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Microbes do not follow the elevational diversity patterns of plants and animals

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Abstract. The elevational gradient in plant and animal diversity is one of the most widely documented patterns in ecology and, although no consensus explanation exists, many hypotheses have been proposed over the past century to explain these patterns. Historically, research on elevational diversity gradients has focused almost exclusively on plant and animal taxa. As a result, we do not know whether microbes exhibit elevational gradients in diversity that parallel those observed for macroscopic taxa. This represents a key knowledge gap in ecology, especially given the ubiquity, abundance, and functional importance of microbes. Here we show that, across a montane elevational gradient in eastern Peru, bacteria living in three distinct habitats (organic soil, mineral soil, and leaf surfaces) exhibit no significant elevational gradient in diversity ($r^2 < 0.17$, $P > 0.1$ in all cases), in direct contrast to the significant diversity changes observed for plant and animal taxa across the same montane gradient ($r^2 > 0.75$, $P < 0.001$ in all cases). This finding suggests that the biogeographical patterns exhibited by bacteria are fundamentally different from those of plants and animals, highlighting the need for the development of more inclusive concepts and theories in biogeography to explain these disparities.

Key words: 16S rRNA genes; bacterial diversity; montane diversity gradient; phyllosphere bacteria; pyrosequencing; soil bacteria.

Introduction

As noted by Linnaeus, Darwin, Wallace, and many others, plant and animal communities are most diverse at the lower portion of mountains with diversity typically decreasing at upper elevations (Lomolino 2001). The elevational patterns for plants and animals generally follow three patterns: diversity decreases monotonically with increasing elevation, diversity is high across the lower elevations, and then decreases at mid- to high elevations (low-elevation plateau), or diversity shows a hump-shaped relationship with a mid-elevational peak in richness (Rahbek 1995, Herzog et al. 2005, McCain 2005, 2009, Rahbek 2005, Cardelus et al. 2006). Montane diversity gradients have been studied extensively with many fundamental concepts in ecology and biogeography derived from research on such gradients (Lomolino 2001, Rahbek 2005, McCain 2009). However, previous research on elevational diversity gradients has focused almost exclusively on plant and animal taxa. To our knowledge, there are no studies of microbial elevational diversity that have thoroughly surveyed communities across a complete elevational gradient. To date, the only microbial study examined diversity in a single taxonomic group of bacteria across a 920-m gradient in the Rocky Mountain alpine and subalpine (i.e., 2460–3380 m; Bryant et al. 2008).

One of the primary interests in repeatedly examining large-scale gradients in species richness (e.g., latitudinal and elevational gradients) for various taxa is to

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determine if there are fundamental rules underlying global patterns in the generation and maintenance of diversity. Latitudinal gradients in diversity are almost ubiquitously uniform: highest richness near the equator and decreasing toward the poles (Gaston 2000, Willig et al. 2003). In contrast, microbes do not appear to follow any predictable trend with latitude (Fierer and Jackson 2006, Corby-Harris et al. 2007, Lauber et al. 2009). Elevational gradients occur across a smaller spatial scale, and therefore we might expect that microbes would exhibit elevational diversity patterns that more closely parallel those observed for plant and animal taxa. If elevational trends for microbes differ significantly from plants and animals along the same gradient, then there would be mounting evidence that microbial diversity patterns are governed by fundamentally different factors.

Latitudinal and elevational diversity have been proposed to be related to five main groups of factors: sampling effort, current climate, spatial factors (e.g., area, mid-domain effect), biotic interactions (e.g., competition, mutualisms), and evolutionary trends (e.g., niche conservatism, diversification) (see Gaston 2000, Willig et al. 2003, and McCain 2009). Climatic factors, particularly temperature and precipitation, show the strongest positive associations with both latitudinal (Currie 1991, Gaston 2000, Hawkins et al. 2003, Willig et al. 2003) and elevational diversity patterns (McCain 2005, 2007, 2009) in plants and animals. Additionally, there is growing support that climatic factors may have mediated various evolutionary diversification trends along large-scale gradients (Chown and Gaston 2000, Wiens and Graham 2005, Wiens et al. 2006, 2007). Soil microbial diversity, on the other hand, appears to be more closely related to trends in pH than to climatic trends across western hemisphere latitudes (Fierer and Jackson 2006, Lauber et al. 2009). However, a stronger test of the similarity in diversity patterns among plants, animals, and microbes would be to study their distribution and diversity along the same detailed gradient. In such a case, the coherence of patterns and the uniformity of various diversity hypotheses could be tested simultaneously.

Here we examine a well-studied elevational gradient in the eastern Andes of Peru (see Plate 1) from 200 to 3400 m above sea level (a.s.l), comparing bacterial diversity in three distinct habitats: organic soil, mineral soil, and leaf surfaces. We use a barcoded pyrosequencing procedure to compare bacterial diversity levels and the composition of the bacterial communities in each of the habitats across the elevation gradient. Previous work has demonstrated that this molecular method can effectively detect pronounced gradients in bacterial diversity that correspond to changes in environmental factors (Jones et al. 2009, Lauber et al. 2009, Rousk et al. 2010). We contrasted the microbial elevational patterns with bird, bat, and tree diversity along the same 3.2-km transect. Additionally, we tested several of the proposed factors potentially underlying global biodiversity trends: climatic, spatial, and soil chemistry to determine if microbes exhibit trends in biodiversity that differ significantly from those commonly observed with plants and animals.

Methods

We collected samples from three distinct microbial habitats across the elevational gradient, organic soil (O-horizon material), mineral soil (A-horizon material), and leaf surfaces. These samples were collected on three separate trips to the area (September 2004 for mineral soils, December 2007 for organic soils, and June 2007 for leaves). Detailed descriptions of the elevational gradient are provided in Girardin et al. (*in press*) and Meier et al. (2010). Organic and mineral soil samples were collected from 5–6 plots that were each 100 m^2 in size arrayed across the gradient, with 10 cores collected from randomly selected locations in each plot and homogenized together. Organic soils were collected from the top 5 cm of the O-horizon (essentially 5 cm from the soil surface) with mineral soils collected from the uppermost 5 cm portion of the A horizon (which was typically 5 to 25 cm below the soil surface). See Table 1 for additional information on the soil characteristics. Leaves (~10 g dry mass) were collected from 2 m above the ground surface within each plot from 3 individual trees of the same genus per elevation: *Weinmannia microphylla* (3250 m, 3450 m), *W. bangii* (2750 m), *W. reticulata* (2500 m), *W. lechleriana* (1800 m, 2000 m). See Table 1 for a description of the sites sampled for each habitat type. Soils were held at 4°C for 5 d prior to DNA extraction with DNA extracted using a technique described previously (Lauber et al. 2009). The collected leaf samples were washed in the field using the technique described in Redford et al. (2010), and the filters were stored in 95% ethanol prior to DNA extraction using the MO BIO PowerSoil DNA isolation kit following the manufacturer's instructions (MO BIO Laboratories, Carlsbad, California, USA). All DNA samples were stored at −80°C prior to polymerase chain reaction (PCR) amplification.

We used a barcoded pyrosequencing procedure targeting bacterial 16S rRNA genes to analyze the diversity and composition of the bacteria in each of the collected samples on a 454 Life Sciences Genome Sequencer FLX (Roche, Branford, Connecticut, USA) instrument at the Environmental Genomics Core Facility at the University of South Carolina, Columbia, USA. The procedure was identical to that described previously (Fierer et al. 2008, 2010, Costello et al. 2009, Lauber et al. 2009). The forward primer (5′-GCC TTG CCA GCC CGC TCA GTC AGA GTT TGA TCC TGG CTC AG-3′) contained the 454 Life Sciences primer B sequence, the broadly conserved bacterial primer 27F, and a two-base linker sequence ("TC"). The reverse primer (5′-GCC TCC CTC GCG CCA TCA GNN NNN NNN NNN NCA TGC TGC CTC CCG TAG GAG T-3′) contained the 454 Life Sciences primer

TABLE 1. Site and sample characteristics.

Site elevation (m)	Latitude (° S)	Longitude (° W)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Soil pH		C (%)		N (%)	
					Organic soils	Mineral soils	Organic soils (g C/ 100 g soil)	Mineral soils (g C/ 100 g soil)	Organic soils (g N/ 100 g soil)	Mineral soils (g N/ 100 g soil)
200	12.83	69.26	25.5	4200	3.4	···	5.7	···	0.40	···
440	12.65	71.23	25.0	4000	···	4.1	···	3.3	···	0.3
860	12.62	71.27	23.2	5000	···	3.6	···	9.4	···	0.7
1000	12.95	71.55	19.6	4500	3.4	···	24.9	···	1.25	···
1800	13.07	71.56	16.1	3850	···	4.1	···	9.3	···	0.6
2000	13.08	71.56	14.9	4500	4.10	4.4	46.57	10.9	2.16	0.8
2500	13.09	71.57	12.4	3700	···	···	···	···	···	···
2750	13.11	71.59	11.1	3000	···	3.8	···	11.2	···	0.7
3020	13.11	71.60	9.8	2200	4.20	···	44.84	···	2.32	···
3250	13.11	71.60	8.9	2100	···	4.1	···	13.4	···	0.9
3450	13.11	71.61	7.7	2600	4.12	···	40.09	···	2.08	···

Notes: Ellipses indicate that samples from a given habitat were not collected from a plot at that elevation. The percentages of carbon and nitrogen were measured on a CE Elantech Model NC2100 elemental analyzer (ThermoQuest Italia, Milan, Italy) with combustion at 900°C. Soil pH was measured after shaking a soil/water (1:1 mass/volume) suspension for 30 min.

A sequence, a unique 12-nt error-correcting Golay barcode used to tag each PCR product (designated by NNNNNNNNNNNN), the broad-range bacterial primer 338R, and a "CA" linker sequence inserted between the barcode and the rRNA primer. PCR reactions were carried out in triplicate 25-μl reactions with 0.6-μM forward and reverse primers, 3-μl template DNA, and 1× of HotMasterMix (5 PRIME, Gaithersburg, Maryland, USA). All dilutions were carried out using certified DNA-free PCR water (MO BIO Laboratories, Carlsbad, California, USA). After visualization, purification, and amplicon quantification, the amplicons were combined in equimolar ratios into a single tube.

Sequences were processed and analyzed following the procedures described previously (Fierer et al. 2008, Hamady et al. 2008, Costello et al. 2009). All low-quality sequences and sequences that were not from bacteria were removed prior to downstream analyses. To determine the amount of dissimilarity (distance) between any pair of bacterial communities, we employed a phylogenetic metric (unweighted UniFrac; [Lozupone et al. 2006, Lozupone et al. 2007]). UniFrac distances are based on the fraction of branch length shared between any pair of communities within a phylogenetic tree constructed from the 16S rRNA gene sequences from all communities being compared. A relatively small UniFrac distance implies that two communities are compositionally similar, harboring lineages sharing a common evolutionary history. We examined the correlation between elevation and the Unifrac distances between the bacterial communities in each habitat using Mantel tests as implemented in PRIMER (Clarke and Gorley 2006), an approach widely used in studies comparing microbial communities across gradients (e.g., Lauber et al. 2009, Rousk et al. 2010). We used two indices to compare community-level diversity between the samples. We compared the number of unique phylotypes (with a phylotype defined at the 97% sequence similarity level) and Faith's phylodiversity index (Faith's PD; Faith 1992) in order to compare taxonomic and phylogenetic diversity levels, respectively, across samples. For both diversity metrics, we used a randomly selected subset of 1300 sequences per sample in order to compensate for differences in sampling effort between samples.

Results and Discussion

Elevation is nearly always a complex, indirect gradient along which many environmental variables are changing. However, many climatic factors, such as moisture availability, that often confound studies of montane diversity gradients are held relatively constant across the gradient studied here. Although mean annual temperatures differed by >16°C across the gradient, moisture availability is high at all elevations with precipitation exceeding evapotranspiration every month of the year (J. M. Rapp, *unpublished data*). In addition, all surface soils are well drained and unlikely to become anaerobic, soil pH within each of the horizons sampled is relatively constant, and all sites are within closed-canopy forest (Table 1). As expected, there are clear changes in the diversity of birds, bats, and trees (shown in Fig. 1A), as well as rodents and marsupials (Patterson et al. 1998), across this gradient. Diversity of these "macro"-scopic taxa increases three- to seven-fold as elevation decreases (Fig. 1).

Regardless of the habitat in question, we found no evidence for an elevational gradient in bacterial diversity as there was no significant relationship between elevation and either the number of bacterial phylotypes (Fig. 1B) or phylogenetic diversity within each community (Appendix A), regardless of the model used (linear, second-order polynomial, or third-order polynomial; $P > 0.1$ in all cases). Likewise, if we compare levels of diversity found within the dominant bacterial phyla found in each habitat (Acidobacteria and Proteobacteria

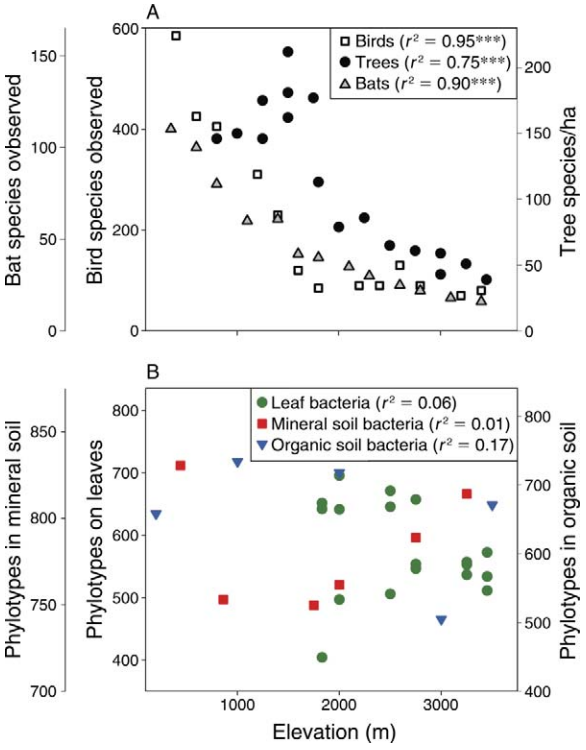

FIG. 1. Changes in plant, animal, and bacterial diversity across the elevational gradient. Asterisks indicate significant regressions. In all cases, results are from best-fit quadratic equations. The number of bacterial phylotypes was estimated from 1300 sequences per sample. Data shown in panel A for birds and bats are from Patterson et al. (1998), and data for trees are from M. Silman (*unpublished data*). These data came from the same transect sampled for this study.

*** $P < 0.001$.

in the soils, Proteobacteria and Cyanobacteria in the phyllosphere) using the same regression models, we did not observe any significant changes in diversity with elevation (Fig. 2). Thus, both at the whole community level and at the level of individual phyla, there was no significant influence of elevation on bacterial diversity, in direct contrast to the patterns observed for plants and animals.

Even with a relatively high level of per-sample surveying effort (a minimum of 1300 bacterial 16S rRNA gene sequences per sample), we have not surveyed the full extent of bacterial diversity in each sample. However, complete surveys are not necessary to compare diversity levels between bacterial communities (Shaw et al. 2008), just as it is not necessary to document every bird species found at a given location to compare diversity levels across plots as long as sampling effort is standardized. This point is demonstrated in Appendix B; in most cases we could resolve differences in diversity between samples, and these differences would have been evident even if we had analyzed fewer sequences per sample. Furthermore, we have shown in previous work that significant differences in bacterial diversity across a range of environmental gradients or sample categories can be readily resolved using the approach described here (Fierer et al. 2008, Costello et al. 2009, Lauber et al. 2009, Rousk et al. 2010). Thus, although it is possible that more in-depth surveys of the collected samples or more detailed surveys of individual bacterial taxa may reveal diversity patterns that more closely parallel those observed for animals and plants (Fig. 1A), we found no evidence for an elevational gradient in bacterial diversity.

Although there were no consistent changes in bacterial diversity across the gradient, each habitat type harbored distinct bacterial communities and the composition of the bacterial communities found in each habitat did change across the gradient (Fig. 3, Appendix

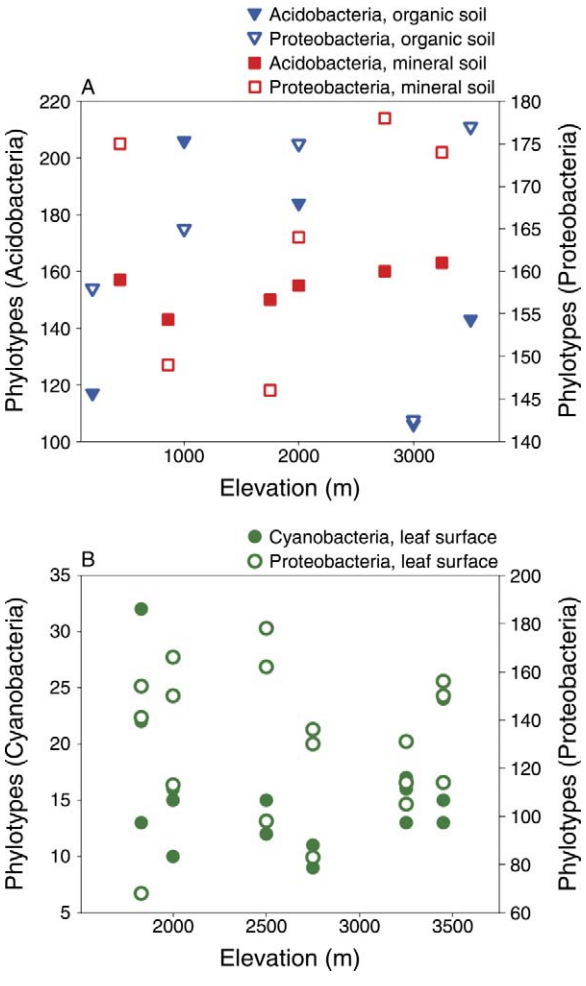

FIG. 2. Changes in the diversity of individual phyla in samples collected from across the elevation gradient. The number of phylotypes was estimated from 300 randomly selected sequences within each phylum per sample. We focused on the most dominant phyla in each habitat: (A) *Proteobacteria* and *Acidobacteria* in soils, and (B) *Proteobacteria* and *Cyanobacteria* on leaves. Regardless of the phylum or habitat type, there was no significant relationship between phylotype numbers and elevation ($P > 0.10$ and $r^2 < 0.20$ in all cases).

C). Regardless of elevation, the soils were dominated by Acidobacteria and Alphaproteobacteria, but the relative abundances of these and other major taxa differed between the mineral and organic soils with the leaf surface communities predominately composed of Cyanobacteria, Actinobacteria, and Alphaproteobacteria (Fig. 3). Within each of these three habitat types, the bacterial communities found at each elevation were significantly different (UniFrac $P < 0.01$ for all pairwise comparisons). For the bacterial communities in the mineral soil and on the leaves, community composition did change with elevation with Mantel tests showing a significant correlation between elevation and the phylogenetic similarity between communities ($r = 0.75$, $P = <0.001$ for mineral soil; $r = 0.70$, $P < 0.001$ for leaf communities). In these two habitats, communities became more phylogenetically distinct with increasing elevational distance as samples collected from similar elevations harbored more similar bacterial communities (see also Appendix C). For the leaf surface communities, the differentiation may be a result of the communities being sampled from different tree species across the gradient. There was no significant correlation between elevation and the phylogenetic distance between communities for the bacterial communities in the organic soil samples ($r = 0.12$, $P = 0.33$). Thus, although there were no detectable effects of elevation on bacterial diversity, the bacterial communities in each habitat were not panmictic across the gradient and, in two of the habitat types, community composition varied in a predictable manner with elevation. As is commonly observed with plant and animal taxa, we observed distinct communities at different elevations; however, the diversity of these bacterial communities does not decrease as we go up in elevation (like birds and bats; Fig. 1) or show a mid-elevation peak (like trees), and, in fact, shows no significant relationship to elevation.

The lack of any apparent elevational gradient in bacterial diversity contrasts with the results reported by Bryant et al. (2008), where the diversity of bacteria within the phylum Acidobacteria was reported to be inversely correlated with elevation from the subalpine to alpine ($r^2 = 0.23$). However, this pattern was likely a product of changes in soil pH, not elevation or temperature per se, given that soil pH decreased monotonically with increasing elevation and diversity levels within this taxonomic group are known to be very sensitive to such changes in soil pH (Jones et al. 2009). More generally, due to the well-recognized influence of soil pH on bacterial community structure (Fierer and Jackson 2006, Baker et al. 2009, Jesus et al. 2009, Lauber et al. 2009), it is important to consider differences in pH when interpreting spatial patterns in soil bacterial diversity. To the best of our knowledge, there are no other studies reporting a significant relationship between bacterial diversity and elevation in the absence of confounding factors.

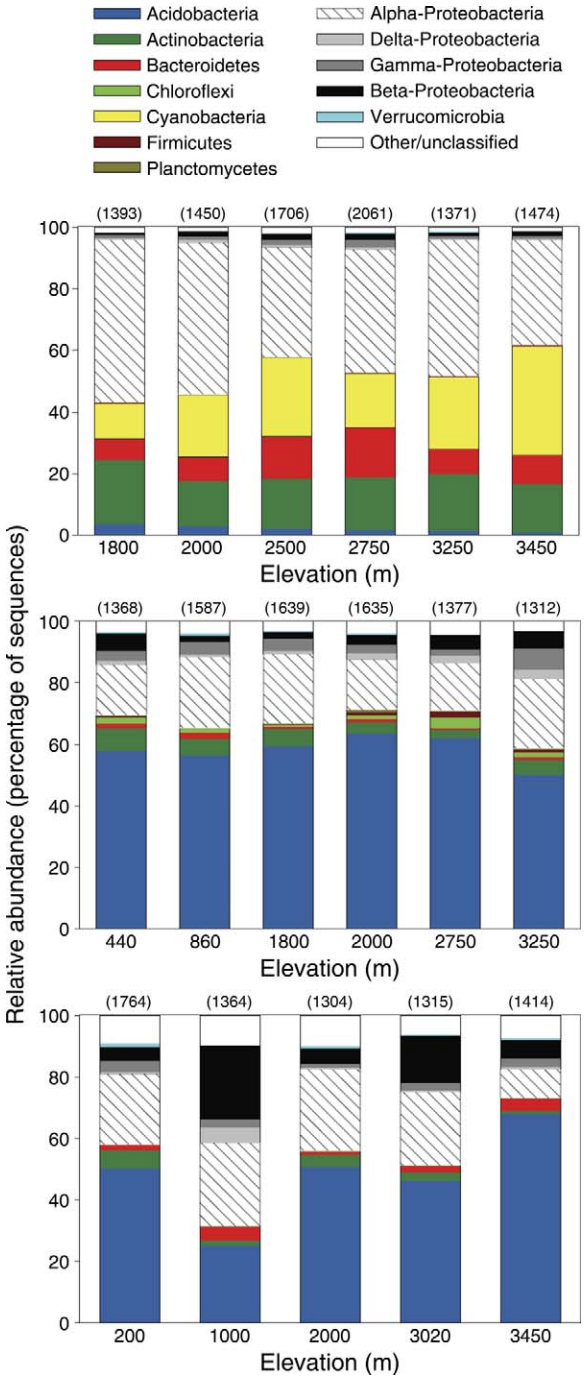

FIG. 3. Changes in bacterial community composition across the gradient in samples collected from (A) leaf surfaces, (B) mineral soil, and (C) organic soil. The number of sequences per sample (or mean number of sequences per sample) is indicated in parentheses. For the leaf surface samples, data represent the mean of the three samples collected per elevation. In all three habitats, samples collected from different elevations harbored significantly different bacterial communities (UniFrac $P < 0.01$ in all cases).

PLATE 1. A view of the elevation transect in eastern Peru from which all samples were collected. The photo was taken from an elevation of 3500 meters, and the view is straight down the ridge that is followed by the transect. The Amazonian lowlands are in the distance, below the clouds, and the tree in the foreground to the left of the photo is a *Weinmannia microphtlla*. Photo credit: J. M. Rapp.

We offer a series of related hypotheses (that are not mutually exclusive) to explain why bacteria exhibit no significant elevational gradient in diversity. First, sampling can be eliminated as the source of the elevational variability in diversity, as survey effort was standardized across the collected samples, a broad gradient in elevations was examined, and because sampling was conducted wholly within undisturbed habitats (McCain 2007, Nogués-Bravo et al. 2008). Second, the strong positive associations between climate and the diversity of birds, bats, and trees (Fig. 1A) were not detected for microbial diversity, or for either temperature or precipitation ($P > 0.1$ in all cases). These results suggest that hypotheses linking diversity patterns directly or indirectly to the temperature dependence of metabolism (Brown et al. 2004) may not apply to bacteria. Third, spatial trends in area and mid-domain effects (MDE) also are unsupported as area decreases exponentially with increasing elevation along this gradient (McCain 2007, 2009) and unimodal peaks in diversity, the primary prediction of MDE (Colwell et al. 2004, McCain 2005), were not detected in any of the three microbial habitats studied. Fourth, given the strong, positive relationship between soil pH and latitudinal microbial diversity trends, soil chemistry may have more important influences on bacterial diversity than either climate or elevation. However, none of the measured soil characteristics (soil pH, percent carbon, percent nitrogen) were strong predictors of bacterial diversity in the habitats studied here. A possible explanation for the lack of a discernable trend with elevation in bacterial diversity is that the spatial heterogeneity within each of the sampling sites may be high, obscuring any inter-site differences in diversity levels across the gradient (Rowe and Lidgard 2009). Alternatively, differences in speciation and extinction rates, or changes in the intensity of competition (Lomolino et al. 2006, McCain 2009) may simply not apply to bacterial communities at the spatial scale we sampled or lack the pertinent evolutionary or ecological data to be sufficiently tested here. We also must consider the possibility that we did not observe any apparent elevational gradient in bacterial diversity because of the molecular methods used to survey bacterial diversity.

Our analyses capture a broader swath of phylogenetic diversity than most plant or animal surveys, and we captured both inactive and active organisms (essentially any bacterial cells with DNA), while plant and animal surveys typically focus only on those organisms that are active at a given point in time. Nonetheless, neither the elevational patterns of microbial diversity, nor the supported factors underlying the elevational changes in diversity, parallel those documented for birds, bats, and trees.

This study, replicated across multiple microbial habitats, adds to the growing body of evidence that bacteria do not necessarily follow the well-established biogeographical patterns that are commonly exhibited by plants and animals. For example, previous work has shown little (Fuhrman et al. 2008) to no relationship (Fierer and Jackson 2006, Corby-Harris et al. 2007, Humbert et al. 2009, Jones et al. 2009, Tedersoo and Nara 2010) between microbial diversity and latitude. Likewise, from previous work we know that the continental-scale structure of bacterial communities does not relate to the general biome classification schemes used by plant and animal biogeographers (Lauber et al. 2009, Chu et al. 2010). Although more research is required, these results presented here suggest that we need more inclusive hypotheses and concepts in the field of biogeography to explain why the well-documented diversity patterns observed for plant and animal diversity differ from those observed for bacteria which represent a considerable portion of the biomass and phylogenetic diversity on Earth.

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APPENDIX A

Changes in overall bacterial diversity in each of the sampled microbial habitats across the elevation gradient (*Ecological Archives* E092-067-A1).

APPENDIX B

Rarefaction curves for the bacterial communities from individual samples collected from the three habitats (*Ecological Archives* E092-067-A2).

APPENDIX C

Percent abundances of bacterial taxa in samples collected from each elevation within each of the three habitats (*Ecological Archives* E092-067-A3).