

1 **Phosphorus fractionation responds to dynamic redox conditions in a humid tropical forest**
2 **soil**

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15

16 **Abstract**

17 Phosphorus (P) is a key limiting nutrient in highly weathered soils of humid tropical forests. A
18 large proportion of P in these soils is bound to redox-sensitive iron (Fe) minerals; however, little
19 is known about how Fe redox interactions affect soil P cycling. In an incubation experiment, we
20 changed bulk soil redox regimes by varying headspace conditions (air vs. N₂ gas), and examined
21 the responses of soil P and Fe species to two fluctuating treatments (4- or 8-day oxic followed by
22 4-day anoxic) and two static redox treatments (oxic and anoxic). A static anoxic headspace
23 increased NaOH-extractable inorganic P (NaOH-P_i) and ammonium oxalate-extractable total P
24 (AO-P_t) by 10% and 38%, respectively, relative to a static oxic headspace. Persistent anoxia also
25 increased NaHCO₃-extractable total P (NaHCO₃-P_t) towards the end of the experiment. Effects
26 of redox fluctuation were more complex and dependent on temporal scales. Ammonium oxalate-
27 extractable Fe and P_i concentrations responded to redox fluctuation early in the experiment, but
28 not thereafter, suggesting a depletion of reductants over time. Immediately following a switch
29 from an oxic to anoxic headspace, concentrations of AO-P_t, AO-Fe, and HCl-extractable Fe(II)
30 increased (within 30 min), but fell back to initial levels by 180 min. Surprisingly, the labile P pool
31 (NaHCO₃-P_i) decreased immediately after reduction events, potentially due to resorption and
32 microbial uptake. Overall, our data demonstrate that P fractions can respond rapidly to changes in
33 soil redox conditions, and in environments where redox oscillation is common, roots and
34 microbes may benefit from these rapid P dynamics.

35

36 **Keywords**

37 Luquillo CZO and LTER, Puerto Rico, Ultisols, plant available phosphorous, Hedley
38 fractionation, Olsen P, redox oscillation, iron reduction

39 **1 Introduction**

40 Phosphorus (P) is a key limiting nutrient in many tropical forest soils that contributes to a
41 wide range of ecosystem functions, including net primary production, litter decomposition, soil
42 respiration, and microbial processes [Cleveland and Townsend, 2006; Herbert and Fownes, 1995;
43 Hobbie and Vitousek, 2000; Nottingham et al., 2015; Vitousek et al., 2010]. Understanding the
44 factors that control soil P availability is central to predicting the responses of tropical forests to
45 environmental change. In highly weathered, low pH soils, a large proportion of P can be bound to
46 secondary minerals that are rich in iron (Fe) and aluminum (Al). Via multiple mechanisms,
47 including adsorption, co-precipitation, and occlusion, Fe and Al oxides, hydroxides, and
48 oxyhydroxides can make P inaccessible to plant roots and microorganisms [Borch and Fendorf,
49 2007; Chacón et al., 2006; McGechan and Lewis, 2002], resulting in extremely low P
50 concentrations in the soil solution [Johnson et al., 2003; Wood et al., 2016]. Thus, Fe and Al
51 minerals play an important role in the dynamics and availability of P in highly weathered tropical
52 forest soils.

53 Humid tropical forest soils are often characterized by dynamic redox conditions [Liptzin et al.,
54 2011; Schuur and Matson, 2001; Silver et al., 1999], which strongly affect Fe mineral phases,
55 microbial communities, and nutrient cycling [DeAngelis et al., 2010; Pett-Ridge and Firestone,
56 2005; Pett-Ridge et al., 2013; Thompson et al., 2006]. The combination of abundant rainfall,
57 warm temperatures, finely textured soils, and high biological activity create redox oscillations
58 that range from fully oxic to anoxic conditions on the order of hours to weeks [Liptzin et al.,
59 2011; Silver et al., 1999]. Changes in redox conditions trigger rapid transformations of Fe
60 minerals [Peretyazhko and Sposito, 2005; Thompson et al., 2011]. Under anoxic conditions,
61 Fe(III) minerals are reduced to Fe(II) species, with a concomitant increase in soil pH and soil
62 organic matter decomposition [Dubinsky et al., 2010; Roden and Wetzel, 2002]. Upon
63 (re)exposure to O₂, these Fe(II) species can be quickly re-oxidized back to Fe(III)-bearing forms

64 by microaerophiles, nitrate-reducing Fe(II) oxidizers, or abiotic processes [Melton *et al.*, 2014].
65 Recent studies in humid tropical forest soils have shown that Fe(II) oxidation is linked to
66 increases in soil CO₂ production and release of dissolved organic matter [Hall and Silver, 2013;
67 Hall *et al.*, 2015]. As a large fraction of soil P is also known to be bound to redox-sensitive Fe
68 minerals, Fe-redox reactions likely play a critical role in regulating soil P cycling.

69 Under reducing conditions, transformation of Fe minerals can have complex effects on soil P
70 availability. On one hand, reductive dissolution of Fe(III) species can release P into soil solution
71 through desorption of P from Fe and other clay minerals and the dissolution and hydrolysis of Fe
72 and Al phosphates [Hutchison and Hesterberg, 2004; Peretyazhko and Sposito, 2005; Sallade
73 and Sims, 1997]. Neutralizing effects of reduction reactions also increase P desorption via
74 deprotonation of hydroxyl groups from mineral surfaces [Barrow, 1984]. On the other hand, even
75 though newly formed Fe(II) species bind phosphate less strongly than Fe(III) (oxyhydr)oxides,
76 they tend to have a higher surface area and potentially more phosphate-sorption sites [Patrick and
77 Khalid, 1974]. Thus, Fe reduction can increase P sorption capacity of soils in some cases
78 [Holford and Patrick, 1979; Zhang *et al.*, 2003]. In addition, the build-up of Fe(II) under anoxic
79 conditions can promote the co-precipitation of Fe(II)-P complexes, consequently reducing P
80 availability [Borch and Fendorf, 2007; Roden and Edmonds, 1997]. Dynamics of these
81 competing mechanisms appear to vary over time [Karathanasis and Shumaker, 2009]. For
82 example, Heiberg *et al.* [2012] reported that soluble P levels in two wetland soils with different
83 soil texture both peaked roughly 30 days after the start of an anoxic incubation as a result of
84 desorption, but then fell back to its initial level due to re-adsorption and precipitation of vivianite.

85 Compared to the relatively rich literature on P cycling under reducing conditions, little is
86 known about how redox *fluctuation* affects P availability in humid tropical forests soils.
87 Reduction and oxidation of Fe (oxyhydr)oxides can occur relatively quickly in humid tropical
88 soils, on timescales of hours to days [Charlet *et al.*, 2013; Hall and Silver, 2013]. In addition,

89 these soils host a diverse microbial community that is sensitive to rapid shifts in redox
90 environments [DeAngelis et al., 2010; Pett-Ridge and Firestone, 2005; Pett-Ridge et al., 2013;
91 Pett-Ridge et al., 2006; Thompson et al., 2006]. Thus, redox oscillation-driven changes in Fe
92 species and associated microbial activities likely result in similarly fast responses within soil P
93 pools. Phosphorus bound to amorphous minerals may be particularly sensitive to redox oscillation
94 given the high redox sensitivity of amorphous Fe [Liptzin and Silver, 2009; Thompson et al.,
95 2006]. Recent studies found that frequent shifts in redox conditions led to more rapid Fe
96 reduction and greater accumulation of Fe(II) [Barcellos et al., 2018; Ginn et al., 2017]. It is likely
97 that these strong Fe reduction responses might also result in greater increases in P solubility under
98 more frequent oscillation regimes. We thus expect that the frequency of redox oscillation affects
99 the redox sensitivity of both Fe and P pools.

100 In this study, we evaluated how dynamic headspace redox regimes alter soil P fractions and
101 how these relate to reactive Fe species. In a laboratory incubation experiment where bulk soil
102 redox status was regulated by controlling microcosm headspace (ambient air vs N₂), soils from a
103 humid tropical forest were exposed to four treatments: two fluctuating regimes (high vs low
104 frequency of redox status shifts); static oxic (ambient air), and anoxic (N₂) headspace conditions.
105 We evaluated the effects of dynamic and static conditions on P and Fe species over a 44-day
106 incubation, as well as during the hours immediately following a shift in headspace redox state.
107 We hypothesized that 1) the *frequency* of redox fluctuation would determine the *magnitude* of
108 response in soil P and Fe pools. We predicted that redox fluctuation would increase Fe reduction
109 and P solubility relative to static oxic conditions, and stronger increases in Fe and P pools would
110 be observed under more frequent redox fluctuation. We also hypothesized that 2) soil P would
111 respond *rapidly* to changes in headspace redox conditions on the scale of hours, mirroring
112 patterns of Fe reduction and re-oxidation.

113

114 **2 Materials and Methods**

115 *2.1. Experimental Design*

116 Soils were collected near the El Verde field station in the Luquillo Experimental Forest (LEF),
117 Puerto Rico (18.3211° N, 65.8175° W). The vegetation is characteristic of the tabonuco
118 (*Dacryodes excelsa*) forest [Thompson *et al.*, 2002] with mean annual temperature of 23 °C and a
119 range of 23.5 to 27 °C [Brown *et al.*, 1983]. Mean annual precipitation is approximately 3.5 m
120 and relatively aseasonal, with no month receiving less than 200 mm precipitation [Scatena, 1989].
121 Soils were collected along a 15° slope. Previous research has shown that topography exerts a
122 strong control on soil moisture, O₂, C, and Fe dynamics in these soils, with slopes experiencing
123 intermediate O₂ concentrations and fluctuations relative to the more anoxic valleys and well
124 aerated ridges [Hall and Silver, 2015; O'Connell *et al.*, 2018; Silver *et al.*, 1999]. Slopes are the
125 dominant landscape position in these ecosystems accounting for 65% of the land area [Scatena
126 and Lugo, 1995]. Soils are classified as Humic Haploperox, relatively well-drained, and
127 developed from volcanoclastic rock with 57 ± 3% sand, 23 ± 1% silt, and 20 ± 3% clay
128 [O'Connell *et al.*, 2018; Soil Survey Staff, 1995]. The average soil C concentration, pH, and soil
129 moisture content (vol.) was 3.4 ± 0.5%, 4.9 ± 0.06, and 51 ± 1%, respectively [values are ± 1
130 standard error; O'Connell *et al.*, 2018].

131 Soil was collected from a 1 x 1 m plot at an upper slope position from the 0-10 cm depth and
132 shipped overnight at ambient temperature to Lawrence Livermore National Laboratory, in
133 Livermore, California. In the lab, soil was homogenized by manually mixing and removing
134 visible plant debris, rocks, and soil macro-fauna. Twenty g (oven dry equivalent weight, ODW)
135 subsamples of moist soil were weighed into glass gas-tight 487 mL incubation vessels (hereafter
136 referred to as microcosms). A total of 178 replicate microcosms were prepared at the outset of our
137 incubation experiment (Table S1). Each microcosm was capped with a gas-tight lid fitted with a
138 GeoMicrobial septum (Geo-Microbial Technologies MT, Ochelata, Oklahoma, USA), Swagelok

139 fitting, two-way stop-stock, and tygon tubing such that each microcosm could be sealed and
140 disconnected from the main gas flushing system. Soils were incubated in the dark. The soil
141 headspace was flushed with either humidified ambient air (oxic) or N₂ gas (anoxic) at a flow rate
142 of 3 ml/min. When switching headspace gas, a higher flow rate (16 ml/min) was used to achieve
143 headspace turnover in roughly 30 minutes. Gas entered the microcosm via the fitting and exited
144 via a syringe needle in the septum. This design allowed us to manipulate the soil headspace,
145 which previous work has shown alters soil redox biogeochemistry, such as headspace O₂, nitrous
146 oxide, and methane concentrations, Fe speciation, nitrogen cycling, and microbial community
147 dynamics [DeAngelis *et al.*, 2010; Pett-Ridge, 2005; Pett-Ridge and Firestone, 2005; Pett-Ridge
148 *et al.*, 2013; Pett-Ridge *et al.*, 2006]. Soil in the microcosms was fresh from the field and
149 uncompacted with small aggregates (≤ 3 mm diameter). Soil formed a thin and even layer within
150 the incubation chambers so that rapid gas exchange was possible between the microcosm
151 headspace and the inter-aggregate pore space. While we did not measure redox potential (Eh)
152 directly, previous experience indicates that Fe oxides strongly poison these Fe-rich soils [Hodges,
153 2017; Whitfield, 1974].

154 Soil was pre-incubated for 16 days in the microcosms under a 4-day ambient air / 4-day N₂
155 (hereafter called 'oxic' and 'anoxic', respectively) regime to allow microbial activity to stabilize.
156 After pre-incubation, microcosms were amended with 0.18 g commercially prepared ground leaf
157 litter. Previous work suggests humid tropical forest soils can quickly deplete labile C stocks
158 during laboratory incubations [Chacón *et al.*, 2006]; thus leaf litter equivalent to 10% of the
159 native soil C pool was added (without mixing) to sustain microbial activity during the
160 experiment. Litter was either ¹³C labeled (97 atom%) or unlabeled ¹²C ryegrass (Cambridge
161 Isotopes Laboratories, Inc.). Isotope tracing results are discussed in a companion paper focused
162 on microbial community dynamics. Statistical analyses revealed no significant effects of litter C
163 isotopic composition on soil P and Fe pools on days when both labeled and unlabeled

164 microcosms were harvested (Table S2), thus the data we present are from pooled ^{12}C and ^{13}C
165 microcosms.

166 Microcosms were divided into four headspace treatment groups: (1) static anoxic, (2) static
167 oxic, (3) 4-day oxic/4-day anoxic (high frequency), (4) 8-day oxic/4-day anoxic (low frequency),
168 and were incubated for 44 days, with 3 replicates per treatment and harvest point (5 replicates for
169 the final harvest), beginning with an oxic phase (air) in the fluctuating treatments (Figure S1). On
170 day 23, both fluctuating treatments were switched to oxic conditions (air headspace) after a
171 slightly shorter time of exposure to N_2 (3 days instead of 4 days) for logistical reasons.

172 *2.2 Soil Sampling and Analyses*

173 At five timepoints (day 12, 20, 23, 36, and 44, Table S1), replicate microcosms from all
174 treatments were destructively harvested, with additional harvests of high and low frequency
175 treatment microcosms on day 16 and day 32, respectively, to examine the responses of soil P and
176 Fe dynamics throughout the experiment. All harvests, with the exception of the final one (day
177 44), coincided with a switch in headspace gas for the two fluctuating treatments. Fluctuating
178 microcosms were harvested immediately prior to switching the headspace gas (0 minute, M0),
179 and then at 30 minutes (M30) and 180 minutes (M180) after switching the headspace gas, in
180 order to examine short-term biogeochemical responses to the change in oxygen availability.
181 Similar rapid changes in soil redox conditions have been observed in humid tropical forest soils
182 [Liptzin *et al.*, 2011]. After opening the microcosms, soil was quickly mixed and subsampled for
183 chemical analyses. For microcosms with an anoxic headspace, microcosms were opened and
184 subsampled within an anaerobic glove box (Coy Laboratory Products, Grass Lake, MI).
185 Extraction reagents used within the anaerobic chamber were degassed with He prior to extraction.

186 Soil P was sequentially extracted with 0.5 M sodium bicarbonate (NaHCO_3 , 1 g ODW fresh
187 soil in 45 ml solution) and 0.1 M sodium hydroxide solution (NaOH , 45 ml solution) [Table 1;

188 *Tiessen and Moir*, 1993]. NaHCO_3 -extractable P is weakly adsorbed on soil particles and thought
189 to be readily available to plants [*Hedley et al.*, 1982]. In contrast, NaOH solubilizes P that is
190 chemically sorbed to secondary Fe- and Al-minerals, and thought to represents a more stable
191 fraction of soil P relative to NaHCO_3 -extractable P [*McLaughlin et al.*, 1977]. Total P in both
192 extracts ($\text{NaHCO}_3\text{-P}_t$ and NaOH-P_i) was determined by measuring PO_4 according to the standard
193 colorimetric method of *Murphy and Riley* [1962] after autoclaving extracted solutions with
194 ammonium persulfate ($(\text{NH}_4)_2\text{S}_2\text{O}_8$) [*Tiessen and Moir*, 1993]. Inorganic P was also determined
195 in the NaOH extract (NaOH-P_i) following *Murphy and Riley* [1962] after acidifying and
196 centrifuging the extractant [*Tiessen and Moir*, 1993]. Organic P in the NaOH extract (NaOH-P_o)
197 was estimated by subtracting inorganic P from total P. Consistent with previous research [*Mage*
198 *and Porder*, 2012], inorganic P in the NaHCO_3 extract was below the detection limit, likely due
199 to the high P sorption capacity of Fe- and Al-minerals, and thus was not reported here.

200 Two assays were used to quantify redox-sensitive soil Fe pools. First, we used an hydrochloric
201 acid (HCl) extraction to solubilize reactive Fe(II) and Fe(III) from short-range order and organo-
202 Fe complexes [*Fredrickson et al.*, 1998]; 1.0 g (ODW) fresh soil was extracted in 5 ml 0.5 M HCl
203 solution. The Fe(II) concentration of the HCl extract (HCl-Fe(II)) was analyzed using a modified
204 Ferrozine assay [*Viollier et al.*, 2000]. Second, we used ammonium-oxalate (AO) to extract
205 chelatable short-range order (oxy)hydroxides and organo-Fe complexes [*McKeague and Day*,
206 1966]. This pool is generally defined as amorphous or poorly crystalline Fe [*Miller et al.*, 2001;
207 *Thompson et al.*, 2006; *Torn et al.*, 1997]. Subsamples of 0.25 g (ODW) fresh soil were extracted
208 with 10 mL of AO solution in the dark at pH 3 [*Loeppert and Inskeep*, 1996]. The Fe, Al, and P
209 concentrations in the filtered supernatant (0.45 μm ; AO-Fe, AO-Al, and AO- P_t , respectively)
210 were measured using a PerkinElmer SCIEX Elan DRC II inductively coupled plasma mass
211 spectrometer.

212 2.3 Statistical Analyses

213 To evaluate the effects of redox treatments on P and Fe pools over the entire experiment, we
214 compiled data collected immediately before the switch in headspace for the fluctuating
215 treatments, corresponding to days 12, 20, 23, 36, and 44 of the experiment. Data were analyzed
216 with a two-way analysis of variance (ANOVA), in which redox treatment and sampling day were
217 two main factors. Sampling day was treated as a categorical variable instead of a continuous
218 variable because many data series did not show clear linear trends over time. Repeated measures
219 ANOVA are not applicable here, as samples were destructively harvested on each sampling day.
220 If the effect of redox treatment was significant at $\alpha = 0.05$ level, we then compared differences
221 among redox treatments using Tukey's tests to control the family-wise error rate at $\alpha = 0.05$ level
222 with the R package 'lsmeans'. When the interaction effect between redox and sampling day was
223 significant, we used one-way ANOVA followed by Tukey's tests to examine the redox effects on
224 individual days. HCl-extractable Fe data were log transformed before statistical analyses to meet
225 the assumptions of ANOVA.

226 For the two fluctuating treatments, we examined how P and Fe pools changed immediately
227 before and after the 4- or 8-day anoxic or oxic phase. In total, we monitored three $N_2 \rightarrow$ ambient
228 air (oxidation) events and four ambient air $\rightarrow N_2$ (reduction) events. For each event, student's T-
229 tests were used to compare the redox effects on P and Fe pools. We did not pool these events to
230 analyze the average effects of redox manipulation due to data overlap. For example, data from
231 day 16 of the high frequency treatment were involved in a reduction event (day 12 to 16) and an
232 oxidation event (day 16 to 20).

233 Finally, we explored the effects of a shift in headspace redox conditions at 0 (M0), 30 (M30),
234 and 180 min (M180) after headspace switches on days 12, 20, 23, and 36. Soil P and Fe data from
235 both fluctuating treatments were pooled and analyzed in two categories, depending on whether
236 data were collected during an $N_2 \rightarrow$ ambient air switch (oxidation) or ambient air $\rightarrow N_2$
237 (reduction) event. In total, there were four oxidation events and four reduction events. Differences

238 in P and Fe species pools with time (0, 30, 180 minutes) were compared using two-way ANOVA
239 followed by multiple comparisons controlled by Tukey's tests. Time and sampling event were
240 treated as two main factors. All statistical analyses were conducted in R ver 3.3.3 [*R Core Team,*
241 2017]. Mean values \pm standard errors and *P* values from Tukey tests are presented unless
242 otherwise noted.

243 **3 Results**

244 *3.1 Static Headspace Treatments*

245 The static anoxic treatment significantly increased the concentrations of most soil P and Fe
246 species over the experiment. Concentrations of NaOH-P_i (inorganic P bound to Fe/Al minerals;
247 Figure 1; *P* < 0.01) and AO-P_t pools (P bound to amorphous Fe/Al minerals; *P* < 0.001) in the
248 static anoxic treatment were $10 \pm 3\%$ and $38 \pm 8\%$ higher than in the static oxic treatment,
249 respectively. The static anoxic treatment also increase the NaHCO₃-P_t pool relative to the static
250 oxic treatment toward the end of the experiment (Figure S2; day 36, *P* < 0.05; day 44, *P* < 0.01),
251 although the effect was not significant when analyzing the experiment as a whole. The
252 concentration of NaOH-P_o (organic P bound to Fe/Al minerals) was not affected by redox
253 treatments. Effects of the static anoxic treatment on Fe species were much stronger than those on
254 P fractions. The static anoxic treatment caused a 15-fold increase in HCl-Fe(II) relative to the
255 static oxic treatment (*P* < 0.001) and also doubled AO-Fe concentrations (*P* < 0.001).

256 *3.2 Fluctuating Redox Treatments*

257 Averaging over the experiment, the low frequency fluctuation treatment increased soil HCl-
258 Fe(II) concentrations relative to the static oxic treatment (Figure 1; *P* < 0.01), but the high
259 frequency treatment did not. The two fluctuating redox treatments did not affect soil AO-Fe
260 concentrations relative to the static oxic treatment. The low frequency fluctuation increased AO-
261 P_t concentrations by $28 \pm 8\%$ relative to the static oxic treatment (*P* < 0.01), but there was no

262 effect of the high frequency treatment. The two fluctuating redox treatments did not affect other
263 soil P pools relative to the static oxic treatment over the experiment.

264 Soil HCl-Fe(II) concentrations were sensitive to the 4-day and 8-day shifts in headspace redox
265 conditions (Figure 2). Concentrations of HCl-Fe(II) consistently decreased during oxidation
266 events and increased during reduction events. The response of HCl-Fe(II) to redox events
267 gradually decreased in magnitude over time. For example, the redox effect was only marginally
268 significant (T-test: $P = 0.09$) during the last oxidation event between day 36 and 44 under the low
269 frequency treatment. In addition, the HCl-Fe(II) concentration under oxic headspace conditions
270 tended to increase over time (linear regression: $P < 0.001$). Similar redox effects were observed in
271 AO-Fe and AO-P_i pools only during the first set of redox events, but not thereafter. Both soil
272 AO-P and AO-Fe concentrations decreased during the first oxidation event (Figures 3 and S3; T-
273 test: both $P < 0.05$). The AO-Fe pool increased during the first reduction event (T-test: $P <$
274 0.001), and AO-P tended to increase (T-test: $P = 0.13$). The soil NaOH-P_i pool decreased during
275 two out of three oxidation events (Figure S4), while it did not respond to reduction events.
276 Neither NaHCO₃-P_i nor NaOH-P_o pool responded to these redox events.

277 3.3 Short-term Effects of Redox Fluctuation

278 To understand the short-term effects of a redox shift on P and Fe pools, we harvested
279 microcosms immediately before, and 30 and 180 minutes following a change in headspace redox
280 conditions. Averaging across sampling events, concentrations of HCl-Fe(II) decreased by $28 \pm$
281 10% (Figure 4; $P < 0.05$) at 180 min following oxidation events, increased by $37 \pm 7\%$ ($P <$
282 0.001) at 30 min following reduction events, and returned to their initial levels by 180 min ($P <$
283 0.01). Similarly, concentrations of AO-Fe and AO-P_i both increased at 30 min following the
284 reduction event (both $P < 0.01$), and returned to their initial levels by 180 min (both $P < 0.05$). In
285 contrast, NaHCO₃-P_i concentrations decreased at 180 min following reduction events ($P < 0.05$).
286 Following oxidation events, soil AO-Fe ($P = 0.07$) and NaOH-P_i pools ($P = 0.11$) tended to

287 decrease at 180 min, but the NaOH-P_i pool did not respond to reduction events. The soil NaOH-
288 P_o pool was not sensitive to either oxidation or reduction events.

289 **4 Discussion**

290 *4.1 Effects of Static Redox Conditions on P and Fe Cycling*

291 The static anoxic treatment elevated the concentrations of NaHCO₃-P_t, NaOH-P_i, and AO-P_t
292 relative to the static oxic treatment. It also led to a significant reduction of ferric (oxyhydr)oxides
293 and accumulation of HCl-Fe(II) and AO-Fe. These results are consistent with previous work
294 using soils from the LEF [Chacón *et al.*, 2006; Liptzin and Silver, 2009], and suggest that static
295 anoxic conditions effectively increased the total amount of extractable P. This increase likely
296 represents a loss of P from other pools (not included in this study) and/or may represent an
297 increase in the extraction efficiency of the solutions (i.e., NaHCO₃, NaOH, and AO). Reduction
298 events can decrease microbial biomass P and convert more recalcitrant P pools (e.g., HCl-
299 extractable P and residual P) to more labile forms [Ajmone-Marsan *et al.*, 2006; Wright *et al.*,
300 2001; Yang *et al.*, 2006; Zhang *et al.*, 1994]. Reducing conditions are also known to decrease the
301 strength of Fe minerals in sorbing P [Chacón *et al.*, 2006; Heiberg *et al.*, 2012; Peretyazhko and
302 Sposito, 2005], which could lead to an increase in the extraction efficiency of NaOH and AO
303 solutions. Elevated Fe(II) could further catalyze the reductive dissolution of crystalline Fe in the
304 presence of organic ligands such as oxalate [Kostka and Luther, 1994; Sulzberger *et al.*, 1989]
305 and likely increase the AO-extractable P.

306 The effects of the static anoxic treatment on Fe species were much greater than those on P
307 fractions, suggesting that Fe-associated soil P was much less abundant relative to the mineral
308 surfaces available for P sorption. It is possible that the static anoxic treatment initially mobilized
309 amorphous Fe minerals that were relatively enriched in P, while continued anoxic conditions
310 transformed the amorphous and crystalline Fe minerals that were P-poor. Re-sorption is another

311 mechanism that could explain the weaker responses of P pools to static anoxic treatments
312 compared to those of Fe pools [Barros *et al.*, 2005; Heiberg *et al.*, 2012]. Reduced Fe minerals,
313 despite weaker P bonding strength than oxidized forms, can still have high surface area for
314 binding P [Borch and Fendorf, 2007; Patrick and Khalid, 1974]. The extremely low P
315 concentration makes the precipitation of metal phosphate, such as vivianite, highly unlikely
316 [Walpersdorf *et al.*, 2013]. Thus resorption is likely responsible for the lack of response in
317 $\text{NaHCO}_3\text{-P}_i$ in the static anoxic treatment before day 36 of the experiment. If indeed $\text{NaHCO}_3\text{-P}_i$
318 was released during reduction events and then quickly re-captured by the mineral matrix, then the
319 gross release of labile P would be greater in magnitude than the net increase observed here.

320 *4.2 Effects of Dynamic Redox Conditions*

321 Effects of redox fluctuation on P fractions were more complex compared to those of static
322 anoxic conditions. Consistent with our first hypothesis, the soil Fe(II) pool was sensitive to redox
323 fluctuation across the experiment. However, contrary to our hypothesis, soil P pools were not
324 consistently affected by fluctuating redox treatments at the weekly time scales. Effects of
325 fluctuating redox on soil Fe(II) were also much less prominent compared to those of static anoxic
326 conditions. The prevalence and persistence of Fe(III) during short-term oscillations in O_2
327 availability likely inhibit changes in P solubility in fluctuating redox treatments, thus decreasing
328 the effects of redox dynamics. Rapid Fe(II) oxidation contributes significantly to the persistence
329 of Fe(III) minerals, as this study and other recent work show declines of Fe(II) within hours after
330 exposure to O_2 [Ginn *et al.*, 2017; Hall and Silver, 2013]. We also found that the response of
331 Fe(II) to redox dynamics decreased in magnitude over the experiment. This reduced sensitivity to
332 redox manipulation could result from the gradual depletion of labile organic matter over time,
333 which served as the reductants of iron reduction [Bhattacharyya *et al.*, 2018]. Our results imply
334 that continuous, strong reducing conditions are needed in order to elevate P solubility at weekly
335 time scales in this Fe-rich tropical soil.

336 Amorphous P and Fe pools also showed signs of a reduced sensitivity to redox dynamics over
337 time, as they only responded to the 4 and 8-day redox events early on in the experiment. Repeated
338 redox oscillation could increase the crystallinity of amorphous minerals [Thompson *et al.*, 2006]
339 and promote the formation of organo-Fe-P complexes that were stable during redox fluctuations
340 [Eusterhues *et al.*, 2014]. We also found that AO-P₁ was increased by the low frequency
341 treatment, but not the high frequency treatment, even though the latter experienced longer anoxic
342 exposure over the entire experiment. This observation is consistent with our interpretation that
343 repeated redox fluctuation can decrease the redox sensitivity of amorphous minerals and
344 stabilized P bound to them. Contrary to expectation, the reduction events between day 20 and 23
345 decreased, rather than increased, AO-P₁ pool in both fluctuating redox treatments. We do not
346 fully understand this phenomenon but speculate that it could be related to the declines of both
347 AO-P₁ and AO-Fe pools in fluctuating and static oxic treatments since the start of the experiment.
348 Our results together suggest complex effects of dynamic redox conditions on the P associated
349 with amorphous minerals.

350 The type of redox environment likely influences the responses of soil P and Fe pools,
351 especially during redox oscillations. By employing anoxic glovebox with 5% H₂ and using soil
352 slurries with addition of organic matter, Barcellos *et al.* [2018] and Ginn *et al.* [2017] showed
353 that repeated redox oscillation increased the redox sensitivity of amorphous Fe minerals,
354 eventually resulting in comparable Fe(II) concentrations between fluctuating redox and static
355 anoxic treatments. Their designs likely stimulated Fe reduction and created stronger reducing
356 conditions than those in our experiment achieved with headspace manipulation. Quantifying
357 redox potential is difficult in Fe-rich soils characterized by heterogeneous microsite dynamics
358 [Silver *et al.*, 1999]. Future breakthroughs on this front would improve the upscaling of
359 laboratory results to an ecosystem level.

360 4.3 Short-term Impacts of Redox Fluctuations

361 Consistent with our second hypothesis, the temporal patterns of HCl-Fe(II), AO-Fe, and AO-
362 P_i followed similar patterns shortly after reduction events: all increased after 30 min, but fell back
363 to initial levels after 180 min. The similar dynamics of Fe and P species over these short time
364 scales suggests that soil P cycling is responsive to redox-induced changes in Fe minerals in these
365 humid tropical soils. These rapid responses highlight the importance of temporal scale in P and Fe
366 cycling with redox fluctuation. For example, dynamic redox conditions have significant but short-
367 lived effects on soil P cycling that could be missed by infrequent sampling. These results also
368 demonstrated that our headspace manipulation was effective in quickly altering soil redox
369 conditions and influencing Fe reduction. The increases in Fe and P species at 30 min were
370 consistent with our expectation that anoxic conditions promoted the reductive dissolution of Fe
371 oxides leading to the subsequent desorption and release of P. The subsequent decreases in these
372 Fe and P species suggest the existence of an active sink that mediates the variation in reduced Fe
373 and P fractions. As discussed above, amorphous minerals may serve as such a sink due to their
374 high sorption potential.

375 Although short-term oxidation events consistently decreased HCl-Fe(II), they did not strongly
376 influence Fe and P pools associated with amorphous minerals. We did find that AO-Fe and
377 NaOH- P_i tended to decrease during these oxidation events. Similar declines of NaOH- P_i were
378 observed during the 4 and 8-day oxidation events. These results suggest that P responses to redox
379 switches are potentially pool-specific and that P bound to crystalline Fe and Al minerals may be
380 more sensitive to oxidation than that bound to amorphous minerals. Iron oxidation led to the
381 formation of Fe (oxyhydr)oxides, which are known to increase the strength of P sorption
382 [Hutchison and Hesterberg, 2004; Sallade and Sims, 1997; Zak *et al.*, 2010] and likely decrease P
383 extractability. Co-precipitation of organo-Fe-P complexes during Fe oxidation might also make
384 some P inaccessible.

385 Interestingly, labile P ($\text{NaHCO}_3\text{-P}_1$) decreased immediately following reduction events. Iron
386 redox dynamics are not a good explanation for this phenomenon, because reductive dissolution of
387 Fe minerals should increase, rather than decrease, P availability. Sorption by organic matter could
388 be one sink of this $\text{NaHCO}_3\text{-P}_1$; a companion study showed that short-term reduction events
389 tripled dissolved organic carbon concentration [*Bhattacharyya et al.*, 2018]. There might also be
390 competition between dissolved organic carbon and P for soil sorption sites, such that release of
391 dissolved organic carbon could increase P sorption by soil minerals [*Guppy et al.*, 2005].
392 Microbial uptake is yet another potential sink, particularly if recently mobilized P diffused into
393 aerobic microsites or was utilized by anaerobic microorganisms. Indeed, *Olander and Vitousek*
394 [2005] found that microbial P uptake can account for a higher proportion of newly added PO_4^-
395 than mineral sorption.

396 Organic P in the NaOH extract accounted for the majority of P measured in these soils, and
397 concentrations of NaOH- P_o and other P fractions we measured are comparable to previous
398 studies conducted in the LEF [*Mage and Porder*, 2012; *McGroddy and Silver*, 2000]. We also
399 found that NaOH- P_o was not sensitive to headspace manipulation at either short or longer time
400 scales, suggesting that if organic P is sorbed on Fe and Al minerals, it is more stable than
401 inorganic P.

402 *4.4 Ecological Implications*

403 Humid tropical forest soils experience periodic anoxic conditions at hourly to weekly time
404 scales [*Liptzin et al.*, 2011; *Silver et al.*, 1999]. Our results demonstrate that effects of redox
405 oscillation on soil P fractions, and potentially P availability to plants and microbes, vary over
406 time. Both labile ($\text{NaHCO}_3\text{-P}_1$) and intermediate (AO- P_1) P pools were highly responsive to a
407 change in bulk redox condition. However, when analyzed at daily to weekly time scales, effects
408 of redox oscillation on soil P pools tended to be less significant. Given that P dynamics are
409 governed by various competing mechanisms, including reductive dissolution, desorption versus

410 adsorption, and biological uptake, the net changes in P fractions we observed at longer time scale
411 (weekly to monthly) likely underestimate the gross flux of P released by dissolution and
412 desorption. In soils that experience regular redox oscillations, such as humid tropical forests, the
413 first several hours following redox shifts may be a critical period for P bioavailability.

414 Our results also suggest that redox fluctuation may increase P accessibility for plants and
415 microbes, since we found that AO-P_i concentrations were enhanced by anoxic conditions at
416 hourly timescales. Periodic redox fluctuation might provide favorable conditions for plants and
417 microbes to acquire soil P that would otherwise be tightly bound to soil Fe minerals. The role of
418 redox fluctuation in enhancing biotic P uptake deserves attention from future research.

419 *4.5 Conclusion*

420 Our study highlights that redox-induced cycling of Fe (oxyhydr)oxides affected several P
421 fractions in humid tropical forest soil. Static anoxic conditions greatly enhanced the reduction of
422 Fe (oxyhydr)oxides and increased the labile (NaHCO₃-P_i) and intermediate (NaOH-P_i and AO-
423 P_i) P pools. Effects of redox fluctuation on P fractions were more complex and dependent on the
424 temporal scale. Redox sensitivity of amorphous Fe and P pools tended to decrease over time. But
425 immediately after a change in redox condition, AO-P_i showed strong coupling with Fe speciation
426 during reduction events. The NaOH-P_i and AO-Fe pools showed a declining trend during
427 oxidation events, similar to that of HCl-Fe(II). Surprisingly, the labile P pool decreased
428 immediately after reduction events, likely mediated by resorption and microbial uptake. These
429 results support our hypothesis that soil P responds *rapidly* to changes in redox condition at the
430 scale of hours. Climate change is likely to affect redox dynamics in humid tropical forests, and
431 this is likely to feed back directly and indirectly on ecosystem-level processes. Effects of redox
432 conditions on soil P availability should thus be considered in predictions of soil fertility and
433 limitations to primary productivity under future climate change scenarios.

434

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456 **References**

457 Ajmone-Marsan, F., D. Côté, and R. R. Simard (2006), Phosphorus transformations under
458 reduction in long-term manured soils, *Plant and Soil*, 282, 239-250, doi:10.1007/s11104-
459 005-5929-6.

460 Barcellos, D., K. T. Cyle, and A. Thompson (2018), Faster redox fluctuations can lead to higher
461 iron reduction rates in humid forest soils, *Biogeochemistry*, doi:10.1007/s10533-018-
462 0427-0.

463 Barros, N. F., Filho, N. B. Comerford, and N. F. Barros (2005), Phosphorus sorption, desorption
464 and resorption by soils of the Brazilian Cerrado supporting eucalypt, *Biomass and*
465 *Bioenergy*, 28(2), 229-236, doi:<https://doi.org/10.1016/j.biombioe.2004.08.005>.

466 Barrow, N. J. (1984), Modeling the effects of pH on phosphate sorption by soils, *European*
467 *Journal of Soil Science*, 35, 283-297, doi:10.1111/j.1365-2389.1984.tb00283.x.

468 Bhattacharyya, A., A. N. Campbell, M. M. Tfaily, Y. Lin, W. L. Silver, P. S. Nico, and J. Pett-
469 Ridge (2018), Redox fluctuations control the coupled cycling of iron and carbon in
470 tropical forest soils, *bioRxiv*, doi:10.1101/312108.

471 Borch, T., and S. Fendorf (2007), Chapter 12 Phosphate Interactions with Iron (Hydr)oxides:
472 Mineralization Pathways and Phosphorus Retention upon Bioreduction, *Developments in*
473 *Earth and Environmental Sciences*, 7(07), 321-348, doi:10.1016/S1571-9197(07)07012-
474 7.

475 Brown, S., A. E. Lugo, S. Silander, and L. Liegel (1983), *Research history and opportunities in*
476 *the Luquillo Experimental Forest*, U.S. Department of Agriculture Forest Service,
477 General Technical Report SO-44, Southern Forest Experiment Station, New Orleans, LA,
478 128 p.

479 Chacón, N., W. L. Silver, E. A. Dubinsky, and D. F. Cusack (2006), Iron reduction and soil
480 phosphorus solubilization in humid tropical forests soils: The roles of labile carbon pools
481 and an electron shuttle compound, *Biogeochemistry*, 78(1), 67-84, doi:10.1007/s10533-
482 005-2343-3.

483 Charlet, L., E. Markelova, C. Parsons, R.-M. Couture, and B. Madé (2013), Redox Oscillation
484 Impact on Natural and Engineered Biogeochemical Systems: Chemical Resilience and
485 Implications for Contaminant Mobility, *Procedia Earth and Planetary Science*, 7, 135-
486 138, doi:10.1016/j.proeps.2013.03.048.

487 Cleveland, C. C., and A. R. Townsend (2006), Nutrient additions to a tropical rain forest drive
488 substantial soil carbon dioxide losses to the atmosphere., *Proceedings of the National*
489 *Academy of Sciences of the United States of America*, 103, 10316-10321,
490 doi:10.1073/pnas.0600989103.

491 DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone (2010), Microbial
492 communities acclimate to recurring changes in soil redox potential status, *Environmental*
493 *Microbiology*, 12(12), 3137-3149, doi:10.1111/j.1462-2920.2010.02286.x.

494 Dubinsky, E. a., W. L. Silver, and M. K. Firestone (2010), Tropical forest soil microbial
495 communities couple iron and carbon biogeochemistry, *Ecology*, 91(9), 2604-2612,
496 doi:10.1890/09-1365.1.

497 Eusterhues, K., A. Hädrich, J. Neidhardt, K. Küsel, T. F. Keller, K. D. Jandt, and K. U. Totsche
498 (2014), Reduction of ferrihydrite with adsorbed and coprecipitated organic matter:
499 microbial reduction by *Geobacter bremensis* vs. abiotic reduction by Na-dithionite,
500 *Biogeosciences*, 11(18), 4953-4966, doi:10.5194/bg-11-4953-2014.

501 Fredrickson, J. K., J. M. Zachara, D. W. Kennedy, H. Dong, T. C. Onstott, N. W. Hinman, and S.
502 M. Li (1998), Biogenic iron mineralization accompanying the dissimilatory reduction of
503 hydrous ferric oxide by a groundwater bacterium, *Geochimica et Cosmochimica Acta*, 62,
504 3239-3257, doi:10.1016/S0016-7037(98)00243-9.

505 Ginn, B., C. Meile, J. Wilmoth, Y. Tang, and A. Thompson (2017), Rapid Iron Reduction Rates
506 Are Stimulated by High-Amplitude Redox Fluctuations in a Tropical Forest Soil,
507 *Environmental Science & Technology*, 51(6), 3250-3259, doi:10.1021/acs.est.6b05709.

508 Guo, F., and R. S. Yost (1999), Quantifying the available soil phosphorus pool with the acid
509 ammonium oxalate method, *Soil Science Society of America Journal*, 63, 651-651,
510 doi:10.2136/sssaj1999.036159950063000300031x.

511 Guppy, C. N., N. W. Menzies, P. W. Moody, and F. P. C. Blamey (2005), Competitive sorption
512 reactions between phosphorus and organic matter in soil: A review, *Australian Journal of*
513 *Soil Research*, 43(2), 189-202, doi:10.1071/SR04049.

514 Hall, S. J., and W. L. Silver (2013), Iron oxidation stimulates organic matter decomposition in
515 humid tropical forest soils, *Global Change Biology*, 19(9), 2804-2813,
516 doi:10.1111/gcb.12229.

517 Hall, S. J., and W. L. Silver (2015), Reducing conditions, reactive metals, and their interactions
518 can explain spatial patterns of surface soil carbon in a humid tropical forest,
519 *Biogeochemistry*, 125(2), 149-165, doi:10.1007/s10533-015-0120-5.

520 Hall, S. J., W. L. Silver, V. I. Timokhin, and K. E. Hammel (2015), Lignin decomposition is
521 sustained under fluctuating redox conditions in humid tropical forest soils, *Global*
522 *Change Biology*, 21(7), 2818-2828, doi:10.1111/gcb.12908.

523 Hedley, M. J., J. W. B. Stewart, and B. Chauhan (1982), Changes in inorganic and organic soil
524 phosphorus fractions induced by cultivation practices and by laboratory incubations, *Soil*
525 *Science Society of America Journal*, 46(5), 970-976.

526 Heiberg, L., C. B. Koch, C. Kjaergaard, H. S. Jensen, and B. H. Hans Christian (2012), Vivianite
527 precipitation and phosphate sorption following iron reduction in anoxic soils, *Journal of*
528 *Environmental Quality*, 41(3), 938-949, doi:10.2134/jeq2011.0067.

529 Herbert, D. A., and J. H. Fownes (1995), Phosphorus limitation of forest leaf area and net primary
530 production on a highly weathered soil, *Biogeochemistry*, 29, 223-235,
531 doi:10.1007/BF02186049.

532 Hobbie, S., and P. M. Vitousek (2000), Nutrient limitation of decomposition in Hawaiian forests,
533 *Ecology*, 81, 1867-1877, doi:10.1890/0012-9658(2000)081[1867:NLODIH]2.0.CO;2.

534 Hodges, C. A. (2017), Drivers and variability of iron redox in upland soils, M.Sc. thesis,
535 University of Georgia, Athens, GA.

536 Holford, I. C. R., and W. H. Patrick (1979), Effects of reduction and pH changes on phosphate
537 sorption and mobility in an acid soil, *Soil Science Society of America Journal*, 43(2), 292-
538 297.

539 Hutchison, K. J., and D. Hesterberg (2004), Dissolution of phosphate in a phosphorus-enriched
540 ultisol as affected by microbial reduction, *Journal of Environmental Quality*, 33(5), 1793-
541 1802.

542 Johnson, A. H., J. Frizano, and D. R. Vann (2003), Biogeochemical implications of labile
543 phosphorus in forest soils determined by the Hedley fractionation procedure, *Oecologia*,
544 135, 487-499, doi:10.1007/s00442-002-1164-5.

545 Karathanasis, A. D., and P. D. Shumaker (2009), Organic and inorganic phosphate interactions
546 with soil hydroxy-interlayered minerals, *Journal of Soils and Sediments*, 9(5), 501-510,
547 doi:10.1007/s11368-009-0116-7.

548 Kostka, J. E., and G. W. Luther (1994), Partitioning and speciation of solid phase iron in
549 saltmarsh sediments, *Geochimica et Cosmochimica Acta*, 58, 1701-1710,
550 doi:10.1016/0016-7037(94)90531-2.

551 Liptzin, D., and W. L. Silver (2009), Effects of carbon additions on iron reduction and
552 phosphorus availability in a humid tropical forest soil, *Soil Biology and Biochemistry*,
553 41(8), 1696-1702, doi:10.1016/j.soilbio.2009.05.013.

554 Liptzin, D., W. L. Silver, and M. Detto (2011), Temporal dynamics in soil oxygen and
555 greenhouse gases in two humid tropical forests, *Ecosystems*, 14, 171-182, doi:DOI
556 10.1007/s10021-010-9402-x.

557 Loeppert, R. H., and W. P. Inskeep (1996), Iron, in *Methods of Soil Analysis, Part 3*, edited by D.
558 L. Sparks, pp. 639-664, ASA and SSSA, Madison, WI.

559 Mage, S. M., and S. Porder (2012), Parent material and topography determine soil phosphorus
560 status in the Luquillo Mountains of Puerto Rico, *Ecosystems*, 16, 284-294,
561 doi:10.1007/s10021-012-9612-5.

562 McGechan, M. B., and D. R. Lewis (2002), Sorption of Phosphorus by Soil, Part 1: Principles,
563 Equations and Models, *Biosystems Engineering*, 82, 1-24, doi:10.1006/bioe.2001.0013.

564 McGroddy, M., and W. L. Silver (2000), Variations in belowground carbon storage and soil CO₂
565 flux rates along a wet tropical climate gradient, *Biotropica*, 32(4), 614-624,
566 doi:10.1646/0006-3606(2000)032[{}0614:VIBCSA]2.0.CO;2.

567 McKeague, J. A., and J. H. Day (1966), Dithionite- and oxalate- extractable Fe and Al As aids in
568 differentiating various classes of soils, *Canadian Journal of Soil Science*, 46, 13-22,
569 doi:10.4141/cjss66-003.

570 McLaughlin, J. R., J. C. Ryden, and J. K. Syers (1977), Development and evaluation of a kinetic
571 model to describe phosphate sorption by hydrous ferric oxide gel, *Geoderma*, 18(4), 295-
572 307, doi:10.1016/0016-7061(77)90038-6.

573 Melton, E. D., E. D. Swanner, S. Behrens, C. Schmidt, and A. Kappler (2014), The interplay of
574 microbially mediated and abiotic reactions in the biogeochemical Fe cycle, *Nature*
575 *Reviews Microbiology*, 12(12), 797-808, doi:10.1038/nrmicro3347.

576 Miller, A. J., E. A. G. Schuur, and O. A. Chadwick (2001), Redox control of phosphorus pools in
577 Hawaiian montane forest soils, *Geoderma*, 102(3-4), 219-237, doi:10.1016/S0016-
578 7061(01)00016-7.

579 Murphy, J., and J. P. Riley (1962), A modified single solution method for the determination of
580 phosphate in natural waters, *Analytica Chimica Acta*, 27, 31-36.

581 Nottingham, A. T., B. L. Turner, A. W. Stott, and E. V. J. Tanner (2015), Nitrogen and
582 phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils,
583 *Soil Biology and Biochemistry*, 80, 26-33, doi:10.1016/j.soilbio.2014.09.012.

584 O'Connell, C. S., L. Ruan, and W. L. Silver (2018), Drought drives rapid shifts in tropical
585 rainforest soil biogeochemistry and greenhouse gas emissions, *Nature Communications*,
586 9, 1348, doi:doi:10.1038/s41467-018-03352-3.

587 Olander, L. P., and P. M. Vitousek (2005), Short-term controls over inorganic phosphorus during
588 soil and ecosystem development, *Soil Biology and Biochemistry*, 37(4), 651-659,
589 doi:10.1016/j.soilbio.2004.08.022.

590 Patrick, W. H., and R. A. Khalid (1974), Phosphate release and sorption by soils and sediments:
591 effect of aerobic and anaerobic conditions, *Science*, 186(4158), 53-55.

592 Peretyazhko, T., and G. Sposito (2005), Iron(III) reduction and phosphorous solubilization in
593 humid tropical forest soils, *Geochimica et Cosmochimica Acta*, 69(14), 3643-3652,
594 doi:10.1016/j.gca.2005.03.045.

595 Pett-Ridge, J. (2005), Rapidly fluctuating redox regimes frame the ecology of microbial
596 communities and their biogeochemical function in a humid tropical soil, University of
597 California, Berkeley.

598 Pett-Ridge, J., and M. K. Firestone (2005), Redox fluctuation structures microbial communities in
599 a wet tropical soil, *Applied and Environmental Microbiology*, 71(11), 6998-7007,
600 doi:10.1128/AEM.71.11.6998.

601 Pett-Ridge, J., D. G. Petersen, E. Nuccio, and M. K. Firestone (2013), Influence of oxic/anoxic
602 fluctuations on ammonia oxidizers and nitrification potential in a wet tropical soil, *FEMS*
603 *Microbiology Ecology*, 85(1), 179-194.

604 Pett-Ridge, J., W. L. Silver, and M. K. Firestone (2006), Redox fluctuations frame microbial
605 community impacts on N-cycling rates in a humid tropical forest soil, *Biogeochemistry*,
606 81, 95-110, doi:10.1007/s10533-006-9032-8.

607 R Core Team (2017), R: A language and environment for statistical computing. R Foundation for
608 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/.Rep>.

609 Richter, D. D., H. L. Allen, J. Li, D. Markewitz, and J. Raikes (2006), Bioavailability of slowly
610 cycling soil phosphorus: Major restructuring of soil P fractions over four decades in an
611 aggrading forest, *Oecologia*, 150(2), 259-271, doi:10.1007/s00442-006-0510-4.

612 Roden, E. E., and J. W. Edmonds (1997), Phosphate mobilization in iron-rich anaerobic
613 sediments: microbial Fe (III) oxide reduction versus iron-sulfide formation, *Archiv für*
614 *Hydrobiologie*, 139(3), 347-378.

615 Roden, E. E., and R. G. Wetzel (2002), Kinetics of microbial Fe(III) oxide reduction in
616 freshwater wetland sediments, *Limnology and Oceanography*, 47(1), 198-211,
617 doi:10.4319/lo.2002.47.1.0198.

618 Sallade, Y. E., and J. T. Sims (1997), Phosphorus transformations in the sediments of Delaware's
619 agricultural drainageways: II. Effect of reducing conditions on phosphorus release,
620 *Journal of Environmental Quality*, 26(6), 1579-1588.

621 Scatena, F. N. (1989), *An introduction to the physiography and history of the Bisley Experimental*
622 *Watersheds in the Luquillo Mountains of Puerto Rico*, US Department of Agriculture,
623 Forest Service, Southern Forest Experiment Station.

624 Scatena, F. N., and A. E. Lugo (1995), Geomorphology, disturbance, and the soil and vegetation
625 of two subtropical wet steep-land watersheds of Puerto Rico, *Geomorphology*, 13, 199-
626 213.

627 Schuur, E. A., and P. A. Matson (2001), Net primary productivity and nutrient cycling across a
628 mesic to wet precipitation gradient in Hawaiian montane forest, *Oecologia*, 128(3), 431-
629 442, doi:10.1007/s004420100671.

630 Silver, W. L., A. E. Lugo, and M. Keller (1999), Soil oxygen availability and biogeochemistry
631 along rainfall and topographic gradients in upland wet tropical forest soils,
632 *Biogeochemistry*, 44(3), 301-328.

633 Soil Survey Staff (1995), *Order 1 Soil Survey of the Luquillo Long-Term Ecological Research*
634 *Grid, Puerto Rico*, U.S. Department of Agriculture, Natural Resources Conservation
635 Service, Lincoln, Nebraska, USA.

636 Sulzberger, B., D. Suter, C. Siffert, S. Banwart, and W. Stumm (1989), Dissolution of
637 Fe(III)(hydr)oxides in natural waters; laboratory assessment on the kinetics controlled by
638 surface coordination, *Marine Chemistry*, 28(1-3), 127-144, doi:10.1016/0304-
639 4203(89)90191-6.

640 Thompson, A., O. A. Chadwick, D. G. Rancourt, and J. Chorover (2006), Iron-oxide crystallinity
641 increases during soil redox oscillations, *Geochimica et Cosmochimica Acta*, 70, 1710-
642 1727, doi:10.1016/j.gca.2005.12.005.

643 Thompson, A., D. G. Rancourt, O. a. Chadwick, and J. Chorover (2011), Iron solid-phase
644 differentiation along a redox gradient in basaltic soils, *Geochimica et Cosmochimica*
645 *Acta*, 75(1), 119-133, doi:10.1016/j.gca.2010.10.005.

646 Thompson, J., N. Brokaw, J. K. Zimmerman, R. B. Waide, E. M. Everham, D. J. Lodge, C. M.
647 Taylor, D. Garcia-Montiel, and M. Fluet (2002), Land use history, environment, and tree
648 composition in a tropical forest, *Ecological Applications*, 12, 1344-1363,
649 doi:10.2307/3099976.

650 Tiessen, H., and J. O. Moir (1993), Characterization of available P by sequential extraction, in
651 *Soil Sampling and Methods of Analysis*, edited, pp. 75-86.

652 Torn, M. S., S. E. Trumbore, O. A. Chadwick, P. M. Vitousek, and D. M. Hendricks (1997),
653 Mineral control of soil organic carbon storage and turnover, *Nature*, 389, 3601-3603,
654 doi:10.1038/38260.

655 Viollier, E., P. W. Inglett, K. Hunter, A. N. Roychoudhury, and P. Van Cappellen (2000), The
656 ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters, *Applied*
657 *Geochemistry*, 15(6), 785-790, doi:10.1016/S0883-2927(99)00097-9.

658 Vitousek, P. M., S. Porder, B. Z. Houlton, and O. a. Chadwick (2010), Terrestrial phosphorus
659 limitation: mechanisms, implications, and nitrogen-phosphorus interactions., *Ecological*
660 *Applications*, 20, 5-15.

661 Walpersdorf, E., C. B. Koch, L. Heiberg, D. W. O'Connell, C. Kjaergaard, and H. C. B. Hansen
662 (2013), Does vivianite control phosphate solubility in anoxic meadow soils?, *Geoderma*,
663 *193-194*, 189-199, doi:10.1016/j.geoderma.2012.10.003.

664 Whitfield, M. (1974), Thermodynamic limitations on the use of the platinum electrode in Eh
665 measurements, *Limnology and Oceanography*, *19*(5), 857-865.

666 Wood, T. E., D. Matthews, K. Vandecar, and D. Lawrence (2016), Short-term variability in labile
667 soil phosphorus is positively related to soil moisture in a humid tropical forest in Puerto
668 Rico, *Biogeochemistry*, *127*(1), 35-43, doi:10.1007/s10533-015-0150-z.

669 Wright, R. B., B. G. Lockaby, and M. R. Walbridge (2001), Phosphorus availability in an
670 artificially flooded southeastern floodplain forest soil, *Soil Science Society of America*
671 *Journal*, *65*(4), 1293-1302, doi:10.2136/sssaj2001.6541293x.

672 Yang, C., L. Yang, and L. Jianhua (2006), Organic phosphorus fractions in organically amended
673 paddy soils in continuously and intermittently flooded conditions, *Journal of*
674 *Environmental Quality*, *35*(4), 1142-1150, doi:10.2134/jeq2005.0194.

675 Zak, D., C. Wagner, B. Payer, J. Augustin, and J. Gelbrecht (2010), Phosphorus mobilization in
676 rewetted fens: the effect of altered peat properties and implications for their restoration,
677 *Ecological Applications*, *20*(5), 1336-1349.

678 Zhang, Y. S., X. Y. Lin, and W. Werner (2003), The effect of soil flooding on the transformation
679 of Fe oxides and the adsorption/desorption behavior of phosphate, *Journal of Plant*
680 *Nutrition and Soil Science*, *166*(1), 68-75, doi:10.1002/jpln.200390014.

681 Zhang, Y. S., W. Werner, H. W. Scherer, and X. Sun (1994), Effect of organic manure on organic
682 phosphorus fractions in two paddy soils, *Biology and Fertility of Soils*, *17*(1), 64-68,
683 doi:10.1007/BF00418674.

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685

686 Table 1. Chemical and biological meanings of studied inorganic and organic P fractions.

Extractant	Chemical characteristics	Presumed biological significance	Referred to as
0.5 M NaHCO ₃	Inorganic P in solution and ion-exchanged forms, and P weakly associated with Fe and Al of mineral surfaces. Easily mineralizable organic P associated with soil organic surfaces and fluvic and humic acids.	Labile P, easily mineralizable organic P, considered available for plant and microbial uptake [<i>Tiessen and Moir, 1993</i>].	“labile P”
0.1 M NaOH	Inorganic P more strongly associated with amorphous and crystalline Fe and Al minerals than what is removed by NaHCO ₃ . Organic P more strongly associated with soil organic surfaces and fluvic and humic acids than what is removed by NaHCO ₃ .	Intermediate P lability. Slowly cycling P available to plants and microbes on a long temporal scale [e.g., months to decades; <i>Richter et al., 2006</i>].	“Fe/Al-bound P” (inorganic or organic)
Ammonium oxalate	Inorganic P specifically associated with amorphous Fe and Al minerals. Organic P specifically associated with soil organic matter bound by amorphous Fe and Al minerals. This pool is less abundant than what is removed by NaOH.	Intermediate P lability. Can be utilized by crops in highly weathered soils [<i>Guo and Yost, 1999</i>].	“amorphous Fe/Al-bound P”

687

688 Figure Captions

689

690 Figure 1. Effects of redox manipulation on (a) NaHCO_3 -extractable total P ($\text{NaHCO}_3\text{-P}_t$), (b)
691 ammonium oxalate extractable total P (AO-P_t), (c) NaOH -extractable inorganic P (NaOH-P_i),
692 (d) HCl -extractable Fe(II) (HCl-Fe(II)), (e) ammonium oxalate extractable Fe (AO-Fe), and (f)
693 NaOH -extractable organic P (NaOH-P_o) of a humid tropical soil from Puerto Rico, averaging
694 across time. Static anoxic, static anoxic treatment; high frequency, high frequency fluctuating
695 treatment (4-day oxic/4-day anoxic); low frequency, low frequency fluctuating treatment (8-day
696 oxic/4-day anoxic); and static oxic treatment. From right to left, treatments increased in their
697 exposure to an oxic headspace (CO_2 -free air). Boxplot whiskers refer to 1.5 times the
698 interquartile range of data. Multiple comparisons were controlled by Tukey's tests. Different
699 letters indicate significant differences at $\alpha = 0.05$.

700

701 Figure 2. Responses of HCl -extractable Fe(II) (HCl-Fe(II)) to oscillating redox conditions.
702 Boxplot whiskers refer to 1.5 times the interquartile range of data. Different upper- and lower-
703 case letters indicate significant differences at $\alpha = 0.05$ and 0.10 derived from Student's T-test,
704 respectively.

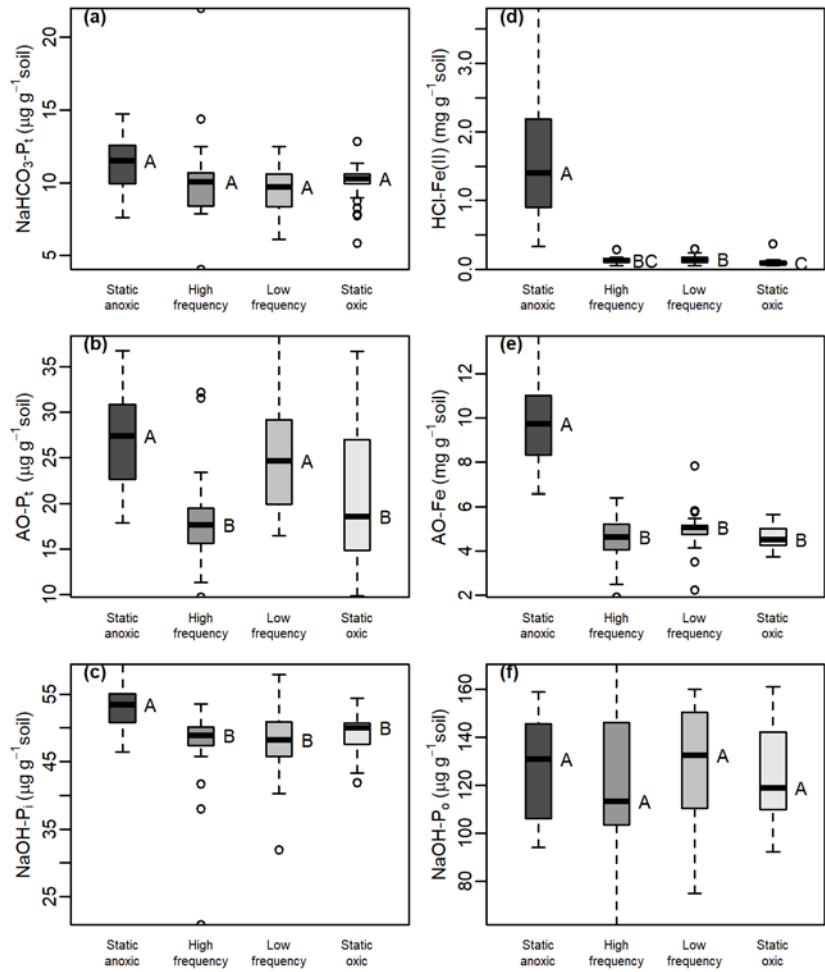
705

706 Figure 3. Responses of ammonium oxalate extractable total P (AO-P_t) to oscillating redox
707 conditions. Boxplot whiskers refer to 1.5 times the interquartile range of data. Different upper-
708 and lower-case letters indicate significant differences at $\alpha = 0.05$ and 0.10 derived from Student's
709 T-test, respectively.

710

711 Figure 4. Responses of on (a) NaHCO_3 -extractable total P ($\text{NaHCO}_3\text{-P}_t$), (b) ammonium oxalate
712 extractable total P (AO-P_t), (c) NaOH -extractable inorganic P (NaOH-P_i), (d) HCl -extractable

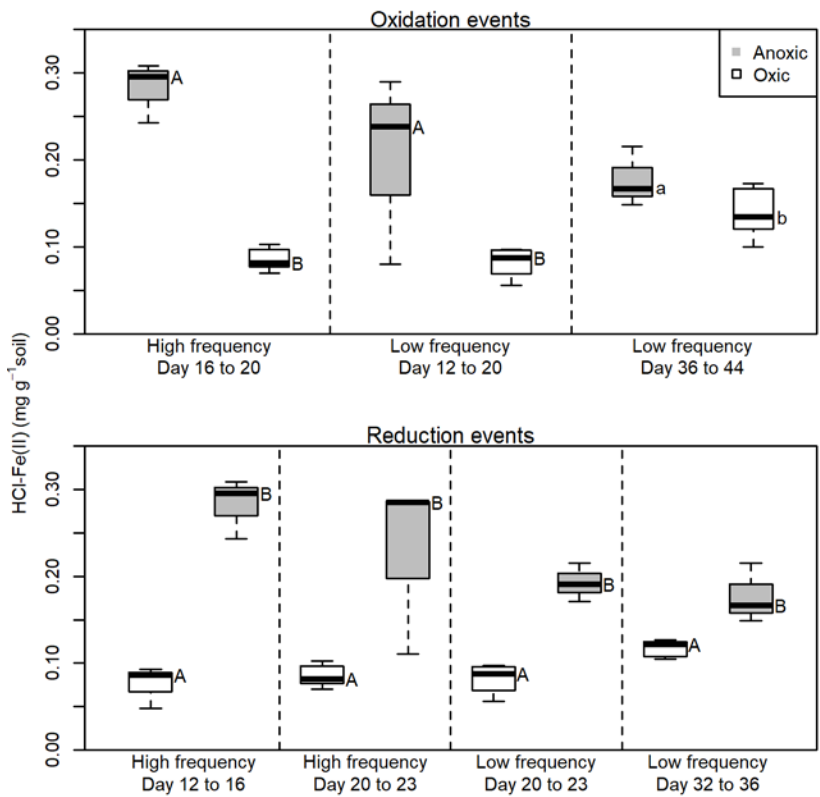
713 Fe(II) (HCl-Fe(II)), (e) ammonium oxalate extractable Fe (AO-Fe), and (f) NaOH-extractable
714 organic P (NaOH-P_o) immediately following oxidation events (N₂ → air) or reduction events (air
715 → N₂) in a humid tropical soil redox oscillation experiment, averaging across multiple events.
716 Boxplot whiskers refer to 1.5 times the interquartile range of data. Multiple comparisons were
717 controlled by Tukey's tests. Different letters indicate significant differences at $\alpha = 0.05$.
718



719

720 Figure 1

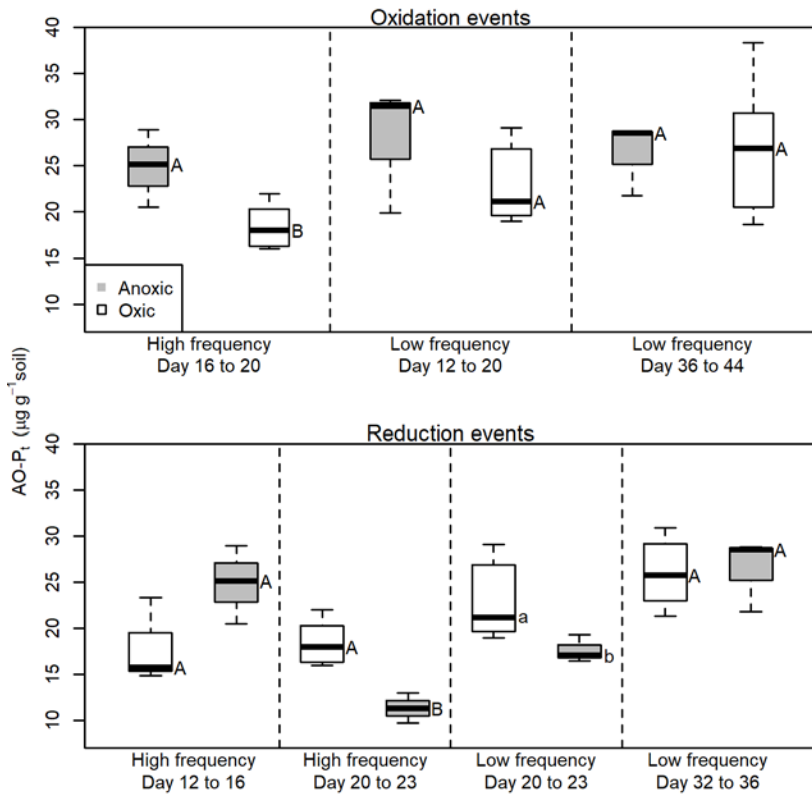
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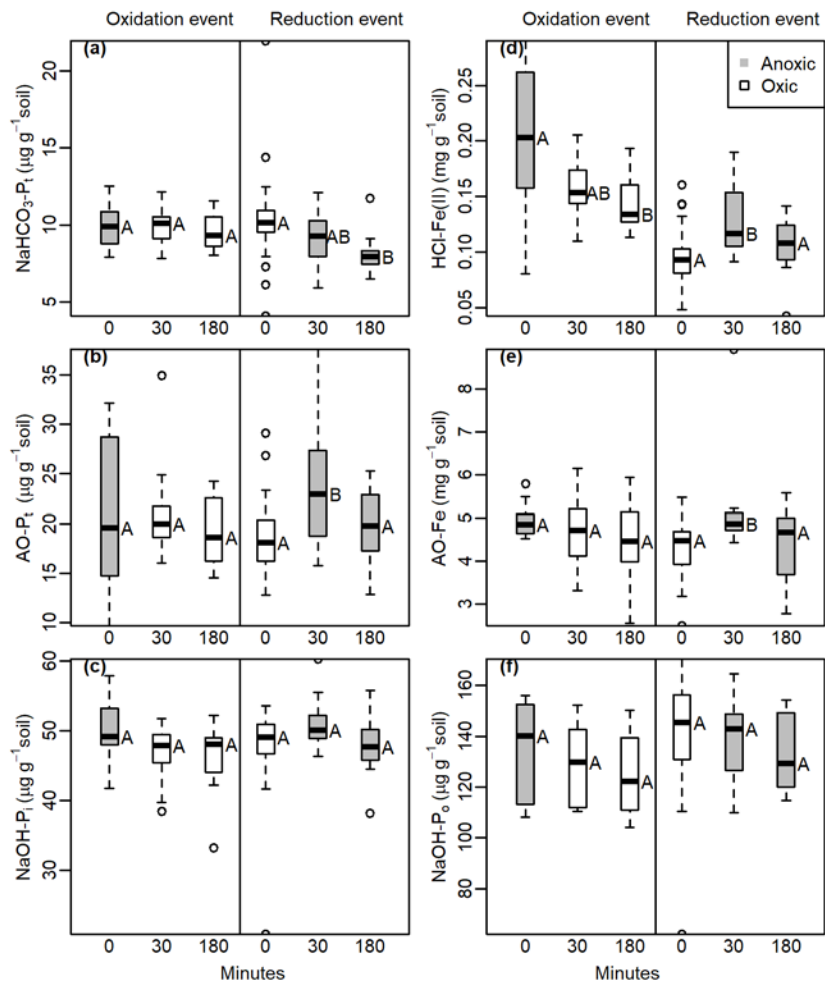
723 Figure 2

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727

728 Figure 4



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Supporting Information for

Phosphorus fractionation responds to dynamic redox conditions in a humid tropical forest soil

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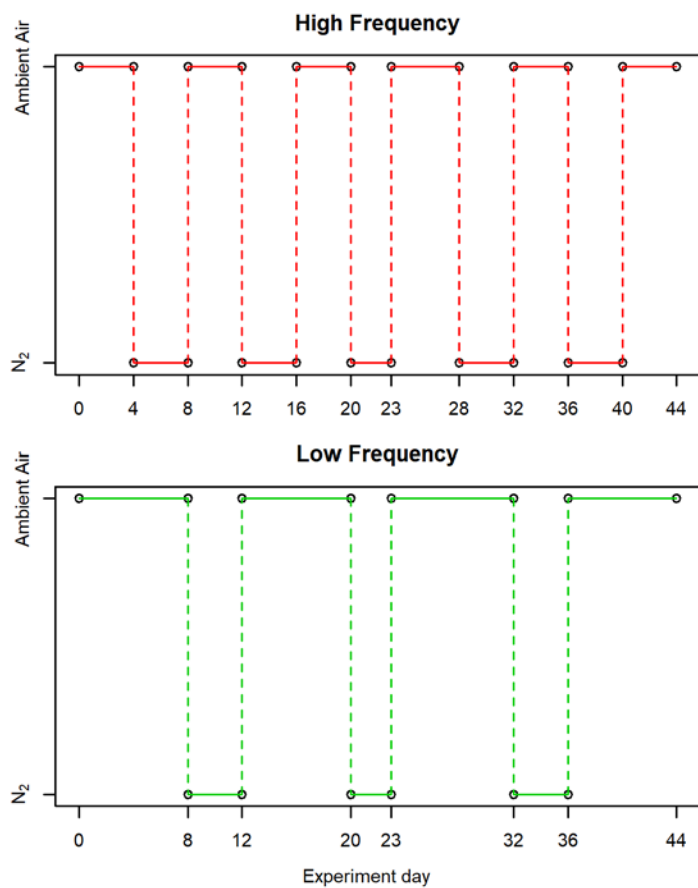


Figure S1. Diagram of the experimental set-up of two fluctuating redox treatments. Dots indicate the times when headspace composition was switched between ambient air and N₂.

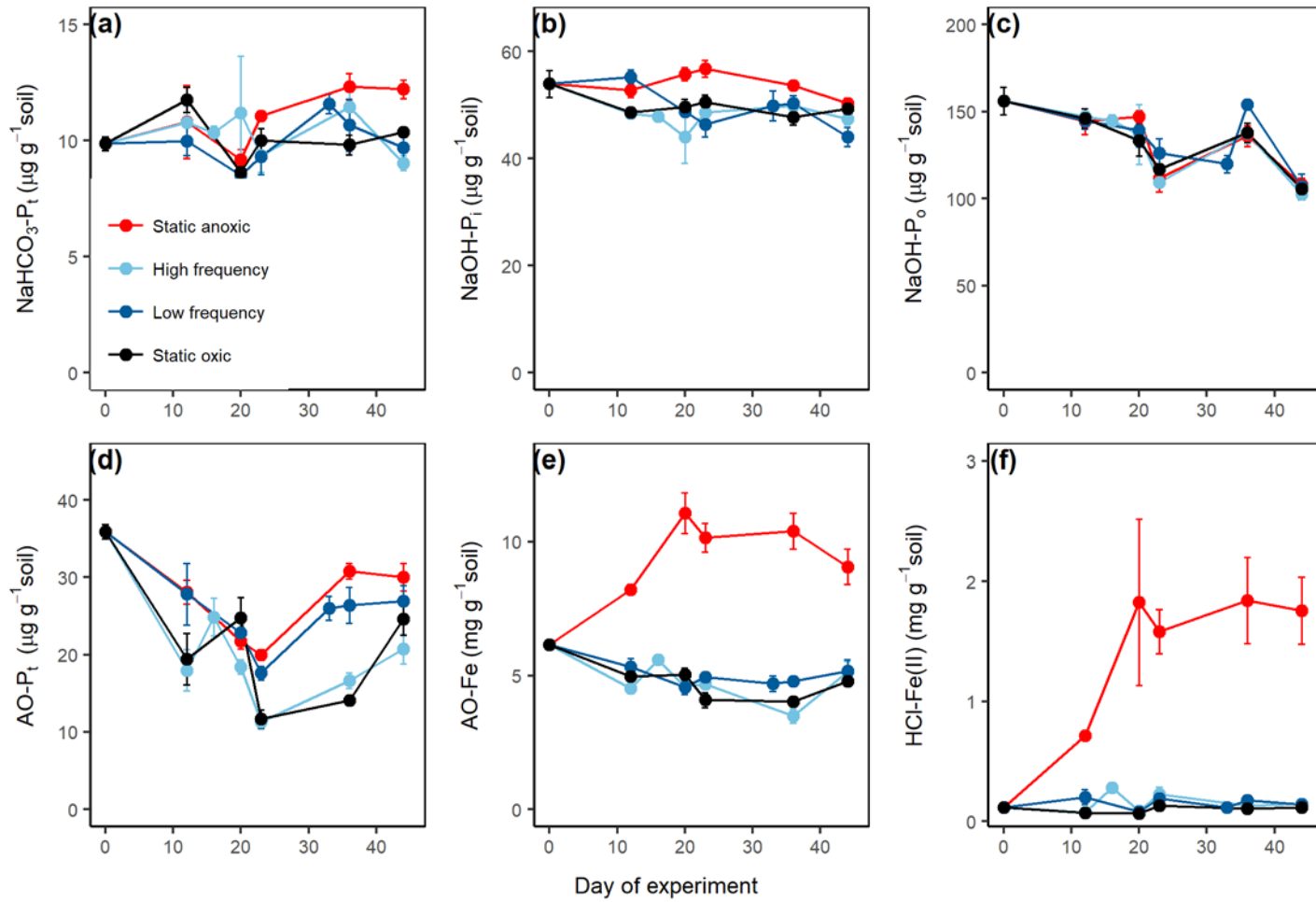


Figure S2. Effects of redox manipulation on (a) NaHCO_3 -extractable total P ($\text{NaHCO}_3\text{-P}_t$), (b) NaOH-extractable inorganic P (NaOH- P_i), (c) NaOH-extractable organic P (NaOH- P_o), (d) ammonium oxalate extractable total P (AO- P_t), (e) ammonium oxalate extractable Fe (AO-Fe), and (f) HCl-extractable Fe(II) (HCl-Fe(II)). Static anoxic, static anoxic treatment; high frequency, high frequency fluctuating treatment (4-day oxic/4-day anoxic); low frequency, low frequency fluctuating treatment (8-day oxic/4-day anoxic); and static oxic, static oxic treatment. Error bars indicate S.E.

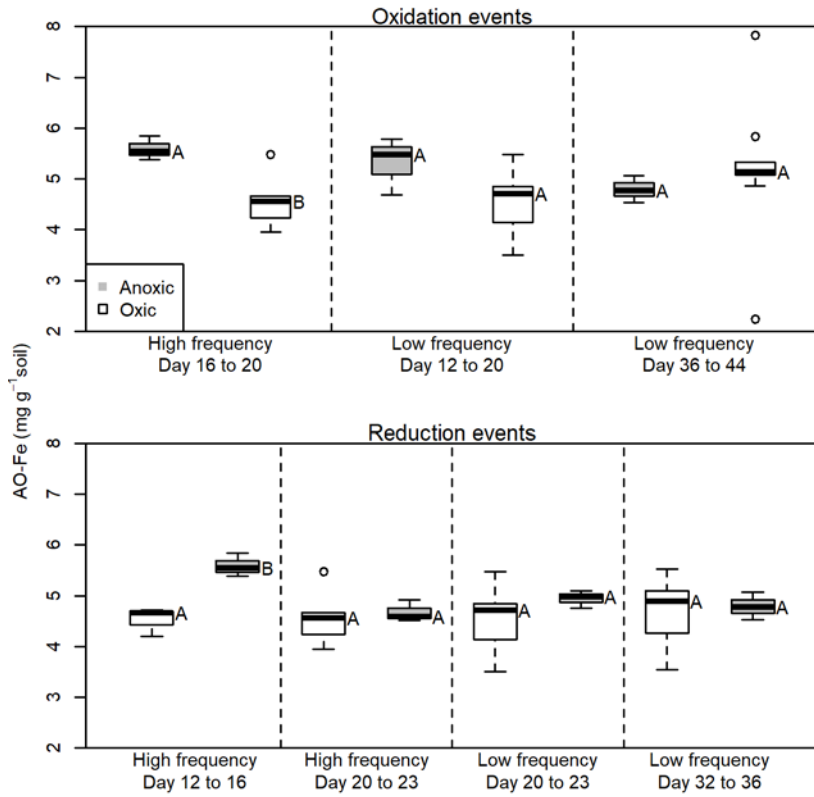


Figure S3. Responses of ammonium oxalate extractable Fe (AO-Fe) to oscillating redox conditions. Boxplot whiskers refer to 1.5 times the interquartile range of data. Different letters indicate significant differences at $\alpha = 0.05$, derived from Student's T-test.

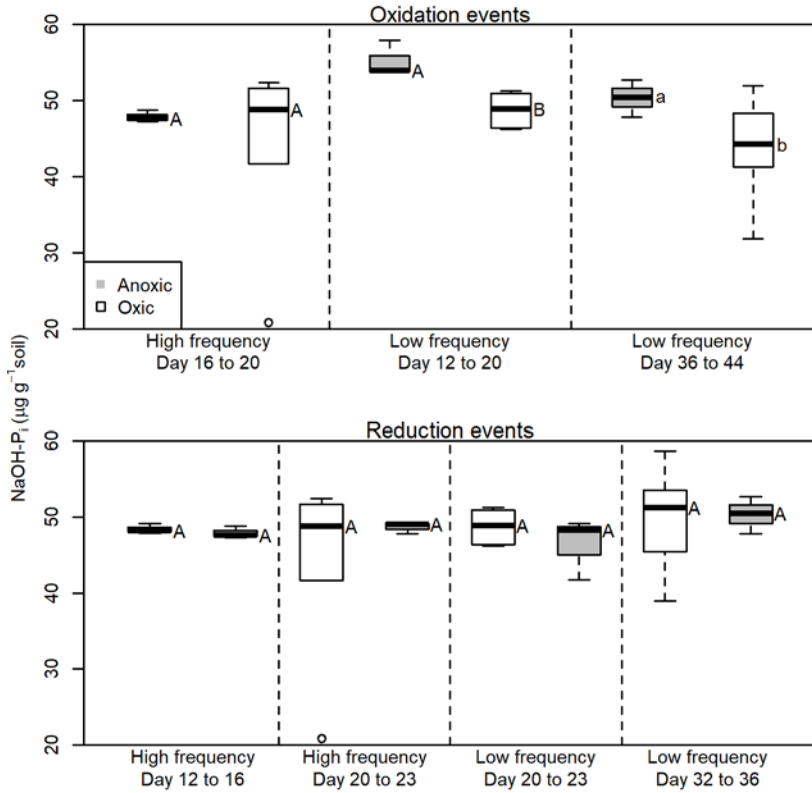


Figure S4. Responses of NaOH-extractable inorganic P (NaOH-P_i) to oscillating redox conditions. Boxplot whiskers refer to 1.5 times the interquartile range of data. Different upper- and lower-case letters indicate significant differences at $\alpha = 0.05$ and 0.10 derived from Student's T-test, respectively.

Table S1. Number of soil microcosms harvested on each sampling day. Microcosms that received labelled rye grass litter were noted separately (¹³C). In the high frequency and low frequency treatments, some microcosms were harvested at 30 (M30) and 180 minutes (M180) after changing headspace composition.

Sampling day	Static anoxic	High frequency	Low frequency	Static oxic
12	3	3+3(M30)+3(M180)	3+3(M30)+3(M180)	3
16	0	3	0	0
20	3+3(¹³ C)	3+3(¹³ C) +3(M30)+3(M180)	3+3(¹³ C) +3(M30)+3(M180)	3+3(¹³ C)
23	3	3+3(M30)+3(M180)	3+3(M30)+3(M180)	3
32	0	0	3+3(¹³ C)	0
36	3+3(¹³ C)	3+3(¹³ C) +3(M30)+3(M180)	3+3(¹³ C) +3(M30)+3(M180)	3+3(¹³ C)
44	5+5(¹³ C)	5+5(¹³ C)	5+5(¹³ C)	5+5(¹³ C)

Table S2. Effects of redox treatment (treatment), isotope labeling (isotope) and their interaction on P and Fe fractions. The P values from analysis of variance (ANOVA) are presented. Analyses were conducted using data from three sampling events where microcosms with both ¹²C- and ¹³C-litter were harvested (day 20, 36, and 44). Sampling day was considered as a random factor.

	Treatment	Isotope	Treatment:Isotope
NaHCO ₃ -extractable total P	0.027	0.569	0.936
NaOH-extractable inorganic P	< 0.001	0.196	0.160
Ammonium oxalate extractable total P	< 0.001	0.695	0.455
HCl-extractable Fe(II)	< 0.001	0.244	0.289
Ammonium oxalate extractable Fe	< 0.001	0.329	0.434

Table S3. Effects of redox treatment (Treatment), sampling day (day), and their interactions on soil P and Fe fractions averaging over the experiment. The P values from analysis of variance (ANOVA) are presented. Data were collected immediately *before* the switch in headspace on days 12, 20, 23, 36, and 44 of the experiment. Results of Tukey's tests were presented in Figure 1.

	Treatment	Day	Treatment:Day
NaHCO ₃ -extractable total P	0.007	0.051	0.098
NaOH-extractable inorganic P	<0.001	0.050	0.023
NaOH-extractable organic P	0.761	<0.001	0.960
Ammonium oxalate extractable total P	<0.001	<0.001	0.013
HCl-extractable Fe(II)	<0.001	<0.001	0.490
Ammonium oxalate extractable Fe	<0.001	0.363	0.011

Table S4. Effects of sampling time (time: 0, 30, and 180 minutes), event, and their interactions on soil P and Fe fractions during short-term oxidation events. The P values from analysis of variance (ANOVA) are presented. Data from four oxidation events were included. Results of Tukey's tests were presented in Figure 4.

	Time	Event	Time: Event
NaHCO ₃ -extractable total P	0.752	0.265	0.769
NaOH-extractable inorganic P	0.090	0.451	0.318
NaOH-extractable organic P	0.324	0.002	0.298
Ammonium oxalate extractable total P	0.350	<0.001	0.008
HCl-extractable Fe(II)	0.025	0.792	0.665
Ammonium oxalate extractable Fe	0.084	<0.001	0.125

Table S5. Effects of sampling time (time: 0, 30, and 180 minutes), event, and their interactions on soil P and Fe fractions during short-term reduction events. The P values from analysis of variance (ANOVA) are presented. Data from four reduction events were included. Results of Tukey's tests were presented in Figure 4.

	Time	Event	Time: Event
NaHCO ₃ -extractable total P	0.049	0.178	0.739
NaOH-extractable inorganic P	0.218	0.210	0.934
NaOH-extractable organic P	0.803	0.741	0.977
Ammonium oxalate extractable total P	0.002	0.014	<0.001
HCl-extractable Fe(II)	<0.001	<0.001	0.347
Ammonium oxalate extractable Fe	0.004	0.157	0.002