Breaking the Enzymatic Latch: Impacts of Reducing Conditions on Hydrolytic Enzyme Activity in Tropical Forest Soils

Steven J. Hall, University of California - Berkeley
Jonathan Treffkorn, University of California - Berkeley
Whendee L. Silver, University of California - Berkeley

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STEVEN J. HALL,1 JONATHAN TREFFKORN, AND WHENDEE L. SILVER

Department of Environmental Science, Policy, and Management, University of California, 107 Mulford Hall, Berkeley 94720 California, USA

Abstract. The enzymatic latch hypothesis proposes that oxygen (O2) limitation promotes wetland carbon (C) storage by indirectly decreasing the activities of hydrolytic enzymes that decompose organic matter. Humid tropical forest soils are often characterized by low and fluctuating redox conditions and harbor a large pool of organic matter, yet they also have the fastest decomposition rates globally. We tested the enzymatic latch hypothesis across a soil O2 gradient in the Luquillo Experimental Forest, Puerto Rico, USA. Enzyme activities expressed on a soil mass basis did not systematically decline across a landscape O2 gradient, nor did phenolics accumulate, the proposed mechanism of the enzymatic latch. Normalizing enzyme activities by C concentrations did suggest a decline in several enzymes as mean soil O2 decreased. However, relationships between hydrolytic enzymes and reducing conditions were scale-dependent: enzymes displayed neutral to strongly positive relationships with reducing conditions and phenolics when comparing samples within sites, and enzyme activities in 18-d anaerobic incubations generally exceeded those in aerobic soils despite a fourfold increase in phenolics. In summary, although O2 availability and the activities of some enzymes appeared to be related at landscape scales after accounting for differences in organic matter, reducing conditions and phenolic compounds did not appear to constrain soil hydrolytic enzyme activity at the scale of soil microsites. We suggest a critical re-examination of mechanisms and the scale dependence of couplings between O2 and decomposition in terrestrial soils.

Key words: decomposition; enzymatic latch; extracellular enzyme; iron reduction; oxygen; phenolics; redox reactions; soil carbon; soil organic matter; tropical forest.

INTRODUCTION

Humid tropical forest soils harbor a substantial portion (~500 Pg) of the global terrestrial C pool, yet simultaneously support the highest litter and root decomposition rates of any biome (Jobbagy and Jackson 2000, Parton et al. 2007). Much previous work has focused on the importance of constraints on microbial decomposition in mineral soils for maintaining C stocks (e.g., Jenny 1950, Schmidt et al. 2011). The availability of O2, in particular, has been proposed as one potential integrator of biophysical constraints on decomposition (Kleber 2010, Davidson et al. 2012). Although most organic matter can ultimately be decomposed anaerobically, O2 limitation (reducing conditions) typically decreases decomposition rates in natural ecosystems (Ponnampерuma 1972, McLatchey and Reddy 1998, Fenner and Freeman 2011), with important consequences for the C balance of soils. In the humid tropics, a combination of finely textured soils and high moisture, temperature, and respiration rates can lead to O2 depletion even in relatively well-drained environments (Silver et al. 1999, 2013, Schuur et al. 2001, Liptzin et al. 2011). However, few studies have explicitly examined relationships between O2 and decomposition in humid tropical soils (Silver et al. 1999, Schuur et al. 2001). Although O2 limitation has long been known to affect decomposition rates in static anaerobic environments (Tenney and Waksman 1930), the relative influence of O2 on decomposition in upland ecosystems remains unclear.

Extracellular enzymes that degrade macromolecular substrates for microbial assimilation often control decomposition rates (Sinsabaugh 1994), and these processes may be linked to O2 availability. Degradation of lignin, for example, may require oxidative enzymes and O2 or H2O2 (Sinsabaugh 2010). The most abundant constituents in organic detritus, however, include compounds such as cellulose and hemicellulose, which can be decomposed by hydrolytic enzymes that do not directly require O2. Hydrolytic enzymes also liberate much of the organic nitrogen (N) and phosphorus (P) ultimately assimilated by plants and microbes, and thus play a critical role in ecosystem productivity; plant roots
may provide a significant source of phosphatase enzymes (Dakora and Phillips 2002), although microbial enzymes are thought to dominate organic matter decomposition overall. Although hydrolytic enzyme activities do not directly require the presence of O$_2$, experiments and theory suggest that reducing conditions may decrease hydrolytic enzyme activities indirectly via at least two mechanisms (McLatchey and Reddy 1998, Freeman et al. 2001, 2004). First, according to the “enzymatic latch” hypothesis (Freeman et al. 2001), O$_2$ limitation may inhibit hydrolytic enzyme activity by promoting the accumulation of phenolic substances, a ubiquitous component of organic matter that interferes with enzyme catalysis (Wetzel 1992, Freeman et al. 2001, Allison 2006, Yao et al. 2009). Polyphenolic compounds accumulate in anaerobic wetland soils because their decomposition presumably requires O$_2$-dependent phenol oxidative enzymes, leading Freeman et al. (2001) to propose that anaerobiosis imposes an enzymatic latch on hydrolytic enzymes due to the accumulation of inhibitory phenolics. Exposing wetland soils to O$_2$ stimulated phenol oxidase activities, decreased phenolic concentrations, and increased hydrolytic enzyme activities (Freeman et al. 2001, 2004, Fenner and Freeman 2011). In tropical montane forest soils, Bruijnzeel et al. (1993) hypothesized (but did not test) that high phenolic concentrations decrease decomposition rates.

A second mechanism postulates relative declines in enzyme synthesis under anaerobic conditions. The adenosine triphosphate (ATP) yield of C mineralization declines in the absence of O$_2$, corresponding with decreased microbial biomass, even in ecosystems that regularly experience anaerobiosis (Unden and Bongaerts 1997, McLatchey and Reddy 1998, Fenner and Freeman 2011). These factors may explain decreased hydrolytic enzyme activities with reducing conditions in wetland soil incubations (McLatchey and Reddy 1998). Generalizable impacts of anaerobiosis on enzyme activities in situ, however, remain uncertain, given the influence of multiple additional factors, including enzyme degradation, stabilization, and inhibition (Allison 2006).

In sum, reducing conditions in wetland ecosystems have been hypothesized to decrease soil hydrolytic enzyme activity by at least two mechanisms; inhibition from phenolic substances, and decreased enzyme production, with important but untested implications for the C cycle of upland soils. Terrestrial soils in the humid tropics potentially exhibit similarities in reducing conditions (Silver et al. 1999), and possibly phenolic concentrations, with flooded wetland ecosystems. We tested these hypotheses by evaluating potential activities of several hydrolytic enzymes that degrade organic C, N, and P within and among six humid tropical forest sites that differed in long-term mean soil O$_2$ availability. These ecosystems exhibit redox heterogeneity within and among sites (Silver et al. 1999, Liptzin et al. 2011), allowing us to assess the impact of reducing conditions on enzymes at multiple spatial and temporal scales. We compared enzyme activities with soil phenolics and with reduced iron (Fe(II)) concentrations as an index of reducing conditions among samples. Concentrations of alternative electron acceptors or their reduced equivalents have frequently been used to infer the relative importance of anaerobic processes in ecosystems (Chapelle et al. 1995). Iron oxides represent the most abundant anaerobic terminal electron acceptor in highly weathered tropical soils, where microbes and humic substances reduce ferric iron (Fe(III)) to ferrous iron (Fe(II)) in the absence of O$_2$ (Chacon et al. 2006, Thompson et al. 2006, Dubinsky et al. 2010). Because Fe(II) rapidly oxidizes to Fe(III) in the presence of O$_2$ (Patrick and Henderson 1980), Fe(II) concentrations provide an integrative metric of O$_2$ availability in soils where aerobic and anaerobic processes occur in close proximity (Hall et al. 2013).

**METHODS**

We sampled soils across gradients of topography and elevation in the Luquillo Experimental Forest, Puerto Rico, USA, a NSF Long-Term Ecological Research and Critical Zone Observatory site (Table 1, Appendix A). These sites encompass a gradient of long-term rainfall and mean soil O$_2$ availability, indicative of the relative importance of anaerobic microsites. Mean volumetric O$_2$ concentrations measured in soil gas wells (10 cm depth) over a multi-year study decreased from ridges to slopes to riparian valleys within a lower montane forest in the Bisley Research Watersheds (19%, 16%, and 10% O$_2$, respectively) and along a montane forest elevation gradient (10% to 8% O$_2$; Silver et al. 1999). Soil O$_2$ concentrations exhibit temporal variability associated with rainfall frequency over weeks to months, and most sites (except the lower montane ridges and slopes) experience periodic bulk soil O$_2$ concentrations <3% (Silver et al. 1999). Our field samples, therefore, provided a representative snapshot of spatial patterns in O$_2$ availability. While it is impossible to strictly isolate effects of O$_2$ on enzymes in the field at the landscape scale due to co-variation in factors such as climate and vegetation, we could assess whether O$_2$ and/or phenolics exerted overarching impacts on enzyme activities under realistic field conditions, as observed across a spectrum of wetland ecosystems (Fenner and Freeman 2011). At a finer spatial scale, we controlled for climate and vegetation by evaluating relationships between reducing conditions and enzyme activities from samples within similar sites (lower montane ridges and slopes). In the laboratory, we conducted controlled experiments to directly examine the influence of soil O$_2$ on enzyme activities over timescales relevant to field conditions (Liptzin et al. 2011), as a further evaluation of the hypotheses.
Elfin 936 18.2702 65.761 0–10 1.71 (0.17) 16.7 (2.0) 3.74 (0.77) 0.69 (0.08) 4.70 (0.05) 5 (1.8)
Colorado 736 18.2942 65.7852 0–10 1.06 (0.05) 7.1 (1.1) 1.35 (0.8) 0.36 (0.13) 4.60 (0.16) 2.5 (0.6)
Palm 614 18.2988 65.7803 0–10 1.82 (0.86) 12.4 (2.3) 1.22 (0.44) 0.28 (0.07) 4.67 (0.08) 10.7 (2.6)
Bisley valleys 210–240 0–10 0.93 (0.03) 4.2 (0.2) 0.38 (0.11) 0.13 (0.03) 5.27 (0.05) 1.2 (0.2)
Palm 936 18.2702 65.761 0–10 1.71 (0.17) 16.7 (2.0) 3.74 (0.77) 0.69 (0.08) 4.70 (0.05) 5 (1.8)
Bisley slopes 220–280 0–10 0.80 (0.03) 3.4 (0.2) 0.43 (0.19) 0.12 (0.04) 5.30 (0.07) 1 (0.2)
Bisley ridges 240–300 18.3157 65.7487 0–10 0.94 (0.04) 5.9 (0.5) 0.7 (0.2) 0.2 (0.05) 4.50 (0.08) 0.4 (0.2)

TABLE 1. Soil characteristics (means with standard errors in parentheses) by site and depth increment.

<table>
<thead>
<tr>
<th>Site</th>
<th>Elevation (m)</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Depth (cm)</th>
<th>Soil moisture (g H$_2$O/g soil)</th>
<th>Carbon (%)</th>
<th>Fe(II) (mg/g soil)</th>
<th>Fe(II)/FeHCl</th>
<th>pH</th>
<th>Soluble phenolics (µg/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisley ridges</td>
<td>240–300</td>
<td>18.3157</td>
<td>65.7487</td>
<td>0–10</td>
<td>0.94 (0.04)</td>
<td>5.9 (0.3)</td>
<td>0.46 (0.04)</td>
<td>0.14 (0.01)</td>
<td>4.31 (0.05)</td>
<td>11 (2)</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>0.74 (0.03)</td>
<td>3.6 (0.2)</td>
<td>0.25 (0.02)</td>
<td>0.08 (0.00)</td>
<td>4.48 (0.04)</td>
<td>6.1 (1.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisley slopes</td>
<td>220–280</td>
<td>0–10</td>
<td>0.95 (0.05)</td>
<td>2.8 (0.2)</td>
<td>0.11 (0.02)</td>
<td>0.06 (0.01)</td>
<td>4.74 (0.06)</td>
<td>2.3 (0.7)</td>
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<tr>
<td></td>
<td>10–20</td>
<td>0.74 (0.04)</td>
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<td></td>
</tr>
<tr>
<td>Bisley valleys</td>
<td>210–240</td>
<td>0–10</td>
<td>0.93 (0.03)</td>
<td>4.2 (0.2)</td>
<td>0.38 (0.11)</td>
<td>0.13 (0.03)</td>
<td>5.27 (0.05)</td>
<td>1.2 (0.2)</td>
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<tr>
<td></td>
<td>10–20</td>
<td>0.80 (0.03)</td>
<td>3.4 (0.2)</td>
<td>0.43 (0.19)</td>
<td>0.12 (0.04)</td>
<td>5.30 (0.07)</td>
<td>1 (0.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado</td>
<td>736</td>
<td>18.2942</td>
<td>65.7852</td>
<td>0–10</td>
<td>1.06 (0.05)</td>
<td>7.1 (1.1)</td>
<td>1.35 (0.8)</td>
<td>0.36 (0.13)</td>
<td>4.60 (0.16)</td>
<td>2.5 (0.6)</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>0.86 (0.08)</td>
<td>4.9 (0.5)</td>
<td>0.7 (0.2)</td>
<td>0.2 (0.05)</td>
<td>4.50 (0.08)</td>
<td>0.4 (0.2)</td>
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</tr>
<tr>
<td>Palm</td>
<td>614</td>
<td>18.2988</td>
<td>65.7803</td>
<td>0–10</td>
<td>1.82 (0.86)</td>
<td>12.4 (2.3)</td>
<td>1.22 (0.44)</td>
<td>0.28 (0.07)</td>
<td>4.67 (0.08)</td>
<td>10.7 (2.6)</td>
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<tr>
<td></td>
<td>10–20</td>
<td>0.88 (0.05)</td>
<td>7 (1.1)</td>
<td>2.69 (1.21)</td>
<td>0.53 (0.13)</td>
<td>4.49 (0.22)</td>
<td>2.2 (0.5)</td>
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<tr>
<td>Elfin</td>
<td>936</td>
<td>18.2702</td>
<td>65.761</td>
<td>0–10</td>
<td>1.71 (0.17)</td>
<td>16.7 (2.0)</td>
<td>3.74 (0.77)</td>
<td>0.69 (0.08)</td>
<td>4.70 (0.05)</td>
<td>5 (1.8)</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>1.33 (0.12)</td>
<td>14.6 (2.0)</td>
<td>4.11 (0.79)</td>
<td>0.71 (0.09)</td>
<td>4.63 (0.07)</td>
<td>3.1 (0.6)</td>
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</table>

Notes: See Appendix A for details on soil analyses; Fe(II)/FeHCl represents the ratio of Fe(II) to total Fe measured in 0.5 mol/L HCl soil extractions.

Field sampling

In June 2011, we sampled four replicate lower montane forest catenas, each containing ridge, slope, and riparian valley topographic zones. In each catena, we established five replicate 0.25-m$^2$ plots in each topographic zone that were randomly stratified within 5–10-m intervals along a 50-m linear transect. We similarly sampled five replicate plots from each of the three montane forest sites on the elevation gradient in February 2012. In each plot, we collected two replicate 6 cm diameter soil cores at depths of 0–10 cm and 10–20 cm (total $n = 149$ cores; one sample could not be collected because of rocks); these depths contain the majority of roots and organic matter in these ecosystems (Silver et al. 1994). Replicate cores from each depth were composited and subsamples assayed for activities of five hydrolytic enzymes by adding fluorescent substrates to buffered soil slurries according to standard methods (German et al. 2011). Cellulohydrolase and β-gluco- side respectively hydrolyze glucose dimers and monomers from cellulose, β-xilosidase yields xylose monomers from xylan (hemicellulose), N-acetyl β-D-glucosaminidase (NAGase) produces amino sugars from peptidoglycan and chitin, and acid phosphatase cleaves phosphate from phosphomonoesters. We also measured concentrations of Fe(II), soluble organic C, phenolics, mineral nitrogen (N), and total C; see Appendix A for details.

Laboratory incubation

We tested the influence of anaerobiosis on enzyme dynamics over an 18-d incubation in replicate soil samples collected from an upland valley site in the lower montane forest that experiences large O$_2$ fluctuations over days to weeks (Liptzin et al. 2011). Samples were exposed to one of four treatments (six replicates each) in a factorial design of headspace (aerobic and anaerobic) and solution addition (water or water + labile C). Enzyme assays were conducted immediately before treatments were imposed and after 6, 12, and 18 d. See Appendix A for further details.

Statistical analysis

We assessed differences in enzyme activity among sites using generalized linear models with a unique variance term for each site. Relationships between enzyme activities, phenolics, and Fe(II) were analyzed using mixed effects models that included transsects, plots (within transects), and transect × depth interactions as potential random effects to account for spatial structure; soil depth was included as a fixed effect. Correlations between enzymes and Fe(II) differed categorically in direction between the lower montane Ridge and Slope soils, on one hand, and the lower montane Valley, Palm, Colorado, and Elfin forest soils, on the other. The former group of sites is characterized by higher mean bulk soil O$_2$ concentrations, whereas the latter sites experience a higher frequency of bulk soil O$_2$ <3%. We thus fitted separate statistical models for these two groups of sites. Variables were normalized by mean and standard deviation to facilitate comparisons, and variables for the low-O$_2$ sites were log transformed to remove residual heteroscedasticity; normality and trends in residuals were assessed graphically. We selected the optimal random effects for each model using Akaike’s information criterion (AIC) on saturated models fit using restricted maximum likelihood (REML), and then selected fixed effects by comparing the AIC of models fit using maximum likelihood. Phenolics could not be assayed for 12 samples, so model selection was conducted on the reduced data set. The laboratory experiment was analyzed similarly, incorporating experimental units as random effects to account for repeated measurements; we report $P$ values for treatment effects based on the $t$ statistic approximation. Labile C additions did not affect enzyme activities, so we pooled the data by headspace treatment. Differences among treatments and days were assessed using Tukey’s honestly significant
difference (HSD) test. We fit models in R (R Development Core Team 2012) using the lmer and lme functions in the lme4 and nlme packages for the field and laboratory data, respectively (Bates et al. 2012, Pinheiro et al. 2013).

**RESULTS**

All soils contained measurable concentrations of Fe(II), indicative of reducing conditions in soil microsites (Fig. 1A, Table 1). Concentrations of Fe(II) increased by more than an order of magnitude with increasing elevation and decreasing mean bulk soil O₂, but were similar among ridges and valleys in the lower montane forest. Soluble phenolics declined with O₂ along the ridge/slope-valley catenas, but did not show consistent trends with O₂ among all sites when expressed on the basis of soil mass (Fig. 1B, Table 1) or soil water content (Appendix B). Soil C and DOC concentrations did not scale linearly with Fe(II) or O₂ among sites (Table 1), nor did mineral N concentrations vary systematically (Appendix B).

*Landscape-scale patterns in hydrolytic enzyme activity*

Enzyme activities did not vary consistently with bulk soil O₂ among sites when expressed on a mass basis. Trends with O₂ were typically stronger when activities were expressed per unit soil C (Fig. 2, Appendix C). Each enzyme showed distinct patterns of variation among sites in the 0–10 cm samples. β-xylosidase showed the most consistent declines in activity with decreasing site O₂. Activities of cellobiohydrolase and β-glucosidase differed between the extremes of the O₂ gradient (Ridge and Elfin sites) but not among sites with intermediate bulk soil O₂ (the Slope, Valley, and Palm sites). When normalized by soil C, cellobiohydrolase showed greater variation among sites, and did not differ significantly across the ridge/slope-valley catenas. Acid phosphatase activity was highest in the Ridge and Slope sites and low in the other four sites, when expressed on both a mass and soil C basis. Conversely, NAGase (mass basis) did not vary across the catenas, and increased as soil O₂ declined in the Palm, Colorado, and Elfin sites. No site trends were evident when NAGase was normalized by soil C.

Subsurface (10–20 cm) soils showed even less significant variation in mass-based enzyme activities among sites, whereas C-normalized activities of cellobiohydrolase, xylosidase, and acid phosphatase declined with decreasing O₂ concentrations (Fig. 2, Appendix C). Subsurface mass-based cellobiohydrolase activity was similar among five of the six sites assayed, whereas β-glucosidase activity was more variable among sites, but was similar among the extremes of the O₂ gradient (Ridge and Elfin sites). When normalized by soil C, β-glucosidase activity was highest in the Valley, a site with intermediate O₂ availability, and NAGase displayed no consistent trends.

*Sample-scale correlates of enzyme activities*

Enzyme activities typically varied by two orders of magnitude within a given site and often displayed significant relationships with Fe(II), but less so with phenolics (Fig. 3, Appendix D). Patterns in enzyme activities fell into two main groupings: the Ridge and Slope sites (higher mean soil O₂), on one hand, and the Valley, Palm, Colorado, and Elfin forests (lower mean soil O₂), on the other. On a soil mass basis, Ridge and Slope soils exhibited strong positive relationships with Fe(II) for all enzymes assayed, with standardized coefficients between 0.36 and 0.61 (Table 2a). Enzymes frequently displayed positive or neutral pairwise relationships with phenolics in these sites (Appendix D), but the inclusion of phenolics seldom improved model AIC. The sites with lower mean O₂ concentrations (Valley, Palm, Colorado, and Elfin) also showed neutral or positive relationships between soil-mass based enzyme activity and phenolics, but enzymes displayed neutral to weakly negative relationships with...
Fe(II) concentrations (Table 2b). Phenolics and soluble organic C concentrations were strongly correlated ($R^2 = 0.72$) across both groups of sites (Appendix D).

Normalizing enzyme activities by soil C concentrations suggested different relationships between enzymes and Fe(II). In the high-O$_2$ sites, normalized acid phosphatase and NAGase activities showed negative relationships with Fe(II), and C-acquisition enzymes showed neutral relationships (Appendix C). In the low-O$_2$ sites, normalized cellobiohydrolase and β-xylosidase displayed weak negative relationships with Fe(II), and acid phosphatase declined more strongly with Fe(II). Phenolics showed no relationship with C-normalized enzymes except for a negative relationship with NAGase in the low-O$_2$ sites.

Fig. 2. Potential activity of five hydrolytic enzymes by depth and across sites with decreasing mean long-term soil O$_2$ concentrations from left to right (Ridge site to Elfin site). Enzyme activities are expressed as the MUB (methylumbelliferone) production rate in terms of soil mass (bulk soil) in column (A) and soil C (carbon-normalized) in column (B). NAGase denotes N-acetyl β-D-glucosaminidase.

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Effects of imposed anaerobiosis on hydrolytic enzyme activities

Imposing anaerobiosis on soils in the laboratory did not suppress enzyme activity relative to aerobic controls for four out of the five enzymes assayed. Rather, enzyme activity in anaerobic soils significantly exceeded activity in aerobic soils at the end of the experiment for three out of five enzymes assayed, which measured 151%, 154%, and 132% of the respective aerobic activities for NAGase ($P = 0.001$), $\beta$-celllobiohydrolase ($P < 0.01$), and $\beta$-xylosidase ($P < 0.001$; Fig. 4). In the case of $\beta$-xylosidase, activity increased under anaerobic conditions relative to the beginning of the experiment ($P < 0.05$), whereas NAGase and celllobiohydrolase showed consistent enzyme activity under anaerobic conditions accompanied by a decline under aerobic conditions ($P < 0.05$ and $P < 0.01$, respectively). $\beta$-glucosidase activity did not significantly differ from initial measurements after 18 days ($P = 0.08$), but activity in anaerobic soils significantly exceeded aerobic controls on day 12 ($P < 0.001$). Acid phosphatase showed highly variable trends over time; activity in anaerobic soils exceeded aerobic soils after 12 days ($P < 0.001$), but this pattern reversed after 18 days due to an increase in aerobic, and a decrease in anaerobic activity ($P < 0.001$). Phenolic compounds measured in soil leachate varied significantly by time, headspace, and C addition treatment. On day 6, phenolics did not differ by treatment and averaged 0.45 mg/L, whereas by day 18 phenolics had significantly increased to 1.27, 1.70, and 2.01 mg/L in the anaerobic, anaerobic + C, and aerobic + C treatments, respectively, while the aerobic treatment decreased to 0.27 mg/L ($P < 0.001$).

**DISCUSSION**

Reducing conditions and the accumulation of phenolics have been shown to decrease soil hydrolytic enzyme activities in wetlands (McLatchey and Reddy 1998, Freeman et al. 2001). We found that reducing conditions and soluble phenolics were ubiquitous in upland surface soils in the Luquillo Mountains, Puerto Rico. In the upper elevation montane forest soils, in particular, Fe(II) concentrations were equivalent to anaerobic wetland sediments (Roden and Wetzel 1996). Normalizing water-extractable soil phenolics by in situ soil moisture levels yielded concentrations similar to peatland porewaters where phenolics inhibited hydrolytic enzymes (Freeman et al. 2004, Fenner and Freeman 2011). In these tropical forest soils, enzyme activities did not consistently decline with reducing conditions or phenolics on a soil mass basis. Normalizing enzyme activities to account for differences in C among soils showed that relative enzyme activities often declined with lower mean O$_2$ availability, but trends were inconsistent across all sites. In the laboratory, we found that anaerobiosis led to an increase in three enzymes relative to aerobic controls, despite an accumulation of soluble phenolics. Together, our data suggest that an important hypothesis for predicting the mechanistic impact of O$_2$ limitation on peatland decomposition may not be widely applicable across terrestrial soils characterized by similarities in reducing conditions, phenolics, and pH. These responses could be driven in part by characteristic differences between flooded wetlands (especially peatlands) and upland humid tropical forests.
specifically the prevalence of moisture and O2 fluctuations, and the importance of a reactive mineral matrix.

Relationships between reducing conditions, enzymes, and phenolic compounds

The enzymatic latch hypothesis proposes that reducing conditions suppress oxidative enzymes, leading to an accumulation of phenolic compounds (Freeman et al. 2001, 2004). Here, we found little relationship between our indices of reducing conditions and phenolics in our field samples. Differences in plant litter chemistry and leaching fluxes among our sites could potentially contribute to decoupling between phenolics and reducing conditions, although previous work in wetlands differing in vegetation and hydrology found an overriding effect of O2 (Fenner and Freeman 2011). Temporal O2 fluctuations over days to weeks (Liptzin et al. 2011) could facilitate periodic degradation of phenolics, contributing to this pattern. High concentrations of reactive minerals in these soils (Dubinsky et al. 2010)

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**TABLE 2.** Optimum-mixed effects models for soil hydrolytic enzyme activity on a soil mass basis (measured as mmol·g⁻¹·hr⁻¹).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Fixed effects</th>
<th>Random effects (variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe(II)</td>
<td>Phenolics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) High-O2 sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobiohydrolase</td>
<td>0.61 (0.08)</td>
<td>–0.27 (0.06)</td>
</tr>
<tr>
<td>β-xylosidase</td>
<td>0.42 (0.10)</td>
<td>0.17 (0.09)</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>0.44 (0.08)</td>
<td>–0.34 (0.07)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>0.36 (0.08)</td>
<td>–0.22 (0.09)</td>
</tr>
<tr>
<td>N-acetyl β-D-glucosaminidase</td>
<td>0.49 (0.09)</td>
<td>–0.30 (0.07)</td>
</tr>
<tr>
<td>b) Low-O2 sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobiohydrolase</td>
<td>–0.54 (0.14)</td>
<td></td>
</tr>
<tr>
<td>β-xylosidase</td>
<td>–0.43 (0.08)</td>
<td></td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>–0.38 (0.14)</td>
<td></td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>–0.33 (0.05)</td>
<td></td>
</tr>
<tr>
<td>N-acetyl β-D-glucosaminidase</td>
<td>0.31 (0.10)</td>
<td>–0.35 (0.12)</td>
</tr>
</tbody>
</table>

**Notes:** Table shows (a) models for the lower montane Ridge and Slope sites (high-O2 sites), and (b) models for the Valley, Palm, Colorado, and Elfin forest sites (low-O2 sites). Fixed effects represent restricted maximum likelihood (REML) model coefficients and standard errors using data normalized by mean and standard deviation. Cells left blank indicate effects not included in the models. Values in parentheses are standard errors.

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**FIG. 4.** Hydrolytic enzyme activities (on a soil mass basis) in soils exposed to aerobic or anaerobic conditions over an 18-d laboratory incubation. Mixed effects models showed significant time by treatment interactions for cellobiohydrolase ($P < 0.01$), β-glucosidase ($P < 0.05$), acid phosphatase ($P < 0.0001$), and NAGase ($P < 0.01$). β-xylosidase showed significant time and treatment effects, respectively ($P < 0.001$ and $P < 0.0001$).
could also sorb or precipitate phenolic compounds, removing them from solution (Kramer et al. 2012). Emerging evidence also suggests that high Fe(II) concentrations can actually increase rates of phenolic decomposition. Van Bodegom et al. (2005) showed that Fe(II) catalyzed phenol oxidative activity under low-O2 conditions in wetland soils; we found a similarly strong relationship between phenol oxidative activity and Fe(II) oxidation in these soils (Hall and Silver 2013). Thus, high concentrations of Fe(II), reactive minerals, and the prevalence of O2 fluctuations might all contribute to a decoupling between phenolics and reducing conditions in humid tropical forest soils, as opposed to wetlands.

The enzymatic latch hypothesis also proposes that phenolic compounds accumulated under reducing conditions suppress hydrolytic enzyme activity. We found largely positive or neutral relationships between phenolic compounds and hydrolytic enzymes in the field when comparing samples grouped according to their mean site O2 dynamics (the higher-O2 sites and the lower-O2 sites). Furthermore, a threefold increase in soluble phenolics in our anaerobic incubation, likely driven by colloid dispersion (Thompson et al. 2006) and inhibition of O2-dependent enzymes (Freeman et al. 2001), did not suppress hydrolytic enzyme activity. Phenolic concentrations in anaerobic soil leachate were similar to those observed to inhibit enzymes in a peatland (2 mg/L; Freeman et al. 2004), but these presumably differed in their chemical composition. These data provide a broader ecological context to the findings of Freeman et al. (2001, 2004) and Allison (2006), where enzyme activities were significantly affected by adding or removing phenolic compounds. Phenolics comprise a biochemically diverse suite of compounds, and positive relationships between phenolics and C substrate availability (as indicated by DOC) might outweigh inhibitory effects at the ecosystem scale.

Scale-dependence of enzyme activities and reducing conditions

Decreasing redox potential has also been shown to affect hydrolytic enzyme activities directly, possibly via impacts on microbial growth (McLatchey and Reddy 1998). We found that some enzymes declined with decreasing site O2 concentrations at the landscape scale, especially when activities were normalized by soil C, supporting the importance of O2 as a coarse index of decomposition rates among ecosystems (Schuur et al. 2001). The mechanistic basis of this relationship remains unclear, however, and could also be driven by covariation in other biogeochemical factors such as organic matter quality, in addition to thermodynamic considerations.

At finer spatial scales, relationships between enzyme activities and Fe(II) varied among samples according to their site and the metric of enzyme activity employed. Several enzymes showed negative relationships with Fe(II) when their activities were normalized by soil C, as a consequence of the fact that most enzymes did not scale linearly with C concentrations. Expressing enzymes on a soil mass basis, however, provides the more relevant metric for assessing potential catalytic rates. In the higher-O2 sites, positive relationships between carbohydrate-degrading enzymes and Fe(II) could reflect the importance of biological O2 demand, coupled with high soil moisture, in generating anaerobic microsites. Increased hydrolytic enzyme activity likely fueled respiration by supplying labile C to microbes, leading to respiratory O2 depletion and Fe reduction. The importance of C availability in driving dissimilatory Fe reduction in these soils was further suggested by the finding that C-degrading enzymes from these sites displayed no relationship with reducing conditions when normalized by soil C concentrations. This is consistent with previous work documenting net Fe reduction in soils exposed to an aerobic atmosphere after labile C addition (Liptzin and Silver 2009). We acknowledge the critical importance of soil moisture in restricting O2 diffusion, yet gravimetric moisture was similar among sites in the lower montane forest, and soil macropores were not water-saturated. Thus, for clay and organic-matter-rich soils with high water-holding capacity, variation in moisture per se may not always provide the most important proximate driver of reducing conditions. This perspective provides nuance to a traditional assumption of wetland ecology, where spatial or temporal variation in moisture is often thought to initiate Fe reduction by limiting O2 supply (Ponnampерuma 1972). In our study, the impact of soil moisture on generating reducing conditions may have been increasingly important in the higher-rainfall sites, characterized by higher soil moisture, lower respiration, and higher Fe(II) concentrations than the lower montane forests.

The strong negative relationship between Fe(II) and acid phosphatase activity evident on both a mass and soil C basis in the lower-O2 sites was an exception to the general pattern. This may reflect the underlying importance of Fe reduction in solubilizing the Fe–phosphorus (P) complexes that control P availability in Fe-oxide rich soils (Chacon et al. 2006, Liptzin and Silver 2009). Declines in acid phosphatase activity with increasing Fe(II), therefore, may actually reflect increased P availability as opposed to constraints on enzyme activity. In support of this interpretation, labile P concentrations were 2.5-fold greater in valleys than ridges in the lower montane soils (Mage and Porder 2013), paralleling similar declines in acid phosphatase. Plant-produced phosphatase enzymes may also have contributed to patterns observed here. Nitrogen availability affects microbial enzyme production (Schimel and Weintraub 2003), although the relatively high extractable ammonium concentrations measured here were not indicative of strong N limitation. Mineral N varied little among sites and was not likely a dominant driver of patterns in enzyme activity.
Enzyme activity under anaerobic conditions

Our laboratory data shows that anaerobiosis did not inhibit hydrolytic enzyme activity over timescales relevant to natural O2 fluctuations in these sites (Liptzin et al. 2011). Our anaerobic incubation decreased the activity of only one enzyme, acid phosphatase, relative to the control; this may have resulted from increased P availability as discussed in the previous section. Activities of the other four enzymes, in contrast, were significantly greater under anaerobic conditions. Potential soil enzyme activity reflects the net effects of enzyme production, stabilization, degradation, and inhibition (Allison 2006). A shift in any or all of these factors could explain the patterns observed here, while leading to the preservation of potential enzyme activity in soils that experience periodic O2 fluctuations.

Conclusions

We tested the hypothesis that hydrolytic enzyme activity declines with reducing conditions and phenolic accumulation in humid tropical forest soils. Soluble phenolics did not appear to affect enzyme activities in the field or laboratory, and indices of reducing conditions showed a variable relationship with enzyme activities depending on site characteristics, spatial scale, and metric of analysis. The activities of some enzymes decreased across a landscape-scale gradient of soil O2 concentrations, especially when they were normalized by C concentrations. This pattern is consistent with theoretical impacts of O2 limitation on metabolism: declining ATP yield of C mineralization could decrease relative enzyme production at the community level. However, enzymes seldom declined with an index of reducing conditions (Fe(II) concentrations) when individual soil samples were compared within similar ecosystems (the high- and low-O2 sites). Furthermore, field-relevant periods of anaerobiosis did not inhibit enzymes in laboratory incubations. We propose an alternative hypothesis: within ecosystems, hydrolytic enzymes contribute to localized hotspots of O2 consumption in humid soils that generate reducing microsites. This leads to a reciprocal relationship between enzymes and O2, as opposed to a simple one-way interaction between environmental conditions and enzyme activities. Our findings challenge the assumption, incorporated in ecosystem models, that decomposition rates vary intrinsically with O2 availability as controlled by soil moisture (Parton et al. 1993, Davidson et al. 2012). Oxygen availability demonstrably affects oxidase enzymes that require O2 as a substrate, but variation in the frequency and extent of reducing conditions in humid tropical soils accompanying climate change (Liptzin et al. 2011) may be insufficient to affect the short-term dynamics of hydrolytic enzymes which mediate a dominant portion of decomposition.

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SUPPLEMENTAL MATERIAL

Appendix A

Study site and soil analysis methods (Ecological Archives E095-255-A1).

Appendix B

Additional soil biogeochemical data (Ecological Archives E095-255-A2).

Appendix C

Additional statistical analyses assessing relationships between enzymes, sites, and soil characteristics (Ecological Archives E095-255-A3).

Appendix D

Additional figures illustrating relationships between enzymes and soil characteristics (Ecological Archives E095-255-A4).