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Long-term litter decomposition controlled by manganese redox cycling

Marco Keiluweit, Peter Nico, Mark E. Harmon, Jingdong Mao, Jennifer Pett-Ridge, and Markus Kleber

Abstract

Decomposition of above-ground plant detritus (litter) is a fundamental process regulating the release of nutrients for plant growth and the formation of soil organic matter (SOM) in forest ecosystems (1). Litter decomposition regulates the proportion of litter-derived carbon (C) that is either retained in the system as SOM or lost as CO₂ (2), thereby influencing net C storage in soils. Although even small decomposition rate increases may accelerate climate change by virtue of increasing storage in soils, the rate is controlled by litter quality, relying on parameters such as lignin content as predictors. However, a strong correlation has been observed between the manganese (Mn) content of litter and decomposition rates across a variety of forest ecosystems. Here, we show that long-term litter decomposition in forest ecosystems is tightly coupled to Mn redox cycling. Over 7 years of litter decomposition, microbial transformation of litter was paralleled by variations in Mn oxidation state and concentration. A detailed chemical imaging analysis of the litter revealed that fungi recruit and redistribute unreactive Mn²⁺-ligands by fresh plant litter to produce oxidative Mn³⁺ species at sites of active decay, with Mn eventually accumulating as insoluble Mn⁴⁺ oxides. Formation of reactive Mn³⁺ species coincided with the generation of aromatic oxidation products, providing direct proof of the previously posited role of Mn⁴⁺-based oxidizers in the breakdown of litter. Our results suggest that the litter-decomposing machinery at our coniferous forest site depends on the ability of plants and microbes to supply, accumulate, and regenerate short-lived Mn²⁺-ligands in the litter layer. This observation indicates that biogeochemical constraints on bioavailability, mobility, and reactivity of Mn in the plant–soil system may have a profound impact on litter decomposition rates.

Litter decomposition is a keystone ecosystem process impacting nutrient cycling and productivity, soil properties, and the terrestrial carbon (C) balance, but the factors regulating decomposition rate are still poorly understood. Traditional models assume that the rate is controlled by litter quality, relying on parameters such as lignin content as predictors. However, a strong correlation has been observed between the manganese (Mn) content of litter and decomposition rates across a variety of forest ecosystems. Here, we show that long-term litter decomposition in forest ecosystems is tightly coupled to Mn redox cycling. Over 7 years of litter decomposition, microbial transformation of litter was paralleled by variations in Mn oxidation state and concentration. A detailed chemical imaging analysis of the litter revealed that fungi recruit and redistribute unreactive Mn²⁺-ligands by fresh plant litter to produce oxidative Mn³⁺ species at sites of active decay, with Mn eventually accumulating as insoluble Mn⁴⁺ oxides. Formation of reactive Mn³⁺ species coincided with the generation of aromatic oxidation products, providing direct proof of the previously posited role of Mn⁴⁺-based oxidizers in the breakdown of litter. Our results suggest that the litter-decomposing machinery at our coniferous forest site depends on the ability of plants and microbes to supply, accumulate, and regenerate short-lived Mn²⁺-ligands in the litter layer. This observation indicates that biogeochemical constraints on bioavailability, mobility, and reactivity of Mn in the plant–soil system may have a profound impact on litter decomposition rates.

Significance

The rate-controlling mechanisms of litter decomposition are of fundamental importance for ecosystem nutrient cycling, productivity, and net carbon (C) balance. Current C cycling models rely primarily on climatic factors and lignin content as the main predictors of litter decomposition rates. Here, we show how the ability of the integrated plant–soil system to promote active redox cycling of manganese (Mn) regulates litter decomposition. Our work suggests that incorporating the coupling of litter decomposition and other elemental cycles, such as the Mn cycle, into conceptual and numerical models may significantly improve our mechanistic understanding and predictions of C cycling in terrestrial ecosystems.

Author contributions: M. Keiluweit, P.N., M.E.H., J.P.-R., and M. Kleber designed research; M. Keiluweit performed research; J.M. contributed new reagents/analytic tools; M. Keiluweit analyzed data; and M. Keiluweit, P.N., J.P.-R., and M. Kleber wrote the paper.

The authors declare no conflict of interest.

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isolated from terrestrial and aquatic systems can do so as well (18, 19). Despite the widespread physiological potential for microbially mediated Mn oxidation, it is unclear to what extent the natural Mn redox cycle is coupled to organic matter degradation in forest ecosystems.

Circumstantial evidence suggests that microbial oxidation may be the main driver of Mn cycling in forest litter layers. Foliage litter is a major source of Mn compared with other plant litter and mineral soils (20). Mn is not only present in its reduced, mobile form (i.e., Mn$^{2+}$), facilitating its supply to photosystem II and other enzymatic systems (21). Foliage litter becomes enriched in Mn relative to fresh plant material and further accumulates Mn during the decomposition process (9, 22). Mn in dead foliage (i.e., litter) was found to be bound in organic complexes (23), but it is unclear whether Mn is present as unreactive Mn$^{2+}$ or the more reactive Mn$^{3+}$ form. A recent spectroscopic investigation showed that foliar tissue contains predominantly Mn$^{3+}$ that is gradually oxidized to Mn$^{4+}$-oxides in the soil upon decomposition (24). This observation led Hernon et al. (24) to suggest that Mn stored in leaves is solubilized upon litter fall but rapidly immobilized as Mn$^{3+}$ oxides after aging in the soil. Others have observed Mn-rich black precipitates on needle litter colonized by fungi (25), resembling Mn$^{3+}$ oxides as observed after fungal Mn oxidation in model systems (26, 27).

Here, we investigated the impact of biotic Mn redox cycling on long-term litter decomposition in forest ecosystems, taking advantage of a field experiment conducted in an old-growth Douglas-fir forest in the Oregon Cascades. During this 7-yr experiment, each year’s litterfall was spatially separated by a nylon mesh that enclosed the forest floor. The mesh created a series of six well-confined litter layers (a “littercake”) containing Douglas-fir needles at increasing decomposition stage. Our approach was to resolve Mn transformation and litter decomposition within the forest ecosystem at two different scales—across the whole soil profile and in microenvironments on needles colonized by fungi. Across the litter layer, we hypothesized that Mn oxidation covaries with the breakdown of aromatic litter components (i.e., lignins and tannins). Changes in Mn chemistry were identified by selective extractions and X-ray absorption near edge structure (XANES) spectroscopy, whereas the alterations in the molecular composition of litter was determined using a combination of Fourier transform infrared (FTIR) spectroscopy, NMR, and laser desorption postionization mass spectrometry (LDP-I-MS). Within microenvironments on needle surfaces, we anticipated that oxidative degradation of aromatic compounds occurs where fungi produce Mn$^{3+}$. To spatially resolve Mn oxidation state and chemical transformations in these microenvironments, we applied a multimodal chemical imaging approach that coupled microscale X-ray fluorescence ($\mu$XRF)/X-ray absorption spectroscopy (XAS) with $\mu$FTIR imaging.

Results

Mn Transformations. With increasing litter age, Mn gradually accumulated and became more oxidized. Total Mn concentrations in decomposing needle litter progressively increased and, on dry-weight basis, became higher than in fresh needles or the underlying mineral horizons (Table S1). The contribution of pyrophosphate-extractable Mn (Mn$_{PP}$; Mn in soluble and organically complex pools) increases most rapidly in the first three layers, before reaching a plateau in layer 4 (Table S1).

Concurrently, Mn XANES absorption maxima shifted from energies associated with Mn$^{2+}$-dominated phases to Mn$^{3+}$ and Mn$^{4+}$-rich phases as litter age increased (Fig. 1). The average oxidation state of Mn calculated from these shifts progressively increased from +2.07 in fresh needles to +2.74 in layer 6 (Fig. 1). The relative amount of Mn$^{3+}$ species increased most rapidly in layers 1 and 2 whereas that of Mn$^{4+}$ species increased strongly from layers 3–6. Needles in the underlying O horizons showed another slight increase in average oxidation state (+2.83). Mn oxidation state (+3.1 and +3.0) and, consequently, the relative contributions of Mn$^{3+}$ and Mn$^{4+}$ were found to be highest in the mineral soil.

Litter Decomposition. Overall, we observed a strong relationship between Mn redox transformations and litter decomposition. The increase of Mn in soluble and organically complexed pools (Mn$_{PP}$; Mn$_{PP}$) was significantly correlated with microbial processing of the litter. The C/N ratio, which might be expected to decrease with increased microbial processing, decreased most rapidly from layers 1–3 (Table S1). This decrease in C/N was correlated with increases in Mn$_{PP}$ (R$^2$ = 0.76, P < 0.05) (see Fig. 3A), but not with changes in organically complexed Al, Ca, or Fe (P > 0.05). Similarly, the FTIR absorbance of amide groups, associated with microbial protein and chitin, increased (Fig. 2A) and is positively correlated with Mn$_{PP}$ (R$^2$ = 0.94, P < 0.05) (Fig. 3B).

Differences in the litter’s molecular composition correspond with increases in Mn oxidation state across the decomposition sequence. FTIR spectra show that the relative absorbance of regions associated with saccharide and aromatic functional groups (Fig. 2A) is significantly correlated with Mn oxidation...
state \((R^2 = 0.79, P < 0.05)\) (Fig. 3C). Similarly, relative changes in the abundance of saccharide and aromatic moieties in the \(^{13}\)C NMR spectra (Fig. 2B) are well correlated with Mn oxidation \((R^2 = 0.98, P < 0.05)\) (Fig. 3D).

To evaluate a link between the chemical alteration of aromatic litter components and Mn transformations, we gathered \(^{13}\)C NMR and synchrotron-LDPI mass spectrometry data. Two different types of aromatic carbons are usually identified in \(^{13}\)C NMR spectra: protonated \((C_{ar}-H)\) and quaternary non-oxygenated \((C_{ar}-C)\) at \(142-110\) ppm, or oxygenated aromatic ring carbons \((C_{ar}-O)\) at \(162-142\) ppm (28). The abundance of oxygenated relative to nonoxygenated aromatic ring carbons \((C_{ar}-O/C_{ar}-R)\), with \(R = C\) or \(H\) in \(^{13}\)C NMR spectra, used here as a proxy for ring oxidation, was strongly correlated with changes in Mn\(_{PYRO}\) \((R^2 = 0.92, P < 0.05)\) (Fig. 3E). Synchrotron-LDPI is a soft-ionization mass spectrometry technique that is particularly sensitive for lignin-derived compounds (29, 30). Inspection of the resulting mass spectra showed that one set of peaks at lower mass-to-charge \((m/z)\) ratios \((\Sigma ar^+; m/z = 340-370)\) gains intensity with increasing litter age whereas the intensity of a second set of peaks at higher \(m/z\) ratios \((\Sigma ar^-; m/z = 396-414)\) declines (Fig. 2C). To confirm that these mass fragments \((i.e., m/z = 340, 370, 396, 400, 406, 406, 414)\) correspond to aromatic structures, we determined ionization energies (IEs) for each of these fragments in a second LDPI experiment as previously described (31, 32) and detailed in SI Materials and Methods. \(\Sigma ar^+\) denotes the sum of normalized intensities of peaks that increase with litter age \((m/z = 340 and 370)\) whereas \(\Sigma ar^-\) represents the sum of normalized intensities of peaks decreasing over time \((m/z = 396, 399, 400, 406, 414)\). Mass spectra presented here were collected with a photon energy of 9.5 eV and were chosen because they showed less fragmentation and greater signal-to-noise ratio.

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**Fig. 2.** Chemical transformations of Douglas-fir needles over 6 y of decomposition. (A) FTIR spectra of needle litter. Absorbance of both (i) amide I and II groups associated with bacterial/fungal protein and chitin increased and (ii) C-O-C groups of polysaccharides increased with litter age whereas (iii) absorbance of COO-stretch of carboxylates and the ar-C-C stretch of aromatic lignin decreased. (B) \(^{13}\)C-NMR spectra of needle litter. Gray lines show spectra obtained by unsel ective CP/TOSS experiments including all carbons, whereas black lines show spectra obtained by selective CP/TOSS-DD sensitive to nonprotonated carbons and mobile carbons \((e.g., -CH\_3 and long-chain -(CH\_2)_n)\). (C) Synchrotron-LDPI mass spectra of needle litter. Fragments with \(m/z\) of 340, 370, 396, 400, 406, and 414 showed ionization energies of \(\leq 8.5\) eV and were thus assigned to aromatic structures. Detailed description of peak assignments can be found in SI Materials and Methods. \(\Sigma ar^+\) denotes the sum of normalized intensities of peaks that increase with litter age \((m/z = 340 and 370)\) whereas \(\Sigma ar^-\) represents the sum of normalized intensities of peaks decreasing over time \((m/z = 396, 399, 400, 406, and 414)\). Mass spectra presented here were collected with a photon energy of 9.5 eV and were chosen because they showed less fragmentation and greater signal-to-noise ratio.
change in aromatic litter components, which showed a strong correlation with Mn oxidation state (Fig. 3F) ($R^2 = 0.88$, $P < 0.05$).

**Mn Form and Distribution on Decomposing Needle Surfaces.** Because Mn chemistry and molecular composition of the litter changed more rapidly in the initial stages of decomposition, we chose needles from layer 1 for detailed imaging analysis. These needles were colonized by fungi forming dense hyphal networks with distinct dark patches hypothesized to be Mn$^{3+/4+}$ oxides (Fig. S1A). Elemental maps of resin-embedded cross-sections from three needles showed that Mn concentrated in diffuse patches on the surface, associated either with individual hyphae or larger particles (Fig. S1B). Other major elements (e.g., Ca, Fe, and Si) were randomly distributed. Mn XANES spectra collected from diffuse patches and discrete particles on the needle surface showed a greater abundance of Mn$^3+$ and Mn$^{4+}$ and, consequently, a higher average oxidation state than those extracted from individual hyphae and the needle tissue (Fig. S1C).

**Manganese Chemistry at the Hyphae–Epidermis Interface.** Photographs of two cryo-sectioned decomposing needles show dark hyphae colonizing the epidermis region (Fig. 4A). Along this interface, μFTIR chemical imaging visually separates needle tissue rich in aromatic structures from amide-rich fungal materials (Fig. 4B). Elemental maps of this interface showed that Mn accumulates where fungal hyphae are in direct contact with the needle epidermis (Fig. 4C). We collected Mn XANES spectra from hotspots (points 3–5, Fig. 4C) along this interface (Fig. 4D). Spectral deconvolution indicated that the hotspots associated with fungi on the needle surface were more oxidized (points 3–5; average oxidation state, 2.6–2.7) than Mn in the needle interior (points 1–2; average oxidation state, 2.1–2.2) (Fig. 4C). Interestingly, these hotspots of oxidized Mn were coloclated with carbonyl groups (1,750–1,680 cm$^{-1}$) as shown in the μFTIR absorbance map (Fig. 4B).

To further investigate the potential chemical alterations due to oxidative Mn species at the site of direct contact between fungal hyphae and the needle surface, we extracted μFTIR spectra along a transect across this interface (red line, Fig. 4B). Spectra taken from fungal tissue, the fungi–epidermis interface (contact zone), the needle epidermis, and mesophyll are presented in Fig. 4E. The spectrum extracted from the infected epidermis (C) showed a lower absorbance of saccharide bands (1,180–950 cm$^{-1}$), as well as enhanced absorbance of bands corresponding to carbonyl (∼1,700 cm$^{-1}$) and aromatic C = C groups (∼1,600 cm$^{-1}$) compared with uninfected epidermis regions (Fig. 4E).

**Discussion**

We initially hypothesized that litter-decomposing fungi repurpose Mn$^{3+}$, naturally present in litter, to produce reactive Mn$^{4+}$ species at the site of oxidative needle degradation. Our results suggest a strong relationship between biotic Mn oxidation and litter decomposition both at macroscales (litter layer) and microscales (fungal needle colonization), the causality of which is discussed in the following.

Across the Douglas-fir litter layer examined here, Mn was progressively transformed. Total Mn increased rapidly in years 1–3, followed by a slower increase in years 4–6, a trend consistent with Mn accumulation in litter layers observed elsewhere (9, 22). Similarly, the amount of pyrophosphate-extractable Mn (Mn$^{PyRO}$) and the proportion of Mn$^{3+}$ as evidenced by XANES increased most rapidly within the first 3 y whereas solid Mn$^{4+}$ phases did not occur until years 4–6 (Table S1 and Fig. 1). Pyrophosphate is expected to complex and extract dissolved and/or organically complexed Mn
which we consider a proxy for bioavailable Mn, but does not differentiate between unreactive Mn$^{2+}$ and oxidative Mn$^{3+}$ species. On the other hand, XANES spectroscopy can detect Mn$^{3+}$, without providing unambiguous information about its physical state. Combined, however, our extraction and spectroscopy results suggest the transformation of litter-borne Mn into bioavailable Mn$^{2+}$ and reactive Mn$^{3+}$ forms in the initial stages of decomposition. In later stages, increasing Mn oxidation resulted in Mn$^{3+/4+}$ forms similar to those observed in solid Mn oxide precipitates (24, 26, 27).

This rapid formation of bioavailable and reactive Mn forms in the first years of decomposition is accompanied by increased microbial processing of the litter. Accumulation of bioavailable and reactive Mn correlated with decreasing C/N ratios and stronger contributions of amide functionalities in the decomposing litter (Fig. 3 A and B). N content (34), fungal biomass (35), and visible fungal colonization of needle litter (36–38) commonly rise within the first 2 y of decomposition. Within a similar time frame, maxima in MnP activity can be observed (35).
Our results support the hypothesis that Mn mobilization and the formation of reactive Mn phases are caused by successive microbial colonization of the litter.

Further, the strong relationship between Mn cycling and chemical transformation of litter points to a direct involvement of Mn in the decomposition process. Mn oxidation is strongly related to the relative loss of saccharides and the transformation of aromatic litter components (Fig. 3). Oxidative transformations of aromatic compounds occurred across the litter layer, reflected in changes in abundance of aromatic compounds (Fig. 2C) and increases in oxygenated aromatic ring carbons (Fig. 2A and B). These transformations were significantly correlated with Mn mobilization and oxidation (Fig. 3E and F), suggesting that decomposing fungi actively cycle Mn for the purpose of using oxidized Mn species in the breakdown of aromatic structures.

Our imaging analysis can be reconciled as evidence that fungi actively promote Mn transport and oxidation during litter colonization. The high concentrations of Mn found in single hyphae and dense hyphal networks (Fig. S1B) imply biotic accumulation and transport of Mn in the litter layer. Greater contribution of Mn<sup>3+</sup> and Mn<sup>4+</sup> forms to overall Mn concentrations in hyphae further indicate active biotic Mn oxidation. In addition to Mn associated with fungal hyphae, we observed larger, Mn-rich particles consisting predominantly of Mn<sup>3+/4+</sup> forms on needle surfaces. A significant fraction of hyphae found on needle surfaces can be assumed dead (36–38), and Blanchette (25) showed dead wood-colonizing hyphae covered in Mn precipitates. These observations suggest that, when the fungal supply of Mn<sup>3+</sup>–stabilizing chelators such as oxalic acid ceases upon cell death, excess Mn<sup>3+</sup> disproportionate and precipitates as Mn<sup>3+/4+</sup> oxides on dead fungal residues. These coprecipitates may then accumulate as larger aggregates observed here and elsewhere (24).

Imaging further showed that reactive Mn<sup>4+</sup> occurs in hotspots at the interface between hyphae and needle epidermis (Fig. 4C). These hotspots showed a mixture of Mn<sup>2+</sup> and reactive Mn<sup>3+</sup> species and almost no signs of Mn<sup>3+/4+</sup> oxide accumulation (Fig. 4A). If microbially produced Mn<sup>3+</sup> engages in oxidation reactions with litter components at this interface, it is reduced back to Mn<sup>2+</sup> in the process. In this scenario, no Mn<sup>3+/4+</sup> oxides accumulate, and fungi continuously reoxidize Mn<sup>2+</sup> to form Mn<sup>3+</sup>. The fact that we find comparable amounts of Mn<sup>2+</sup> and Mn<sup>3+</sup> is therefore consistent with active cycling of the Mn<sup>2+/3+</sup> couple and its involvement in oxidation reactions.

Evidence for chemical alterations of saccharide and aromatic components at infected sites with high Mn<sup>3+</sup> concentrations reveal a direct involvement of Mn in litter decomposition. First, decreasing absorbance of saccharides in spectra taken from the contact zone between hyphae and epidermis (Fig. 4E) is in good agreement with bulk saccharide loss across the litter layers (Table S1) and preferential (hemi)cellulose removal from the site of attack. Second, increased absorbance of bands arising from conjugated C=O and C=C bonds can be attributed to aromatic decomposition products released during the oxidative breakdown of plant material (39, 40). Removal of saccharides and concurrent oxidative alteration of aromatic lignin structures is a widely noted fungal decay pattern, where hyphae generate oxidized trenches in cell walls to liberate and release (hemi) cellulose components without invading the cell interior (41).

In summary, our study reveals the mechanistic link between microbial Mn cycling and the transformations of organic compounds during litter decomposition (Fig. 5A). Collectively, our bulk and microscale results show that litter-decomposing fungi first recruit and accumulate reduced Mn<sup>2+</sup> in the litter layer, transform it into oxidative Mn<sup>4+</sup> forms at the site of oxidative litter decomposition, and later accumulate it as Mn<sup>3+/4+</sup> oxide precipitates (Fig. 5B). Moreover, we find that Mn oxidation—specifically the formation of reactive Mn<sup>3+</sup> species—in this ecosystem is not incidental, but tightly coupled to the oxidative degradation of aromatic structures in litter. These findings provide a mechanistic basis for the highly significant “Mn-dependence” (9–11) of litter decomposition across a wide variety of forest ecosystems. Our results suggest that this relationship largely rests on the ability of decomposer organisms to recruit Mn in the litter layer and oxidize it at the site of litter decay. This insight demonstrates that Mn bioavailability and oxidation rate should be recognized as major determinants of litter decomposition in forest ecosystems. Furthermore, it provides strong support for a new ecological concept of litter decomposition: That the ability of decomposer organisms to recruit Mn in the litter layer and oxidize it at the site of litter decay is, in large part, controlled by availability of, and access to, resources critical for the biochemical breakdown of litter.

Our results support the hypothesis that plant–soil systems in forest biomes have coevolved to optimize the “cell-wall degrading machinery” (42), thereby maximizing litter decomposition

**Fig. 5.** Macro- and microscale coupling of C and Mn cycles in forest ecosystems. (A) Mn cycling through the entire plant–soil system and its link to litter decomposition and SOM formation. (B) Microscale Mn cycling by litter-decomposing fungi and its role in the oxidative breakdown of lignin or other aromatic litter components.
(and thus the recycling of Mn) by ensuring the availability of key resources such as Mn (Fig. 5A). Because aspects of global change also impact ecosystem Mn fluxes, bioavailability in soils (43, 44), plant uptake, and foliar litter concentrations (45), the tight coupling we demonstrate between Mn cycling and litter decomposition suggests that further research on regulators of ecosystem Mn fluxes is warranted.

Materials and Methods

Litter Decomposition State. This study took advantage of an ongoing litter decomposition experiment at the H. J. Andrews Experimental Forest (HJA) in Oregon, United States. About 90% of the area’s annual precipitation falls from October to April, with the wettest period in December and peak drought conditions occurring in July. Within the HJA, the study was conducted at a site in watershed no. 8, at an elevation of 982 m and slope aspect of 223 degrees. Mean annual temperature at the nearby headquarters averaged 8.8 °C, and annual precipitation was 2,200 mm during 1974–2003 (46). The soil underlying a dense cover of old-growth Douglas fir (Pseudotsuga menziesii) is an Andic Dystrudept and shows abundant patches of dense ectomycorrhizal mats (47), which can result in peroxidase activities 28–126 times greater than that of nonmat soils (48). We therefore expected participation by fungi at this site.

A “rolling” litter decomposition study spanning the years 2005–2011 was conducted at this site to examine the temporal and spatial variation in litter decomposition across individual layers. Nylon mesh panels (1-mm netting, 60 × 60-cm frames) were placed on the litter surface at this site annually. Litter samples were collected over time to delineate each year’s litter fall from the next without confining processes by artificial closure. Upon harvest at the end of the dry season in November 2011, the whole litter was placed on a supporting sheet and placed in a sealed plastic container. Fresh needles and underlaying O, A, and B horizon material were also collected and transported anaerobically in capped amber vials containing dry ice. Samples were transported at 4 °C and immediately returned to the laboratory. In the laboratory, individual 60 × 60-cm litter layers were separated. Decaying needles were manually isolated from other forms of litter such as cones and twigs, and mixed. Soils were pulled through a 2-mm sieve and mixed thoroughly. Needle litter and soil samples were then stored for further analyses as described in SI Materials and Methods.

Manganese Chemistry. Total Mn (as well as Fe, Ca, and Al) content was quantified with X-ray fluorescence spectrometry (XRF: HE XR fluorometer; SPECTRO Analytical Instruments). To determine the amount of bioavailable Mn, we additionally conducted Na-pyrophosphate extractions (49), with extractable Mn (Mnpyroph) taken to originate predominantly from soluble and organically complexed Mn (33). Extracted Mn concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS). Mn oxidation state was determined using Mn XANES (50). Litter and soil samples were dried and hand-ground in an anaerobic glove box and sealed with X-ray transparent Kapton tape. Mn XANES spectra were recorded at the wigglcer beamline 4–3 at the Stanford Synchrotron Radiation Lightsourse (SSRL) (51).

Litter Decomposition State. Total C and N content was determined using a Europa Scientific 20/20 isotope ratio mass spectrometer. Changes in the organic composition of the decomposing litter were determined using FTIR spectroscopy. FTIR spectra of the samples pressed in KBr pellets were recorded from 4,000 to 650 cm⁻¹ with a resolution of 4 cm⁻¹ on a Thermo Nicolet Nexus 670 FTIR spectrometer (Thermo Fisher Scientific).

13C-NMR experiments were performed on a Bruker Avance 400 spectrometer at 100 MHz (400 MHz 1H frequency). All experiments were performed with 4-mm sample rotors in a double-resonance probe head. Semiquantitative structural information on all carbon atoms in the sample was obtained by 13C cross-polarization/totai sideband suppression (CP/TOS). The corresponding subspectrum of carbon signals of mobile groups such as rotating CH3 was obtained by 13C CP/TOS combined with 40-ms dipolar dephasing (DD).

To identify molecular changes in aromatic components of the litter (lignins and tannins), synchrotron-based laser desorption post ionization (LDPI) mass spectrometry of fresh needles and litter layers was performed on a modified time-of-flight secondary ion mass spectrometer (TOF-SIMS V; IonTOF) coupled to a synchrotron UV light path at beamline 9.0.2 of the Advanced Light Source (ALS) (29, 31). To identify peaks in the resulting mass spectra that correspond to aromatic structures, ionization energies for each of the most prominent mass peaks were determined as described in ref. 31 and detailed in SI Materials and Methods. Based on the low IEs of larger aromatic systems (29, 30), peaks with IEs of less than 8.5 eV were attributed to aromatic moieties.

Chemical Imaging Analyses. Needles from all layers were visibly colonized by fungal hyphae, frequently concentrated around dark infections of the surface. To determine Mn distribution and oxidation state associated with these fungal infections, elemental maps and Mn pXANES spectra of cross-sectional needle litter were obtained using X-ray fluorescence mapping and absorption spectroscopy (XRF/XAS) at ALS beamline 10.3.2. To this end, individual needles taken from the top layer were imaged in epoxy (Spurr; TedPella) and cured. Cross-sections were obtained by cutting the resin block and polishing the exposed surface using sandpaper and diamond paste.

To obtain high-resolution maps of the functional group chemistry at the hypha–needle interface, thin sections (<5 μm) of infected needles from the top layer were also prepared using a cryostat (Leica 1950 Cryostat; Leica Instruments) without the use of carbon-based resins and transferred to gold-coated (IR reflective) microscope slides. High-resolution infrared maps of these locations were acquired using synchrotron FTIR (μFTIR) spectromicroscopy at ALS beamline 1.4.3. After completion of the μFTIR analysis, XRF/XAS maps of the same regions and Mn pXANES spectra of selected points within that region were collected at ALS beamline 10.3.2. Further details on sample preparation, analytical procedures, and data processing can be found in SI Materials and Methods.

Data Analysis. All statistical analyses were performed using OriginPro (OriginLab Corp.). Reported SEs are based on three analytical replicates.

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