

10-2016

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Drivers and Patterns of Iron Redox Cycling from Surface to Bedrock in a Deep Tropical Forest Soil: A New Conceptual Model

Abstract

Iron (Fe) reduction and oxidation are important biogeochemical processes coupled to decomposition, nutrient cycling, and mineral weathering, but factors controlling their rates and spatial distribution with depth are poorly understood in terrestrial soils. In aquatic ecosystems, Fe reduction often occurs below a zone of oxic sediments. We tested an alternative conceptual model for Fe redox cycling in terrestrial soils using a deep humid tropical forest soil profile. We hypothesized that Fe reduction in anaerobic microsites scales with depth variation in labile C and Fe availability, as opposed to bulk oxygen (O₂). We measured bulk O₂ at multiple depths from 0.1 to 5 m quasi-continuously over 18 months and sampled soils from surface to bedrock (~7 m). Median O₂ mixing ratios declined from 19.8 ± 1.2 % at 0.25 m to 16.1 ± 1.0 % at 1 m, but did not consistently decrease below 1 m, challenging a recent model of regolith development. Reduced Fe (Fe(II)) extractable in 0.5 M hydrochloric acid was greatest in 0–0.1 m soil and declined precipitously with depth, and did not correspond with visible gleying in B horizons. We observed similar depth trends in potential Fe reduction under anaerobic conditions. Depth trends in Fe(II) also closely mirrored short-term soil respiration and bulk soil C. Labile C stimulated Fe reduction at 0–0.1 m depth, whereas addition of short-range-ordered Fe oxides had no effect. Cultivable Fe-reducing bacterial abundance was four orders of magnitude greater in surface soil (0–0.1 m) than below 1 m. Although cultivable Fe oxidizing bacteria were typically also more abundant in surface soil, addition of labile C and nitrate stimulated Fe oxidizers in deep soil by two orders of magnitude under anaerobic conditions. This implies that infiltration of nitrate (and possibly C) from shallow soil water could potentially promote biotic Fe oxidation, a critical step in bedrock weathering, 7 m below. Together, these data suggest that C, Fe, and nutrient availability increase microbial Fe reduction and oxidation in surface (vs deeper) soil microsites despite high bulk O₂, in contrast to the depth segregation of electron accepting processes often observed in aquatic ecosystems. Furthermore, the greatest capacity for Fe redox cycling can occur in A horizons that do not display gleying or mottling.

Keywords

Critical zone, Iron oxidation, Iron reduction, Mineral weathering, Oxygen, Redox, TEAP

Disciplines

Biogeochemistry | Ecology and Evolutionary Biology | Terrestrial and Aquatic Ecology

Comments

This is a manuscript of an article from *Biogeochemistry* 130 (2016): 177, doi: [10.1007/s10533-016-0251-3](https://doi.org/10.1007/s10533-016-0251-3).
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1 **Drivers and patterns of iron redox cycling from surface to bedrock in a deep tropical forest**
2 **soil: a new conceptual model**

3

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17 TEAP

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19 **Abstract**

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22 spatial distribution with depth are poorly understood in terrestrial soils. In aquatic ecosystems, Fe
23 reduction often occurs below a zone of oxic sediments. We tested an alternative conceptual
24 model for Fe redox cycling in terrestrial soils using a deep humid tropical forest soil profile. We
25 hypothesized that Fe reduction in anaerobic microsites scales with depth variation in labile C and
26 Fe availability, as opposed to bulk oxygen (O₂). We measured bulk O₂ at multiple depths from
27 0.1–5 m quasi-continuously over 18 months and sampled soils from surface to bedrock (~7 m).
28 Median O₂ mixing ratios declined from 19.8 ± 1.2% at 0.25 m to 16.1 ± 1.0% at 1 m, but did not
29 consistently decrease below 1 m, challenging a recent model of regolith development. Reduced
30 Fe (Fe(II)) extractable in 0.5 M hydrochloric acid was greatest in 0 – 0.1 m soil and declined
31 precipitously with depth, and did not correspond with visible gleying in B horizons. We observed
32 similar depth trends in potential Fe reduction under anaerobic conditions. Depth trends in Fe(II)
33 also closely mirrored short-term soil respiration and bulk soil C. Labile C stimulated Fe
34 reduction at 0 – 0.1 m depth, whereas addition of short-range-ordered Fe oxides had no effect.
35 Cultivable Fe-reducing bacterial abundance was four orders of magnitude greater in surface soil
36 (0 – 0.1 m) than below 1 m. Although cultivable Fe oxidizing bacteria were typically also more
37 abundant in surface soil, addition of labile C and nitrate stimulated Fe oxidizers in deep soil by
38 two orders of magnitude under anaerobic conditions. This implies that infiltration of nitrate (and
39 possibly C) from shallow soil water could potentially promote biotic Fe oxidation, a critical step
40 in bedrock weathering, 7 m below. Together, these data suggest that C, Fe, and nutrient
41 availability increase microbial Fe reduction and oxidation in surface (vs. deeper) soil microsites
42 despite high bulk O₂, in contrast to the depth segregation of electron accepting processes often

43 observed in aquatic ecosystems. Furthermore, the greatest capacity for Fe redox cycling can
44 occur in A horizons that do not display gleying or mottling.

45

46 **Introduction**

47 Iron (Fe) oxidation and reduction driven by microbial and/or abiotic processes are
48 coupled to the biogeochemical cycling of carbon (C), phosphorus (P), nitrogen (N), and cations
49 over ecological timescales, and contribute to mineral weathering and soil evolution over
50 pedogenic timescales. Dissimilatory Fe reduction coupled to C oxidation is an important
51 anaerobic microbial respiratory process, and dark Fe oxidation coupled to oxygen (O₂) or nitrate
52 (NO₃⁻) reduction can also support microbial growth (Weber et al. 2006; Melton et al. 2014). The
53 ecosystem-scale importance of Fe redox cycling and its relationships to other elemental cycles
54 have received greatest attention in aquatic sediments and wetland soils (Ponnamperuma 1972;
55 Lovley 1995; Thamdrup 2000; Weber et al. 2006; Cheng et al. 2010). Yet, Fe redox cycling can
56 also influence organic matter decomposition, nutrient dynamics, and mineral weathering in
57 relatively well-drained surface soils of terrestrial ecosystems (Chacón et al. 2006; Thompson et
58 al. 2006; Fimmen et al. 2008; Dubinsky et al. 2010; Hall and Silver 2013; Yang and Liptzin
59 2015). These dynamics are especially relevant in humid tropical soils, which are often rich in
60 short-range-ordered Fe oxides and organic C. In these ecosystems, rates of soil Fe redox cycling
61 and pools of reduced Fe (Fe(II)) often equal or exceed wetland sediments (Dubinsky et al. 2010;
62 Thompson et al. 2011; Hall and Silver 2015). Yet, understanding the spatial distribution and
63 controls on Fe reduction and oxidation in terrestrial soils remains an important knowledge gap
64 hampering the incorporation of Fe redox cycling into quantitative and conceptual models of short

65 term (i.e., minutes – months) ecosystem dynamics, and long-term (i.e., centennial – millennial)
66 weathering and pedogenic processes.

67 In aquatic sediments and groundwater, a dominant conceptual model proposes that
68 respiratory terminal electron accepting processes exhibit an approximate segregation with depth
69 according to their thermodynamic favorability. That is, given sufficient supply of C or other
70 reductants, O₂ is rapidly reduced near the sediment surface, followed by the reduction of nitrate,
71 manganese oxides, and Fe oxides in progressively deeper zones (Froelich et al. 1979; Chapelle et
72 al. 1995; Roden and Wetzel 1996; Emerson and Hedges 2003). Thus, in undisturbed sediments
73 we would typically expect respiratory Fe reduction to commence at depths where most O₂ has
74 been depleted (Fig. 1a)—although sediment redox gradients can also be disrupted by
75 bioturbation (Norkko et al. 2011). In terrestrial environments, spatial and temporal heterogeneity
76 in O₂ availability is a common feature of soils undergoing fluctuations in moisture, C inputs, and
77 biological activity. The importance of microsite-scale (mm – cm) redox gradients for stimulating
78 denitrification in well-drained surface soils is widely acknowledged, and has been contrasted
79 with the depth stratification of redox reactions in aquatic sediments (Seitzinger et al. 2006). Iron
80 reduction also appears to be relatively commonplace in many well-drained surface soils (Silver
81 et al. 1999; DeAngelis et al. 2010; Liptzin et al. 2011; Yang and Liptzin 2015), and even gross
82 methane production can sometimes be measured in these systems (von Fischer and Hedin 2007).
83 Despite these observations, patterns in the depth distribution of Fe redox cycling vis a vis
84 availability of O₂ and other potential drivers (C, Fe, and N) have received much less attention in
85 terrestrial soils.

86 Spatial interactions between controls on physical O₂ supply and biological O₂ demand
87 may be crucial for understanding trends in Fe redox cycling with depth in terrestrial soils.

88 Macropore carbon dioxide (CO₂) concentrations typically increase with soil depth (Cerling 1991),
89 corresponding to a stoichiometric decrease in O₂ that could stimulate Fe reduction with depth.
90 Indeed, several studies have documented Fe reduction in subsoils (0.5 – 1.5 m) using
91 morphological observations and geochemical analyses (Veneman et al. 1976; Fimmen et al.
92 2008; Schulz et al. 2016). The combination of periodically perched water and/or root C inputs to
93 clay-rich subsurface horizons appeared to promote Fe reduction and oxidation in these studies
94 (ibid.), generating prominent visual features of gleying and mottling indicative of Fe redox
95 cycling. However, significant rates of Fe reduction can also occur in surface (A) soil horizons
96 from a broad range of ecosystems (Chacón et al. 2006; Thompson et al. 2006; Dubinsky et al.
97 2010; Buettner et al. 2014; Yang and Liptzin 2015). At the surface, development of aggregates
98 with tortuous diffusion paths allows anaerobic processes to occur in close spatial proximity to
99 macropores with near-atmospheric O₂ concentrations (Sexstone et al. 1985). As a consequence,
100 measurements of O₂ in soil macropores (defined here as “bulk O₂”) do not necessarily reflect the
101 prevalence of anaerobic microsites at small (mm – cm) spatial scales, despite their utility when
102 comparing among sites over larger (m – km) spatial scales (Silver et al. 2013; Hall and Silver
103 2015; Liptzin and Silver 2015). Because both the availability of O₂ in soil macropores as well as
104 total biological O₂ demand generally decrease with depth (Cerling 1991), the overall relationship
105 between soil depth and Fe reduction remains unclear.

106 The availability of short-range-ordered Fe oxides, organic C, and co-limiting nutrients
107 could also have a crucial impact on the depth distribution of Fe reduction. Humid tropical soils
108 are often rich in Fe oxides, especially goethite and hematite, as a consequence of extensive
109 weathering and desilication (Sanchez 1976; White et al. 1998). Yet, a relatively small fraction of
110 total Fe may be readily accessible to Fe-reducing microbes. Iron reduction rates often scale with

111 the surface area and solubility of Fe oxide phases (Roden and Zachara 1996; Bonneville et al.
112 2009). The short-range-ordered Fe phases that dominate reducible Fe pools (Hyacinthe et al.
113 2006) may decline with depth (Thompson et al. 2011; Hall and Silver 2015), potentially limiting
114 Fe reduction. Organic C availability may also limit Fe reduction with depth. Humid tropical
115 forests are characterized by high C availability that fuels heterotrophic activity in surface soils
116 (Raich and Schlesinger 1992), and rates of Fe reduction appear tightly coupled with the
117 availability of dissolved organic C (Chacón et al. 2006; Fuss et al. 2010). Other nutrients such as
118 nitrogen (N) could also limit Fe reduction/oxidation, especially in deeper soil horizons with low
119 organic matter content. Even in comparatively N-rich tropical forests, N additions can enhance
120 particulate organic matter decomposition (Cleveland and Townsend 2006; Cusack et al. 2011),
121 and nutrient limitation may be exacerbated in comparatively resource-poor subsoils (Stone et al.
122 2014).

123 In deep soils, Fe(II) oxidation is also a crucial step in bedrock weathering, where
124 minerals such as hornblende and biotite provide a source of Fe(II) that can be oxidized via biotic
125 or abiotic mechanisms coupled to O₂ or NO₃⁻ (Buss et al. 2005; Fletcher et al. 2006; Liermann et
126 al. 2015). It has been hypothesized that O₂ availability limits mineral weathering at the interface
127 between bedrock and saprolite, and thus may play a key role in controlling landscape evolution
128 (Fletcher et al. 2006; Brantley and White 2009; Bazilevskaya et al. 2013; Behrens et al. 2015).
129 Oxygen concentrations often decrease with depth, reflecting a balance between diffusive supply
130 and the biological and geochemical processes that consume O₂. Fletcher et al. (2006) proposed
131 that depth-dependent decreases in O₂ served as a negative feedback on bedrock weathering,
132 given that increasing regolith thickness would presumably result in decreased O₂ supply at the

133 weathering front. Alternative oxidants such as NO_3^- could also potentially contribute to Fe(II)
134 oxidation (Böhlke et al. 2002; Liermann et al. 2015), especially under O_2 -limited conditions.

135 Few studies have examined trends in O_2 and biogeochemical processes across deep soil
136 profiles. In Amazonian forests and pastures, soil CO_2 concentrations increased monotonically
137 with soil depth, implying a corresponding stoichiometric decline in O_2 from ~ 19 % above 1 m to
138 ~ 12 % at 8 m (Nepstad et al. 1994). Similarly, in a highly weathered Puerto Rican forest soil, O_2
139 declined from ~18% above 2 m to ~13% at 7 m (Liermann et al. 2015). It is unclear whether
140 changes in O_2 availability of this magnitude might impact biotic Fe(II) oxidation at depth, and
141 whether Fe-oxidizing microbial abundance might respond to availability of O_2 , NO_3^- , or organic
142 matter.

143 We tested the hypothesis that microbial capacity for Fe reduction and oxidation across a
144 deep tropical forest soil profile correlates with depth variation in the availability of C and short-
145 range-ordered Fe as opposed to bulk soil O_2 . In accordance with this hypothesis, we predicted
146 greater rates of Fe reduction and oxidation potential in surface as opposed to deeper soils, despite
147 a predicted decline in bulk soil O_2 with depth (Fig. 1b). This conceptual framework contrasts
148 with patterns often observed in flooded wetland soils and sediments (Fig. 1a). We predicted that
149 our terrestrial soils would deviate from this spatial segregation of aerobic and anaerobic
150 processes with depth, because of the importance of electron donor supply (i.e., organic C) in
151 generating anaerobic microsites where Fe reduction can occur within a porous soil matrix, as
152 well as an increased abundance of short-range-ordered Fe in surface soils. We also assessed the
153 degree to which addition of C, Fe, and NO_3^- affected Fe reduction and oxidation capacity across
154 the depth gradient. We predicted that NO_3^- addition would increase Fe oxidation capacity in deep

155 soils, where bedrock supplies ample Fe(II) but oxidant (O₂, NO₃⁻) availability may limit rates of
156 Fe(II) oxidation.

157

158 **Methods**

159 *Site description*

160 Samples were collected from the Guaba Ridge (18°17'02"N, 65°47'20"W) in the Río
161 Icacos Watershed of the Luquillo Experimental Forest, Puerto Rico. This humid montane
162 tropical forest ecosystem has mean annual temperature and precipitation of 22 °C and 4200 mm,
163 respectively (White et al. 1998). Despite high precipitation, surface soils (0 - 10 cm) remain well
164 drained due to high porosity (~75 %; White et al. 1998) and bioturbation. Parent material is
165 quartz diorite from the Rio Blanco stock, dominated by plagioclase feldspar and quartz (White et
166 al. 1998). Soils in the watershed include Oxisols, Ultisols, and Inceptisols, and vary according to
167 topographic position (Soil Survey Staff 2002; Johnson et al. 2015). The Guaba Ridge separates
168 two first-order streams that discharge to the Río Icacos at approximately 650 m elevation. The
169 soil sampled here was recently characterized as a Plinthic Haplohumult (Yi-Balan et al. 2014),
170 similar to the Humic Hapludox described by the Soil Survey Staff (2002). The B horizons
171 transition to saprolite at a depth of approximately 1 m (White et al. 1998; Yi-Balan et al. 2014).
172 Gleying indicative of Fe reduction was especially prominent between 0.2 and 0.4 m. Fine root
173 biomass was greatest from 0 – 10 cm and declined precipitously with depth, and was absent
174 below 80 cm (Johnson et al. 2015; Hall and Silver 2015). Organic C declined with depth from 2
175 – 3 % C by mass from 0 – 0.1 m (S. J. Hall, unpublished data), to 1.6, 1.5, 1.2, and 1.1 % C at
176 depths of 0.15, 0.3, 0.45, and 0.6 m, respectively. Below 0.6 m, C was typically < 0.2 % (Buss et

177 al. 2005). Clay-sized particles were most abundant (42 %) at 0.3 m, and measured between 16
178 and 30 % in other samples to 5 m depth (Buss et al. 2005). Total Fe oxide content (as Fe₂O₃)
179 increased from ~ 4 % at the surface to > 7 % at depth (White et al. 1998). Site vegetation was
180 evergreen tropical montane forest locally described as the “palo colorado” forest, after the
181 dominant species *Cyrilla racemiflora* L. (Weaver and Murphy 1990).

182 *Soil sampling*

183 Soils were sampled on two separate occasions using a 7.6 cm diameter stainless steel
184 bucket auger and extensions. Samples at a given depth interval represent composites from three
185 separate augured holes collected within a radius of 15 m. The 2010 samples were collected from
186 depth increments of 0 – 0.15, 1.5 – 1.8, and 6.9 – 7.2 m. The 2012 samples were collected from 0
187 – 0.1, 0.1 – 0.2, 0.2 – 0.5, 0.5 – 1, 1 – 2, 2 – 3, 3 – 4, and 4 – 5 m. Samples were stored at field
188 moisture in sealed polyethylene bags at ambient temperature (22 – 25 °C).

189 *Oxygen measurements*

190 We installed O₂ sensors (Apogee SO-110, Logan UT) at depths of 0.1, 0.25, 0.5, 1, 2, 3, 4,
191 and 5 m in June 2010 and monitored them until February 2012. Sensors were calibrated at 100 %
192 relative humidity prior to installation and upon retrieval, and corrected for linear drift over time.
193 Each sensor was installed in a separate hole augured to the depth of installation. Holes were
194 separated laterally by > 1 m. Sensors were deployed inside 10 cm lengths of 5.1 cm diameter
195 polyvinylchloride pipe sealed with a cap on the top and bottom and perforated on the sides with
196 0.5 cm diameter holes to allow gas exchange with the adjacent soil atmosphere. After lowering a
197 sensor to the bottom of the augured hole, soil was refilled and tamped above the sensor to
198 approximate field bulk density using a stainless steel rod. The initial week of data was discarded,

199 after which point O₂ concentrations (atmospheric mixing ratios) established pseudo steady-state
200 values at deeper depths. Data were recorded at hourly intervals on a datalogger (CR1000,
201 Campbell Scientific, Logan UT) during most of the 21-month period. Continuous measurements
202 were not possible due to remote nature of the field site and associated battery failure.

203 *Chemical analyses and laboratory experiments*

204 We measured Fe and trace gas production in the laboratory at U. C. Berkeley shortly after
205 soils were sampled, and during the course of two laboratory experiments. Iron(II) and (III) were
206 measured in 0.5M HCl extractions using a 1:10 mass ratio of soil to solution, denoted as Fe(II)_{HCl}
207 and Fe(III)_{HCl}. Soils were extracted for two hours on a rotary shaker, centrifuged at 3200 rcf, and
208 the supernatant solution filtered to 0.2 μm. Solutions were analyzed using a modified ferrozine
209 method (Viollier et al. 2000). Here, we used Fe(III)_{HCl} as an index of short-range-ordered Fe
210 oxides. Our previous work at nearby sites showed a strong correlation between Fe(III)_{HCl} and Fe
211 extracted via reductive dissolution with citrate-ascorbate solution, although Fe(III)_{HCl} was always
212 of smaller magnitude (Hall and Silver 2015). Citrate-ascorbate extractable Fe is thought to be
213 closely correlated with microbially-reducible Fe (Hyacinthe et al. 2006). We measured
214 production of carbon dioxide (CO₂) using gas chromatography (Shimadzu 14A, Columbia MD)
215 as described previously (Hall et al. 2013).

216 We tested relationships between bulk soil O₂, trace gas production, and Fe_{HCl} across soils
217 from 0 – 5 m depth (0 – 0.1, 0.1 – 0.2, 0.2 – 0.5, 0.5 – 1, 1 – 2, 2 – 3, 3 – 4, and 4 – 5 m).
218 Samples (~15 g dry mass equivalent) were incubated in glass jars for 24 hours in darkness under
219 an ambient atmosphere (20.9 % O₂) within 7 days of sample collection, with three replicates per
220 depth. We report Fe(II)_{HCl} and Fe(III)_{HCl} extracted immediately prior to the trace gas

221 measurements. Next, we incubated a subset of these soils (0.1 – 0.2, 0.5 – 1, 2 – 3, 3 – 4, 4 – 5 m
222 depths) under hypoxic (N₂ headspace) and aerobic conditions (~20.9 % O₂) over 10-days to
223 assess potential rates of Fe reduction (n = 3 per depth and headspace).

224 Together, these measurements identified surface soil horizons as a dominant zone of
225 actual and potential Fe reduction. We then tested the importance of labile C and short-range-
226 ordered Fe availability as controls on Fe reduction in 0 – 0.1 m soil using a full factorial
227 experiment (n = 3 per treatment) conducted under hypoxic conditions (N₂ headspace) to simulate
228 the presence of reducing microsites under field conditions. Short-range-ordered Fe as hydrous
229 ferric oxide (HFO) was prepared as described previously (Yang et al. 2012) and gently
230 homogenized with soil subsamples (~ 15 g dry mass equivalent) at concentrations of 0, 0.1, 0.5,
231 and 1 mg Fe g soil⁻¹. Labile C was added as glucose dissolved in deionized water at
232 concentrations of 0, 50, 100, and 200 µg C g soil⁻¹. Glucose was used given that it can be
233 fermented to multiple compounds that support Fe reduction (Lovley 1995).

234 Finally, we used separate samples spanning the soil surface to bedrock to further test
235 environmental controls on Fe reduction, as well as the abundance of Fe reducing and oxidizing
236 bacteria using most probable number (MPN) analyses as described by Dubinsky et al. (2010).
237 Soils from 0 – 0.15, 1.5 – 1.8, and 6.9 – 7.2 m depths were combined in a 1:2 ratio with
238 deionized water and incubated under anaerobic conditions for 8 days. This experiment was
239 designed to assess controls on Fe cycling under anaerobic conditions, to test impacts of NO₃⁻
240 availability on Fe oxidation, and to compare with an aerobic pre-treatment control. Soil solutions
241 were amended with either sodium nitrate (NO₃⁻; 1 mM final concentration), Fe as ferrous
242 chloride (2 mM), Fe + NO₃⁻, sodium acetate (0.5 mM), and acetate + NO₃⁻, or deionized water,
243 and incubated in an anaerobic chamber (90% N₂, 8% CO₂, and 2% H₂ headspace). Acetate was

244 used in this experiment given the precedence of studies that successfully cultivated Fe oxidizers
245 and reducers (Lovley 1995; Straub et al. 1996). To enumerate anaerobic Fe reducing and
246 oxidizing bacteria, soils were extracted in buffer containing 0.1% sodium pyrophosphate and
247 0.03% Tween 80 in basal microbiological medium (BMM). BMM consisted of (per L) 5.0 g 2-
248 (N-morpholino)ethanesulfonic acid (MES) buffer and 10 ml mineral solution, with 0.80 g NaCl,
249 1.0 g NH₄Cl, 0.1 g KCl, 0.1 g KH₂PO₄, 0.2 g MgCl₂·6H₂O, and 0.04 g CaCl₂·2H₂O (per L).
250 After autoclaving, the media pH was adjusted to 5.5 and amended with 1 ml SL12 trace elements
251 solution, 2.5 ml trace metal solution (Widdel and Bak 1992), and 1 ml vitamin solution (Pfennig
252 and Trüper 1992) per L. Media was dispensed into 96-well microplates, and soil subsamples
253 added in ten-fold dilutions from 10⁻² to 10⁻¹³ with four biological replicates and three technical
254 replicates per depth per amendment. Plates were incubated in the dark for 30 days with negative
255 controls including soil extract buffer only (no soil). Positive growth of Fe(III) reducers was
256 visualized by adding ferrozine solution, which turns purple in the presence of Fe(II). Formation
257 of reddish-brown precipitates was used to verify positive results for Fe(II) oxidizers. Cell counts
258 per gram of soil were calculated using the Most Probable Number Calculator version 4.04 (Klee
259 1996).

260 For all experiments, statistical differences among treatments and/or depths were assessed
261 using ANOVA and post-hoc Tukey comparisons using R v. 3.2.0. To account for temporal
262 autocorrelation in the O₂ data, we used a generalized linear model with an autoregressive error
263 term implemented using the glm function.

264 **Results**

265 Median bulk soil O₂ concentrations (mixing ratios) exceeded 16 % at all depths measured
266 between 0.10 and 5 m (Fig. 2a). All depths significantly differed ($p < 0.05$) from each other with
267 the exception of the 1 and 4 m depths. However, differences in O₂ were often small: below 0.5 m,
268 median O₂ concentrations differed by < 0.3 % and did not consistently decrease with depth (Fig.
269 2a). Oxygen was most dynamic at 0.25 and 0.5 m depths, where O₂ varied by as much as 10 %
270 over time (Fig. 2a). In contrast, depths below 0.5 m showed much less variability (< 1.5 % O₂)
271 relative to median values.

272 Concentrations of Fe(II)_{HCl} and Fe(III)_{HCl} measured on soils sampled in May 2012
273 showed different patterns from bulk soil O₂ (Fig. 2). Iron(II)_{HCl} was greatest in 0 – 0.1 m soil and
274 declined precipitously with depth, and was negligible below 1 m (Fig. 2b). Concentrations of
275 Fe(III)_{HCl} showed very similar depth trends as Fe(II)_{HCl} (Fig. 2c). Patterns of short-term CO₂
276 production closely mirrored both Fe(II)_{HCl} and Fe(III)_{HCl} (Fig. 2d), and rates declined > 10 -fold
277 between the 0 – 0.1 and 0.5 – 1 m depths. Ten-day anaerobic incubations of a subset of these
278 soils confirmed that potential Fe reduction was greatest in the most shallow soil tested (0.1 – 0.2
279 m), declined by an order of magnitude between 0.5 and 3 m, and was undetectable (not different
280 from zero, $p > 0.05$) from 3 to 5 m (Fig. 3). Samples incubated under an *aerobic* atmosphere
281 displayed no significant net change in Fe(II) concentrations over this time period (data not
282 shown).

283 Factorial incubation experiments with 0 – 0.1 m soil indicated that Fe reduction generally
284 increased with increased rates of labile C addition (glucose) under anaerobic conditions.
285 Production of Fe(II)_{HCl} was significantly greater ($p < 0.05$) in samples that received the highest
286 glucose concentrations (100 and 200 $\mu\text{g C g soil}^{-1}$) and no or minimal Fe(III) addition (0 and 0.1
287 mg Fe g soil⁻¹). Iron(III) addition had no significant effect on Fe(II)_{HCl} production in the

288 treatments with no or minimal glucose addition (0 and 50 $\mu\text{g C g soil}^{-1}$). However, the treatments
289 with the highest Fe concentrations decreased $\text{Fe(II)}_{\text{HCl}}$ production relative to the controls. This
290 effect depended on the amount of added glucose (Fig. 4; treatment interaction $p < 0.0001$).
291 Addition of 1 mg Fe g soil^{-1} decreased $\text{Fe(II)}_{\text{HCl}}$ production in the presence of 100 $\mu\text{g glucose C}$
292 g soil^{-1} , whereas 0.5 mg Fe g soil^{-1} decreased $\text{Fe(II)}_{\text{HCl}}$ production with 200 $\mu\text{g glucose C g soil}^{-1}$.

293 In our final experiment, we incubated soil slurries from surface, intermediate, and deep
294 samples (0 – 0.15, 1.5 – 1.8, and 6.9 – 7.2 m) to test the factors controlling the abundance of Fe
295 reducing and oxidizing organisms under anaerobic conditions, simulating anaerobic microsites in
296 the field. Iron reduction rates were two orders of magnitude greater in surface than deeper
297 samples ($p < 0.001$), although lower but significant rates of Fe reduction were also detectable in
298 some of the intermediate and deep samples under these experimental conditions (Fig. 5a, note the
299 log scale). Trends in Fe reduction rates with depth were corroborated by MPN analyses of
300 cultivable Fe reducers, which were four orders of magnitude greater in surface than deeper
301 samples (Fig. 5b). Experimental amendments (acetate, Fe, and NO_3^-) did not significantly affect
302 Fe reduction rates in surface (0 – 0.15 m) samples. However, Fe + NO_3^- addition increased the
303 abundance of cultivable Fe reducers three-fold relative to the controls in these samples ($p < 0.05$;
304 Fig. 5b). Nitrate addition alone doubled mean cultivable Fe reducer abundance relative to the
305 control in surface soil, but this difference was not statistically significant. In the intermediate
306 depth samples (1.5 – 1.8 m), addition of Fe, Fe + NO_3^- , acetate, and acetate + NO_3^- all
307 significantly stimulated Fe reduction rates relative to the control, but had no significant impact
308 on Fe reducer MPN (Fig. 5a,b), where abundances were low across all treatments. Iron + NO_3^-
309 addition stimulated Fe reduction rates in the intermediate depth samples to the greatest extent (p

310 < 0.05). In the deep soil samples (6.9 – 7.2 m), Fe and Fe + NO₃⁻ stimulated Fe reduction relative
311 to the controls, whereas the other treatments had no significant effects.

312 Iron(II) oxidizers were most abundant in surface soils at the beginning of the experiment,
313 and Fe oxidizer MPN was not significantly affected by experimental amendments in either the
314 surface or intermediate samples (Fig. 5c). In the deep samples, addition of acetate + NO₃⁻ (but
315 not acetate or NO₃⁻ alone) significantly stimulated Fe oxidation MPN by two orders of
316 magnitude relative to the other treatments. Notably, Fe oxidizer MPN values were not inhibited
317 by anaerobiosis per se at any depth, as they did not decrease relative to initial values under any
318 treatment despite incubation under anaerobic conditions.

319

320 **Discussion**

321 Our data support the hypothesis that labile C and Fe availability (Fig. 1b), as opposed to
322 variation in bulk O₂ with depth (Figs. 1a, 2), controlled Fe reduction across this deep humid
323 tropical forest soil profile. Actual and potential Fe reduction and cultivable Fe reducer abundance
324 were greatest in surface soils where bulk O₂ concentrations were also highest (Figs. 2,3). Iron
325 reduction declined by two orders of magnitude below 1 m despite decreased bulk soil O₂, and
326 cultivable Fe reducer abundance declined by four orders of magnitude. Soil respiration (Fig. 2)
327 and bulk soil C (Buss et al. 2005) showed a depth pattern similar to Fe reduction, and addition of
328 labile C (but not Fe) significantly enhanced Fe reduction in surface soil (Fig. 4). Apparent
329 decreases in Fe(II)_{HCl} production at the highest concentrations of added Fe and glucose (Fig. 4)
330 may represent Fe(II)-catalyzed transformation of short-range-ordered Fe into more crystalline
331 phases that occluded the newly-produced Fe(II) (Jeon et al. 2003). Although these data represent

332 a single site, they support our proposed conceptual framework as well as the need to more
333 broadly reconsider the controls and impacts of Fe redox cycling with depth, as discussed below.

334 *Contrasting depth distribution of Fe redox cycling in wetlands and uplands*

335 A dominant conceptual model in aquatic sediments posits that terminal electron accepting
336 processes are segregated with depth according to their thermodynamic favorability (Emerson and
337 Hedges 2003; Fig. 1a). However, our data suggest that this model does not necessarily explain
338 depth variation in Fe reduction and oxidation potential at our site, and perhaps also in other well-
339 drained terrestrial soils where high Fe reduction capacity has been documented (Yang and
340 Liptzin 2015). Bulk O₂, Fe(II) concentrations, and potential Fe reduction were all greatest at the
341 surface, concomitant with greatest labile C availability. These observations, combined with our
342 experimental data, suggest that patterns in electron donor (i.e., organic C) availability provide a
343 proximate control on the depth distribution of Fe reduction in this soil.

344 Although bulk O₂ concentrations declined with depth below 0.25 m, the abundance of
345 anaerobic microsites also declined along with C availability, as reflected by lower concentrations
346 of Fe(II), lower potential Fe reduction rates, and lower abundance of Fe-reducing and oxidizing
347 microbes. Subtly increased O₂ concentrations at 0.25 m relative to 0.1 m likely reflected lateral
348 spatial heterogeneity in soil O₂ (Liptzin et al. 2011; Hall et al. 2013) as opposed to a consistent
349 trend with depth. Contrasting patterns between the depth distribution of terminal electron
350 accepting processes in aquatic sediments vs. this terrestrial forest point to the importance of
351 microsite-scale anaerobic processes within the largely aerobic soil matrix (Sexstone et al. 1985;
352 Hall and Silver 2015; Keiluweit et al. 2016). Localized inputs of labile C to fuel O₂ consumption
353 may be a critical regulator of the abundance of anaerobic microsites (Chacón et al. 2006),

354 evidenced by recent reports of Fe reduction associated with the rhizosphere (Fimmen et al. 2008;
355 Schulz et al. 2016). In many ecosystems, including this humid tropical forest, root biomass C
356 inputs are greatest near the soil surface (Jobbagy and Jackson 2000; Hall and Silver 2015). Thus,
357 we predict that Fe redox cycling and the numerous processes linked to these dynamics—e.g.,
358 sorption and desorption of P and organic matter (Peretyazhko and Sposito 2005; Chacón et al.
359 2006; Thompson et al. 2006; Buettner et al. 2014; Hall et al. 2016), microbial respiration
360 (Dubinsky et al. 2010), and production of reactive oxygen species (Hall and Silver 2013)—may
361 also be most significant near the surface of many other terrestrial soils, despite the fact that
362 moisture and visual indicators of Fe redox cycling often increase with depth.

363 *Cryptic Fe redox cycling in surface soils*

364 Our finding of greater Fe reduction capacity in surface vs. subsurface soils shows that
365 trends in soil coloration and moisture are not necessarily reliable indicators of potential *rates* of
366 Fe redox cycling. The greatest Fe(II) concentrations, potential rates of Fe reduction, and
367 abundance of cultivable microbial Fe reducers and oxidizers occurred in surface A horizon (0 –
368 0.1 m) soil, where porosity was high (~ 75 %) and moisture rarely approached saturation (White
369 et al. 1998). In this soil, gleying was visible throughout the B horizons, but not in the A horizon
370 (Yi-Balan et al. 2014). Surface horizons were rarely saturated, and water content was typically
371 greatest near the soil-saprolite interface (White et al. 1998). Investigations of Fe reduction in
372 terrestrial soils have often focused on Fe redox dynamics in relatively deeper (> 0.5 m) B
373 horizons associated with periodic moisture saturation and rhizosphere gleying (Veneman et al.
374 1976; Fimmen et al. 2008; Schulz et al. 2016). Mottling and gleying provide important visual
375 evidence of Fe reduction, translocation, and oxidation (Veneman et al. 1976; Fimmen et al. 2008;
376 Schulz et al. 2016). Although these features are *sufficient* to indicate the occurrence of Fe redox

377 cycling, they are not obligate indicators, as demonstrated by our data. Several studies have
378 similarly demonstrated high rates of Fe reduction at the soil surface (Chacón et al. 2006;
379 Dubinsky et al. 2010; Yang and Liptzin 2015) but did not assess trends with depth. Visual
380 evidence of Fe redox cycling in 0 – 0.1 m soil may have been obscured by high organic matter
381 content at the surface, which imparts a dark color. Surface soil horizons typically contain the
382 highest stocks of root biomass and organic C across a broad range of terrestrial ecosystems
383 (Jobbagy and Jackson 2000). As a consequence of abundant C inputs that generate anaerobic
384 microsites yet obscure the visual effects of Fe reduction, we suggest that cryptic Fe reduction in
385 terrestrial surface soils may be a more commonplace phenomenon than is implied by visible
386 gleying and mottling.

387 In surface soils of humid tropical forests and other terrestrial ecosystems, hotspots of Fe
388 reduction in surface soil microsites are likely generated due to the confluence of several critical
389 factors. High clay content decreases gas-phase diffusivity and O₂ supply, high temperature
390 decreases O₂ solubility while increasing biological O₂ consumption, a high density of live and
391 dead roots provides abundant C inputs, and large pools of Fe are maintained in short-range-
392 ordered minerals (Silver et al. 1999; Buss et al. 2005; Thompson et al. 2011; Johnson et al. 2015;
393 Hall and Silver 2015). The maintenance of short-range-ordered Fe appears critical in that these
394 phases exhibit greater rates of reduction than crystalline Fe (Roden and Wetzel 2002).
395 Interactions between Fe and organic matter likely retard the formation of crystalline minerals
396 (Schwertmann et al. 1988), despite the fact that redox cycling can potentially lead to formation
397 of Fe with greater crystalline structure (Thompson et al. 2006). At depth, lower organic matter
398 concentrations may facilitate the formation of more crystalline Fe minerals during redox cycling
399 (Jeon et al. 2003; Thompson et al. 2006), consistent with previous Fe isotope measurements at

400 this site (Buss et al. 2010). This coincides with our finding that Fe addition stimulated Fe
401 reduction at depth (Fig. 5), despite the presence of a large total Fe oxide pool (White et al. 1998).

402 *Implications of O₂ depth distribution for bedrock weathering*

403 Bulk soil O₂ concentrations did not consistently decrease with depth below 1 m, the
404 approximate depth of the soil/saprolite transition, but rather fluctuated around similar median
405 values (~ 16 %; Fig. 2). Previous work hypothesized that regolith depth controls weathering rates
406 by constraining O₂ supply, given that O₂ availability may limit oxidation of Fe in primary
407 bedrock minerals (Fletcher et al. 2006; Brantley and White 2009; Behrens et al. 2015). A key
408 assumption of this hypothesis is that O₂ concentrations decrease monotonically with regolith
409 depth, facilitating a negative feedback between regolith development and weathering rates. Our
410 data show that bulk soil O₂ does not necessarily decrease consistently or significantly within the
411 saprolite profile. Rather, the asymptotic trend in bulk soil O₂ observed here at depths below 1 m
412 is consistent with analytical models of soil CO₂ production and diffusion (Cerling 1991; Fig. 1b)
413 validated by measurements in shallower soil profiles (< 1.5 m depth) from other ecosystems (e.g.
414 Solomon and Cerling 1987; Bowling et al. 2015). Bulk O₂ concentrations observed here at 5 m
415 depth (~16 %), as well as other tropical forests (~12 – 17 %; Nepstad et al., 1994; Liermann et al.,
416 2015), were relatively high. If these data and models are broadly representative, regolith depth
417 per se may not necessarily influence gas-phase O₂ supply to the bedrock/saprolite interface.
418 Rather, the presence and depth of perched water tables at the bedrock/saprolite interface (White
419 et al. 1998) may be more important in controlling diffusive O₂ supply for primary mineral
420 weathering.

421 *Potential importance of anaerobic Fe oxidation at depth*

422 Although bulk soil O₂ concentrations were relatively high in deep soils, anaerobic
423 microsites are likely to occur (Silver et al. 1999), particularly in the presence of perched water
424 tables at the bedrock interface (White et al. 1998; Schulze and White 1999). Iron(II) oxidation by
425 NO₃⁻ can be a significant process in shallow groundwater (Böhlke et al. 2002). As a consequence,
426 availability of both O₂ and NO₃⁻ could potentially influence rates of Fe(II) oxidation at the
427 bedrock/saprolite weathering front. In our study, Fe(II) oxidation at intermediate depths was
428 likely limited by Fe(II) supply from primary minerals (Buss et al. 2005) and the abundance of
429 cultivable Fe(II) oxidizers was unaffected by our experimental treatments. In deep soils,
430 cultivable Fe(II) oxidizer abundance was initially similar to intermediate depths, but responded
431 strongly to additions of NO₃⁻ and acetate under anaerobic conditions—increasing by two orders
432 of magnitude relative to initial aerobic conditions. This finding suggests significant capacity for
433 anaerobic, microbially-mediated Fe(II) oxidation in deep soils.

434 We note that our results are likely conservative in that the MPN enumeration method
435 used here yielded *cultivable* anaerobic Fe reducers and oxidizers, and likely underestimates their
436 total populations (Dubinsky et al. 2010). Previous work in nearby soils found that cultivable Fe
437 reducers represented 0.7 – 5.7 % of total bacterial abundance, but that the relative abundances of
438 the canonical Fe reducers *Shewanella* and *Geobacter* assessed were low when assessed using
439 quantitative PCR (Dubinsky et al. 2010; DeAngelis et al. 2010). The composition of Fe reducing
440 and oxidizing microbial communities in humid tropical soils remains poorly understood.

441 The finding that acetate stimulates anaerobic Fe(II) oxidizers suggests that heterotrophic
442 or mixotrophic Fe oxidizers contribute to Fe(II) oxidation and related bedrock weathering at
443 depth. Previous studies similarly found that C addition enhanced rates of Fe(II) oxidation (Straub
444 et al. 1996; Kappler et al. 2005). The stimulatory effect of acetate on Fe(II) oxidizer abundance

445 may reflect the importance of mixotrophy in preventing the deleterious effects of cell
446 encrustation by the newly-formed Fe(III) oxides (Kappler et al. 2005). However, the finding that
447 acetate stimulated Fe(II) oxidizer abundance presents an interesting conundrum: previous
448 measurements suggested that deep dissolved organic C concentrations may be extremely low at
449 this site (Schulz and White 1999), supporting the hypothesis that microbial communities are
450 dominated by autotrophs reliant on bedrock Fe(II) supply and are decoupled from surface C
451 inputs (Liermann et al. 2015). Yet, the strong response of cultivable Fe oxidizers to C addition in
452 deep soils also suggests that heterotrophic microbial communities are poised to respond to C
453 inputs, either from co-occurring autotrophs or possibly from surface soils.

454 The observation of high NO_3^- concentrations ($\sim 20 \mu\text{M}$) in deep soils from this site and
455 another nearby site (Schulz and White 1999; Liermann et al. 2015) is indicative of hydrologic
456 NO_3^- supply from surface soils 7 m above, as the parent material does not contain significant N
457 (White et al. 1998). Surface soil biological processes do not appear strongly N limited in this
458 ecosystem (Cusack et al. 2011), thus infiltration of surface soil NO_3^- to deep soils appears
459 plausible. The potential for surface soil dissolved organic matter to reach the weathering front at
460 7 m depth without being sorbed or mineralized in transit may be more tenuous. Couplings
461 between surface-derived nutrients and bedrock weathering remain an important but poorly
462 explored topic in the context of landscape evolution, and biogeochemical connections between
463 surface and deep subsurface soils merit further exploration.

464

465 **Conclusions**

466 Trends in potential Fe reduction and oxidation varied systematically with depth in this
467 terrestrial humid tropical forest soil but showed distinctly different trends compared to the
468 standard conceptual model for saturated sediments. Although mean bulk O₂ declined overall with
469 depth, it was most variable and sporadically reached the lowest values at shallow depths (0.25
470 and 0.5 m). Biotic Fe reduction and oxidation capacity were greatest at the surface and declined
471 precipitously with depth. At intermediate and deep depths, Fe reduction and oxidation appeared
472 strongly limited by C, NO₃⁻, and/or labile Fe, despite high total Fe. However, biotic Fe oxidation
473 potential increased at the saprolite/bedrock interface in response to acetate and NO₃⁻ addition,
474 likely as a consequence of increased Fe(II) supply from primary Fe(II)-rich minerals, which had
475 been depleted from shallower saprolite (intermediate depths). Shallow surface soils may play an
476 underappreciated role as hotspots of coupled Fe reduction and oxidation, even when visible
477 gleying is not apparent. Furthermore, our data suggest that the total depth of soil profiles may
478 have less influence on bulk O₂ supply to bedrock than previously proposed, given the observed
479 asymptotic trend in O₂ with depth. In addition to O₂ availability, we showed that the supply of
480 NO₃⁻ from surface soils could play an important role in bedrock weathering by stimulating Fe(II)
481 oxidizing microbial communities. Although labile C amendments stimulated the growth of Fe(II)
482 oxidizers, it remains uncertain whether surface soil inputs provide a significant C source at 7 m
483 depth.

484

485 **Acknowledgements**

486 Data associated with this manuscript will be available on the Luquillo CZO data repository
487 (<http://criticalzone.org/luquillo/data/>) after publication. We thank Heather Dang and Andrew

488 McDowell for crucial help in the lab, and Manual Rosario for data collection. We thank Aaron
489 Thompson for providing valuable insights on this work. This work was supported by NSF grant
490 DEB-1457805 to WLS and SJH, and the NSF Luquillo Critical Zone Observatory (EAR-
491 0722476) and LTER (DEB-0620910).

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682 **Figure captions:**

683 Figure 1: Idealized depth profiles of bulk O₂ (solid line) and Fe(II) (dashed line) with depth.

684 Panel **a** represents a standard conceptual model for aquatic sediments, where Fe reduction occurs

685 at depths below which O₂ and other oxidants (not shown for clarity) have been reduced. Panel **b**

686 shows an alternative model for terrestrial soils where bulk O₂ decreases slightly with depth, and

687 total Fe reduction peaks in anaerobic microsites near the soil surface as a consequence of greater
688 availability of C and short-range-ordered Fe. Oxygen profiles were modeled after Cerling (1991),
689 assuming a stoichiometric relationship between CO₂ and O₂ and an exponential decline in CO₂
690 production with depth; diffusivity differs for panels **a** and **b**. Iron(II) trends are hypothetical but
691 consistent with previous work at this site; labile Fe(II) may increase near bedrock due to supply
692 from primary minerals (Buss et al. 2005).

693 Figure 2: Soil O₂ concentrations (**a**) measured at hourly intervals along the Guaba ridge depth
694 profile from June 2010 to February 2012. Boxes represent medians and the first and third
695 quartiles. Whiskers represent the furthest value less than 1.5 times the box length measured from
696 the box edge; more extreme points are denoted as circles. Mean (\pm SE) concentrations of
697 Fe(II)_{HCl} (**b**), Fe(III)_{HCl} (**c**), and soil respiration (**d**) were measured shortly after sampling (n = 3
698 per depth). Means with different letters differed significantly (p < 0.05, Tukey comparison).

699 Figure 3: Net Fe(II) production by depth (\pm SE) over a 10-day incubation of intact (non-slurried)
700 soils under anaerobic conditions (n = 3 per depth). Note that bar widths are not proportional to
701 soil depths (as in Fig. 2) because a subset of depths was measured.

702 Figure 4: Iron reduction during three-day anaerobic incubations of intact (non-slurried) soils
703 from 0 – 0.1 m depth, incubated with varying concentrations of labile C (glucose) and short-
704 range-ordered Fe(III). Treatments with different letters differed significantly (all possible
705 pairwise comparisons were evaluated), and whiskers represent standard errors (n = 3 per
706 treatment).

707 Figure 5: Rates of Fe reduction (**a**) and most-probable-number (MPN) analyses of Fe reducers
708 (**b**) and oxidizers (**c**) measured before and after anaerobic incubations of soil slurries. Samples

709 were amended with NO_3^- , Fe as ferrous chloride, $\text{NO}_3^- + \text{Fe}$, acetate, or acetate + NO_3^- . Means
710 with different letters within a given depth increment differed significantly (n = 4 per treatment).

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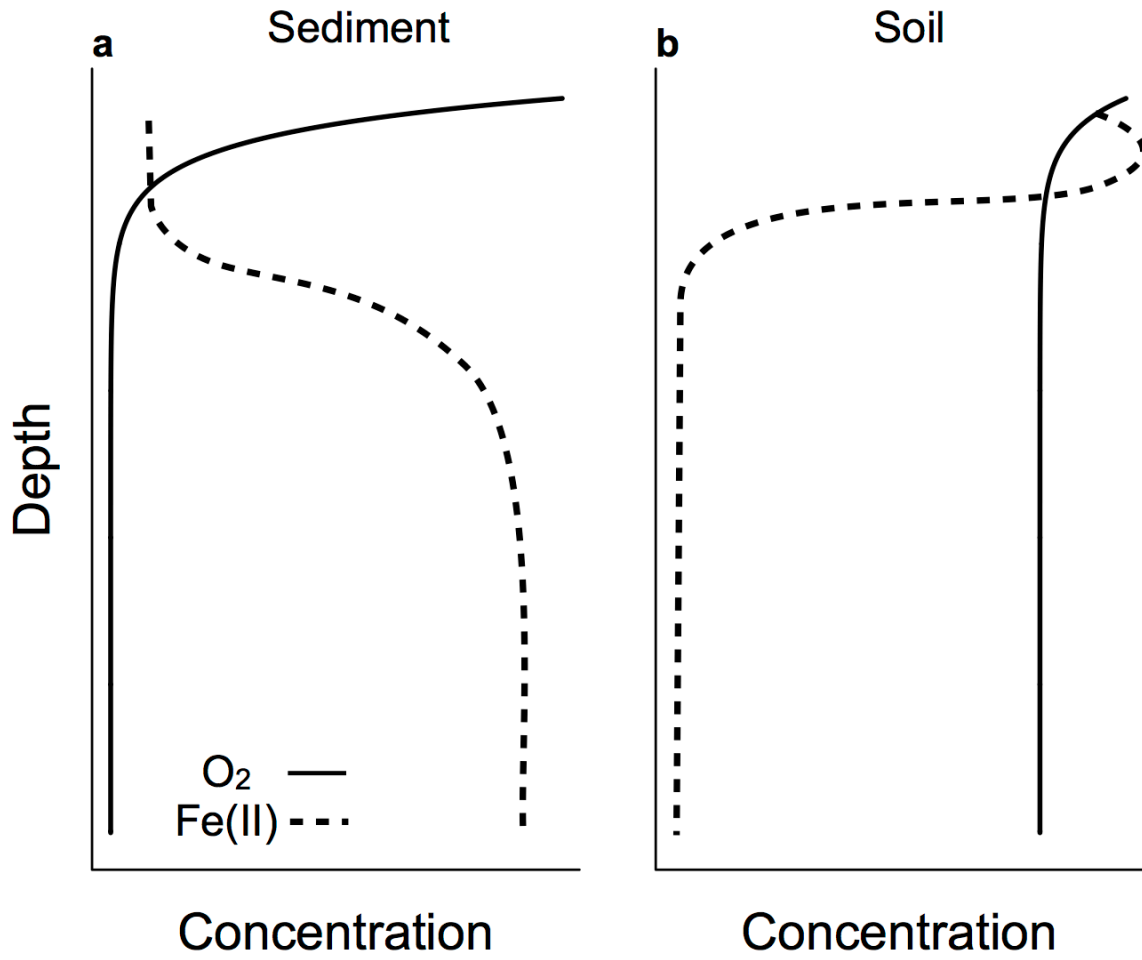
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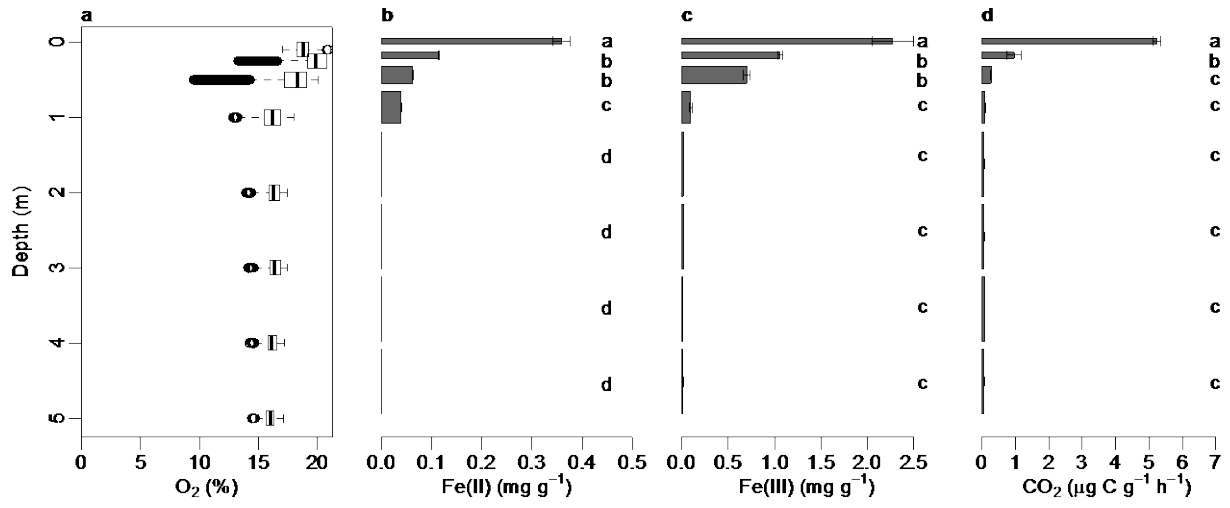
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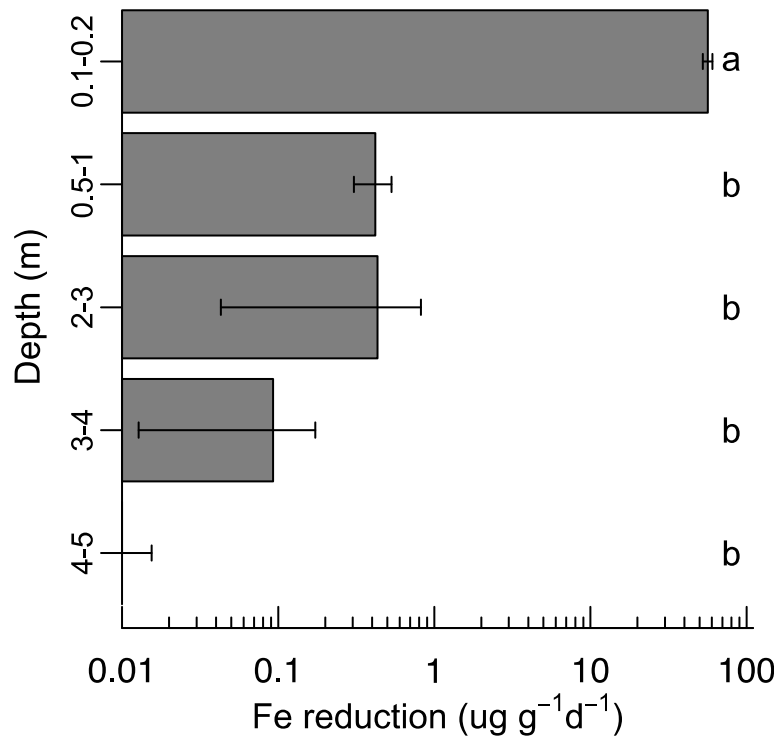
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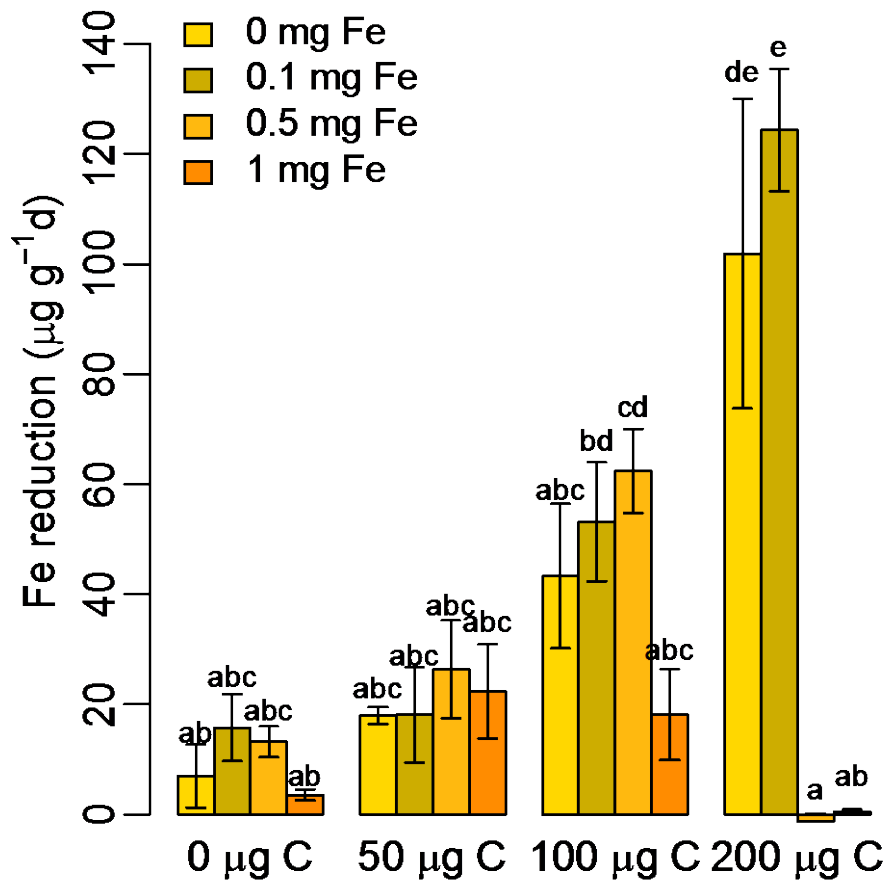
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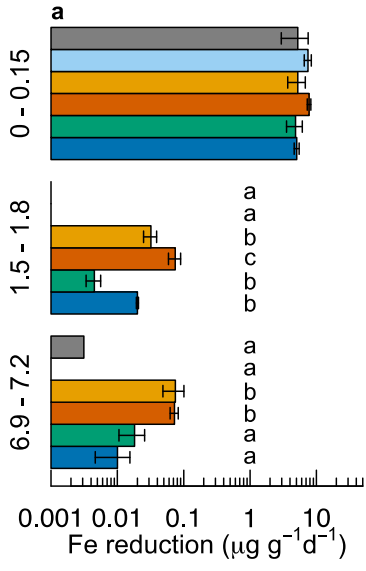
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