Ecological Bulletins 54: 87–100, 2013

Differential abundance of microbial functional groups along the elevation gradient from the coast to the Luquillo Mountains

Sharon A. Cantrell, D. Jean Lodge, Carlos A. Cruz, Luis M. García, José R. Pérez-Jiménez and Marirosa Molina

Microbial communities respond to multiple abiotic and biotic factors that change along elevation gradients. We compare changes in microbial community composition in soil and review previous research on differential abundance of microbial functional groups along an elevation gradient in eastern Puerto Rico. Previous studies within the Luquillo Mountains showed that activity of methanogenic bacteria increased significantly with elevation, whereas diversity, abundance or activity decreased with elevation in 'slime molds', microbial nitrogen-fixing activity (nitrogenase), and abundance of basidiomycete fungi that degrade lignin in leaf litter. Our results, based on fatty acid (FA) composition and TRFLP analyses from a longer gradient (dry coastal forest to elfin rainforest) produced humped distributions for Shannon diversity of FA, fungal to bacteria (F:B) ratios, fungi, Myxomycetes, G– FA cy19:0 and sulfate reducing bacteria (SRB) 10Me18:0. Soil microbial communities differed significantly among forest types using ANCOVA. TRFLP were more frequently unique to forest types in fungi than bacteria, but we found unique and diverse sulfidogenic and crenarchaeal assemblages in some forest types, with highest diversity in high elevation palm and elfin forests. In multiple linear regression (MLR) models, soil moisture was predictive for all but Actinomycete FA abundance, and forest type contributed significantly to these same models for F:B ratios and all FA fractions except for G– SRB 10Me18:0, and G+ bacteria 15:0. F:B ratio peaked at mid-elevation, then declined with increasing moisture at higher elevation. Since most G– and G+ bacterial FA were positively related to soil pH in MLR models, lower pH in mid-elevation tabonuco forest soil may suppress bacteria and contribute to higher F:B ratios in this forest type.

S. A. Cantrell (scantrel@suagm.edu), C. A. Cruz, L. M. García and J. R. Pérez-Jiménez, Science and Technology, Univ. del Turabo, PO Box 3030, Gurabo, PR 00778, USA. – D. J. Lodge, Center for Forest Mycology Research, USDA Forest Service, Northern Research Station, PO Box 1377, Luquillo, PR 00773, USA. – M. Molina, U.S. Environmental Protection Agency, ORD, 960 College Station Road, Athens, GA 30605, USA.

Microorganisms mediate most of the biogeochemical transformations that are critical for the functioning of ecosystems, including making nutrients available to plants through decomposition of organic matter, and transformations in the nitrogen cycle. There are relatively few studies, however, of microbial diversity or abundance along elevation gradients. Bryant et al. (2008) found that acidobacteria declined with elevation in the northwestern USA using PCR of 16S ribosomal DNA while Adair and Schwartz (2008) found that ammonia oxidizing bacteria varied with precipitation in semi-arid soils in Arizona, USA. In contrast, Collins and Cavigelli (2003) found that the number of fatty acids increased with elevation in arid southern California. Several studies of slime molds along elevation gradients in Puerto Rico and elsewhere showed that diversity and abundance were inversely related to elevation (Stephenson et al. 1999, 2004, Novozhilov et al. 2001, Schnittler and Stephenson 2002, Landolt et al. 2006, Rojas and Stephenson 2008, Ndiritu et al. 2009a, b). Similarly, several basidiomycete fungal groups were found to have lower diversity at high elevations including neotropical Tricholomataceae (Dennis 1970) and polypores (Lindblad 2001). In contrast, Bailey et al. (2002) found higher fungal:bacteria ratios and higher fungal activity with increasing elevation in the dry northwestern USA. These studies show that microbial groups behave differently along elevational gradients in response to environmental (abiotic and biotic) factors, and that microbial distribution patterns differ among studies depending on the range of environments encompassed.

Microbial communities comprise various functional groups that mediate ecosystem processes, such as lignolytic

and cellulolytic decomposers; nitrogen-fixers, nitrifiers and denitrifiers; methanogens and methane oxidizers; sulfidogenic bacteria and dissimilatory Fe(III)-reducers. Microbivorous 'slime molds' prey primarily on bacteria and yeasts, and can thus increase the rate of nutrient mineralization. Differential abundances of these microbial groups or rates of the processes they mediate have been studied along all or part of the elevation gradient from the coast in northeastern Puerto Rico to the top of the Luquillo Mountains. New data on microbial communities in litter and soil were obtained along the elevation gradient using terminal restriction fragment length polymorphisms (TRFLP) of selected genes and fatty acids, and are presented with a review of previously published research.

Lignin-degrading basidiomycetes in leaf litter

Ligninolytic (white-rot) basidiomycete fungi are the most efficient biodegraders of low-quality leaf litter because they can remove the recalcitrant lignin to expose the assimilable cellulose and hemicelluloses. A few ascomycetes also have this ability, but are generally much less efficient than white-rot basidiomycetes. Bacteria, by contrast, have little, if any ability to decompose lignocellulose (Eriksson et al. 1990). Early decomposition of leaf litter from middle-elevation tabonuco forest in the Luquillo Mountains was accelerated by 15–22% in the presence of white-rot basidiomycetes and the proportion of the ground covered by white-rot basidiomycete colonies decreased with increasing elevation (Lodge et al. 2008b). The decrease in abundance of litter basidiomycetes corresponded to decreases in net primary production (NPP) and litterfall production, which are correlated with increases in annual rainfall along the elevation gradient in the Luquillo Mountains (Weaver and Murphy 1990). Basidiomycete colonies were most abundant on steep slopes and migrated uphill because fresh litter accumulated on the upslope side of terrestrial debris dams including litter matted together by fungi (Lodge et al. 2008b). Similarly, basidiomycete fungi that decay wood are also known to respond to resource quantity (Boddy 1993). The presence of litter bound into mats by basidiomycete fungi protects the underlying soil surface, so the abundance of fungal-litter mats on steep slopes makes them especially important in preventing the loss of soil nutrients via erosion in the steep terrain of the Luquillo Mountains (Lodge et al. 2008b). While abundance of litter mats formed by white-rot fungi generally decreased with elevation, there was a significant dip at 600 m at the lower boundary of the colorado forest that corresponded to the level of the cloud base and abrupt changes in plant communities. Many plant species have distributions that begin or end at this interface (Barone et al. 2008); thus white-rot basidiomycetes may respond to changes in plant litter composition as well as litterfall rates. Lodge et al. (2008a) found three species of *Xylaria* (ascomycetes) that delignify fine litter were restricted to cloud forests in Puerto Rico and elsewhere in the neotropics.

Soil organic matter decomposer activity

Soil organic matter (SOM) generally accumulates with increasing elevation in the Luquillo Mountains (Wang et al. 2004). Silver et al. (1999) hypothesized, based on soil gas measurements, that soil oxygen limitation to decomposers at high elevation was the likely driver for SOM accumulation. Soil frequently has low concentrations of O_2 at high elevation in the Luquillo Mountains (Silver et al. 1999, 2013). Differences in the chemical and physical nature of the litter have also been found to reduce invertebrates at high elevation (Richardson et al. 2005). Microbial decomposers are known to respond similarly to litter quality (Santana et al. 2005). Thus plant community composition and litter quality may contribute to accumulation of soil organic carbon at high elevation. NPP is inversely related to elevation and rainfall in the Luquillo Mountains (Weaver and Murphy 1990, Wang et al. 2002), so the rate of organic matter input is unlikely to contribute to soil carbon accumulation at high elevation.

Microbivorous slime mold diversity and abundance

Microbivorous 'slime molds' include dictyostelid (cellular), protestelid (amoeboid) and myxomycetes (plasmodial) slime molds. Their primary foods include bacteria, myxobacteria and yeasts (Schnittler and Stephenson 2002), though myxomycetes have also been observed feeding on mycelial fungi (Lodge 1996). Microbivores tend to increase rates of nutrient cycling and mineralization (Zak et al. 1994). In tabonuco forest of the Luquillo Mountains, the highest species richness of dictyostelid slime molds were found in disturbed areas (Stephenson et al. 1999), which coincided with highest functional diversity of their bacterial prey (Willig et al. 1996, Lodge 1997). Both diversity and abundance of microbivorous myxomycetes and cellular and plasmodial slime molds decreased with elevation in the Luquillo Mountains of Puerto Rico (Stephenson et al. 1999, Novozhilov et al. 2001, Schnittler and Stephenson 2002). The same result was found elsewhere in the tropics (Schnittler and Stephenson 2002, Stephenson et al. 2004, Ndiritu et al. 2009b), a warm temperate forest (Landolt et al. 2006) and a desert (Ndiritu et al. 2009a).

Mediators of biogeochemical transformations

Several distinct functional groups of archeae, bacteria, cyanobacteria and crustose lichens that are involved in ni-

trogen cycling have been studied on the elevation gradient in the Luquillo Mountains of Puerto Rico. Cusack et al. (2009) found lower inputs from biological nitrogen fixation in high elevation colorado forest than in middle elevation tabonuco forest despite the presence of N-fixation by leaf epiphyll at high but not in middle elevation sites. Despite low nitrogenase activity on a per-gram substrate basis, Cusack et al. (2009) found that free-living soil microbes contributed the most to N-fixation, but rates of nitrogenase activity were highest in mosses. Nitrogenase activity in mosses was likely from cyanobacteria associated with them as has previously been found for another species of moss in boreal forest (DeLuca et al. 2002). Biological nitrogen fixation may have been slowed by lower temperatures at high elevation, and was significantly positively correlated with moisture content in litter and soil but not in wood (Cusack et al. 2009). Litter quality may have contributed to the patterns found by Cusack et al. (2009) as low-lignin substrates support higher rates of heterotrophic nitrogen fixation in tropical leaf litter (Vitousek and Hobbie 2000) and leaf litter lignin concentrations were higher in the dominant tree species at the higher elevation (*Cyrilla racemiflora*, 22.1%) than litter from the dominant tree species at mid-elevation (*Dacryodes excelsa*, 16.6%) in the Luquillo Mountains.

Several studies have been published on rates of transformation by nitrifying and denitrifying microorganisms in the Luquillo Mountains, but except for Silver et al. (1999), these have not compared rates of nitrogen transformation along the elevation gradient. Silver et al. (1999) showed that N_2O concentrations in soil gas increased significantly with elevation, but it is produced through activity of both nitrifiers and denitrifiers. Templer et al. (2008) found that TRFLP bacterial fingerprints in upper elevation colorado forest were weakly correlated with N-cycling processes. Pett-Ridge and Firestone (2005), and Pett-Ridge et al. (2006) showed that fluctuating redox potential controlled rates of nitrogen cycling through its effect on soil microbial communities in upper elevation colorado forest sites. Rates of dissimilatory nitrate reduction to ammonium contributed significantly to rates of nitrogen transformations (25% of gross NH_4^+ production and 35% of gross nitrification) in these forests (Templer et al. 2008). The conversion of NO_3^- to NH_4^+, a form that is less susceptible to leaching, led to nitrogen conservation at higher elevations in the Luquillo Mountains (Templer et al. 2008). Studies in mid-elevation tabonuco forest found that activity of nitrifiers was stimulated by low rates of plant nutrient uptake following disturbance (Silver and Vogt 1993). Disturbance-related increase in NO_3^- concentrations led to high rates of denitrification in mid-elevation tabonuco forest following hurricane Hugo (Steudler et al. 1991).

Activity of methanogenic and methane consuming microbes has been compared along the elevation gradient in the Luquillo Mountains by Silver et al. (1999) and Teh et al. (2005, 2008). They found that methane production from upland tropical forest contributed unexpectedly high quantities of this greenhouse gas that is important in global climate change. Methane efflux from windward facing ridge sites was inversely correlated with soil O_2 concentrations, and increased significantly with elevation and the rainfall gradient (Silver et al. 1999). Soil O_2 in ridge sites decreased significantly with elevation and was correlated with mean annual rainfall (Silver et al. 1999, 2013). Teh et al. (2008) found in high elevation elfin sites that activity of dissimilatory Fe(III)-reducing bacteria suppressed methanogenesis by competing with methanogens for a carbon substrates (acetate) and hydrogen. Methane consumption exceeded methanogenesis in oxygenated ridge top soils in tabonuco forest (Silver et al. 1999), but only 48–78% of the methane generated was oxidized in high elevation colorado forest soils (Teh et al. 2005).

While previous studies of microbes and microbial processes along the elevation gradient in northeastern Puerto Rico have been informative, they were highly focused on particular microbes or their biogeochemical processes, and often covered only part of the elevation gradient from the coast to the top of the mountain. The new data presented here differ in providing a microbial community analysis along the entire gradient using fatty acid analyses and TRFLP data. In addition, we provide the first documentation of sulfate-reducing bacteria (SRB) from the coast to the Luquillo Mountains.

Methods

Study sites

The study area was located along an elevation gradient from 0 to 1000 m from Las Cabezas de San Juan, Fajardo to the top of the Luquillo Mountains in El Yunque National Forest in northeastern Puerto Rico. Sites for molecular sampling were selected in five different forest types: dry coastal (deciduous semievergreen co-dominants *Acacia farnesiana*, *Bursera simaruba*, *Capparis hastata*, *Cassine xylocarpa*, *Erythroxylum brevipes* and *Gymnanthes lucida*), tabonuco (evergreen canopy co-dominants *Dacryodes excelsa*, *Sloanea berteriana* and *Manilkara bidentata*), palo colorado (characterized by *Cyrilla racemiflora*), palm (canopy of sierra palm *Prestoea montana*) and elfin (cloud forest 3–4 m tall on peaks). The distribution of the constituent species in the latter four forest types along the elevation gradient were analyzed by Weaver (1991) and Barone et al. (2008). Barone et al. (2008) used vegetation plots distributed at regular elevation intervals along two transects in the Luquillo Mountains and found distinct breaks in plant communities at 500, 700 and 900 m elevation where upper limits of numerous plant species distributions converged, corresponding to the lower limits of the palo colorado, palm and elfin forests.

There are several types of soil parent material found along the elevation gradient, but this experiment was not designed to address this factor. The site in the dry coastal forest was on sedimentary volcanoclastic breccia (Weaver et al. 1999), whereas the sites in the Luquillo Mountains were on soils formed from quartz-diorite or andesite (Ping et al. 2013). Although palo colorado forest is generally associated with outcrops of quartz-diorite in the Luquillo Mountains, Weaver (1996) found that its characteristic canopy tree species, *C. racemiflora*, occurred on many types of soils throughout its range. Furthermore, Weaver (1996) and Guariguata (1990) found that *C. racemiflora* is a gap species that often colonizes landslides; thus the correlation of this characteristic tree species of the palo colorado forest with quartz-diorite in Puerto Rico may result from the high rate of landslides associated with this type of bedrock rather than soil chemical or physical characteristics. Sierra palm and palo colorado forests occur on the same soils that were formed in colluvium due to slope failure or slump (Ping et al. 2013). As a result, most of the soils horizons in palm and colorado forests have 20–70% angular granodiorite and volcanic rocks with sizes from cobblestone to boulder and the distribution in the profile has no relation to depth (Ping et al. 2013). Vegetation has a major influence on soil properties in the uppermost horizon, especially soil carbon content (Ping et al. 2013), and consequently vegetation and soil properties are often confounded. Johnston (1992) found that sierra palm soils were higher in Ca^{2+} and Mg^{2+} in addition to being wetter; Johnston showed this palm concentrated Ca^{2+} and Mg^{2+} and it may thus influence the abundance of soil cations through litter cycling. Soil organic carbon was found to be lower in young than old secondary tabonuco forest with the same elevation and rainfall in the Luquillo Mountains, and differences in fungal and bacterial biovolumes between soil samples between these sites were directly proportional to soil organic carbon (Lodge and Ingham 1991). Higher soil carbon (Wang et al. 2002) and higher N to C ratios with increasing elevation and rainfall and decreasing temperature (Cox et al. 2002, Wang et al. 2002) were shown in the Luquillo Mountains of Puerto Rico, and attributed to slower decomposition with high rainfall and low temperatures.

Basidiomycete litter mat cover transects

Thirty-four line intercepts were used in ridge-to-valley transects to assess percent cover by white rot basidiomycete mats in the litter layer. Transects were located in three watersheds at 50 m elevation intervals along the elevation gradient in the Luquillo Mountains. The number of transects per forest type was 16 in tabonuco, 5 in colorado, 3 in palm and 10 in elfin. Transect length varied with slope length, from 500 to 2009 m. Extent of fungal mat colonies was determined by pulling on leaves in the litter layer and recording the type of fungal attachments between leaves (rhizomorphs, cords, hyphal strands, and sticky pads). The distance at the beginning and end of each mat crossed by the meter tape was recorded, and the sum of the diameters of each colony transected by the meter tape was divided by the total transect length.

Soil sampling for microbial analyses

In each forest type, five plots of 5 × 5 m were established and soil samples were collected every three months starting in March 2003 thru December 2004. A polyvinyl chloride plastic tube 5 cm in diameter and 10 cm in length was used to take a random core sample from each plot after removal of surface litter. A total of 150 soil cores were analyzed but only 79 of these yielded usable DNA extractions. Samples were stored in sterile plastic bags at –20°C for fatty acid or DNA extraction. Each sample was subdivided by depth into 0–5 and >5–10 cm strata, and pH, temperature, and percent soil moisture content (using a gravimetric method) were determined. Rainfall data were obtained from the Luquillo LTER Database available at <http://luq.lternet.edu/>.

Fatty acid analyses

We used cellular and molecular markers to study the diversity of different microbial groups such as Eubacteria (Gram positives – G+, Gram negatives – G–, sulfur reducing bacteria – SRB, actinomycetes), Archaea (Crenarchaeota) and fungi. Fatty acids are cellular markers that characterize microbial groups based on their cell membranes (Kaur et al. 2005) and the molecular markers (16S rDNA, ITS and *dsrAB*) are widely used to characterize microbial diversity in natural ecosystems.

Fatty acids were extracted directly from 3 g of soil using the method described by Schutter and Dick (2000) for ester linked fatty acid methyl ester (EL-FAME). Briefly, the method used a mild alkaline methylation followed by pH neutralization with 1M acetic acid and FAME extraction using hexane. Extracts were cleaned using a NH_2 column to remove humic substances. Samples were stored at –20°C until analyzed. Samples were analyzed in a GC-MS (Hewlett Packard 6890). Table 1 summarizes the fatty acids used to characterize the microbial communities.

TRFLP community structure analysis

Soil samples were homogenized and DNA was extracted from 0.3 g using MoBio Ultraclean Soil DNA extraction kit. DNA quality was assessed by electrophoresis with 1% agarose gel and DNA concentration was calculated with a biophotometer. TRFLP community analysis was conducted to determine structure and distribution of microbes in

Table 1. Classification of fatty acid markers used in the present study (from Kaur et al. 2005).

Functional group	Fatty acid markers
Bacteria	br16:0
Gram positive	i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, br18:0
Gram negative	16:1w7c, cy19:0
Sulfate-reducing bacteria (SRB)	cy17:0, 10Me18:0
Actinomycetes	10Me16:0
Fungi	18:2w6

the different forest types (Lord et al. 2002, Pérez-Jiménez and Kerkhof 2005). Table 2 shows the four functional groups studied with corresponding genetic markers, primers and restriction enzymes used for each guild. Briefly, selected genetic markers were PCR amplified separately from genomic DNA extracts using forward primer fluorescently labeled. Amplicons were enzymatically digested with specific restriction enzymes, ethanol precipitated, and dried. Samples were re-suspended in formamide with GenScan Liz size standard and analyzed in an ABI 3130 Genetic Analyzer.

Statistical analyses

A 1-way ANOVA with post-hoc Tukey's test ($p<0.05$) was performed to determine if forest types differed in percent cover by litter that was bound into mats by basidiomycete fungi. Data are presented as means and standard deviations of percent cover from all transects within each forest type.

The Shannon diversity index of fatty acid (FA) composition was calculated for each forest type using the formula $\sum p_i \ln p_i$, where p_i is the peak area of the ith peak divided by the area of all peaks (Kaur et al. 2005). ANOVA was used to test for diversity differences between forest types, year and seasons using SPSS10 followed by 1-way ANOVA with post-hoc Bonferroni tests for pair wise comparisons between forest types. A principal component analysis with ANOVA was done in MINITAB (release 14).

A pair-wise correlation matrix was used to combine highly correlated dependent variables (FA fractions) prior to ANCOVA and multiple linear regression analyses. Strongly correlated fatty acids may represent the same bacterial communities. Correlations between G+ i15:0 and i17:0 fractions had an R^2 of 0.77 and were treated as a single group. Similarly, the R^2 values for correlations between G– i16:0, a17:0 and br18:0 fractions were 0.74–0.88, and they were treated as a group. Although the 0–5 and >5–10 cm soil strata were analyzed separately, ANOVA, PCA and paired student t-tests showed that only the br16:0 and Cy19 FA fractions changed with soil strata; we therefore used the mean values of the two strata from each soil core to represent the microbial communities in the entire core from 0–10 cm depth for those FA without a significant effect of soil strata. Log transformation of mean fungi and mean G+ bacteria (i16:0, a17:0, br18:0) was used to linearize the data and equalize the distribution of the variance. An inverse transformation of G+ bacteria a15:0 was used to convert it to a convex curve. The arcsine-square root conversion was used to linearize the fungal:bacteria ratios and equalize variances; two soil cores were excluded because they had exceptionally high ratios that skewed the results. High fungal biovolumes in soils have been found previously in the Luquillo Mountains when samples were collected near decaying wood (Lodge 1993). Soil moisture was distributed linearly with equal variance so it was not transformed. Separate ANCOVA were performed in SPSS10 for each dependent variable (FA, FA group and fungal:bacteria ratios), with three fixed factors (forest type, soil strata and season), and four covariates (soil pH, soil temperature, % soil moisture and % soil moisture^2). Similar stepwise backwards and forwards multiple linear regression analyses (MLR) were performed in SPSS10 to determine which environmental factors were predictive of individual FA or FA groups ($p<0.05$ for variables to enter and $p>0.10$ for variables to be removed). The independent variables included in the MLR models were: forest type, which were numbered from low to high elevation

Table 2. Microbial functional groups studied using T-RFPL analysis along an elevation gradient.

Functional group	Primer set	Genetic marker	Restriction enzyme
All fungi	ITS1F/ITS4[a]	Internal transcribed spacer region	*Hae*III
All Eubacteria	27F/1525R[b]	16S rDNA	*Mnl*I
Crenarchaeota	89Fb/915R[c]	16S rDNA	*Hha*I
Sulfate-reducers	DSR1F/DSR4R[d]	dissimilatory sulfite reductase	*Nde*II

[a]Anderson and Carney (2004).
[b]Lane (1991).
[c]Buckley et al. (1998).
[d]Pérez-Jiménez and Kerkhof (2005).

as follows: 1) dry coastal; 2) tabonuco; 3) palo colorado; 4) palm; 5) elfin; soil temperature, percent soil moisture and squared percent moisture. Half of the data for mean rainfall in the prior month were missing, which resulted in loss of half of the data in models in which it was included. Mean rainfall (mm) during the prior month was therefore only included if it improved the adjusted R^2 and equalized the variance better than the simple and quadratic terms for soil moisture. In addition, year (2003 or 2004), and soil pH were also included. Standardized Beta coefficients for variables contributing significantly to the predictive model are presented with their probabilities together with the adjusted model R^2 and probability for the overall model. In addition, all FA or FA groups were included as independent variables in an MLR to determine which could predict forest type.

For each molecular marker, the abundance (the sum of the number of observed TRFLP peaks across all samples), the richness (the number of different TRFLP peaks) and the number of unique TRFLP peaks was calculated for each forest type. Each TRFLP peak represents a phylotype, which also represents a category independent of taxonomy. Four presence/absence matrices were constructed for each molecular marker and the different forest types. These matrices were used for cluster analyses using Bray–Curtis similarity index with PAST ver. 2.03 (Hammer et al. 2001).

Results and discussion

Microbial community changes along the elevation gradient

Overall, microbial diversity decreased along the elevation gradient because of significantly higher diversity in the dry coastal forest (ANOVA, Shannon diversity index, model $p<0.001$; Fig 1c). The Shannon diversity index (H') differed significantly among forest types ($F=11.57$, $p<0.001$), year ($F=38.06$, $p<0.001$) and season ($F=7.73$, $p<0.001$). The greatest diversity of fatty acids was extracted from soil in dry coastal forest (H' 3.1) and the lowest diversity in palo colorado forest (H' 2.87) (Fig. 1c). Similarly, Lindblad (2001) found lower diversity of polypores at high el-

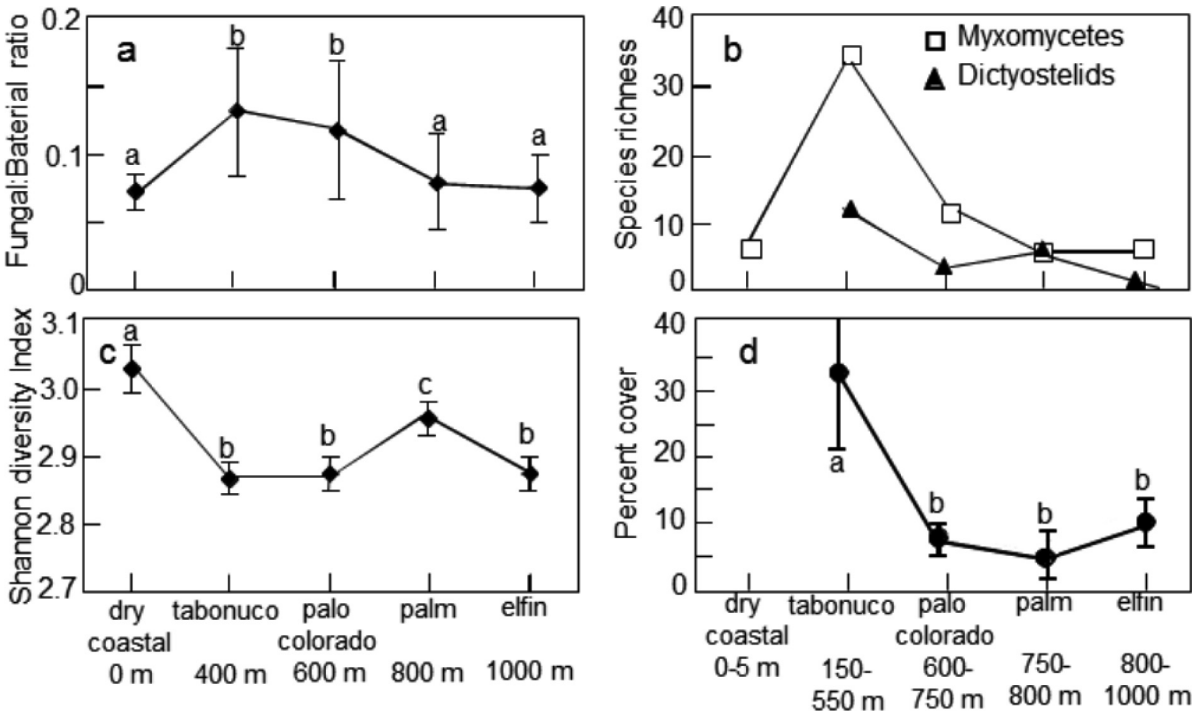

Figure 1. Microbial patterns on the elevation gradient from the coast to the peak of the Luquillo Mountains of Puerto Rico. (a) Fungal:bacterial ratio based on fatty acids from soil. (b) Species richness of microbivorous slime molds. Myxomycete data are from Novozhilov et al. (2001) and Schnittler and Stephenson (2002) while the disctyostelid data are from Stephenson et al. 1999. (c) Shannon diversity index of microbial fatty acids from soil. (d) Percent ground cover by basidiomycete fungal mats that cause white rot (delignification) of leaf litter. Figures (a) and (c) were based on five replicate samples from a single site per forest type – elevations 0 m for dry coastal; 400 m for tabonuco, 600 m for palo colorado, 800 m for palm and 1000 m for elfin forests. Figures (b) and (d) were based on data from several sites within an elevation range – 0–5 m for dry coastal, 150–550 m for tabonuco, 600–750 m for palo colorado, 750–800 m for palm and 800–1000 m for elfin forests. Letters above bars represent pairwise comparisons between forest types.

evations in Costa Rica. In contrast, Collins and Cavigelli (2003) found in a drier gradient that diversity, as indicated by the number of FAME, generally increased with elevation in the Sonoran Desert in southern California, corresponding to increased precipitation and percent of plant cover, higher C and N fractions and mineralization, and decreased sand content and pH.

Microbial biomass and diversity are often correlated. Zalamea and González (2007) found a decline in total microbial biomass with increasing elevation and moisture corresponding to our decrease in diversity along part of the gradient (dry coastal, moist forest and wet tabonuco forest) using a substrate induced respiration technique. In contrast, myxomycete species richness and the dictyostelid slime mold species richness tabulated from previous studies (Fig. 1b) were highest in wet tabonuco forest at middle elevation and then declined at higher elevations. Concentrations of fatty acid fractions declined in the higher elevation forest types, except for sulfate-reducing bacteria 10Me18:0 and G+ bacteria a15:0.

Fierer and Jackson (2006) found that soil pH was very important in determining bacterial diversity; soil samples with neutral pH (arid and semiarid ecosystems) clustered together as well as samples with acidic pH (temperate and tropical forest ecosystems). In our study, five of the 12 groups of fatty acid fractions increased with pH. Our palm and colorado forest plots occurred on the same soil type, but Johnston's (1992) study in the Luquillo Mountains found higher calcium content (and pH) in soil under palm – a species that concentrates calcium. Correspondingly, we found a significantly higher abundance of the bacterial G+ markers i16:0, a17:0 and br18:0 in palm than in colorado forest ($p<0.001$ in Bonferroni tests, Fig. 2), and soil pH was a significant predictor of FA abundance for this group (Table 3).

The fungal to bacterial ratio was low at low elevation, peaked in tabonuco and colorado forest, and then decreased with elevation along the gradient in the Luquillo Mountains (Fig. 1a). This indicates that the microbial community had greater bacterial dominance at low and high elevations than at mid-elevation. Lodge (1996) found that in mid-elevation tabonuco forest, fungal biomass was dominant in the litter layer although bacterial biomass was dominant in the upper 10 cm of soil. The peak we found in fungal to bacteria ratio corresponds with the peak in myxomycete species richness and the highest dictyostelid slime mold species richness (Fig. 1b), though the previous studies had reported a monotonic decline in slime mold diversity and abundance along elevation gradients in Puerto Rico and elsewhere (Stephenson et al. 1999, 2004, Novozhilov et al. 2001, Schnittler and Stephenson 2002, Landolt et al. 2006, Rojas and Stephenson 2008, Ndiritu et al. 2009a, b).

Similarly, litter mats formed by basidiomycete colonies were also most abundant in midelevation tabonuco forests (Fig. 1d). The differences in white rot basidiomycete litter mat cover among forest types was highly significant ($p<0.001$) in our ANOVA, but only tabonuco forest was significantly different from the other forests according to Bonferroni tests. Lodge et al. (2008b), however, had used both slope and elevation in their analysis of the same data set, and found a stronger, significant dip in litter mat cover in the colorado zone. Barone et al. (2008) suggested that the distribution of plant species along the elevation gradient in the Luquillo Mountains was strongly influenced by the extension of the cloud cover and proposed that the decrease at middle–upper elevations was related to the lower boundary of the cloud cover. The peak in the fungi to bacteria ratio occurred in tabonuco forest, where an earlier study at a site with 3500 mm annual rainfall showed that

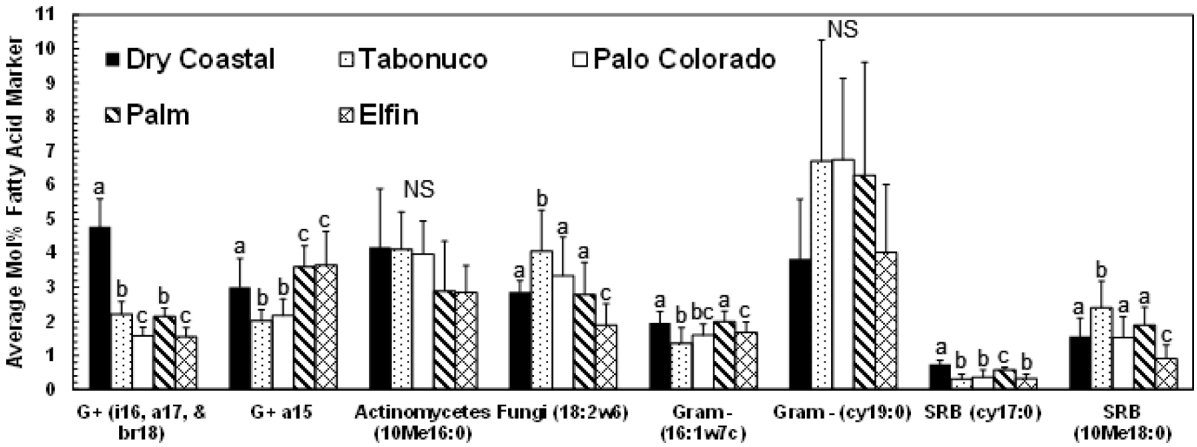

Figure 2. Comparisons of the different forest types based on different bacterial and fungal fatty acid (FAME) markers. (G+ Gram positive and G– Gram negative bacteria; SRB – sulfate-reducing bacteria). Elevations were 0 m for dry coastal; 400 m for tabonuco, 600 m for palo colorado, 800 m for palm and 1000 m for elfin forests. Letters above bars represent pairwise comparisons between forest types for each fatty acid marker. (NS = not significant.)

Table 3. Summary of standardized beta coefficients and significance levels from stepwise multiple linear correlation analyses to determine which environmental variables are predictive for concentrations of fatty acids associated with particular microbial groups (sulfate-reducing bacteria – SRB; inverse (Inv) and log (Log) transformations were used, as noted).

Variable	Forest type	Year	mm rain prior month	Season	Soil temp °C	soil pH	% soil moisture	Squared % moisture	Adjusted model R^2
Actinomycetes	–0.668***	.	.	.	–0.325**	.	.	.	**0.335***
Log fungi 18:2w6	–0.886***	–0.342***	.	0.243*	–0.371***	.	0.329*	.	**0.376***
G– bacteria 16:1w7c	–0.504***	.	.	.	.	0.271**	–0.376***	.	**0.529***
G– SRB cy17:0	–0.560***	.	.	.	.	.	1.810***	–1.071*	**0.327***
G– SRB 10Me18:0	.	2.225*	.	–0.214*	.	0.247*	–2.103***	1.534**	**0.342***
Mean G+ i15:0, i17:0	–0.609***	–0.280**	.	.	.	0.337***	–1.746***	1.553***	**0.618***
Log mean G+ bacteria i16:0, a17:0, br18:0	–0.560***	.	.	–0.119	.	0.287***	–1.983***	1.579***	**0.770***
Inv G+ bacteria a15:0	–0.068***	.	.	0.216*	.	.	2.017***	–1.495**	**0.406***
G+ bacteria 15:0	.	.	.	.	.	.	–0.243*	.	0.047

*p<0.05; **p<0.01; ***p<0.001.

fungal biovolume was strongly and significantly positively correlated with previous rainfall and soil moisture content (Lodge 1993). In this study, we found a significant positive relationship of percent soil moisture and the fungal FA 18:2w6 (Table 3). Fungi, however, may be inhibited at higher elevations that can have 5000 mm or more annual rainfall plus cloud condensation by saturated soils that are often anoxic (Silver et al. 1999, 2013). A decline in fungi to bacteria ratio and fungal FA between middle and upper elevation forests in the Luquillo Mountains is evident in Fig. 1a and 2. While the decline from mid to high elevation does not seem to be significant in Fig. 1 based on the high standard error bars, a mixed model ANCOVA that included soil strata, pH, temperature and moisture revealed significant differences between tabonuco and palo colorado versus palm and elfin forest types. In contrast to the Luquillo Mountains gradient, Bailey et al. (2002) found the fungal to bacterial ratio increased with moisture along an elevation gradient in a drier, desert ecosystem in Washington State. Thus, responses of fungi to moisture along elevation gradients depend on the range of conditions comprising the gradient.

Distribution patterns of microbial groups vary along elevation gradients in temperate and desert ecosystems and little is known for tropical regions. Some microbial groups increase or decrease with elevation presenting a monotonic distribution, while others have unimodal or bimodal distributions with peak diversity at lower, mid or high elevations (Bryant et al. 2008, Xu et al. 2010). All of the MLR models were highly significant except for G+ bacteria FA 15:0, and the amount of variation explained by the models was in the range of one to three fourths in the FA fractions or FA groups (Table 3). All of the FA fractions and groups presented in Table 3 showed no difference between the 0–5 and >5–10 cm soil strata, so the means of the two strata were analyzed for each core. Data for G– bacteria FA cy19:0 differed significantly between strata and is not included in Table 3, but the model was also highly significant. The G– bacteria FA br16:0 was only found in dry forest; MLR indicated that in addition to forest type ($p<0.001$), br16:0 was predicted by rainfall in the prior month ($p<0.01$), percent soil moisture ($p<0.001$) and soil pH ($p<0.001$) (adjusted model $R^2=0.536$). A monotonic distribution with a decrease in relative abundance as a function of elevation was observed for actinomycete marker 10Me16:0 (Fig. 2). The difference between elfin and other forest types was significant ($p<0.01$), and forest type plus soil temperature contributed significantly to predicting actinomycete FA abundance (Table 3). The fungal marker 18:2w6 was most abundant at middle elevations in tabonuco forest presenting a unimodal distribution (Fig. 2); differences among forest types were significant ($p<0.01$). Forest type, soil moisture and season all contributed significantly to predicting fungal FA abundance. A similar unimodal distribution for myxomycete species richness was found in Puerto Rico using records from Stephenson et al. 1999, Novozhilov et al. 2001, and Schnittler and Stephenson 2002 (Fig. 1b). A unimodal pattern was also observed for the SRB marker 10Me18:0, which had a significant peak in tabonuco forest ($p<0.001$) and the lowest value in elfin forest (the small peak in palm forest was not statistically significant). Both the linear and quadratic terms for percent soil moisture were significant in the best-fit multiple linear regression model (Table 3). The reverse pattern was evident for a15:0, a G+ marker, which decreased signifi-

cantly ($p<0.001$) at middle elevations (Fig. 2). Forest type and both the linear and quadratic soil moisture terms contributed significantly to the multiple linear regression model with an inverse transformation of a15:0 FA (Table 3). The G– bacteria (marker cy19:0) had a unimodal distribution with the relative abundance being significantly ($p<0.05$) higher in tabonuco, palo colorado, and palm forests than in dry coastal or elfin forest (Fig. 2). Forest type, soil strata, season, and percent soil moisture contributed significantly to the MLR model ($p<0.001$ for forest type and soil moisture, and $p<0.05$ for soil strata and season; adjusted model $R^2=0.355$). Bimodal distributions were observed for the group of Gram positive markers i16:0, a17:0 and br18:0 with a large decrease in abundance from dry coastal to palo colorado, followed by a slight increase in palm, and then a decrease in elfin forest (all $p<0.001$ between adjacent forest types in Bonferroni tests). Forest type, season, soil pH and both the linear and quadratic moisture terms contributed significantly to the linear regression model for this group of G+ FA (Table 3). A similar pattern was observed for SRB markers cy17:0 and G– marker 16:1w7c except that the only significant differences were between dry coastal and tabonuco forests ($p<0.001$) and between palm and elfin forests ($p<0.001$ for cy17:0, and $p<0.01$ for 16:1w7c). Forest type and soil moisture contributed significantly to linear regression models for both FA, but soil pH also contributed to the model for 16:1w7c whereas the quadratic soil moisture term contributed to the cy17:0 model (Table 3). The G+ bacteria FA marker, 15:0, was not significantly related to the independent variables (Table 3). Although the distributions of actinomycetes (10Me16:0) and G– bacteria (cy19:0) did not differ significantly among forest types using simple 1-way ANOVA ($p<0.05$), they had highly significant multiple linear regression models ($p<0.001$, Table 3 for actinomycetes) in which forest type contributed significantly ($p<0.001$). The standardized beta coefficients and their probabilities for the best fit model of the G– bacteria Cy19:0 (adjusted $R^2=0.355$, $p<0.001$) included forest type (–0.549, $p<0.001$), soil strata (0.140, $p<0.05$), season (–0.158, $p<0.05$) and percent soil moisture (0.745, $p<0.001$). These results indicate that forest type is a significant factor but that other contributing factors need to be accounted for in the model to detect the effect.

Percent soil moisture was significant in predicting the abundance of all fatty acid fractions except for actinomycetes (Table 3). However, the relationship varied depending on the microbial group. Fatty acid fractions for fungi increased with soil moisture, whereas G– bacteria 16:1w7c and cy19:0, and G+ bacteria 15:0 were inversely related to soil moisture. Most bacterial groups, however, had hump shaped distributions on the elevation gradient (Fig. 2) and both the linear and quadratic terms for soil moisture were significant in MLR models (Table 3). Cusack et al. (2009) also found that nitrogen fixation was significantly correlated with moisture in soil and leaf litter. Forest type together with soil moisture were strong predictors of fatty acid abundances for most groups suggesting possible biotic controls related to vegetation (Table 3). The correlation of colorado forest with quartz-diorite bedrock, however, is a partially confounding factor that constrains interpretation. The higher calcium and magnesium concentrations found by Johnston (1992) in palm than in colorado forest soils, despite being formed from the same quartz-diorite parent material suggests a plant effect on soil through nutrient cycling, and this may be related to distributions of some of the bacterial groups we detected. While our data cannot detect the influence of vegetation at the same elevation, Richardson et al. (2005) previously found biotic effects of vegetation on invertebrates along the elevation gradient using paired palm versus dicotyledonous tree plots. Lodge et al. (2008a) found three host-specific species of *Xylaria* that were restricted to neotropical cloud forests including those in Puerto Rico. They concluded that high moisture inhibited fungal growth in large, waterlogged woody debris while favoring *Xylaria* species that grow in small debris (i.e. leaves, twigs and fruits) – a group that is predominantly host-specific.

The analysis to determine if FA fractions could predict forest type was highly significant (multiple $R^2=0.92$, $p<0.001$), but the only FA that contributed significantly to the model were SRB 10Me18:0 ($F=5.414$, $p=0.023$), and the G+ bacteria fractions 15:0 ($F=21.7$, $p<0.001$), group i15:0 and i17:0 ($F=16.682$, $p<0.001$) and group i16:0, a17:0 and br18:0 ($F=52.149$, $p<0.001$). Dry coastal forest was dominated by G+ bacteria, and a unique fatty acid maker br16:0 was found in this forest type that might be useful as a bacterial indicator (Fig. 2). Collins and Cavigelli (2003) reported high diversity of G+ bacteria in the Sonoran desert particularly for samples taken between plants and not under plants. Bacterial communities surviving in dry environments need to be able to survive extensive desiccation periods. In general among the non-spore forming bacteria, G+ bacteria tend to be highly tolerant to desiccation (Potts et al. 2005), which may explain the abundance of G+ markers in dry coastal forest. The abundance of G– bacteria in the wet high elevation forests supports previous studies by Silver et al. (1999), Pett-Ridge and Firestone (2005), Teh et al. (2005, 2008), Pett-Ridge et al. (2006), Templer et al. (2008). In these locations, the authors cited above observed higher levels of nitrification, denitrification, methanogenesis and iron reduction – processes that are primarily mediated by G– Proteobacteria. Principal component (PC) analysis showed that dry coastal and elfin forests had distinct microbial communities, whereas microbial communities in palm, palo colorado and tabonuco forests resembled each other (Fig. 3). Based on an ANOVA of the principal component analysis, only the variable forest type was significant. Dry coastal and palm forests were significantly different from tabonuco, palo

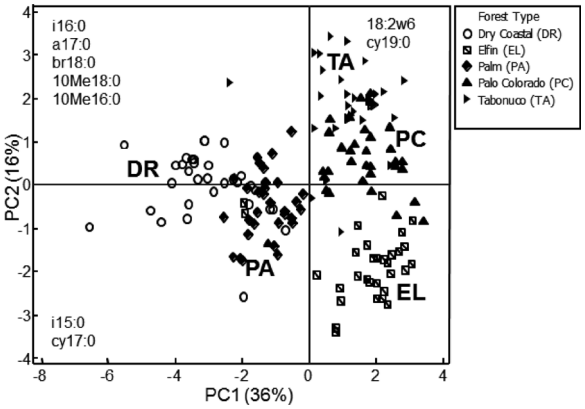

Figure 3. Principal component analysis of the bacterial and fungal community in the different forest types based on fatty acid (FAME) markers.

colorado and elfin ($p<0.001$) for PC1. For PC2, elfin and tabonuco forests were significantly different from the other three ($p<0.001$).

The results obtained from TRFLP analysis show partial support for the fatty acids results. TRFLP analyses of the eubacterial 16S rDNA gave the total abundance of phylotypes (the sum of the number of observed peaks) for all five forest types as 1531. The highest abundance was observed in palm forest with 419 phylotypes, followed by tabonuco with 341 (Fig. 4a). The total richness (the number of different peaks) was 307, and the highest richness was observed in dry coastal forests with 230 (Fig. 4a). Only a total of 69 phylotypes were found to be unique across the different forest types, representing 22.5% of the 307. This indicates that most of the eubacterial phylotypes were shared among forest types. Results of clustering analysis of TRFLP data resembled results from fatty acid markers: dry coastal and elfin forests differed from palm, palo colorado and tabonuco forests (Fig. 5a). Chan et al. (2008) using TRFLP of the bacterial 16S rDNA found that the structure of soil bacterial community in moist forests of southwestern China was determined by vegetation and not climate along a temperate to tropical gradient.

TRFLP analyses of the fungal ITS region of the rDNA yielded 713 phylotypes across the different forests (Fig. 4b). The total fungal phylotype richness was 254, with greatest diversity in palo colorado forest (118) and the lowest in elfin forest (62) (Fig. 4b). A total of 119 phylotypes were unique across forest types, representing 47% of the 254. This means that contrary to bacteria, fungal communities have higher alpha and beta diversity in these forests. Nevertheless, results of clustering analysis of fungal TRFLP data showed a pattern similar to Eubacteria: dry coastal and elfin forests differed significantly from palm, palo colorado and tabonuco forests (Fig. 5b).

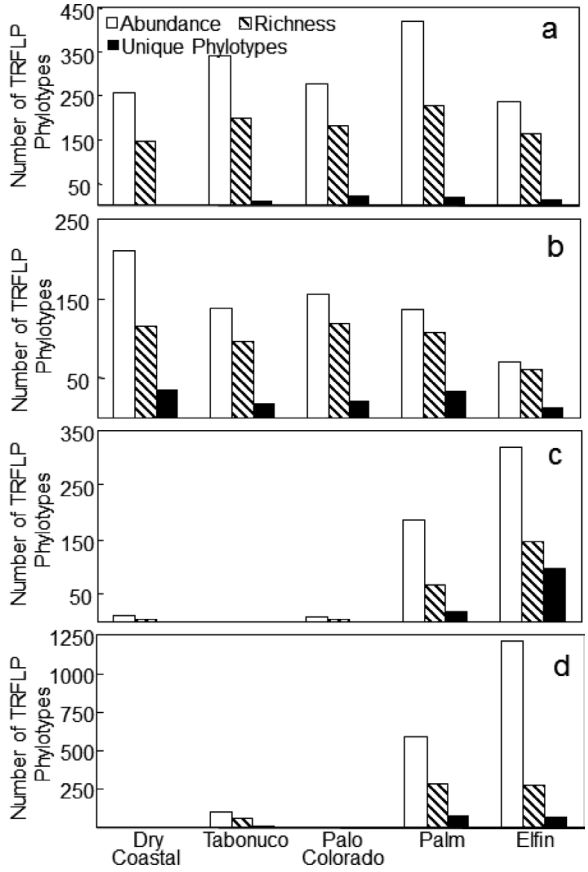

Figure 4. Comparisons between different forest types based on abundance, richness and unique TRFLP phylotypes. (a) Eubacterial 16S rDNA amplified with primers 27F/1525R and digested with the restriction enzymes *Mnl*I. (b) Fungal ITS region amplified with primers ITS1/ITS4 and digested with the restriction enzymes *Hae*III. (c) Crenarchaeotal 16S rDNA region amplified with primers 89Fb/915R and digested with the restriction enzymes *Hha*I. (d) SRB *dsrAB* gene amplified with primers DSR1F/DSR4R and digested with the restriction enzymes *Nde*II. Elevations were 0 m for dry coastal, 400 m for tabonuco, 600 m for palo colorado, 800 m for palm, and 1000 m for elfin forests. The missing data indicate that no PCR amplification was obtained probably meaning that this functional group was not present and therefore is interpreted as zero.

Sulfidogenic and crenarchaeal communities detected at higher elevations

This is the first documentation of sulphate-reducing bacteria (SRB) and Crenarchaeota along an elevation gradient in the Luquillo Mountains. We detected bacterial groups relevant to biogeochemical cycling of sulfur and nitrogen at both high and low elevations. Sulfate-reducing (SRB) are prevalent in marine or coastal environment due to the abundance of sulfate in seawater. In Puerto Rico among mangroves, the sulfidogenic community demonstrated

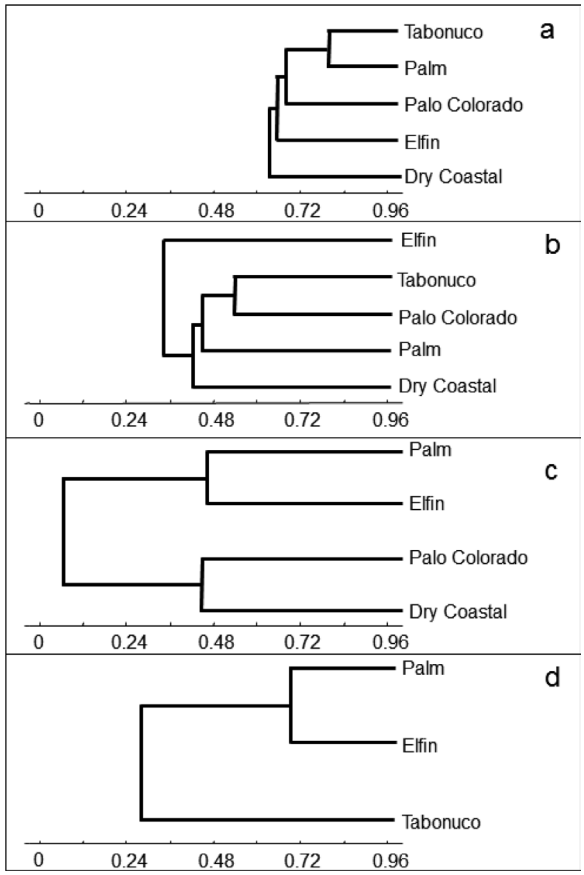

Figure 5. Clustering analysis comparing the different forest types and the soil microbial communities. (a) Eubacteria. (b) Fungi. (c) Crenarchaeota. (d) SRB. (Scale bars represent the Bray–Curtis similarity index.)

greater heterogeneity in respect to geographical location than plant species (Pérez-Jiménez 2003). Sulfate derived from plant decomposition, mineral content, and microbial processes may be used as the electron acceptor in sulfate respiration. Alternatively, many SRB can thrive based on fermentative metabolism. Both processes ensure energy for the strict anaerobic lifestyle of SRB.

Crenarchaeota, initially characterized as thermal extremophiles, have been found in mesophilic habitats, including temperate forests, and arid dessert soils (Jurgens et al. 1997, Buckley et al. 1998, Beman and Francis 2006). More recently, a significant role in ammonia oxidation has been attributed to the crenarchaeal communities in soils, and their numbers surpass the contribution of bacterial ammonia oxidizers. The process transforms ammonia into nitrate that is preferred for plant metabolism and is used as electron acceptor for facultative anaerobic bacteria.

Richness and distribution were assessed simultaneously for sulfidogenic and crenarchaeal communities along the elevation gradient in the Luquillo Mountains. Unique and diverse sulfidogenic and crenarchaeal assemblages were evident at higher elevation forests (palm and elfin) (Fig. 4c, d), corresponding to low redox potentials in those forests (Pett-Ridge and Firestone 2005, Pett-Ridge et al. 2006). We obtained a total richness of 167 crenarchaeotal phylotypes, of which 96 (57%) were unique or endemic of elfin forest (Fig. 4c). A total of 371 SRB phylotypes were obtained, of which 79 (21%) and 74 (20%) were unique or endemic to palm and elfin forests, respectively (Fig. 4d). Each forest type harbored a unique SRB and Crenarchaeota community as shown in the clustering analysis (Fig. 5c, d). Both SRB and Crenarchaeota communities were similar between the elfin and palm forests. Preliminary results from the elfin forests clone library revealed novel lineages of Crenarchaeota and SRB (data not shown). The Crenarchaeota clones analyzed revealed a novel clusters within the mesophilic Crenarchaeota. The SRB clones analyzed showed novel clusters and similarities with *Desulfobacca acetoxidans* and *Desulfomonile tiedjei*.

The heterogeneity of sulfidogenic and crenarchaeal communities in the elevation gradient at Luquillo Mountains may be affected by soil moisture, leaf litter quality, nutrient concentrations, and redox potential faced by microbes in their particular microhabitats. The application of DNA-based molecular approaches has provided insight on the community structure of selected guilds relevant to the ecosystem that are very difficult to cultivate or may elude detection using traditional methodologies. A deeper documentation of presence and activity of microbial guilds will assist in understanding the overall functioning of the ecosystem and assess responses to disturbances.

Closing remarks

Dramatic changes in temperature, relative humidity, UV radiation and vegetation can occur along elevation gradients. Even though changes in plant and animal diversity with elevation gradients are well documented, little is known about microbial diversity (Bryant et al. 2008). The limited information available on microbes and microbial processes along elevation gradients is primarily from temperate areas and very few studies are from tropical regions. The limited information on microbial diversity along elevational gradients represents a serious gap in our understanding of biodiversity particularly since microbes are extremely diverse, are key players in ecosystems processes and will likely respond very rapidly to climate change (Bryant et al. 2008). This study documents changes in microbial diversity, and abundance and composition of microbial functional groups along a subtropical elevation gradient, how different microbial groups behave along the gradient and which environmental variables are related to microbial changes.

Increasing elevation in the Luquillo Mountains is correlated with increases in rainfall, decrease in temperature,

changes in soil types, and changes in vegetation including decreasing plant species richness, tree height, tree dbh (diameter at breast height) and complexity (Brown et al. 1983). The decrease of some microbial groups in palo colorado forest corresponded to the lower boundary of the cloud cover – a factor suggested by Barone et al. (2008) as important in controlling the upper distribution of many plant species along the elevation gradient. Our results showed that forest type was a strong predictor of microbial communities and that only some of the variation was related to soil moisture, temperature or soil pH. This suggests that the microbial communities may be responding to biotic controls related to the composition and quality of the vegetation in addition to the environmental factors that vary along the elevation gradient. Other studies have concluded that vegetation is a key factor controlling microbial communities in soil (Collins and Cavigelli 2003, Carney and Matson 2006, Chan et al. 2008). Overall, fungi were most abundant at middle elevation tabonuco forest (~300 m) while bacteria, which were dominant in soil across the entire gradient, were proportionately more abundant at the low, dry and high, wet ends. There was high overlap in bacterial communities along the elevation gradient, but communities of fungi, Crenarchaeota and sulfate-reducing bacteria were distinct for each forest type with many phylotypes unique to each forest. Our study clearly demonstrates that even though overall microbial diversity decreased with elevation along the gradient we studied, different microbial groups behaved differently. While not a novel concept, it is important to note that it is not elevation per se, but a combination of abiotic and biotic factors that influence microbial diversity. These data can be used as a benchmark for monitoring changes in the microbial community along the elevation gradient caused by natural and anthropogenic disturbances, as well as global and regional climate changes.

Acknowledgements – The authors thank the Luquillo LTER for their support. This paper has been reviewed in accordance with the USEPA and USDA-Forest Service's peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by USEPA or USDA. This paper was prepared by US Government employees and is thus in the public domain and not subject to copyright.

References

Adair, K. L. and Schwartz, E. 2008. Evidence that ammonia-oxidizing Archaea are more abundant than ammonia-oxidizing bacteria in semiarid soils of northern Arizona, USA. – Microb. Ecol. 56: 420–426.

Anderson, I. C. and Cairney, J. W. G. 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. – Environ. Microbiol. 6: 769–779.

Bailey, V. L. et al. 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. – Soil Biol. Biochem. 34: 997–1007.

Barone, J. A. et al. 2008. Metacommunity structure of tropical forest along an elevational gradient in Puerto Rico. – J. Trop. Ecol. 24: 525–534.

Beman, J. M. and Francis, C. A. 2006. Diversity of ammonia-oxidizing Archaea and bacteria in the sediments of a hyper nutrified subtropical estuary: Bahía del Tóbari, Mexico. – Appl. Environ. Microbiol. 72: 7767–7777.

Boddy, L. 1993. Cord-forming fungi: warfare strategies and other ecological aspects. – Mycol. Res. 97: 641–655.

Brown, S. et al. 1983. Research history and opportunities in the Luquillo Experimental Forest. – General Technical Report SO-44. USDA Forest Service, Southern Forest Experiment Station, New Orleans, LA.

Bryant, J. A. et al. 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. – Proc. Natl Acad. Sci. USA 105 suppl. 1: 11505–11511.

Buckley, D. H. et al. 1998. Phylogenetic analysis of nonthermophilic members of the kingdom Crenarchaeota and their diversity and abundance in soils. – Appl. Environ. Microbiol. 64: 4333–4339.

Carney, K. M. and Matson, P. A. 2006. The influence of tropical plant diversity and composition on soil microbial communities. – Microb. Ecol. 52: 226–238.

Chan, O. C. et al. 2008. Vegetation cover of forest, shrub and pasture strongly influences soil bacterial community structure as revealed by 16S rDNA gene T-RFLP analysis. – FEMS Microb. Ecol. 64: 449–458.

Collins, H. P. and Cavigelli, M. A. 2003. Soil microbial community characteristics along an elevation gradient in the Laguna Mountains of southern California. – Soil Biol. Biochem. 35: 1027–1037.

Cox, S. B. et al. 2002. Variation in nutrient characteristics of surface soils from the Luquillo Experimental Forest of Puerto Rico: a multivariate perspective. – Plant Soil 247: 189–198.

Cusack, D. F. et al. 2009. Biological nitrogen fixation in two tropical forests: ecosystem-level patterns and effects of nitrogen fertilization. – Ecosystems 12: 1299–1315.

DeLuca, T. H. et al. 2002. Quantifying nitrogen fixation in feather moss carpets of boreal forests. – Nature 419: 917–920.

Dennis, R. W. G. 1970. Fungi venezuelani: IV Agaricales. – Kew Bull. 15: 67–156.

Eriksson, K.-E. et al. 1990. Microbial degradation of wood and wood components. – Springer.

Fierer, N. and Jackson, B. 2006. The diversity and biogeography of soil bacterial communities. – Proc. Natl Acad. Sci. USA 103: 626–631.

Guariguata, M. R. 1990. Landslide disturbance and forest regeneration in the upper Luquillo Mountains of Puerto Rico. – J. Ecol. 78: 814–832.

Hammer, Ø. et al 2001. PAST: paleontological statistics software package for education and data analysis. – Palaeontol. Electron. 4, <http://palaeo-electronica.org/2001_1/past/issue1_01.htm>.

Johnston, M. K. 1992. Soil–vegetation relationships in a tabonuco forest community in the Luquillo Mountains of Puerto Rico. – J. Trop. Biol. 8: 253–263.

Jurgens, G. et al. 1997. Novel group within the kingdom Crenarchaeota from boreal forest soil. – Appl. Environ. Microbiol. 63: 803–805.
Kaur, A. et al. 2005. Phospholipid fatty acid – a bioindicator of environment monitoring and assessment in soil ecosystem. – Curr. Sci. 89: 1103–1112.
Landolt, J. et al. 2006. Distribution and ecology of dictyostelid cellular slime molds in Great Smoky Mountains National Park. – Mycologia 98: 541–549.
Lane, D. J. 1991. 16S/23S rRNA sequencing. – In: Stackebrandt, E. G. M. (ed.), Nucleic acid techniques in bacterial systematics. Wiley, pp. 115–148.
Lindblad, I. 2001. Diversity of poroid and some corticioid wood-inhabiting fungi along the rainfall gradient in tropical forests, Costa Rica. – J. Trop. Ecol. 17: 353–369.
Lodge, D. J. 1993. Nutrient cycling by fungi in wet tropical forests. – In: Isaac, S. et al. (eds), Aspects of tropical mycology. Cambridge Univ. Press, BMS Symp. Ser. 19: 37–57.
Lodge, D. J. 1996. Microorganisms. – In: Regan, D. P. and Waide, R. B. (eds), The food web of a tropical forest. Univ. of Chicago Press, pp. 53–108.
Lodge, D. J. 1997. Factors related to diversity of decomposer fungi in tropical forests. – Biol. Conserv. 6: 681–688.
Lodge, D. J. and Ingham, E. R. 1991. A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. – Agric. Ecosyst. Environ. 34: 131–144.
Lodge, D. J. et al. 2008a. Montane and cloud forest specialists among neotropical *Xylaria* species. – N. Am. Fungi 3: 193–213.
Lodge, D. J. et al. 2008b. Distribution and role of mat-forming saprobic basidiomycetes in a tropical forest. – In: Boddy, L. and Frankland, J. C. (eds), Ecology of saprobic basidiomycetes. Academic Press, pp. 195–208.
Lord, N. S. et al. 2002. Assessment of fungal diversity using terminal restriction fragment (TRF) pattern analysis: comparison of 18S and ITS ribosomal regions. – Microb. Ecol. 42: 327–337.
Ndiritu, G. G. et al. 2009a. Distribution and ecology of assemblages of myxomycetes associated with major vegetation types in Big Bend National Park, USA. – Fungal Ecol. 2: 168–183.
Ndiritu, G. G. et al. 2009b. First records and microhabitat assessment of protostelids in the Aberdare region, central Kenya. – J. Eukaryot. Microbiol. 56: 148–158.
Novozhilov, Y. K. et al. 2001. Myxomycetes from different forest types in Puerto Rico. – Mycotaxon 77: 285–299.
Pérez-Jiménez, J. R. 2003. Molecular characterization of sulfidogenic communities in polluted and pristine environments based on the dissimilatory sulfite reductase genes (*dsrAB*). – PhD thesis, Rutgers Univ.
Pérez-Jiménez, J. R. and Kerkhof, L. J. 2005. Phylogeography of sulfate-reducing bacteria among disturbed sediments, disclosed by analysis of the dissimilatory sulfite reductase genes (*dsrAB*). – Appl. Environ. Microbiol. 71: 1004–1011.
Pett-Ridge, J. and Firestone, M. K. 2005. Redox fluctuation structures microbial communities in a wet tropical soil. – Appl. Environ. Microbiol. 71: 6998–7007.
Pett-Ridge, J. et al. 2006. Redox fluctuations in a humid tropical forest soil impact N-cycling rates by framing the composition of the soil microbial communities. – Biogeochemistry 81: 95–110.
Ping, C. L. et al. 2013. Soil characteristics, carbon stores and nutrient distribution in eight forest types along an elevation gradient, eastern Puerto Rico. – Ecol. Bull. 54: 67–86.
Potts, M. et al. 2005. Desiccation tolerance of Prokaryotes: application of principles to human cells. – Integr. Comp. Biol. 45: 800–809.
Richardson, B. A. et al. 2005. Separating the effects of forest type and elevation on the diversity of litter invertebrate communities in a humid tropical forest in Puerto Rico. – J. Anim. Ecol. 74: 926–936.
Rojas, C. and Stephenson, S. L. 2008. Myxomycete ecology along an elevation gradient on Cocos Island, Costa Rica. – Fungal Divers. 29: 117–127.
Santana, M. et al. 2005. Relationship of host recurrence in fungi to rates of tropical leaf decomposition. – Pedobiologia 49: 549–564.
Schnittler, M. and Stephenson, S. L. 2002. Inflorescences of Neotropical herbs as a newly discovered microhabitat for myxomycetes. – Mycologia 94: 6–20.
Schutter, M. E. and Dick, R. P. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. – Soil Sci. Soc. Am. J. 64: 1659–1668.
Silver, W. L. and Vogt, K. A. 1993. Fine root dynamics following single and multiple disturbances in a subtropical wet forest ecosystem. – J. Ecol. 81: 729–738.
Silver, W. L. et al. 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. – Biogeochemistry 44: 301–328.
Silver, W. L. et al. 2013. Soil redox dynamics and biogeochemistry along a tropical elevation gradient. – Ecol. Bull. 54: 195–210.
Stephenson, S. L. et al. 1999. Protestelids, dictyostelids, and myxomycetes in the litter microhabitat of the Luquillo Experimental Forest, Puerto Rico. – Mycol. Res. 103: 209–214.
Stephenson, S. L. et al. 2004. Ecological characterization of a tropical myxomycete assemblage – Maquipucuna Cloud Forest Reserve, Ecuador. – Mycologia 96: 488–497.
Steudler, P. A. et al. 1991. The effects of natural and human disturbances on soil nitrogen dynamics and trace gas fluxes in a Puerto Rican wet forest. – Biotropica 23: 356–363.
Teh, Y. A. et al. 2005. Oxygen effects on methane production and oxidation in humid tropical forest soils. – Global Change Biol. 11: 1283–1297.
Teh, Y. A. et al. 2008. Suppression of methanogenesis by dissimilatory Fe(III)-reducing bacteria in tropical rain forest soils: implications for ecosystem methane flux. – Global Change Biol. 14: 413–422.
Templer, P. H. et al. 2008. Plant and microbial controls on nitrogen retention and loss in a humid tropical forest. – Ecology 89: 3030–3040.
Vitousek, P. M. and Hobbie, S. 2000. Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. – Ecology 81: 2366–2376.
Wang, H. Q. et al. 2002. Modeling the spatial and temporal variability in climate and primary productivity across the Luquillo Mountains, Puerto Rico. – For. Ecol. Manage. 179: 69–94.
Weaver, P. L. 1991. Environmental gradients affect forest composition in the Luquillo Mountains of Puerto Rico. – Interciencia 16:142–151.

Weaver, P. L. 1996. *Cyrilla racemiflora* L. Swamp Cyrilla, Cyrillaceae, Cyrilla Family. – USDA-Forest Service Publication SO-ITF-SM78.
Weaver, P. L. and Murphy, P. G. 1990. Forest structure and productivity in Puerto Rico's Luquillo Mountains. – Biotropica 22: 69–82.
Weaver, P. L. et al. 1999. Las Cabezas de San Juan Nature Reserve (El Faro). – USDA-Forest Service Publication Gen. Tech. Rep. IITF-5.
Willig, M. R. et al. 1996. Functional diversity of soil bacterial communities in the tabonuco forest: interaction of anthropogenic and natural disturbance. – Biotropica 28: 471–483.
Xu, Y. et al. 2010. Distribution pattern of plant species diversity in the mountainous region of Ili River Valley, Xinjiang. – Environ. Monit. Assess. doi: 10.1007/s10661-010-1665-3
Zak, J. C. et al. 1994. Functional diversity of microbial communities: a quantitative approach. – Soil Biol. Biochem. 26: 1101–1108.
Zalamea, M. and González, G. 2007. Substrate-induced respiration in Puerto Rican soils: minimum glucose amendment. – Acta Científica 21: 11–13.