Iron-mediated organic matter decomposition in humid soils can counteract protection

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Abstract
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Iron-mediated organic matter decomposition in humid soils can counteract protection

Chunmei Chen¹, Steven J. Hall², Elizabeth Coward³ & Aaron Thompson⁴

Soil organic matter (SOM) is correlated with reactive iron (Fe) in humid soils, but Fe also promotes SOM decomposition when oxygen (O₂) becomes limited. Here we quantify Fe-mediated OM protection vs. decomposition by adding ¹³C dissolved organic matter (DOM) and ⁵⁷FeII to soil slurries incubated under static or fluctuating O₂. We find Fe uniformly protects OM only under static oxic conditions, and only when Fe and DOM are added together: de novo reactive FeIII phases suppress DOM and SOM mineralization by 35 and 47%, respectively. Conversely, adding ⁵⁷FeII alone increases SOM mineralization by 8% following oxidation to ⁵⁷FeIII. Under O₂ limitation, de novo reactive ⁵⁷FeIII phases are preferentially reduced, increasing anaerobic mineralization of DOM and SOM by 74% and 32–41%, respectively. Periodic O₂ limitation is common in humid soils, so Fe does not intrinsically protect OM; rather reactive Fe phases require their own physiochemical protection to contribute to OM persistence.
The net balance of soil carbon (C) accrual vs. loss is central to future climate predictions. Accumulating research has demonstrated that geochemical factors, such as secondary clay minerals and short-range-ordered (SRO) iron (Fe), and aluminum (Al) phases, in particular, are vital determinants of C accrual. Mineral-associated organic matter (MAOM) is thought to persist because organic matter (OM) can form strong chemical bonds to minerals and can be physically protected in microaggregates or co-precipitates. Once the initial association of OM with minerals has occurred, soil structural conditions (aggregate formation, macro-scale shifts in fluid flowpaths, etc.) can further isolate and compartmentalize OM from decomposer organisms and restrict the diffusion of oxygen (O2), thus further protecting soil organic matter (SOM) against decomposition. These features can lead to longer turnover times for MAOM than for particulate organic matter, and may explain MAOM residence times of centuries–millenia. A large portion of MAOM in soils and sediments is adsorbed or co-precipitated with Fe minerals. However, soil Fe plays multiple roles in ecosystem biogeochemistry aside from C protection, some of which also drive C loss.

Soil Fe serves three categorical roles in ecosystem function: the first is a structural role, where Fe (as FeIII) forms connective cements that bind minerals and SOM together in nano-, micro-, and macro-aggregates; the second is a sorbent role, whereby nutrients and OM adsorb or co-precipitate with FeIII minerals or FeIII surface coatings; and the third is an electron-transfer role, whereby FeIII accepts electrons from microbes or electron shuttles, or FeII donates electrons to various oxidants, such as O2, NO3−, or H2O2. The relative impacts of these Fe functional roles on soil C cycling remain unclear.

The sorbent and structural roles of Fe may increase soil C stocks by decreasing the availability of OM to extracellular enzymes and heterotrophic microbes. A commonly accepted mechanism for MAOM formation is for dissolved organic matter (DOM) of plant or microbial origin to sorb or co-precipitate with existing and de novo minerals. One particularly important route of MAOM formation involves the oxidation of FeII to FeIII at redox interfaces and its rapid hydrolysis to SRO FeIII (oxyhydr)oxides, which co-precipitate with DOM. This can occur wherever FeII-bearing anoxic solutions come in contact with O2, such as in periodically flooded soil horizons or across redox gradients within aggregates in upland soils. High rates of Fe reduction have been observed in surface soils during periods of elevated moisture and high biological activity, leading to a heterogeneous distribution of Fe in the soil profile.

**Fig. 1 Schematic of Fe-mediated C transformation.** Under oxic conditions, FeIII phases sorb C (a). Under anoxic conditions, FeIII can be reduced to FeII coupled with C mineralization, releasing dissolved organic matter (DOM) (b). Fe2+ oxidation yields reactive O2 species driving CO2 and DOM production (c). d Conceptual diagram of contrasting positive (blue arrows) and negative (red arrows) changes in CO2 production linked to a given mechanism.
iron within soil profiles. Iron reduction appears to be a ubiquitous soil biochemical process across a broad range of terrestrial ecosystems. Across these ecosystems, C:Fe molar ratios of Fe–C associations point to the dominance of co-precipitation vs. adsorption. These lines of evidence place the epicenter of Fe-associated OM formation at these dynamic anoxic-oxic interfaces in surface soils.

However, the biogeochemical factors linked to Fe-associated C formation could also contribute to its decomposition. Fe electron transfer reactions can drive C solubilization, depolymerization, and loss as CO₂. During anoxic periods, microbial use of Fe^{II} as an electron acceptor directly produces CO₂ from the metabolic coupling of OM oxidation to Fe reduction, but also releases Fe from OM coprecipitates and OM occluded in Fe^{III}-cemented micro-aggregates. In soils that experience frequent redox fluctuations, microbial Fe reduction can account for up to 44% of anaerobic OC mineralization. Therefore, significant portions of C protected by complexation under oxidic conditions can be released and decomposed following Fe reduction. Conversely, the abiotic oxidation of Fe^{II} by O₂ can also produce CO₂. This is a consequence of reactive oxygen species production (Fenton chemistry), which can directly produce CO₂ or cleave organic polymers to increase OM availability.

Despite evidence for Fe-stimulated decomposition, the common perception of iron’s role in SOM has largely focused on Fe-mediated OM protection via adsorption, co-precipitation, or aggregation. While it is also recognized that Fe-OM associations are formed during Fe redox cycling, and that Fe oxidation and reduction can promote C release and mineralization, these processes are rarely explored concurrently. In fact, few studies have directly measured the microbial availability of Fe-associated OM in soils, and studies highlighting Fe-associated C in anoxic zones do not examine why these Fe^{III} minerals persist despite being thermodynamically poised for reductive dissolution—this is a topic of separate studies explaining Fe^{III} stability based on the thermodynamic constraints that OM composition places on Fe^{III} respiration. Examining these competing functional roles together remains a critical knowledge gap.

In this study, we quantified the relative contributions of Fe in retarding and accelerating C loss in the initial stages of MAOM formation, where physical constraints (macroaggregation, etc.) on decomposition were minimized using soil slurries. We hypothesized that the electron transfer roles of Fe, which accelerate C mineralization, counteract C protection by Fe’s sorbent roles during and shortly following MAOM formation. To test this, we amended soil slurries with ⁵⁷Fe^{II} and/or ¹³C-DOM under anoxic conditions and formed Fe–MAOM by introducing O₂, simulating a primary mechanism of Fe–MAOM formation in humid soils. The soil slurries were incubated under either static or alternating oxic/anoxic treatments under pH-buffered humid soils. The soil slurries were incubated under either static or alternating oxic/anoxic treatments under pH-buffered humid soils. The soil slurries were incubated under either static or alternating oxic/anoxic treatments under pH-buffered humid soils.

Results and discussion

Synopsis. Consistent with a protective role, under static oxic conditions we found that Fe^{II} oxidation in the presence of added ¹³C-DOM resulted in SRO Fe–C associations that not only inhibited the mineralization of ¹³C-DOM by 35% relative to controls, but also suppressed the priming of native SOM mineralization by 47%, which consequently decreased overall CO₂ production by 22% (Fig. 1d). However, when ¹³C-DOM was not added, Fe^{II} oxidation and the production of reactive oxygen species stimulated mineralization of native SOM by 8% relative to the controls (Fig. 1d). Thus, the formation of additional SRO–Fe phases did not provide net protection to SOM unless there was additional DOM present. As might be expected, the protective role of Fe was reversible under anoxic conditions. Although CO₂ production from non-Fe amended treatments during the anoxic period was 68–70% lower than in the static oxic treatment (Fig. 1d), the de novo SRO Fe–MAOM formed via Fe^{II} oxidation was disproportionately vulnerable to subsequent reduction. This consequently stimulated the mineralization of both added ¹³C-DOM and the native SOM by 74% and 32–41%, respectively, and thus increased overall CO₂ production by 41–49% relative to both non-Fe amended treatments (with or without added DOM, Fig. 1d). As a result of Fe-stimulated C mineralization, the anerobic ¹³C-DOM mineralization was 81% greater than the oxic control. Below we provide details on the production of the Fe–MAOM, discuss the data supporting Fe protection of C along with the data supporting Fe stimulation of C loss, and then provide a synthesis of the work.

Generation of Fe^{III}-(oxyhydr)oxides. The oxidation of ⁵⁷Fe^{II} after a 1-d equilibration with the soil under anoxic conditions generated SRO Fe^{III}-(oxyhydr)oxides that impacted C cycling. Exposure to O₂ (day 1–6) led to the oxidation of Fe^{II}, with aqueous Fe^{II} completely oxidized within 6 h. The sorbed Fe^{II} substantially decreased by 91% over the first day and slowly declined thereafter (Fig. 2). The treatment with both ⁵⁷Fe and ¹³C-DOM added had 10% more adsorbed ⁵⁷Fe than the ⁵⁷Fe-only treatment before oxidation (Fig. 2a and b), likely due to co-sorption of the Fe^{2+}–DOM complex, as observed previously. The variable-temperature Mössbauer spectroscopy technique that we use to track the mineral composition of the ⁵⁷Fe additions, gives excellent information on the crystallinity of the Fe phases, with high crystallinity phases order at higher temperatures. Both ⁵⁷Fe addition treatments led to the formation of de novo SRO ⁵⁷Fe^{III} phases of lower crystallinity (lower Mössbauer ordering temperature) than the bulk soil Fe (Table 1; Supplementary Fig. 2 and Fig. 3), resulting in a 26–31 mmol kg⁻¹ increase in lepidocrocite and 3–14 mmol kg⁻¹ increase in nano-goethite and very-disordered Fe^{III}-(oxyhydr)oxides that preclude assignment (Fig. 3; Supplementary Table 1). The addition of ⁵⁷Fe and ¹³C-DOM together resulted in the formation of even lower crystallinity SRO ⁵⁷Fe^{III}-(oxyhydr)oxides than the ⁵⁷Fe-only treatment as illustrated by the lower 35K/5K and 12K/5K assignment (Fig. 3; Supplementary Table 1). The oxidation of ⁵⁷Fe^{II} with high crystallinity phases order at higher temperatures. Both ⁵⁷Fe addition treatments led to the formation of de novo SRO ⁵⁷Fe^{III} phases of lower crystallinity (lower Mössbauer ordering temperature) than the bulk soil Fe (Table 1; Supplementary Fig. 2 and Fig. 3), resulting in a 26–31 mmol kg⁻¹ increase in lepidocrocite and 3–14 mmol kg⁻¹ increase in nano-goethite and very-disordered Fe^{III}-(oxyhydr)oxides that preclude assignment (Fig. 3; Supplementary Table 1).

Iron protection of organic matter. In the static oxic treatment, addition of ⁵⁷Fe suppressed the mineralization of ¹³C-DOM by...
35% ($p < 0.01$, Figs. 1d, 4 and Table 2): cumulative CO$_2$ production was $25.6 \pm 0.8$ and $39.5 \pm 1.1$ mmol C kg$^{-1}$ with and without $^{57}$Fe, respectively, equivalent to 17.1% and 26.3% of the added $^{13}$C-DOM (Fig. 4c and Table 2). Although $^{57}$Fe addition also inhibited net $^{13}$C-DOM mineralization in the fluctuating redox treatments ($p < 0.01$, Fig. 4c and Table 2), this inhibition was confined to the oxic portions (days 1–6 and days 17–22) of the incubation and was partly offset by a 74% enhanced $^{13}$C-DOM mineralization during the anoxic phase (days 6–17) relative to the treatment without added Fe (Figs. 1d, 4b, and Table 2, see below).

The generation of low crystallinity SRO–Fe$^{III}$ (oxyhydr)oxides from the oxidation of $^{57}$Fe$^{II}$ in the presence of $^{13}$C-DOM resulted in a lower DOC concentration than in the $^{13}$C-DOM-only treatment and a concurrent increase in solid-phase $^{13}$C content (Fig. 5). This likely reflects the formation of SRO Fe–C complexes with $^{13}$C-DOM adsorbing or co-precipitating with the newly-formed SRO lepidocrocite and nanogoethite phases (Fig. 3). It is generally assumed that SRO Fe phases contribute to soil C persistence by protecting it against microbial mineralization$^5$, but few studies have directly measured the bioavailability of Fe-associated OM$^{39}$. Our study provides evidence that de novo formation of SRO Fe–C complexes inhibit the mineralization of fresh DOM inputs to soil. Others have also observed a large decrease in OM decomposition when glucose or fulvic acid sorbed to synthetic Fe minerals (ferrihydrite/goethite) was added to soils, as compared to additions of the free organic compounds$^{40,41}$. The bioavailability of mineral-associated OM is generally thought to be linked to C loadings (e.g., C/Fe ratios), with a maximum adsorption capacity occurring at a C/Fe molar

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**Fig. 2** Fe$^{II}$ concentration and $^{57}$Fe to total Fe ratio. Time-dependent **a** aqueous Fe$^{II}$, **b** sorbed (HCl-extractable) Fe$^{II}$, and **c** dissolved $^{57}$Fe to total Fe ratio in the anoxic phase in the redox-fluctuating treatment. Fe$^{II}$ and $^{57}$Fe were undetectable during day 1.5–6 and 17.5–22. The gray shaded region represents the anoxic phase of the redox-fluctuating treatment. Error bars indicate s.e.m. ($n = 3$).
ratio of about one\(^{46}\). Co-precipitation could result in Fe–OM associations with much higher C/Fe ratios\(^{11,12,19}\). In our study, the initial C/Fe molar ratio of the added\(^{13}\)C-DOM and \(^{57}\)Fe was 2.1. If we assume that all DOM that was removed from the solution during the Fe\(^{II}\) oxidation event sorbed to the newly-formed Fe\(^{III}\) (oxyhydr)oxides, the C/Fe ratio of those OM–Fe\(^{III}\) (oxyhydr)oxide complexes would be \(\sim 1.7\). Thus, there was likely \(^{13}\)C-DOM with a low affinity for Fe\(^{III}\) (oxyhydr)oxides that remained as unprotected \(^{13}\)C-DOM in the aqueous phase and this likely led to our observation of significant \(^{13}\)C-DOM mineralization even in the presence of de novo Fe\(^{III}\) (oxyhydr)oxides (Fig. 4).

Labile C inputs are often observed to alter the decomposition of extant SOM, defined as priming\(^{47,48}\). During the oxic periods of the experiment, \(^{57}\)Fe\(^{II}\) oxidation in the presence of added \(^{13}\)C-DOM not only suppressed the mineralization of the amended \(^{13}\)C-DOM, but also partially inhibited the priming of native SOM decomposition compared to the DOM-only treatment (Fig. 6; Table 2; Supplementary Table 2). In the static oxic treatment, addition of \(^{13}\)C-DOM alone or together with \(^{57}\)Fe increased native SOM-derived CO\(_2\) production compared to the soil-only control (priming effect) \((p < 0.01, \text{Fig. 6a, c and e; Table 2; Supplementary Table 2})\). However, adding \(^{13}\)C-DOM and \(^{57}\)Fe together resulted in a significantly smaller priming effect on native SOM mineralization than adding \(^{13}\)C-DOM alone under the static oxic treatment \((p < 0.01, \text{Fig. 6e, Table 2 and Supplementary Table 2})\). With the addition of \(^{13}\)C-DOM, cumulative primed CO\(_2\) from native SOM under the static oxic treatment measured \(10.2 \pm 1.2\) and \(19.1 \pm 0.9\ \text{mmol C kg}^{-1}\) with and without \(^{57}\)Fe addition, respectively (Fig. 6e and Supplementary Table 2). Cumulatively, adding

| Treatments | Sample time | Magneticily ordered Fe\(^{III}\)-(oxyhydr)oxides (%) | Crystallinity index |
|------------|-------------|-----------------------------------------------|-------------------|
| Initial soil | 77 K | 57.7 (2.2) | 0.75 |
| Added \(^{57}\)Fe | 35 K | 66.8 (2.1) | 0.87 |
| \(^{57}\)Fe\(^{II}\)-only addition | 12 K | 70.6 (2.4) | 0.92 |
| | 5 K | 76.4 (3.2) | |
| 57Fe\(^{II}\) and \(^{13}\)C-DOM addition | 77 K/5 K | 0.20 | |
| | 35 K/5 K | 0.29 | |
| | 12 K/5 K | 0.71 | |

| | 77 K | 35 K | 12 K | 5 K |
|---|---|---|---|---|
| Initial soil | 8.1 (0.9) | 11.4 (1.0) | 29.2 (1.4) | 41.2 (1.0) |
| Added \(^{57}\)Fe | 18.5 (2.4) | 62.4 (4.1) | 84.3 (2.8) | |
| \(^{57}\)Fe\(^{II}\)-only addition | 3.5 (0.7) | 15.3 (0.9) | 57.9 (3.1) | 79.1 (1.0) |
| | 14.7 (1.7) | 20.8 (1.3) | 65.7 (1.9) | 85.6 (2.0) |
| 57Fe\(^{II}\) and \(^{13}\)C-DOM addition | 6.2 (0.8) | 8.7 (0.7) | 16.0 (1.1) | 34.7 (1.9) |
| | 13.1 (0.8) | 13.7 (2.8) | 74.0 (3.4) | 0.71 |
| | 5.2 (0.7) | 7.9 (0.6) | 21.0 (1.0) | 42.0 (3.1) |
| | 11.7 (0.9) | 15.7 (1.0) | 48.3 (3.4) | 77.8 (2.4) |

Numbers in parenthesis represent standard errors associated with Mössbauer data modeling.

Fig. 3 Solid-phase speciation of added \(^{57}\)Fe. \(^{57}\)Fe partition was calculated from respective Mössbauer spectra (corrected to exclude the signal from the native soil Fe) for the (a) \(^{57}\)Fe-only and (b) \(^{13}\)C-DOM-\(^{57}\)Fe addition treatments, prior to the oxic phase (day 1) and at the end of the 1st oxic (day 6), anoxic (day 17) and 2nd oxic (day 22) phases. Error bars represent standard errors associated with Mössbauer data modeling (see Supplementary Information).
Iron stimulation of DOM and SOM mineralization. Only in the treatment where $^{13}$C-DOM and $^{57}$Fe were added together were we able to confirm that Fe had an overall protective effect on OM, and that protection was limited to the oxic portions of the experiment. Below, we quantified the impact of Fe on OM mineralization via Fe-stimulated Fenton chemistry during the first few days of oxic exposure and via Fe reduction-mediated reactions during the anoxic periods.

Adding $^{57}$Fe alone strongly stimulated CO$_2$ production from native SOM during the first 3 days of the static oxic treatment (and the oxic portions of the fluctuating redox treatment) relative to the soil-only control ($p < 0.01$), with no stimulatory impact afterwards (Figs. 6a and e). Cumulatively, Fe$^{II}$ oxidation stimulated CO$_2$ production by 8% (Fig. 1d and Table 2). To confirm the role of Fenton chemistry, we performed a parallel experiment with added terephthalate—an effective hydroxyl radical scavenger—and found similar CO$_2$ production between Fe$^{II}$-added treatments and soil-only controls (Supplementary Fig. 4). Recent studies have similarly shown that Fe$^{II}$ oxidation is linked to increases in soil CO$_2$ production via the generation of radical oxygen species, which facilitate the breakdown of complex biopolymers to produce labile substrates for microbial respiratory processes. Others have also attributed increased CO$_2$ production following Fe$^{II}$ oxidation to an increase in acidity that can promote DOC release. Given that we conducted these experiments in a strong ionic buffer at a constant pH, the increased CO$_2$ production following Fe$^{II}$ oxidation was most likely derived from the production of reactive oxygen species such as the hydroxyl radical.

Soil C mineralization rates typically decrease as O$_2$ becomes limiting. In our soil-only control, CO$_2$ production from native SOM during the anoxic period was 70% lower than in the static oxic treatment (Figs. 1d, 6b and f; Table 2). However, during the anoxic portions of the experiment, Fe addition stimulated native SOM mineralization relative to the no-Fe treatment (Fig. 6b and f; Table 2). In the $^{57}$Fe-addition treatment, the degree of anoxic suppression of CO$_2$ production decreased from 70 to 58% of that under oxic conditions ($p < 0.05$; Table 2), as a result of a 41% higher anoxic native SOM-derived CO$_2$ production in the Fe addition treatment than in the no-addition control ($p < 0.05$, Table 2; Figs. 1d, 6b, d and f). This likely resulted from enhanced microbial use of Fe$^{II}$ as an electron acceptor in the $^{57}$Fe addition treatments. Following the transition from oxic to anoxic conditions in the fluctuating redox treatments, substantial Fe$^{III}$ reduction occurred (day 6–17, Fig. 2a and b) and adding $^{57}$Fe increased the total Fe$^{II}$ production rates (2.9 mmol kg$^{-1}$ d$^{-1}$) compared to the soil-only control (1.6 mmol kg$^{-1}$ d$^{-1}$). This was most likely due to the facile reduction of de novo $^{57}$Fe SRO lepidocrocite and nanogoethite, which had a much lower crystallinity (and thus a higher reactivity) than native soil Fe$^{III}$ (oxyhydr)oxides. In our soil-only control, CO$_2$ production from native SOM during the anoxic period was 70% lower than in the static oxic treatment (Figs. 1d, 6b and f; Table 2). However, during the anoxic portions of the experiment, Fe addition stimulated native SOM mineralization relative to the no-Fe treatment (Fig. 6b and f; Table 2). 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This was most likely due to the facile reduction of de novo $^{57}$Fe SRO lepidocrocite and nanogoethite, which had a much lower crystallinity (and thus a higher reactivity) than native soil Fe$^{III}$ (oxyhydr)oxides.
The availability of native SRO FeIII phases likely limits Fe reduction in this subtropical agricultural soil, and the de novo SRO FeIII phases were preferentially utilized as electron acceptors for microbial respiration as evidenced by the preferential release of 57FeII in the aqueous phase (Fig. 2c) and the measured decrease of these 57Fe mineral phases following reduction (Fig. 3a and Table 1; Supplementary Table 1).

Iron’s stimulation of C mineralization during anoxic periods was greatly enhanced when 57Fe and 13C-DOM were added together, yielding increases in mineralization of native SOM (anoxic priming) and 13C-DOM by 32 ± 3% and 74 ± 7%, respectively, relative to adding 13C-DOM alone (p < 0.05; Table 2; Figs. 1d, 4b, 4c, 6b, d and f). In fact, when 13C-DOM and 57Fe were added together, anaerobic 13C-DOM mineralization in the fluctuating redox treatment was greatly enhanced, with increases in mineralization of native SOM (anoxic priming) and 13C-DOM by 58 ± 4% and 84 ± 6%, respectively, relative to adding 13C-DOM alone (p < 0.05; Table 2; Figs. 1d, 4b, 4c, 6b, d and f).

### Table 2 Cumulative CO2 production in the fluctuating redox and static oxic treatments for all soils.

| Substrate treatment | Time | Redox treatment | SOM-derived CO2 (mmol C kg⁻¹) | 13C DOM-derived CO2 (mmol C kg⁻¹) | Total CO2 (mmol C kg⁻¹) |
|---------------------|------|-----------------|-----------------------------|---------------------------------|------------------------|
| Soil-only           | 1-6  | 1st-oxic/fluctuating | 11.4 (0.4) | 11.4 (0.4) | 22.8 (0.8) |
|                     | 6-17 | Anoxic/oxic      | 6.6 (0.5)  | 6.6 (0.5)  | 13.2 (1.0) |
|                     | 17-22| 2nd-oxic/fluctuating | 9.8 (0.8) | 9.8 (0.8)  | 19.4 (1.6) |
|                     | Sum  | Fluctuating      | 27.8 (0.6) | 27.8 (0.6) | 55.6 (1.2) |
| FeII-added soils    | 1-6  | 1st-oxic/fluctuating | 14.1 (0.9) | 14.1 (0.9) | 28.2 (1.8) |
|                     | 6-17 | Anoxic/oxic      | 9.3 (0.8)  | 9.3 (0.8)  | 18.6 (1.6) |
|                     | 17-22| 2nd-oxic/fluctuating | 22.2 (1.2) | 22.2 (1.2) | 44.4 (2.4) |
|                     | Sum  | Fluctuating      | 43.1 (0.9) | 43.1 (0.9) | 86.2 (1.8) |
| DOM-added soils     | 1-6  | 1st-oxic/fluctuating | 20.1 (0.9) | 31.2 (1.2) | 51.3 (1.5) |
|                     | 6-17 | Anoxic/oxic      | 6.7 (0.5)  | 4.6 (0.4)  | 11.3 (0.9) |
|                     | 17-22| 2nd-oxic/fluctuating | 9.5 (0.3) | 1.4 (0.1)  | 10.9 (0.4) |
|                     | Sum  | Fluctuating      | 36.3 (0.6) | 37.2 (1.2) | 73.5 (1.7) |
| DOM- and FeII-added soils | 1-6 | 1st-oxic/fluctuating | 15.7 (0.7) | 20.2 (0.9) | 35.9 (1.5) |
|                     | 6-17 | Anoxic/oxic      | 8.9 (0.5)  | 8.0 (0.3)  | 16.9 (0.9) |
|                     | 17-22| 2nd-oxic/fluctuating | 8.2 (0.7) | 1.4 (0.1)  | 9.6 (0.9)  |
|                     | Sum  | Fluctuating      | 32.8 (0.8) | 29.6 (0.9) | 62.4 (1.7) |

Numbers in parenthesis represent standard errors (n = 3 per treatment).

**Fig. 5** Dissolved organic carbon concentration and solid-phase 13C enrichment. **a** Dissolved organic carbon concentration (MES buffer concentration was subtracted) and **b** solid-phase 13C atom fraction from 13C-DOM addition treatments. The gray shaded region represents the anoxic phase of the redox-fluctuating treatment. Error bars represent s.e.m. (n = 3).
even 81% greater than the aerobic $^{13}$C-DOM mineralization in the static oxic treatment at the same point in time ($p < 0.01$; Fig. 4b; Table 2). The stimulation of $^{13}$C-DOM mineralization under anoxic conditions was linked in part to its molecular composition, given that thermodynamic constraints on Fe reduction limit metabolism to relatively oxidized C substrates$^{22,42,43}$. During the anoxic periods, the mineralization of $^{13}$C-DOM over the native SOM (in both the $^{13}$C-DOM only and DOM-Fe addition treatments) was 2–3 times higher than that in the static oxic treatment at the same point (Fig. 4a). Characterization of the molecular composition of $^{13}$C-DOM and water-extractable native SOM using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) revealed that the $^{13}$C-DOM had significantly less lignin-derived materials and much more aliphatic formulae than the water-extractable native SOM (Supplementary Table 3, Figs. 5 and 6), which represents the most bioavailable fraction of native SOM$^{54}$. The preferential anaerobic mineralization of $^{13}$C-DOM over SOM may be due to a lower abundance of lignin-derived compounds, which are not readily depolymerized under anoxic conditions$^{55}$. In addition, compared to water-extractable native SOM, $^{13}$C-DOM contains compounds with higher nominal oxidation state of C (NOSC values $> 0.5$, Supplementary Fig. 6), which are associated with a higher likelihood of thermodynamic favorability ($-\Delta G_c$) when coupled to Fe$^{44}$ reduction than the bioavailable fraction of

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**Fig. 6 Native soil C mineralization.** CO$_2$ production rates from soil C under a static-oxic and b redox-fluctuating conditions, cumulative CO$_2$ production from soil C under c static-oxic and d redox-fluctuating conditions, and priming of soil C under e static-oxic and f redox-fluctuating conditions. The gray shaded region represents the anoxic phase of the redox-fluctuating treatment. Error bars indicate s.e.m. ($n = 3$).
Fe reduction was also stimulated by the addition of 13C-DOM alone (producing 2.3 mmol kg⁻¹ d⁻¹ of FeII compared to the soil-only control rate of 1.6 mmol kg⁻¹ d⁻¹) (Fig. 2a and b), consistent with prior work.26 However, when Fe and DOM were added together, Fe reduction was greatly increased to 7.4 mmol kg⁻¹ d⁻¹, which was even greater than the additive effect of separate 13C-DOM (2.3 mmol kg⁻¹ d⁻¹) and 57Fe reductions (2.9 mmol kg⁻¹ d⁻¹) (Fig. 2a and b). This was because compared to oxidation of 57FeII alone, oxidizing 57FeIII in the presence of 13C-DOM led to formation of even less-crystalline SRO lepidocrocite and nanogoethite phases (all ordering at <35 K in the Mössbauer spectra, Table 1 and Supplementary Fig. 3). These SRO 57Fe-13C-OM phases exhibited high rates of Fe reduction, releasing significant 57FeII(aq) and 13C-OM (Figs. 2 and 5) when exposed to anoxic conditions, leaving the solid phase depleted in its lowest crystallinity Fe phases (Fig. 3 and Table 1; Supplementary Table 1 and Fig. 7), and preferentially stimulating anaerobic mineralization of the added 13C-DOM (Fig. 4a).

Fe reduction can solubilize significant amounts of OM adsorbed or coprecipitated with FeIII (oxyhydr)oxides directly, as shown in our experiment, or indirectly because of an increase in pH30,32,56. This re-mobilized 13C-DOM often includes biochemically labile C32,57, and may potentially offset the kinetic/thermodynamic constraints often limiting anaerobic decomposition.

We find that collectively, the reduction of SRO FeIII phases offset O2 limitations on C mineralization by 24 ± 3% relative to the non-Fe amended treatment (Table 2).

**Synthesis.** A recent survey of over 5500 soil profiles spanning continental scale environmental gradients found that SRO Fe and Al (oxyhydr)oxide abundance was the best predictor of C content in humid soils, among the geochemical and climate variables that were available.45 This is consistent with other work showing that SRO FeIII phases are broadly implicated in the persistence of OM in soil.13,59 However, the nature of the relationship between Fe and C in humid soils—and redox dynamic soils in general, which would include floodplain and perennal wetland soils from all climatic regions—is far from straightforward. Humid soils are replete with microsites that undergo dynamic anoxia in response to high labile C loads during periods of high moisture and experience appreciable FeIII reduction rates.23,25,60,61. Oxidation of the FeII generated from FeIII reduction is a common mechanism for MAOM formation in humid and redox-dynamic soils, yet Fe is also responsible for OM loss and our work here illustrates two principal refinements in this regard.

First, the production of SRO Fe–MAOM via FeII oxidation will likely increase CO2 production in the short-term. Only when we formed MAOM in the presence of DOM and maintained strict oxic conditions was there a net decrease in C mineralization (both in the added 13C-DOM and the native SOM, i.e. via decreased priming). When we simply generated MAOM via FeII oxidation without added DOM, Fenton chemistry caused an 8% increase in C mineralization (Fig. 1d). Upon the inevitable return to periodic anoxia in humid soils, our work suggests that C mineralization would be accelerated by 41–49% by Fe reduction (Fig. 1d), thus counteracting the stabilization effect on OM of SRO Fe phases. The magnitude of these counteracting mechanisms may also be influenced by soil structure, which we largely eliminated in our study by conducting experiments in soil slurries. Hence, direct application of our results to in situ soil environments is tentative. However, the general principles of our work are also likely to be applicable to structurally complex soil systems. For example, Fe mineral-associated C is often released in natural soils under in-situ flow conditions as a consequence of dissimilatory Fe reduction (e.g.,62) and thus becomes more vulnerable to microbial decomposition. In our study, we even found that the added DOM was preferentially degraded under anoxic conditions relative to the oxic control (Fig. 4), which highlights how the thermodynamic constraints of anaerobic metabolism and the molecular composition of C sources can influence the fate of fresh DOM inputs.22,24,43. Consequently, the net effect of Fe–C interactions in dynamic redox environments likely hinges in part on the composition of DOM inputs, a worthy topic for further research.

Second, our work here suggests that the initial SRO Fe–C associations are not likely to persist without protection from periodic Fe reduction events. Several researchers have identified or produced SRO–FeIII–OM colloids that are resistant to either microbial or chemical reduction63–67, however, the key components conferring this protection are variable and/or elusive. Some work has identified that SRO–FeIII–OM co-precipitates with low C/Fe ratios provide resistance to microbial reduction63,64, whereas other work has emphasized structural properties (conformation and micro-aggregation) as the mechanism that retards dissolution65–67. SRO Fe–OM phases are often co-precipitated with Al and Si ions68—which can lead to recrystallization69—and given the co-association of Al and Fe with OM in humid soils, Al is a strong candidate for protecting Fe against reduction. However, studies that have examined Al and Si co-precipitated Fe–(oxyhydr)oxides found those ions also make the co-precipitates more susceptible to reductive dissolution70. Coward et al. recently proposed several mechanisms by which SRO FeIII–OM phases could become resistant to reductive dissolution, including acquiring reduction-resistant surface coatings, or becoming embedded in a composite aggregate structure.6 Such a protective coating could even come from higher crystallinity Fe (oxyhydr)oxides. Hall et al. recently found that 14C-derived C residence time in humid soils was positively correlated with Fe phase crystallinity.71 Consistent with that, we find here that in contrast to the initial oxidation event, the 2nd oxidation event generated more crystalline 57Fe phases (Table 1; Supplementary Fig. 8) and did not stimulate additional C mineralization (Fig. 6 and Table 2). It may be that during repeated redox fluctuations a substantial portion of the co-precipitated OM would be lost, but a core Fe–MAOM structure would remain protected from reductive dissolution.

Perhaps most compelling is the growing evidence that various aggregation, conformation, and structural characteristics of soils confer protection for OM5–7,10. Even the protective surface coatings66,67 or conformational changes in OM at low C/Fe ratios64 discussed above are examples of micro-aggregate structures not unlike the encasement of SRO Fe–OM phases by aluminosilicate clays or other processes that generate micro-aggregates of minerals and OM during pedogenesis.5,7,10,72. These aggregation processes can structure microaggregates with core SRO Fe phases and outer aluminosilicate or other phases that are not susceptible to reductive dissolution—as observed in Andisols by dithionite-resistant SRO–Fe phases.66. Our soil slurry approach was designed to minimize the physical constraints (macro-pore flow, spatial arrangement of microbes, minerals and OM, and the development of aggregates) on C decomposition and thereby isolate the sorbent and electron-transfer roles of Fe in C dynamics (Supplementary Fig. 1). Under these conditions, we find that Fe does not confer intrinsic protection for OM in redox-dynamic soils. In an in situ soil environment—where MAOM emerges in a dynamic three-dimensional space—structural and physical protection of MAOM is thus likely a key protective mechanism for reconciling the comparatively large proportions of SRO-OM associations in soil of very old age based on 14C-dating1,4,5,59. Future studies should thus assess the extent that the formation and destruction of Fe-cemented microaggregates contribute to OM persistence in redox-dynamic soils. Our work demonstrates that the inherent persistence of SRO Fe-associated C cannot be guaranteed. Biological and geochemical context is critical.

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for understanding the long-term fate of FeII-associated SOM under a changing climate, given the dual roles of FeIII phases in both accelerating and inhibiting OM decomposition.

Methods

Approach. We employed a dual isotope approach in a soil slurry to test our hypothesis that the electron transfer roles of Fe that accelerate C mineralization counterpoint Fe’s sorbent and electron-transfer roles. Our dual isotope approach allowed us to distinguish between native SOM and fresh plant-derived DOM via 13C labeling, as well as between neo-formed reactive Fe minerals formed in situ and the different forms of Fe minerals in the native soil via 57Fe labeling coupled with 57Fe Mössbauer spectroscopy.

Preparation of 13C-labeled plant-derived DOM. 13C-DOM was extracted from 13C-labeled bermudagrass. DOM is inherently heterogeneous, diverse and dynamic in composition2, and here we used bermudagrass-extracted DOM to encompass a mixture of molecular organic representatives of those that derive from early stage herbaceous litter decomposition. A pulse-labeling method was used to label Tifton-85 bermudagrass (Cynodon dactylon x Cynodon nlemfuensis) with 13CO2 (99.999 atom%), Cambridge Isotope Laboratories Inc; see Supplementary Methods for additional information). After labeling, aboveground biomass was harvested, immediately frozen, freeze-dried, and then ground using a Wiley mill to <1 mm. DOM extractions were conducted in a shaker at 140 rpm for two days with a solid-to-water ratio of 1:5, followed by centrifugation. The supernatant was filtered through a 0.2 µm membrane filter. The derived DOM solution had 10.3% 13C. Characterization of the molecular composition using ultrahigh resolution mass spectrometry (FTICRMS) revealed that this 13C-DOM was comprised of predominantly aliphatic compounds (76%) and lignin-derived/carboxyl-rich alicyclic molecules (23%), with mean population O/C, H/C and DBE values of 0.44 ± 0.12, 1.60 ± 0.22, and 6.31 ± 3.04, respectively (Supplementary Fig. 6 and Table 3). Compared to water-extractable natural SOM, the bermudagrass-derived DOM had significantly more aliphatic compounds with less lignin-derived material (Supplementary Notes). In addition, the 13C-containing population of DOM formulates displayed chemical composition distribution indistinguishable from that of 13C-only formulae, suggesting no preferential incorporation of 13C atoms across molecular compounds (Supplementary Fig. 5 and Table 3).

Study site and soil sampling. Our study site is located in the Calhoun Critical Zone Observatory (CZO) in Union County, South Carolina, USA (34.611 N; 81.727, IGSN: IEJCA0013). This site has a humid warm temperate climate, with mean annual precipitation and mean annual temperature of about 1212 mm and 17 °C, respectively (Southeast Regional Climate Center, 2016). The soil used is derived from granitic gneiss. We collected soils from cultivated land on an intercropped corn (Zea mays, IGSN: IEGI0007) and field pea (Vicia sativa, IGSN: IGSB0029) field located at the site. Surface soils (initial depth of 0–20 cm) were dug by backhoe. Surface soils (0–20 cm) were collected and transported overnight to the University of Georgia under ambient conditions. Soils were homogenized and visible plant debris, rocks, and soil macrofauna were removed manually.

Total OC content and its 13C content were measured via an elemental analyzer-stable isotope ratio mass spectrometer (EA-IRMS) EA-IRMS) were 2.1% and −22.9‰, respectively. Water-extractable OM was extracted by mixing field soils with high purity water (see details in Supplementary Methods), which was 33.8 mg Cg−1. Water-extractable native SOM, extracted by mixing field soils with high purity water (see details in Supplementary Methods), which was 33.8 mg Cg−1. Water-extractable native SOM was comprised of largely polymeric aromatic (21.5%), lignin-derived/carboxyl-rich alicyclic compounds (49.1%) and aliphatic compounds (22.5%). The water-extractable native SOM and field soils had CH and CNO values of 0.21 ± 0.09, 4.01 ± 2.40, 0.01 ± 0.2, and 0.20 ± 0.15 and 0.13 ± 0.7 ± 3.71, respectively (Supplementary Fig. 6 and Table 3). Total soil Fe content, measured by ICP-MS following li-metaborate fusion (Acme Labs, Kelowna, BC Canada), was 308 mmol kg−1. The 13C atom fraction of CO2 from soil呼吸, and acid-extractable iron measured by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, Elan 9000). Soil slurries were sampled at the end of anoxic incubations for C and isotope analysis and centrifuged at 14,000 rcf for 10 mins. The supernatant was carefully removed and filtered through a 0.2 µm membrane filter for DOC analysis. DOC was measured with a TOC analyzer. The pico-TOC was washed with distilled water three times, freeze-dried and analyzed for C and 13C analysis by EA-IRMS.

Gas sampling and measurements. Each reactor was flushed with CO2-free air or N2 gas for 15 min at 500 mL min−1 every 4 h to 1 d during the oxic phases and every 2–3 days during the anoxic period immediately following each headspace gas measurement. We collected gas samples for measurements of CO2 and their 13C values immediately prior to flushing, enabling us to quantify cumulative CO2 losses and their 13C values over the entire experiment. A 0.5 mL gas sample was collected with gastight syringes and injected to pre-evacuated 3 mL glass vials (Exetainer, Labcon Inc., UK) for CO2 concentration analysis. A 30 mL gas sample was collected from each reactor and stored in helium-purged and evacuated 20 mL glass serum bottles with teflon septa sealed with aluminum crimps for 13C measurements. Concentrations of CO2 were measured with gas chromatography and thermal conductivity detector (Shimadzu, Kyoto, Japan). Dissolved CO2 in the slurry was calculated based on Henry’s law. The 13C/12C isotopic ratio of CO2 was determined by injecting 20 mL gas using a gas-tight syringe to Picarro G2120-i via an ultra-zero grade CO2-free air carrier gas. 13C of CO2 from the 57Fe-added soils and soil-only controls was corrected using three CO2 tank standards with 13C values of −10.0‰, −23.8‰, and −39.7‰, respectively. The 13C atom fraction of CO2 from 13C-DOM-added soils was calibrated using 5 standards varying from 2 to 18% (13C). These standards were created by mixing 99% 13C Na2CO3 with natural abundance Na2CO3 (13C = 1.42‰), digesting with an excess of 12 M HCl and removing aliquots of headspace. Concentrations of CH4 were analyzed by gas chromatography with a flame ionization detector (Shimadzu, Kyoto, Japan). However, CH4 production in this experiment was minimal, accounting for <1% of total C mineralization. Therefore the effect of CH4 production on 13C mass balance was negligible.

The percent contribution of added 13C-DOM to CO2 respiration (PDOM) was calculated using a two-source mixing model:

\[ P_{\text{DOM}} = \left( \frac{1}{k_{\text{DOM}} - 1} \right) \times \frac{\delta_{\text{DOM}} - \delta_{\text{water}}}{\delta_{\text{water}} - \delta_{\text{DOM}}} \times \frac{(1 - k_{\text{DOM}})}{k_{\text{DOM}}} \times 100 \]

where \( k_{\text{DOM}} \) is the fraction of total C mineralization. Therefore the effect of CH4 production on 13C mass balance was negligible.
where $^{13}CO_2_{ls}$ and $^{13}CO_2_{ls}$ are atom fraction $^{13}C$ of CO$_2$ respired in the $^{13}$C-DOM amended soils and the treatments with no C addition, respectively; $^{13}CO_2$ is the initial atom fraction $^{13}C$ of $^{13}$C-DOM and $^{13}CO_2$ is the initial soil $^{13}C$. The fraction of CO$_2$ derived from SOM was calculated by difference:

$$P_{\text{soil}} = 100 - P_{\text{DOM}}$$

Fluxes of CO$_2$ derived from the added DOM and native SOM were calculated by multiplying total CO$_2$ fluxes by their fractional contributions. We calculated priming as the difference in soil-derived CO$_2$ losses between treatments that received $^{13}$C-DOM and/or $^{57}$Fe additions and soil-only control treatment:

$$C_{\text{primed}} = C_{\text{sO amended}} - C_{\text{sO control}}$$

$^{57}$Fe Mössbauer analysis. Fe speciation was determined using $^{57}$Fe Mössbauer analysis. Use of $^{57}$Fe isotopes allows us to track the amended $^{57}$Fe using Mössbauer spectroscopy, which detects only $^{57}$Fe atoms and no other Fe isotopes. The Mössbauer spectra of the amended $^{57}$Fe was calculated as the difference between the spectra from the $^{57}$Fe-enriched treatment and the baseline spectrum from soils with natural isotopic abundance, after taking into account the different total $^{57}$Fe concentrations in the $^{57}$Fe-enriched treatment and the control soils.

The results of the Mössbauer analysis were calculated using the anoxic glove box following centrifugation at 14,000 rpm for 10 min, preserved between layers of O$_2$-impermeable Kapton tape, and immediately frozen in a $-20^\circ$C freezer

Data availability. The data that support the findings of this study are available in a compressed Source Data file accompanying the paper. Other data are included in the Supplementary Materials. Pre-processed data is available upon request.

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Author contributions
C.C. and A.T. conceived of this study, C.C. performed research and analyzed data. E.C. carried out FTICR-MS analysis and data interpretation. C.C., A.T., and S.J.H. wrote the paper with the input of E.C.

Competing interests
The authors declare no competing interests.

Additional information
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