esa ECOSPHERE

Spatial patterns in oxygen and redox sensitive biogeochemistry in tropical forest soils

DANIEL LIPTZIN[1],† AND WHENDEE L. SILVER

Department of Environmental Science, Policy, and Management, University of California at Berkeley,
Berkeley, California 94720 USA

Citation: Liptzin, D., and W. L. Silver. 2015. Spatial patterns in oxygen and redox sensitive biogeochemistry in tropical forest soils. Ecosphere 6(11):211. http://dx.doi.org/10.1890/ES14-00309.1

Abstract. Humid tropical forest soils are characterized by warm temperatures, abundant rainfall, and high rates of biological activity that vary considerably in both space and time. These conditions, together with finely textured soils typical of humid tropical forests lead to periodic low redox conditions, even in well-drained upland environments. The relationship between redox and biogeochemical processes has been studied for decades in saturated environments like wetlands and sediments, but much less is known about redox dynamics in upland soils. The goal of this study was to understand the spatial variability of redox sensitive biogeochemistry within and across two forest types at the ends of a high rainfall gradient (3500 to 5000 mm y^{-1}) in the Luquillo Experimental Forest, Puerto Rico. The two sites differed significantly in average soil chemical and physical properties, but the scale of variability was similar across sites, with greater variability in soil gas concentrations than extractable Fe and P. Soil P and Fe pools and trace gas concentrations were more strongly correlated with each other and exhibited more spatial structure at the wetter site. While the within-site relationships among these redox sensitive variables were typically weak, the relationships across sites were much stronger. We provide a conceptual model that elucidates how the strength of the relationships between indicators of redox-sensitive biogeochemical processes depends on the spatial scale of analysis.

Key words: iron reduction; Puerto Rico; soil phosphorus; soil redox; spatial heterogeneity.

Received September 2014; revised 20 February 2015; accepted 18 March 3015; **published** 9 November 2015. Corresponding Editor: Y. Pan.

[1] Present address: INSTAAR, University of Colorado, Boulder, Colorado 80309 USA.

† **E-mail:** liptzin@colorado.edu

INTRODUCTION

The reduction and oxidation potential (redox) in soils is a strong driver of biogeochemical cycling in terrestrial ecosystems. Redox dynamics are well studied in flooded environments, where patterns in water movement and the distribution of electron donors and acceptors create strong spatial gradients in redox (Chapelle et al. 1995). Redox is less well studied in upland systems, but evidence suggests that it varies at multiple spatial scales. Redox gradients occur at the scale of a single soil aggregate, where oxygen (O_2) concentrations typically decrease from the surface towards the center, providing a reducing environment even in seemingly well-aerated soils (Sexstone et al. 1985, Hojberg et al. 1994). At an ecosystem scale, redox can vary across topographic gradients as factors such as soil texture, degree of saturation, and soil depth influence gaseous diffusion and drainage (McKeague 1965, Silver et al. 1999, Yu et al. 2006). Redox status also varies along climate gradients at landscape and regional scale, where redox potential tends to decline under high rainfall conditions (Silver et al. 1999, 2014, Schuur and Matson 2001). While the importance of soil redox has been investigated at small (i.e., soil aggregate) and larger (i.e., topographic and climate gradients) scales in soils, spatial variability in soil redox has not, except with rare exceptions (e.g., Lipson et al. 2013), been investigated at the plot scale (centimeters to meters). It is at this scale that most biogeochemical processes are measured in the field.

Spatial patterns in redox and associated biogeochemistry may be particularly important in wet tropical forest soils where abundant precipitation and high levels of biological activity can create heterogeneous patches of electron donors and acceptors (Lodge et al. 1994, Townsend et al. 2008, Silver et al. 2014). Redox potential is likely to exert significant direct and indirect control over key biogeochemical processes in humid tropical forests. One of the most important indirect mechanisms of redox control in wet tropical forests is the relationship between iron (Fe) species and phosphorus (P) availability. Humid tropical forests on highly weathered soils are generally thought to be limited by P availability (Vitousek and Sanford 1986); aboveground net primary productivity, decomposition rate, and soil respiration have been shown to decline with decreasing soil P availability (Cleveland et al. 2011). A primary driver of low P availability in these Fe-rich soils is the propensity of Fe and Al hyd(oxides) to bind P in semi-stable forms (Porder and Hilley 2011) that are thought to be biologically unavailable (Sanchez 1976). However, reduction of Fe(III) can liberate P from Fe-P bonds increasing P availability (Baldwin and Mitchell 2000, Peretyazhko and Sposito 2005, Chacon et al. 2006). Soil P pools vary across environmental gradients, and circumstantial evidence provides linkages with redox (Schimel et al. 1985, McGroddy and Silver 2000, Litaor et al. 2005, Mage and Porder 2013). For example, P availability generally increases when soils become saturated (Ponnamperuma 1972) and temporal changes in labile P associated with the onset of the rainy season in seasonal tropical rain forests has been linked to changes in redox (Vandecar et al. 2011). Similarly, both labile P (Miller et al. 2001) and resin available P (Schuur and Matson 2001) were greatest at the wettest site along a precipitation gradient in Hawaii.

The production and consumption of greenhouse gases are derived from redox reactions, and thus soil redox conditions have important implications for greenhouse gas emissions. For example, the oxidation of organic C to CO_2 in soils is one of the largest annual fluxes of the terrestrial C cycle, and tropical forests have the highest rates of soil CO_2 emissions globally (Raich and Schlesinger 1992). Methane (CH_4) is the product of several different redox reactions, and is a frequent end product of the anaerobic decomposition of organic matter (Ponnamperuma 1972). Although CH_4 effluxes typically exhibit high spatial variability, humid tropical forests are generally considered a net source of CH_4 (Frankenberg et al. 2008). Given the spatial heterogeneity in O_2 availability and redox in soils, both production and consumption of CH_4 can occur simultaneously (Teh et al. 2005). Humid tropical forests are the largest global source of N_2O from natural ecosystems (Zhuang et al. 2012). Like CH_4, there is high spatial variability in N_2O emissions from tropical forest soils (e.g., Wood and Silver 2012). Greenhouse gas emissions vary predictably with soil redox in controlled settings, with the maximum rates for at high redox for CO_2, intermediate redox for N_2O, and low redox for CH_4 (Yu and Patrick 2004). However, there is considerable variability in tropical soils including anaerobic sources of CO_2 (Chacon et al. 2006, Liptzin and Silver 2009, Dubinsky et al. 2010). A better understanding of the variability in soil redox conditions is needed to help predict patterns in greenhouse gas fluxes.

Our goal was to understand the spatial context of redox sensitive biogeochemistry in humid tropical forests. Previous work described patterns in soil O_2, Fe species, P, N and C cycling along known redox gradients of topography and climate (Silver et al. 1999, 2014, Teh et al. 2005, Dubinsky et al. 2010, Hall and Silver 2013). Here we explored patterns within a given topographic zone in two tropical forest types that occupy the endpoints of a high rainfall gradient in the Luquillo Experimental Forest, Puerto Rico. These sites both have fluctuating soil redox environments with evidence of anaerobic processes such as Fe reduction and CH_4 and N_2O emissions (Silver et al. 1999, 2014, Dubinsky et al. 2010, Liptzin et al. 2011, Hall et al. 2013). We compared the soil concentrations of O_2, trace gases (CO_2,

Table 1. Site characteristics with standard errors in parentheses. Climate data are from Brown et al. (1983) and Weaver and Murphy (1990).

Characteristic	Bisley	Cloud forest
Life zone	subtropical wet	lower montane rain
Forest type	tabonuco	cloud
Air temperature (°C)	21	19
Precipitation (mm)	3500	5000
Gravimetric water content (%)	73 (1.3)	111 (4.8)
Total soil carbon (%)	3.1 (0.07)	16.0 (0.05)
Total soil nitrogen (%)	0.27 (0.005)	0.70 (0.027)
Soil pH	5.0 (0.03)	4.7 (0.08)

N_2O, and CH_4), reduced Fe, and available P within and between these two forests, and explored the bivariate relationships among these variables. Finally, we evaluated the role of spatial heterogeneity in terms of variability and spatial dependence for the concentration of each measured soil variable.

METHODS

Study area

This study was conducted in two forest types within the Luquillo Experimental Forest (LEF) in northeastern Puerto Rico (Lat. 18°8′ N; Long. 65°50′ W). The lower elevation site (350 m asl) was located in the Bisley Research Watersheds (hereafter Bisley) and occurs within the Tabonuco forest type. The upper elevation site (1050 m asl) was located in the cloud forest zone (hereafter cloud forest). These represent the lowermost and uppermost primary forest types along an elevation gradient of plant communities in the LEF (Table 1; Brown et al. 1983). The mesotopography at both sites has been classified as relatively level ridges and valleys connected by steep and slightly convex slopes (Scatena 1989). The Tabonuco forest site was located in a valley bottom and the cloud forest site was located on a ridge. In both of these sites, there are small topographic features at the scale of centimeters to meters, but the sites are relatively flat. Chambers at each site were located on both high points and low points of these small topographic features. Both sites occur on highly weathered soils derived from volcaniclastic sediments: in Bisley the soils are Ultisols and Oxisols in the Cristal/Humatus/Zarzal complex with a typical solum ~2 m deep, while in the cloud forest the soil are classified as a Humic Haplaquox (oxisols) and the soils are typically shallower (Scatena 1989, USDA NRCS 2002). Precipitation is relatively evenly distributed throughout the year and increases from 3500 mm yr^{-1} to 5000 mm yr^{-1}, while annual temperature decreases from 21°C to 19°C from Bisley to the cloud forest with little intra-annual variation (Brown et al. 1983, Weaver 1994).

Field and laboratory methods

Soil equilibration chambers were installed at 0.5–1 m spacing, and the spatial location of each chamber was recorded. At the Bisley site the chambers were located within an area of ~50 m^2. A larger area, ~100 m^2, was required in the cloud forest because the trees have dense mats of aerial roots which interfered with the insertion of the chambers. This phenomenon has been observed in many cloud forests ecosystems and is assumed to be an adaptation to the high water content of the soils (Schawe et al. 2010). The chambers were 15 cm long and constructed of 5 cm diameter polyvinylchloride (PVC), open on the bottom and capped on top. They were fitted with copper tubing and sealed with a 3-way stopcock for gas sampling. All seams and connections were furthered sealed with automotive goop, which is inert to the chemical species measured here (Silver et al. 1999). To insert the chambers, 10 cm of soil was removed with a metal corer and chambers were pushed into the ground 5 cm deeper so that the cap was below the soil surface and to assure good contact with soil. After insertion, the removed soil was used to fill the gap between the chamber and the surrounding intact soil. The chambers were sampled for gases weekly for five months in Bisley and nine months in the cloud forest with results reported in Liptzin et al. (2011). The gas sampling reported here represents the conditions on the last day with the chambers in the ground at each site, just prior to chamber removal and extraction of the soils within to chambers.

Approximately half (14 in Bisley, 15 in the cloud forest) of the chambers were equipped with sensors (Apogee Instruments, Logan, Utah, USA) to measure hourly O_2 concentrations. Each sensor was individually calibrated, but no special calibration was done for concentrations below 1%. Soil O_2 was measured on twelve additional chambers without O_2 sensors by extracting 30 mL of headspace and injecting gas into a YSI O_2 meter. The O_2 electrode was adapted with an airtight cell with two ports to allow for flushing with soil gas and sample analysis without exposure to air (Silver et al. 1999). At the Bisley site, a 30 ml gas sample from the chambers with sensors was also measured with the YSI meter verifying that the two of measurements typically differed by less than 1% O_2. To measure trace gases (CO_2, CH_4, N_2O) a 30 mL sample was extracted from the chamber and injected into a sealed, pre-evacuated 20 mL glass Wheaton vial sealed with aluminum crimps and thick Geo-Microbial septa (GMT, Ochelata, Oklahoma, USA). At the end of the experiment the chambers were removed from the ground and the soil from within the chamber was collected for chemical analyses. Three replicates for each standard gas (CH_4, N_2O and CO_2) were also injected into evacuated vials to test for effects of storage and shipping on sample quality. Vials were shipped to University of California-Berkeley where they were analyzed by gas chromatography (GC) on a Shimadzu GC-14A (Shimadzu Scientific, Columbia, Maryland, USA), equipped with a Porapak-Q column, using a flame ionization detector for CH_4 detection, and an electron capture detector for N_2O detection, and a thermal conductivity detector for CO_2.

Soils were collected one time at each site from within the PVC chambers. All of the soil extractions were performed on fresh soils within 24 hours of removal from the field. Reduced iron (Fe) concentrations were measured with a 0.5 M HCl extraction. To minimize oxidation of reduced Fe species, soils were added to pre-weighed centrifuge tubes with 30 mL of 0.5 M HCl in the field. These extracts were shaken for 1 hour, centrifuged and the supernatant syringe filtered through a 0.45 μm filter. Ferrous Fe was quantified with a modified Ferrozine method because of the high concentrations of Fe(III) in the extracts (Liptzin and Silver 2009). Poorly crystalline Fe was extracted with 0.2 M sodium citrate/0.05 M sodium ascorbate solution, which is less sensitive to Fe(II) concentrations than the acid ammonium oxalate extraction (Reyes and Torrent 1997). Total Fe in these extracts was quantified on an inductively coupled plasma atomic emission spectrometer (Perkin-Elmer, USA). Phosphorus (P) was quantified following Tiessen and Moir (1993) with two exceptions: (1) the resin P step was omitted because the concentrations are typically very low for these forest soils (McGroddy and Silver 2000), and (2) fresh soils were extracted to prevent any binding of P via Fe oxidation during the air drying process. Labile P was quantified with a $NaHCO_3$ extraction followed by a sequential extraction with 0.1 M NaOH to quantify the P associated Al or Fe (Al/Fe P). The $NaHCO_3$ extracts were analyzed colorimetrically for inorganic P and total P following digestion with in acid ammonium persulfate. The NaOH extracts were analyzed colorimetrically for inorganic P and with the ICP for total P. Soil pH was quantified on 1:1 soil to water slurry. Total soil C and N were measured on a CE Instruments NC 2100 Elemental Analyzer (Rodano, Milano, Italy) on soils that were air dried and ground.

Statistical analysis

Differences between forest types were tested with separate one-way analysis of variance (ANOVA) for all variables. The relationships between O_2 and other soil biogeochemical properties were tested with analysis of covariance (ANCOVA) with O_2 as a continuous variable and forest type as a categorical variable. The relationships among all the variables within forest types were tested with correlation analyses. The variables were transformed as needed to meet the assumptions of heterogeneity of variance and normality. These tests were performed in SAS v9.1.3.

We tested the spatial structure of each variable in each forest type by calculating Moran's I using the Excel Add-in ROOKCASE (Sawada 1999). Moran's I is a metric of spatial dependence and ranges in value from −1 to +1. The null hypothesis is a random distribution and a Moran's I value of zero. Positive values indicate

Table 2. Mean (1 SE) for soil extracts and gas concentrations and the *P* value for one-way ANOVA comparing sites.

Characteristic	Units	Bisley	Cloud forest	*P*
Available P	$\mu g\ P \cdot g\ soil^{-1}$	21 (0.7)	33 (1.1)	<0.0001
Al/Fe P	$\mu g\ P \cdot g\ soil^{-1}$	194 (4.0)	109 (3.7)	<0.0001
Reduced Fe	$\mu g\ Fe(II) \cdot g\ soil^{-1}$	123 (11)	2155 (342)	<0.0001
Poorly crystalline Fe	$mg\ Fe \cdot g\ soil^{-1}$	6.2 (0.4)	3.8 (0.5)	0.0004
O_2	%	7.9 (1.2)	2.6 (0.75)	0.0005
CO_2	%	7.1 (0.6)	9.9 (0.6)	0.0013
CH_4	ppm	1.4 (0.2)	8967 (6960)	<0.0001
N_2O	ppm	28 (7.3)	0.071 (0.04)	<0.0001

spatial autocorrelation and negative values indicate dispersion. The distance classes were created for this irregularly spaced dataset such that each contained at least 30 sample pairs and the greatest distance class was no more than two-thirds of the maximum distance between samples. If the global Moran's *I* was significant, each distance class was tested separately with a Monte Carlo permutation test. For each variable within forest types we estimated the number of samples needed for a range of degrees of precision based on the sampled soils (Peterson and Calvin 1986). First, the distribution was tested for normality using the Shapiro-Wilk test. The following equation was applied

$$n = \left\{\frac{t\alpha s}{r}\right\}^2$$

where n is the number of samples required, *t* the *t*-statistic for a given confidence level ($\alpha = 0.05$) and degrees of freedom, *s* the standard deviation, and *r* the width of the desired interval about the mean (i.e., within ±10% of the mean). Data were transformed when necessary to meet the assumptions of the statistical procedures used. Values in the text are reported as means and standard errors. For the transformed variables, the back transformed means are shown and only the standard error greater than then mean is shown for all variables for consistency.

Results and Discussion

Biogeochemical indicators of redox conditions

There were clear differences between forest types in the redox sensitive variables. The range of soil O_2 concentrations was similar at both sites (0–18% in Bisley and 0–16% in the cloud forest), but the mean O_2 concentration was significantly higher in Bisley (7.9 ± 1.2%) compared to the cloud forest (2.6 ± 0.8%; Table 2). Previous work in these forest types showed that temporal patterns in soil O_2 were related to the timing of precipitation, with periods of higher soil O_2 associated with precipitation-free periods of four days or longer (Liptzin et al. 2011). The more frequent precipitation at the cloud forest site and higher soil moisture were likely responsible for the lower average soil O_2 measured.

The mean soil concentrations of CO_2 (9.9 ± 0.6 vs. 7.1 ± 0.6%) and CH_4 (8967 + 6957 vs. 1.4 + 0.2 ppm) were significantly higher while mean N_2O (0.1 + 0.04 vs. 28 + 7.3 ppm) was significantly lower at the cloud forest site compared to the Bisley site (Table 2). These results are consistent with lower redox potential in the higher rainfall environment. While all of these differences between sites were statistically significant, it should be noted that the magnitude of the difference varied widely among the gases: CO_2 was 25% higher, N_2O was two 2 orders of magnitude lower and CH_4 was almost four orders of magnitude higher at the cloud forest site. The concentrations of available P, Al/Fe P, reduced Fe, and poorly crystalline Fe also differed significantly between the two forest types (Table 2). Available P (33 ± 1.1 vs. 21 ± 0.7 $\mu g\ P \cdot g\ soil^{-1}$) and reduced Fe (2155 + 342 vs. 123 + 10.8 $\mu g\ Fe(II) \cdot g\ soil^{-1}$) were significantly higher while Al/Fe P (109 ± 3.7 vs. 194 ± 4.0 $\mu g\ P \cdot g\ soil^{-1}$) and poorly crystalline Fe (3.8 ± 0.5 vs. 6.2 ± 0.4 $mg\ Fe \cdot g\ soil^{-1}$) were significantly lower at the cloud forest site compared to the Bisley site. Similar to the gases, the site differences for the soil extracts were all statistically significant, but the magnitude of the difference varied among the extracts: the reduced Fe concentration was more than 10 times higher in the cloud forest. Compared to the total Fe hydr(oxide)

Table 3. Pearson correlation coefficients for Bisley (a) and Cloud forest (b).

Characteristic	Available P	Fe/Al P	Reduced Fe	Poorly crystalline Fe	O_2	CH_4	CO_2	N_2O
a) Bisley								
Available P	1	0.20	0.17	0.13	0.21	0.15	−0.21	0.11
Fe/Al P		1	−0.08	0.01	−0.25	**0.54**	0.16	−0.06
Reduced Fe			1	−0.03	*−0.37*	0.23	0.30	−0.12
Poorly crystalline Fe				1	−0.07	0.07	0.02	0.24
O_2					1	−0.26	**−0.94**	0.03
CH_4						1	0.23	−0.22
CO_2							1	0.00
N_2O								1
b) Cloud forest								
Available P	1	**0.71**	0.17	−0.11	−0.06	0.07	0.09	−0.10
Fe/Al P		1	0.13	−0.04	−0.02	−0.08	0.20	0.08
Reduced Fe			1	**0.62**	−0.30	**0.58**	**0.56**	**−0.48**
Poorly crystalline Fe				1	−0.17	*0.33*	**0.59**	**−0.39**
O_2					1	**−0.60**	**−0.58**	**0.56**
CH_4						1	**0.63**	**−0.66**
CO_2							1	**−0.66**
N_2O								1

Note: Significant correlations ($P < 0.05$) are shown in bold with marginal significance ($P < 0.10$) in italics.

content in the cloud forest compared to Bisley (19.2 vs. 30.7 mg Fe·g soil^{-1}), reduced Fe accounted for more than 10% of the total (Dubinsky et al. 2010). The more reducing soils at the cloud forest site were associated with greater labile P and reduced Fe concentrations. Similar patterns have been observed in flooded soils (Ponnamperuma 1972) and along a precipitation gradient in Hawaii where available P increased while Al/Fe P and poorly crystalline Fe increased as soil redox decreased with increasing precipitation (Miller et al. 2001).

Bivariate relationships

Redox indicators followed predictable patterns at the cloud forest site. Soil O_2 concentrations were positively correlated with N_2O concentrations and soil CO_2 was positively correlated with CH_4 concentrations; as expected, these two groups were negatively correlated to each other (Table 3). These relationships were even more clear when the gas concentrations were compared during the entire months-long chamber experiments at each site (Liptzin et al. 2011). Carbon dioxide and O_2 were tightly coupled with the sum of CO_2 and O_2 essentially a constant consistent with the conversion of O_2 into CO_2 during aerobic respiration. The strong negative relationship between CH_4 and CO_2 is likely indirect as CO_2 increases with decreasing O_2. The strong negative relationship between O_2 and CH_4 is not a surprise since methanogenesis is an anaerobic process. However, in the LEF soils, where some O_2 is always present, CH_4 production and consumption are both occurring (Teh et al. 2005) with the net balance favoring production in the cloud forest and consumption in Bisley. Of all of the bivariate relationships with O_2, the relationship between O_2 and N_2O is least understood. Nitrous oxide can be produced during nitrification (an aerobic process) or denitrification (an anaerobic process) and is predicted to be most associated with intermediate moisture (Davidson et al. 2000). Perhaps the multiple pathways that lead to N_2O can explain the positive linear relationship between N_2O and O_2 observed in the present study and the quadratic relationship (maximum N_2O at intermediate O_2 concentrations) reported by McSwiney et al. (2001) from a different watershed in the LEF. Reduced Fe was significantly positively correlated with both CO_2 and CH_4 and negatively correlated with N_2O. At the Bisley site, the only significant correlation among the gases was the strong negative relationship between O_2 and CO_2. In addition, O_2 was weakly correlated with reduced Fe ($p < 0.07$), but Fe and P species were not correlated.

The strength of these bivariate relationships may depend on a complex set of interactions that independently affect the substrate and products of redox-sensitive biogeochemical processes. For example, laboratory experiments showed that increased Fe reduction was associated with greater microbial P in these soils, but not with increased exchangeable P (Liptzin and Silver

Table 4. Significance (*P* values) of ANCOVA with site as a categorical variable and oxygen as a continuous variable and their interaction.

Variable	Site	Oxygen	Interaction
Available P	<0.0001	ns	ns
Al/Fe P	<0.0001	ns	ns
Reduced Fe	<0.0001	0.02	ns
Poorly crystalline Fe	0.02	ns	ns
CO_2	ns	<0.0001	ns
CH_4	<0.0001	0.007	0.0003
N_2O	<0.0001	0.0013	0.0019

2009). This suggests that the residence time of labile P is short in Bisley soils, with P only available briefly after the onset of reducing conditions. Reduced Fe may persist for longer time periods prior to leaching, precipitation, or re-oxidation. Spatial heterogeneity in redox and soil biogeochemical dynamics is also likely to be important in this ecosystem. While redox likely varies at several spatial scales, the steepest redox gradients in upland soils may occur within soil aggregates where O_2 concentrations can decrease from atmospheric concentrations to anoxic over a distance of 1 mm (Sexstone et al. 1985). This raises important issues for how soils are typically collected and processed. For example, collecting and homogenizing soil cores likely differentially alters biogeochemical processes by mixing soils with high and low redox conditions (Teh and Silver 2006). Homogenized and slurried soils typically used in laboratory incubations can artificially increase extractable P when tightly coupled with changes in Fe species (Chacon et al. 2006, Peretyazhko and Sposito 2005). The difference between the two forest types in the strength of these relationships may ultimately be a result of climate. In the cloud forest, soil O_2 concentrations commonly average below 5%, while more frequent rain-free periods in Bisley resulted in higher mean concentrations of soil O_2 (Liptzin et al. 2011). Soil O_2 concentrations were a better predictor of other redox sensitive processes at the cloud forest site perhaps in part because the redox environment was more homogenous in space and time.

When both sites were included together in the bivariate analysis, the relationships were much stronger. This would be expected if the combined dataset increases the range of redox drivers and alternative electron acceptors. All three of the soil gases as well as reduced Fe were related to O_2 concentration using an ANCOVA (Table 4). There was no site effect for CO_2 concentrations in the ANCOVA model, unlike all the other variables. This was likely due to the strong and direct relationship of O_2 consumption and CO_2 production during aerobic respiration (Liptzin et al. 2011). In some cases, biogeochemical relationships were only apparent when a wider range of values was considered. For example, the relationships between available P and reduced Fe were weak within sites ($R^2 = 0.02$ for Bisley and $R^2 = 0.03$ for the cloud forest). However, when both sites were included, reduced Fe concentration explained 57% of the variance in available P (Fig. 1). Previous work in the LEF has suggested that available P does not vary significantly across known redox gradients (Silver et al. 1994, 1999, Cox et al. 2002, Mage and Porder 2013). However, these studies measured P from air-dried soils, which can result in the formation of relatively insoluble Fe-P bonds associated with Fe oxidation (Chacon et al. 2006, Wood and Silver 2012). The P concentrations from air-dried soils have been shown to differ from soils under field conditions (Xu et al. 2011).

Spatial variability

The relative variability (as measured by the coefficient of variation; CV) in biogeochemical properties was generally similar at the two sites. The CV was less than 20% for soil extracts while for soil gases the CV was often greater than 100% (Table 5). Of the greenhouse gases, soil CO_2 concentrations were less variable than N_2O or CH_4. Chemical species in the soil extracts could be characterized within 20% of the mean by collecting fewer than 10 samples within forest types, but the soil gases exhibited much greater variability requiring tens to hundreds of samples to achieve similar precision. Lower variability in the efflux of CO_2 relative to CH_4 and N_2O has been observed in other tropical soils (Verchot et al. 1999, 2000, Davidson et al. 2000, Konda et al. 2010). For CO_2 measurements, Davidson et al. (2002) suggested that 6–8 large chambers would be sufficient to characterize a site. However, a larger number of samples would be required to achieve a similar characterization of N_2O or CH_4 fluxes. There is a growing body of evidence that a large number of samples is needed to quantify

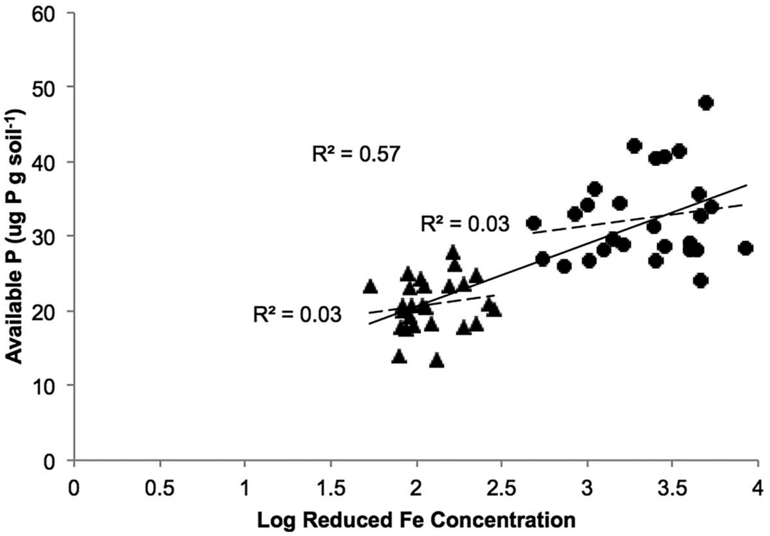

Fig. 1. Relationship between reduced Fe and available P. Within a site the variability in available P cannot be explained by the reduced Fe concentration ($R^2 = 0.02$ for Bisley and $R^2 = 0.03$ for the cloud forest sites). When data from both sites were combined, reduced Fe concentration can explain 57% of the variance in the available P. Dashed lines represent the best linear fit for the individual sites and the solid line represents the best linear fit for both sites combined. Original units for Fe concentration were (μg Fe · g soil^{-1}).

the mean flux rates of greenhouse gases; however, there is still limited evidence on what factors control the spatial variability at the scale of meters (Hall and Silver 2013).

The two sites differed in their degree of spatial dependence. There was no evidence for any spatial structure for any variable at the Bisley site. In contrast, most of the variables showed spatial dependence in the cloud forest (Fig. 2). Both reduced and poorly crystalline Fe exhibited evidence of patchiness as Moran's I was positive at short and long distances and negative at intermediate distances (Fig. 2). For soil P concentrations, Moran's *I* was highest at distances ≤7 m and decreased to zero with increasing distance. Carbon dioxide exhibited a patchy distribution, but the other gases showed no evidence of spatial structure. Our understanding of the spatial structure of measures of redox sensitive biogeochemistry in tropical forest soils is still relatively limited. Spatial structure at scales of tens of meters to kilometers has been reported for available P (e.g., Gonzalez and Zak 1994, Sun et al. 2003, Yavitt et al. 2009), but much less is known at smaller spatial scales. Further, there is nothing known about the spatial structure of soil redox at the scale of meters in wet tropical soils. While many saturated environments have predictable vertical gradients in redox sensitive processes (e.g., Roden and Wetzel 1996), there appears to be high variability and low predictability at the scale of meters in the surface soils of these wet tropical forests.

The predictive ability of soil O_2 for redox sensitive variables differed between the two sites. Our results show that there was spatial structure in the variables as well as significant relationships between soil O_2 and the other soil gases at the lower redox cloud forest site. In contrast, at the Bisley site, with higher mean as well as more heterogeneous O_2 concentrations, soil O_2 concentrations were not a good predictor of redox processes at this scale of resolution. The difference between the sites could be due to differences in the temporal and spatial variability at the two sites. In the cloud forest site, the spatial structure and lower variability in soil O_2 suggests that a single soil core will contain soils with a more homogenous redox environment; in Bisley, the lack of spatial structure means that a soil core could contain a unpredictable mixture of high and low redox sites. Further, the more frequent rapid temporal fluctuations in soil O_2 at the Bisley site (Liptzin et al. 2011), may lead to a

Table 5. Number of samples required to achieve various degrees of precision (within ±10–50%) based on sample distributions for both sites.

Characteristic	Interval around mean ±10%	±20%	±30%	±40%	±50%	CV (%)
Bisley						
Available P	12	3	2	1	1	17
Al/Fe P	5	2	1	1	1	10
Reduced Fe	4	1	1	1	1	46
Poorly crystalline Fe	11	3	2	1	1	31
O_2	269	68	30	17	11	80
CH_4	179	45	20	12	8	193
CO_2	74	19	9	5	3	42
N_2O	52	13	6	4	3	198
Cloud forest						
Available P	14	4	2	1	1	18
Al/Fe P	14	4	2	1	1	18
Reduced Fe	5	2	1	1	1	69
Poorly crystalline Fe	153	39	17	10	7	68
O_2	980	245	109	62	40	153
CH_4	245	62	28	16	10	130
CO_2	40	10	5	3	2	30
N_2O	297	75	33	19	12	307

Notes: Reduced iron, poorly crystalline Fe, and N_2O were log transformed and CH_4 was square root transformed in order to meet the assumption of normality based on the Shapiro-Wilk test ($P < 0.01$). No transformation was possible for O_2 at either site or CH_4 at Bisley, but data are still shown for untransformed data (O_2) or square root transformed (CH_4). The coefficients of variation (CV) based on untransformed data are also shown.

temporal decoupling between soil O_2 and other redox sensitive events. That is, the rapid decreases in soil O_2 fluctuations are largely associated with the physical process of filling of soil pores after rainfall events decreasing O_2 diffusion into soils; in contrast, the rates of redox-sensitive biological reactions (e.g., Fe reduction, methanogenesis) may change more slowly than the soil O_2 concentration. Such a temporal lag would be more likely to preclude significant bivariate relationships at the Bisley site where redox fluctuations are more common. While the within site relationships among redox-sensitive variables was not always evident, especially at Bisley, the differences among the sites were quite predictable. The cloud forest site, with its consistently lower soil O_2 concentrations, had CH_4 concentrations several orders of magnitude higher than Bisley, but also significantly higher Fe(II) and available P concentrations.

Based on our observations as well as previous literature we propose a conceptual model of the role of spatial scale on redox-sensitive biogeochemistry (Fig. 3). We predict a strong relationship (dashed lines) between variables when individual soil aggregates are sampled from the edge to the center as described by Sexstone et al. (1985). However, at the scale of a typical sampling plot (i.e., meter scale), the ability to detect bivariate relationships from samples collected as soil cores will depend upon the frequency and magnitude of low redox events. Both physical (e.g., leaching) and biological (e.g., microbial or plant uptake) processes can mask the important role of redox in structuring soil properties, particularly in well-aerated environments, or those that experience frequent high magnitude redox oscillations. Relatively few samples are needed to describe the mean and variability of redox sensitive variables (solid ovals) in a plot. Finally, we predict strong relationships (solid line) at the landscape scale, such as comparing sites along the elevation gradient in the present study. This strong relationship could be associated with physical drivers like climate or topography, as well as large scale patterns in C (as an electron donor) or availability of electron acceptors (i.e., derived from geologic, hydrologic, or topographic processes). In summary, we suggest that the role of redox in structuring soil properties can be readily detected at small and large spatial scales. At intermediate spatial scales, the scale at which most ecosystem research is conducted, the frequency and magnitude of redox fluctuations will determine our ability to detect patterns in

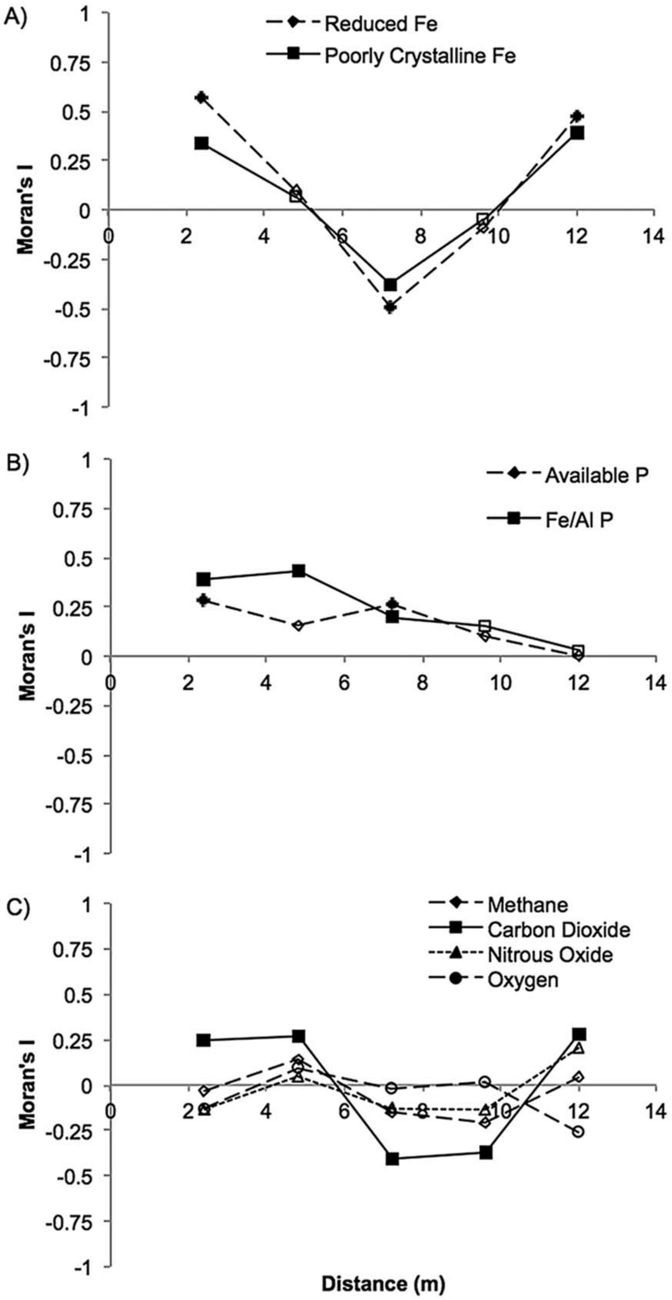

Fig. 2. Spatial correlograms (Moran's *I*) for (A) soil Fe, (B) soil P, and (C) soil gases at the Cloud Forest site. Values range from +1 (perfect spatial autocorrelation) to −1 (perfect dispersion) with a value of 0 indicating a random pattern. Solid symbols indicate Moran's *I* values that differ significantly from 0 (i.e., non-random spatial pattern) while open symbols are not significantly different from 0. No data is shown for Bisley as none of the variables indicated significant spatial structure at any lag distance.

redox sensitive biogeochemical processes.

This study is one of a growing number directly linking soil O_2 or redox potential directly to nutrient and carbon cycling in upland wet tropical forest soils (e.g., Silver et al. 1999, Miller et al. 2001, Schuur and Matson 2001, Hall et al. 2013). There also may be indirect consequences of soil redox status in shaping the structure and

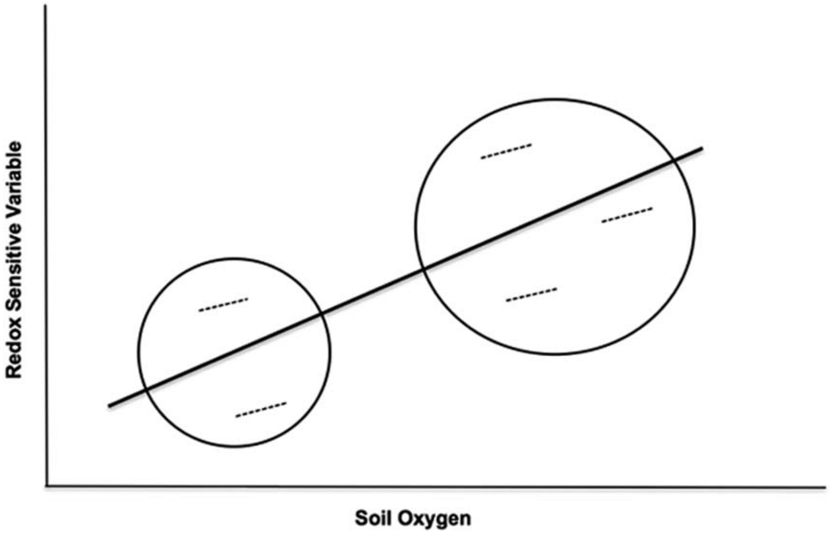

Fig. 3. Conceptual model of the role of spatial scale on the strength of the relationship between redox sensitive variables. The range of values within a typical sampling plot (e.g., Bisley or Cloud forest) is represented by the solid ovals. At this scale there is high variability among soil cores and weak bivariate relationships between the two variables (see Table 3), but the plot level variability (i.e., the size of the oval) can be determined with relatively few samples (see Table 5). The solid line represents the linear relationship when comparing plot averages along a gradient such as the climate gradient in this study. At this landscape scale, there are strong relationships in redox-sensitive variables. Finally, dashed lines represent the strong relationship between variables predicted when sampling within individual soil aggregates. Thus, at the microscale (i.e., μm scale) as well as the landscape scale (i.e., km scale), there are strong (in this case positive) relationships between soil O_2 and another redox sensitive variable while at the plot scale (i.e., m scale) the relationships are not discernible.

function of wet tropical forest ecosystems. For example, soil redox, likely through its effects on nutrient dynamics, has been proposed as a mechanism to explain differences in plant communities and primary productivity along precipitation gradients in the LEF (Waide et al. 1998) and in Hawaii (Schuur and Matson 2001). Our results suggest that soil redox can also have effects at smaller spatial scales in upland wet tropical forests. The sites in the present study clearly differed in the degree and spatial structure of variability in soil O_2 and the other redox sensitive variables, but the relationships among variables were not always clear within a site. Careful and continuous measurements of soil redox dynamics at the scale of centimeters to meters may provide more insight into the spatial and temporal dynamics of redox sensitive biogeochemistry, but could also help resolve important issues in tropical forest ecology. Measuring on the appropriate temporal scale to capture the release of P associated with redox fluctuations may help explain how these forests exhibit such high plant productivity when available P concentrations are so low. Similarly, a better understanding of the spatial variability in soil redox status may help predict where high emissions of greenhouse gas will occur.

Conclusions

Soil redox is an important driver of biogeochemical processes in upland tropical forest soils. In this study there were predictable patterns of redox-sensitive variables and predictable relationships among them when examined across forests at either end of a high rainfall gradient. However, at the scale of individual soil samples within the study plots, the predicted relationships among variables differed between the sites: the bivariate relationships were less predictable in the soils with more variable and higher average soil O_2 availability. Trace gas concentrations were highly variable at both sites, but soil chemical properties were predictable with reasonable sampling protocols within plots. We suggest that improving our understanding the mechanisms that control the spatial patterns of redox in tropical soils will improve our ability to predict rates of redox sensitive biogeochemical processes as well as providing a greater understanding of tropical forest ecology.

ACKNOWLEDGMENTS

We thank B. Quintero, T. Wood, C. Torrens, and A. Thompson for assistance in the lab and in the field and S. Hall for comments on an early version of this manuscript. This research was supported by grants DEB 0963447 from NSF to the Institute for Tropical Ecosystem Studies, University of Puerto Rico, and to the International Institute of Tropical Forestry USDA Forest Service, as part of the Luquillo Long-Term Ecological Research Program and in cooperation with the University of Puerto Rico. The U.S. Forest Service (Dept. of Agriculture) and the University of Puerto Rico gave additional support. Support was also provided by NSF grants DEB-0543558, DEB-0842385, EAR-1331841 and EAR-0722476 to W. L. Silver.

LITERATURE CITED

Baldwin, D. S., and A. M. Mitchell. 2000. The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river–floodplain systems: a synthesis. Regulated Rivers: Research and Management 16:457–467.

Brown, S., A. E. Lugo, S. Silander, and L. Liegel. 1983. Research history and opportunities in the Luquillo Experimental Forest. General Technical Report SO-44. USDA Forest Service, Southern Forest Experiment Station, New Orleans, Louisiana, USA.

Chacon, N., W. L. Silver, E. A. Dubinsky, and D. F. Cusack. 2006. Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. Biogeochemistry 78:67–84.

Chapelle, F. H., P. B. McMahon, N. M. Dubrovsky, R. F. Fujii, E. T. Oaksford, and D. A. Vroblesky. 1995. Deducing the distribution of terminal electron-accepting processes in hydrologically diverse groundwater systems. Water Resources Research 31:359–371.

Cleveland, C. C., et al. 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. Ecology Letters 14:939–947.

Cox, S. B., M. R. Willig, and F. N. Scatena. 2002. Variation in nutrient characteristics of surface soils from the Luquillo Experimental Forest of Puerto Rico: a multivariate perspective. Plant and Soil 247:189–198.

Davidson, E. A., K. Savage, L. V. Verchot, and R. Navarro. 2002. Minimizing artifacts and biases in chamber-based measurements of soil respiration. Agricultural and Forest Meteorology 113:21–37.

Davidson, E. A., L. V. Verchot, J. H. Cattânio, I. L. Ackerman, and J. Carvalho. 2000. Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. Biogeochemistry 48:53–69.

Dubinsky, E. A., W. L. Silver, and M. K. Firestone. 2010. Tropical forest soil microbial communities couple iron and carbon biogeochemistry. Ecology 91:2604–2612.

Frankenberg, C., P. Bergamaschi, A. Butz, S. Houweling, J. F. Meirink, J. Notholt, A. K. Petersen, H. Schrijver, T. Warneke, and I. Aben. 2008. Tropical methane emissions: a revised view from SCIAMACHY onboard ENVISAT. Geophysical Research Letters 35:L15811.

Gonzalez, O. J., and D. R. Zak. 1994. Geostatistical analysis of soil properties in a secondary tropical dry forest, St. Lucia, West Indies. Plant and Soil 163:45–54.

Hall, S. J., W. H. McDowell, and W. L. Silver. 2013. When wet gets wetter: decoupling of moisture, redox biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. Ecosystems 16:576–589.

Hall, S. J., and W. L. Silver. 2013. Iron oxidation stimulates organic matter decomposition in humid tropical forest soils. Global Change Biology 19:2804–2813.

Hojberg, O., N. P. Revsbach, and J. M. Tiedje. 1994. Denitrification in soil aggregates analyzed with microsensors for nitrous oxide and oxygen. Soil Science Society of America Journal 58:1691–1698.

Konda, R., S. Ohta, S. Ishizuka, J. Heriyanto, and A. Wicaksono. 2010. Soil biology and biochemistry. Soil Biology and Biochemistry 42:1512–1522.

Lipson, D. A., T. K. Raab, D. Goria, and J. Zlamal. 2013. The contribution of Fe(III) and humic acid reduction to ecosystem respiration in drained thaw lake basins of the Arctic Coastal Plain. Global Biogeochemical Cycles. 27:399–409.

Liptzin, D., and W. L. Silver. 2009. Effects of carbon addition on iron and phosphorus in a tropical soil. Soil Biology and Biochemistry 41:1696–1702.

Liptzin, D., W. L. Silver, and M. Detto. 2011. Temporal dynamics in soil oxygen and greenhouse gases in two humid tropical forests. Ecosystems 14:171–182.

Litaor, M. I., T. R. Seastedt, M. D. Walker, M. Carbone, and A. Townsend. 2005. The biogeochemistry of phosphorus across an alpine topographic/snow gradient. Geoderma 124:49–61.

Lodge, D. J., W. H. McDowell, and C. P. McSwiney.

1994. The importance of nutrient pulses in tropical forests. Trends in Ecology and Evolution 9:384–387.

Mage, S. M., and S. Porder. 2013. Parent material and topography determine soil phosphorus status in the Luquillo Mountains of Puerto Rico. Ecosystems 16:284–294.

McGroddy, M., and W. L. Silver. 2000. Variations in belowground carbon storage and soil CO_2 flux rates along a wet tropical climate gradient1. Biotropica 32:614–624.

McKeague, J. A. 1965. Properties and genesis of three members of the Uplands catena. Canadian Journal of Soil Science 45:63–77.

McSwiney, C. P., W. H. McDowell, and M. Keller. 2001. Distribution of nitrous oxide and regulators of its production across a tropical rainforest catena in the Luquillo Experimental Forest, Puerto Rico. Biogeochemistry 56:265–286.

Miller, A. J., E. A. Schuur, and O. A. Chadwick. 2001. Redox control of phosphorus pools in Hawaiian montane forest soils. Geoderma 102:219–237.

Peretyazhko, T., and G. Sposito. 2005. Iron(III) reduction and phosphorous solubilization in humid tropical forest soils. Geochimica et Cosmochimica Acta 69:3643–3652.

Peterson, R. G., and L. D. Calvin. 1986. Sampling. Pages 33–52 *in* A. Klute, editor. Methods of soil analysis. Part 1. Physical and mineralogical methods. Agronomy, 2nd Edition, Number 9. Soil Science Society of America, Madison, Wisconsin.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Plant and Soil 24:29–96.

Porder, S., and G. E. Hilley. 2011. Linking chronosequences with the rest of the world: predicting soil phosphorus content in denuding landscapes. Biogeochemistry 102:153–166.

Raich, J. W., and W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44:81–99.

Reyes, I., and J. Torrent. 1997. Citrate-ascorbate as a highly selective extractant for poorly crystalline iron oxides. Soil Science Society of America Journal 61:1647–1654.

Roden, E. E., and R. G. Wetzel. 1996. Organic carbon oxidation and suppression of methane production by microbial Fe (III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnology and Oceanography 41:1733–1748.

Sanchez, P. A. 1976. Properties and management of soils in the tropics. Wiley, New York, New York, USA.

Sawada, M. 1999. ROOKCASE: an Excel 97/2000 Visual Basic (VB) add-in for exploring global and local spatial autocorrelation. Bulletin of the Ecological Society of America 80:231–234.

Scatena, F. N. 1989. An introduction to the physiography and history of the Bisley Experimental Watersheds in the Luquillo Mountains of Puerto Rico. General Technical Report SO-72. USDA Forest Service, Southern Forest Experiment Station, New Orleans, Louisiana, USA.

Schawe, M., G. Gerold, K. Bach, and S. R. Gradstein. 2010. Hydrometeorological patterns in relation to forest structure and plant species diversity along an elevational gradient in a montane cloud forest in the Yungas, Bolivia. Pages 199–207 *in* L. A. Brijnzeel, F. N. Scatena, and L. S. Hamilton, editors. Tropical montane cloud forests. Cambridge University Press, New York, New York, USA.

Schimel, D., M. A. Stillwell, and R. G. Woodmansee. 1985. Biogeochemistry of C, N, and P in a soil catena of the shortgrass steppe. Ecology 66:276–282.

Schuur, E. A. G., and P. A. Matson. 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. Oecologia 128:431–442.

Sexstone, A. J., N. P. Revsbech, T. B. Parkin, and J. M. Tiedje. 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. Soil Science Society of America Journal 49:645–651.

Silver, W. L., D. Liptzin, and M. Almara. 2014. Soil redox dynamics and biogeochemistry along a tropical elevation gradient. Pages 195–209 *in* G. González and M. R. Willig, editors. Ecological gradient analyses in a tropical landscape. Ecological Bulletins 54:195–209.

Silver, W. L., A. E. Lugo, and M. Keller. 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44:301–328.

Silver, W. L., F. N. Scatena, A. H. Johnson, T. G. Siccama, and M. J. Sanchez. 1994. Nutrient availability in a montane wet tropical forest: spatial patterns and methodological considerations. Plant and Soil 164:129–145.

Sun, B., S. Zhou, and Q. Zhao. 2003. Evaluation of spatial and temporal changes of soil quality based on geostatistical analysis in the hill region of subtropical China. Geoderma 115:85–99.

Teh, Y. A., and W. L. Silver. 2006. Effects of soil structure destruction on methane production and carbon partitioning between methanogenic pathways in tropical rain forest soils. Journal of Geophysical Research: Biogeosciences 111:2156–2202.

Teh, Y. A., W. L. Silver, and M. E. Conrad. 2005. Oxygen effects on methane production and oxidation in humid tropical forest soils. Global Change Biology 11:1283–1297.

Tiessen, H., and J. O. Moir. 1993. Characterization of available phosphorus by sequential extraction. Pages 75–86 *in* M. R. Carter, editor. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science. Lewis, Boca Raton, Florida, USA.

Townsend, A., G. Asner, and C. Cleveland. 2008. The biogeochemical heterogeneity of tropical forests. Trends in Ecology and Evolution 23:424–431.

USDA NRCS. 2002. Soil survey of Caribbean National Forest and Luquillo Experimental Forest. Commonwealth of Puerto Rico. USDA, Natural Resources Conservation Service, Washington, D.C., USA.

Vandecar, K. L., D. Lawrence, and D. Clark. 2011. Phosphorus sorption dynamics of anion exchange resin membranes in tropical rain forest soils. Soil Science Society of America Journal 75:1520.

Verchot, L. V., E. A. Davidson, J. H. Cattânio, and I. L. Ackerman. 2000. Land-use change and biogeochemical controls of methane fluxes in soils of eastern Amazonia. Ecosystems 3:41–56.

Verchot, L. V., E. A. Davidson, J. H. Cattanio, I. L. Ackerman, H. E. Erickson, and M. Keller. 1999. Land use change and biogeochemical controls of nitrogen oxide emissions from soils in eastern Amazonia. Global Biogeochemical Cycles 13:31–46.

Vitousek, P. M., and R. L. Sanford. 1986. Nutrient cycling in moist tropical forest. Annual Review of Ecology and Systematics 17:137–167.

Waide, R. B., J. K. Zimmerman, and F. N. Scatena. 1998. Controls of primary productivity: lessons from the Luquillo Mountains in Puerto Rico. Ecology 79:31–37.

Weaver, P. L. 1994. Baño de Oro Natural Area, Luquillo Mountains, Puerto Rico. USDA Forest Service, Southern Forest Experiment Station, New Orleans, Louisiana, USA.

Weaver, P. L., and P. G. Murphy. 1990. Forest structure and productivity in Puerto Rico's Luquillo Mountains. Biotropica 22:69–82.

Wood, T. E., and W. L. Silver. 2012. Strong spatial variability in trace gas dynamics following experimental drought in a humid tropical forest. Global Biogeochemical Cycles 26. http://dx.doi.org/10.1029/2010GB004014

Xu, G., J. N. Sun, R. F. Xu, Y. C. Lv, H. B. Shao, K. Yan, L. H. Zhang, and M. Blackwell. 2011. Effects of air-drying and freezing on phosphorus fractions in soils with different organic matter contents. Plant Soil and Environment 57:228–234.

Yavitt, J. B., K. E. Harms, M. N. Garcia, S. J. Wright, F. He, and M. J. Mirabello. 2009. Spatial heterogeneity of soil chemical properties in a lowland tropical moist forest, Panama. Australian Journal of Soil Research 47:674.

Yu, K., and W. H. Patrick. 2004. Redox window with minimum global warming potential contribution from rice soils. Soil Science Society of America Journal 68:2086–2091.

Yu, K., S. P. Faulkner, and W. H. Patrick, Jr. 2006. Redox potential characterization and soil greenhouse gas concentration across a hydrological gradient in a Gulf coast forest. Chemosphere 62:905–914.

Zhuang, Q., Y. Lu, and M. Chen. 2012. Atmospheric environment. Atmospheric Environment 47:66–75.