



Hall, S. J., Liptzin, D., Buss, H., DeAngelis, K., & Silver, W. L. (2016). Drivers and patterns of iron redox cycling from surface to bedrock in a deep tropical forest soil: a new conceptual model. *Biogeochemistry*, 130(1), 177-190. <https://doi.org/10.1007/s10533-016-0251-3>

Peer reviewed version

Link to published version (if available):  
[10.1007/s10533-016-0251-3](https://doi.org/10.1007/s10533-016-0251-3)

[Link to publication record in Explore Bristol Research](#)  
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Springer at <http://link.springer.com/article/10.1007%2Fs10533-016-0251-3>. Please refer to any applicable terms of use of the publisher.

## University of Bristol - Explore Bristol Research

### General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: <http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

1 **Drivers and patterns of iron redox cycling from surface to bedrock in a deep tropical forest**  
2 **soil: a new conceptual model**

3

4 Steven J. Hall\*<sup>1</sup>, Daniel Liptzin<sup>2</sup>, Heather L. Buss<sup>3</sup>, Kristen DeAngelis<sup>4</sup>, Whendee L. Silver<sup>5</sup>

5 <sup>1</sup>Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 251 Bessey  
6 Hall, Ames, Iowa, 50011, USA

7 <sup>2</sup>INSTAAR, University of Colorado - Boulder, Boulder, CO, USA

8 <sup>3</sup>School of Earth Sciences, University of Bristol, Wills Memorial Building, Queens Road, Bristol  
9 BS8 1RJ, UK

10 <sup>4</sup>Department of Microbiology, University of Massachusetts Amherst, Amherst, Massachusetts  
11 USA

12 <sup>5</sup>Department of Environmental Science, Policy, and Management, University of California-  
13 Berkeley, Berkeley, California USA

14 \* Corresponding author: [stevenjh@iastate.edu](mailto:stevenjh@iastate.edu), 515-294-7650

15

16 *Key words:* Critical zone, Iron oxidation, Iron reduction, Mineral weathering, Oxygen, Redox,  
17 TEAP

18

19 **Abstract**

20 Iron (Fe) reduction and oxidation are important biogeochemical processes coupled to  
21 decomposition, nutrient cycling, and mineral weathering, but factors controlling their rates and  
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 (O<sub>2</sub>). We measured bulk O<sub>2</sub> 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 O<sub>2</sub> 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 O<sub>2</sub>, 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 (O<sub>2</sub>) or nitrate  
52 (NO<sub>3</sub><sup>-</sup>) 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, O<sub>2</sub> 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 O<sub>2</sub> 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 O<sub>2</sub> 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 O<sub>2</sub> and other potential drivers (C, Fe, and N) have received much less attention in  
85 terrestrial soils.

86 Spatial interactions between controls on physical O<sub>2</sub> supply and biological O<sub>2</sub> demand  
87 may be crucial for understanding trends in Fe redox cycling with depth in terrestrial soils.

88 Macropore carbon dioxide (CO<sub>2</sub>) concentrations typically increase with soil depth (Cerling 1991),  
89 corresponding to a stoichiometric decrease in O<sub>2</sub> 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 O<sub>2</sub> concentrations (Sexstone et al. 1985). As a consequence,  
100 measurements of O<sub>2</sub> in soil macropores (defined here as “bulk O<sub>2</sub>”) 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 O<sub>2</sub> in soil macropores as well as  
104 total biological O<sub>2</sub> 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 O<sub>2</sub> or NO<sub>3</sub><sup>-</sup> (Buss et al. 2005; Fletcher et al. 2006; Liermann et  
126 al. 2015). It has been hypothesized that O<sub>2</sub> 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 O<sub>2</sub>. Fletcher et al. (2006) proposed  
131 that depth-dependent decreases in O<sub>2</sub> served as a negative feedback on bedrock weathering,  
132 given that increasing regolith thickness would presumably result in decreased O<sub>2</sub> supply at the

133 weathering front. Alternative oxidants such as  $\text{NO}_3^-$  could also potentially contribute to Fe(II)  
134 oxidation (Böhlke et al. 2002; Liermann et al. 2015), especially under  $\text{O}_2$ -limited conditions.

135 Few studies have examined trends in  $\text{O}_2$  and biogeochemical processes across deep soil  
136 profiles. In Amazonian forests and pastures, soil  $\text{CO}_2$  concentrations increased monotonically  
137 with soil depth, implying a corresponding stoichiometric decline in  $\text{O}_2$  from ~ 19 % above 1 m to  
138 ~ 12 % at 8 m (Nepstad et al. 1994). Similarly, in a highly weathered Puerto Rican forest soil,  $\text{O}_2$   
139 declined from ~18% above 2 m to ~13% at 7 m (Liermann et al. 2015). It is unclear whether  
140 changes in  $\text{O}_2$  availability of this magnitude might impact biotic Fe(II) oxidation at depth, and  
141 whether Fe-oxidizing microbial abundance might respond to availability of  $\text{O}_2$ ,  $\text{NO}_3^-$ , 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  $\text{O}_2$ . 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  $\text{O}_2$  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  $\text{NO}_3^-$  affected Fe reduction and oxidation capacity across  
154 the depth gradient. We predicted that  $\text{NO}_3^-$  addition would increase Fe oxidation capacity in deep



155 soils, where bedrock supplies ample Fe(II) but oxidant (O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>) 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 Fe<sub>2</sub>O<sub>3</sub>)  
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 O<sub>2</sub> 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 O<sub>2</sub> 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)<sub>HCl</sub>  
207 and Fe(III)<sub>HCl</sub>. 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)<sub>HCl</sub> as an index of short-range-ordered Fe  
210 oxides. Our previous work at nearby sites showed a strong correlation between Fe(III)<sub>HCl</sub> and Fe  
211 extracted via reductive dissolution with citrate-ascorbate solution, although Fe(III)<sub>HCl</sub> 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 (CO<sub>2</sub>) using gas chromatography (Shimadzu 14A, Columbia MD)  
215 as described previously (Hall et al. 2013).

216 We tested relationships between bulk soil O<sub>2</sub>, trace gas production, and Fe<sub>HCl</sub> 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 % O<sub>2</sub>) within 7 days of sample collection, with three replicates per  
220 depth. We report Fe(II)<sub>HCl</sub> and Fe(III)<sub>HCl</sub> 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 (N<sub>2</sub> headspace) and aerobic conditions (~20.9 % O<sub>2</sub>) 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 (N<sub>2</sub> 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<sup>-1</sup>. Labile C was added as glucose dissolved in deionized water at  
232 concentrations of 0, 50, 100, and 200 µg C g soil<sup>-1</sup>. 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 NO<sub>3</sub><sup>-</sup>  
240 availability on Fe oxidation, and to compare with an aerobic pre-treatment control. Soil solutions  
241 were amended with either sodium nitrate (NO<sub>3</sub><sup>-</sup>; 1 mM final concentration), Fe as ferrous  
242 chloride (2 mM), Fe + NO<sub>3</sub><sup>-</sup>, sodium acetate (0.5 mM), and acetate + NO<sub>3</sub><sup>-</sup>, or deionized water,  
243 and incubated in an anaerobic chamber (90% N<sub>2</sub>, 8% CO<sub>2</sub>, and 2% H<sub>2</sub> 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 NH<sub>4</sub>Cl, 0.1 g KCl, 0.1 g KH<sub>2</sub>PO<sub>4</sub>, 0.2 g MgCl<sub>2</sub>·6H<sub>2</sub>O, and 0.04 g CaCl<sub>2</sub>·2H<sub>2</sub>O (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<sup>-2</sup> to 10<sup>-13</sup> 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 O<sub>2</sub> data, we used a generalized linear model with an autoregressive error  
263 term implemented using the glm function.

## 264 **Results**

265 Median bulk soil O<sub>2</sub> 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 O<sub>2</sub> were often small: below 0.5 m,  
268 median O<sub>2</sub> 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 O<sub>2</sub> varied by as much as 10 %  
270 over time (Fig. 2a). In contrast, depths below 0.5 m showed much less variability ( $< 1.5$  % O<sub>2</sub>)  
271 relative to median values.

272 Concentrations of Fe(II)<sub>HCl</sub> and Fe(III)<sub>HCl</sub> measured on soils sampled in May 2012  
273 showed different patterns from bulk soil O<sub>2</sub> (Fig. 2). Iron(II)<sub>HCl</sub> 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)<sub>HCl</sub> showed very similar depth trends as Fe(II)<sub>HCl</sub> (Fig. 2c). Patterns of short-term CO<sub>2</sub>  
276 production closely mirrored both Fe(II)<sub>HCl</sub> and Fe(III)<sub>HCl</sub> (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)<sub>HCl</sub> 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<sup>-1</sup>). Iron(III) addition had no significant effect on Fe(II)<sub>HCl</sub> 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  $\text{g soil}^{-1}$  decreased  $\text{Fe(II)}_{\text{HCl}}$  production in the presence of 100  $\mu\text{g glucose C}$   
292  $\text{g soil}^{-1}$ , whereas 0.5 mg Fe  $\text{g soil}^{-1}$  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  $\text{NO}_3^-$ ) did not significantly affect  
302 Fe reduction rates in surface (0 – 0.15 m) samples. However, Fe +  $\text{NO}_3^-$  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 +  $\text{NO}_3^-$ , acetate, and acetate +  $\text{NO}_3^-$  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 +  $\text{NO}_3^-$   
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 + NO<sub>3</sub><sup>-</sup> 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 + NO<sub>3</sub><sup>-</sup> (but  
315 not acetate or NO<sub>3</sub><sup>-</sup> 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 O<sub>2</sub> 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 O<sub>2</sub> concentrations were also highest (Figs. 2,3). Iron  
325 reduction declined by two orders of magnitude below 1 m despite decreased bulk soil O<sub>2</sub>, 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)<sub>HCl</sub> 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 O<sub>2</sub>, 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 O<sub>2</sub> 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 O<sub>2</sub> concentrations at 0.25 m relative to 0.1 m likely reflected lateral  
348 spatial heterogeneity in soil O<sub>2</sub> (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 O<sub>2</sub> 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 O<sub>2</sub> supply, high temperature  
390 decreases O<sub>2</sub> solubility while increasing biological O<sub>2</sub> 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 O<sub>2</sub> depth distribution for bedrock weathering*

403 Bulk soil O<sub>2</sub> 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 O<sub>2</sub> supply, given that O<sub>2</sub> 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 O<sub>2</sub> concentrations decrease monotonically with regolith  
409 depth, facilitating a negative feedback between regolith development and weathering rates. Our  
410 data show that bulk soil O<sub>2</sub> does not necessarily decrease consistently or significantly within the  
411 saprolite profile. Rather, the asymptotic trend in bulk soil O<sub>2</sub> observed here at depths below 1 m  
412 is consistent with analytical models of soil CO<sub>2</sub> 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 O<sub>2</sub> 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 O<sub>2</sub> 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 O<sub>2</sub> supply for primary mineral  
420 weathering.

#### 421 *Potential importance of anaerobic Fe oxidation at depth*

422           Although bulk soil O<sub>2</sub> 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   NO<sub>3</sub><sup>-</sup> can be a significant process in shallow groundwater (Böhlke et al. 2002). As a consequence,  
426   availability of both O<sub>2</sub> and NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> 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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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 O<sub>2</sub> 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, NO<sub>3</sub><sup>-</sup>, 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 NO<sub>3</sub><sup>-</sup> 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 O<sub>2</sub> supply to bedrock than previously proposed, given the observed  
479 asymptotic trend in O<sub>2</sub> with depth. In addition to O<sub>2</sub> availability, we showed that the supply of  
480 NO<sub>3</sub><sup>-</sup> 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).

## 492 **References**

- 493 Bazilevskaya E, Lebedeva M, Pavich M, et al (2013) Where fast weathering creates thin regolith  
494 and slow weathering creates thick regolith. *Earth Surf Process Landf* 38:847–858. doi:  
495 10.1002/esp.3369
- 496 Behrens R, Bouchez J, Schuessler JA, et al (2015) Mineralogical transformations set slow  
497 weathering rates in low-porosity metamorphic bedrock on mountain slopes in a tropical  
498 climate. *Chem Geol* 411:283–298. doi: 10.1016/j.chemgeo.2015.07.008
- 499 Böhlke JK, Wanty R, Tuttle M, et al (2002) Denitrification in the recharge area and discharge  
500 area of a transient agricultural nitrate plume in a glacial outwash sand aquifer, Minnesota.  
501 *Water Resour Res* 38:10–1. doi: 10.1029/2001WR000663
- 502 Bonneville S, Behrends T, Van Cappellen P (2009) Solubility and dissimilatory reduction  
503 kinetics of iron(III) oxyhydroxides: A linear free energy relationship. *Geochim*  
504 *Cosmochim Acta* 73:5273–5282. doi: 10.1016/j.gca.2009.06.006
- 505 Bowling DR, Egan JE, Hall SJ, Risk DA (2015) Environmental forcing does not induce diel or  
506 synoptic variation in the carbon isotope content of forest soil respiration. *Biogeosciences*  
507 12:5143–5160. doi: 10.5194/bg-12-5143-2015
- 508 Brantley SL, White AF (2009) Approaches to modeling weathered regolith. *Rev Mineral*  
509 *Geochem* 70:435–484. doi: 10.2138/rmg.2009.70.10
- 510 Buettner SW, Kramer MG, Chadwick OA, Thompson A (2014) Mobilization of colloidal carbon  
511 during iron reduction in basaltic soils. *Geoderma* 221–222:139–145. doi:  
512 10.1016/j.geoderma.2014.01.012
- 513 Buss HL, Bruns MA, Schultz MJ, et al (2005) The coupling of biological iron cycling and  
514 mineral weathering during saprolite formation, Luquillo Mountains, Puerto Rico.  
515 *Geobiology* 3:247–260. doi: 10.1111/j.1472-4669.2006.00058.x
- 516 Buss HL, Mathur R, White AF, Brantley SL (2010) Phosphorus and iron cycling in deep  
517 saprolite, Luquillo Mountains, Puerto Rico. *Chem Geol* 269:52–61. doi:  
518 10.1016/j.chemgeo.2009.08.001



- 519 Cerling TE (1991) Carbon dioxide in the atmosphere; evidence from Cenozoic and Mesozoic  
520 Paleosols. *Am J Sci* 291:377–400. doi: 10.2475/ajs.291.4.377
- 521 Chacon N, Silver WL, Dubinsky EA, Cusack DF (2006) Iron reduction and soil phosphorus  
522 solubilization in humid tropical forest soils: The roles of labile carbon pools and an  
523 electron shuttle compound. *Biogeochemistry* 78:67–84. doi: 10.1007/s10533-005-2343-3
- 524 Chapelle FH, McMahon PB, Dubrovsky NM, et al (1995) Deducing the distribution of terminal  
525 electron-accepting processes in hydrologically diverse groundwater systems. *Water*  
526 *Resour Res* 31:359–371.
- 527 Cheng L, Zhu J, Chen G, et al (2010) Atmospheric CO<sub>2</sub> enrichment facilitates cation release  
528 from soil. *Ecol Lett* 13:284–291. doi: 10.1111/j.1461-0248.2009.01421.x
- 529 Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial  
530 soil carbon dioxide losses to the atmosphere. *Proc Natl Acad Sci* 103:10316–10321. doi:  
531 10.1073/pnas.0600989103
- 532 Cusack DF, Silver WL, Torn MS, McDowell WH (2011) Effects of nitrogen additions on above-  
533 and belowground carbon dynamics in two tropical forests. *Biogeochemistry* 104:203–225.  
534 doi: 10.1007/s10533-010-9496-4
- 535 DeAngelis KM, Silver WL, Thompson AW, Firestone MK (2010) Microbial communities  
536 acclimate to recurring changes in soil redox potential status. *Environ Microbiol* 12:3137–  
537 3149. doi: 10.1111/j.1462-2920.2010.02286.x
- 538 Dubinsky EA, Silver WL, Firestone MK (2010) Tropical forest soil microbial communities  
539 couple iron and carbon biogeochemistry. *Ecology* 91:2604–2612. doi: 10.1890/09-1365.1
- 540 Emerson S, Hedges J (2003) Sediment diagenesis and benthic flux. *Treatise Geochem* 6:293–319.  
541 doi: 10.1016/B0-08-043751-6/06112-0
- 542 Fimmen RL, Richter D deB, Vasudevan D, et al (2008) Rhizogenic Fe–C redox cycling: a  
543 hypothetical biogeochemical mechanism that drives crustal weathering in upland soils.  
544 *Biogeochemistry* 87:127–141.
- 545 Fletcher RC, Buss HL, Brantley SL (2006) A spheroidal weathering model coupling porewater  
546 chemistry to soil thicknesses during steady-state denudation. *Earth Planet Sci Lett*  
547 244:444–457. doi: 10.1016/j.epsl.2006.01.055
- 548 Froelich PN, Klinkhammer GP, Bender ML, et al (1979) Early oxidation of organic matter in  
549 pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim*  
550 *Cosmochim Acta* 43:1075–1090. doi: 10.1016/0016-7037(79)90095-4
- 551 Fuss CB, Driscoll CT, Johnson CE, et al (2010) Dynamics of oxidized and reduced iron in a  
552 northern hardwood forest. *Biogeochemistry* 104:103–119. doi: 10.1007/s10533-010-  
553 9490-x

- 554 Hall SJ, McDowell WH, Silver WL (2013) When wet gets wetter: Decoupling of moisture, redox  
555 biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. *Ecosystems*  
556 16:576–589. doi: 10.1007/s10021-012-9631-2
- 557 Hall SJ, Silver WL (2013) Iron oxidation stimulates organic matter decomposition in humid  
558 tropical forest soils. *Glob Change Biol* 19:2804–2813. doi: 10.1111/gcb.12229
- 559 Hall SJ, Silver WL (2015) Reducing conditions, reactive metals, and their interactions can  
560 explain spatial patterns of surface soil carbon in a humid tropical forest. *Biogeochemistry*  
561 125:149–165. doi: 10.1007/s10533-015-0120-5
- 562 Hall SJ, Silver WL, Timokhin VI, Hammel KE (2016) Iron addition to soil specifically stabilized  
563 lignin. *Soil Biol Biochem* 98:95–98. doi: 10.1016/j.soilbio.2016.04.010
- 564 Hyacinthe C, Bonneville S, Van Cappellen P (2006) Reactive iron(III) in sediments: Chemical  
565 versus microbial extractions. *Geochim Cosmochim Acta* 70:4166–4180. doi:  
566 10.1016/j.gca.2006.05.018
- 567 Jeon B-H, Dempsey BA, Burgos WD (2003) Kinetics and mechanisms for reactions of Fe(II)  
568 with iron(III) oxides. *Environ Sci Technol* 37:3309–3315. doi: 10.1021/es025900p
- 569 Jobbagy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation  
570 to climate and vegetation. *Ecol Appl* 10:423–436. doi: 10.1890/1051-  
571 0761(2000)010[0423:TVDOSO]2.0.CO;2
- 572 Johnson AH, Xing HX, Scatena FN (2015) Controls on soil carbon stocks in El Yunque National  
573 Forest, Puerto Rico. *Soil Sci Soc Am J* 79:294. doi: 10.2136/sssaj2014.05.0199
- 574 Kappler A, Schink B, Newman DK (2005) Fe(III) mineral formation and cell encrustation by the  
575 nitrate-dependent Fe(II)-oxidizer strain BoFeN1. *Geobiology* 3:235–245. doi:  
576 10.1111/j.1472-4669.2006.00056.x
- 577 Keiluweit M, Nico PS, Kleber M, Fendorf S (2016) Are oxygen limitations under recognized  
578 regulators of organic carbon turnover in upland soils? *Biogeochemistry* 1–15. doi:  
579 10.1007/s10533-015-0180-6
- 580 Klee AJ (1996) Most Probable Number Calculator. US Environmental Protection Agency, Risk  
581 Reduction Engineering Laboratory, Cincinnati, OH, USA
- 582 Liermann LJ, Albert I, Buss HL, et al (2015) Relating microbial community structure and  
583 geochemistry in deep regolith developed on volcanoclastic rock in the Luquillo  
584 Mountains, Puerto Rico. *Geomicrobiol J* 32:494–510. doi:  
585 10.1080/01490451.2014.964885
- 586 Liptzin D, Silver WL (2015) Spatial patterns in oxygen and redox sensitive biogeochemistry in  
587 tropical forest soils. *Ecosphere* 6:1–14. doi: 10.1890/ES14-00309.1

- 588 Liptzin D, Silver WL, Detto M (2011) Temporal dynamics in soil oxygen and greenhouse gases  
589 in two humid tropical forests. *Ecosystems* 14:171–182. doi: 10.1007/s10021-010-9402-x
- 590 Lovley DR (1995) Microbial reduction of iron, manganese, and other metals. *Adv Agron*  
591 54:175–231.
- 592 Melton ED, Swanner ED, Behrens S, et al (2014) The interplay of microbially mediated and  
593 abiotic reactions in the biogeochemical Fe cycle. *Nat Rev Microbiol* 12:797–808. doi:  
594 10.1038/nrmicro3347
- 595 Nepstad DC, de Carvalho CR, Davidson EA, et al (1994) The role of deep roots in the  
596 hydrological and carbon cycles of Amazonian forests and pastures. *Nature* 372:666–669.  
597 doi: 10.1038/372666a0
- 598 Norkko J, Reed DC, Timmermann K, et al (2011) A welcome can of worms? Hypoxia mitigation  
599 by an invasive species. *Glob Change Biol* 18:422–434. doi: 10.1111/j.1365-  
600 2486.2011.02513.x
- 601 Peretyazhko T, Sposito G (2005) Iron(III) reduction and phosphorous solubilization in humid  
602 tropical forest soils. *Geochim Cosmochim Acta* 69:3643–3652. doi:  
603 10.1016/j.gca.2005.03.045
- 604 Pfennig N, Trüper HG (1992) The family Chromatiaceae. In: Balows A, Trüper HG, Dworkin M,  
605 et al. (eds) *The Prokaryotes*. Springer, New York, USA, pp 3200–3221
- 606 Ponnampetuma FN (1972) The chemistry of submerged soils. *Adv Agron* 24:29–96.
- 607 Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its  
608 relationship to vegetation and climate. *Tellus B* 44:81–99. doi: 10.1034/j.1600-  
609 0889.1992.t01-1-00001.x
- 610 Roden E, Wetzel R (1996) Organic carbon oxidation and suppression of methane production by  
611 microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland  
612 sediments. *Limnol Oceanogr* 41:1733–1748.
- 613 Roden EE, Wetzel RG (2002) Kinetics of microbial Fe(III) oxide reduction in freshwater  
614 wetland sediments. *Limnol Oceanogr* 47:198–211.
- 615 Roden EE, Zachara JM (1996) Microbial reduction of crystalline Iron(III) oxides: influence of  
616 oxide surface area and potential for cell growth. *Environ Sci Technol* 30:1618–1628. doi:  
617 10.1021/es9506216
- 618 Sanchez PA (1976) *Properties and Management of Soils in the Tropics*. John Wiley and Sons,  
619 New York
- 620 Schulz M, Stonestrom D, Lawrence C, et al (2016) Structured heterogeneity in a marine terrace  
621 chronosequence: upland mottling. *Vadose Zone J* 15:0. doi: 10.2136/vzj2015.07.0102

- 622 Schulz MS, White AF (1999) Chemical weathering in a tropical watershed, Luquillo Mountains,  
623 Puerto Rico III: quartz dissolution rates. *Geochim Cosmochim Acta* 63:337–350. doi:  
624 10.1016/S0016-7037(99)00056-3
- 625 Schwertmann U, Murad E, others (1988) The nature of an iron oxide-organic iron association in  
626 a peaty environment. *Clay Miner* 23:291–299.
- 627 Seitzinger S, Harrison JA, Böhlke JK, et al (2006) Denitrification across landscapes and  
628 waterscapes: a synthesis. *Ecol Appl* 16:2064–2090. doi: 10.1890/1051-  
629 0761(2006)016[2064:DALAWA]2.0.CO;2
- 630 Sexstone A, Revsbech N, Parkin T, Tiedje J (1985) Direct measurement of oxygen profiles and  
631 denitrification rates in soil aggregates. *Soil Sci Soc Am J* 49:645–651.
- 632 Silver WL, Liptzin D, Almaraz M (2013) Soil redox dynamics and biogeochemistry along a  
633 tropical elevation gradient. In: Gonzalez G, Willig MR, Waide RB (eds) *Ecological*  
634 *Gradient Analyses in a Tropical Landscape*. Wiley, NJ, USA,
- 635 Silver WL, Lugo AE, Keller M (1999) Soil oxygen availability and biogeochemistry along  
636 rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry*  
637 44:301–328. doi: 10.1023/A:1006034126698
- 638 Soil Survey Staff (2002) Soil survey of Caribbean National Forest and Luquillo Experimental  
639 Forest, Commonwealth of Puerto Rico. United States Department of Agriculture, Natural  
640 Resources Conservation Service
- 641 Solomon DK, Cerling TE (1987) The annual carbon dioxide cycle in a montane soil:  
642 Observations, modeling, and implications for weathering. *Water Resour Res* 23:2257–  
643 2265. doi: 10.1029/WR023i012p02257
- 644 Stone MM, DeForest JL, Plante AF (2014) Changes in extracellular enzyme activity and  
645 microbial community structure with soil depth at the Luquillo Critical Zone Observatory.  
646 *Soil Biol Biochem* 75:237–247. doi: 10.1016/j.soilbio.2014.04.017
- 647 Straub KL, Benz M, Schink B, Widdel F (1996) Anaerobic, nitrate-dependent microbial  
648 oxidation of ferrous iron. *Appl Environ Microbiol* 62:1458–1460.
- 649 Thamdrup B (2000) Bacterial manganese and iron reduction in aquatic sediments. In: Schink B  
650 (ed) *Advances in Microbial Ecology*. Springer, Boston, MA, pp 41–84
- 651 Thompson A, Chadwick OA, Boman S, Chorover J (2006) Colloid mobilization during soil iron  
652 redox oscillations. *Environ Sci Technol* 40:5743–5749. doi: 10.1021/es061203b
- 653 Thompson A, Rancourt D, Chadwick O, Chorover J (2011) Iron solid-phase differentiation along  
654 a redox gradient in basaltic soils. *Geochim Cosmochim Acta* 75:119–133. doi:  
655 10.1016/j.gca.2010.10.005

- 656 Veneman PLM, Vepraskas MJ, Bouma J (1976) The physical significance of soil mottling in a  
657 Wisconsin toposequence. *Geoderma* 15:103–118. doi: 10.1016/0016-7061(76)90081-1
- 658 Viollier E, Inglett P, Hunter K, et al (2000) The ferrozine method revisited: Fe(II)/Fe(III)  
659 determination in natural waters. *Appl Geochem* 15:785–790. doi: 10.1016/S0883-  
660 2927(99)00097-9
- 661 von Fischer JC, Hedin LO (2007) Controls on soil methane fluxes: Tests of biophysical  
662 mechanisms using stable isotope tracers. *Glob Biogeochem Cycle* 21:9. doi: Gb2007  
663 10.1029/2006gb002687
- 664 Weaver PL, Murphy PG (1990) Forest structure and productivity in Puerto Rico’s Luquillo  
665 Mountains. *Biotropica* 22:69–82. doi: 10.2307/2388721
- 666 Weber KA, Achenbach LA, Coates JD (2006) Microorganisms pumping iron: anaerobic  
667 microbial iron oxidation and reduction. *Nat Rev Microbiol* 4:752–764. doi:  
668 10.1038/nrmicro1490
- 669 White AF, Blum AE, Schulz MS, et al (1998) Chemical weathering in a tropical watershed,  
670 Luquillo Mountains, Puerto Rico: I. Long-term versus short-term weathering fluxes.  
671 *Geochim Cosmochim Acta* 62:209–226. doi: 10.1016/S0016-7037(97)00335-9
- 672 Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: Balows A,  
673 Trüper HG, Dworkin M, et al. (eds) *The Prokaryotes*. Springer New York, pp 3352–3378
- 674 Yang WH, Liptzin D (2015) High potential for iron reduction in upland soils. *Ecology* 96:2015–  
675 2020. doi: 10.1890/14-2097.1
- 676 Yang WH, Weber KA, Silver WL (2012) Nitrogen loss from soil through anaerobic ammonium  
677 oxidation coupled to iron reduction. *Nat Geosci* 5:538–541. doi: 10.1038/ngeo1530
- 678 Yi-Balan SA, Amundson R, Buss HL (2014) Decoupling of sulfur and nitrogen cycling due to  
679 biotic processes in a tropical rainforest. *Geochim Cosmochim Acta* 142:411–428. doi:  
680 10.1016/j.gca.2014.05.049

681

682 **Figure captions:**

683 Figure 1: Idealized depth profiles of bulk O<sub>2</sub> (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 O<sub>2</sub> and other oxidants (not shown for clarity) have been reduced. Panel **b**  
686 shows an alternative model for terrestrial soils where bulk O<sub>2</sub> 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 CO<sub>2</sub> and O<sub>2</sub> and an exponential decline in CO<sub>2</sub>  
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 O<sub>2</sub> 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)<sub>HCl</sub> (**b**), Fe(III)<sub>HCl</sub> (**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  $\text{NO}_3^-$ , Fe as ferrous chloride,  $\text{NO}_3^- + \text{Fe}$ , acetate, or acetate +  $\text{NO}_3^-$ . Means  
710 with different letters within a given depth increment differed significantly (n = 4 per treatment).

711

712

713

714

715

716

717

718

719

720

721

722

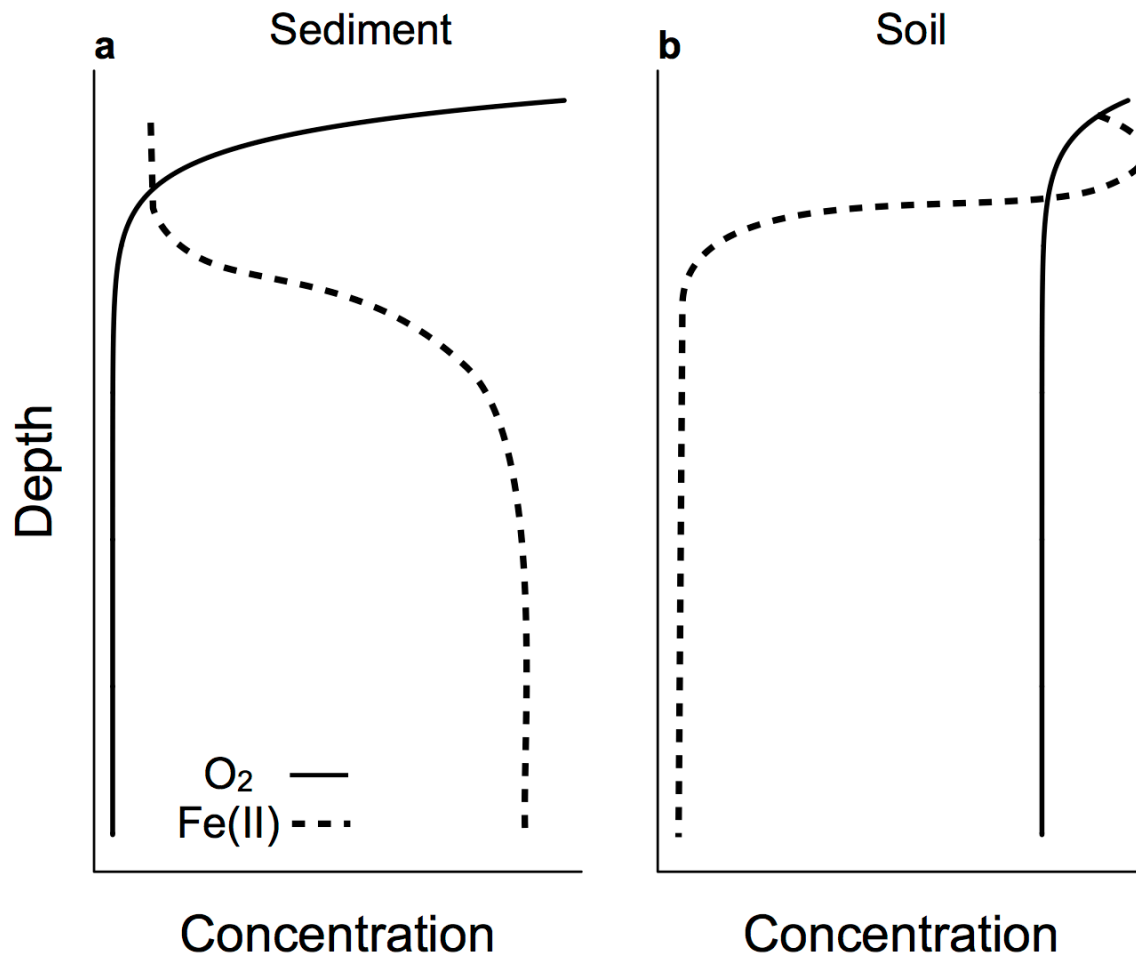
723

724

725

726

727 Figure 1:



728

729

730

731

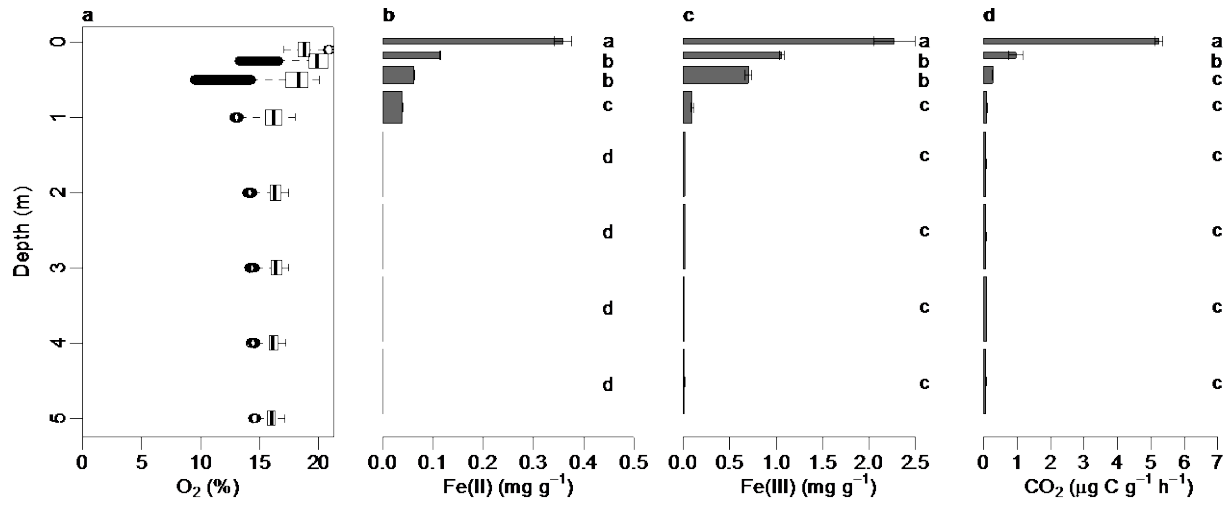
732

733

734



735 Figure 2:



736

737

738

739

740

741

742

743

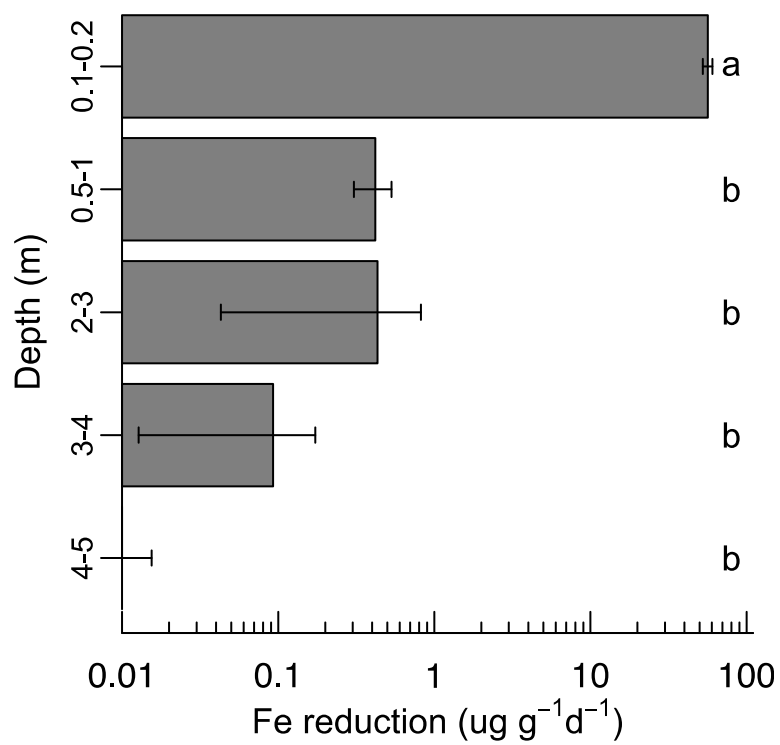
744

745

746

747

748 Figure 3:



749

750

751

752

753

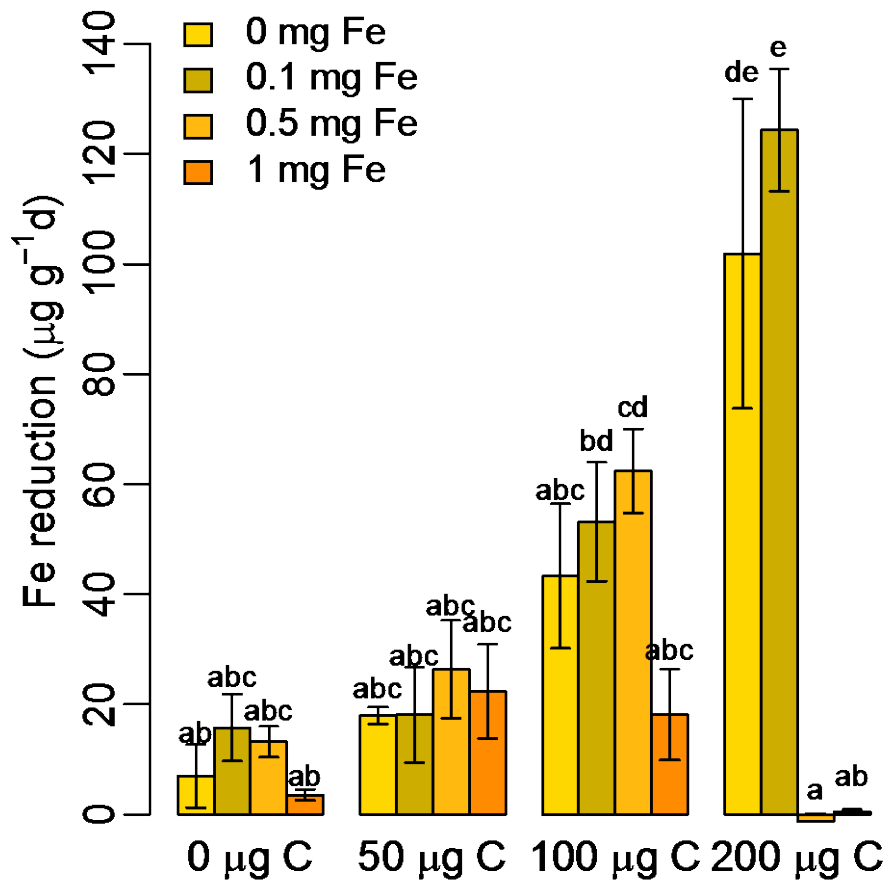
754

755

756

757

758 Figure 4:



759

760

761

762

763

764

765

766

