

Enrichment of Lignin-Derived Carbon in Mineral-Associated Soil Organic Matter

Wenjuan Huang,^{†,¶} Kenneth E. Hammel,^{‡,§} Jialong Hao,^{||} Aaron Thompson,[⊥] Vitaliy I. Timokhin,[#] and Steven J. Hall^{*,†,¶}

[†]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011, United States

[‡]US Forest Products Laboratory, Madison, Wisconsin 53726, United States

[§]Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706, United States

^{||}Key Laboratory of Earth and Planetary Physics, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing 100029, China

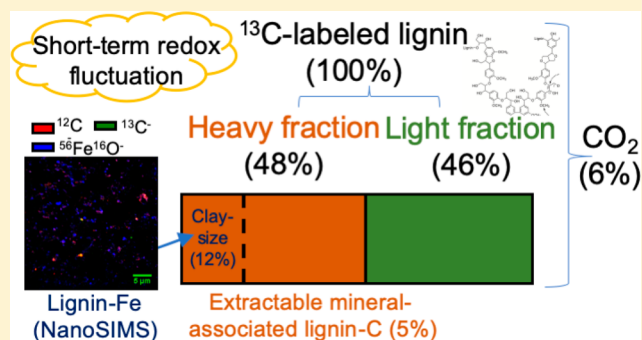
[⊥]Department of Crop and Soil Sciences, The University of Georgia, Athens, Georgia 30602, United States

[#]University of Wisconsin, Wisconsin Energy Institute, DOE Great Lakes Bioenergy Research Center, Madison, Wisconsin 53706, United States

[¶]Key laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Supporting Information

ABSTRACT: A modern paradigm of soil organic matter proposes that persistent carbon (C) derives primarily from microbial residues interacting with minerals, challenging older ideas that lignin moieties contribute to soil C because of inherent recalcitrance. We proposed that aspects of these old and new paradigms can be partially reconciled by considering interactions between lignin decomposition products and redox-sensitive iron (Fe) minerals. An Fe-rich tropical soil (with C₄ litter and either ¹³C-labeled or unlabeled lignin) was pretreated with different durations of anaerobiosis (0–12 days) and incubated aerobically for 317 days. Only 5.7 ± 0.2% of lignin ¹³C was mineralized to CO₂ versus 51.2 ± 0.4% of litter C. More added lignin-derived C (48.2 ± 0.9%) than bulk litter-derived C (30.6 ± 0.7%) was retained in mineral-associated organic matter (MAOM; density >1.8 g cm⁻³), and 12.2 ± 0.3% of lignin-derived C vs 6.4 ± 0.1% of litter C accrued in clay-sized (<2 μm) MAOM. Longer anaerobic pretreatments increased added lignin-derived C associated with Fe, according to extractions and nanoscale secondary ion mass spectrometry (NanoSIMS). Microbial residues are important, but lignin-derived C may also contribute disproportionately to MAOM relative to bulk litter-derived C, especially following redox-sensitive biogeochemical interactions.



INTRODUCTION

Lignin is critically linked to our understanding of terrestrial carbon (C) cycling given its importance in controlling initial rates of litter decomposition and its high overall C loading to the environment; lignin is the second most abundant plant-derived organic substance after cellulose.^{1,2} Lignin was historically thought to contribute disproportionately to soil organic matter (SOM) relative to other organic compounds as a consequence of its complex macromolecular structure,³ which requires strong, nonspecific oxidants for depolymerization.⁴ However, a modern paradigm of SOM dynamics^{5,6} proposes that environmental, biological, and geochemical controls, rather than molecular structure alone, determine the persistence of SOM. The Microbial Efficiency-Matrix Stabilization (MEMS) hypothesis proposes that C derived

from labile constituents of plant biomass (e.g., unprotected cellulose) which are efficiently converted to microbial biomass and necromass (i.e., compounds with high C-use efficiency) tend to accrue in SOM by interacting with mineral surfaces and facilitating aggregate formation.^{5,7–10} In contrast, C from complex polymers such as lignin tends to have poor microbial C-use efficiency (as low as 1%^{11,12} to 8–31%¹³) and may be more likely to be lost as CO₂ than to contribute to mineral-associated organic matter (MAOM).⁸ However, this framework does not fully consider the impacts of compound-specific

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interactions between SOM constituents and minerals, which may be especially important in soils with large proportions of reactive secondary minerals.¹⁴

Fe (oxyhydr)oxides and short-range-ordered (SRO) Fe phases in particular are widely understood to protect SOM from microbial decomposition via sorption and coprecipitation, and these phases may preferentially associate with aromatic lignin decomposition products relative to other organic compounds.^{15–19} Soils undergoing short-term redox fluctuations can generate significant amounts of SRO Fe phases,^{20–22} which would potentially increase the capacity for adsorbing or coprecipitating lignin-derived C. Although lignin decomposition can be sustained under fluctuating redox environments,²³ sorption of lignin decomposition products to freshly formed SRO phases^{17,18} may provide a mechanism for incorporation of lignin-derived C in MAOM. Lignin decomposition products can potentially contribute significantly to MAOM,^{15,16} yet the fates of lignin during decomposition in soil remain poorly understood. Here, we propose that lignin represents a more important contributor to MAOM than recently acknowledged due to interactions with Fe mineral phases, and that these interactions vary significantly as a function of soil redox conditions.

Analytical challenges have presented major barriers in resolving ongoing controversies about lignin decomposition in soil. The common assays of lignin residues using cupric oxide oxidation can potentially obscure the importance of lignin in MAOM due to failure to recover significant amounts of lignin sorbed on soil minerals.^{24,25} Nuclear magnetic resonance analyses of SOM frequently require removal of Fe by strong acid pretreatment, causing potentially significant loss and possible chemical fractionation of SOM.²⁶ Solution-phase analyses of SOM such as ultrahigh resolution spectroscopy (FT-ICR-MS) can be very powerful,¹⁵ but quantitative solubilization of SOM is difficult.⁵ The addition of isotope-labeled synthetic lignins to soil can surmount these challenges by allowing unambiguous measurements of lignin mineralization to CO₂ and incorporation of lignin-derived C in microbial biomass.^{11,12,27} The potential flux of lignin-derived C through microbial biomass can also be constrained using measurements of lignin mineralization and estimates of microbial C-use efficiency, and residual lignin-derived C in the solid phase can be quantified by combustion of solid organic matter to CO₂. However, isotope-labeled synthetic lignins have not been widely used in soils since the seminal work of Haider and colleagues.^{11,12,27}

Here, we tested the hypotheses that (1) added lignin-derived C contributes disproportionately to MAOM relative to C derived from bulk litter, due to differential sorption or coprecipitation with Fe and (2) a short-term redox fluctuation has lasting impacts on the fate of added lignin-derived C. We collected soil from a tropical forest (C₃ vegetation) rich in reactive Fe minerals that experiences frequent O₂ fluctuations driven by episodic rainfall and high rates of microbial metabolism.^{28,29} The soil was amended with leaf litter from a C₄ grass and either synthetic ¹³C-labeled or unlabeled lignin, which enabled us to discriminate between three different C sources (soil-C, bulk litter-C, and lignin-C labeled at C_β of the propyl side chain). Release of lignin ¹³C_β as CO₂ demonstrates unequivocally that the polymer was cleaved.⁴ Soil samples were pretreated with either 12, 8, 4, and 0 days of anaerobiosis to generate a gradient of redox conditions representative of field

conditions²⁹ and were subsequently exposed to an aerobic headspace for 317 days.

MATERIALS AND METHODS

Sample Preparation. Soil (A horizon, 0–10 cm) was collected from an upland valley in a perhumid tropical forest near the El Verde field station of the Luquillo Experimental Forest (18°17'N, 65°47'W), Puerto Rico. Leaves and fine roots of the dominant tree species at this site contain significant lignin (12–29% by mass³⁰), as is typical for most higher plants.¹ This soil experiences temporal O₂ fluctuations in surface horizons, which in turn stimulate Fe redox cycling,²⁹ and is an Oxisol developed from basaltic to andesitic volcanoclastic sediments.²¹ The sample was shipped overnight to Iowa State University and passed through an 8 mm sieve to remove coarse roots while retaining microaggregate structure (rocks are absent in the <8 mm fraction of this soil). Soil was gently mixed and brought to field moisture capacity (1.0 g H₂O g^{−1} soil). Subsamples (1 g dry mass equivalent) were incubated according to three substrate treatments to partition respiration from soil, litter (senesced leaves of *Andropogon gerardii*; see the [Supporting Information \(SI\)](#) for further details), and the C_β position of the added ¹³C-labeled lignin: (1) soils alone (control); (2) soils amended with litter and synthetic lignin (soil + litter + unlabeled lignin), and (3) soils amended with litter and synthetic lignin labeled with 99 atom % ¹³C at the C_β position of each lignin C₉ substructure (soil + litter + ¹³C_β-labeled lignin). The synthetic lignin had been fractionated prior to the experiment by gel permeation chromatography to obtain polymeric lignins with a molecular mass greater than 1 kDa. Similar to natural lignin, macromolecular synthetic lignin is insoluble in water until it is depolymerized to lower-molecular-weight constituents during decomposition.³¹ Each subunit had an average molecular mass of 197, such that the ¹³C label represented 13/197 of the added synthetic lignin mass. The lyophilized unlabeled or labeled lignins were precipitated in a 1:21 mass ratio on dried and finely ground leaf litter of *Andropogon gerardii* (big bluestem, a C₄ grass), which provided an isotopic contrast with C₃-derived soil C (see [SI](#) for more details). Leaf litter of *Andropogon gerardii* (no stem) was collected shortly after senescence and had 41.3% C and 1.1% N. Soils were gently homogenized with the litter + lignin mixtures in a 9:1 ratio (0.9 g dry soil mass mixed with 99 mg litter and 4.74 mg lignin for each soil sample). The ratio of added C (litter + lignin mixtures) to SOC was ~42:31 for each sample. In this paper, “lignin-derived C” hereafter refers to the ¹³C_β moiety of the synthetic lignin added to the litter; the *A. gerardii* litter also contained natural lignin.

Headspace Treatments. Soil samples were incubated in the dark for 329 days in total at laboratory temperature (23 ± 1 °C) in glass jars (946 mL) sealed with Viton gaskets and aluminum lids with butyl septa. Over the first 12 days of the experiment, samples were exposed to CO₂-free air and/or dinitrogen (N₂) headspaces to impose varying durations of anaerobiosis at staggered intervals (12, 8, 4, and 0 days). We refer to the first 12 days as the “anaerobic pre-treatment” phase of the experiment, which mimics the short-term deprivation of oxygen that soils frequently experience in this ecosystem.^{28,29} Specifically, during the pretreatment, samples were exposed to aerobic conditions for 12 days (0-day anaerobic pretreatment, control), for 8 days followed by a 4-day anaerobic phase (4-day anaerobic pretreatment), for 4 days followed by an 8-day

anaerobic phase (8-day anaerobic pretreatment), and for 0 days (12-day anaerobic pretreatment). After day 12, all samples were exposed to an aerobic headspace for the subsequent 317 days. Anaerobic conditions were attained by flushing jars with ultrapure N_2 , and aerobic conditions were attained using CO_2 -free air. Jars were refilled with the appropriate gas following periodic headspace sampling as described below. Soil moisture was monitored by recording the mass of each sample, and water was added as necessary throughout the experiment to replace moisture lost during headspace flushing. Blank jars (without soil) were similarly treated as the samples.

Partitioning of CO_2 Sources. Headspace gas was initially measured every 2 days for soils with litter + lignin and every 4 days for control soils (to ensure sufficient accumulation of CO_2 for precise analyses in the latter treatment).³² After 47 days, samples were measured weekly, or after 189 days, every 2 weeks. We measured CO_2 concentrations and their $\delta^{13}C$ values immediately prior to flushing the headspace. Gas was sampled via a 5 mL syringe with stopcock and immediately injected into a carrier gas (CO_2 -free air) flowing into a tunable diode laser absorption spectrometer (TDLAS, TGA200A, Campbell Scientific, Logan, UT). Concentrations of CO_2 and $\delta^{13}C$ values were calibrated following Hall et al.³² The production of CO_2 derived from soil, litter, and $^{13}C_{\beta}$ -labeled lignin was calculated by mixing models³² described in the SI. Additional headspace gas samples (20 mL) were collected for CH_4 measurements during the first 105 days, after which CH_4 production was negligible. Concentrations of CH_4 were analyzed by gas chromatography with a flame ionization detector (GC-2014, Shimadzu, Columbia, MD). However, impacts of CH_4 production on ^{13}C mass balance were negligible in this experiment as CH_4 accounted for <1% of total C mineralization.³³ The CO_2 production from soil, litter, and lignin was expressed relative to dry mass of the whole sample (soil + litter + lignin). All other element fluxes or concentrations were expressed similarly.

Carbon in Different Soil Fractions. At the end of this experiment, a combined density and particle size fractionation was conducted to remove free light organic matter and separate high-density fractions ($>1.8\text{ g cm}^{-3}$) and the clay-sized subset of the high-density fractions ($<2\text{ }\mu\text{m}$) in the samples with litter and unlabeled/ $^{13}C_{\beta}$ -labeled lignin ($n = 24$) to quantify the lignin-and litter-derived C in different soil fractions (see SI for more details). Briefly, sodium polytungstate solution (density 1.8 g cm^{-3})³⁴ was used to remove light fractions from heavy fractions. Samples were shaken overnight to gently disperse aggregates rather than the application of sonication, which can cause organo-mineral associations to be released in lighter fractions.³⁵ The clay-sized fractions were then separated from remainder of the heavy fractions by centrifugation, and masses of each were recorded. The C concentrations and $\delta^{13}C$ values of both the clay-sized and nonclay-sized portions of the heavy fractions in the $^{13}C_{\beta}$ -labeled lignin samples were analyzed at the UC Davis Stable Isotope Facility using an elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), and unlabeled samples were similarly analyzed at Iowa State University (ThermoFinnigan Delta Plus XL, Waltham, MA). We used previous data to estimate $\delta^{13}C$ values of the initial dense fraction SOM (-27.6‰) in the mixing model.³⁶

Selective Extractions. Subsamples of bulk (nondensity-fractionated) soil were extracted by sodium dithionite ($Na_2S_2O_4$, used to reductively dissolve Fe phases) and subsequently by sodium pyrophosphate ($Na_4P_2O_7$, used to complex Fe and Al)^{37,38} to examine anaerobic pretreatment effects on Fe (oxyhydr)oxides and associated organic C at the end of incubation, which was partitioned among soil, litter, and lignin using $\delta^{13}C$ values of DOC released in the extraction (see SI for more details).

^{57}Fe Mossbauer Spectroscopy. Iron phases were characterized at the end of the experiment in a subsample of bulk (nondensity-fractionated) soil from one replicate from each headspace treatment by ^{57}Fe Mossbauer spectroscopy. This method has been previously used to investigate soil Fe abundance and composition and their relationships with SOM.^{20,21,36,39} We recorded spectra at 140, 77, and 5K to evaluate the crystallinity continuum of Fe^{III} (oxyhydr)oxide phases. We calculated a relative crystallinity index for each sample using the ratio of sextet areas measured at 77K and 5K.^{36,39} More information on the ^{57}Fe Mossbauer spectroscopy can be found in SI.

NanoSIMS Analysis. Finally, to assess spatial relationships among mineral and organic phases, the high-density clay-sized fractions of samples ($<2\text{ }\mu\text{m}$) that received litter and $^{13}C_{\beta}$ -labeled lignin ($n = 12$) were analyzed by NanoSIMS (Cameca NanoSIMS 50L, Gennevilliers, France) at the Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing, China (see SI for a detailed description). We used the $<2\text{ }\mu\text{m}$ dense fraction for NanoSIMS for two reasons. First, larger particles decrease the uniformity of the sample surface on the silicon analysis wafer (see SI) and can decrease the resolution of the method. Second, we sought to be conservative in our analysis of organo-mineral associations, acknowledging the potential for incomplete dispersion of microaggregates during density fractionation. Therefore, our NanoSIMS data do not necessarily represent the dense fraction as a whole. NanoSIMS images were analyzed using ImageJ (1.52d version) with the Open MIMS Image plugin.⁴⁰ In the NanoSIMS images, the presence of $^{12}C^-$, $^{13}C^-$, and $^{56}Fe^{16}O^-$ ion masses indicated the presence of organic C and Fe minerals, respectively. On the basis of the $^{56}Fe^{16}O^-$ images, the regions of interest (ROIs) were selected using the threshold option of the ImageJ software with the Otsu method.⁴¹ The same ROIs were simultaneously selected from the other elemental images. We extracted areas and means of secondary ion masses for each ROI from all images. Areas greater than 10 pixels in the ROIs were used for further calculations.

Data Analysis. Differences in CO_2 production from soils, litter, and lignin among the anaerobic pretreatments were examined using mixed-effects models, which included treatments as fixed effects and samples as a random effect (to account for temporal correlation within sampling units) using the lmer function in R.⁴² Effects of anaerobic pretreatments on cumulative CO_2 production and soil chemical properties were tested by ANOVA, with Duncan tests to compare significant differences among treatments. Relationships between soil chemical properties at the end of the experiment and the duration of anaerobic pretreatments were also analyzed by linear regression models using the lm function in R. Mean values followed by standard errors were reported. All statistical analyses were conducted with R.⁴³

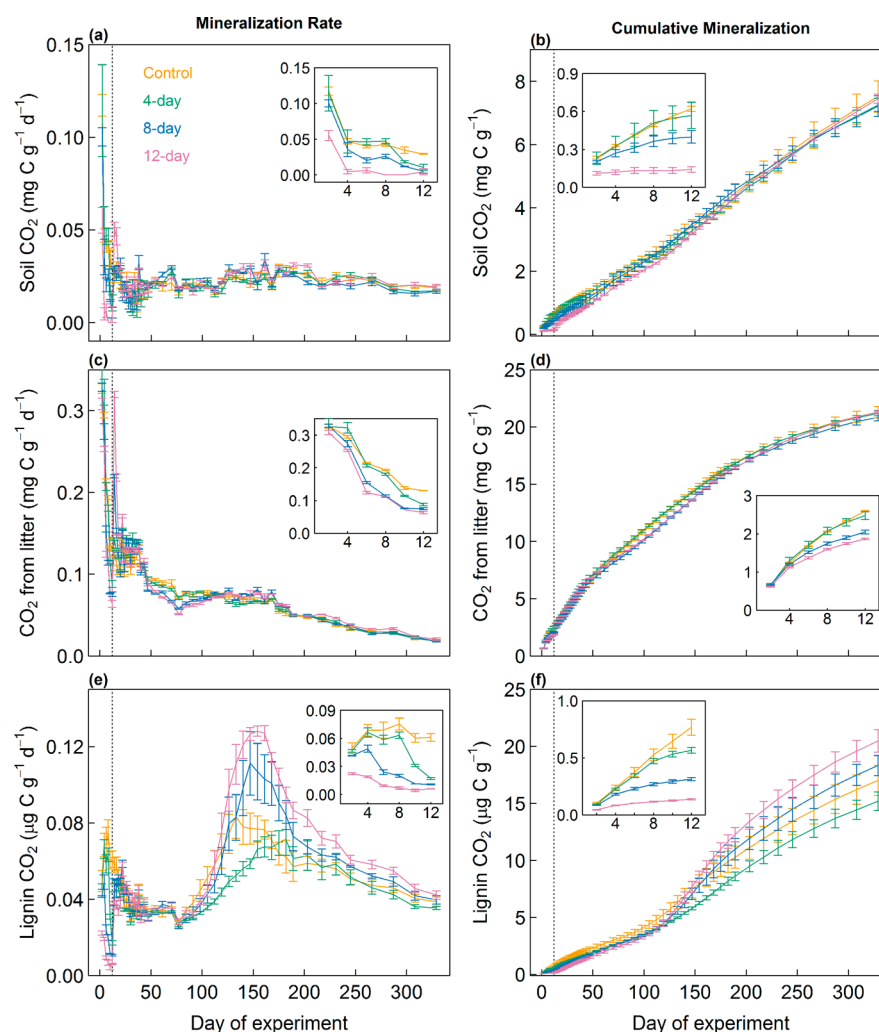


Figure 1. Carbon mineralization (CO_2 production) from soil (a, b), litter (c, d), and synthetic lignin (e, f) in whole soil, expressed relative to dry mass of the whole sample. Insets are expanded views of CO_2 production over the first 12 days. The dotted lines denote the end of anaerobic phase of the pretreatments at 12 days. The error bars indicate SE ($n = 3$ for each treatment). Control: continuous aerobic phase during the pretreatment; 4-day: 8-day aerobic phase followed by 4-day anaerobic phase during the pretreatment; 8-day: 4-day aerobic phase followed by 8-day anaerobic phase during the pretreatment; 12-day: 12-day anaerobic phase during the pretreatment.

RESULTS

Mineralization of Different C Sources. Longer anaerobic periods depressed CO_2 production (C mineralization) from soil, litter, and especially lignin over the first 12 days (Figure 1); however, mineralization of lignin C increased to a greater extent than did mineralization of soil or litter C following the reintroduction of O_2 (day 12 of this experiment). During 12 to 22 days, longer anaerobic pretreatments resulted in a pulse of greater C mineralization from soil, litter, and lignin after the reintroduction of O_2 . Notably, after 119 days, C mineralization from lignin (but not soil or litter) increased once again and diverged markedly among the anaerobic pretreatments (12-day > control and 4-day, $P < 0.01$; 8-day > 4-day, $P < 0.05$). At the end of the incubation (329 days), the 12-day anaerobic pretreatment had the highest cumulative lignin C mineralization ($20.48 \pm 0.98 \mu\text{g C g}^{-1}$), followed by the 8-day anaerobic pretreatment ($18.32 \pm 0.88 \mu\text{g C g}^{-1}$), control ($17.03 \pm 1.31 \mu\text{g C g}^{-1}$), and 4-day anaerobic pretreatment ($15.19 \pm 0.82 \mu\text{g C g}^{-1}$) (Figure 1f). The value in the 12-day anaerobic pretreatments significantly differed from the control ($P = 0.05$) and 4-day anaerobic pretreatments

($P < 0.01$). The C mineralization from soil and litter was similar among the treatments ($7.36 \pm 0.14 \text{ mg C g}^{-1}$ and $21.15 \pm 0.15 \text{ mg C g}^{-1}$ on average, respectively).

Fates of Added Lignin and Litter C. We found that a greater proportion of synthetic lignin-derived C contributed to MAOM than did litter-derived C, across all incubation treatments. Mean C mineralization from lignin accounted for only $5.7 \pm 0.2\%$ of initial added lignin ^{13}C , whereas that from litter was $51.2 \pm 0.4\%$ of total added litter C (Figure 2). The high-density fractions and their clay-sized subsets ($<2 \mu\text{m}$) contained significantly higher proportions of the added lignin-derived C ($48.2 \pm 0.9\%$ and $12.2 \pm 0.3\%$, respectively) than of litter-derived C ($30.6 \pm 0.7\%$ and $6.4 \pm 0.1\%$, respectively, $P < 0.01$; Figure 2). The anaerobic pretreatments did not significantly affect the masses of soil in the high-density fractions or their corresponding total lignin- or litter-derived C stocks. However, we found a trend of greater clay-sized fraction lignin-derived C in the 4-day ($42.5 \pm 1.6 \mu\text{g C g}^{-1}$) than the 12-day ($36.9 \pm 2.6 \mu\text{g C g}^{-1}$; $P = 0.08$) anaerobic pretreatment, which was the opposite of that observed for cumulative lignin C mineralization.

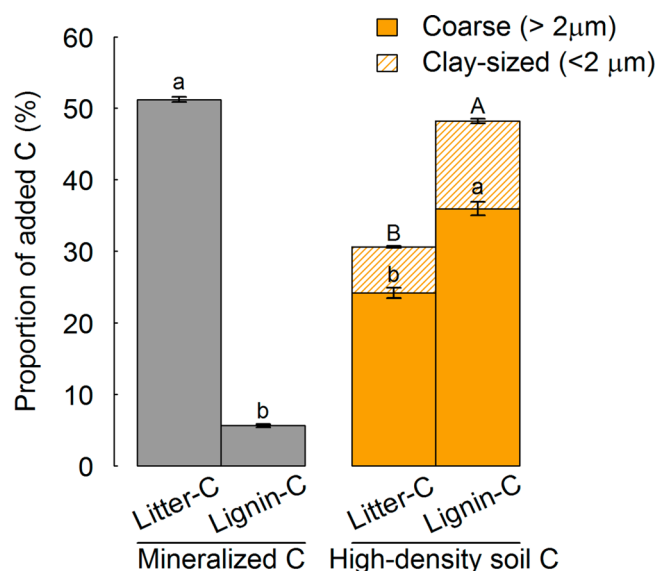


Figure 2. Fates of added lignin and litter C after 329 days. The coarse (>2 μm) and fine (<2 μm) portions of the high-density fractions are indicated by solid and hatched bars, respectively. The error bars indicate SE ($n = 12$). Different lowercase letters indicate significant differences between the litter-C and lignin-C at $P < 0.05$. Different uppercase letters indicate significant differences between the litter-C and lignin-C in the fine portion of the high-density fractions at $P < 0.05$.

Lignin-Derived C Associated with Fe Minerals. The mean proportions of added lignin-derived C extracted by dithionite and subsequent pyrophosphate were $1.37 \pm 0.06\%$ and $3.31 \pm 0.11\%$, respectively. Increasing the duration of anaerobiosis from 0 to 12 days during the pretreatment phase yielded significantly more lignin-derived C associated with Fe minerals (and co-occurring Al) at the end of incubation (Figure 3), with no change in litter-derived or soil C. Specifically, lignin-derived C extracted by dithionite and subsequent pyrophosphate significantly increased with the duration of anaerobiosis (3.65 ± 0.22 and $9.18 \pm 0.59 \mu\text{g C g}^{-1}$ in the control vs 4.91 ± 0.34 and $10.98 \pm 0.58 \mu\text{g C g}^{-1}$ in the 12-day anaerobic pretreatment, $P < 0.01$ and $P < 0.05$, respectively; Figure 3). In contrast to lignin, soil and litter-derived C extracted by dithionite (4.17 ± 0.06 and $2.03 \pm 0.06 \text{ mg C g}^{-1}$, respectively) and subsequent pyrophosphate (7.99 ± 0.09 and $2.31 \pm 0.08 \text{ mg C g}^{-1}$, respectively) were similar among treatments.

No significant differences in concentrations of Fe (oxyhydr)-oxides or Fe phase composition were observed among treatments at the end of the experiment, according to the dithionite and secondary pyrophosphate extractions (61.95 ± 0.80 and $2.44 \pm 0.16 \text{ mg Fe g}^{-1}$, respectively) and ^{57}Fe Mossbauer spectroscopy (Tables S1–4 and Figures S3–6). The dominant Fe phase in soil was nanogoethite, comprising $82.6 \pm 0.2\%$ of total Fe, while a ferrihydrite-like phase accounted for $10.5 \pm 0.3\%$. The Fe in primary silicate minerals, silicate clays, and/or surface complexes could not be distinguished by Mossbauer. The Fe(III) and Fe(II) in these phases represented <7% and <0.5% of total Fe, respectively (Table S1–4 and Figure S3–6).

NanoSIMS measurements showed that the 8- and 12-day anaerobic pretreatments increased the amount of lignin-derived C associated with Fe minerals in the high-density clay-sized fractions at the end of the incubation (Figures 4 and

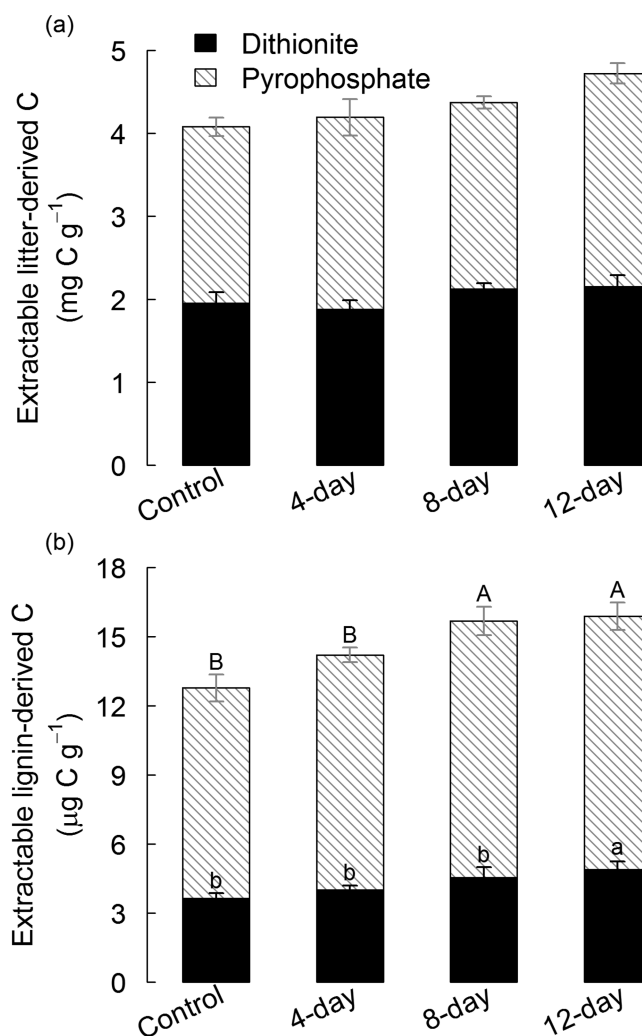


Figure 3. Litter-derived C (a) and lignin-derived C (b) in dithionite and subsequent pyrophosphate extractions of whole soil after 329 days. The error bars indicate SE ($n = 3$ for each treatment). Different lowercase and uppercase letters indicate significant differences among the treatments at $P < 0.05$ for the dithionite and pyrophosphate extractions, respectively. Control: continuous aerobic phase during the pretreatment; 4-day: 8-day aerobic phase followed by 4-day anaerobic phase during the pretreatment; 8-day: 4-day aerobic phase followed by 8-day anaerobic phase during the pretreatment; 12-day: 12-day anaerobic phase during the pretreatment.

S7), despite the similarities in bulk Fe phase compositions among treatments. The regions of interest (ROIs) selected using the $^{56}\text{Fe}^{16}\text{O}^-$ images showed that the 12- and 8-day anaerobic pretreatments had higher ratios of $(^{12}\text{C}^- + ^{13}\text{C}^-)/^{56}\text{Fe}^{16}\text{O}^-$ and were more ^{13}C associated with Fe minerals than the other treatments ($P < 0.01$; Figure S7).

DISCUSSION

A modern paradigm of soil organic matter posits that lignin-derived C is a relatively minor contributor to soil organic matter,⁶ due in part to its low microbial C-use efficiency.^{8,9} However, methodological challenges may have obscured the contributions of lignin-derived C to MAOM.^{24,25} Although low C-use efficiency of lignin would decrease the retention of lignin-derived C in microbial biomass and necromass, lignin degradation products could also potentially sorb to soil minerals. Consistent with these ideas, we found that increasing

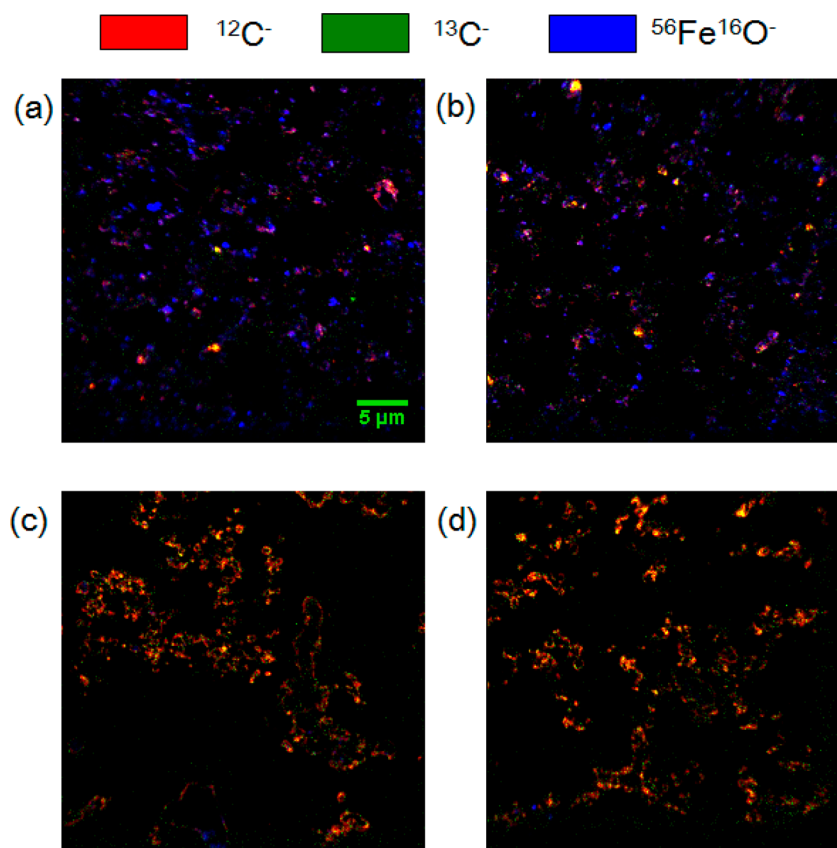


Figure 4. Representative NanoSIMS images of $^{12}\text{C}^-$, $^{13}\text{C}^-$, $^{56}\text{Fe}^{16}\text{O}^-$ from different anaerobic pretreatments ($35 \times 35 \mu\text{m}$ with a 256×256 pixel resolution). (a) Control: continuous aerobic phase during the pretreatment; (b) 4-day anaerobic pretreatment: 8-day aerobic phase followed by 4-day anaerobic phase during the pretreatment; (c) 8-day anaerobic pretreatment: 4-day aerobic phase followed by 8-day anaerobic phase during the pretreatment; (d) 12-day anaerobic pretreatment: 12-day anaerobic phase during the pretreatment. The mixture of red and blue yields magenta. The mixture of red and green yields yellow, which indicates lignin-derived C.

the duration of a single anaerobic event from 0 to 12 days had dual and long-lasting impacts on the decomposition of lignin and significantly impacted its association with Fe mineral phases after a subsequent 317-day aerobic incubation. On one hand, lignin was depolymerized to a greater extent following longer durations of anaerobic pretreatments, as indicated by cumulative $^{13}\text{CO}_2$ production from lignin C_β (Figure 1f). The extent of lignin mineralization was inversely related to its solid-phase partitioning, supporting the concept that lignin can be readily decomposed in the absence of physicochemical protection.⁶ On the other hand, longer durations of anaerobic pretreatments also led to increased association of lignin-derived C with Fe (oxyhydr)oxides at the end of the experiment, as indicated by extractions of bulk soil and NanoSIMS analyses of high-density clay-sized fractions (Figures 3 and 4). These findings indicated that the fragmentation and decomposition of lignin stimulated after the initial redox fluctuation also increased the preferential association of lignin fragments with Fe minerals. Our results can thus reconcile the short-term stimulation of lignin decomposition in fluctuating redox environments²³ with the preservation of lignin-derived C by Fe oxides observed previously.^{15,17,18,44} Although the turnover times of Fe-associated lignin-derived C remain unknown, these coupled mechanisms could potentially explain recent observations of preferential accumulation of lignin decomposition products relative to other organic C compounds in Fe-rich tropical forest soils.^{15,16} These studies included samples from the

Oxisol, Andisol, Ultisol, and Inceptisol soil orders with varying Fe content and mineral compositions.

Long-term Impacts of Short-term Anaerobiosis on Lignin Decomposition. Lignin decomposition was suppressed during the anaerobic pretreatment but rapidly recovered following the reintroduction of O_2 . The immediate source of increased litter- and soil- CO_2 during 12 to 22 days was probably DOC which progressively accumulated during anaerobiosis in parallel with Fe reduction (Figures S1 and S2). Added lignin was not a likely source of the accumulated DOC given that lignin depolymerization is suppressed in the absence of O_2 .⁴⁵ The fact that treatment differences in lignin decomposition did not manifest until four months later is consistent with progressive degradation of lignin macromolecules (>1000 Da) over time. Following polymer cleavage, low-molecular weight lignin fragments can be mineralized to CO_2 at much greater rates than initial high-molecular weight lignin since many bacteria, in addition to lignin-degrading fungi, can assimilate or oxidize low-molecular weight lignin derivatives.^{27,31} In addition, this time lag might be related to the depletion of easily assimilable substrates (e.g., cellulose and proteins) and changes in microbial community composition or abundance after the anaerobic pretreatments. Coincidentally, fungal diversity peaked after four months in another study of litter decomposition,⁴⁶ the same period when we observed highest mineralization rates of added lignin. Along with changes in microbial composition, hydroxyl radical (OH) production via the oxidation of Fe(II) by hydrogen peroxide

(H_2O_2), known as the Fenton reaction, may also have contributed to variation in lignin depolymerization among treatments. This reaction can contribute to lignin decomposition because OH nonspecifically cleaves the relatively stable ether bonds of lignin.^{4,47} Iron-rich soils undergoing redox fluctuations may promote Fenton chemistry given that Fe(II) is produced via microbial metabolism, and H_2O_2 can be generated via numerous enzymatic reactions or by the abiotic oxidation of Fe(II) by O_2 ($\text{Fe(II)} + \text{O}_2 + \text{H}^+ \rightarrow \text{Fe(III)} + \text{HO}_2$; $\text{Fe(II)} + \text{H}^+ + \text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{Fe(III)}$).^{23,48} Relative to the control, higher Fe(II) concentrations were generated in the longer anaerobic pretreatments after 12 days of anaerobiosis and slowly declined concomitant with Fe(II) oxidation over the subsequent weeks (Figures S1 and S2). However, added lignin residues were also susceptible to sorption to mineral surfaces, and lignin-derived C in the high-density clay-sized fractions was altered by the anaerobic pretreatments. The difference in lignin-derived C in the clay-sized fraction between the 4- and 12-day anaerobic pretreatments ($5.6 \mu\text{g C g}^{-1}$) was very similar to differences in lignin decomposition to CO_2 ($5.3 \mu\text{g C g}^{-1}$). Thus, alteration of lignin sorption by the anaerobic pretreatments most likely controlled lignin cleavage and mineralization of lignin C_β .

Preferential Association of Lignin-Derived C with Fe Minerals. Consistent with our first hypothesis, our ^{13}C measurements directly showed that lignin-derived C can provide significant and relatively rapid contributions to MAOM which were disproportionately greater than C from bulk litter. Our findings challenge the MEMS hypothesis,^{8,9} where biochemically labile compounds that are efficiently incorporated into microbial biomass are thought to preferentially accumulate in MAOM relative to more biochemically complex plant compounds.⁴⁹ We acknowledge that microbial residues are clearly important contributors to MAOM.⁵⁰ However, the lignin decomposition products observed in the dense fraction and its clay-sized subset were unlikely to have substantially been derived from microbial biomass given the small extent of cumulative lignin mineralization to CO_2 (5.7%) relative to the much larger proportion of lignin-derived C in the high-density fractions (clay-sized high-density fraction + coarse high-density fraction, 48.2%; Figure 2). Two arguments can be made to support this conclusion. First, using the usual definition of microbial C-use efficiency⁵¹ and even assuming an unusually high value of 50% for added lignin propyl side chain C_β ,^{11,13} only 5.7% of added lignin C_β would have accumulated in microbial biomass. Second, stoichiometric calculations have shown that even for the readily utilized substrate glucose, the ratio of biomass produced to glucose mass respired for energy cannot exceed approximately 7:3.⁵² Thus, assuming that the 5.7% mineralization of added lignin C_β reflects its use for energy production, no more than 13–14% of the added C could have accumulated in microbial biomass, even under ideal conditions. In fact, the proportion of lignin C_β in biomass would have to be considerably smaller, because the most efficient mechanism for microbial cleavage of lignin's abundant arylglycerol- β -aryl ether structure releases C_β as the aldehyde moiety of glycolaldehyde,⁵³ which is a more oxidized C source than glucose is. Our direct evidence of disproportionate lignin-derived C inputs to MAOM relative to bulk litter-derived C bolsters other recent work suggesting that plant-derived compounds can substantially contribute to MAOM.^{54–56}

The results from dithionite and subsequent pyrophosphate extractions and NanoSIMS images indicated that the

accumulation of lignin-derived C in MAOM can be due in part to its preferential association with Fe minerals relative to the litter-derived C, which supported our second hypothesis. Compared with the litter-derived C, the increased lignin-derived C in dithionite extractions of soil from the longer anaerobic pretreatments, together with the evidence of increased lignin degradation but no changes in reactive Fe phases, demonstrated preferential sorption of lignin-derived C to Fe oxides.^{15,17} We observed even greater lignin release in the subsequent pyrophosphate extraction, echoing a previous finding that sequential extraction by dithionite and pyrophosphate release distinct C pools.³⁷ The lignin-derived C extracted by dithionite and pyrophosphate solutions likely contained low-molecular-weight decomposition products (e.g., modified alkyl side chain moieties with and without attached aromatics) given the poor solubility of lignin macromolecules in water.³¹ However, a much larger proportion of remaining mineral-associated lignin-derived C resisted extraction in dithionite and pyrophosphate and was therefore likely to have remained in poorly soluble phenylpropanoid polymers after the Fe phases were dissolved in the extractants.

The NanoSIMS data from the clay-sized dense fractions provided additional evidence that this large pool of nonextractable lignin-derived C also showed a specific association with Fe at the nanoscale relative to litter-derived C and that these interactions were enhanced following short-term anaerobiosis. Previous studies by NanoSIMS demonstrated that mineral particles in the clay-sized fraction can act as nuclei for litter-derived organic C accumulation.^{34,57} Analogously, the SRO Fe phases regenerated following Fe reduction and Fe(II) oxidation in the longer anaerobic pretreatments (8- and 12-day; Figure S1) likely provided new mineral surfaces to sorb lignin C. Lignin-derived C incorporation in MAOM was enhanced with increasing durations of short-term anaerobiosis (from 0 to 12 days). Given that many ecosystems in addition to Fe-rich tropical forests have been shown to experience short-term soil O_2 variability and associated Fe reduction (e.g., Mediterranean grassland, drained peatland, Midwestern cropland, montane meadow, urban lawn, temperate forest)^{58–61} the redox-driven accrual of lignin-derived C in MAOM could represent a generally important mechanism.

Role of Molecular Composition in SOM Paradigms.

Our results show that lignin can represent a significant source of MAOM and that its fate is sensitive to environmental factors which have already been altered by climate change in this tropical forest, where an unusually severe drought recently caused a large increase in soil O_2 availability.⁶² A lack of O_2 is increasingly acknowledged to influence the persistence of SOM via impacts on the bioenergetics of decomposition and the consequent accumulation of reduced compounds,^{63,55} but our results demonstrate that geochemical impacts of O_2 deprivation may be equally important. Our findings indicated that the fragmentation and decomposition of lignin stimulated after the initial redox fluctuation (and associated Fe redox cycling) also increased the association of lignin fragments with Fe minerals. Frequent O_2 fluctuations can potentially alter soil Fe composition by promoting Fe reduction and increasing or decreasing Fe crystallinity,^{21,64} which could conceivably lead to net accrual or release of lignin-derived C. It thus remains uncertain how Fe-lignin associations might be affected by frequent O_2 fluctuations in the field, although aromatic C was shown to be selectively released during hematite reduction in a

laboratory incubation.⁶⁵ Soils similar to those used in this experiment showed positive correlations among SRO Fe, a proxy for aromatic C content, and turnover rates of mineral-associated C.³⁶ More research is needed to evaluate the persistence of Fe-associated lignin in soils that experience O₂ fluctuations. To sum up, in a partial reconciliation between aspects of old and new paradigms of SOM, we show that lignin-derived C can indeed represent a disproportionate source of MAOM relative to bulk litter, in contrast to recent work which hypothesized a minor role for lignin-derived C in MAOM.^{7–9} However, the persistence of lignin was not due to inherent recalcitrance: we also found that the contribution of lignin-derived C to MAOM varied crucially as a function of ecosystem context (i.e., redox-sensitive biogeochemical reactions), a key tenet of recent thinking about SOM.⁶ The further resolution of when, where, and how long different organic compounds can persist in soil along the decay continuum is key to improving our understanding of SOM persistence under global change.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.9b01834](https://doi.org/10.1021/acs.est.9b01834).

Sample preparation, data processing calculations, details about the selected chemical properties of soil samples, additional data on Fe reduction and DOC, additional data on NanoSIMS analysis, additional Mossbauer spectra and Mossbauer spectral fitting parameters (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (01) 515-294-7650. E-mail: stevenjh@iastate.edu.

ORCID

Aaron Thompson: [0000-0001-6301-7377](https://orcid.org/0000-0001-6301-7377)

Steven J. Hall: [0000-0002-7841-2019](https://orcid.org/0000-0002-7841-2019)

Author Contributions

All authors contributed to research. The manuscript was primarily written by W.H. and S.J.H. with contributions from K.E.H. and A.T. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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