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

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Abstract

Phosphorus (P) is a key limiting nutrient in highly weathered soils of humid tropical forests. A large proportion of P in these soils is bound to redox-sensitive iron (Fe) minerals; however, little is known about how Fe redox interactions affect soil P cycling. In an incubation experiment, we changed bulk soil redox regimes by varying headspace conditions (air vs. N_2 gas), and examined the responses of soil P and Fe species to two fluctuating treatments (4- or 8-day oxic followed by 4-day anoxic) and two static redox treatments (oxic and anoxic). A static anoxic headspace increased NaOH-extractable inorganic P (NaOH-P_i) and ammonium oxalate-extractable total P (AO-P_t) by 10% and 38%, respectively, relative to a static oxic headspace. Persistent anoxia also increased $NaHCO_3$-extractable total P ($NaHCO_3$-P_t) towards the end of the experiment. Effects of redox fluctuation were more complex and dependent on temporal scales. Ammonium oxalate-extractable Fe and P_t concentrations responded to redox fluctuation early in the experiment, but not thereafter, suggesting a depletion of reductants over time. Immediately following a switch from an oxic to anoxic headspace, concentrations of AO-P_t, AO-Fe, and HCl-extractable Fe(II) increased (within 30 min), but fell back to initial levels by 180 min. Surprisingly, the labile P pool ($NaHCO_3$-P_t) decreased immediately after reduction events, potentially due to resorption and microbial uptake. Overall, our data demonstrate that P fractions can respond rapidly to changes in soil redox conditions, and in environments where redox oscillation is common, roots and microbes may benefit from these rapid P dynamics.

Keywords

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

1 Introduction

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

Humid tropical forest soils are often characterized by dynamic redox conditions [*Liptzin et al.*, 2011; *Schuur and Matson*, 2001; *Silver et al.*, 1999], which strongly affect Fe mineral phases, microbial communities, and nutrient cycling [*DeAngelis et al.*, 2010; *Pett-Ridge and Firestone*, 2005; *Pett-Ridge et al.*, 2013; *Thompson et al.*, 2006]. The combination of abundant rainfall, warm temperatures, finely textured soils, and high biological activity create redox oscillations that range from fully oxic to anoxic conditions on the order of hours to weeks [*Liptzin et al.*, 2011; *Silver et al.*, 1999]. Changes in redox conditions trigger rapid transformations of Fe minerals [*Peretyazhko and Sposito*, 2005; *Thompson et al.*, 2011]. Under anoxic conditions, Fe(III) minerals are reduced to Fe(II) species, with a concomitant increase in soil pH and soil organic matter decomposition [*Dubinsky et al.*, 2010; *Roden and Wetzel*, 2002]. Upon (re)exposure to O_2, these Fe(II) species can be quickly re-oxidized back to Fe(III)-bearing forms by microaerophiles, nitrate-reducing Fe(II) oxidizers, or abiotic processes [*Melton et al.*, 2014]. Recent studies in humid tropical forest soils have shown that Fe(II) oxidation is linked to increases in soil CO_2 production and release of dissolved organic matter [*Hall and Silver*, 2013; *Hall et al.*, 2015]. As a large fraction of soil P is also known to be bound to redox-sensitive Fe minerals, Fe-redox reactions likely play a critical role in regulating soil P cycling.

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

Compared to the relatively rich literature on P cycling under reducing conditions, little is known about how redox *fluctuation* affects P availability in humid tropical forests soils. Reduction and oxidation of Fe (oxyhydr)oxides can occur relatively quickly in humid tropical soils, on timescales of hours to days [*Charlet et al.*, 2013; *Hall and Silver*, 2013]. In addition, these soils host a diverse microbial community that is sensitive to rapid shifts in redox environments [*DeAngelis et al.*, 2010; *Pett-Ridge and Firestone*, 2005; *Pett-Ridge et al.*, 2013; *Pett-Ridge et al.*, 2006; *Thompson et al.*, 2006]. Thus, redox oscillation-driven changes in Fe species and associated microbial activities likely result in similarly fast responses within soil P pools. Phosphorus bound to amorphous minerals may be particularly sensitive to redox oscillation given the high redox sensitivity of amorphous Fe [*Liptzin and Silver*, 2009; *Thompson et al.*, 2006]. Recent studies found that frequent shifts in redox conditions led to more rapid Fe reduction and greater accumulation of Fe(II) [*Barcellos et al.*, 2018; *Ginn et al.*, 2017]. It is likely that these strong Fe reduction responses might also result in greater increases in P solubility under more frequent oscillation regimes. We thus expect that the frequency of redox oscillation affects the redox sensitivity of both Fe and P pools.

In this study, we evaluated how dynamic headspace redox regimes alter soil P fractions and how these relate to reactive Fe species. In a laboratory incubation experiment where bulk soil redox status was regulated by controlling microcosm headspace (ambient air vs N_2), soils from a humid tropical forest were exposed to four treatments: two fluctuating regimes (high vs low frequency of redox status shifts); static oxic (ambient air), and anoxic (N_2) headspace conditions. We evaluated the effects of dynamic and static conditions on P and Fe species over a 44-day incubation, as well as during the hours immediately following a shift in headspace redox state. We hypothesized that 1) the *frequency* of redox fluctuation would determine the *magnitude* of response in soil P and Fe pools. We predicted that redox fluctuation would increase Fe reduction and P solubility relative to static oxic conditions, and stronger increases in Fe and P pools would be observed under more frequent redox fluctuation. We also hypothesized that 2) soil P would respond *rapidly* to changes in headspace redox conditions on the scale of hours, mirroring patterns of Fe reduction and re-oxidation.

2 Materials and Methods

2.1. Experimental Design

Soils were collected near the El Verde field station in the Luquillo Experimental Forest (LEF), Puerto Rico (18.3211° N, 65.8175° W). The vegetation is characteristic of the tabonuco (*Dacryodes excelsa*) forest [*Thompson et al.*, 2002] with mean annual temperature of 23 °C and a range of 23.5 to 27 °C [*Brown et al.*, 1983]. Mean annual precipitation is approximately 3.5 m and relatively aseasonal, with no month receiving less than 200 mm precipitation [*Scatena*, 1989]. Soils were collected along a 15^o slope. Previous research has shown that topography exerts a strong control on soil moisture, O_2, C, and Fe dynamics in these soils, with slopes experiencing intermediate O_2 concentrations and fluctuations relative to the more anoxic valleys and well aerated ridges [*Hall and Silver*, 2015; *O'Connell et al.*, 2018; *Silver et al.*, 1999]. Slopes are the dominant landscape position in these ecosystems accounting for 65% of the land area [*Scatena and Lugo*, 1995]. Soils are classified as Humic Haploperox, relatively well-drained, and developed from volcaniclastic rock with 57 ± 3% sand, 23 ± 1% silt, and 20 ± 3% clay [*O'Connell et al.*, 2018; *Soil Survey Stuff*, 1995]. The average soil C concentration, pH, and soil moisture content (vol.) was 3.4 ± 0.5%, 4.9 ± 0.06, and 51 ± 1%, respectively [values are ± 1 standard error; *O'Connell et al.*, 2018].

Soil was collected from a 1 x 1 m plot at an upper slope position from the 0-10 cm depth and shipped overnight at ambient temperature to Lawrence Livermore National Laboratory, in Livermore, California. In the lab, soil was homogenized by manually mixing and removing visible plant debris, rocks, and soil macro-fauna. Twenty g (oven dry equivalent weight, ODW) subsamples of moist soil were weighed into glass gas-tight 487 mL incubation vessels (hereafter referred to as microcosms). A total of 178 replicate microcosms were prepared at the outset of our incubation experiment (Table S1). Each microcosm was capped with a gas-tight lid fitted with a GeoMicrobial septum (Geo-Microbial Technologies MT, Ochelata, Oklahoma, USA), Swagelok fitting, two-way stop-stock, and tygon tubing such that each microcosm could be sealed and disconnected from the main gas flushing system. Soils were incubated in the dark. The soil headspace was flushed with either humidified ambient air (oxic) or N_2 gas (anoxic) at a flow rate of 3 ml/min. When switching headspace gas, a higher flow rate (16 ml/min) was used to achieve headspace turnover in roughly 30 minutes. Gas entered the microcosm via the fitting and exited via a syringe needle in the septum. This design allowed us to manipulate the soil headspace, which previous work has shown alters soil redox biogeochemistry, such as headspace O_2, nitrous oxide, and methane concentrations, Fe speciation, nitrogen cycling, and microbial community dynamics [*DeAngelis et al.*, 2010; *Pett-Ridge*, 2005; *Pett-Ridge and Firestone*, 2005; *Pett-Ridge et al.*, 2013; *Pett-Ridge et al.*, 2006]. Soil in the microcosms was fresh from the field and uncompacted with small aggregates (≤ 3 mm diameter). Soil formed a thin and even layer within the incubation chambers so that rapid gas exchange was possible between the microcosm headspace and the inter-aggregate pore space. While we did not measure redox potential (Eh) directly, previous experience indicates that Fe oxides strongly poise these Fe-rich soils [*Hodges*, 2017; *Whitfield*, 1974].

Soil was pre-incubated for 16 days in the microcosms under a 4-day ambient air / 4-day N_2 (hereafter called ‘oxic’ and ‘anoxic’, respectively) regime to allow microbial activity to stabilize. After pre-incubation, microcosms were amended with 0.18 g commercially prepared ground leaf litter. Previous work suggests humid tropical forest soils can quickly deplete labile C stocks during laboratory incubations [*Chacón et al.*, 2006]; thus leaf litter equivalent to 10% of the native soil C pool was added (without mixing) to sustain microbial activity during the experiment. Litter was either ^{13}C labeled (97 atom%) or unlabeled ^{12}C ryegrass (Cambridge Isotopes Laboratories, Inc.). Isotope tracing results are discussed in a companion paper focused on microbial community dynamics. Statistical analyses revealed no significant effects of litter C isotopic composition on soil P and Fe pools on days when both labeled and unlabeled microcosms were harvested (Table S2), thus the data we present are from pooled ^{12}C and ^{13}C microcosms.

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

2.2 Soil Sampling and Analyses

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

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

Tiessen and Moir, 1993]. $NaHCO_3$-extractable P is weakly adsorbed on soil particles and thought to be readily available to plants [*Hedley et al.*, 1982]. In contrast, NaOH solubilizes P that is chemically sorbed to secondary Fe- and Al-minerals, and thought to represents a more stable fraction of soil P relative to $NaHCO_3$-extractable P [*McLaughlin et al.*, 1977]. Total P in both extracts ($NaHCO_3$-P_t and NaOH-P_t) was determined by measuring PO_4 according to the standard colorimetric method of *Murphy and Riley* [1962] after autoclaving extracted solutions with ammonium persulfate ($(NH_4)_2S_2O_8$) [*Tiessen and Moir*, 1993]. Inorganic P was also determined in the NaOH extract (NaOH-P_i) following *Murphy and Riley* [1962] after acidifying and centrifuging the extractant [*Tiessen and Moir*, 1993]. Organic P in the NaOH extract (NaOH-P_o) was estimated by subtracting inorganic P from total P. Consistent with previous research [*Mage and Porder*, 2012], inorganic P in the $NaHCO_3$ extract was below the detection limit, likely due to the high P sorption capacity of Fe- and Al-minerals, and thus was not reported here.

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

2.3 Statistical Analyses

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

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

Finally, we explored the effects of a shift in headspace redox conditions at 0 (M0), 30 (M30), and 180 min (M180) after headspace switches on days 12, 20, 23, and 36. Soil P and Fe data from both fluctuating treatments were pooled and analyzed in two categories, depending on whether data were collected during an $N_2 \rightarrow$ ambient air switch (oxidation) or ambient air $\rightarrow N_2$ (reduction) event. In total, there were four oxidation events and four reduction events. Differences in P and Fe species pools with time (0, 30, 180 minutes) were compared using two-way ANOVA followed by multiple comparisons controlled by Tukey's tests. Time and sampling event were treated as two main factors. All statistical analyses were conducted in R ver 3.3.3 [*R Core Team*, 2017]. Mean values ± standard errors and *P* values from Tukey tests are presented unless otherwise noted.

3 Results

3.1 Static Headspace Treatments

The static anoxic treatment significantly increased the concentrations of most soil P and Fe species over the experiment. Concentrations of NaOH-P_i (inorganic P bound to Fe/Al minerals; Figure 1; $P < 0.01$) and AO-P_t pools (P bound to amorphous Fe/Al minerals; $P < 0.001$) in the static anoxic treatment were 10 ± 3% and 38 ± 8% higher than in the static oxic treatment, respectively. The static anoxic treatment also increase the $NaHCO_3$-P_t pool relative to the static oxic treatment toward the end of the experiment (Figure S2; day 36, $P < 0.05$; day 44, $P < 0.01$), although the effect was not significant when analyzing the experiment as a whole. The concentration of NaOH-P_o (organic P bound to Fe/Al minerals) was not affected by redox treatments. Effects of the static anoxic treatment on Fe species were much stronger than those on P fractions. The static anoxic treatment caused a 15-fold increase in HCl-Fe(II) relative to the static oxic treatment ($P < 0.001$) and also doubled AO-Fe concentrations ($P < 0.001$).

3.2 Fluctuating Redox Treatments

Averaging over the experiment, the low frequency fluctuation treatment increased soil HCl-Fe(II) concentrations relative to the static oxic treatment (Figure 1; $P < 0.01$), but the high frequency treatment did not. The two fluctuating redox treatments did not affect soil AO-Fe concentrations relative to the static oxic treatment. The low frequency fluctuation increased AO-P_t concentrations by 28 ± 8% relative to the static oxic treatment ($P < 0.01$), but there was no effect of the high frequency treatment. The two fluctuating redox treatments did not affect other soil P pools relative to the static oxic treatment over the experiment.

Soil HCl-Fe(II) concentrations were sensitive to the 4-day and 8-day shifts in headspace redox conditions (Figure 2). Concentrations of HCl-Fe(II) consistently decreased during oxidation events and increased during reduction events. The response of HCl-Fe(II) to redox events gradually decreased in magnitude over time. For example, the redox effect was only marginally significant (T-test: $P = 0.09$) during the last oxidation event between day 36 and 44 under the low frequency treatment. In addition, the HCl-Fe(II) concentration under oxic headspace conditions tended to increase over time (linear regression: $P < 0.001$). Similar redox effects were observed in AO-Fe and AO-P_t pools only during the first set of redox events, but not thereafter. Both soil AO-P and AO-Fe concentrations decreased during the first oxidation event (Figures 3 and S3; T-test: both $P < 0.05$). The AO-Fe pool increased during the first reduction event (T-test: $P < 0.001$), and AO-P tended to increase (T-test: $P = 0.13$). The soil NaOH-P_i pool decreased during two out of three oxidation events (Figure S4), while it did not respond to reduction events. Neither $NaHCO_3$-P_t nor NaOH-P_o pool responded to these redox events.

3.3 Short-term Effects of Redox Fluctuation

To understand the short-term effects of a redox shift on P and Fe pools, we harvested microcosms immediately before, and 30 and 180 minutes following a change in headspace redox conditions. Averaging across sampling events, concentrations of HCl-Fe(II) decreased by 28 ± 10% (Figure 4; $P < 0.05$) at 180 min following oxidation events, increased by 37 ± 7% ($P < 0.001$) at 30 min following reduction events, and returned to their initial levels by 180 min ($P < 0.01$). Similarly, concentrations of AO-Fe and AO-P_t both increased at 30 min following the reduction event (both $P < 0.01$), and returned to their initial levels by 180 min (both $P < 0.05$). In contrast, $NaHCO_3$-P_t concentrations decreased at 180 min following reduction events ($P < 0.05$). Following oxidation events, soil AO-Fe ($P = 0.07$) and NaOH-P_i pools ($P = 0.11$) tended to decrease at 180 min, but the NaOH-P_i pool did not respond to reduction events. The soil NaOH-P_o pool was not sensitive to either oxidation or reduction events.

4 Discussion

4.1 Effects of Static Redox Conditions on P and Fe Cycling

The static anoxic treatment elevated the concentrations of $NaHCO_3$-P_t, NaOH-P_i, and AO-P_t relative to the static oxic treatment. It also led to a significant reduction of ferric (oxyhydr)oxides and accumulation of HCl-Fe(II) and AO-Fe. These results are consistent with previous work using soils from the LEF [*Chacón et al.*, 2006; *Liptzin and Silver*, 2009], and suggest that static anoxic conditions effectively increased the total amount of extractable P. This increase likely represents a loss of P from other pools (not included in this study) and/or may represent an increase in the extraction efficiency of the solutions (i.e., $NaHCO_3$, NaOH, and AO). Reduction events can decrease microbial biomass P and convert more recalcitrant P pools (e.g., HCl-extractable P and residual P) to more labile forms [*Ajmone-Marsan et al.*, 2006; *Wright et al.*, 2001; *Yang et al.*, 2006; *Zhang et al.*, 1994]. Reducing conditions are also known to decrease the strength of Fe minerals in sorbing P [*Chacón et al.*, 2006; *Heiberg et al.*, 2012; *Peretyazhko and Sposito*, 2005], which could lead to an increase in the extraction efficiency of NaOH and AO solutions. Elevated Fe(II) could further catalyze the reductive dissolution of crystalline Fe in the presence of organic ligands such as oxalate [*Kostka and Luther*, 1994; *Sulzberger et al.*, 1989] and likely increase the AO-extractable P.

The effects of the static anoxic treatment on Fe species were much greater than those on P fractions, suggesting that Fe-associated soil P was much less abundant relative to the mineral surfaces available for P sorption. It is possible that the static anoxic treatment initially mobilized amorphous Fe minerals that were relatively enriched in P, while continued anoxic conditions transformed the amorphous and crystalline Fe minerals that were P-poor. Re-sorption is another mechanism that could explain the weaker responses of P pools to static anoxic treatments compared to those of Fe pools [*Barros et al.*, 2005; *Heiberg et al.*, 2012]. Reduced Fe minerals, despite weaker P bonding strength than oxidized forms, can still have high surface area for binding P [*Borch and Fendorf*, 2007; *Patrick and Khalid*, 1974]. The extremely low P concentration makes the precipitation of metal phosphate, such as vivianite, highly unlikely [*Walpersdorf et al.*, 2013]. Thus resorption is likely responsible for the lack of response in $NaHCO_3$-P_t in the static anoxic treatment before day 36 of the experiment. If indeed $NaHCO_3$-P_t was released during reduction events and then quickly re-captured by the mineral matrix, then the gross release of labile P would be greater in magnitude than the net increase observed here.

4.2 Effects of Dynamic Redox Conditions

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

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

The type of redox environment likely influences the responses of soil P and Fe pools, especially during redox oscillations. By employing anoxic glovebox with 5% H_2 and using soil slurries with addition of organic matter, Barcellos *et al. [2018]* and Ginn *et al. [2017]* showed that repeated redox oscillation increased the redox sensitivity of amorphous Fe minerals, eventually resulting in comparable Fe(II) concentrations between fluctuating redox and static anoxic treatments. Their designs likely stimulated Fe reduction and created stronger reducing conditions than those in our experiment achieved with headspace manipulation. Quantifying redox potential is difficult in Fe-rich soils characterized by heterogeneous microsite dynamics [*Silver et al.*, 1999]. Future breakthroughs on this front would improve the upscaling of laboratory results to an ecosystem level.

4.3 Short-term Impacts of Redox Fluctuations

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

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

Interestingly, labile P ($NaHCO_3$-P_t) decreased immediately following reduction events. Iron redox dynamics are not a good explanation for this phenomenon, because reductive dissolution of Fe minerals should increase, rather than decrease, P availability. Sorption by organic matter could be one sink of this $NaHCO_3$-P_t; a companion study showed that short-term reduction events tripled dissolved organic carbon concentration [*Bhattacharyya et al.*, 2018]. There might also be competition between dissolved organic carbon and P for soil sorption sites, such that release of dissolved organic carbon could increase P sorption by soil minerals [*Guppy et al.*, 2005]. Microbial uptake is yet another potential sink, particularly if recently mobilized P diffused into aerobic microsites or was utilized by anaerobic microorganisms. Indeed, *Olander and Vitousek* [2005] found that microbial P uptake can account for a higher proportion of newly added PO_4^- than mineral sorption.

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

4.4 Ecological Implications

Humid tropical forest soils experience periodic anoxic conditions at hourly to weekly time scales [*Liptzin et al.*, 2011; *Silver et al.*, 1999]. Our results demonstrate that effects of redox oscillation on soil P fractions, and potentially P availability to plants and microbes, vary over time. Both labile ($NaHCO_3$-P_t) and intermediate (AO-P_t) P pools were highly responsive to a change in bulk redox condition. However, when analyzed at daily to weekly time scales, effects of redox oscillation on soil P pools tended to be less significant. Given that P dynamics are governed by various competing mechanisms, including reductive dissolution, desorption versus adsorption, and biological uptake, the net changes in P fractions we observed at longer time scale (weekly to monthly) likely underestimate the gross flux of P released by dissolution and desorption. In soils that experience regular redox oscillations, such as humid tropical forests, the first several hours following redox shifts may be a critical period for P bioavailability.

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

4.5 Conclusion

Our study highlights that redox-induced cycling of Fe (oxyhydr)oxides affected several P fractions in humid tropical forest soil. Static anoxic conditions greatly enhanced the reduction of Fe (oxyhydr)oxides and increased the labile ($NaHCO_3$-P_t) and intermediate (NaOH-P_i and AO-P_t) P pools. Effects of redox fluctuation on P fractions were more complex and dependent on the temporal scale. Redox sensitivity of amorphous Fe and P pools tended to decrease over time. But immediately after a change in redox condition, AO-P_t showed strong coupling with Fe speciation during reduction events. The NaOH-P_i and AO-Fe pools showed a declining trend during oxidation events, similar to that of HCl-Fe(II). Surprisingly, the labile P pool decreased immediately after reduction events, likely mediated by resorption and microbial uptake. These results support our hypothesis that soil P responds *rapidly* to changes in redox condition at the scale of hours. Climate change is likely to affect redox dynamics in humid tropical forests, and this is likely to feed back directly and indirectly on ecosystem-level processes. Effects of redox conditions on soil P availability should thus be considered in predictions of soil fertility and limitations to primary productivity under future climate change scenarios.

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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 [*Tiessen and Moir*, 1993].	"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; *Richter et al.*, 2006].	"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 [*Guo and Yost*, 1999].	"amorphous Fe/Al-bound P"

Figure Captions

Figure 1. Effects of redox manipulation on (a) $NaHCO_3$-extractable total P ($NaHCO_3$-P_t), (b) ammonium oxalate extractable total P (AO-P_t) , (c) NaOH-extractable inorganic P (NaOH-P_i), (d) HCl-extractable Fe(II) (HCl-Fe(II)), (e) ammonium oxalate extractable Fe (AO-Fe), and (f) NaOH-extractable organic P (NaOH-P_o) of a humid tropical soil from Puerto Rico, averaging across time. 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 treatment. From right to left, treatments increased in their exposure to an oxic headspace (CO_2-free air). Boxplot whiskers refer to 1.5 times the interquartile range of data. Multiple comparisons were controlled by Tukey's tests. Different letters indicate significant differences at $\alpha = 0.05$.

Figure 2. Responses of HCl-extractable Fe(II) (HCl-Fe(II)) 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.

Figure 3. Responses of ammonium oxalate extractable total P (AO-P_t) 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.

Figure 4. Responses of on (a) $NaHCO_3$-extractable total P ($NaHCO_3$-P_t), (b) ammonium oxalate extractable total P (AO-P_t) , (c) NaOH-extractable inorganic P (NaOH-P_i), (d) HCl-extractable

Fe(II) (HCl-Fe(II)), (e) ammonium oxalate extractable Fe (AO-Fe), and (f) NaOH-extractable organic P (NaOH-P_o) immediately following oxidation events (N_2 → air) or reduction events (air → N_2) in a humid tropical soil redox oscillation experiment, averaging across multiple events. Boxplot whiskers refer to 1.5 times the interquartile range of data. Multiple comparisons were controlled by Tukey’s tests. Different letters indicate significant differences at $\alpha = 0.05$.

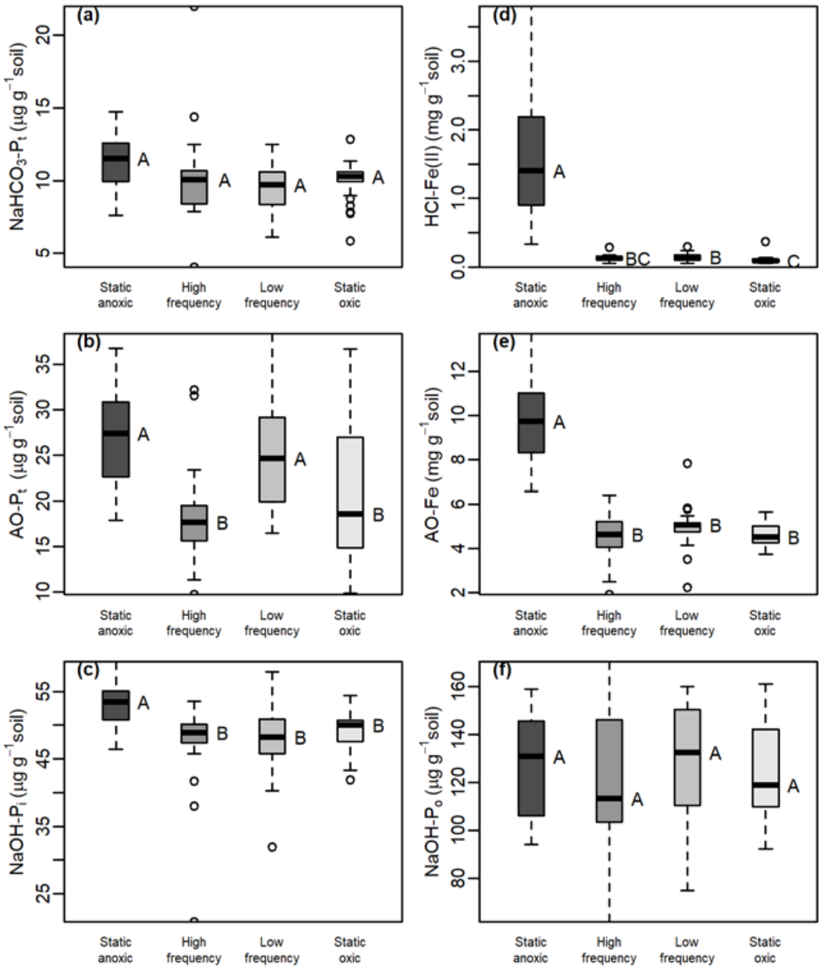
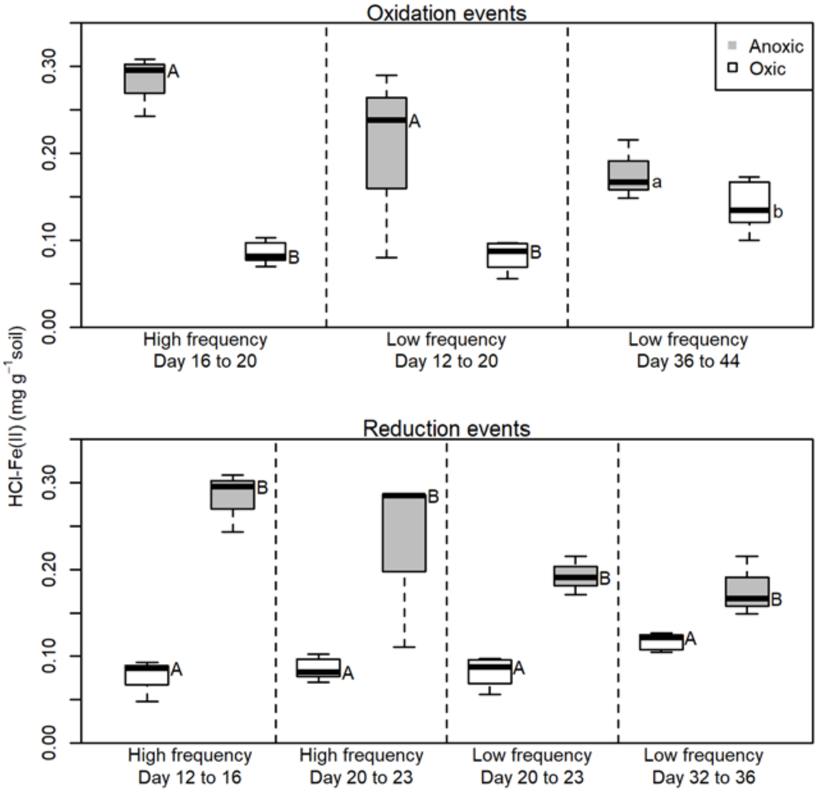
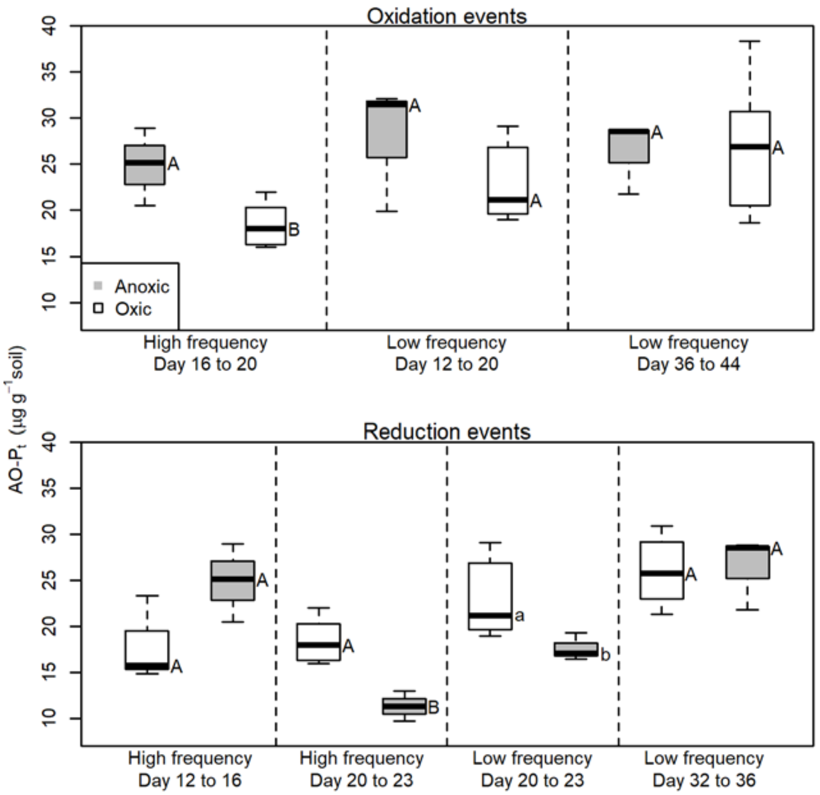

(a)
NaHCO$_3$-P$_t$ (µg g^{-1} soil)
5
10
15
20
A
A
A
A
(b)
AO-P$_t$ (µg g^{-1} soil)
10
15
20
25
30
35
A
B
A
B
(c)
NaOH-P$_i$ (µg g^{-1} soil)
25
35
45
55
A
B
B
B
(d)
HCl-Fe(II) (mg g^{-1} soil)
0.0
1.0
2.0
3.0
A
BC
B
C
(e)
AO-Fe (mg g^{-1} soil)
2
4
6
8
10
12
A
B
B
B
(f)
NaOH-P$_o$ (µg g^{-1} soil)
80
100
120
140
160
A
A
A
A
Static anoxic
High frequency
Low frequency
Static oxic

Figure 1



Oxidation events
Anoxic
Oxic
HCl-Fe(II) (mg g^{-1} soil)
0.00
0.10
0.20
0.30
A
B
A
B
a
b
High frequency
Day 16 to 20
Low frequency
Day 12 to 20
Low frequency
Day 36 to 44
Reduction events
A
B
A
B
A
B
A
B
High frequency
Day 12 to 16
High frequency
Day 20 to 23
Low frequency
Day 20 to 23
Low frequency
Day 32 to 36

Figure 2


Oxidation events
Reduction events
AO-P_t (µg g^{-1}soil)
40
35
30
25
20
15
10
■ Anoxic
□ Oxic
A
B
A
A
A
A
A
A
B
a
b
A
A
High frequency
Day 16 to 20
Low frequency
Day 12 to 20
Low frequency
Day 36 to 44
High frequency
Day 12 to 16
High frequency
Day 20 to 23
Low frequency
Day 20 to 23
Low frequency
Day 32 to 36

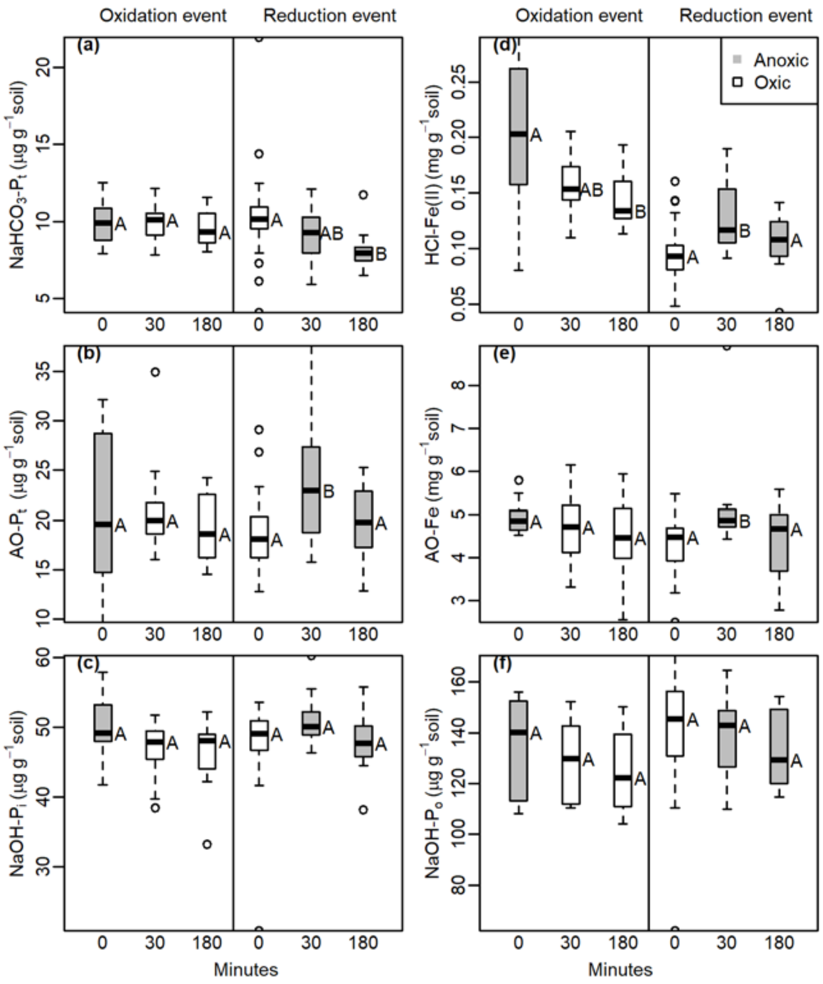

Oxidation event
Reduction event
Oxidation event
Reduction event
(a)
$NaHCO_3$-P_t (µg g^{-1} soil)
(b)
AO-P_t (µg g^{-1} soil)
(c)
NaOH-P_i (µg g^{-1} soil)
(d)
HCl-Fe(II) (mg g^{-1} soil)
(e)
AO-Fe (mg g^{-1} soil)
(f)
NaOH-P_o (µg g^{-1} soil)
Anoxic
Oxic
A
AB
B
0
30
180
Minutes
Minutes

Figure 4

Journal of Geophysical Research - Biogeosciences

Supporting Information for

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

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Contents of this file

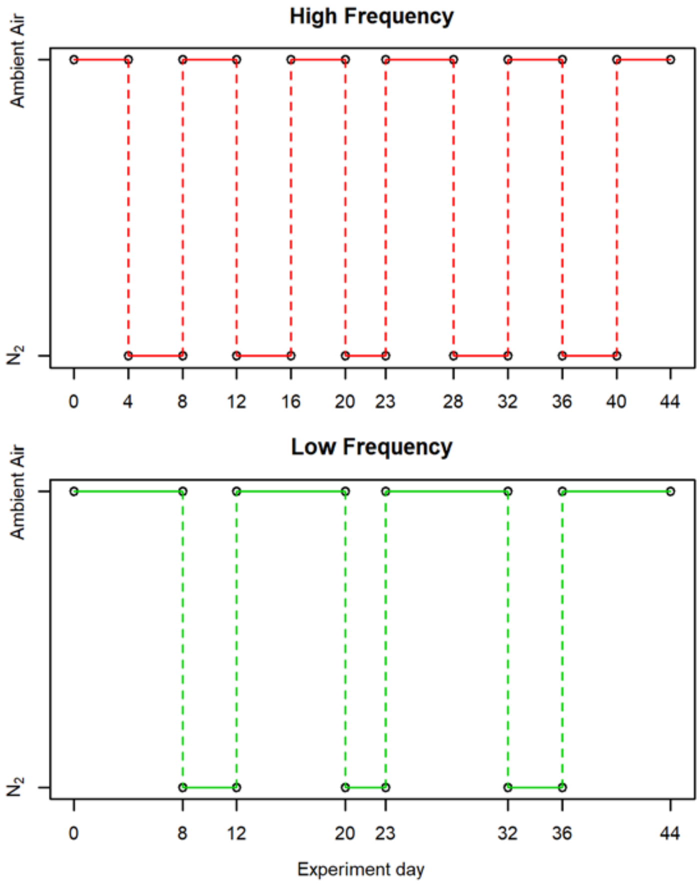

Figure S1. Diagram of the experimental set-up of two fluctuating redox treatments. Dots indicate the times when headspace composition was switched between ambient air and N_2.

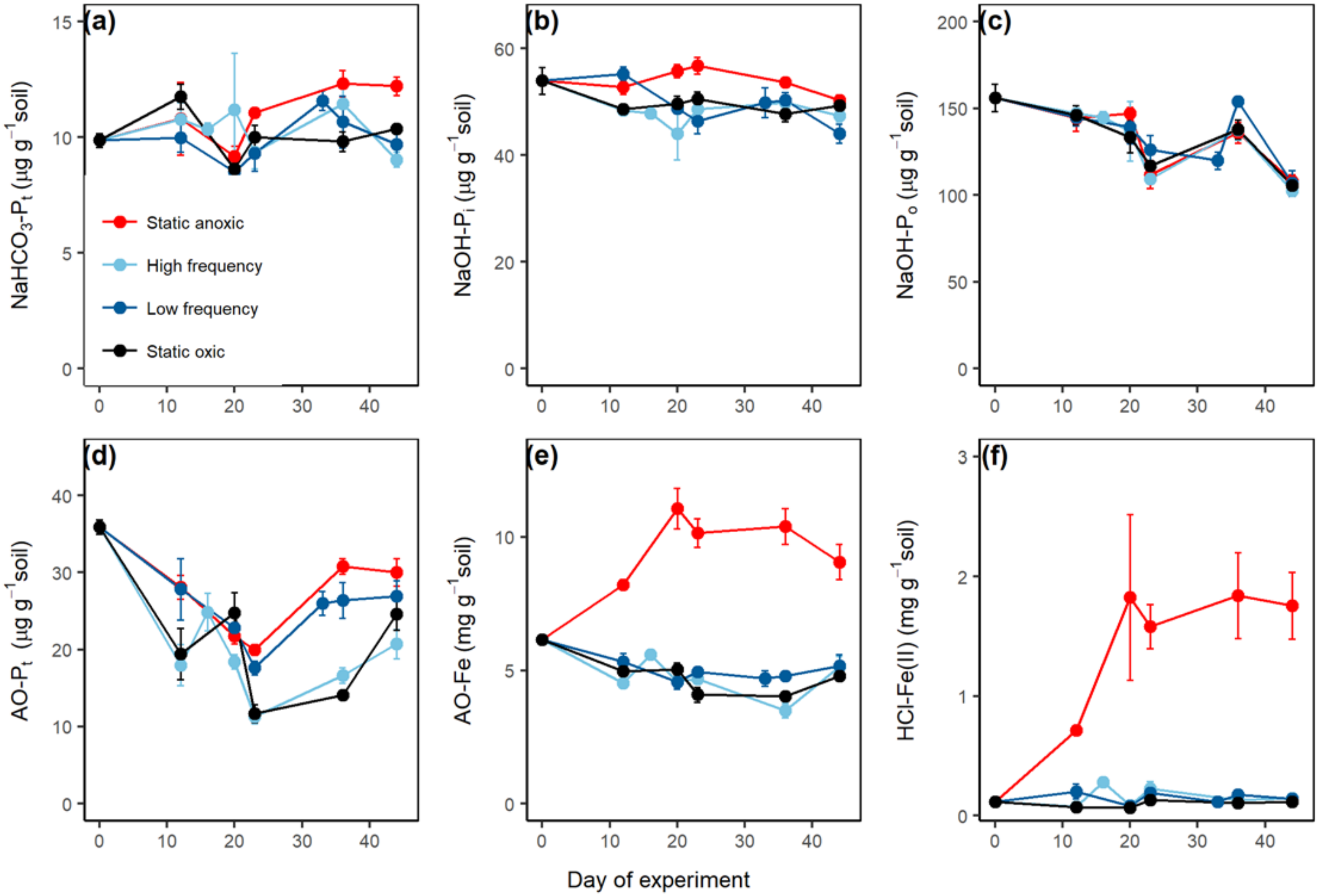
(a)
NaHCO$_3$-P$_t$ (μg g^{-1}soil)
15
10
5
0
Static anoxic
High frequency
Low frequency
Static oxic
(b)
NaOH-P$_i$ (μg g^{-1}soil)
60
40
20
0
(c)
NaOH-P$_o$ (μg g^{-1}soil)
200
150
100
50
0
(d)
AO-P$_t$ (μg g^{-1}soil)
40
30
20
10
0
(e)
AO-Fe (mg g^{-1}soil)
10
5
0
(f)
HCl-Fe(II) (mg g^{-1}soil)
3
2
1
0
0
10
20
30
40
Day of experiment

Figure S2. Effects of redox manipulation on (a) $NaHCO_3$-extractable total P ($NaHCO_3$-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.

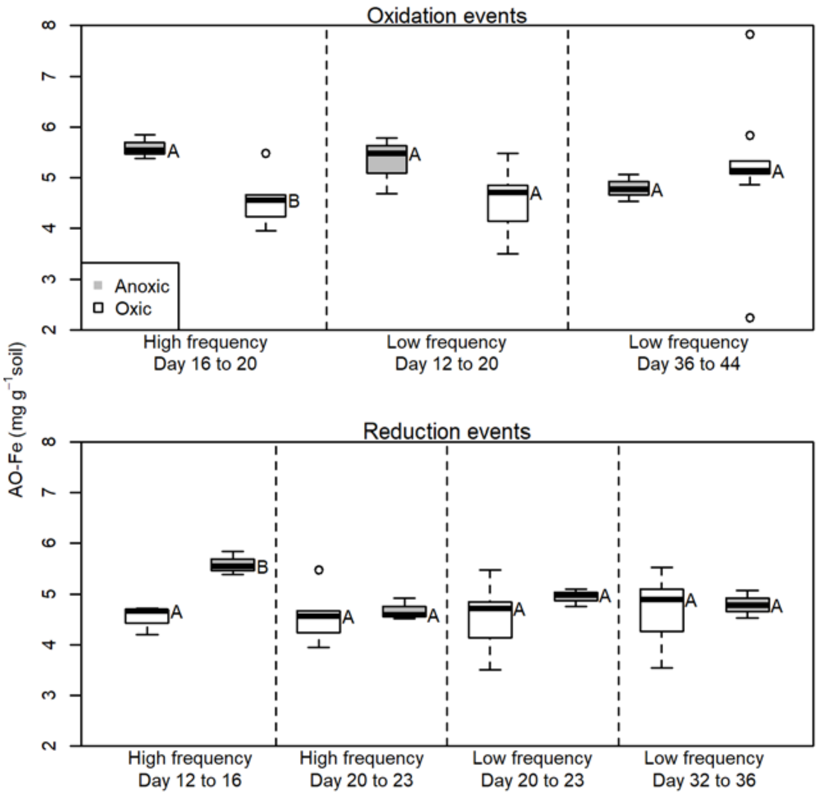

Figure S3. Responses of ammonium oxalate extractable Fe (AO-Fe) to oscillating redox conditions. Boxplot whiskers refer to 1.5 times the interquartile range of data. Different letters indicate significant differences at α = 0.05, derived from Student's T-test.

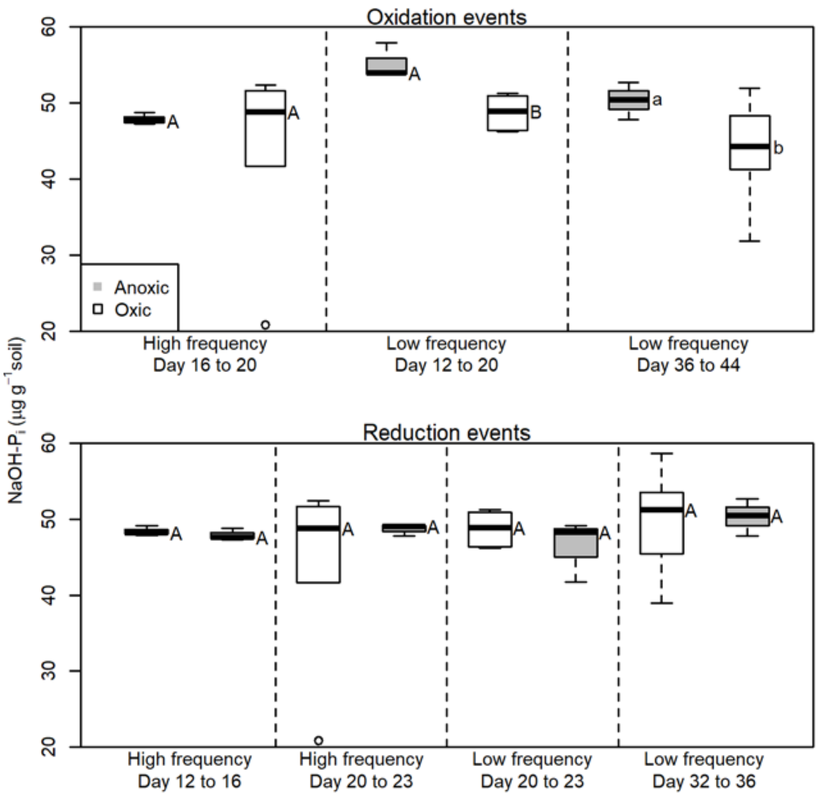

Figure S4. Responses of NaOH-extractable inorganic P (NaOH-P$_i$) to oscillating redox conditions. Boxplot whiskers refer to 1.5 times the interquartile range of data. Different upper- and lower-case letters indicate significant differences at $\alpha = 0.05$ and 0.10 derived from Student's T-test, respectively.

Table S1. Number of soil microcosms harvested on each sampling day. Microcosms that received labelled rye grass litter were noted separately (^{13}C). 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 ^{12}C- and ^{13}C-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**