

Short communication

## Iron addition to soil specifically stabilized lignin



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### ABSTRACT

The importance of lignin as a recalcitrant constituent of soil organic matter (SOM) remains contested. Associations with iron (Fe) oxides have been proposed to specifically protect lignin from decomposition, but impacts of Fe-lignin interactions on mineralization rates remain unclear. Oxygen (O<sub>2</sub>) fluctuations characteristic of humid tropical soils drive reductive Fe dissolution and precipitation, facilitating multiple types of Fe-lignin interactions that could variably decompose or protect lignin. We tested impacts of Fe addition on <sup>13</sup>C methoxyl-labeled lignin mineralization in soils that were exposed to static or fluctuating O<sub>2</sub>. Iron addition suppressed lignin mineralization to 21% of controls, regardless of O<sub>2</sub> availability. However, Fe addition had no effect on soil CO<sub>2</sub> production, implying that Fe oxides specifically protected lignin methoxyls but not bulk SOM. Iron oxide-lignin interactions represent a specific mechanism for lignin stabilization, linking SOM biochemical composition to turnover via geochemistry.

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The new paradigm of soil organic matter (SOM) dynamics posits that biochemical differences among compounds that influence short-term decomposition do not impact long-term persistence in SOM (Grandy and Neff, 2008; Marschner et al., 2008; Schmidt et al., 2011). These findings challenged the doctrine of humus formation, namely that recalcitrant biomolecules derived from lignin contribute disproportionately to SOM (Bollag et al., 1997). In support of the new paradigm, several studies reported that lignin might decompose as fast or faster than bulk SOM (Dignac et al., 2005; Gleixner et al., 1999; Guggenberger et al., 1994; Heim and Schmidt, 2007; Kiem and Kögel-Knabner, 2003). Yet, these findings are difficult to reconcile with other studies demonstrating preferential association of aromatic lignin constituents with minerals, especially Fe oxides (Chorover and Amistadi, 2001; Gu et al., 1995; Hernes et al., 2013; Huang et al., 1977; Kaiser, 2003; Riedel et al., 2013). These associations would presumably decrease lignin mineralization, as has been observed for coprecipitates of lignin and ferrihydrite (Eusterhues et al., 2014). Thus, a fundamental question remains: can associations with minerals, particularly Fe oxides, decrease the decomposition of lignin relative to bulk SOM?

Iron-lignin interactions in soils are also complicated by potential stimulation of decomposition by solid-phase and soluble Fe. Fenton reactions driven by coupled biotic/abiotic Fe redox cycling can decompose lignin (Yelle et al., 2011), and may be important in Fe-rich soils that experience fluctuating O<sub>2</sub> (Hall et al., 2015a; Hall and Silver, 2013). Also, Fe minerals such as goethite can abiotically oxidize SOM (Chorover and Amistadi, 2001). Finally, Fe reduction dominates anaerobic metabolism in many Fe-rich soils (Dubinsky et al., 2010), solubilizing Fe and exposing adsorbed or coprecipitated organic matter to enzymatic attack.

Our previous work (Hall et al., 2015a) demonstrated that redox fluctuations increased the contribution of methoxyl groups in lignin to respiration from a tropical forest soil relative to static aerobic conditions. Here, we report an additional experiment where soils were amended with aqueous Fe(II) to simulate a highly reduced microsite, and then exposed to aerobic or fluctuating aerobic/hypoxic conditions. Added Fe(II) reacted with O<sub>2</sub> to form Fe(III) oxides, a portion of which was again reduced under subsequent hypoxic conditions. We evaluated three alternative hypotheses: H1) the precipitation of Fe oxides following Fe(II) oxidation disproportionately protects lignin relative to bulk SOM; H2) precipitation of Fe oxides protects lignin and bulk SOM equally; H3) oxidation of added Fe(II) stimulates organic matter decomposition.

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Soil was sampled from a tropical montane forest in the Luquillo Experimental Forest, Puerto Rico (Bisley Watershed), with 3500 mm of annual precipitation and mean annual temperature of 22 °C (Hall and Silver, 2015). This Aquic Hapludox was derived from volcanoclastic sediments and contained substantial short-range-order Fe oxides (24 mg Fe g<sup>-1</sup>) and Fe(II) (0.2–0.8 mg g<sup>-1</sup>). Iron(II) concentrations were much higher in reducing microsites, occasionally exceeding 5 mg g<sup>-1</sup> (Hall and Silver, 2013).

A macromolecular synthetic guaiacyl lignin labeled with <sup>13</sup>C at the methoxyl position was produced (Kirk et al., 1975) to provide a sensitive index of mineralization. Lignin was precipitated onto ground leaf litter and gently homogenized with soils (20 mg lignin and 1000 mg soil) following Hall et al. (2015a). We used a synthetic <sup>13</sup>C-labeled lignin because these polymers yield similar long-term mineralization estimates as plant-derived lignins (Haider and Martin, 1981), while eliminating the labeled polysaccharides that inevitably contaminate labeled lignins produced in *planta* (Crawford, 1981). We recognize that the physical state of synthetic lignins does not completely represent that of natural lignins, which are intimately associated with other organic compounds (e.g. hemicellulose) in plant biomass. However, the fact that lignin biodegradation is a highly nonspecific oxidative process (Ruiz-Dueñas and Martínez, 2009) indicates that the precise spatial orientation of the lignin is probably not a critical factor, especially considering that extensive fragmentation of litter occurs during its breakdown in soils.

Samples were incubated under one of four headspace and Fe addition treatment combinations (n = 5 per treatment): static aerobic + aqueous Fe(II), static aerobic + water, fluctuating aerobic/hypoxic + aqueous Fe(II), fluctuating aerobic/hypoxic + water. The fluctuating aerobic/hypoxic treatment experienced three days of aerobic conditions followed by four days of hypoxia (N<sub>2</sub> atmosphere). Before the experiment, samples received either N<sub>2</sub>-flushed water (controls), or 5 mg Fe(II) g soil<sup>-1</sup> as aqueous FeCl<sub>2</sub> (Fe addition treatment) in an anaerobic chamber. Total CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub> production were measured by gas chromatography and isotope ratio mass spectrometry over 3–4 day intervals. Additional subsamples were destructively sampled for Fe(II) analysis in 0.5M HCl using a modified ferrozine method (Hall et al., 2015a).

In the Fe addition treatment, most (>70%) of added Fe(II) oxidized to Fe(III) or precipitated in non-HCl extractable phases within the first three days (Fig. 1). Iron addition increased subsequent Fe(II) production during the hypoxic phase of the fluctuating treatment 2–5 fold relative to the control (Fig. 1), likely due to the facile reduction of newly-formed short-range-ordered Fe(III) oxides (i.e., phases with nm-scale structure). This indicated that the availability of short-range-ordered Fe phases likely limited Fe reduction even in this Fe-rich soil. Iron(II) decreased over time in all treatments, and was ultimately similar between Fe addition and control treatments.

Iron addition consistently and significantly (p < 0.0001) decreased the contribution of lignin methoxyl C to soil respiration (P<sub>lignin</sub>) to 15–55% of the control (Fig. 2a). Hypoxia also decreased P<sub>lignin</sub> in both treatments on days 7 and 14 (p < 0.05; Fig. 2a). However, O<sub>2</sub> effects were dwarfed by Fe addition, especially for cumulative mineralization (Fig. 2b). Iron-amended soils cumulatively mineralized only 21% of lignin methoxyls relative to the controls (p < 0.0001). Cumulative mineralization did not differ between O<sub>2</sub> treatments (Fig. 2b).

We modeled cumulative lignin methoxyl mineralization in the aerobic treatments (Fig. 2b) using first-order decay after discarding the first observation (R<sup>2</sup> = 0.93 and 0.92, for Fe addition and controls, respectively). A two-pool model did not improve the Akaike Information Criterion. Decomposition constants for Fe addition and control soils measured 0.0191 ± 0.0003 and 0.0762 ± 0.0009 y<sup>-1</sup>,

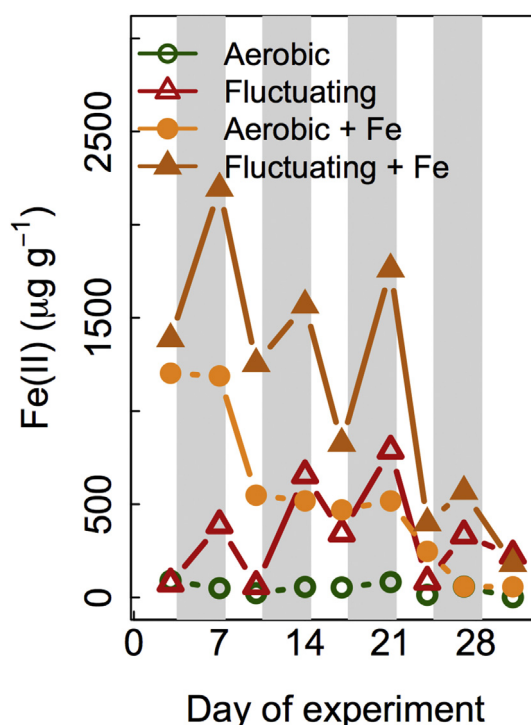
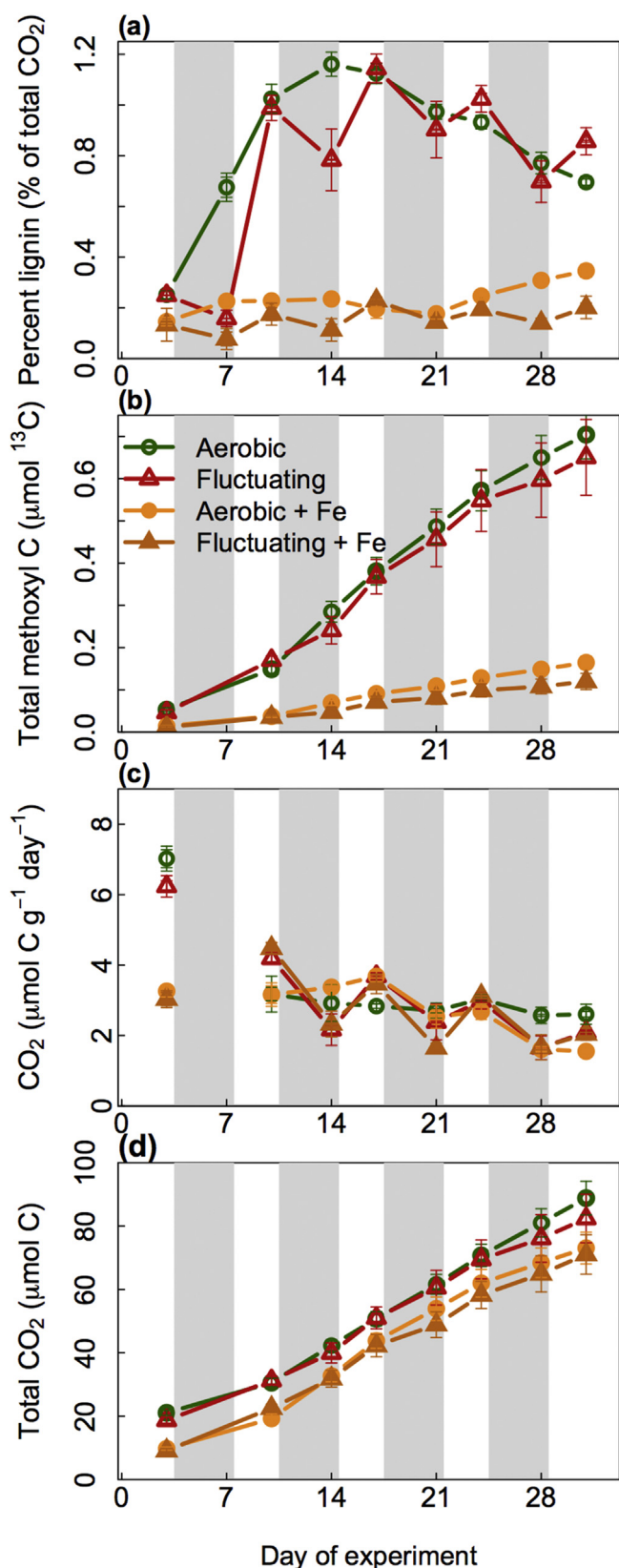


Fig. 1. Soil Fe(II) concentrations in 0.5 M HCl extractions by treatment and day of experiment (n = 1 per treatment/day). The grey bars represent the hypoxic phase of the fluctuating headspace treatment. In the Fe addition treatments, 5000 µg Fe(II) g<sup>-1</sup> soil were added at the beginning of the experiment.

respectively, implying mean turnover times of 52 and 13 y. These were similar to previous estimates of total lignin turnover in other ecosystems (10–40 y; Bahri et al., 2008; Heim and Schmidt, 2007; Rasse et al., 2006), and of slow-pool mineral-associated C in this ecosystem (11–26 y; Hall et al., 2015b). Because we measured the most labile constituent of lignin, its methoxyl group (Kirk et al., 1975), our results suggest that the more recalcitrant aromatic C of lignin could persist in soils for decades because of interactions with Fe oxides.

Trends in soil respiration differed from those of lignin mineralization (Fig. 2c,d). Total CO<sub>2</sub> production from Fe-amended soils was less than controls on day 3 (p < 0.0001), potentially due to direct toxicity of soluble Fe or reactive oxygen species produced during Fe(II) oxidation. Both mechanisms may have been important given the Fe(II) concentrations added here (Dunning et al., 1998). However, by day 10 Fe addition did not affect CO<sub>2</sub> production (Fig. 1c). Cumulative CO<sub>2</sub> production was similar between Fe addition and controls (p = 0.15; Fig. 1d). The similar CO<sub>2</sub> production from Fe addition and control treatments suggests no general suppression of organic matter mineralization upon interaction with Fe oxides as observed elsewhere (Eusterhues et al., 2014). Rather, availability of Fe(III) oxides as a terminal electron acceptor (Sutton-Grier et al., 2011), or shifts in pH, dissolved organic C, or reactive oxygen species known to be associated with Fe reduction and oxidation (Hall and Silver, 2013; Thompson et al., 2006) might have sustained CO<sub>2</sub> production despite protective effects of Fe–C interactions.

Our data thus support H1: Fe addition disproportionately inhibited the mineralization of lignin methoxyls relative to bulk SOM, irrespective of O<sub>2</sub> availability. This is consistent with preferential interactions between Fe oxide minerals and aromatic lignin constituents (Gu et al., 1995; Kaiser, 2003; Riedel et al., 2013). Given that short-range-order Fe oxides are present in most soils (Reyes



**Fig. 2.** a) Production of CO<sub>2</sub> from <sup>13</sup>C methoxyl lignin as a percent of total soil respiration. b) Total mineralization of methoxyl lignin C. c) Soil CO<sub>2</sub> production by sampling date. d) Cumulative CO<sub>2</sub> production. Bars represent standard errors with n = 5 per treatment. See the text for a description of the treatments.

and Torrent, 1997), Fe-lignin interactions deserve attention as contributors to “hidden lignin” pools that have potentially been obscured due to methodological artifacts associated with common lignin measurements (Hernes et al., 2013). Lignins synthesized with labeled isotopes provide one method to surmount these analytical challenges. We propose that consideration of specific interactions between recalcitrant biomolecules and soil geochemical constituents may prove useful in bridging old and new paradigms of SOM.

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