Soil Biology & Biochemistry 66 (2013) 60–68

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Changes in microbial dynamics during long-term decomposition in tropical forests

Kristen M. DeAngelis [a,b,*], Dylan Chivian [c,d], Julian L. Fortney [e], Adam P. Arkin [c,d], Blake Simmons [b,f], Terry C. Hazen [b,e,g,h], Whendee L. Silver [g,i]

[a] Microbiology Department, University of Massachusetts, Amherst, MA, USA
[b] Deconstruction Division, DOE Joint BioEnergy Institute, Emeryville, CA, USA
[c] Technologies Division, DOE Joint BioEnergy Institute, Emeryville, CA, USA
[d] Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
[e] Department of Civil and Environmental Engineering, University of Tennessee, Knoxville, TN 37996, USA
[f] Sandia National Laboratory, Livermore, CA, USA
[g] Ecology Department, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
[h] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[i] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA, USA

ARTICLE INFO

Article history:
Received 4 April 2013
Received in revised form
13 June 2013
Accepted 14 June 2013
Available online 14 July 2013

Keywords:
Anaerobic decomposition
Fluctuating redox potential
Small subunit ribosomal rRNA gene
community analysis
Hydrolytic and oxidative enzyme activity
Population dynamics
Microbial succession

ABSTRACT

Humid tropical forest soils are characterized by low and fluctuating redox, conditions which are thought to inhibit organic matter degradation by microbes. However, evidence suggests that soil microbial communities are adapted to the redox conditions in these ecosystems. In this study we tested the hypothesis that soil oxygen (O_2) availability as an index of redox conditions structures patterns in litter decomposition and associated microbial community dynamics over space and time in humid tropical forests. We conducted a two year decomposition experiment on a common litter substrate in four sites along an elevational gradient with well described climate and redox dynamics. Microbial community sequencing, potential enzyme activities, and litter chemistry measurements were made on litter and soil to determine the relationship between soil and litter communities and biogeochemistry. Decomposition was slowest in the upper elevation site, which was the wettest and had the lowest average soil O_2 availability. However, soil hydrolytic and litter phenol oxidase activities were greatest at this site. Small subunit ribosomal RNA genes were sequenced with universal primers for bacteria, archaea and eukaryotes, yielding 40,850 unique taxa after quality filtering and clustering. Across all sites, microbial succession was observed as increasing litter richness, converging bacterial community profiles, and diverging fungal community profiles. Initial decomposers (1–4 weeks) included many r-selected bacteria, including *Alpha-*, *Beta-* and *Gammaproteobacteria*, *Clostridia, Bacteroidetes*. We also found evidence of anaerobic fungi such as *Cryptococcus*, as well as the plant-associated *Phialocephala* and *Phyllachora* species, suggesting that anaerobic and plant-associated fungi are prevalent later in decomposition in soils with low and fluctuating redox conditions. Because of the striking similarities between sites in functional potential despite differences in wet tropical soil decomposing communities and litter chemistry, we suggest that future climate-driven disruptions to redox fluctuations could significantly alter the terrestrial carbon (C) cycle in tropical forests.

1. Introduction

Humid tropical forests have the fastest rates of litter decomposition globally (Parton et al., 2007; Cusack et al., 2009), providing nutrients and energy to support the abundant microbial life typical of these ecosystems. These rapid decomposition rates occur in soils that are characterized by low and fluctuating redox conditions which permit denitrification, iron reduction, and methanogenesis (Silver et al., 1999, 2013; Schuur and Matson, 2001; Liptzin et al., 2011). The warm wet climate typical of humid tropical forests coupled with finely textured soils and high biological O_2 demand leads to conditions where O_2 can be quickly and completely consumed from the soil atmosphere (Silver et al., 1999). Soil O_2

* Corresponding author. Microbiology Department, University of Massachusetts, 639 N Pleasant St., 203 Morrill IVN, UMass, Amherst, MA 01003, USA.
E-mail address: kristen@post.harvard.edu (K.M. DeAngelis).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.06.010

concentrations in these ecosystems provide a good index of redox potential, with the concentrations of reduced species increasing with decreasing O_2 availability (Chacon et al., 2006; DeAngelis et al., 2010; Liptzin et al., 2011; Hall and Silver, 2013). This juxtaposition of low redox events and rapid C cycling presents an apparent dichotomy: low redox conditions are thought to inhibit microbial C degradation in most environments, leading to slow decomposition and the build up of partially decomposed organic matter (Bridgham et al., 1998; Freeman et al., 2001; Bridgham et al., 1998; Freeman et al., 2001). Rapid redox dynamics in humid tropical forest soils have lead to microbial adaptations to low and fluctuating redox potential in these soils (Pett-Ridge and Firestone, 2005; DeAngelis et al., 2010). Further, frequent O_2 limitation coupled to high rates of decomposition suggests that anaerobic or facultative microbes are active decomposers.

One of the primary mechanisms proposed for the inhibition of decomposition in anaerobic soils is the "enzymatic latch" – the hypothesis that oxidative activity is constrained by the absence of molecular O_2 (Freeman et al., 2001). Oxidative enzymes such as phenol oxidase and peroxidase play a key role in patterns of litter colonization over time, (Sinsabaugh, 2010), though Freeman et al. (2001) found that phenol oxidase exhibited decreased in activity when exposed to low redox conditions, slowing the overall rates of decomposition. However, anaerobic inhibition of phenol oxidase activity may be limited to substrates that are high in lignin or low in available N (Bragazza et al., 2006; Bragazza and Freeman, 2007). High N availability has been observed to stimulate the activity of phenol oxidase and increase the rate of decomposition (Waldrop et al., 2004), and humid tropical forests on clay-rich soils are typically thought to have high N availability (Vitousek and Sanford, 1986; Vitousek and Matson, 1988). The effects of low and fluctuating redox dynamics on microbial enzyme activities in this context are not well understood.

Redox dynamics are also likely to affect the time it takes for litter to decompose, where chemical, physical and biological processes conspire to mineralize litter, resulting in formation of soil organic matter (Swift et al., 1979; Kögel-Knabner, 2002). Constraints on soil organic matter dynamics often differ from those of litter decomposition due to changes in the chemical and physical properties of the material as well as the physical location of the substrate (Parton et al., 1987). Initial decomposition is driven by dissolution of organic matter (Reed et al., 2007; Wieder et al., 2009), which can occur over days to weeks (Bastian et al., 2009; Marschner et al., 2011; Strickland et al., 2012). Soluble labile C recruits microbial decomposers, beginning a course of microbial succession that can be characterized by function, where initial decomposition (in days) is catalyzed by opportunists able to quickly access soluble labile C along with partial breakdown of lignin to improve access to cellulose. This is followed by early decomposition (over weeks) where specialist decomposers are able to access the more recalcitrant cellulosic fractions; finally late stage decomposition (over months) is catalyzed by miners equipped with enzymes for lignin degradation (Moorhead and Sinsabaugh, 2006). Bacteria capable of fast growth on more labile C sources are generally able to out-compete fungi during the first days of decomposition (Meidute et al., 2008; Rousk et al., 2010), while late-stage decomposition tends to be dominated by fungi due to their ability to access the more recalcitrant litter C fractions (Stemmer et al., 2007; Marschner et al., 2011). An early dominance of fast-growing (r-selected) species such as *Proteobacteria* spp. and *Neurospora* sp. has been observed, with a later dominance of slow-growing (K-selected) species such as *Actinobacteria* and *Deltaproteobacteria* (Stevenson and Schmidt, 2004; Fierer et al., 2007; Bastian et al., 2009).

The low and fluctuating redox conditions in wet tropical soils may lead to different successional dynamics over decomposition than has been observed in temperate, more oxic forest soils. There is ample evidence that bacteria should have an advantage over fungi under conditions of low or fluctuating redox potentials: bacteria are both faster growers and better equipped for growth in anaerobic and fluctuating redox conditions than fungi (Conrad, 1996). A decomposition study in Costa Rican tropical soils showed that bacterial community profiles explained 46% of the variation in soil response to litter addition (Leff et al., 2012). Bacterial communities in wet tropical soils are adapted to fluctuating redox potentials (Pett-Ridge and Firestone, 2005; DeAngelis et al., 2010), and in general low or fluctuating redox potentials should favor bacterial over fungal decomposers. Low O_2 availability has been demonstrated to inhibit white rot fungi (Basidiomycetes), which are generally thought to be intolerant of anaerobic conditions (Baldrian and Valášková, 2008; Rawls, 2009; Seo and DeLaune, 2010). However, anaerobic fungi have recently been observed in tropical forest soils (Štursová et al., 2012), and are active in lignocellulose degradation in rumen environments (Bauchop, 1979; Leschine, 1995; Brulc et al., 2009) though they make up only a small (1–2%) fraction of this bacterially-dominated community. Similar relative abundances have been observed in forest soil (Damon et al., 2012), with biomass measures showing fungal dominance over bacteria in most systems (though tropical soils were not included in this meta-analysis) (Joergensen and Wichern, 2008). There is also evidence that bacterial decomposers perform better in the presence of fungi (Romaní et al., 2006; Meidute et al., 2008).

In this study we tested the hypothesis that soil O_2 availability and associated redox conditions structure patterns in litter decomposition and related microbial community dynamics over space and time in humid tropical forests. We predicted that (1) decomposition would be slowest in sites with lower cumulative O_2 availability (as an index of redox conditions) according to the enzyme latch hypothesis, (2) that oxidative enzyme activity would be highest in the well-aerated sites, and (3) that bacteria would be dominant throughout decomposition relative to fungi. To evaluate our predictions, we measured indices of biochemical and microbiological succession during the decomposition of a common litter substrate across a range of climate and redox conditions in humid tropical forests. Changes in oxidative and hydrolytic enzyme activities were used as indices of microbial activity. We also measured changes in microbial community structure of litter and soil by sequencing the small subunit ribosomal RNA gene, and the use of universal primers permits identification of bacteria, archaea, fungi and non-fungal eukaryotes. Phylogenetic markers like 16S rRNA cannot explicitly inform functional roles of taxa in communities, but have functional coherence to phylogenetic groupings (Philippot et al., 2010). Soils were followed along with litter to characterize the larger microbial community pool associated with decomposition processes.

2. Methods

2.1. Site description

The study was conducted in the Luquillo Experimental Forest, Puerto Rico USA (18.3′ N, 65.80′ W), part of the NSF-sponsored Long Term Ecological Research Program. Four sites were chosen along a landscape-scale rainfall gradient to encompass a range in soil moisture and concomitant O_2 availability (Table 1, (Silver et al., 1999, 2013)). Mean annual precipitation ranged from 2900 mm y^{-1} to just under 5000 mm y^{-1} along the rainfall gradient (Table 1). Mean annual temperature differed only slightly from 19.5 to 21 °C with little within or across year variation (Brown and others, 1983; Silver et al., 2013). Soils are derived from volcanoclastic sediments,

Table 1
Site characteristics.

Site	Bisley ridge	Bisley valley	Colorado forest	Cloud forest
Short name	BisR	BisV	COL	SCF
Elevation (masl)	275	250	750	1050
MAP (mm)	2900	3500	4500	5000
Soil moisture (%)[a]	86 (2.8)	97 (4.0)	109 (4.3)	196 (9.6)
Litter moisture (%)	52 (3.2)	67 (3.9)	68 (3.5)	104 (5.1)
% Observations <3% soil O_2[b]	0.21	15	7	25
% Observations <10% soil O_2[b]	0.21	49	35	68
Soil O_2 concentration (%)[b]	19 (0.05)	10 (0.18)	13 (0.21)	8 (0.19)
Final mass remaining (%)[a]	27.8 (4.9)	21.0 (3.8)	24.7 (8.4)	47.7 (5.0)
k (y^{-1})	0.600	0.728	0.952	0.374
Adjusted R value	0.433	0.771	0.804	0.587
$t_{1/2}$ (y)	1.13	0.95	0.73	1.85

[a] Data presented are mean (standard error).
[b] "Obs.", observations; from Silver et al. (1999).

are clay-rich, and classified as Ultisols (Scatena, 1989; Silver et al., 2013). Several factors in addition to soil O_2 availability and rainfall varied across the study sites, *e.g.* plant community composition, NPP (Weaver and Murphy, 1990; Weaver, 1994) and could impact patterns in decomposition and microbial community dynamics. These factors can also affect and be affected by redox potential. The goals of this study were to determine if we could identify patterns in decomposition and associated microbial community dynamics using redox environment as an organizing principle. The short cloud forest (SCF) site was located at approximately 1027 m above sea level (18°18′ N, 65°50′ W) and experienced a high frequency of low soil O_2 availability (≤5% soil O_2 (Silver et al., 2013)). The Colorado forest (COL) site was located in a subtropical lower montane wet forest approximately 750 m above sea level (18° 30'N, 65° 80'W) and experienced intermediate redox conditions (10–15% average soil O_2). The lower elevation sites in the Bisley watershed (ridge (BisR) and valley (BisV)) were located in a lower montane wet tropical forest at approximately 270 and 250 m above sea level, respectively (18°18′ N, 65°45′ W) (Scatena, 1989). The soils at this site fluctuate between anoxic and oxic (Silver et al., 1999, 2013; Liptzin et al., 2011), with greater amplitude fluctuations in the valleys and generally well-aerated soils on the ridges (Silver et al., 1999).

2.2. Experimental design

We used a full factorial design with six replicate litter bags each for six collection periods at each site over approximately 23 months (n of 144 litter samples, with 144 soil samples collected adjacent to the litter at the same time). Litter bags were arrayed six to a post with each post representing a complete decomposition sequence. Replicates ($n = 6$) for each time point were collected as single bags from each of six posts per site. Posts were randomly located and were approximately 2–3 m apart. We used oven-dried switchgrass (*Panicum virgatum* L.) as a common substrate litter because it is not native to any of the forest sites studied (thus there was no site bias); it has also been well studied as a biofuels feedstock (Perlack, 2005) and model feedstock used for optimization of lignocellulose deconstruction for biofuels development (Blanch et al., 2008; Schmer et al., 2008). Litter bags contained approximately 4 g dry weight of switchgrass, cut into 2 cm lengths consisting of equal parts leaves and stems. Litter bags were constructed of 2 mm mesh and measured 10 cm by 12 cm. To assure good contact with soil, bags were placed within the top 10 cm of soil, in pairs, one for microbial community analysis and one for decomposition analysis; each pair was considered one sample ($n = 144$). At each collection period, adjacent soil cores (0–10 cm depth) were sampled within two centimeters of the litter bag. The litter bags were placed in the field on June 17, 2008, and the time points were as follows: T1 June 23, 2008 (1 week), T2 July 22, 2008 (4 weeks), T3 September 14, 2008 (13 weeks), T4 January 12, 2009 (30 weeks), T5 August 3, 2009 (60 weeks), T6 April 13 2010 (96 weeks). At each collection point, plant material and soils were shipped overnight in coolers at ambient temperature to U. C. Berkeley for analysis. Within 48 h of sampling, enzyme activities were obtained and subsamples were preserved by freezing at −80 °C.

2.3. Decomposition rates and litter C and N

Litter samples were dried at 55 °C until mass was constant. Decomposition rates were calculated as the exponential loss of mass over time as $k = [\ln(C_o/C_t)]/t$, where k is the decay constant, C_o is the original mass, C_t is the amount of mass remaining, and t is the time (Wieder and Lang, 1982). Mass of litter remaining was measured by dry weights of ash-free mass. Ash content was measured as differential mass after combustion in a muffle furnace at 500 °C. A subsample of dried litter was ground to 0.44 mm using a Wiley mill and analyzed for total N and total C on a CE Elantech CN analyzer (Lakewood, New Jersey) using alanine as a standard.

2.4. Enzyme assays

Potential hydrolytic and oxidative enzyme activities were assayed on soil and litter at each of the six time points based on previously published protocols (Sinsabaugh et al., 2008; DeForest, 2009; DeAngelis et al., 2011). Approximately 1 g soil or litter was added to 50 mM acetate buffer solution pH 5.5, mixed by stirring for 2 min, then the buffer extract was analyzed for enzyme activity at 27 °C. We performed oxidative enzyme assays using a colorimetric method for phenol oxidase (EC 1.10.3.2) L-dihydroxyphenylalanine (L-DOPA), and peroxidase (EC 1.11.1.7) DOPA plus 0.3% H_2O_2. Data collected as absorbance units 460 nm and were converted to mM L-DOPA based on the published extinction coefficient of 3388 (Mason, 1948; Waite, 1976). We also performed enzyme assays using the fluorogenic detection molecule methylumbylliferyl (MUB): MUB-beta-d-glucopyranoside for beta-glucosidase (EC 3.2.1.21); MUB-cellobioside for cellobiohydrolase (EC 3.2.1.91); MUB-beta-xylopyranoside for beta-xylosidase (EC 3.2.1.37); MUB-N-acetyl-beta-glucosaminidase for chitinase (EC 3.2.1.30). Plates were read at excitation 365 nm and emission 442 nm. Rates are reported as mean and standard error of six biological replicates of amount of substrate evolved per unit time per gram dry soil or litter.

2.5. Extraction of nucleic acids

Decomposing plant litter and soils were ground frozen and homogenized using a TissueLyser (Qiagen) in stainless steel grinding jars twice at 27 Hz for one minute each, re-freezing in liquid nitrogen in between beats. We then extracted with bead beating (FastPrep) using CTAB extraction buffer and phenol, followed by a chloroform extraction and isopropanol precipitate and finally the AllPrep DNA/RNA extraction kit (Qiagen), as previously described (DeAngelis et al., 2010). Briefly, frozen samples were added to CTAB extraction buffer, phenol, and aluminum ammonium sulfate in Lysing Matrix E tubes (Qbiogene), bead beaten in a FastPrep instrument (Bio101), followed by a chloroform extraction, isopropanol precipitate, and the AllPrep DNA/RNA extraction kit (Qiagen). DNA yields were calculated by measuring absorbance at 260 nm using a Nanodrop (Thermo Scientific, Waltham, Ma).

2.6. Amplicon pyrosequencing

The full factorial experiment based on a subset of four biological replicates was sequenced for small subunit (SSU) rRNA genes using high-throughput amplicon pyrosequencing. The universal primers 926F (5′-aaactYaaaKgaattgacgg-3′) and 1391R (5′-acgggcggtgtgtRc-3′) were used to amplify the V8 variable region of the small subunit rRNA gene from bacteria, archaea and eukarya (Engelbrektson et al., 2010). Emulsion PCR and sequencing of the PCR amplicons was performed following manufacturer's instructions for the Roche 454 GS FLX Titanium technology, with the exception that the final dilution was 1e-8. Sequencing tags were analyzed using the software tool PyroTagger (http://pyrotagger.jgi-psf.org/), which filters by removing low-quality sequences from the set based on the qual file, trims using a 225 bp sequence length threshold, dereplicates, clusters at the OTU level based on 97% identity, then classifies (Kunin and Hugenholtz, 2010). Classification was based on the Greengenes database of ribosomal RNA genes (DeSantis et al., 2006) for bacterial and archaeal amplicons, and the SILVA database for eukaryotic amplicons (Pruesse et al., 2007). Because of incomplete classification in some sequences in these databases, not all sequences are classified to the species level. Sequences were submitted to the public repository MG-RAST under the ID 4519119.3.

2.7. Community analysis

Species accumulation curves were determined using a randomization approach, subsampling without replacement. Because richness estimates are skewed by total number of reads (Gihring et al., 2012), richness estimates are based on randomly rarefied communities, obtained using sampling without replacement to 1053 taxa (the smallest sample size). Normalization of library size reduces common errors associated with estimating species diversity (Gotelli and Colwell, 2001). Total richness was reported using Chao's estimator, which extrapolates total richness based on taxa detected and likely taxa undetected based on singletons and doubletons (Colwell and Coddington, 1994) Ordination of whole community detected by pyrosequencing was performed using Principle Co-ordinates Analysis based on phylogenetic distance. Phylogenetic trees were constructed in Qiime using FastTree (Price et al., 2009; Caporaso et al., 2010), and phylogenetic community analysis was performed using Fast UniFrac (Hamady et al., 2009). Bidirectional clustering was used to determine how the litter and soil samples clustered (in one direction) as well as the dominant taxa (in the other direction), where dominant taxa had abundance of 1% or greater in at least one sample in the set. Additionally, we determined taxa that were enriched in litter vs. soil samples, either by greater presence in litter samples (Table S1) or by elevated average abundance (Table 3). High values were arbitrarily set at a ratio of 1.5 for enriched presence and a difference of 1% for enriched average abundance.

2.8. Statistical analyses

The experimental design included six biological replicates, although four replicates were chosen for the sequencing efforts due to cost and difficulty; these were chosen randomly. We used analysis of variance (ANOVA) to determine the effect of site on experimental variables; significance was reported at $P < 0.05$ using Tukey's Honestly Significant Difference as a means separation test. When appropriate, *P*-values were corrected for repeated measures (Benjamini and Hochberg, 1995). Regression analysis was performed to determine the effect of time (in weeks) on experimental variables; Pearson's correlation coefficient *R* is reported at a

Table 2
Enzyme activities by site and time.

Factor:	Site		Time	
	P[a]	Trend	*P*[a]	Trend
Litter enzyme activities				
b-Glucosidase	n.s.		***	$R = 0.365$
Cellobiohydrolase	n.s.		***	$R = 0.393$
N-Acetylglucosaminidase	n.s.		.	
Xylosidase	n.s.		***	$R = 0.587$
Soil enzyme activities				
b-Glucosidase	***	SCF > Col, BisV, BisR	***	$R = 0.320$
Cellobiohydrolase	***	SCF > Col, BisV, BisR	**	$R = 0.259$
N-Acetylglucosaminidase	***	SCF > Col, BisV, BisR	*	$R = 0.212$
Xylosidase	***	SCF > Col, BisV, BisR	**	$R = 0.247$
Litter oxidative enzyme activities				
Phenol oxidase	*	SCF > BisV	***	$R = 0.430$
Peroxidase	n.s.		***	$R = 0.433$
Soil oxidative enzyme activities				
Phenol oxidase	n.s.		n.s.	
Peroxidase	n.s.		***	$R = 0.443$

[a] *P*-values are indicated by. $P < 0.10$,*$P < 0.05$,**$P < 0.01$,***$P < 0.001$, n.s., not significant.

Table 3
Litter-enriched dominant taxa for initial, early, and late-stage decomposition.

Abundance delta[a]	Domain[b]	Phylum (class)	Taxon ID	Best hit (% identity)
Initial (1 and 4 weeks)				
5.67	B	Gammaproteobacteria	Cluster18	(100%) *Enterobacter* sp. str. MB-2-2-4
4.66	B	Gammaproteobacteria	Cluster7	(100%) *Xanthomonas* sp. str. SPk
3.91	E	Stramenopiles (Oomycetes)	Cluster6	(98.33%) *Phytophthora megasperma*
2.94	B	Bacteroidetes (Sphingobacteria)	Cluster13	(98.89%) *Chitinophaga* sp. str. CC-SG1B
2.31	B	Gammaproteobacteria	Cluster35	(100%) *Pseudomonas fulva* str. PTPful-1
1.87	B	Gammaproteobacteria	Cluster62	(100%) *Klebsiella oxytoca* str. W-6
1.80	B	Gammaproteobacteria	Cluster33	(100%) *Salmonella* sp. str. DAP8
1.78	B	Alphaproteobacteria	Cluster46	(100%) *Novosphingobium* sp. str. CC-TPE-1
1.60	B	Betaproteobacteria	Cluster47	(100%) soil isolate Ellin321
1.26	B	Firmicutes (Clostridia)	Cluster32	(100%) *Clostridium* sp. str. UsS101-1
1.11	B	Firmicutes (Clostridia)	Cluster31	(100%) *Clostridium* sp. str. BL-22
1.08	B	Betaproteobacteria	Cluster58	(100%) *Comamonas* sp. str. SS-1
1.04	B	Alphaproteobacteria	Cluster157	(100%) *Rhizobium sullae* str. CCBAU 45230
1.02	E	Fungi (Dikarya)	Cluster8	(96.13%) *Cryptococcus gilvescens*
Early (13 and 30 weeks)				
3.71	E	Fungi (Dikarya)	Cluster8	(96.13%) *Cryptococcus gilvescens*
1.14	E	Fungi (Dikarya)	Cluster16	(99.44%) *Phialocephala sphaeroides*
Late (60 and 96 weeks)				
3.74	E	Fungi (Dikarya)	Cluster22	(100%) *Phyllachora graminis*
1.38	E	Viridiplantae (Streptophyta)	Cluster1	(100%) *Mitella integripetala*
1.00	B	Alphaproteobacteria	Cluster21	(100%) *Bradyrhizobium elkanii* str. CCBAU 33026

[a] Abundance delta is ratio of relative abundance in litter over soil.
[b] Domain (Dom. taxa is abbreviated as "B" for Bacteria and "E" for Eukaryotes; no Archaea were detected in this analysis.

significance level of $P < 0.05$. In most cases, data are presented as means plus or minus standard error; in others, boxplots display medians and ranges. Bidirectional clustering was performed using MeV (Saeed et al., 2006) using the covariance similarity measure and average linkage clustering, with ordering optimized for samples. All statistics were performed in *R* (R Development Core Team, 2010) using the packages vegan (Oksanen et al., 2011) and plotrix (Lemon et al., 2007).

3. Results

3.1. Litter decomposition

Rates of litter mass loss varied spatially and temporally. At all sites the litter decomposed rapidly in the beginning of the experiment, and slowed substantially by week 30 (Fig. 1A). Rates of mass loss were slowest in the lowest redox site (SCF) ($P < 0.05$). Half-lives for litter were estimated at 1.13 years for BisR, 0.95 years for BisV, 0.73 years for COL, and 1.85 years for SCF (Table 1). At the end of the experiment, the percent mass remaining varied from 21 to 28% in the BisR, BisV, and COL, to 48% in the SCF ($P < 0.05$). Carbon to nitrogen ratios (C:N) decreased significantly over time, and by the end of the experiment C:N ranged from 12.2 to 28.0 (Fig. 1B). The final C:N ratios were highest in the SCF site, followed by Col, BisV then BisR sites; this was due to both N depletion from litter (for total N, SCF > BisV, BisR > COL, $P < 0.001$), as well as more complete C loss in the drier sites (for total C, SCF > COL, BisV, BisR, $P < 0.001$). There was a significant linear trend in total litter C over time ($R = -0.371$, $P < 0.001$) as well as mass ($R = -0.658$, $P < 0.001$) and C:N ratio ($R = -0.499$, $P < 0.001$), but no trend in total N over time.

3.2. Enzyme activities

Potential phenol oxidase activity in litter was significantly higher for SCF compared to BisV (Table 2, $P < 0.05$), and overall potential soil hydrolytic enzyme activities were also significantly higher in the SCF site compared to the other sites (Table 2, $P < 0.001$). Measurements in potential enzyme activities suggested that over the course of litter decomposition, there was a prevalence of oxidative activities earlier (phenol oxidase and peroxidase) and hydrolytic activities later (β-galactosidase, cellobiohydrolase, N-acetylglucosaminidase and xylosidase). Hydrolytic enzyme activities peaked around 30 weeks then declined slightly (Fig. S1), while oxidative enzyme activities were elevated by 13 weeks and remained high until the end of the experiment at 96 weeks (Fig. S2). The temporal trend was also observed in community analysis, but enzyme activities showed little predictive power for patterns in decomposition by site.

3.3. Microbial community analysis

Pyrosequencing detected 40,850 taxa that included 37,871 bacteria, 588 archaea, 831 fungi and 1560 non-fungal eukaryotes. Species accumulation curves showed that although the number of eukaryotic, fungal and archaeal sequences observed were an order of magnitude fewer than those for bacteria, the archaeal communities were the most completely sampled with this primer set, followed by the fungal and then the eukaryotic communities. The bacterial communities were the least well sampled as evidenced by the steepest accumulation curves (Fig. S3).

To examine differences in alpha diversity by site, we examined both total richness (by observed taxa only) and estimated richness (by Chao's estimator, which calculates predicted under-sampling based on taxa whose sequences are only detected once or twice). There was no change in total soil community richness over time, and at the beginning of the experiment, litter samples were much less rich compared to soil. This difference was diminished by the end of the experiment, due to a significant increase in litter community richness between T3 and T4 ($P < 0.001$; Fig. 2A). Sites did not differ significantly in total richness of soil or litter communities (Fig. 2B). Chao's richness estimated $4–7 \times 10^3$ different species, with higher estimated soil richness in the lower redox sites from the upper elevation compared to the BisV and BisR sites.

Over time, patterns of bacterial community dynamics in soil and litter converged, but patterns of fungal communities did not. During the early-stages of decomposition, fungal communities were similar in soil and litter by our first sampling after 1 week, then began to diverge after 30 weeks (Fig. 3A). In contrast, bacterial litter communities differed strongly from soil but were indistinguishable after 30 weeks (Fig. 3B). While effects of time and site were both significant for bacterial and fungal beta diversity, there were no significant trends for the archaeal or non-fungal eukaryotic communities (Fig. S4). Bacterial communities were grouped for sites that experience the highest cumulative low redox events (SCF & BisV) in contrast to the more aerobic sites (Col & BisR), whereas for the fungal communities higher rainfall sites grouped together (SCF & Col) in contrast to the drier sites (BisV & BisR).

Dominant taxa, defined as having at least 1% relative abundance within a given sample, were examined to identify patterns in relative abundance on litter and soil. Dominant taxa consisted mostly of bacteria and a few mostly fungal eukaryotes. Bidirectional

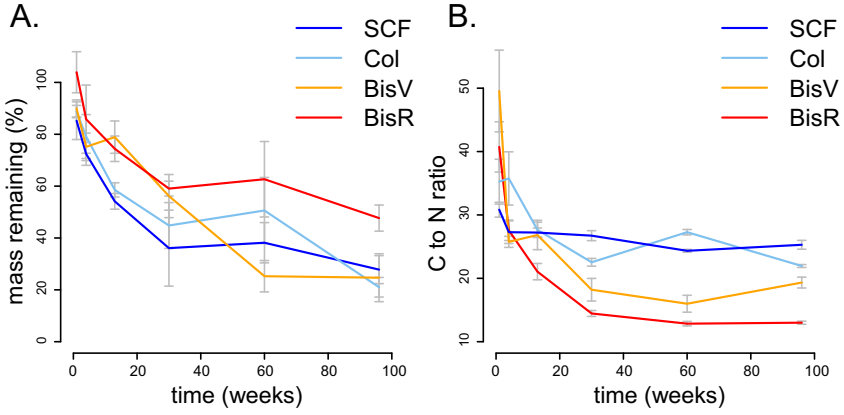

Fig. 1. (A) Mass loss decomposition of litter is shown for the course of the 96-week experiment. (B) Carbon to nitrogen ratios for litter collected at each site over the course of the experiment. Means of six biological replicates are reported with standard error bars displayed per site per time point ($n = 6$).

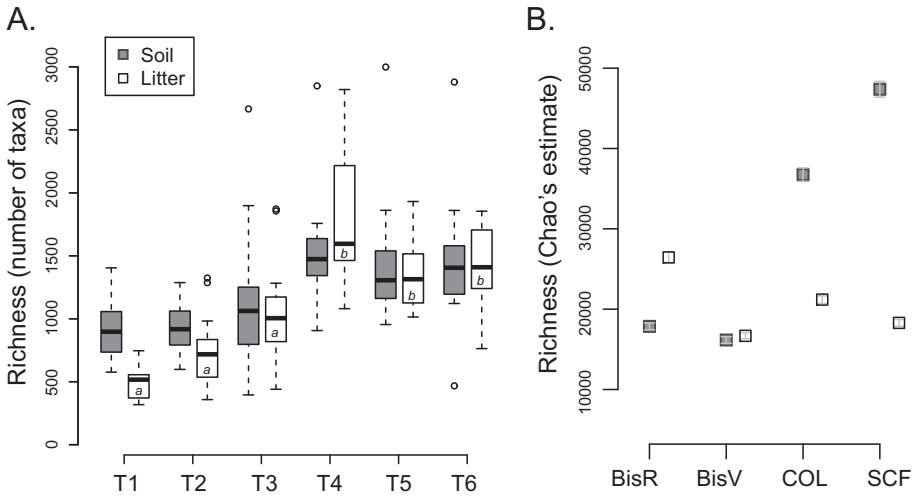

Fig. 2. (A) Total richness was calculated based on all taxa detected over time for all sites. Box plots denote medians and range, and lower-case letters display significant differences between time points. (B) Richness estimates are shown for soil and litter samples across all time points using Chao's estimator, which considers singletons and doubletons in estimates in rarefied communities. For samples from each site, mean estimated richness is shown plus or minus standard error ($n = 24$).

clustering based on differences in presence-absence revealed strong separation between soil and litter populations during initial decomposition (1–4 weeks), that declined by the early-stage (13–30 weeks) and were virtually indistinguishable by late-stage decomposition (60–96 weeks). Two groups of taxa were initially enriched on litter compared to soil: one group enriched on litter in all sites, and one group enriched on litter in the lowest redox sites only (SCF and BisV). The litter-enriched dominant taxa common to all sites included *Bacteroidetes*, α-, β-, and γ-*Proteobacteria*, while the litter-enriched dominant taxa in the low redox sites (SCF & BisV) included *Acidobacteria*, *Spirochaetes* and the eukaryotes *Stramenopiles* (*Phytopthora* sp.) and *Alveolata* (*Tetrahymena* sp.). Presence-absence analysis of dominant taxa confirmed the prevalence of classical fast-growing bacteria on litter during initial decomposition (Table 3). By 13–30 weeks, there was less separation between litter and soil, and the fungal *Dikarya* and eukaryotic *Metazoa* were among the taxa enriched. By late-stage decomposition (60 and 96 weeks), there were few dominant taxa. These included *Clostridia* (phylum *Firmicutes*) and the archaeon *Methanobrevibacter*, which were enriched in the BisV and SCF litter compared to soils (Fig. S5, Table S1). The presence of a Bradyrhizobium OTU that was present in litter but not detected in soil at late-stage decomposition suggests a role for this bacteria as a specialist decomposer or other K-strategist bacteria (Table 3). We also detected evidence of anaerobic fungi enriched on litter, includin. *Cryptoccous* (yeast) in initial and early-stage decomposition, as well as the known plant-associating fungi *Phialocephala* and *Phyllachora* in early and late decomposition, respectively. Among all dominant taxa, there was an evident succession over the 96 weeks at all sites, where the trend emerged as a transition from habitat (litter versus soil) to site as the dominant driver of community structure.

4. Discussion

4.1. Redox and soil moisture control on decomposition

This study was designed to test the hypothesis that soil O_2 availability structures patterns in litter decomposition and associated microbial community dynamics in humid tropical forests. We predicted that sites with lower cumulative soil O_2 availability would have slower decomposition rates. As expected, the SCF site had both the slowest decomposition rates and the most mass remaining at the end of the two-year experiment. However, we did not see strong patterns among the other sites even though they differed in the average soil O_2 concentrations as well as patterns in O_2 availability over time. This may be due to the influence of other site factors on decomposition (*e.g.* soil chemistry, neighboring litter quality and quantity). The rapid decomposition rate in COL and BisV sites was particularly surprising given the relatively high proportion of low redox events in these ecosystems.

One potential explanation for rapid rates of decomposition under fluctuating redox regimes could be iron (Fe) cycling, where dissimilatory iron reduction fuels microbial activity and organic C degradation, with redox potential fluctuations to aerobic conditions abiotically re-oxidize the iron, regenerating the electron acceptors (Lovley et al., 2004; Weber et al., 2006). Soils at the field sites were rich in poorly crystalline iron (Fe) minerals (Chacon et al., 2006), typical of many highly weathered tropical soils (Sanchez, 1976). Frequent periods of low redox potential could facilitate Fe(III) reduction coupled to C oxidation, with periodic oxic events regenerating the reductant (Dubinsky et al., 2010; Liptzin et al., 2011; Hall and Silver, 2013). Iron oxidation can also stimulate decomposition via hydroxyl radical and super oxides in tropical forest soils (Hall and Silver, 2013). Fluctuating redox, and Fe redox cycling in particular, thus have the potential to fuel considerable C cycling in wet tropical ecosystems.

Patterns in litter C:N ratio followed the moisture and soil redox gradient and was somewhat decoupled from patterns in mass loss. The wide range in C:N ratios at the end of the experiment may reflect the differences in the microbial capacities during decomposition, as patterns in N and C loss differed among the sites. Taken together, these data provide further support for the hypothesis that dissimilatory iron reduction or other anaerobic metabolisms could support carbon mineralization rates in wet tropical forest soils that are as high as those for aerobic, temperate soil systems (DeAngelis et al., 2010; Dubinsky et al., 2010).

4.2. Redox and moisture control on enzyme activity

Enzyme activity turned out to be a reasonable predictor of microbial succession over the course of decomposition, though the potential rates measured were highly variable. Oxidative capacity peaked earlier than hydrolytic enzymes, reflective of the observed

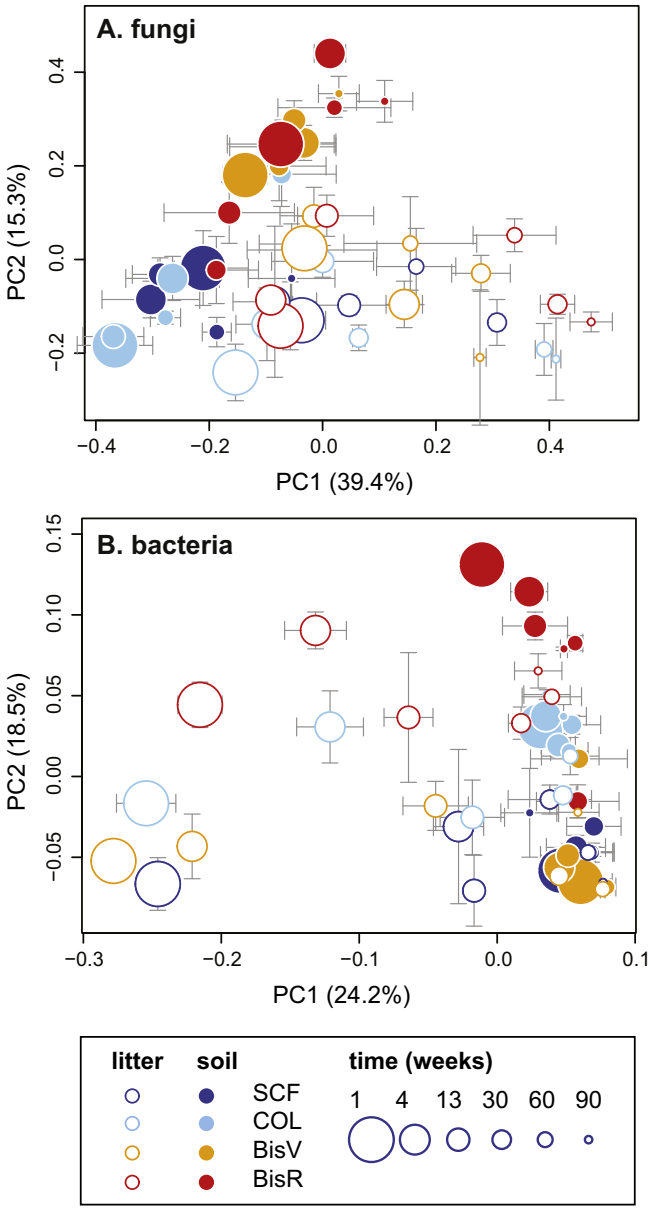

Fig. 3. Microbial community profiles are shown for fungi (A) and bacteria (B), where each point is the average and gray bars denote standard error ($n = 4$). Profiles are based on ordination by principle coordinates analysis of phylogenetic distances (calculated using UniFrac distance) and weighted by taxa abundance. Symbols are for soil (filled circles) and litter (open circles), where the progress of time is depicted by circle size (T1 samples are the largest circles, and get progressively smaller over time). Sites are coded by color: Short Cloud Forest (SCF, dark blue), Colorado forest (Col, light blue), Bisley watershed valley site (BisV, orange), and Bisley watershed ridge site (BisR, red). The amount of variance in the data that the model explains is reported for each principle coordinate (axis), as a percentage (%) of the total variance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

early colonization of litter by fungi. Phenol oxidase and peroxidase enzymes are not as stable as hydrolytic enzymes (Sinsabaugh, 2010), so presence of oxidative enzyme activity is more likely to reflect recent environmental conditions compared to hydrolytic potential activities. Enzyme activity turned out to be a poor predictor of redox effect on decomposition across sites, possibly because of abiotic C oxidation coupled to Fe oxidation as well as the fact that measurements from all sites were of potential activities, made under similar controlled laboratory conditions. Oxidative enzyme activities and Fe-coupled abiotic oxidative capacity require molecular oxygen for decomposition of organic carbon (Zibilske and Bradford, 2007; Hall and Silver, 2013). That there was so much more potential oxidative activity in the most anaerobic site was likely due to higher levels of enzyme abundances, to account for the lower frequency with which they have an opportunity to be active.

4.3. Patterns in microbial community succession during decomposition

Measures of alpha diversity over time revealed that soils were initially much richer than litter, and that after 30 weeks richness was similar. Measures of dominance in litter also declined over time, whether calculated by changing relative abundance (Table 3, Fig. S5) or changing presence-absence (Table S1). Litter in all sites was rapidly colonized by taxa that have been classified as typical of fast-growing r-strategists (Stevenson and Schmidt, 2004; Fierer et al., 2007), which are known to respond quickly to labile, soluble C inputs in soil (Cleveland et al., 2007; España et al., 2011). This is consistent with other observations of α-, β-, and γ-Proteobacteria as successful initial colonizers of litter (Aneja et al., 2006; Bastian et al., 2009), and high ribosomal RNA operon copy number tends to be well-conserved among the Proteobacteria and Firmicutes (Rastogi et al., 2009; Kembel et al., 2012). Gram-positive bacteria have been shown to be able to out-compete fungi as soon as nitrogen becomes limiting (Esperschütz et al., 2011). It seems likely that N became limiting between 13 and 30 weeks, since this is when the litter communities switched from fungal to bacterially-dominated. There is considerable overlap between the r-strategist bacteria and the bacterial groups that have known Fe-reducing capabilities, which include many members of the Proteobacteria and Firmicutes (Lovley et al., 2004; Weber et al., 2006). Though we did not measure iron reduction in this study, these and prior data support a hypothesis that iron reduction fuels considerable carbon mineralization in wet tropical soils (Chacon et al., 2006; Dubinsky et al., 2010).

The fungi that were significantly enriched on litter after nearly 2 years decomposition were phylogenetically distinct from taxa identified as important to decomposition in temperate systems. Metaproteome data from decomposing litters of different nutrient contents in a temperate forest identified only fungal hydrolases though bacteria were otherwise detected (Schneider et al., 2012). A species of *Cryptomycota* was detected as a dominant community member and significantly enriched in the litter during both initial and early decomposition. In a separate study, stable isotope probing using ^{13}C-cellulose turned up a variety of fungi in addition to bacteria, including the Cryptomycetes, suggesting that anaerobic fungi may play a role in below-ground primary decomposition (Šťursová et al., 2012; Eichorst and Kuske, 2012). The litter-enrichment of the fungi *Phialocephala* and *Phyllachora* also suggests that plant-associated fungi may be contributing nutrients to late-stage decomposers (Seaver, 1928; Jones et al., 2011). While the non-fungal eukaryotes *Tetrahymena* and *Phytopthora* are best known as pathogens, they likely became enriched on litter as a direct or indirect beneficiary of the litter nutrients released from the primary decomposers. Though studies that used high-resolution sequencing to understanding the populations involved in decomposition in tropical soils have so far focused on bacteria (Cleveland et al., 2007; Leff et al., 2012), we know that fungi play a critical role in litter decomposition aboveground (Lodge and Cantrell, 1995). Our work suggests an important role for anaerobic fungi in below-ground decomposition in tropical soils during initial and early-stage decomposition.

We observed two consistent statistically significant patterns in microbial community composition, where either the higher

elevation sites (SCF and Col) grouped separately from the lower elevation sites (BisV and BisR), or the lower redox sites (SCF and BisV) grouped separately from the drier sites with higher average soil oxygen concentration (COL and BisR). The beta diversity of bacterial and fungal communities are a reflection of the sum environmental pressures on each group, where fungal community profiles clustered by sites that differed by elevation and rainfall, while bacterial community profiles clustered by sites that differed by aspect and soil redox potential. The fungi were likely strongly driven by edaphic effects, though the proximity of the BisV and BisR sites were reflected in their similar profiles. Bacterial beta diversity was likely strongly driven by redox potential, where the two lower redox sites saw many more frequent observations of less than 3% or less than 10% soil O_2 compared to other sites (Table 1; (Silver et al., 1999)). Further research will determine how two types of environmental stresses divide our observations into those more strongly influenced by immediate and frequently fluctuating environmental conditions, such as soil redox, and differentiate these from those influenced by more long-term, stable underlying conditions like soil minerals, total precipitation at an annual scale, and others.

5. Conclusions

Fast rates of decomposition in low and fluctuating redox conditions of wet tropical forest soils were observed across a landscape-scale rainfall (elevational) gradient, and revealed that the site with the consistently lowest redox had slower and the most mass remaining in tropical forest soils after two years. Litter decomposition at sites that experience fluctuating redox was as fast as at the oxic site, suggesting a role for iron cycling in expediting litter decomposition under fluctuating redox conditions. Potential enzyme activities were highest in the high elevation site, potentially due to increased enzyme abundances to compensate for the lower frequency of conditions permissible for enzyme activity. Finally, microbial community analysis by the use of high-throughput sequencing revealed a succession of microbial decomposers that decreased significantly in richness over time. Initial decomposition was dominated by fast-growing bacteria, with a large degree of phylogenetic overlap with known iron reducing groups. Early and late-stage decomposition had communities with fewer dominant taxa overall, and litter enriched with anaerobic fungi and non-fungal eukaryotes. This work suggests the importance of r-selected and likely iron-reducing bacteria as well as anaerobic fungi in catalyzing fast rates of decomposition in wet tropical forest soils.

Acknowledgments

This work was conducted by the Joint BioEnergy Institute and was supported by the Office of Science, Office of Biological and Environmental Research, of the U. S. Department of Energy under Contract No. DE-AC02-05CH11231. The research was also partially supported by Contract No. DEB-0620910 from NSF to the Institute of Tropical Ecosystem Studies, University of Puerto Rico, and the International Institute of Tropical Forestry (IITF) as part of the Luquillo LTER program, and by NSF Grants EAR-08199072 and DEB-0842385 to WLS, and the NSF Luquillo Critical Zone Observatory (EAR-0722476). We would also like to thank Gail Hazen for helping with materials construction, and Dr. Ken Vogel of the USDA for switchgrass used in this study. Susannah Tringe, Tijana Glavina Del Rio, and Stephanie Malfatti of the Joint Genome Institute are acknowledged for their assistance in obtaining pyrosequencing data, which was conducted by the Joint Genome Institute and is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.06.010.

References

Aneja, M., Sharma, S., Fleischmann, F., Stich, S., Heller, W., Bahnweg, G., Munch, J., Schloter, M., 2006. Microbial colonization of beech and spruce litter—influence of decomposition site and plant litter species on the diversity of microbial community. Microbial Ecology 52, 127–135.

Baldrian, P., Valášková, V., 2008. Degradation of cellulose by basidiomycetous fungi. FEMS Microbiology Reviews 32, 501–521.

Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biology and Biochemistry 41, 262–275.

Bauchop, T., 1979. Rumen anaerobic fungi of cattle and sheep. Applied and Environmental Microbiology 38, 148–158.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 289–300.

Blanch, H.W., Adams, P.D., Andrews-Cramer, K.M., Frommer, W.B., Simmons, B.A., Keasling, J.D., 2008. Addressing the need for alternative transportation fuels: the Joint BioEnergy Institute. ACS Chemical Biology 3, 17–20.

Bragazza, L., Freeman, C., 2007. High nitrogen availability reduces polyphenol content in sphagnum peat. Science of The Total Environment 377, 439–443.

Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, N., Ellis, T., Gerdol, R., Hájek, M., Hájek, T., Iacumin, P., Kutnar, L., Tahvanainen, T., Toberman, H., 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. Proceedings of the National Academy of Sciences 103, 19386–19389.

Bridgham, S.D., Updegraff, K., Pastor, J., 1998. Carbon, nitrogen, and phosphorous mineralization in northern wetlands. Ecology 79, 1545–1561.

Brown, S.K., others, 1983. Research History and Opportunities in the Luquillo Experimental Forest. US Dept. of Agriculture, Forest Service, Southern Forest Experiment Station.

Brulc, J.M., Antonopoulos, D.A., Miller, M.E.B., Wilson, M.K., Yannarell, A.C., Dinsdale, E.A., Edwards, R.E., Frank, E.D., Emerson, J.B., Wacklin, P., Coutinho, P.M., Henrissat, B., Nelson, K.E., White, B.A., 2009. Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proceedings of the National Academy of Sciences 106, 1948–1953.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Chacon, N., Silver, W.L., Dubinsky, E.A., Cusack, D.F., 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.

Cleveland, C., Nemergut, D., Schmidt, S., Townsend, A., 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82, 229–240.

Colwell, R.K., Coddington, J.A., 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 345, 101–118.

Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiological Reviews 60, 609–640.

Cusack, D.F., Chou, W.W., Yang, W.H., Harmon, M.E., Silver, W.L., 2009. Controls on long-term root and leaf litter decomposition in neotropical forests. Global Change Biology 15, 1339–1355.

Damon, C., Lehembre, F., Oger-Desfeux, C., Luis, P., Ranger, J., Fraissinet-Tachet, L., Marmeisse, R., 2012. Metatranscriptomics reveals the diversity of genes expressed by Eukaryotes in forest soils. PloS One 7, e28967.

DeAngelis, K.M., Silver, W.L., Thompson, A.W., Firestone, M.K., 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology 12, 3137–3149.

DeAngelis, K.M., Allgaier, M., Chavarria, Y., Fortney, J.L., Hugenholtz, P., Simmons, B., Sublette, K., Silver, W.L., Hazen, T.C., 2011. Characterization of trapped lignin-degrading microbes in tropical forest soil. PloS One 6, e19306.

DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. Soil Biology and Biochemistry 41, 1180–1186.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72, 5069–5072.

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

Eichorst, S.A., Kuske, C.R., 2012. Identification of cellulose-responsive bacterial and fungal communities in geographically and edaphically different soils by using stable isotope probing. Applied and Environmental Microbiology 78, 2316–2327.

Engelbrektson, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman, H., Hugenholtz, P., 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. The ISME Journal 4, 642–647.
España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G.D., Cadisch, G., 2011. Identification of active bacteria involved in decomposition of complex maize and soybean residues in a tropical vertisol using 15N-DNA stable isotope probing. Pedobiologia 54, 187–193.
Esperschütz, J., Welzl, G., Schreiner, K., Buegger, F., Munch, J.C., Schloter, M., 2011. Incorporation of carbon from decomposing litter of two pioneer plant species into microbial communities of the detritusphere. FEMS Microbiology Letters 320, 48–55.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Freeman, C., Ostle, N., Kang, H., 2001. An enzymic "latch" on a global carbon store. Nature 409, 149.
Gihring, T.M., Green, S.J., Schadt, C.W., 2012. Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. Environmental Microbiology 14, 285–290.
Gotelli, N.J., Colwell, R.K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4, 379–391.
Hall, S.J., Silver, W.L., 2013. Iron oxidation stimulates organic matter decomposition in humid tropical forest soils. Global Change Biology. http://dx.doi.org/10.1111/gcb.1222.
Hamady, M., Lozupone, C., Knight, R., 2009. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. The ISME Journal 4, 17–27.
Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology and Biochemistry 40, 2977–2991.
Jones, M.D.M., Forn, I., Gadelha, C., Egan, M.J., Bass, D., Massana, R., Richards, T.A., 2011. Discovery of novel intermediate forms redefines the fungal tree of life. Nature 474, 200–U234.
Kembel, S.W., Wu, M., Eisen, J.A., Green, J.L., 2012. Incorporating 16S gene copy number information improves estimates of microbial diversity and abundance. PLoS Comput Biol 8, e1002743.
Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139–162.
Kunin, V., Hugenholtz, P., 2010. PyroTagger: a fast, accurate pipeline for analysis of rRNA amplicon pyrosequence data. The Open Journal 1, 1.
Leff, J., Nemergut, D., Grandy, A., O'Neill, S., Wickings, K., Townsend, A., Cleveland, C., 2012. The effects of soil bacterial community structure on decomposition in a tropical rain forest. Ecosystems 15, 284–298.
Lemon, J., Bolker, B., Oom, S., Klein, E., Rowlingson, B., Wickham, H., Tyagi, A., Eterradossi, O., Grothendieck, G., Toews, M., others, 2007. Plotrix: Various Plotting Functions. URL http://cran.r-project. org/src/contrib/Descriptions/plotrix.html.
Leschine, S.B., 1995. Cellulose degradation in anaerobic environments. Annual Reviews in Microbiology 49, 399–426.
Liptzin, D., Silver, W.L., Detto, M., 2011. Temporal dynamics in soil oxygen and greenhouse gases in two humid tropical forests. Ecosystems, 1–12.
Lodge, D.J., Cantrell, S., 1995. Fungal communities in wet tropical forests: variation in time and space. Canadian Journal of Botany 73, 1391–1398.
Lovley, D.R., Holmes, D.E., Nevin, K.P., 2004. Dissimilatory Fe (iii) and Mn (iv) reduction. Advances in Microbial Physiology 49, 219–286.
Marschner, P., Umar, S., Baumann, K., 2011. The microbial community composition changes rapidly in the early stages of decomposition of wheat residue. Soil Biology and Biochemistry 43, 445–451.
Mason, H.S., 1948. The chemistry of melanin; mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase. The Journal of Biological Chemistry 172, 83.
Meidute, S., Demoling, F., Bååth, E., 2008. Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. Soil Biology and Biochemistry 40, 2334–2343.
Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76, 151–174.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. Vegan: Community Ecology Package.
Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173–1179.
Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C., others, 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315, 361.
Perlack, R.D., 2005. Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-ton Annual Supply. DTIC Document.
Pett-Ridge, J., Firestone, M., 2005. Redox fluctuation structures microbial communities in a wet tropical soil. Applied and Environmental Microbiology 71, 6998.
Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nature Reviews Microbiology 8, 523–529.
Price, M.N., Dehal, P.S., Arkin, A.P., 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Molecular Biology and Evolution 26, 1641–1650.
Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research 35, 7188–7196.
R Development Core Team, 2010. R: a Language and Environment for Statistical Computing, ISBN 3-900051-07-0.
Rastogi, R., Wu, M., DasGupta, I., Fox, G., 2009. Visualization of Ribosomal RNA Operon Copy Number Distribution.
Rawls, M., 2009. An Evaluation of Bacterial and Fungal Contributions to Organic Matter Decomposition along a Soil Moisture Gradient.
Reed, S.C., Cleveland, C.C., Townsend, A.R., 2007. Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. Biotropica 39, 585–592.
Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87, 2559–2569.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340–1351.
Saeed, A.I., Bhagabati, N.K., Braisted, J.C., Liang, W., Sharov, V., Howe, E.A., Li, J., Thiagarajan, M., White, J.A., Quackenbush, J., 2006. TM4 microarray software suite. Methods in Enzymology 411, 134–193.
Sanchez, P.A., 1976. Properties and Management of Soils in the Tropics, p. 629.
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.
Schmer, M., Vogel, K.P., Mitchell, R.B., Perrin, R.K., 2008. Net energy of cellulosic ethanol from switchgrass. Proceedings of the National Academy of Sciences 105, 464.
Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. The ISME Journal 6, 1749–1762.
Schuur, E., Matson, P., 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. Oecologia 128, 431–442.
Seaver, F.J., 1928. Studies in tropical Ascomycetes: V. species of Phyllachora. Mycologia 20, 214–225.
Seo, D.C., DeLaune, R.D., 2010. Effect of redox conditions on bacterial and fungal biomass and carbon dioxide production in Louisiana coastal swamp forest sediment. Science of The Total Environment 408, 3623–3631.
Silver, W.L., Lugo, A., Keller, M., 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44, 301–328.
Silver, W.L., Liptzin, D., Almaraz, M., 2013. Soil redox dynamics and biogeochemistry along a tropical elevational gradient. Ecological Bulletins (in press).
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404.
Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264.
Stemmer, M., Watzinger, A., Blochberger, K., Haberhauer, G., Gerzabek, M.H., 2007. Linking dynamics of soil microbial phospholipid fatty acids to carbon mineralization in a ^{13}C natural abundance experiment: impact of heavy metals and acid rain. Soil Biology and Biochemistry 39, 3177–3186.
Stevenson, B.S., Schmidt, T.M., 2004. Life history implications of rRNA gene copy number in *Escherichia coli*. Applied and Environmental Microbiology 70, 6670–6677.
Strickland, M.S., Wickings, K., Bradford, M.A., 2012. The fate of glucose, a low molecular weight compound of root exudates, in the belowground foodweb of forests and pastures. Soil Biology and Biochemistry 49, 23–29.
Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. University of California Press.
Vitousek, P.M., Matson, P.A., 1988. Nitrogen transformations in a range of tropical forest soils. Soil Biology and Biochemistry 20, 361–367.
Vitousek, P.M., Sanford, R.L.J., 1986. Nutrient cycling in moist tropical forest. Annual Review of Ecology and Systematics 17, 137–167.
Waite, J.H., 1976. Calculating extinction coefficients for enzymatically produced *o*-quinones. Analytical Biochemistry 75, 211–218.
Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry 36, 1443–1451.
Weaver, P.L., 1994. Bano de Oro Natural Area Luquillo Mountains. USDA. Forest Service. Southern Forest Experiment Station, Puerto Rico.
Weaver, P.L., Murphy, P.G., 1990. Forest structure and productivity in Puerto Rico's Luquillo Mountains. Biotropica, 69–82.
Weber, K.A., Achenbach, L.A., Coates, J.D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. Nature Reviews Microbiology 4, 752–764.
Wieder, R.K., Lang, G.E., 1982. A critique of the analytical methods used in examining decomposition data obtained from litter bags. Ecology 63, 1636–1642.
Wieder, W.R., Cleveland, C.C., Townsend, A.R., 2009. Controls over leaf litter decomposition in wet tropical forests. Ecology 90, 3333–3341.
Zibilske, L.M., Bradford, J.M., 2007. Oxygen effects on carbon, polyphenols, and nitrogen mineralization potential in soil. Soil Science Society of America Journal 71, 133.
Šturšová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiology Ecology 80, 735–746.