of mycorrhizal necromass, utilising the Spruce and Peatland Response Under Changing Environments (SPRUCE) experiment. Warming significantly increased necromass decomposition rates but was strongest in normally submerged microsites where warming caused water table drawdown. Necromass chemistry exerted the strongest control on the decomposition, with initial nitrogen content strongly predicting early decay rates (3 months) and initial melanin content determining mass remaining after 2 years. Collectively, our results suggest that as global temperatures rise, variation in species biochemical traits as well as microsites where mycorrhizal necromass is deposited will determine how these important inputs contribute to the belowground storage of carbon in boreal peatlands.

Keywords

Bog microtopography, carbon cycling, decomposition, microbial residues, nitrogen cycling.

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INTRODUCTION

A shift of boreal ecosystems from being global sinks to sources for atmospheric carbon (C) would strongly exacerbate the rising air temperatures associated with increased atmospheric greenhouse gas concentrations (Davidson & Janssens 2006). Boreal peatlands are of particular concern because their soils hold a disproportionate amount of C relative to land area coverage (Aselmann & Crutzen 1989). The accumulation of C in peatlands is the result of a perched water table, causing anoxic conditions that significantly slow the decomposition rates of soil organic matter (Moore & Basiliko 2006). Unlike many soils where organic C can be protected from decomposition via physical sequestration within soil aggregates or via sorption to mineral surfaces (Schmidt *et al*. 2011), C stored in peatland soils is thought to be very sensitive to changes in the level of submersion by the water table (Moore & Basiliko 2006). Since climate can have dramatic consequences on evapotranspiration dynamics leading to water table drawdown in these systems, predicted near-future climate change scenarios may have strong negative impacts on C stored in peatlands, particularly in high-latitude systems where a substantial amount of C is stored (Gorham 1991; Belyea & Malmer 2004; Davidson & Janssens 2006). Although basic thermodynamic and kinetic principles predict that decomposition and respiration rates will increase with temperature, there remains considerable uncertainty about the many possible abiotic and biotic interactions that may alter the flux rate and form in which C leaves these systems (Belyea & Malmer 2004). In particular, the vulnerability of complex assemblages of belowground biota to climate change and the associated consequences on biogeochemical cycling is largely unknown (Bardgett *et al*. 2008).

Globally, plants allocate a considerable amount of fixed C belowground to fine roots (McCormack *et al*. 2015) and mycorrhizal fungal symbionts (Godbold *et al*. 2006; Hobbie 2006; Ekblad *et al*. 2013). The death of this biomass (hereafter referred to as necromass) represents a large flux of C into soil biogeochemical cycles (Fernandez *et al*. 2016). Conservative estimates indicate that on average 7% of net primary productivity is allocated to mycorrhizal fungi in forest ecosystems (Ekblad *et al*. 2013), but measured values exceeding 20% are not uncommon (Hobbie 2006; Allen & Kitajima 2013). Both ectomycorrhizal (EM) and ericoid mycorrhizal (ERM) associations are common in high-latitude peatland systems and because of the large amount of C allocated to these fungi in high-latitude systems, understanding the decomposition dynamics of necromass may be particularly important in boreal soil C cycles (Clemmensen *et al*. 2013, 2015). Currently, however, knowledge of the ecological factors governing these processes is limited to only a few ecosystems (Fernandez *et al*. 2016). The decomposition rates of mycorrhizal fungal necromass have been shown to vary widely across species and to be linked to intrinsic biochemical traits (Fernandez & Koide 2012, 2014). Specifically, both melanin and nitrogen concentration appear to be important controls on fungal necromass decomposition (Fernandez & Koide 2014). The potential for interactions with exogenous factors, such as climate change scenarios, have yet to be examined, representing a major gap in understanding controls on decomposition dynamics of fungal necromass (Fernandez & Kennedy 2015).

Recently, ^{14}C bomb dating has revealed that mycorrhizal inputs contribute disproportionately (*c*. 50–70%) to soil C stocks compared to aboveground inputs along a boreal forest chronosequence (Clemmensen *et al*. 2013). Efforts towards

quantifying residence times of both mycorrhizal biomass (production and death) and necromass (resistance to decomposition) represent key areas that will improve our understanding of the mycorrhizal contribution to C storage in soils (Clemmensen *et al*. 2013; Ekblad *et al*. 2013; Fernandez *et al*. 2016). Beyond the effects of initial biochemistry on decomposition rates, the persistence of fungal biochemical components in necromass inputs over long-term incubations is unknown. Generally, fungal necromass has faster rates of decomposition compared to plant residues (Koide *et al*. 2011), but this observation is largely based on short-term litter bag incubations and it is unclear at what point, if any, the decomposition rates of fungal necromass reach a point of stabilisation as seen in plant litter and if it is related to biochemical composition (e.g. melanin content). *In situ* measurements of ectomycorrhizal root persistence via minirhizotron imaging have demonstrated that roots colonised by the heavily melanised EM fungus, *Cenococcum geophilum* Fr., persist in the soil between 4 and 10 times longer than roots colonised by other non-melanised EM fungi (Fernandez *et al*. 2013). It is unclear, however, which biochemical components remain in the necromass.

The primary objective of this study was to fill key knowledge gaps associated with effects of warming and elevated atmospheric carbon dioxide (CO_2) on the decomposition of fungal necromass in peatland systems. Our second objective was to assess the decomposition dynamics and chemical changes of fungal necromass, which varies in their biochemical makeup, over the course of a long-term (i.e. multi-year) incubation. To our knowledge, all previous studies have examined fungal necromass decomposition over short time scales (mostly < 3 months) and have not thoroughly examined the chemical composition of the mass remaining post-incubation. To address these critical knowledge gaps, we conducted a 2-year fungal necromass litter bag decomposition study at the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment in the Marcell Experimental Forest in northern Minnesota, USA. We hypothesised that (1) elevated temperatures will increase the decay rates of the necromass, (2) temperature-driven increases in decay rates will be most pronounced in hollow microtopography as a result of water table drawdown and increased oxic conditions, 3) initial melanin and nitrogen contents of the necromass will control the short- and long-term decomposition dynamics of the fungal necromass and (4) the remaining mass in the necromass after an initial decay period will be mostly composed of melanin.

METHODS

Study site

The SPRUCE project is an ecosystem climate manipulation experiment situated in a forested peatland in the USDA Forest Service's Marcell Experimental Forest in Northern, MN, USA. The climate change treatments are located in the S1 bog in the Marcell Experimental Forest (N47° 30.4760′, W93° 27.1620′), which is a weakly ombotrophic peatland with a perched water table (Sebestyen *et al*. 2011). The tree communities are dominated by *Picea mariana* (Mill.) Britton, Sterns & Poggenb. and *Larix laricina* (Du Roi) K. Koch, both of which are well-colonised by EM fungi at the site (Kennedy *et al*. 2018). The understory shrub communities are primarily composed of ericaceous shrubs such as *Rhododendron groenlandicum* Oeder and *Chamaedaphne calyculata* (L.) Moench that all host ERM fungi (Kennedy *et al*. 2018). The bryophyte layer is dominated by *Sphagnum angustifolium* (C.E.O. Jensen ex Russow) C.E.O. Jensen and *S. fallax* (Klinggr.) Klinggr. in hollows (i.e. low-lying peatland microsites that are in direct contact with the perched water table) and *S. magellanicum* Brid. on hummocks (i.e. elevated peatland microsites that are rarely in contact with the perched water table). The +0, +2.25, +4.5, +6.75 and +9°C warming treatments are crossed with a CO_2 treatment at either ambient or +500 ppm above ambient reference plots (*c*. 900 ppm) in large open-top chambers (12 m in diameter). Further environmental and experimental details associated with SPRUCE are documented in Hanson *et al*. (2017).

Necromass generation

We generated necromass from four mycorrhizal fungal species ('necromass types') that naturally occur and associate with either the EM tree hosts or ERM shrubs in the S1 site and also vary in melanin content: *Cenococcum geophilum* Fr. (EM + high melanin), *Suillus grisellus* (Peck) H. Engel & Klofac (EM + low melanin), *Meliniomyces bicolor* Hambleton & Sigler (ERM + high melanin) and *Oideodendron griseum* Robak (ERM + low melanin). Fungal isolates were grown in 50 mL of half-strength potato dextrose broth (PDB, Difco, BD Products, Franklin Lakes, New Jersey, USA) in 125 mL flasks shaken on orbital shakers at 80 RPM at room temperature for 30 days. Fungal colonies were then harvested, rinsed in deionised water and dried at 27°C in a drying oven for 24 h. Nylon mesh litter bags (*c*. 3 × 3 cm, 53 μm nylon mesh (Elko, Minneapolis, MN, USA)) containing known dry masses of the four necromass types were heat sealed (American International Electric Inc., City of Industry, CA, USA). The average dry mass of the necromass placed in each litter bag was 42 mg.

Necromass incubation

We chose a litter bag approach to assess necromass decomposition dynamics and associated chemical compositional changes. We recognise the limitations associated with litter bags, most notably the fact that under natural circumstances fungal residues are deposited in a heterogeneous soil matrix, but, like many other studies, we chose this approach because it is a practical way of assessing these difficult-to-measure processes. Litter bags containing necromass of each type were individually incubated *in situ c*. 5 cm deep into the sphagnum in both hummocks and hollows within 3 blocks in each of the treatment chambers (5 temperature and 2 CO_2 treatment levels) for 3, 12 and 24 months (N = 720). The decomposition incubations were initiated on 1 June 2016 and harvested 2 September 2016 (3 months) and 7 June 2017 (12 months), and 5 June 2018 (24 months). Upon harvest, litter bags were immediately placed in plastic ziptop bags that were placed on

ice in a cooler and transported back to the laboratory within 24 h for processing. Incubated necromass was then placed in the drying oven set at 27 °C until mass readings stabilised. Following drying, dry mass of the necromass was measured.

Necromass chemistry

The initial melanin content from each necromass type was assessed using a quantitative colorimetric assay (as in Fernandez & Koide 2014). This assay utilises Azure A dye, which has a strong binding affinity to melanins, allowing for the quantification of changes in absorbance once a solution has been in contact with melanin. Known amounts of melanin extracted from *Cenococcum geophilum* mycelia by selective acid hydrolysis were used to generate a standard curve (Butler & Lachance 1986). The initial C and N contents of each necromass type was determined using isotope ratio mass spectrometry (vario PyroCube, Elementar, Mt. Laurel, NJ, USA) at the IRMS facility at the University of Minnesota. Fourier transform infrared (FTIR) spectroscopy was used to investigate the biochemical composition of the remaining necromass. Briefly, we subsampled 2 mg from each necromass type sample to get representation of all factors included in the model (N = 160). Those subsamples were then ground until homogenous with an agate mortar and pestle in 100 mg KBr and stored in 20 ml glass vials until the samples were analysed. After homogenisation, samples were pressed into discs and transmission FTIR spectra were recorded using a Thermo Scientific Nicolet iS5 spectrometer with an iD1 Transmission accessory. Sixty-four scans were averaged across the 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}. Background subtraction was applied using a pure KBr spectrum, and a baseline correction was applied to remove baseline distortions. Both background subtraction and baseline correction were done in OMNIC, version 9 (Thermo Fisher Scientific Inc.), while peak heights were normalised by calculating z-scores prior to final analysis. Some of the subsamples (N = 34) were excluded because not enough mass remained after the incubations. To link the treatment effects with residual necromass chemistry, we identified peaks corresponding with chemical bonds in common organic polymers (Table S2) and used normalised peak height values in statistical analyses.

Statistical analyses

Differences in initial C, N and melanin contents among the mycorrhizal necromass of the four necromass types were assessed with one-way ANOVAs. We used 4-way ANOVA tests for each incubation time to test the main effects of necromass type, microtopography, temperature, CO_2 treatments and their interactions on necromass mass loss. Because of *Sphagnum* overgrowth and the need to keep disturbance in the plots minimal, many of the litterbags that were scheduled for harvest at the 24-month timepoint could not be located and recovered (N = 108). For this reason, we lost the statistical power needed to run a full factorial model ANOVA model and therefore only examined the differences in mass loss among the necromass types for the 24-month timepoint. To more specifically assess the nature of the necromass decay dynamics and the effect of altered climatic conditions, we fit exponential decay models to the mass loss data using factor groupings that were identified as significant in the ANOVA models. We tested the fit of single pool ($X = e^{kt}$), asymptotic ($X = A + (1 - A)e^{kt}$) and two-pool exponential decay models ($= Ce^{k_1 t} + (1 - C)e^{k_2 t}$), where X is the proportion of the mass remaining at time t, and k is the decay rate. In the two-pool model, there are two decay rates, one associated with the 'fast' pool (k_1) and the other with the 'slow' pool (k_2). In the asymptotic model A is the asymptote. Model selection was based on lowest Bayesian Information criterion (BIC) score (Table S3; S4). In all cases, the asymptotic model was the best fit for the data. Principal component analysis was used to visualise and analyse the effect of incubation time, climate change treatments and microtopography on necromass FTIR peak chemistry data. Mass loss and initial chemistry vectors for the samples were also mapped on to the ordination space. Finally, we performed linear regression analyses to investigate the relationship between initial chemistry, FTIR peak intensity data, mass remaining data and decay parameter estimates from the exponential decay models. All analyses were run in JMP v14 (Cary, NC, USA) and considered significant at $P < 0.05$.

RESULTS

Initial melanin concentrations were significantly different among the four necromass types (Fig. 1; ANOVA; $F_{3,11}$: 89.17, $P < 0.0001$) and were consistent with the *a priori* visual assessment. The C and N contents also differed significantly among types (ANOVA Carbon: $F_{3,11}$: 9.86, $P = 0.0046$; Nitrogen: $F_{3,11}$: 17.20, $P = 0.0008$), due largely to *Meliniomyces bicolor*, which had the highest C content and lowest N content (Fig. 1). FTIR analysis indicated that the high C and low N contents of *M. bicolor* were likely due to high lipid concentrations rather polysaccharide or melanin constituents (Figure S5). Proportional mass losses were significantly different among the four necromass types at both 3 ($P < 0.0001$; Table S1) and 12 months ($P < 0.0001$; Table S1), being highest for *S. grisellus* and lowest for *C. geophilum* (Figure S2). Per cent mass loss increased with elevated temperature, but the effect of temperature interacted significantly with microtopography (3 months: $P = 0.022$, 12 months: $P = 0.014$; Table S1; Figure S1). In particular, necromass mass loss rates in hummocks were relatively consistent across temperatures, particularly after 3 months, whereas mass loss was significantly greater in the warmed compared to ambient temperature hollows (Fig. 2). There was also a significant temperature × CO_2 treatment interaction on necromass decomposition after 12 months, but this effect was minor and driven by the contrast between the CO_2 treatment under ambient temperature (+0°C) (Figure S3). Initial melanin-to-nitrogen ratio of the necromass was negatively correlated with proportional mass loss of the necromass (Figure S4). Across all experimental treatments, all four necromass types had an initial period of rapid mass loss followed by stabilisation after 3 months (Fig. 3). The mean mass lost during the first 3 months across all treatments and necromass types was 81%, but decreased to < 5% on average during the following 21 months of incubation.

In the decay models, initial necromass N content was significantly correlated with the initial decay rate of the 'labile fraction' (k; $P = 0.021$; $R^2 = 0.96$), while initial melanin content was significantly correlated with the 'recalcitrant fraction' (A) ($P = 0.013$; R^2: 0.97; Fig. 4). Initial necromass melanin content was also positively correlated with the FTIR peak intensity at wavelength 1620 cm^{-1} of incubated necromass, which corresponds with C=C and C=N bonds found in aromatic compounds (Figure S6; 3 months: $P < 0.0001$, R^2: 0.70; 12 months: $P < 0.0001$, R^2: 0.90). Peak intensity at the 1620 cm^{-1} wavenumber explained *c*. 21% of the variation in mass remaining in necromass at 3 months and 64% of the variation after 12 months (Figure S7). A principal component analysis of the full FTIR data indicated that PC1 accounted for 58.3% of the variation in the incubated necromass, mostly driven by melanin, protein and polysaccharide constituents, while PC2 accounted for 23.5% of the remaining variation and was primarily driven by lipid constituents (Fig. 5).

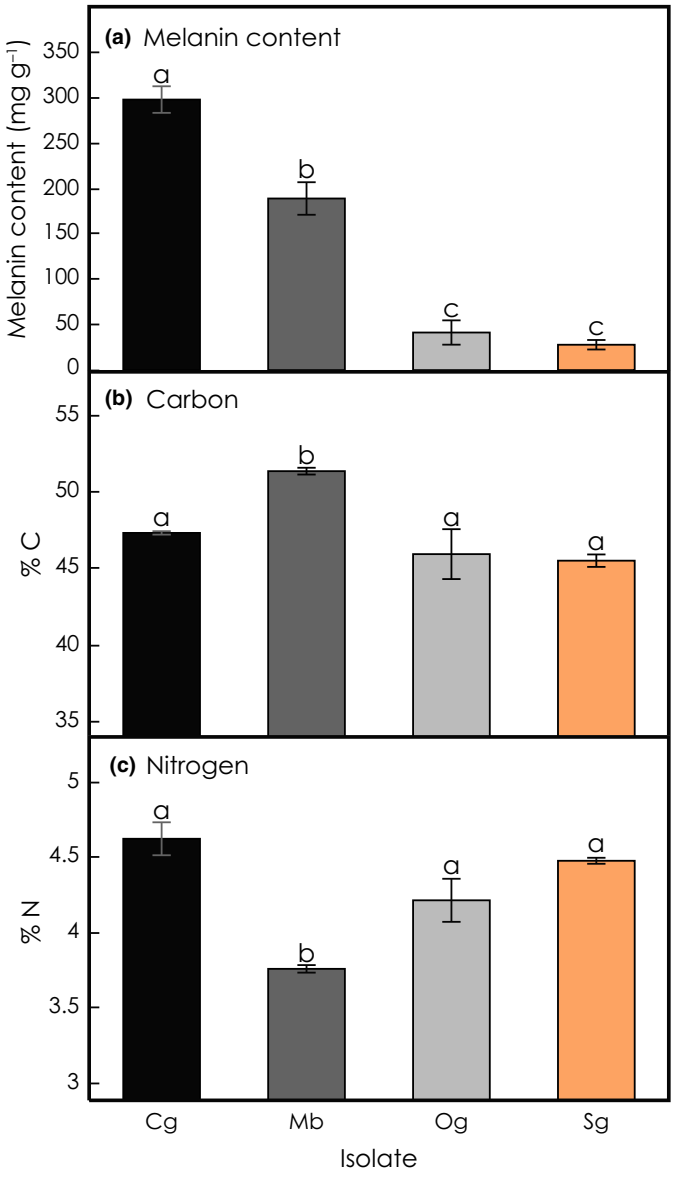

Figure 1 Initial melanin content (a), carbon (b), and nitrogen (c) content of the mycorrhizal necromass for *Cenococcum geophilum* (Cg), *Meliniomyces bicolor* (Mb), *Oideodendron griseum and Suillus grisellus* (Sg). Significant differences from Tukey's HSD test are indicated with different letters.

DISCUSSION

We found that elevated temperatures significantly increased the decomposition rates of mycorrhizal necromass, but that the response to temperature was strongest when the necromass was incubated in the hollows. Since hollows are submerged by the water table under ambient conditions, this interaction was likely the result of water table drawdown with increased temperatures that increased the oxygen availability, which would thereby increase decomposition rates (Freeman *et al*. 1996). Supporting that scenario, we observed that necromass mass loss was negatively correlated with mean water table depth (during the growing season) at both 3- and 12-month sampling points (Figure S8). This depth-dependent response of necromass decomposition has significant consequences for the stabilisation of mycorrhizal-derived C. Unlike aboveground inputs, roots and associated mycorrhizal biomass are born and subsequently decomposed at different positions in the soil profile (Schmidt *et al*. 2011). The abundance of saprotrophic bacteria and fungi are also known to vary across the soil profile because of depletion of labile C and steep oxygen gradients (Lindahl *et al*. 2007). In peatland systems, potential shifts in microbial decomposers with temperature and water table depth (Asemaninejad *et al*. 2017) may have important consequences on the decomposition of necromass inputs. Mycorrhizal inputs deposited in hollows of peatlands that would otherwise be protected from decomposition by submersion could be susceptible to increased decomposition rates under elevated temperatures if this reduced water levels. Based on our decay model, we estimate that necromass organic matter stabilisation in hollows will be reduced by *c*. 39 and 45% under the +4.5 °C and +9.0 °C scenarios, respectively. Like other complex aromatic compounds, the breakdown of melanin requires the production of oxidative enzymes (Butler & Day 1998), which are rate limited by the availability of O_2. Because of the possibility of lower oxidative enzyme activity in hollow microsites, we predicted that melanin constituents would be relatively protected from decomposition under ambient conditions but upon warming would become vulnerable to oxidative attack. While we did not find direct evidence of the temperature treatment reducing melanin content with the FTIR analyses, it is possible that these effects are not detectable until later in the decomposition process.

The initial decay rates of the mycorrhizal necromass were rapid in this peatland system compared to findings from studies in upland forests. For instance, the average mass loss after 3 months of decomposition was *c*. 30% higher in this study compared to the average from a temperate Pine-dominated system reported in Fernandez & Koide (2014). The faster initial decay rates may be the consequence of fungal necromass being rich in N, and this peatland system is extremely nutrient poor, which may lead to rapid mineralisation of the labile

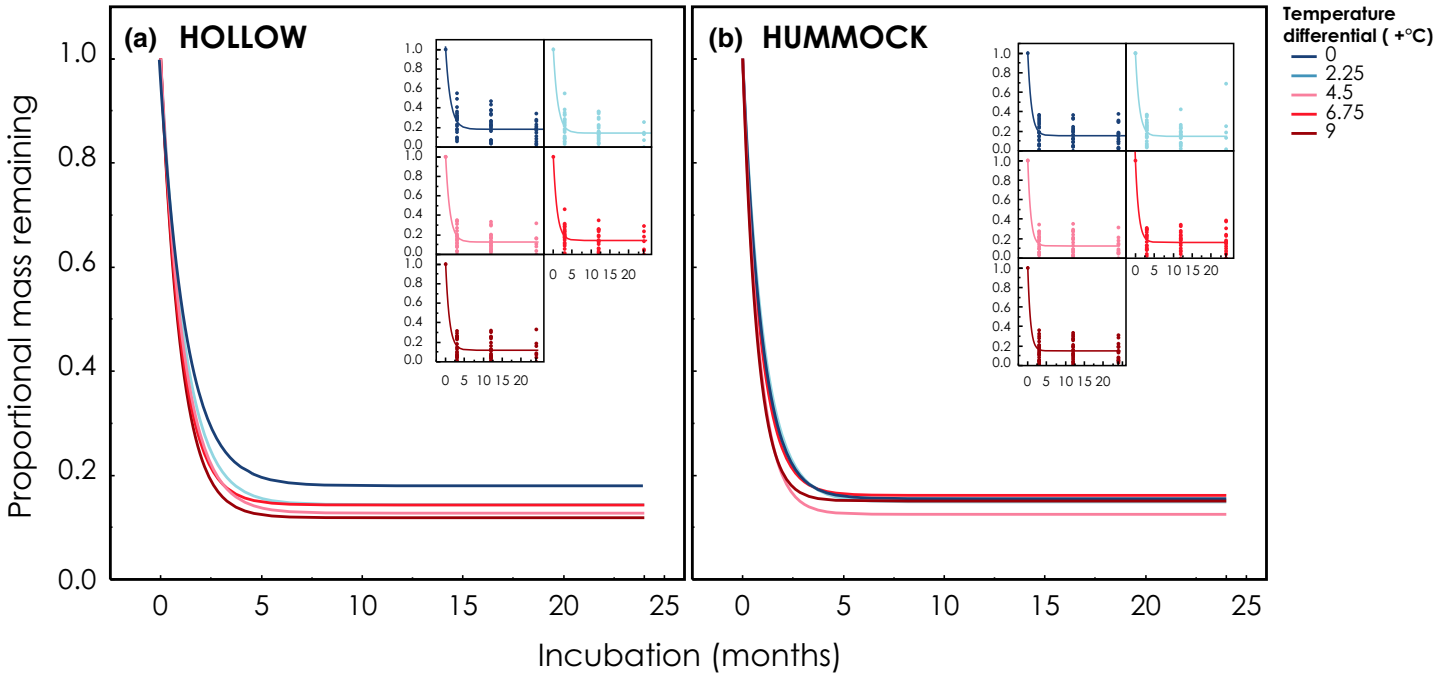

Figure 2 Asymptotic non-linear exponential decay model fits for temperature treatment in hollow (a) and hummock (b) microtopography microsites. See Table S3 for model parameters estimates. Data points and associated non-linear curve for each temperature treatment are presented in the inset panels.

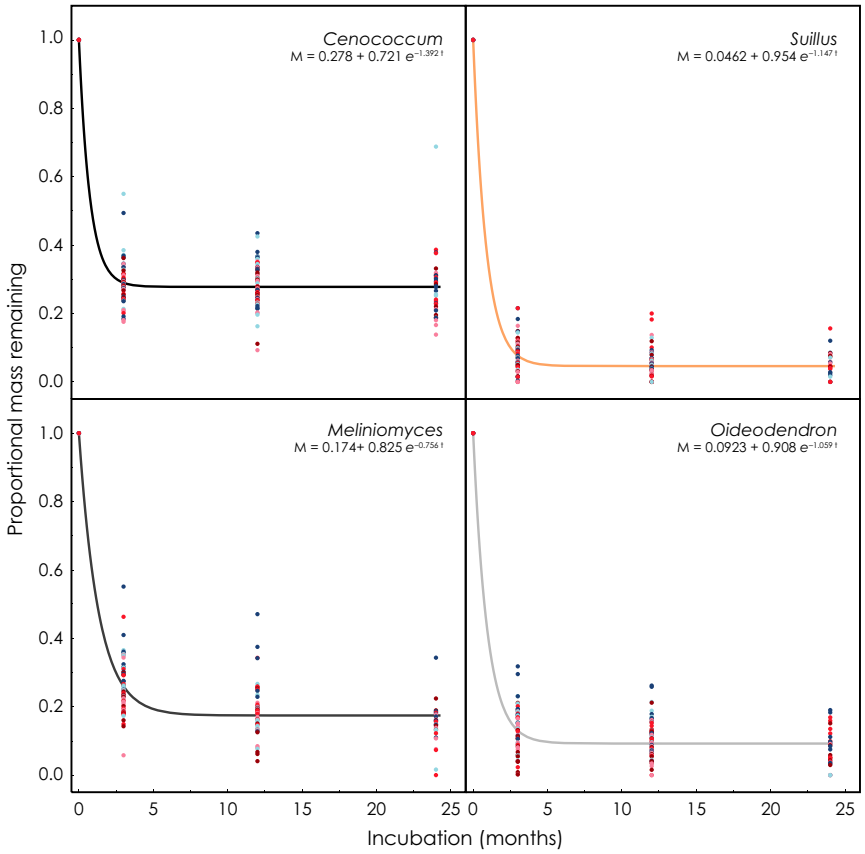

Figure 3 Exponential decay model fits for *Cenococcum*, *Suillus*, *Meliniomyces* and *Oideodendron* necromass. Data points represent a single sample and are colour coded by temperature treatment (N = 606)

compounds found in the necromass. Consistent with previous findings, we show that nitrogen and melanin contents are the primary biochemical controls on the decomposition of fungal necromass (Koide & Malcolm 2009; Fernandez *et al*. 2013; Fernandez & Koide 2014; Lenaers *et al*. 2018; Fernandez & Kennedy 2018). Since previous studies used relatively short-term incubations (< 3 months), our understanding of how these chemical constituents may influence the decomposition dynamics of fungal necromass over longer incubation times has remained unclear. In this study, we implemented a longer-term incubation (24 months), which revealed that N content of the necromass largely controls the initial decomposition rates of the labile fraction, while melanin content has little effect initially but ultimately determined the size and rate of decay of the recalcitrant fraction. For each necromass type, the decay rates stabilised at points where the mass remaining in the necromass corresponded strikingly to initial melanin content, suggesting that most of the hydrolysable fraction, such as polysaccharides and proteins, is rapidly mineralised leaving behind a recalcitrant fraction composed of melanin. Evidence from previous studies examining bacterial and fungal decomposer communities associated with fungal necromass decomposition suggests they are dominated early by bacteria and fast-growing moulds that likely take advantage of soluble and hydrolysable fractions and are strongly N limited (Brabcová *et al*. 2016; Fernandez & Kennedy 2018). Later in necromass incubation, these taxa are replaced by slower growing basidiomycete fungi that can presumably break down the more recalcitrant fraction (Fernandez & Kennedy 2018). Since the proportion of necromass mass not accounted for by melanin content is rapidly mineralised, our findings do not support the previously hypothesised possibility that melanin may form complexes with labile components, akin to lignocellulosic plant residues, that would inhibit their decomposition (see Fernandez *et al*. 2016). That said, more long-term studies in other systems and with necromass from more isolates are needed to confirm this result.

It is critical to understand not only what governs the decomposition of mycorrhizal necromass, but also the amount of C allocated to mycorrhizal fungi, the composition and abundance of functional traits present in the associated mycorrhizal communities, and the response of both elements to climate change to fully understand the impacts on C cycling in these systems. Mycorrhizal fungal communities will undoubtedly respond to changes in climate (Mohan *et al*. 2014), both in terms of community structure as well as spatial and temporal deposition of biomass. Mycorrhizal fungi are intimately linked to plant host C supply (Pena *et al*. 2010), and warming can have negative impacts on boreal tree host photosynthetic capacity (Reich *et al*. 2015; Dusenge *et al*. 2018), which has been linked to changes in mycorrhizal community composition (Fernandez *et al*. 2017). Direct effects of elevated temperatures can ultimately cause shifts the abundances of mycorrhizal fungal taxa with traits known or hypothesised to control necromass decomposition (e.g. biochemistry; morphology) that may increase or further reduce C stabilisation (Koide *et al*. 2014). In this peatland system, there are also notable differences among the two ectomycorrhizal hosts in how they are responding to elevated temperatures, with *Picea mariana* responding negatively and *Larix laricina* being tolerant (Hanson *et al*. 2018). Since the ectomycorrhizal communities of *L. laricina* are dominated by *Suillus* spp. in this peatland (Kennedy *et al*. 2018), it is plausible to expect increased non-melanised necromass inputs that are highly labile compared to the recalcitrant inputs produced by *Cenococcum geophilum*. These host-mediated responses to warming, along with those of the ericaceous shrubs, may also play a key role in determining the amount of necromass C that is quickly mineralised versus the amount entering stable belowground C pools. Additionally, the distribution of fine roots in this system is driven by the water table depth and under ambient conditions are mostly relegated to zones no deeper than 10 cm below the average water table depth (Iversen *et al*. 2018). Since fine roots are the primary C sources for mycorrhizal fungi, the drawdown of the water table is likely to increase the vertical range of where mycorrhizal inputs are deposited in the peat profile across hummocks and hollows. Unlike aboveground inputs, and even root inputs, estimating mycorrhizal fungal inputs is incredibly challenging to obtain because of the fineness of the fungal hyphae and their heterogenous distribution in soils. However, methods using in-growth cores or bags filled with sterile sand have been used to estimate mycorrhizal biomass production and turnover (death) (Wallander *et al*. 2001; Hagenbo *et al*. 2017) and will be important to include to better understand the mycorrhizal biomass production dynamics in these systems.

Based on our results, elevated temperatures associated with climate change will increase necromass decomposition rates, particularly when deposited in hollows. While increases in

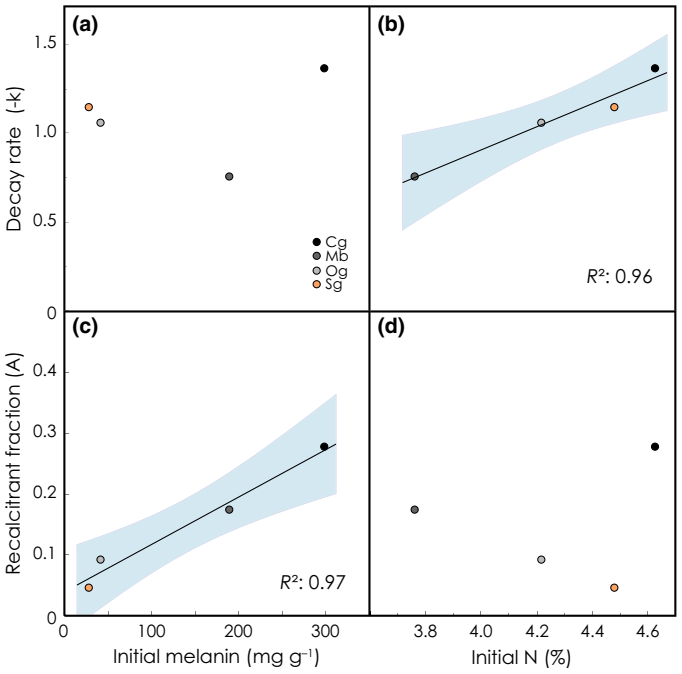

Figure 4 Mean initial necromass melanin (a and c) and nitrogen (b and d) content and relationships with decay rate (-*k*) and asymptotic parameter (*A*) from non-linear model fit from Table S4. Best fit line Decay rate ($-k$) = −1.695 + 0.65* Initial Nitrogen; $P = 0.021$; R^2: 0.96. Best fit line Recalcitrant fraction (A) = 0.391 + 0.0007* Initial melanin; $P = 0.014$; R^2: 0.97.

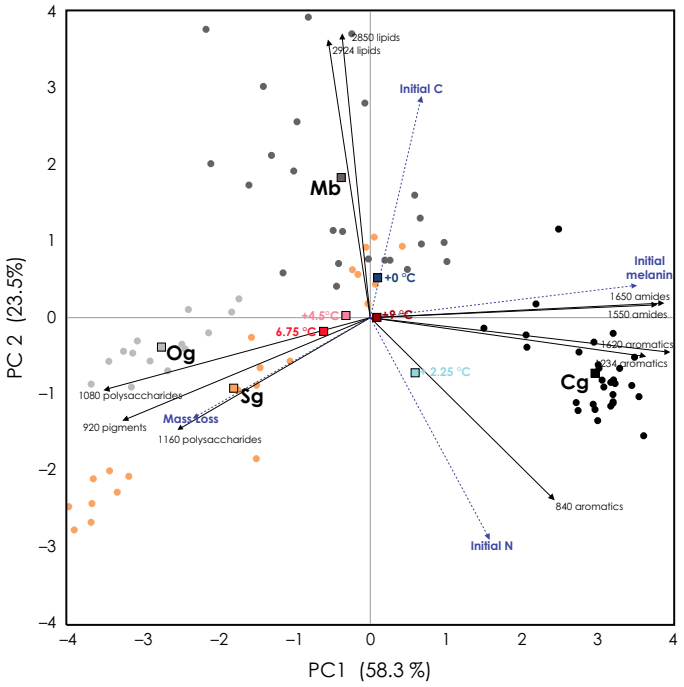

Figure 5 Principal component analysis biplot for FTIR wavenumber relative absorbance data (black vectors) for incubated *Cenococcum geophilum* (Cg), *Suillus grisellus* (Sg), *Meliniomyces bicolor* (Mb) and *Oideodendron griseum* (Og) necromass. Centroids for necromass type and temperature treatments indicated with square points and are colour coded. Blue vectors represent initial chemistry measurements and proportional mass loss of the necromass and are projected on to the PCA biplot.

decay rates across temperature treatments were small, because of the flux size (Ekblad *et al.* 2013) and the frequent turnover of mycorrhizal biomass (death) (Allen & Kitajima 2013), these differences may have considerable consequences on the contribution to C stocks in these systems. In addition, while it is well documented that fungal necromass has an initial period of rapid decay, our results indicate that, like plant litter, these rates significantly slow down after these initial labile materials are degraded and more recalcitrant compounds such as melanin remain. In our data set, the mass loss rates stabilised for all the necromass types after 3 months. As such, it is highly important to design the next generation of necromass decomposition studies with longer incubation times in order to better estimate decay rates of this recalcitrant second pool.

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AUTHOR CONTRIBUTIONS

CWF led the experimental design, field and laboratory work, data analysis and manuscript preparation; KH carried out the FTIR analyses and assisted in manuscript preparation; RK assisted with SPRUCE site operations, fieldwork and manuscript preparation; PGK assisted with experimental design, fieldwork, data analysis and manuscript writing.

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SUPPORTING INFORMATION

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

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