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# Low Redox Decreases Potential Phosphorus Limitation on Soil Biogeochemical Cycling Along a Tropical Rainfall Gradient

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## ABSTRACT

Humid tropical forests on highly weathered soils are often characterized by low bioavailable phosphorus (P) concentrations. These ecosystems also often experience low and fluctuating redox conditions. Little is known about how soil redox conditions affect P availability and how this might feedback on biogeochemical cycling. Here we used soils from a wet tropical rainfall gradient in Puerto Rico to explore the effects of redox on P bioavailability and associated biogeochemical processes. Concentrations of soil carbon (C) and poorly crystalline iron (Fe) and aluminum (Al) minerals increased at least twofold with increasing rainfall, reflecting stronger anaerobic conditions at wetter sites and associated declines in decomposition. The fraction of the total P pool in the NaOH-extractable organic form also generally increased with increasing rainfall. In a laboratory incubation experiment using three sites along the gradient, P amendment increased aerobic $CO_2$ production. However, anaerobic processes, including anaerobic respiration, Fe reduction, and methanogenesis, increased with P amendment at the driest site only. Microbial biomass C:P ratios decreased with P amendment under anoxic conditions at the driest site, an indicator of possible microbial P limitation at this site. Both microbial biomass C and P concentrations were lower under anoxic conditions than under oxic conditions across all soils, suggesting that anoxic conditions could be a more limiting factor to microbes than P concentrations. Overall, our results demonstrate that redox conditions regulate the extent of P limitation to biogeochemical processes in tropical forest soils. Phosphorus limitation was pronounced in aerated environments with low mean annual rainfall, whereas low redox conditions or associated factors under high rainfall conditions may have a stronger impact on biogeochemical cycling than P availability.

**Key words:** Luquillo CZO and LTER; Oxisols; Labile phosphorus; Nutrient limitation; Carbon use efficiency.

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## INTRODUCTION

Soil phosphorus (P) availability has profound impacts on belowground carbon (C) cycling in humid tropical forests (Huang and others 2013; Reed and others 2015; Wright 2019; Jiang and others 2020). Labile P concentrations are often low in these ecosystems, as warm, perhumid conditions promote weathering processes that release P from primary minerals and facilitate leaching losses that exceed weathering rates over geologic time (Miller and others 2001; Porder and others 2007). Strongly weathered Fe and Al minerals also have high P sorption capacity that can lower P solubility (McGechan and Lewis 2002; Olander and Vitousek 2004; Lin and others 2020). Thus, soil P availability is commonly thought to limit primary productivity, which provides C inputs into belowground environments (Tanner 1998; Vitousek and others 2010; Turner and others 2018). Soil P availability also limits a wide range of microbially derived biogeochemical processes, such as respiration, organic matter decomposition, and nitrogen mineralization (Camenzind and others 2017).

Redox conditions represent another important environmental control of belowground biogeochemical cycling in humid tropical forests. Many upland soils in these environments experience frequent low redox conditions on the time scales of hours to weeks (Silver and others 1999; Schuur and others 2001; Liptzin and Silver 2015; Barcellos and others 2018b). The prevailing redox regimes can be important predictors of soil C storage and persistence at a given site, as hypoxia and anoxia has been shown to inhibit organic matter decomposition (Greenwood 1961; Davidson and others 2012; Ping and others 2013; Keiluweit and others 2016). However, reducing conditions also trigger Fe reduction that may subsequently increase the solubility and mineralization of soil organic matter (Dubinsky and others 2010; Chen and others 2018; Huang and others 2020). Reducing conditions may indirectly affect soil C cycling by altering P dynamics. Several studies have reported increased P mobility with Fe reduction, as reduced Fe species are thought to have lower Fe–P bonding strength (Peretyazhko and Sposito 2005; Chacón and others 2006; Lin and others 2018). In theory, P limitation on biogeochemical processes might be relieved with increased P mobility and utilization.

The extent of microbial P limitation is likely to vary across sites with different redox regimes. Reducing conditions can inhibit phosphatase enzyme activity (Kang and Freeman 1999; Hall and others 2014), and thus, P mineralization may be low at sites experiencing frequent low redox events. Redox regimes may also affect soil mineralogy and mineral-P associations. For example, rainfall regimes regulated the concentrations of poorly crystalline minerals in soils by affecting leaching intensity (Miller and others 2001). Given the importance of these reactive minerals as P sinks, changes in their concentrations would likely affect the competition between mineral and biological P sinks (Olander and Vitousek 2004; Gross and others 2018). How these processes vary across rainfall and redox gradients, and how they ultimately affect biogeochemical processes, is poorly understood.

In the Luquillo Mountains of Puerto Rico, increasing elevation corresponds to higher mean annual precipitation (Garcia-Martino and others 1996; Murphy and others 2017). Field studies of soil $O_2$ concentrations and redox-sensitive biogeochemistry over time and space have revealed more prevalent reducing conditions with increasing rainfall (Silver and others 1999, 2013; Liptzin and Silver 2015). In a recent study, Gross and others (2020) explored the effects of anoxia on microbial P uptake and microbial C use efficiency (CUE) in tropical forests and found that anoxia inhibited P uptake into microbial cells and lowered their CUE. Here, we explored the effects of rainfall and associated redox conditions on P fractions along a landscape-scale tropical palm forest gradient. Our gradient was parallel to the one studied in Gross and others (2020), but unlike that study, this one controlled for forest type and explored biogeochemical impacts on C fluxes, Fe reduction, and reactive Fe and Al. Our overarching hypothesis was that long-term rainfall regimes affect the extent of P limitation by regulating mineral-P association and soil P composition. We hypothesized that low P availability limits biogeochemical processes under both oxic and anoxic conditions, because reactive Fe and Al minerals act as P sinks under both redox conditions (Lin and others 2020). Anaerobic processes, including $CO_2$ production, methanogenesis, and Fe reduction, would increase with P amendment as an indicator of release from P limitation. We predicted that increasing mean annual rainfall and its effect on soil redox conditions lead to an accumulation of organic matter, organic P, poorly crystalline Fe and Al minerals, and NaOH-extractable P, which is commonly thought to be associated with Fe and Al minerals (Tiessen and Moir 1993; Gross and others 2020). As high concentrations of organic P and reactive minerals increase the cost of P acquisition and the strength of mineral-P sink, respectively, biogeochemical processes would be more limited by P availability at wetter than drier sites.

## MATERIALS AND METHODS

### Study Sites

This study was conducted in the Luquillo Experimental Forest (LEF) in northeastern Puerto Rico, part of the NSF-sponsored Long-Term Ecological Research (LTER) and Critical Zone Observatory (CZO) networks, and the DOE funded Next Generation Ecosystem Experiment (NGEE-Tropics). The Luquillo Mountains rise steeply from approximately 250 m above sea level to its highest peak, El Toro (1075 m, a.s.l.) in less than 10 km (Weaver and Murphy 1990). Mean annual precipitation (MAP) increases with increasing elevation. A recent assessment of the rainfall records from the LEF showed that MAP changed from approximately 3200 mm at 300 m to approximately 4800 mm at 1000 m (Murphy and others 2017). Estimated MAP correlated linearly with elevation ($r > 0.99$) (Murphy and others 2017). This rainfall gradient also features associated changes in soil redox conditions, with more frequent and intensive reducing events at wetter sites (Silver and others 1999, 2013; Liptzin and others 2011; Hall and Silver 2015; Liptzin and Silver 2015). Studies have reported soil $O_2$ concentrations and associated indicators of redox conditions (for example, Fe(II) concentrations, methane and nitrous oxide concentrations and production) along the Luquillo elevation gradient (Silver and others 1999, 2013; Liptzin and others 2011; Hall and Silver 2015; Liptzin and Silver 2015), including an 8-year study by Silver and others (2013) that found that mean monthly soil $O_2$ had a strong negative correlation ($R^2 = 0.80$) with mean precipitation along the elevation gradient. Mean annual temperature (MAT) decreases slightly with increasing elevation. Field measurements of MAT ranged from approximately 23 °C at 350 m to 19 °C at 930 m (Brown and others 1983; Weaver and Murphy 1990).

This study was conducted along a transect naturally dominated by one species, sierra palm (*Prestoea acuminata*), in the Sonadora River watershed (Figure S1) (Barone and others 2008; Willig and others 2011). The transect ranged in elevation from 300 to 1000 m and occurred on volcaniclastic parent material from the Hato Puerco and Tabonuco formations that weathered into soils predominantly in the orders of Oxisols and Ultisols (USDA Natural Resources Conservation Service 2002). Soils are moderately or strongly acidic with pH ranging from 4.0 to 6.0 and high contents of kaolinite and Fe and Al oxides and (oxy)hydroxides (Beinroth 1982; Porder and others 2015). Palm forest occurs throughout the elevation gradient in the LEF. In addition to *P. acuminata*, the palm forest often includes low densities of *Henriettea squamulosa*, *Eugenia borinquensis*, *Cyathea arborea*, and *Cecropia schreberiana* (Gould and others 2006; Shiels and Walker 2013). The palm forests are a stable plant community in the LEF that occur on slopes, likely as a late successional stage/climax community following a historical disturbance (Guariguata 1990).

### Soil Sampling

Soils were collected from the transect every 100 m from 300 to 1000 m above sea level. At each elevation, a 0.1 ha (50 × 20 m) plot was established with the long side of the plot perpendicular to the slope (Willig and others 2011). Each plot was then divided into ten 10 × 10 m subsections. One 10 cm long soil core was obtained using an auger with 5 cm diameter in each 10 × 10 m subsection. All soil cores were taken at upper topographic positions to avoid valleys that experienced more prevalent reducing conditions and the accumulation of finer soil material from downslope movement (Silver and others 1999; Hall and Silver 2015; O'Connell and others 2018). Previous work along the elevation gradient on volcaniclastic soils showed that ridges and slopes had similar P fractions (that is, no significant differences, Mage and Porder 2012). The litter layer was removed prior to sampling the mineral soil. Soil cores were combined and homogenized from two adjacent 10 × 10 m subsections in parallel to the short side of the 0.1 ha plot, resulting in five soil samples per elevation level, for a total of 40 soil samples (8 elevation levels × 5 replicates). Samples were collected in two 3-day campaigns that were one week apart and then shipped overnight to the University of California, Berkeley at the end of each campaign. Statistical analysis did not reveal significant effects of sampling time. In the laboratory, roots, rock fragments, and soil macrofauna were removed by hand and samples were gently mixed to homogenize.

### Soil Analyses

Soil C and N concentrations were measured in duplicate on an elemental analyzer (NC 2100, CE Instruments) using oven-dried (105 °C until the weight was stable) and ground 20 mg subsamples. To measure dissolved organic carbon (DOC), we used a potassium sulfate ($K_2SO_4$) index (Vance and others 1987), commonly used as a measure of DOC (for example, Park and Matzner 2003; Zeglin and others 2007; Rousk and Jones 2010). Approximately 10 g fresh soil (oven-dry equivalent [ODE] weight) was mixed with 40 ml 0.5 M $K_2SO_4$ solution for 1 h and then filtered through Whatman # 42 filter paper (Park and Matzner 2003; Rousk and Jones, 2010). Total organic C concentrations of the extractants were measured using a TOC analyzer (Model 1010, O-I-Analytical). Acid-soluble Fe was extracted by mixing 4 g (ODE) fresh soil in 40 ml of 0.5 M hydrochloric acid (HCl) solution and shaking for 1 h followed by centrifugation. The HCl-extractable Fe(II) and Fe(III) was determined using a modified ferrozine assay (Viollier and others 2000). The HCl solution solubilizes sorbed Fe(II) and a reactive fraction of poorly crystalline Fe(III) minerals (Hall and Silver 2015). Poorly crystalline Fe and Al minerals were extracted by mixing 1.5 g (ODE) fresh soil with 45 ml citrate ascorbate solution over 16 h (Reyes and Torrent 1997; Hall and Silver 2015); this extraction provides an index of Fe oxides and (oxy)hydroxides that are potentially reducible by microorganisms (Hyacinthe and others 2006). We also used an ammonium oxalate extraction in the dark at pH 3 to provide another set of estimates of the poorly crystalline Fe and Al minerals. Ammonium oxalate measures the Fe and Al oxides and (oxy)hydroxides that can be chelated. For the ammonium oxalate extraction, we followed a standardized protocol (Loeppert and Inskeep 1996) that used 1.0 g air-dried soils and 1:40 soil-to-solution ratio shaken over 2 h. Inductively coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer Optima 5300 DV) was used to measure the Fe and Al concentrations of the citrate ascorbate and ammonium oxalate extractants. Soil gravimetric water content and ODE was determined by oven drying 30 g subsamples at 105 °C until the weight was stable. Soil pH was measured in a 1:2 soil to water slurry.

Compared to the companion study (Gross and others 2020) that focused on microbial uptake and the organic P fraction, this study aimed to characterize soil total P and several P fractions that were predicted to differ along the gradient. We used a 0.1 M sodium hydroxide solution (NaOH) to extract P that is associated with organic matter and chemically sorbed to Fe and Al minerals (Tiessen and Moir 1993). We expected that the NaOH solution solubilized most of the labile P fractions that could be recovered by anion exchange resin and 0.5 M $NaHCO_3$ solution in the Hedley protocol (Hedley and others 1982), as the NaOH solution is a more effective extractant and the labile P concentrations were low in these soils under field-like conditions (McGroddy and Silver 2000; Lin and others 2018). One gram (ODE) of fresh soil was shaken overnight with 45 mL of NaOH solution. Total NaOH-extractable P was determined after autoclaving the NaOH extractant with ammonium persulfate ($(NH_4)_2S_2O_8$) (Tiessen and Moir 1993). The NaOH-extractable inorganic P (NaOH Pi) was determined after acidifying and centrifuging the non-autoclaved extractant (Tiessen and Moir 1993). The NaOH-extractable organic P (NaOH Po) was calculated as the difference between the total and inorganic P fractions. Finally, we measured soil P following ignition of 0.8 g air-dried soil at 550 °C for 1 h and then extraction with sulfuric acid (that is, ignition total P or ITP; 1 M $H_2SO_4$, 1:50 soil-to-acid ratio over 16 h) (Saunders and Williams 1955; Walker and Adams 1958; Turner and Engelbrecht 2011; Stahr and others 2017). Phosphorus concentrations in the above analyses were all determined colorimetrically following Murphy and Riley (1962). We did not extract soil P with 1 M HCl as in the Hedley protocol because this pool is negligible based on past research from the LEF (Mage and Porder 2012). Soil residual P was calculated by subtracting the NaOH-extractable total P from the ITP. Residual P is assumed to represent the P fraction that is not extractable by NaOH and held tightly to minerals or protected by occlusion and coprecipitation.

## Incubation Experiment

The incubation experiment followed a factorial design with two levels of P addition (control vs. P amendment), two levels of redox conditions (oxic vs anoxic), and soils from three sites (400, 800, and 1000 m elevation). A total of 144 microcosms were established to enable three destructive sampling events over 29 days (on days 1, 8, and 29; four replicates per treatment per day). Fresh soil (0–10 cm) was collected near the 0.1 ha plots ($< 20$ m) in a separate sampling trip and shipped overnight to the University of California, Berkeley. Upon arrival, soil was manually sorted to remove large rocks, roots, and fauna. To build the microcosms, 20 g (ODE) of soil was placed in each pint-size glass jar and closed with a gas-tight lid that included a septum for gas sampling. Soil water content was also determined from a set of subsamples ($n = 4$ per soil type) using the method described above. Phosphorus was added in the form of $NaH_2PO_4$ at the rate of 200 μg P $g^{-1}$ soil, an amount similar to the residual P concentrations and comparable to past studies (for example, Cleveland

and others 2002; Ginn and others 2017; Soong and others 2018). This amount was deemed necessary to increase labile P availability due to the high sorption capacity in these soils (Lin and others 2020). Control soils received NaCl to account for changes in ionic strength. Anoxic conditions were created by evacuation and refilling with $N_2$ for three times before transferring microcosms to a glovebox with 2% $H_2$ and 98% $N_2$ (Coy Laboratory Products, Grass Lake, MI). Microcosms were sealed inside the glovebox and flushed every two or three days. Oxic conditions were maintained by venting microcosms under ambient air at the same time when anoxic microcosms were flushed. The experiment lasted 29 days, which was equivalent to strong low redox events in the field (Liptzin and others 2011; Silver and others 2013). It was also long enough to deplete the reducible Fe pool and relieve the limitation of Fe reduction on methanogenesis according to previous studies with soils from the LEF (Ginn and others 2017; Bhattacharyya and others 2018; Lin and others 2018) and our preliminary results.

Measurements of greenhouse gas production were initiated by collecting a set of headspace samples right after microcosms were flushed. Using the septum on the lid, 30 ml of headspace was taken using a syringe and needle and injected into pre-evacuated 20 ml glass vials. Another set of headspace samples was collected after sealing the microcosms for 3 h. Concentrations of $CO_2$ and $CH_4$ (an index of low redox conditions) were measured by a thermal conductivity detector and a flame ionization detector, respectively, equipped on a gas chromatograph (GC-14A, Shimadzu, Columbia, MD). Gas production rates were determined by calculating the differences in their concentrations before and after the sealed period assuming linear flux rates over 3 h.

On days 1, 8, and 29 after the initiation of the experiment, a set of microcosms ($n = 4$ per treatment) was destructively harvested to analyze for soil microbial biomass C and P and chemical properties. The high concentrations of Fe and Al minerals contributed to strong P sorption in these soils (Lin and others 2020), so we adopted the modified fumigation-extraction method to measure microbial biomass P (Kouno and others 1995). The extraction system includes fresh soil (5 g, ODE), DI water (40 ml), and four strips of anion exchange resin membrane ($\sim 4$ $cm^2$ per strip; AMI-7001S, Membranes International; Wuenscher and others 2015). For each microcosm, one set of extraction systems received 1 ml of chloroform ($CHCl_3$) as a fumigant, while the other set did not and served as a control. After the 24-h extraction, the membrane was rinsed with DI water and mixed with 20 ml 0.25 M nitric acid ($HNO_3$) for 1 h to recover the released P. The P concentration of the $HNO_3$ solution was determined following Murphy and Riley (1962). Microbial biomass P was estimated by calculating the difference in P concentration between fumigated and non-fumigated samples and adjusted for the efficiency of fumigation in releasing P with a conversion ratio of 0.4 (Brookes and others 1982). We also followed the convention of considering the resin-extractable P concentration in the non-fumigated samples (or resin P, in short) as an index of readily soluble and exchangeable soil P (Tiessen and Moir 1993). Microbial biomass C was measured using the $CHCl_3$ fumigation method (Vance and others 1987). Fresh soil subsamples (10 g ODE) were fumigated in $CHCl_3$ for three days and then extracted with 40 ml of 0.5 M $K_2SO_4$ solution for 1 h. The DOC concentrations of the $K_2SO_4$ extracts were measured using a TOC analyzer (Model 1010, O-I-Analytical). Microbial biomass C was estimated as the difference in DOC between the fumigated and non-fumigated samples, after correcting for the unrecovered biomass with a conversion ratio of 0.45 (Vance and others 1987). The microbial biomass C:P ratio was calculated on a molar basis. Microbial biomass C and the C:P ratio was analyzed after day 1 of the experiment.

Acid-soluble Fe was measured using the method described above. In the anoxic treatment, rates of Fe reduction were estimated by calculating the differences in HCl-Fe(II) concentrations before and after the 29-day experiment and standardizing them over time. Concentrations of citrate ascorbate-extractable Fe and Al (CA-Fe and CA-Al) were analyzed using the method described above.

## Statistical Analyses

We used the concentrations and the ratios of P fractions (that is, NaOH P and residual P) to ITP to compare their relative abundances along the rainfall gradient. We explored the relationships between rainfall and the measured soil variables via Pearson’s correlation. We also used the Davies test to explore potential piecewise linear relationships along the rainfall gradient with the ‘segmented’ package in R (Davies 1987; Muggeo 2008). A significant Davies test ($\alpha = 0.1$) suggests the existence of potential breakpoints in the patterns of soil variables along the gradient.

For the incubation experiment, the effects of redox and P treatments on the cumulative production of $CO_2$ and $CH_4$ were assessed using two-way analysis of variance (ANOVA) after checking data for the assumption of homogeneity of variance and normality of residuals. For microbial and chemical characteristics, responses to the redox and P treatments were evaluated using two-way ANOVA in each combination of soil type and sampling day. When there was a significant interaction effect between the redox and P treatments, Tukey's tests were used to conduct multiple comparisons among all combinations of redox and P treatments. All statistical analyses were conducted in R ver. 3.6.1 (R Core Team 2019). Values in the text are means ± standard errors unless otherwise noted.

# RESULTS

## Rainfall Gradient

The results from the field sampling along the gradient showed significant accumulation of soil C and poorly crystalline minerals with increasing mean annual rainfall (Figure 1). Surface soil C concentrations increased from 4.80 ± 0.49% at the driest site to 11.48 ± 1.78% at the wettest site, resulting in a positive correlation with mean annual rainfall ($P < 0.001$, $r = 0.79$). Similar to soil C concentrations, poorly crystalline Al minerals extracted by the citrate ascorbate solution (CA-Al) increased linearly from 1.0 ± 0.1 mg $g^{-1}$ soil at the driest sites to 4.4 ± 0.7 mg $g^{-1}$ soil at the wettest sites ($P < 0.001$, $r = 0.75$). Concentrations of CA-Fe were approximately four times higher than CA-Al and were also positively correlated with rainfall ($P < 0.001$, $r = 0.70$). Concentrations of ammonium oxalate extractable Fe and Al minerals were positively correlated with mean annual rainfall as well (Figure S2; both $P < 0.001$, $r = 0.69$ and 0.80, respectively).

Patterns of DOC and HCl-extractable Fe pools were better described by piecewise linear relationships rather than linear correlations (Figure S3). According to Davies test, a breakpoint around 4650 mm rainfall existed in the patterns of DOC concentrations along the rainfall gradient ($P < 0.001$, regression $R^2 = 0.64$): DOC concentration first increased sharply toward the breakpoint and then decreased toward the wettest sites. Similarly, HCl–Fe and HCl–Fe(II) concentrations could be described by piecewise linear relationships with breakpoints around 4500–4700 mm rainfall (both $P < 0.05$; regression $R^2 = 0.44$ and 0.38 for HCl–Fe and HCl–Fe(II), respectively). As the 800 m site appeared to have a big impact on these nonlinear patterns, we analyzed the data without the 800 m site. Davies test again identified a breakpoint around 4500–4700 mm rainfall in DOC data ($P < 0.001$), but not in HCl–Fe(II).

Averaging across the rainfall gradient, soil NaOH Po concentrations were approximately twice as high as NaOH Pi concentrations (123.6 ± 8.5 vs. 66.1 ± 5.9 µg P $g^{-1}$ soil; Table 1). Soil NaOH Po concentrations had a weak positive correlation with rainfall ($P < 0.05$, $r = 0.41$). Soil NaOH Pi concentrations were higher at 500 m than at other sites except the 600 m and 1000 m sites (Tukey's tests, all $P < 0.10$). Soil ITP peaked at the 500 m site, and its concentration was significantly higher than those from other sites except the 600 m (Tukey's tests, all $P < 0.05$; Figure S3). Residual P did not follow a significant trend with rainfall.

The proportion of NaOH-extractable P (that is, NaOH Po and NaOH Pi) showed a weak, but significant trend along the rainfall gradient (Figure 2) when we used the ratio of P fractions (NaOH and residual) to ITP to account for the variability of ITP. Soil NaOH Po accounted for a higher proportion of the ITP at wetter sites, resulting in a weak positive correlation with rainfall ($P < 0.05$, $r = 0.41$). This correlation became stronger after removing the wettest site ($P < 0.001$, $r = 0.65$). In contrast, the ratio of NaOH Pi to ITP had a weak negative correlation with rainfall ($P < 0.05$, $r = -0.35$), which also became stronger ($P < 0.001$, $r = -0.77$) after removing the wettest site. The results from piecewise regressions were consistent with these nonlinear trends, as a breakpoint around 4600–4800 m rainfall could be found in both ratios (both Davies tests: $P < 0.05$). Ratios of residual P to ITP did not show a clear trend along the rainfall gradient.

## Incubation Experiment

Redox conditions affected how soil $CO_2$ production responded to P amendment (Figure 3). Under oxic conditions, P amendment increased cumulative soil $CO_2$ production in all three soils by at least 21% ($P < 0.01$ in 400 and 1000 m soils; $P < 0.10$ in 800 m soil). Under anoxic conditions, however, P amendment only increased cumulative soil $CO_2$ production in the 400 m elevation soil ($P < 0.001$). In fact, P amendment decreased $CO_2$ production in the 800 m elevation soil under anoxic conditions ($P < 0.10$). Pooling data from both P treatments, the anoxic treatment decreased the cumulative $CO_2$ production relative to oxic treatment by 15.3 ± 3.4% and 51.5 ± 1.7% in the 400 and 1000 m soils, respectively (all $P < 0.001$). The anoxic treatment increased cumulative $CO_2$ pro-

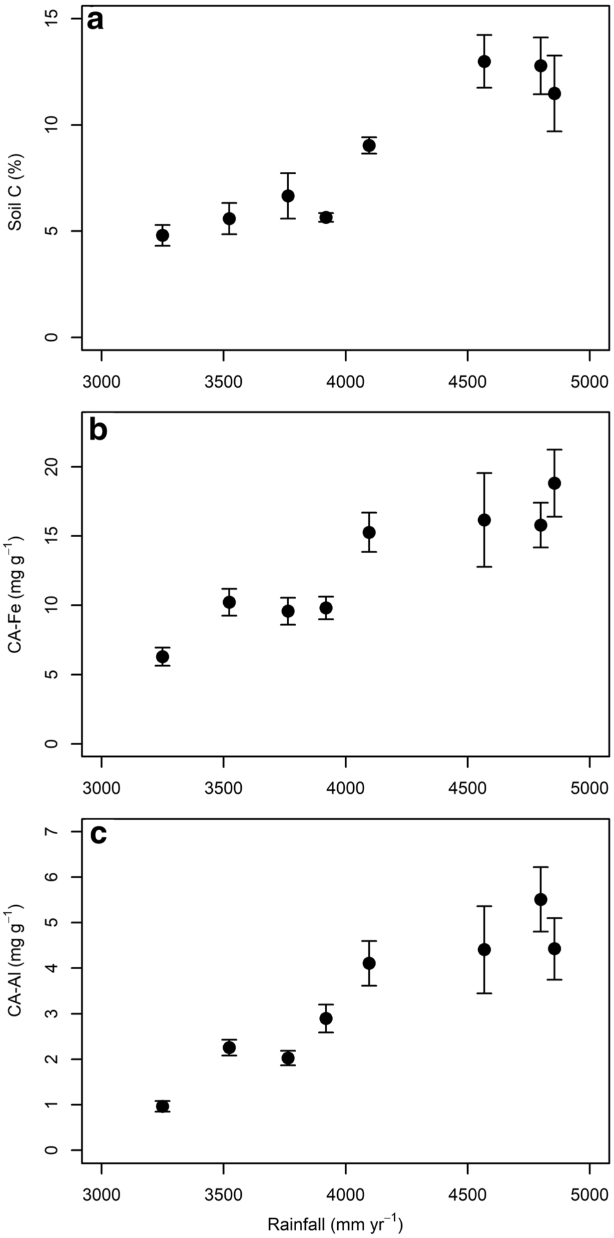

**Figure 1.** Effects of rainfall on soil C concentration (upper panel), citrate ascorbate-extractable iron (CA-Fe, middle panel), and citrate ascorbate-extractable aluminum (CA-Al, lower panel) along the rainfall gradient in the Luquillo Experimental Forest, Puerto Rico. Means and S.Es. are shown.

duction by 18.5 ± 4.2% in the 800 m soil ($P < 0.001$).

Methanogenesis and Fe reduction, both anaerobic processes, showed strong responses to P treatments in the 400 m soil (Figure 4). Under anoxic conditions, P amendment increased cumulative $CH_4$ production and Fe reduction rates relative to the control by 224 ± 14% ($P < 0.001$) and 508 ± 62% ($P < 0.01$), respectively. The P amendment did not affect cumulative $CH_4$ production or Fe reduction in the other two soils. The accumulation of HCl-extractable Fe(II) in the anoxic treatment over time was correlated with increasing concentrations of DOC in all three soils (Figure 5; all $P < 0.001$, $r = 0.65$–$0.81$).

Regardless of P treatments, anoxic conditions generally resulted in lower microbial biomass C and P concentrations when compared to the oxic treatment. On days 8 and 29, the soils from the 800 and 1000 m in the anoxic treatment had lower microbial biomass C than those in the oxic treatment (Figure S4; all $P < 0.05$). On day 8, the an-

**Table 1.** Concentrations of Soil NaOH-extractable Inorganic P (NaOH Pi), NaOH-extractable Organic P (NaOH Po), Residual P, and Ignition Total P (ITP).

| Elevation | Precipitation (mm) | NaOH Pi (μg P $g^{-1}$ soil) | NaOH Po (μg P $g^{-1}$ soil) | Residual P (μg P $g^{-1}$ soil) | ITP (μg P $g^{-1}$ soil) |
|---|---|---|---|---|---|
| 300 | 3250 | 65.5 ± 4.6 | 79.6 ± 10.1 | 166.6 ± 23.8 | 312.3 ± 22.9 |
| 400 | 3523 | 64.7 ± 5.9 | 60.0 ± 13.9 | 175.3 ± 16.0 | 300.9 ± 9.2 |
| 500 | 3764 | 118.8 ± 12.3 | 135.0 ± 16.9 | 282.8 ± 57.0 | 647.3 ± 52.1 |
| 600 | 3919 | 74.0 ± 8.9 | 172.1 ± 20.1 | 243.9 ± 17.2 | 491.2 ± 19.0 |
| 700 | 4096 | 35.4 ± 5.1 | 128.6 ± 13.2 | 194.5 ± 47.7 | 359.7 ± 56.7 |
| 800 | 4568 | 38.4 ± 4.3 | 161.2 ± 17.3 | 197.9 ± 26.2 | 399.4 ± 32.3 |
| 900 | 4799 | 39.1 ± 7.9 | 165.4 ± 22.9 | 231.9 ± 54.2 | 396.3 ± 30.8 |
| 1000 | 4855 | 97.7 ± 26.9 | 99.9 ± 15.4 | 217.3 ± 38.3 | 415.8 ± 73.3 |
| Mean | | 66.1 ± 5.9 | 123.6 ± 8.5 | 212 ± 13.1 | 409.4 ± 20.8 |

*Means and SE are shown.*

oxic treatment also decreased the microbial biomass P relative to the oxic treatment in the 800 and 1000 m soils (Figure S5; both $P < 0.05$). On day 29, the anoxic treatment decreased the microbial biomass P in the 400 and 800 m soils (both $P < 0.05$).

Redox conditions also influenced how microbial biomass stoichiometry responded to P treatments. Under oxic conditions, P amendment decreased microbial biomass C:P ratios relative to the control in the 400 and 1000 m soils by the end of the experiment (Figure 6; both $P < 0.10$) due to increases of microbial biomass P in response to P amendment (Figure S5; both $P < 0.01$). Under anoxic conditions, P amendment only decreased microbial biomass C:P ratios in the 400 m soil ($P < 0.05$). On day 8, neither P amendment nor redox conditions had significant impacts on microbial biomass C:P ratios (Figure S6).

Averaging across all sampling days, P amendment increased the resin-extractable P concentration relative to control by 33.3 ± 6.8, 21.8 ± 3.9, 65.2 ± 5.5 μg $g^{-1}$ soil in the 400 m, 800 m, and 1000 m elevation soils, respectively (Figure S7; all $P < 0.001$). The increase in resin P was larger in the anoxic than in the oxic treatment in the 1000 m soil ($P < 0.05$); however, no interaction effects between redox and P treatments on resin P were found in the 400 m or 800 m soil. The concentration of CA-Fe did not respond to either redox or P treatments in any of the three soils, nor did CA-Al concentrations.

# DISCUSSION

## Biogeochemical Coupling Induced by High Rainfall

Our research along the rainfall gradient revealed concurrent accumulation of soil C, poorly crystalline minerals, and organic P with increasing precipitation. Accumulation of organic matter at high rainfall sites is consistent with previous field observations conducted in the LEF where soils from high elevations experienced more persistent and frequent low redox events (Silver and others 1999, 2013; Liptzin and Silver 2015). Building upon our companion study (Gross and others 2020), we found accumulation of organic P and depletion of inorganic P at wetter sites, which were not derived from changes in total soil P. Reducing conditions suppressed organic matter decomposition and might also have inhibited the mineralization of organic P compounds, as the production and potential activities of soil phosphatase enzymes can decrease in low redox environments (Kang and Freeman 1999; Hall and others 2014). In contrast, the inorganic P sorbed by soil minerals was expected to cycle more rapidly in wet sites, as the solubility of inorganic P is well documented to increase during anaerobic events (Peretyazhko and Sposito 2005; Chacón and others 2006; Rakotoson and others 2014; Maranguit and others 2017; Lin and others 2018). Thus, rainfall is likely to differentially affect the turnover times of organic and inorganic P fractions.

Prevalent reducing conditions caused organo-metal complexes to become net sinks of C, as evidenced by the positive correlation between soil C concentration and mean annual rainfall. Although short-term redox events can facilitate C losses (for

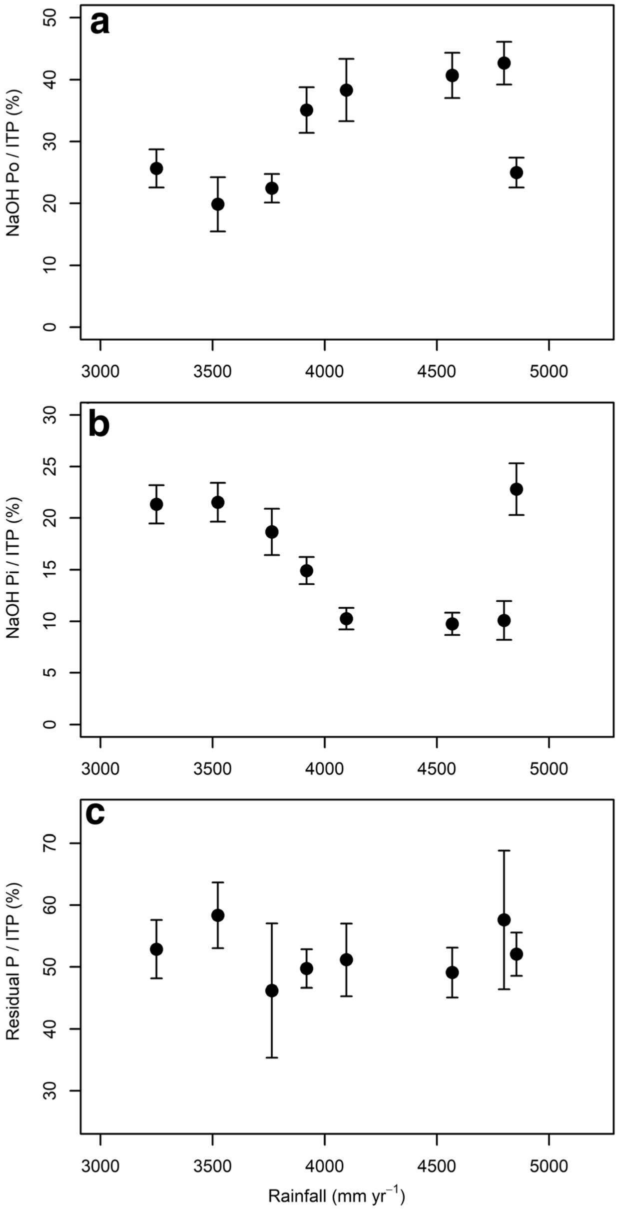

**Figure 2.** Effects of rainfall on the ratio of NaOH-extractable organic P (NaOH Po) to ignition total P (ITP, upper panel), the ratio of NaOH-extractable inorganic P (NaOH Pi) to ITP (middle panel), and the ratio of residual P to ITP (bottom panel) along the rainfall gradient in the Luquillo Experimental Forest, Puerto Rico. Means and S.Es. are shown.

example, the coupling between Fe reduction and DOC observed in our incubation experiment, or increased $CO_2$ production via Fe reduction), C accumulation exceeded C losses at the higher elevations. High molar ratios between C and reactive Fe (5–30) are consistent with the formation of organo-Fe and Al complexes or chelates that are primarily organic structures with reactive Fe or Al species binding organic compounds (Guggenberger and Kaiser 2003). The accumulation of poorly crystalline minerals at wetter sites is in contrast to the findings from an Hawaiian rainfall gradient, where these minerals and residual P all decreased at and above 3500 mm y$^{-1}$ rainfall (Miller and others 2001). The differential degree of weathering may be responsible for the discrepancy between the two studies. The age of soil and underlying saprolite at our site is younger than that of the Maui study (40–60 ky vs. 400 ky) due to the rapid soil production rate at the Puerto Rico field site (Dosseto and others 2012). Higher rainfall might have enhanced the erosion and renewal of minerals from

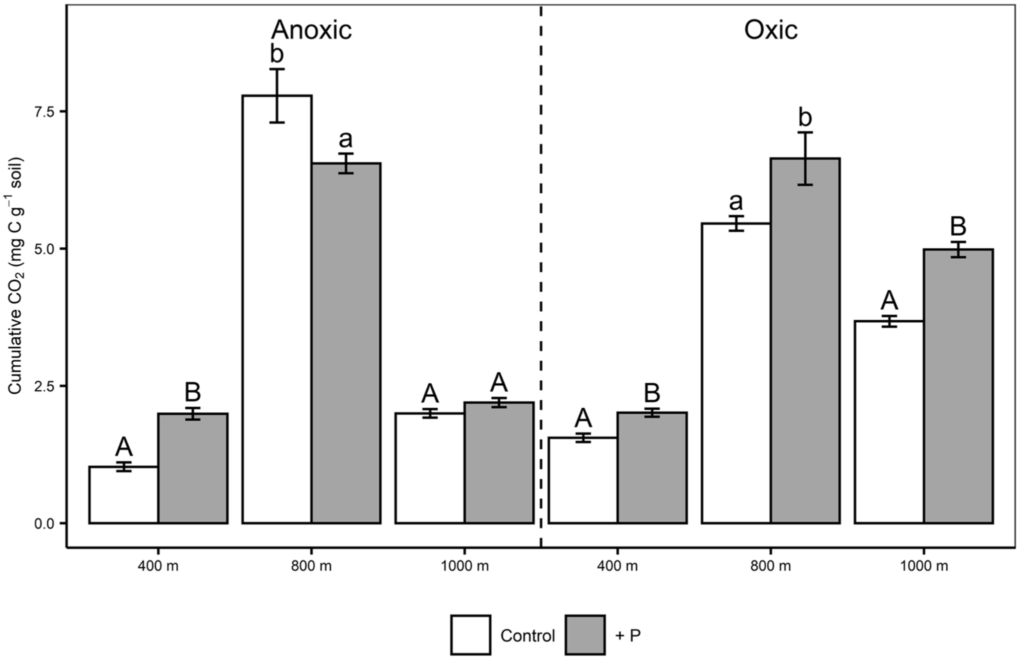

**Figure 3.** Effects of P amendment (control vs. + P) and redox conditions (anoxic vs. oxic) on the cumulative $CO_2$ production from three soils collected at 400, 800, and 1000 m elevations along the rainfall gradient. Upper- and lower-case letters indicate significant differences in each combination of redox and soil types at $\alpha = 0.05$ and 0.10, respectively.

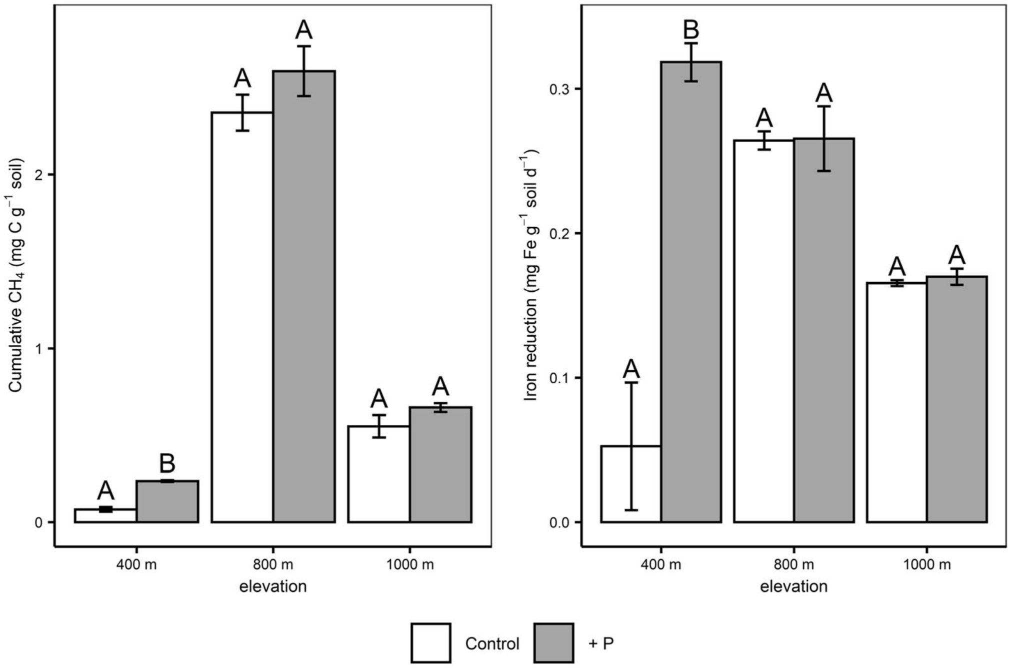

**Figure 4.** Effects of P amendment (control vs. + P) on the cumulative $CH_4$ production (left panel) and soil iron reduction (right panel) under anoxic conditions from three soils collected at 400, 800, and 1000 m elevations along the rainfall gradient. Letters indicate significant differences in each soil type at $\alpha = 0.05$.

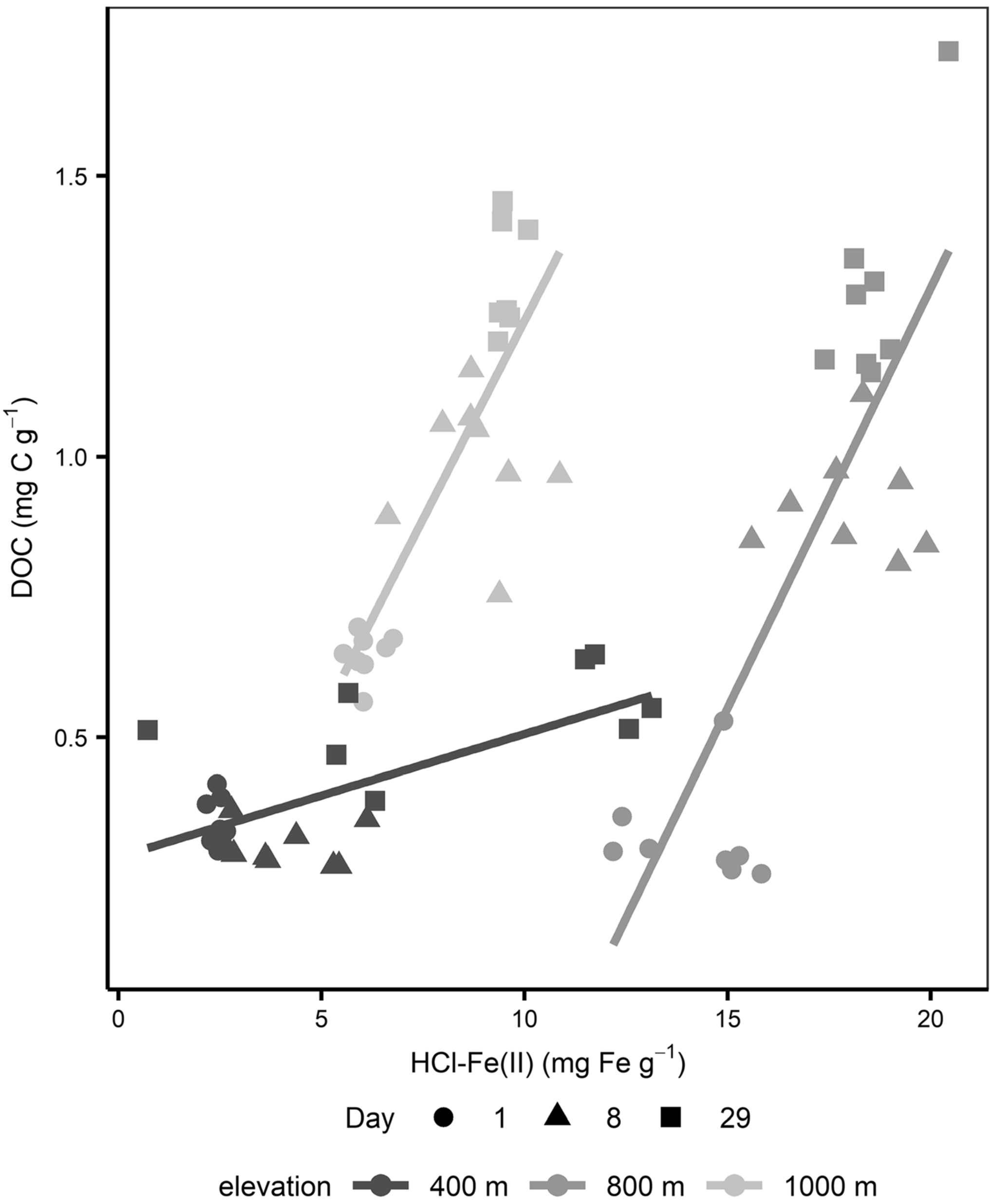

**Figure 5.** Correlations between HCl-extractable Fe(II) (HCl–Fe(II)) and dissolved organic carbon (DOC) during the anoxic incubation of three soils collected at 400, 800, and 1000 m elevations along the rainfall gradient.

deeper saprolite. Dust deposition can also introduce significant amounts of P into the LEF (Pett-Ridge 2009). High mineral inputs might have offset the effects of leaching losses and erosion in removing poorly crystalline minerals.

Similarly, input from weathering and dust might have helped to balance P leaching loss and stabilize ITP pool along the rainfall gradient. The accumulation of P in organic form also helped to maintain total P stocks at wetter sites (Miller and others 2001). The peak of ITP at 500 m site was associated with high levels of NaOH Pi and residual P, indicative of higher sorption, coprecipitation, or occlusion at this site. This might have resulted from a combination of climate and mineralogy, which are likely related to each other, albeit nonlinearly, along the gradient. For example, it is possible that the intermediate and fluctuating redox conditions of this elevation (Silver and others 2013) provide the necessary conditions to yield high P retention via mineral associations relative to higher rainfall/lower redox environments.

The wettest site often behaved differently than the other sites. We suspect that a different set of

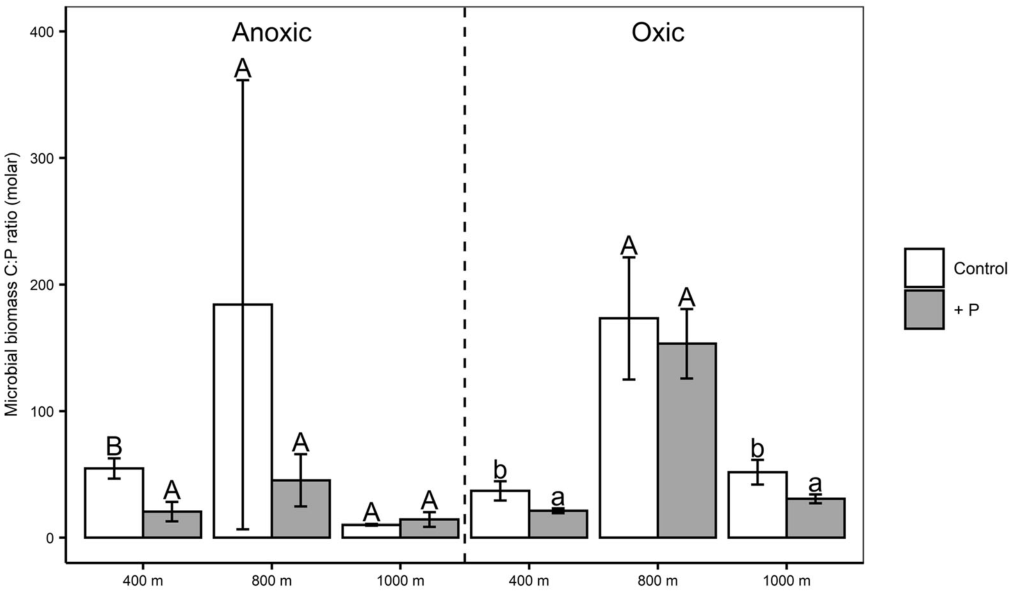

**Figure 6.** Effects of P amendment (control vs. + P) and redox conditions (anoxic vs. oxic) on the microbial biomass C:P ratios measured on day 29 of the incubation experiment using three soils collected at 400, 800, and 1000 m elevations along the rainfall gradient. Upper- and lower-case letters indicate significant differences in each combination of redox and soil types at $\alpha = 0.05$ and 0.10, respectively.

factors are driving biogeochemical cycles in these C-rich soils, even though they are still categorized as Oxisols (Humic Haplaquox; USDA Natural Resources Conservation Service 2002). Concentrations of DOC and HCl-Fe pools were depleted at the wettest sites relative to the adjacent wet forests suggesting a potential rainfall threshold. We speculate that some soluble Fe and C have been leached out of the wettest soils over time, although the leaching was not strong enough to deplete the poorly crystalline Fe as observed by Miller and others (2001). Stressful conditions such as light limitation, wind disturbance, or anoxic soil conditions at the wettest site could propel plants to invest more on structural compounds such as aromatics and lignin (Richardson and others 2005; Yang and others 2007). Low-quality litter, reduced decomposition rates, and slow nutrient cycling could all be parts of a positive feedback loop that was reinforced by prevailing reducing conditions (Vitousek and others 1988, 1992; Silver 1998; Schuur and others 2001; Werner and Homeier 2015). Soils from the wettest sites had similar P composition as those from the driest sites. Selective leaching of soluble C and Fe compounds might have removed some organic P (Kalbitz and others 2000), whereas inorganic P was retained by poorly crystalline Fe and Al minerals (McGechan and Lewis 2002). Overall, there appears to be a threshold of soil accumulation of P, C, Fe dynamics along the gradient culminating at 900 m and then declining in the wettest site. Future studies are needed to better understand the exact underlying mechanisms for the patterns observed.

## Phosphorus and Redox Interactions on Soil Biogeochemistry

Our results show that the combination of P concentrations and redox conditions influenced soil biogeochemistry. Under oxic conditions, P amendment consistently increased soil respiration, indicative of P limitation on decomposition. This result is consistent with the idea that P limitation on aerobic processes is prevalent among tropical forests (Camenzind and others 2017). However, P availability limited anaerobic processes, including $CO_2$ emissions, methanogenesis, and Fe reduction, in only one of the three soils examined (that is, 400 m elevation). These results suggest that while P may limit some aerobic biogeochemical process, it may not be the primary control on anaerobic biogeochemical cycling.

Anoxic conditions could be a more limiting factor for microbial processes than low P availability. We found lower microbial biomass P concentrations under anoxic conditions than under oxic conditions across all three soils, in line with findings from Gross and others (2018) in a lower montane tropical forest. Anoxic conditions also consistently reduced microbial biomass C among these soils, while maintaining respiration rates. These microbial responses likely reflect the physiological stress induced by anoxic conditions (Newman and Reddy 1993). The results also suggest that anoxic conditions led to a partial decoupling of respiration from growth of soil microbes, resulting in 'overflow respiration' (Hessen and Anderson 2008) and lower microbial CUE (Zheng and others 2019; Gross and others 2020). Soils from 800 and 1000 m elevations did not show signs of microbial P limitation under anoxic condition, while soils from the drier site did. Given that reducing events were more prevalent in the higher elevation sites (Silver and others 2013), anoxic conditions likely inhibited the ability of microbes to respond to added P. Our results further suggest that mean annual rainfall or redox regimes likely influenced the degree of apparent P limitation of anaerobic biogeochemical processes. Our incubation used relatively static headspace conditions, similar to periods of strong reducing conditions experienced at the field sites. Frequency and intensity of low redox events could impact the extent of P limitation, given its importance in regulating Fe reduction and P dynamics (Ginn and others 2017; Barcellos and others 2018a).

The data presented here indicated that anaerobic decomposers were surprisingly effective in $CO_2$ production under anoxic conditions. We found that the 800 m soil emitted more $CO_2$ under anoxic conditions than under oxic conditions. Past studies demonstrated that the soil microbial community from this landscape was capable of adapting to or tolerating low and fluctuating redox conditions (Pett-Ridge and Firestone 2005; DeAngelis and others 2010; Pett-Ridge and others 2013). In particular, the high abundance and activities of Fe reducers (DeAngelis and others 2010; Dubinsky and others 2010) point to the importance of Fe reduction in organic matter decomposition under low redox conditions in these ecosystems. In environments with periodic $O_2$ limitation, alternative terminal electron acceptors (for example, Fe(III)) can become the dominant driver of organic matter oxidation and decomposition (Postma and Jakobsen 1996; Fredrickson and others 1998). Regarding the 800 m soil, we observed significant decline in microbial biomass between Days 8 and 29 in the anoxic treatment, but not in the oxic treatment. The dead microbes might have been a new C source that was coupled to Fe reduction, subsequently enabling $CO_2$ production. This hypothesis is consistent with the observation that the decline in microbial biomass over time was comparable to the differences in $CO_2$ production between anoxic and oxic treatments. These mechanisms help to explain the high $CO_2$ production rates observed under anoxic conditions (Teh and others 2005; Bhattacharyya and others 2018).

Anoxia may regulate the extent of P limitation by affecting microbial P uptake. We found that in treatments where P amendment increased microbial biomass P, it also increased microbial respiration, regardless of the redox conditions. This result indicates that enhanced P uptake enabled microbes to upregulate their metabolic activities. Under oxic conditions, the increases in soil respiration induced by P amendment corresponded to decreases in biomass C:P ratios in two of the three soils. Under anoxic conditions, a decrease in microbial biomass C:P ratio in response to P amendment was only found in the soil that showed signs of P limitation (that is, 400 m elevation). These results add further support to the theory that the extent of microbial P limitation depends on whether microbes were capable of utilizing added P. Changes in microbial enzyme activities and/or community composition could also be responsible for the observed changes in microbial stoichiometry and/or processes, as microbes including Fe-reducing bacteria, Fe-oxidizing bacteria, and ammonia-oxidizing archaea thrived during low redox events in the LEF soils (Pett-Ridge and Firestone 2005; Dubinsky and others 2010; Pett-Ridge and others 2013). Phosphorus sorption to minerals was not likely to be responsible for these changes in biogeochemical processes or characteristics, as neither redox nor P treatments had meaningful effects on the concentrations of poorly crystalline minerals, which are a main predictor of P sorption capacity in strongly weathered soils (Roy and others 2017; Lin and others 2020).

## Ecological Implications and Conclusions

Our results indicate that anaerobic biogeochemical cycling may not be as P limited as aerobic processes in tropical forest soils. Our study identified mean annual rainfall and associated redox regimes as important environmental factors that influence the extent of P limitation on biogeochemical processes, as P limitation becomes less severe at sites with high rainfall and prevalent low redox conditions. Mean annual rainfall also played a critical role in inducing a coupling of organic matter, poorly crystalline minerals, and organic P across the surface soils in the LEF.

Our findings have important implications for predicting the biogeochemical dynamics of tropical forests in a changing climate. For soils that will experience significantly more rainfall in the future, accumulation of soil organic C and organic P would be expected if reducing events become stronger and more frequent. Reduced rainfall intensity and drought events, such as the severe Caribbean drought in 2015, likely decrease $O_2$ limitation on organic matter decomposition (O'Connell and others 2018) and consequently increase the sensitivity of biogeochemical processes to P availability (Wood and Silver 2012; Wood and others 2013; Bouskill and others 2016). Mineralization of organic P would also increase in this scenario and might contribute to increased P availability, especially if biological uptake is more competitive for P than mineral sorption (McGroddy and others 2008).

Our findings also have implications for incorporating P dynamics and redox dynamics in ecosystem modeling. Low P availability limits biogeochemical processes in several new models that included P cycling (Zhu and others 2016; Fleischer and others 2019; Yu and others 2020). We recommend caution in assigning the same degree of P limitation to aerobic and anaerobic processes. Our study highlights the importance and complexity of the redox-P interactions in regulating belowground biogeochemistry that warrants further research.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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