Fate of Decomposed Fungal Cell Wall Material in Organic Horizons of Old-Growth Douglas-fir Forest Soils

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Organic horizons of old-growth Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] forests are colonized by ectomycorrhizal fungi, which can form large patches or mats. Respiration, N mineralization, chitinase activity, and amino sugar C and N turnover are higher in soils colonized by ectomycorrhizal mats. To test if microbial activity in mat soil is driven by decomposition of fungal biomass, we incubated mat and non-mat soil with $^{13}C^{15}N$-labeled, chitin-enriched fungal cell wall material. Cell wall material was readily decomposed during 22-d incubations in both soils, and cell-wall-derived ^{13}C and ^{15}N were traced into mineralized pools, extractable organic pools, microbial biomass, and microbial phospholipid fatty acids (PLFAs). Basal microbial activity was higher in mat soils. Although total respiration was higher in mat soils with cell walls added, more cell wall C was mineralized in non-mat than mat soils; this was inferred to reflect greater preferential respiration of cell wall C over soil organic matter (SOM). Mineralization of N from cell wall material was low, with no difference between soils, and positive priming of N mineralization from SOM occurred after 3 wk. Both the proportion of cell wall N assimilated and N growth yield efficiency (GYE) were higher than for cell wall C. The relative abundance of fungi was higher in cell-wall-amended soils, and cell-wall-derived ^{13}C was found in fungal and Gram-negative bacterial PLFAs. Cell wall C assimilation and C GYE were higher in mat soils, which could be linked to greater mineralization of SOM or to differential activity of select microbial taxonomic groups. Chitin-rich fungal cell wall material was utilized as both a microbial N and C source; N was predominantly assimilated and C was predominantly metabolized. It is possible that fungal necromass may contribute to N stabilization in these soils.

Abbreviations: EcM, ectomycorrhizal; EOC, extractable organic carbon; EON, extractable organic nitrogen; GYE, growth yield efficiency; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PLFA, phospholipid fatty acid; SOM, soil organic matter.

Mycorrhizal fungi utilize plant-fixed C to grow into and acquire nutrients from the soil matrix. Some ectomycorrhizal (EcM) fungi develop substantial networks of rhizomorphic mycelial tissue to enhance nutrient acquisition for their plant associates (Agerer, 2001). Ectomycorrhizal biomass can comprise up to one-third of the total microbial biomass in forest mineral soils (Högberg and Högberg, 2002), and an EcM biomass may account for the majority of fungal biomass in organic layer soils (Bååth et al., 2004). In old-growth Douglas-fir forests, EcM mats can cover 20 to 40% of the forest floor area and display significantly higher N mineralization and respiration rates than non-mat soils (Griffiths et al., 1996, 1991; Phillips et al., 2012; Zeglin et al., 2013). Thus, in forest soils, ectomycorrhizae comprise a large proportion of total microbial biomass and contribute significantly to rates of soil nutrient cycling. The importance of decomposition of this EcM biomass is less well studied, however.

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Ectomycorrhizae clearly influence soil C and N turnover, but it is less clear whether they are associated directly with SOM decomposition (Read and Perez-Moreno, 2003; Talbot et al., 2008). Certainly, they can produce a variety of extracellular enzymes (Courty et al., 2005; Griffiths and Caldwell, 1992), including peptidases, phosphatases, cellulases, and oxidative enzymes, that may contribute to the degradation of SOM (Schimel and Weintraub, 2003). In old-growth Douglas-fir soils, however, only chitinase activity was elevated in EcM mat colonized soils relative to non-mat soils (Kluber et al., 2010). Chitinase activity is associated with hydrolysis of amino sugars from microbial cell wall material (Sinsabaugh and Moorhead, 1994), primarily fungal chitin in these organic layer soils (Zhang and Amelung, 1996). This leads to the hypothesis that fungal biomass turnover, not SOM decomposition, might be associated with higher respiration and N mineralization rates in EcM mat soils.

In fact, a prior study confirmed that chitin stimulated respiration, N mineralization, and microbial biomass growth and that chitin was degraded more rapidly in EcM mat soils than non-mat soils (Zeglin et al., 2013). Although chitin is an insoluble compound (Gooday, 1990), it may also represent a significant soil N pool, particularly in low-N old-growth forest floor soils (Sollins et al., 1980). Based on a higher N than C microbial GYE following chitin amendment, Zeglin et al. (2013) suggested that chitin was utilized primarily as an N substrate and secondarily as a C substrate for microbial growth in the N-limited study soils. In other studies, the N and chitin content of fungal necromass was positively correlated to the necromass decomposition rate (Fernandez and Koide, 2012; Koide and Malcolm, 2009), and both saprotrophic and mycorrhizal fungi may assimilate fungal necromass C and N (Drigo et al., 2012; Kerley and Read, 1997). Although chitin is of particular interest as a nitrogenous compound, in fungal cell walls chitin exists complexed with glucans and other C-containing molecules, all of which may be mineralized and assimilated during cell wall decomposition (Drigo et al., 2012). In soils with different standing pools of fungal biomass, e.g., EcM mat and non-mat soils, differences in microbial C and N cycling may reflect the breakdown of differing quality organic matter.

Because it is important to understand the fate of fungal cell wall C and N during decomposition, particularly in these EcM mat and non-mat soils, we designed an experiment to directly measure fungal cell wall C and N movement through the soil C and N pools using $^{13}C^{15}N$-labeled fungal cell wall material. Because chitin is hypothesized to be the substrate that drives elevated microbial activity in the study soils (Zeglin et al., 2013), we isolated protein-free, chitin-rich fungal cell wall material from fungi grown on ^{13}C-glucose and $^{15}NH_4$ in the laboratory (Kirchman and White, 1999) and amended this material to EcM mat and non-mat soils from an old-growth Douglas-fir forest. We predicted that microbial transformation (solubilization via enzyme-driven depolymerization and mineralization) and assimilation of fungal cell wall material would be more rapid in mat soil than non-mat soil and that N from cell wall material would be assimilated in preference to C.

MATERIALS AND METHODS

Site Description and Soil Sampling

Soil was collected in December 2009 from the organic horizons of an old-growth Douglas-fir stand located at the H.J. Andrews Experimental Forest, western Cascade Mountains, Oregon (44°13′ N, 122°15′ W, 450 m elevation). Annual precipitation at the site averages 230 cm, mainly as rainfall between November and March; snow rarely accumulates at this elevation. Soils are classified as coarse-loamy, mixed, mesic Typic Hapludands, with a mean O horizon of 6 cm (Dixon, 2003). The O horizon has a pH of 4.5 to 4.8, is 40 to 41% C and 1.1 to 1.2% N by mass (molar C/N ratio = 40–42), and contains 13 to 16 g kg^{-1} dry soil of acid-hydrolyzable amino sugars (Zeglin et al., 2013).

A previous study on EcM mat colonization of soils at the H.J. Andrews Experimental Forest found *Piloderma* spp. (Atheliaceae) to be the most common and widespread EcM associate of old-growth Douglas-fir trees, where it forms rhizomorphic mats in the O horizon (Dunham et al., 2007). We identified EcM mats using the same criteria as Dunham et al. (2007) and confirmed that they were colonized by only *Piloderma* spp. (Zeglin et al., 2013). Soil from the O horizon was collected from within the boundary of three rhizomorphic colonization ("mat" soils) and adjacent uncolonized areas ("non-mat" soils). Soils were stored intact at 4°C to preserve mycelial structure until the experimental incubations were begun. Immediately before the start of the experiment, soils were sieved (4 mm) and allowed to rest for 24 h. The sieved field replicates were combined into one composite batch of mat soil and one batch of non-mat soil and allotted into four independent incubation replicates per soil by treatment combination.

Experimental Setup and Production of Labeled Cell Wall Material

To produce $^{13}C^{15}N$-labeled fungal cell wall material, baker's yeast (*Saccharomyces cerevisiae*) was grown in batch culture on Difco C- and N-free yeast base medium with 10 g L^{-1} sucrose, 5 g L^{-1} $(NH_4)_2SO_4$, 10 mg L^{-1} L-histidine HCl (hydrous), 20 mg L^{-1} DL-methionine, 20 mg L^{-1} DL-tryptophan, 0.05 g L^{-1} 99 atom% ^{13}C-glucose, and 0.41 g L^{-1} 10.84 atom% ^{15}N-$(NH_4)_2SO_4$ (final enrichment level of medium was approximately 1.58 atom% ^{13}C and 1.16 atom% ^{15}N). After harvest, cell pellets were washed and chemically treated to isolate insoluble chitin-enriched cell wall material (Kirchman and White, 1999; Roff et al., 1994). Before each extraction step, cells were bead-beaten and washed three times with deionized water to remove water-soluble molecules. The cells were incubated in 4% sodium dodecyl sulfate (SDS) for 2 h at 90°C, suspended in acetate buffer (0.01 mol L^{-1} NaOAc and 0.05 mol L^{-1} CaOAc), and incubated with 1 g L^{-1} proteinase K overnight at 37°C. Cell material was suspended in 4% SDS and incubated again for 2 h at 90°C. The cell material was then extracted twice with a 1:1 mix-

ture of chloroform/methanol at room temperature. This protocol breaks cell walls and releases cytoplasmic molecules, removing all water- and organic-soluble cell debris and cell wall glycoproteins. Finally, because insoluble *S. cerevisiae* cell wall polymers are dominated by glucans (Cabib and Bowers, 1971; Watson et al., 2009), the remaining material was incubated with 1 g L^{-1} β-1-3-glucanase overnight at 37°C, thus enriching the material in chitin. The final material, presumably a mixture of insoluble polymeric chitin, chitosan, and glucan, was washed three times in deionized water, air dried, and suspended in deionized water.

The total C and N content and ^{13}C and ^{15}N enrichment of labeled cell wall material was determined by isotope ratio mass spectrometry at the Oregon State University Stable Isotope Research Unit. The cell wall material had a $\delta^{13}C$ value of 151‰ (atom% $^{13}C_{cw}$ = 1.277) and a $\delta^{15}N$ value of 501‰ (atom% $^{15}N_{cw}$ = 0.549). The molar C/N ratio of this cell wall material was 19.5, implying an approximate 2:1 ratio of glucan (6 C) to amino sugar (8 C/1 N) molecules; this was confirmed by amino sugar quantification, which found that the labeled cell wall material was approximately 39% amino sugars by mass. Treated soils were amended with 9.9 g C and 0.6 g N kg^{-1} dry soil as chitin-enriched insoluble cell wall material; this amendment corresponds to an approximately equivalent amount of C and N as found in the soil microbial biomass, and mean increases from 40.5 to 41.5% soil total C, from 1.16 to 1.22% soil total N, and from 14.5 to 19.1 g amino sugars kg^{-1} dry soil.

To quantify the movement of C and N from chitin-enriched fungal cell wall material into other soil C and N pools, $^{13}C^{15}N$-labeled cell wall material was added to mat and nonmat soils and the resulting dynamics were measured during the course of 22-d incubations. For each incubation replicate, 30 g of soil at field moisture content (approximately 10 g dry soil) was contained in an airtight 500-mL canning jar equipped with a gas sampling septum. Cell wall material was added as a suspension; control soils received an equal amount of water (4 mL) as the amended soils. The $^{13}CO_2$–C respiration was monitored continuously, and the experiment was terminated when cell wall substrate respiration began to plateau, after 22 d (t_3). In addition, temporal incubation replicates were destructively sampled at t_0 and 2 (t_1) and 7 d (t_2) after the cell wall material was added (n = 4 per time point). At each destructive sampling point, soils were harvested from all replicate jars and divided for analysis of exchangeable organic C and inorganic and organic N, microbial biomass C and N, and chitinase activity. Soil nutrient chemistry and microbial biomass samples were processed immediately, and soil subsamples were frozen and stored at −20°C until chitinase activity assays could be run.

Total and Carbon-13 Respiration Responses

Respiration rates were calculated as the accumulation of CO_2–C in the headspace of incubation chambers during a 2- to 3-h period. The chamber headspace was flushed with room air before and after every data collection point or every 72 h. Both $^{12}CO_2$–C and $^{13}CO_2$–C concentrations were measured using a Picarro cavity ring-down spectrometer, and atom% ^{13}C was derived from these values. The amount of cell wall (CW)-derived C respired in treatment soils was calculated as $CO_2\text{–}C_{treatment\text{-}CW}$ = [(atom% $^{13}CO_2\text{–}C_{treatment}$ − atom% $^{13}CO_2\text{–}C_{control}$)/(atom% C_{CW} − atom% C_{SOM})] × (g CO_2–C kg^{-1} soil). The amount of SOM-derived C respired in treatment soils was calculated as $CO_2\text{–}C_{treatment\text{-}SOM}$ = ($CO_2\text{–}C_{treatment}$ − $CO_2\text{–}C_{treatment\text{-}CW}$) and the C priming percentage was calculated as [($CO_2\text{–}C_{treatment\text{-}SOM}$ − $CO_2\text{–}C_{control}$)/$CO_2\text{–}C_{control}$) × 100. For mass balance purposes, $CO_2\text{–}C_{treatment\text{-}CW}$ was also expressed as a proportion of the total C added as cell wall material.

Total and Nitrogen-15 Mineralization Responses

Extractable soil N was obtained by shaking soil in a 0.05 mol L^{-1} K_2SO_4 solution (40 mL per 3 g dry soil) for 1 h. The inorganic N concentration within the extract solution was measured colorimetrically using an Astoria-Pacific autoanalyzer. After reduction to NO_2^- on a Cd column, NO_3^- + NO_2^-–N was quantified using the sulfanilamide/*N*-1-naphthylethylenediamine dihydrochloride assay. Ammonium N was measured as NH_3 using the alkaline salicylate–hypochlorite method. Nitrogen mineralization rates were calculated from the change in total inorganic N concentrations during the course of the 22-d incubation. Spiked diffusions were conducted to measure soil solution $^{15}NO_3$–N and $^{15}NH_4$–N (Stark and Hart, 1996). The amount of cell-wall-derived N mineralized in the treatment soils was calculated as N $min_{treatment\text{-}CW}$ = [(atom% ^{15}N $min_{treatment}$ − atom% ^{15}N $min_{control}$)/(atom% $^{15}N_{CW}$ − atom% $^{15}N_{SOM}$)] × (g $NH^+ + NO_3^-$–N kg^{-1} soil). The amount of SOM-derived inorganic N in treatment soils was calculated as N $min_{treatment\text{-}SOM}$ = (N $min_{treatment}$ − N $min_{treatment\text{-}CW}$). The N priming percentage could not be calculated because of the net negative mineralization status of the control incubations, but the qualitative direction of priming (positive or negative) was evident when comparing the amount of SOM mineralized in treatment vs. control soils. For mass balance purposes, N $min_{treatment\text{-}CW}$ was also expressed as a proportion of the total N added as cell wall material.

Total and Carbon-13, Nitrogen-15 Biomass and Solubilization Responses

Microbial biomass C (MBC) and N (MBN) were estimated as the extractable organic C (EOC) and N (EON) liberated after a 24-h chloroform fumigation of the soil. The EOC and EON were extracted from fumigated and unfumigated soils by shaking in a 0.05 mol L^{-1} K_2SO_4 solution (13.3 L kg^{-1} dry soil) for 1 h and was quantified using a Shimadzu total C and N analyzer. The EOC and EON of unfumigated soils were calculated as standalone variables, and MBC and MBN values are reported using extraction coefficients: K_{EC} = 0.45 and K_{EN} = 0.56 (Brookes et al., 1985; Vance et al., 1987). Subsamples (1.5 mL) of each soil extract, plus a spike of 225 to 450 μg C and 33 to 65 μg N as glu-

tamic acid with known atom% ^{13}C and ^{15}N, were dried down to quantify the mass of $EO^{13}C$, $EO^{15}N$, $MB^{13}C$, and $MB^{15}N$ relative to dry-down spike controls. The cell-wall-derived amounts of C and N solubilized and C and N assimilated into microbial biomass are reported as the percentage of cell wall material C or N added using calculations analogous to those described above for C and N mineralization. Substrate-derived C GYE was calculated as $C_{assimilated}/(C_{assimilated} + C_{solubilized} + C_{mineralized})$; N GYE was calculated similarly.

Carbon-13 Phospholipid Fatty Acid Analysis

Total PLFAs were extracted from 0.5-g subsamples of all control and treatment soil replicates collected when ^{13}C assimilation peaked at 7 d of incubation (Bligh and Dyer, 1959; Frostegård et al., 1993). Soils were incubated in a 2:1:0.67 mixture of methanol/chloroform/citrate buffer, and phospholipids were isolated from the chloroform phase using 3-mL Supelco Supelclean LC-S1 SPE columns, then saponified and methylated. The resulting fatty acid methyl esters (FAMEs) were analyzed using an Agilent 6890 gas chromatograph with a 30-m HP Innowax column connected to a Europa ORCHID on-line combustion interface in line with a Europa 20–20 isotope ratio mass spectrometer, and their relative abundance and ^{13}C content were determined by comparing soil extract FAMEs with the retention time profiles of FAME standards of known composition and concentration (Brant et al., 2006; Butler et al., 2003). The $\delta^{13}C$ of FAMEs was corrected to account for the C added during methylation to derive the $\delta^{13}C$ of the original PLFAs. The origins of soil PLFAs were classified as: Gram-positive bacteria (iso15:0, anteiso15:0, iso16:0, iso17:0, anteiso17:0), Gram-negative bacteria (16:1ω9, 17:1ω9, cyclo17:0, 18:1ω7, 18:1ω9t, cyclo19:0), actinobacteria (10Me16:0), fungi (18:2ω6), and protozoa (20:4ω6). The sum of the mole percentage of C in these particular PLFAs was accepted as a proxy for the relative abundance of each taxonomic group, and the amount of total bacterial relative to fungal PLFAs was utilized as a fungal/bacterial biomass ratio (F/B).

Chitinase Potential Activities

Potential *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30) enzyme activity was measured colorimetrically using *p*-nitrophenol–*N*-acetyl-β-D-glucosaminidine (pNP–NAG, N-9736, Sigma Chemicals). Soil slurries with pNP–NAG substrates added at saturating concentrations, and appropriate controls, were incubated at 30°C for 1 to 2 h (Parham and Deng, 2000). A pNP standard solution was used as calibrant, and the amount of color accumulation during the assay incubation time was used to calculate the chitinase activity rate (in μmol pNP kg^{-1} dry soil h^{-1}).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare basal rates and pool sizes between mat and non-mat soils at t_0. Two-way ANOVA was used to evaluate the independent and interactive influence of soil (mat and non-mat) and sampling time on total and cell-wall-derived C and N transformations and on the relative abundance and $\delta^{13}C$ of each PLFA taxonomic group. Three-way ANOVA was used to compare the level of C and N assimilation and GYE responses in both soils and all time points. Three-way ANOVA was also used to compare C mineralization patterns, inorganic N pools, and chitinase activity rates among soils, sampling times, and control vs. treatment soils (for mineralization data, *source* was designated to include the comparison between SOM mineralized in control soils, total SOM + cell wall material mineralized in treatment soils, and SOM mineralized in treatment soils). For all respiration data, which were collected continuously on the same four experimental replicates, repeated-measures ANOVA was utilized; where there was a significant interaction with time, the effects of soil and treatment were evaluated using one- or two-way ANOVA on data from each individual time point. All relevant post-hoc multiple comparisons were evaluated using Tukey's honest significant difference. The significance of any test was accepted at $\alpha \leq 0.05$. When necessary, dependent variables were natural logarithm transformed to meet assumptions of normality. All statistics were run using R (R Development Core Team, 2010).

RESULTS

Ambient Soil Characteristics

Carbon and N pools and microbial activity rates differed between mat and non-mat soils before the initiation of the experiment (Table 1). Extractable NH_4^+–N, EOC, EON, MBC, and MBN pools were all larger in mat soils than non-mat soils. Also, basal respiration, net nitrification, and chitinase activity rates were higher in mat soils than non-mat soils. The MBC/N ratio was significantly greater in mat soils than non-mat soils, but the F/B PLFA ratio was not significantly different ($P = 0.17$).

Respiration Response to Addition of Cell Wall Material

Chitin-enriched fungal cell wall amendment significantly elevated respiration in both mat and non-mat soils at many time points (Fig. 1a and1b), and respiration of the cell wall material began to plateau after 22 d (Fig. 1c). There were interaction effects between soil and time and among respiration sources (i.e., control soil [SOM] respiration, total respiration with cell walls added, or SOM respiration with cell walls added), but no three-way interaction (Table 2). Cumulative respiration with cell wall material added was higher in mat than non-mat soils after 2 d of incubation ($16.6 \pm 0.8 > 15.3 \pm 0.4$ g C kg^{-1} dry soil at 22 d), and less respiration was derived from the SOM in soils amended with cell wall material than the control through 15 d for both mat and non-mat soils, indicating a negative priming effect (Fig. 1a and 1b). There was also an interaction between time and soil effects on cell wall C mineralization and C priming (Table 3). The percentage of cell wall C respired was higher in non-mat soils, and the negative priming effect was stronger in non-mat soils, but only in the intermediate times of the incubation course (Fig. 1c and 1d).

Nitrogen Mineralization Response to Addition of Cell Wall Material

In both mat and non-mat soils, N immobilization occurred in the control incubations throughout the experiment (Table 1; Fig. 2) and in the treatment soils during the first week of incubation. Mineralization of N from the added cell wall material was very low during the first week of incubation but clearly detectable by the end of the experiment, peaking at 1.2 ± 0.05% (mat soil) and 1.3 ± 0.07% (non-mat soil) of N added (Fig. 3d; Table 2). Soil inorganic N pools were dynamic throughout the incubation time course, showing a three-way interaction among soil, mineralization source, and time (Fig. 2; Table 2). In treatment soils, N in excess of that derived from the added cell wall material was immobilized during the first week and was mineralized by the end of the experiment; thus N priming switched from a negative to a positive effect sometime between 7 and 22 d of incubation. This priming effect could not be calculated as a percentage, however, due to the net N immobilization (negative N mineralization) status in the control soils. No net changes in the NO_3^-–N pools were detected in response to substrate amendment.

Microbial Biomass Carbon and Nitrogen Response to Addition of Cell Wall Material

Assimilation of C from cell wall material occurred by 2 d, accounted for up to 5.8 ± 0.5% (mat soils) or 4.2 ± 0.2% (non-mat soils) of C added, and was significantly greater in mat soils throughout the experiment (Table 3; Fig. 3c). The C GYE decreased with time and was also higher in mat soils than non-mat soils (Table 3; Fig. 4a). Net assimilation of N from cell wall material occurred by 2 d and peaked at 22 d at 7.4 ± 1.2% (mat soils) or 6.8 ± 0.6% (non-mat soils) of N added (Table 3; Fig. 3f). Neither total N assimilation nor N GYE differed between mat and non-mat soils ($P > 0.3$; Table 3), but the N GYE decreased with time (Table 3; Fig. 4b). There was a difference between the amount of C and N assimilated, and between the C and N GYE, both of which showed an interaction with time ($F > 20.5$, $P < 0.001$) but not soil ($F \leq 2.3$, $P > 0.14$). Post-hoc tests showed that the percentage of N assimilated at 22 d was higher than the amount of C or N assimilated at all other time points and also overlapped with the peak level of C assimilated at 7 d ($P \leq 0.005$). Also, the N GYE (0.77 ± 0.03 to 0.96 ± 0.01) was higher than the C GYE (0.06 ± 0.0005 to 0.55 ± 0.05) for all time points, and both decreased as time elapsed ($P < 0.001$). The ratio of N GYE to C GYE ranged from 1.5 ± 0.05 (mat soil at 2 d) to 12.6 ± 0.2 (non-mat soil at 22 d).

Solubilized Carbon and Nitrogen and Chitinase Enzyme Activity

The ^{13}C enrichment of EOC showed that a small amount of the labeled cell wall material was solubilized, peaking 7 d into the incubation at 0.4 ± 0.06% of C added (Fig. 3b). Similarly, the ^{15}N enrichment of EON indicated that a small amount of cell-wall-derived N (on average, 0.6 ± 0.2% of N added) was present in the solubilized pool throughout the incubation (Fig.

Table 1. Soil chemical and microbiological characteristics, including NO_3^-–N, NH_4^+–N, extractable organic N (EON) and C (EOC), microbial biomass C (MBC) and N (MBN), microbial biomass C/N ratio (MBC/N), fungal/bacterial ratio (F/B) net nitrification and net N mineralization during the 22-d control soil incubation, basal respiration, and chitinase potential activity.

Soil	NO_3^-–N	NH_4^+–N	EON	EOC	MBC	MBN	MBC/N	F/B	Net nitrification	Net N mineralized	Respiration	Chitinase
	mg kg^{-1} dry soil				g kg^{-1} dry soil				mg N kg^{-1} dry soil d^{-1}		mg C kg^{-1} dry soil h^{-1}	μmol pNP† kg^{-1} dry soil h^{-1}
Mat soil	3.5 (1.0)‡	36.8 a§ (0.2)	46.6 a (0.4)	921 a (5)	6.5 a (0.01)	0.81 a (0.004)	9.30 a (0.05)	0.14 (0.004)	0.010 a (0.01)	−1.29 a (0.05)	27.0 a (0.6)	17.2 a (3.7)
Non-mat soil	1.6 (0.2)	25.6 b (1.1)	33.6 b (1.0)	578 b (6)	5.2 b (0.06)	0.71 b (0.006)	8.63 b (0.06)	0.13 (0.006)	−0.032 b (0.02)	−0.90 b (0.07)	24.3 b (0.7)	11.0 b (3.5)

† pNP, p-nitrophenol.
‡ Mean with SE in parentheses of the four time zero experimental replicate values (or control incubations for N cycling rates) for each soil type.
§ Means of a property followed by different letters for mat and non-mat soils are significantly different (by pairwise ANOVA, $\alpha = 0.05$).

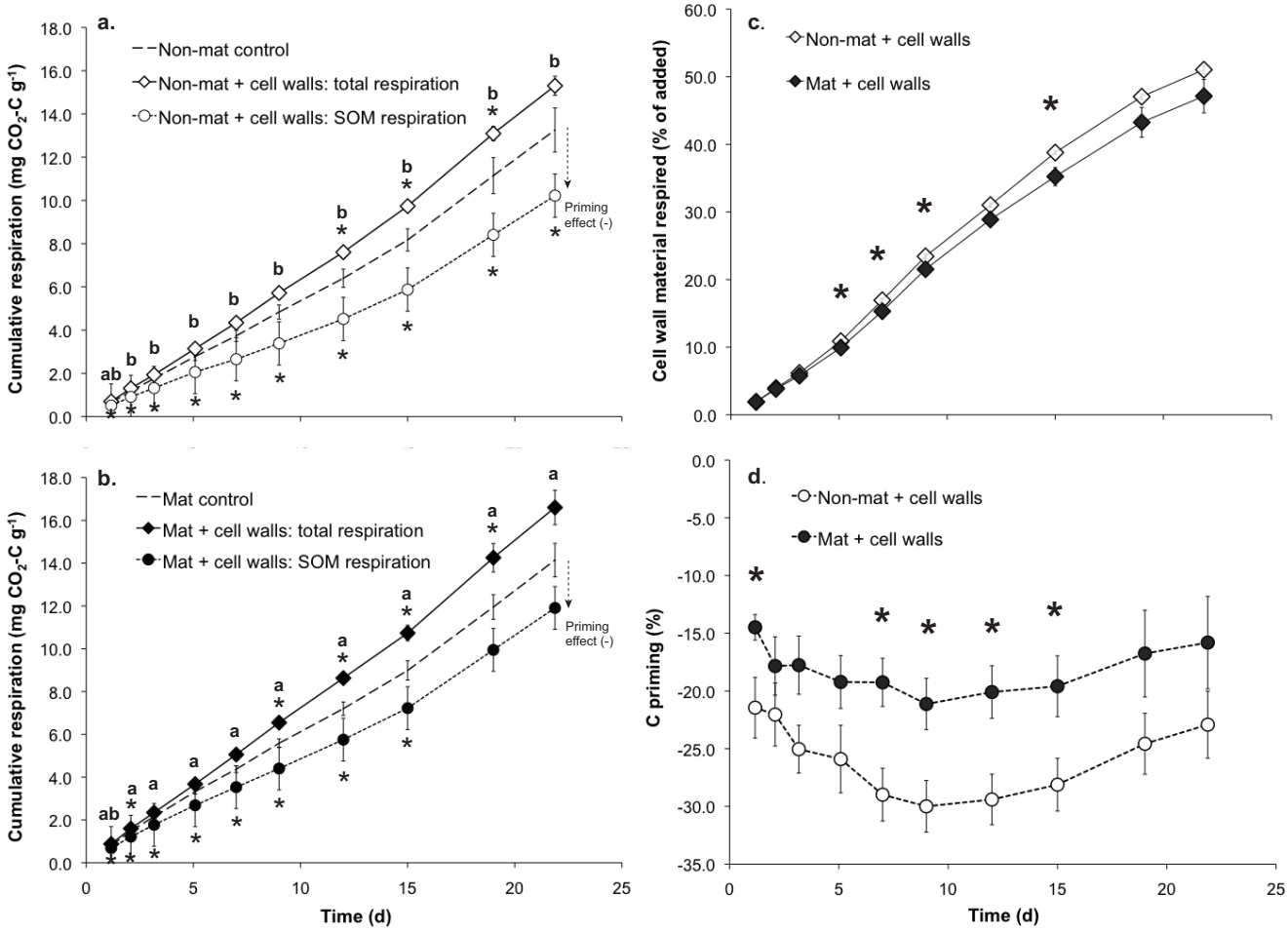

Fig. 1. Cumulative respiration in experimental incubations: Total C respired with time in control soils (dashed lines), soil with cell walls added (diamonds), and soil organic matter (SOM)-derived respiration only in soil with cell walls added (circles) in (a) non-mat and (b) mat soils, with the priming effect noted as the discrepancy between respiration of C in control incubations and SOM-derived C in treatment incubations; (c) cumulative cell-wall-derived C respired as a percentage of C added in non-mat (open symbols) and mat (filled symbols) soils; and (d) percentage of C priming with time in non-mat (open symbols) and mat (filled symbols) soils. Error bars represent ±1 SE. For each time point in (a) and (b), the comparative grouping of mat and non-mat soil respiration is noted using lowercase letters, and a significant difference from the control soil is noted by an asterisk. For each time point in (c) and (d), a significant difference between mat and non-mat soil is noted by an asterisk.

Table 2. Results of three-way ANOVA tests to evaluate cumulative respiration and total inorganic N pool size responses to cell wall material amendment in mat and non-mat soils. Respiration data were analyzed using a repeated-measures ANOVA. Data comprise four experimental replicates for each soil type and each of four (total inorganic N) or 10 (respiration) time points; *P* values in bold type are statistically significant (*P* < 0.05).

	F statistic, *P* value	
Parameter	Cumulative respiration	Total inorganic N
Soil	32.6, **0.011**	0.039, 0.844
Source†	203, **<0.001**	12.5, **<0.001**
Time	540, **<0.001**	123, **<0.001**
Soil × source	0.289, 0.759	1.16, 0.321
Soil × time	9.34, **<0.001**	8.59, **<0.001**
Source × time	189, **<0.001**	80.9, **<0.001**
Soil × source × time	0.446, 0.976	4.88, **0.002**

† Source contrasts soil organic matter (SOM) mineralized in control soils, total SOM + cell wall material mineralized in treatment soils, and SOM mineralized in treatment soils.

3e). Changes in the EOC and EON pools did not differ between mat and non-mat soils (Table 3).

Throughout the incubation experiments, chitinase activities were variable (Fig. 5). A three-way ANOVA of chitinase activity with soil type, time, and treatment as factors showed that rates remained higher in mat soils throughout the experiment (main soil effect, $P < 0.001$), that there was no significant main effect of time or treatment and no significant interactions among soil and any other factors ($P > 0.05$), but that there was a treatment × time interaction effect ($P = 0.032$). Initially (2 d), chitinase activity appeared to be higher than the control in non-mat soils with cell wall material added and to be lower than the control in mat soils amended with cell wall material at 22 d, but no significant post-hoc groups emerged to pinpoint time or treatment effects with certainty ($P > 0.05$).

Phospholipid Fatty Acid Community Composition

The relative abundance of different soil microbial groups after 7 d of incubation with chitin-enriched cell wall material

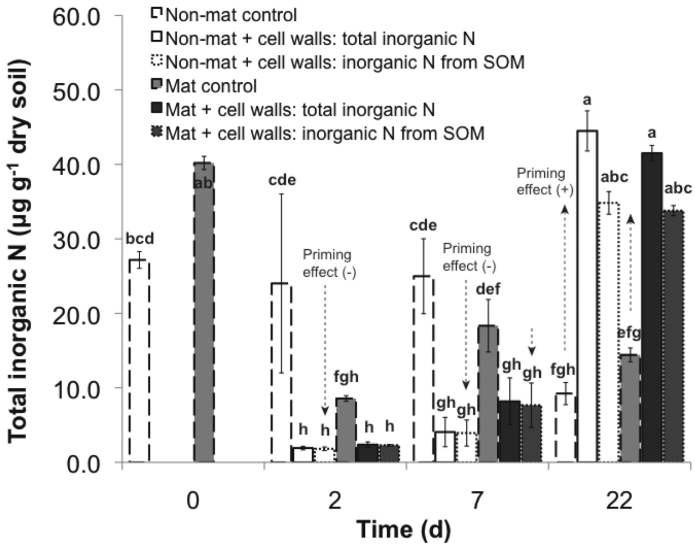

Fig. 2. Inorganic N (NH_4^+ + NO_3^-) pools in experimental incubations. Total inorganic N at each time point during the experiment in control soils (dashed lines), soil with cell walls added (solid lines) and soil organic matter (SOM)-derived inorganic N only in soil with cell walls added (dotted lines) in non-mat (white) and mat (dark gray) soils, with the priming effect noted as the discrepancy between inorganic N in control incubations and SOM-derived inorganic N in treatment incubations. Error bars represent ±1 SE. Groupings resulting from multiple comparisons of means across the data are noted using lowercase letters.

changed as the relative abundance of fungal markers increased by 44% (Fig. 6a, $P = 0.003$). Correspondingly, PLFA F:B increased from 0.13 ± 0.01 to 0.26 ± 0.04 ($P = 0.009$). Independent of PLFA abundance, different groups of markers were significantly enriched with ^{13}C from the cell wall material, including fungi and Gram-negative bacteria ($P < 0.001$, Fig. 6b). There were no significant differences in PLFA composition or ^{13}C enrichment between mat and non-mat soils, and no soil by treatment interactions ($P > 0.14$).

DISCUSSION

Chitin-enriched fungal cell wall material was significantly decomposed in both EcM mat and non-mat organic layer forest soils. After 3 wk of incubation, 51.2 ± 2.5% of the C and 9.1 ± 0.6% of the N added as cell wall material could be found in either the mineralized, solubilized, or biomass C and N pools. Carbon from cell wall material was mineralized to a much greater extent than N (49.2 ± 1.4% of cell-wall C mineralized, >1.2 ± 0.5% of cell-wall N mineralized). Nitrogen assimilation was higher than C assimilation at the experiment's end (7.1 ± 0.7% of cell-wall N assimilated, >3.5 ± 0.1% of cell-wall C assimilated), although peak C assimilation at 7 d (5.1 ± 0.4%) was not significantly different from peak N assimilation at 22 d. Of course, because gross N mineralization was not measured, the total amount of cell-wall-derived N that moved through the mineral pool during the experiment is unknown. Still, after 3 wk of soil incubation, mineralization was the dominant fate of decomposed cell-wall C and net assimilation was the dominant fate of decomposed cell-wall N (Fig. 3). These results support the original hypothesis that N from cell wall material would be assimilated in preference to

Table 3. Results of ANOVA tests for all fates of C and N derived from cell wall material. Experimental responses to soil type (mat or non-mat) and time point were tested using a two-way ANOVA, except for C mineralization and priming, which were analyzed using a repeated-measures two-way ANOVA. Data comprise four experimental replicates for each soil type and time point (2 , 7, and 22 d for destructive samples, 10 time points for nondestructive [repeated measures] data); *P* values in bold type are statistically significant ($P < 0.05$).

	F statistic, *P* value									
Parameter	C mineralization	C priming	N mineralization	C solubilization	N solubilization	C assimilation	N assimilation	C GYE†	N GYE†	Total C utilization	Total N utilization
Soil	7.35, 0.073	11.1, **0.045**	0.017, 0.897	0.421, 0.525	2.29, 0.154	5.38, **0.033**	0.056, 0.817	5.00, **0.039**	1.25, 0.284	1.32, 0.265	1.43, 0.253
Time	1019, <**0.001**	6.95, <**0.001**	456.6, <**0.001**	9.50, **0.002**	0.900, 0.430	2.80, 0.089	15.1, <**0.001**	98.9, <**0.001**	9.69, **0.003**	426.4, <**0.001**	79.2, <**0.001**
Soil × time	3.76, **0.004**	2.47, **0.034**	1.32, 0.298	0.259, 0.775	0.910, 0.427	0.532, 0.597	0.630, 0.548	0.920, 0.418	0.705, 0.514	2.46, 0.114	0.384, 0.521

† GYE, growth yield efficiency.

C; also, the N GYE was consistently greater than C GYE (Fig. 4). Together, these results suggest that fungal cell wall material N, is primarily utilized as a microbial growth substrate (Sinsabaugh and Moorhead, 1994; Zeglin et al., 2013). Particularly in these N-limited (C/N = 40) organic layer soils (Hart and Stark, 1997), microbial "recycling" of N may be an important N conservation strategy.

Although less partitioned into microbial growth, C derived from insoluble fungal cell wall material clearly supported a significant amount of microbial metabolism (Fig. 1a and 1b). In fact, the negative C priming effect suggests that cell-wall C was respired preferentially to SOM in both mat and non-mat soils (Fig. 1d). In this experiment, fungal cell-wall C was added predominantly as glucans and amino sugars; these compounds, despite being in polymeric and insoluble form, appear to be more bioavailable than the bulk SOM. Within 7 d, 21.6 ± 0.3% of cell wall C was depolymerized and solubilized, mineralized, or assimilated. This rapid decomposition is in agreement with a recent nuclear magnetic resonance based study that measured the largest amount of hyphal glucan-chitin C decomposition within the first 7 d following addition of hyphae to soil, accompanied by significant C incorporation into the DNA of saprotrophic fungal taxa (Drigo et al., 2012). This study also measured the rapid decomposition of other organic compounds comprising intact fungal necromass, including glycogen, lipids, and protein. In intact fungal biomass, there may be a larger N payoff for cell-wall decomposition—mycorrhizal fungal biomass has an mean C/N ratio of 14 (range 3–51; Strickland and Rousk, 2010), the C/N ratio of pure chitin is 8, but the insoluble cell wall material used in our experiment had a C/N ratio of 19.5. Thus, it is possible that the level of C mineralization measured in this experiment was higher (and the C GYE lower) than it would be for pure necromass or chitin;

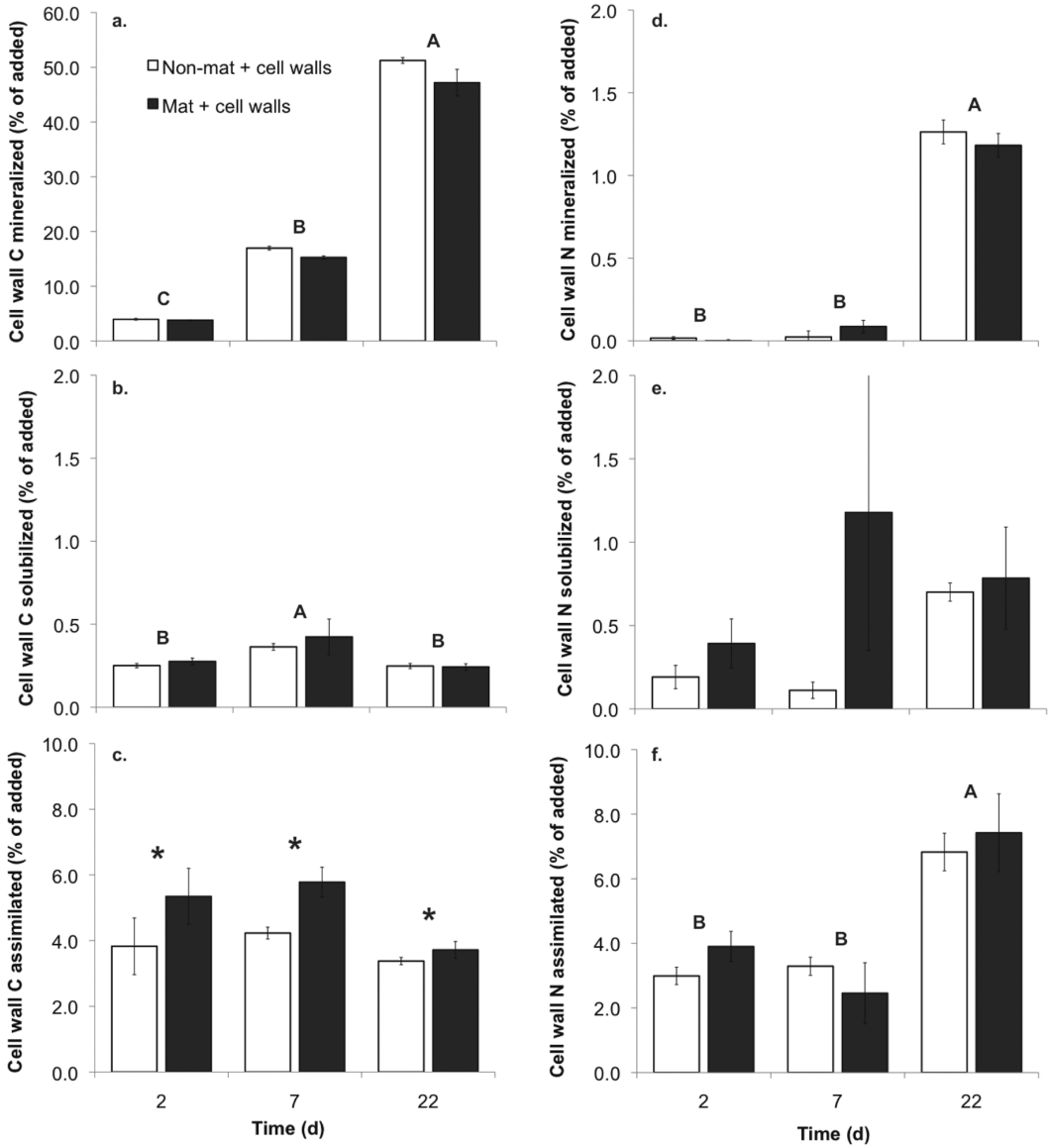

Fig. 3. Cell wall derived (a,b,c) C and (d,e,f) N that was found to be (a,d) mineralized, (b,e) solubilized, or (c,f) assimilated with time as a percentage of C or N added in experimental incubations in non-mat (white bars) and mat (black bars) soils. Error bars represent ±1 SE. For each response variable, significant multiple comparisons groupings of means by time are designated by uppercase letters, and a significant difference between mat and non-mat soil is designated by an asterisk.

however, our $^{13}C^{15}N$-labeled fungal cell wall material was comprised of natural microbial residues, unlike pure chitin, so may more realistically reflect quantitative in situ soil transformations of fungal necromass C and N than previous experiments.

The responses to cell wall material amendment differed less between mat and non-mat soils than we predicted. Cumulative total respiration (Fig. 1a and 1b), cell-wall-derived C assimilation (Fig. 3c), and overall chitinase potential activity rates (Fig. 5) were higher in mat than non-mat soils, as predicted; however, these differences were not large. Nitrogen cycling and PLFA responses were generally similar in mat and non-mat soils. The amount of cell-wall-derived C mineralization was slightly higher in non-mat soils than mat soils (Fig. 1c), contrary to our prediction; correspondingly, there was a greater C GYE (Fig. 4a) and a stronger C priming effect in non-mat soils (Fig. 1d), as well as a stronger N priming effect (Fig. 2). The predicted differential response between mat and non-mat soils was predicated in part by higher ambient microbial biomass and activity rates in mat soils (e.g., Griffiths et al., 1996; Kluber et al., 2010; Phillips et al., 2012), which were also detected in the study soils (Table 1). In our previous study, which showed larger response differences between mat and non-mat soils, incubation times and the ranges of amendment rate (particularly of N) were greater (Zeglin et al., 2013), so there are technical reasons that may explain the stronger responses in that study. Most importantly, the fate of the fungal cell wall material was traced directly using stable isotopes of C and N rather than inferred from pool size changes, revealing priming effects that could not be detected using unlabeled substrate amendments.

Priming effects appear to be driven by preferential decomposition of cell wall material over SOM. The mineralization of cell wall N showed initial (2–7 d) negative priming of N mineralization, followed by positive priming of N mineralization by 22 d (Fig. 2a), which follows the hypothesized trajectory of preferential substrate utilization (Blagodatskaya and Kuzyakov, 2008). The observation of negative C priming (Fig. 1d) also supports this mechanism, as noted above. In addition, 1 wk after cell wall material was added, fungal PLFA relative abundance was elevated and significant levels of ^{13}C from fungal cell wall material were detected in the lipids of fungi and Gram-negative bacteria in all treatment soils. This suggests that fungi metabolized cell wall C and used it most efficiently for growth, and certain bacteria also metabolized the fungal cell wall C, perhaps for cell turnover or division, but did not grow to an extent that significantly impacted population size. Although it is somewhat surprising that Gram-positive actinomycetes, classically considered to be the predominant group of chitinolytic bacteria (Hsu and Lockwood, 1975), did not utilize fungal cell wall ^{13}C, it is known that other bacterial taxa (including Gram-negative *Pseudomonas* spp., *Stenotrophomonas* spp., and *Janthinobacterium* spp.) carry chitinase genes (Terahara et al., 2009; Williamson et al., 2000) or increase in abundance in response to chitin amendment (Gould et al., 1981; Kielak et al., 2013). Also, sensitivity in the ^{13}C PLFA response may be relatively low, partially due to the relatively low atom% ^{13}C enrichment of the amended cell wall material. Overall, the observed priming effects may be mediated by the respiration and growth of specific microbial taxa that can better utilize fungal cell wall material (De Nobili et al., 2001).

In addition, negative C priming effects were stronger in non-mat soils than mat soils. This, in combination with greater

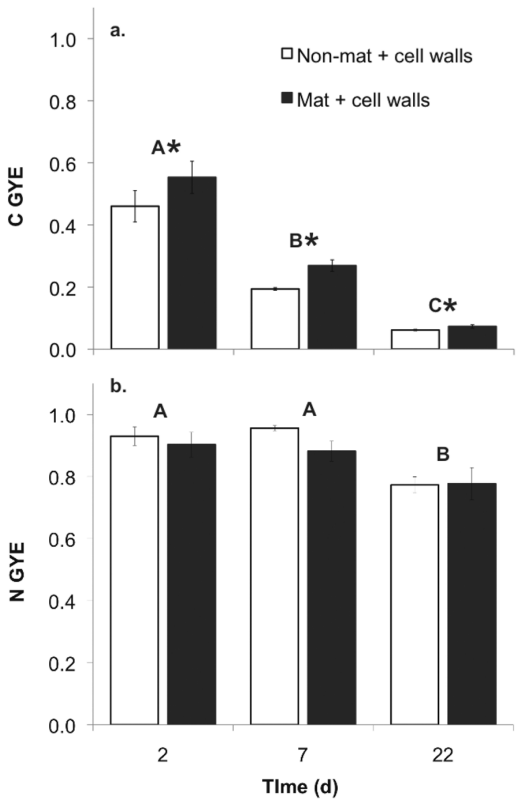

Fig. 4. The growth yield efficiency (GYE) of cell-wall-derived (a) C and (b) N with time in experimental incubations in non-mat (white bars) and mat (black bars) soils. Error bars represent ±1 SE. For each response variable, significant multiple comparisons groupings of means by time are designated by uppercase letters and a significant difference between mat and non-mat soil is designated by an asterisk. Note that C mineralization proportions are a subset of those data shown in Fig. 1c.

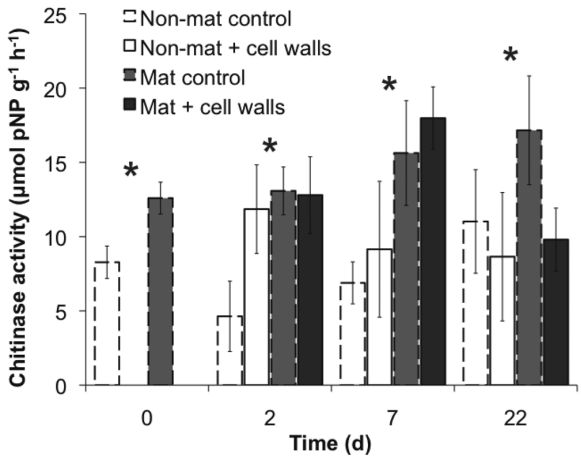

Fig. 5. Chitinase potential activity with time in non-mat (white bars) and mat (dark gray bars) soils in control soils (dashed lines) and treatment soils with cell wall material added (solid lines). Error bars represent ±1 SE. A significant difference between mat and non-mat soil is designated by an asterisk.

cell wall C assimilation in mat soils, is linked to greater C GYE in mat soils. Beyond assimilation of cell wall material, total biomass accumulation was also greater in mat soils: during the incubation period, total MBC and MBN increased by 0.30 ± 0.09 g C and 0.07 ± 0.03 g N kg^{-1} dry soil in the mat soils but only by 0.09 ± 0.06 g C g^{-1} dry soil and an insignificant amount of N in the non-mat soils. Individual fungal and bacterial taxa also may respond with different levels of growth or respiration when chitin is supplied as a substrate within a whole soil matrix (Gould et al., 1981). Because both saprophytic fungal and bacterial community composition differs between EcM mat and non-mat study soils (Kluber et al., 2011), the growth efficiency of specific microbial taxa, not detectable at the coarse resolution of PLFAs, may be linked to the higher integrated fungal cell-wall C GYE in EcM mat soils. In addition, it is possible that SOM in mat soils is more bioavailable, feasibly due to elevated levels of chitinase activity (Table 1; Fig. 5) or some other mechanism.

Another commonly predicted or detected priming mechanism is the stimulation of the production of extracellular enzymes by substrate amendment, which could also explain increased N mineralization from SOM by 22 d of incubation (Fontaine et al., 2003; Kuzyakov and Bol, 2006). Although we did not observe any increase in chitinase enzyme activity during the experiment (Fig. 5), we did not conduct any assay of peptidase activity, so this mechanism cannot be ruled out. It was somewhat surprising to see no substrate-induced increase in chitinase activity; however, this result is consistent with the observations made following monomeric and polymeric amino sugar addition to these soils (Zeglin et al., 2013). In fact, in the past we observed a significant decrease in chitinase activity in EcM mat soils with chitin amendment, and there was also a trend toward lower chitinase activity in cell-wall-amended mat soils in this experiment (although the result was nonsignificant). The hypothesis that chitinase production is downregulated by increased soluble organic or inorganic N concentrations in mat soils, and that there is some degradation of protein in response to microbial N demand, is still viable.

Although fungal cell wall material seems to be valuable as a substrate for microbial N assimilation and C metabolism, it may also play a role in SOM accumulation. The positive N priming effect was equivalent to a substrate-induced 22-d cumulative N mineralization of 19 ± 2 to 26 ± 2 mg N kg^{-1} dry soil (the amount of mineral N derived from SOM in excess of that in control mat and non-mat soils, respectively; Fig. 2), whereas the amount of cell-wall N not recovered in mineral, extractable organic, or biomass pools after 22 d was 540 ± 3 mg N kg^{-1} dry soil. Although we cannot distinguish "stabilized" from nonmineralized cell wall N in this manner, the calculations indicate that the potential for stabilization of fungal cell-wall N may be up to an order of magnitude greater than the potential for fungal cell wall material to stimulate N mineralization from SOM. If this is true, then the higher soil microbial biomass present in EcM rhizomorphic mat structures may influence SOM dynamics via stabilization of fungal necromass-derived N. The total amount of amino sugars recovered from the study soils after acid hydrolysis was 25 to 500 times the amount contained in the microbial biomass (Zeglin et al., 2013), suggesting that some stabilization of amino sugars does occur in these organic horizon soils. Interestingly, combined scanning transmission x-ray spectromicroscopy and nanoscale imaging secondary ion mass spectrometry of EcM mat soils incubated with labeled cell wall material for 22 d showed significant physical association of organic ^{15}N with Fe-rich particles (Keiluweit et al., 2012). This organic ^{15}N showed an amide bonding profile rather than an amino sugar bonding profile, suggesting that the cell-wall ^{15}N had been altered into a proteinaceous form before becoming stabilized on Fe mineral surfaces. Because a portion of cell-wall ^{13}C was modified from glucan-chitin form to lipid form within 7 d of amendment, it is also feasible that some cell-wall ^{15}N was microbially modified from chitin to protein within 22 d of amendment and subsequently stabilized by Fe minerals.

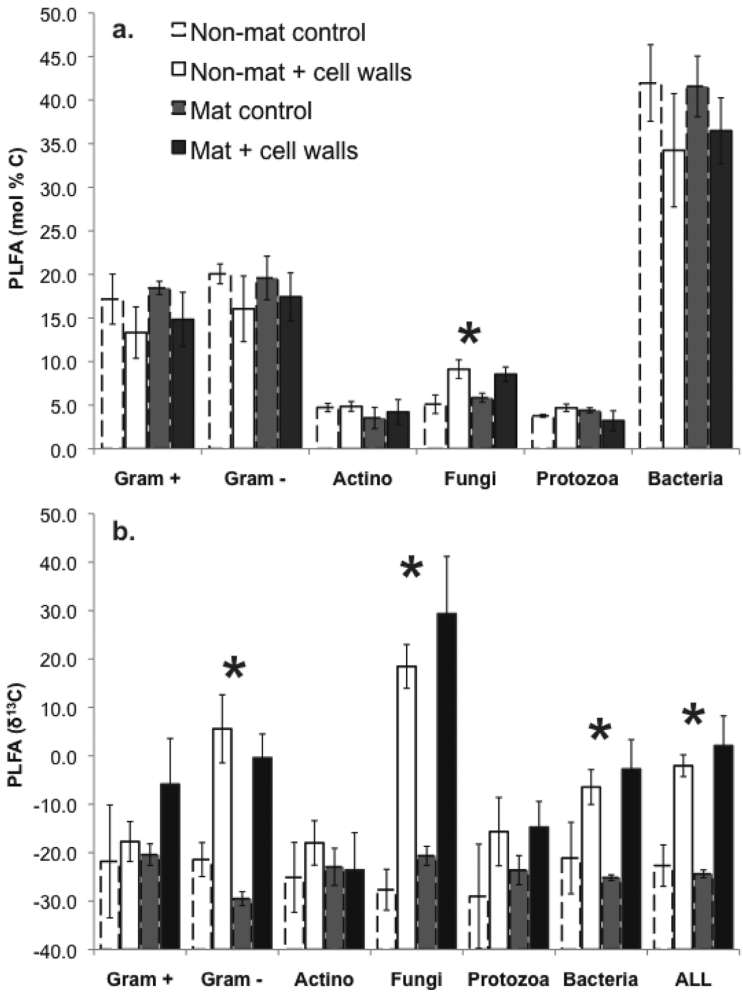

Fig. 6. Phospholipid fatty acid (PLFA) (a) relative abundance and (b) incorporation of cell-wall-derived ^{13}C in different microbial groups after 7-d incubation of non-mat (white bars) and mat (dark gray bars) control soils (dashed lines) and soils with cell wall material added (solid lines). Error bars represent ±1 SE. Data means within a taxonomic category that showed a treatment response are designated by an asterisk.

Overall, chitin-enriched fungal cell material was significantly decomposed during the 22-d incubation period in old-growth Douglas-fir organic layer soils, both colonized and uncolonized by rhizomorphic mycorrhizal mats. Using stable isotope tracer techniques, it was apparent that cell-wall-derived C was predominantly mineralized, while cell-wall-derived N was predominantly assimilated. Cell wall C assimilation and C GYE were higher in mat soils, possibly due to more efficient growth of specific microbial taxa or a relatively greater reliance on SOM for maintenance. Fungi clearly grew selectively using cell wall C, and Gram-negative bacteria (but not actinomycetes) also metabolized the amended substrate. Also, both C and N priming patterns reflected a preferential utilization of cell wall material over the SOM, and chitinase potential activity did not respond significantly to the addition of chitin-enriched cell wall material. Finally, there is the potential that N from the cell wall material may be stabilized within the SOM. It is clear that fungal cell wall material is an important source of both C and N that drives microbial nutrient cycling in old-growth forest organic soils. Further experiments may elucidate whether different populations of the soil microbiota are linked to the turnover and retention of C and N in organic soil horizons dominated by EcM mats.

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REFERENCES

Agerer, R. 2001. Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114. doi:10.1007/s005720100108

Bååth, E., L.O. Nilsson, H. Goransson, and H. Wallander. 2004. Can the extent of degradation of soil fungal mycelium during soil incubation be used to estimate ectomycorrhizal biomass in soil? Soil Biol. Biochem. 36:2105–2109. doi:10.1016/j.soilbio.2004.06.004

Blagodatskaya, E., and Y. Kuzyakov. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. Biol. Fertil. Soils 45:115–131. doi:10.1007/s00374-008-0334-y

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917. doi:10.1139/o59-099

Brant, J.B., D.D. Myrold, and E.W. Sulzman. 2006. Root controls on soil microbial community structure in forest soils. Oecologia 148:650–659. doi:10.1007/s00442-006-0402-7

Brookes, P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17:837–842. doi:10.1016/0038-0717(85)90144-0

Butler, J.L., M.A. Williams, P.J. Bottomley, and D.D. Myrold. 2003. Microbial community dynamics associated with rhizosphere carbon flow. Appl. Environ. Microbiol. 69:6793–6800. doi:10.1128/AEM.69.11.6793-6800.2003

Cabib, E., and B. Bowers. 1971. Chitin and yeast budding: Localization of chitin in yeast bud scars. J. Biol. Chem. 246:152–159.

Courty, P.E., K. Pritsch, M. Schloter, A. Hartmann, and J. Garbaye. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytol. 167:309–319. doi:10.1111/j.1469-8137.2005.01401.x

De Nobili, M., M. Contin, C. Mondini, and P.C. Brookes. 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol. Biochem. 33:1163–1170. doi:10.1016/S0038-0717(01)00020-7

Dixon, J.J. 2003. Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek valley, western Cascades, Oregon. M.S. thesis. Oregon State Univ., Corvallis.

Drigo, B., I.C. Anderson, G.S.K. Kannangara, J.W.G. Cairney, and D. Johnson. 2012. Rapid incorporation of carbon from ectomycorrhizal mycelial necromass into soil fungal communities. Soil Biol. Biochem. 49:4–10. doi:10.1016/j.soilbio.2012.02.003

Dunham, S.M., K.H. Larsson, and J.W. Spatafora. 2007. Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. Mycorrhiza 17:633–645. doi:10.1007/s00572-007-0141-6

Fernandez, C.W., and R.T. Koide. 2012. The role of chitin in the decomposition of ectomycorrhizal fungal litter. Ecology 93:24–28. doi:10.1890/11-1346.1

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: A question of microbial competition? Soil Biol. Biochem. 35:837–843. doi:10.1016/S0038-0717(03)00123-8

Frostegård, A., A. Tunlid, and E. Bååth. 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol. 59:3605–3617.

Gooday, G.W. 1990. Physiology of microbial degradation of chitin and chitosan. Biodegradation 1:177–190. doi:10.1007/BF00058835

Gould, W.D., R.J. Bryant, J.A. Trofymow, R.V. Anderson, E.T. Elliott, and D.C. Coleman. 1981. Chitin decomposition in a model soil system. Soil Biol. Biochem. 13:487–492. doi:10.1016/0038-0717(81)90039-0

Griffiths, R.P., and B.A. Caldwell. 1992. Mycorrhizal mat communities in forest soils. In: D.J. Read et al., editors, Mycorrhizas in ecosystems. CAB Int., Wallingford, UK. p. 98–105.

Griffiths, R.P., G.A. Bradshaw, B. Marks, and G.W. Lienkaemper. 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant Soil 180:147–158. doi:10.1007/BF00015421

Griffiths, R.P., E.R. Ingham, B.A. Caldwell, M.A. Castellano, and K. Cromack. 1991. Microbial characteristics of ectomycorrhizal mat communities in Oregon and California. Biol. Fertil. Soils 11:196–202. doi:10.1007/BF00335767

Hart, S.C., and J.M. Stark. 1997. Nitrogen limitation of the microbial biomass in an old-growth forest soil. Ecoscience 4:91–98.

Högberg, M.N., and P. Högberg. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol. 154:791–795. doi:10.1046/j.1469-8137.2002.00417.x

Hsu, S.C., and J.L. Lockwood. 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl. Microbiol. 29:422–426.

Keiluweit, M., J.J. Bougoure, L.H. Zeglin, D.D. Myrold, P.K. Weber, J. Pett-Ridge, M. Kleber, and P. Nico. 2012. Nano-scale investigation of the association of microbial N residues with iron (hydr)oxides in a forest soil O-horizon. Geochim. Cosmochim. Acta 95:213–226. doi:10.1016/j.gca.2012.07.001

Kerley, S.J., and D.J. Read. 1997. The biology of mycorrhiza in the Ericaceae: XIX. Fungal mycelium as a nitrogen source for the ericoid mycorrhizal fungus *Hymenoscyphus ericae* and its host plants. New Phytol. 136:691–701. doi:10.1046/j.1469-8137.1997.00778.x

Kielak, A.M., M.S. Cretoiu, A.V. Semenov, S.J. Sørensen, and J.D. van Elsas. 2013. Bacterial chitinolytic communities respond to chitin and pH alteration in soil. Appl. Environ. Microbiol. 79:263–272. doi:10.1128/AEM.02546-12

Kirchman, D.L., and J. White. 1999. Hydrolysis and mineralization of chitin in the Delaware estuary. Aquat. Microb. Ecol. 18:187–196. doi:10.3354/ame018187

Kluber, L.A., J.E. Smith, and D.D. Myrold. 2011. Distinctive fungal and bacterial communities are associated with mats formed by ectomycorrhizal fungi. Soil Biol. Biochem. 43:1042–1050. doi:10.1016/j.soilbio.2011.01.022

Kluber, L.A., K.M. Tinnesand, B.A. Caldwell, S.M. Dunham, R.R. Yarwood, P.J. Bottomley, and D.D. Myrold. 2010. Ectomycorrhizal mats alter forest soil biogeochemistry. Soil Biol. Biochem. 42:1607–1613. doi:10.1016/j.soilbio.2010.06.001

Koide, R.T., and G.M. Malcolm. 2009. N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. Fungal Ecol. 2:197–202.

doi:10.1016/j.funeco.2009.06.001
Kuzyakov, Y., and R. Bol. 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biol. Biochem. 38:747–758. doi:10.1016/j.soilbio.2005.06.025
Parham, J.A., and S.P. Deng. 2000. Detection, quantification and characterization of beta-glucosaminidase activity in soil. Soil Biol. Biochem. 32:1183–1190. doi:10.1016/S0038-0717(00)00034-1
Phillips, C.L., L.A. Kluber, J. Martin, B.A. Caldwell, and B.J. Bond. 2012. Contributions of ectomycorrhizal fungal mats to forest soil respiration. Biogeosciences 9:2099–2110. doi:10.5194/bg-9-2099-2012
Read, D.J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems: A journey towards relevance? New Phytol. 157:475–492. doi:10.1046/j.1469-8137.2003.00704.x
Roff, J.C., J.T. Kroetsch, and A.J. Clarke. 1994. A radiochemical method for secondary production in planktonic Crustacea based on rate of chitin synthesis. J. Plankton Res. 16:961–976. doi:10.1093/plankt/16.8.961
Schimel, J.P., and M.N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. Soil Biol. Biochem. 35:549–563. doi:10.1016/S0038-0717(03)00015-4
Sinsabaugh, R.L., and D.L. Moorhead. 1994. Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. Soil Biol. Biochem. 26:1305–1311. doi:10.1016/0038-0717(94)90211-9
Sollins, P., C.C. Grier, F.M. McCorison, K. Cromack, and R. Fogel. 1980. The internal element cycles of an old-growth Douglas-fir ecosystem in western Oregon. Ecol. Monogr. 50:261–285. doi:10.2307/2937252
Stark, J.M., and S.C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci. Soc. Am. J. 60:1846–1855. doi:10.2136/sssaj1996.03615995006000060033x
Strickland, M.S., and J. Rousk. 2010. Considering fungal:bacterial dominance in soils: Methods, controls, and ecosystem implications. Soil Biol. Biochem. 42:1385–1395. doi:10.1016/j.soilbio.2010.05.007
Talbot, J.M., S.D. Allison, and K.K. Treseder. 2008. Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct. Ecol. 22:955–963. doi:10.1111/j.1365-2435.2008.01402.x
R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Terahara, T., S. Ikeda, C. Noritake, K. Minamisawa, K. Ando, S. Tsuneda, and S. Harayama. 2009. Molecular diversity of bacterial chitinases in arable soils and the effects of environmental factors on the chitinolytic bacterial community. Soil Biol. Biochem. 41:473–480. doi:10.1016/j.soilbio.2008.11.024
Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19:703–707. doi:10.1016/0038-0717(87)90052-6
Watson, H.R., D.C. Apperley, and D.P. Dixon. 2009. An efficient method for ^{15}N-labeling of chitin in fungi. Biomacromolecules 10:793–797. doi:10.1021/bm8012814
Williamson, N., P. Brian, and E.M.H. Wellington. 2000. Molecular detection of bacterial and streptomycete chitinases in the environment. Antonie van Leeuwenhoek 78:315–321. doi:10.1023/A:1010225909148
Zeglin, L.H., L.A. Kluber, and D.D. Myrold. 2013. The importance of amino sugar turnover to C and N cycling in organic horizons of old-growth Douglas-fir forest soils colonized by ectomycorrhizal mats. Biogeochemistry (in press). doi:10.1007/s10533-012-9746-8
Zhang, X.D., and W. Amelung. 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biol. Biochem. 28:1201–1206. doi:10.1016/0038-0717(96)00117-4