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Title: Convergence and contrast in the community structure of Bacteria, Fungi and Archaea along a tropical elevation-climate gradient

Running Title: Community Change Along A Tropical Climate Gradient

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KGP, EC and HT carried out data analysis, and all authors contributed to writing of the final manuscript.

Conflict of interest

The authors declare no conflict of interest

Abstract:

Changes in species richness along climatological gradients have been instrumental in developing theories about the general drivers of biodiversity. Previous studies on microbial communities along climate gradients on mountainsides have revealed positive, negative and neutral richness trends. We examined changes in richness and composition of Fungi, Bacteria and Archaea in soil along a 50-1000 m elevation, 280-3280 mm/yr precipitation gradient in Hawai'i. Soil properties and their drivers are exceptionally well understood along this gradient. All three microbial groups responded strongly to the gradient, with community ordinations being similar along axes of environmental conditions (pH, rainfall) and resource availability (nitrogen, phosphorus). However, the form of the richness-climate relationship varied between Fungi (positive-linear), Bacteria (unimodal), and Archaea (negative-linear). These differences were related to resource-ecology and limiting conditions for each group, with fungal richness increasing most strongly with soil carbon, ammonia-oxidizing Archaea increasing with nitrogen mineralization rate, and Bacteria increasing with both carbon and pH. Responses to the gradient became increasingly variable at finer taxonomic scales and within any taxonomic group individual OTUs occurred in narrow climate-elevation ranges. These results show that microbial responses to climate

gradients are heterogeneous due to complexity of underlying environmental changes and the diverse ecologies of microbial taxa.

Keywords:

Biogeochemistry, Climate Change, Elevation, Diversity-Productivity, Hawai'i, pH, Soil
Climosequence

Introduction

Environmental gradients have been instrumental in developing hypotheses about the ecological and evolutionary factors controlling biodiversity (Pianka 1966, Chown and Gaston 2000). The consistent decrease in species richness with increasing altitude (Gaston 2000) along with parallel changes in climate (Körner 2000) suggest the existence of general principles controlling biodiversity. However, the studies that have examined microbial diversity along altitudinal gradients (Bryant et al. 2008, Fierer et al. 2011, Corneo et al. 2013, Looby et al. 2016, Wu et al. 2017) have shown variable results. The lack of consistent microbial diversity patterns along climate gradients has suggested new and/or more inclusive hypotheses about the underlying drivers of biodiversity are necessary (Fierer et al. 2011). Thus, two major challenges in microbial ecology are determining the extent to which microbes show coordinated patterns in their distributions across major climate and environment gradients, and identifying hypotheses to explain these patterns.

Tropical elevational gradients may be particularly useful for understanding the controls over microbial biodiversity because they encompass large changes in climate and the environment over relatively compact spatial scales, without the confounding biogeographic changes that occur along latitudinal gradients (Malhi et al. 2010). The Hawaiian islands, and the Island of Hawai'i,

in particular, have been studied extensively to understand nutrient cycling and ecosystem development (Vitousek 2004) – and as a result, major gradients of soil properties and nutrient availability are well understood, as are their underlying climate and geological drivers (Chadwick et al. 2003). On the northwest coast of the Island of Hawai'i, annual precipitation ranges from <300 to >3000 mm and annual temperatures range from 16 °C to 23 °C along a 14 km transect that reaches from sea level to 1000 m. Previous studies on this gradient have demonstrated the importance of pedogenic thresholds (i.e. locations where soil properties or processes change abruptly and/or non-linearly with a small increment in climate), and conversely of domains within which soil properties and processes change relatively little across a wide range of key climate variables (Vitousek and Chadwick 2013). However, the composition of the microbial communities present in these soils, and the framework that shapes their community development has yet to be explored.

We hypothesize that within the soil microbial community there will be a variety of responses to elevation due to the complexity of environmental change with climate along this elevation gradient and the ecological diversity of microbial taxa. To test this hypothesis, we examined microbial communities along the elevational transect on Hawai'i to determine the extent that microbial taxa show coordinated changes in community structure - both composition (i.e. species identity) and diversity (i.e. numbers of species) - and to identify the specific environmental variables (i.e. rainfall, relief, and nutrient availability) that explain these changes. We use high-throughput DNA sequencing to identify Fungi, Bacteria and Archaea from topsoils at 46 sites along this elevation transect and present comparative distributions of these microbial communities, coupled with climate and soil biogeochemistry measurements, to infer how the communities respond to changing environmental conditions. Given the large changes along this

gradient in soil biogeochemical factors (e.g. pH, nitrogen, phosphorus) that are known to influence microbial communities (Treseder 2004, Fierer and Jackson 2006, Gubry-Rangin et al. 2011) we expected that there would be large changes in richness and composition of microbes along this elevation transect. However, because of the widely differing ecologies and evolutionary histories of the taxonomic groups involved, we also hypothesized that the specific nature of community change along the transect would vary greatly between microbial taxa in ways related to their resource acquisition strategies. For example, at the broadest taxonomic scale we expected that bacteria would respond most strongly to soil pH, fungi to soil carbon concentrations, and archaea to pH and nitrogen availability.

Methods

Sites

Field sites were located along a transect on the leeward slope of Kohala Volcano on the Island of Hawai'i (approximately 20.15° N, -155.8° W), where topsoils developed in parent material of the (ca. 150 kyr) Hawi Volcanic Formation. The transect was approximately 14 km in length, from 50 to 1000 m elevation, over which mean annual precipitation increased from 280-3280 mm/yr and mean annual temperature decreased from 16 – 23°C (Giambelluca et al. 2013). A majority of the transect parallels a transect previously evaluated by Chadwick et al. (2003), also on Hawi Formation substrate, and it was included within the larger set of sites summarized by Vitousek and Chadwick (2013). Past vegetation, soil dynamics, and the land use history of the region are discussed in Chadwick et al. (2007) and Kagawa and Vitousek (2012). All rainfalls reported are derived from Giambelluca et al. (2013). Because these sites occur over a compact spatial scale and have developed on identical parent material, soil chemical differences between sites arise

primarily from effects of climate on weathering and biological activity. Temperature and precipitation are highly correlated across the elevation gradient ($r = 0.988$, $p < 0.001$; von Sperber, et al. 2017) making it impossible to statistically separate these two factors. While studies have generally viewed elevational gradients from the perspective of temperature, in this system evidence suggests that precipitation is the most important driver of geochemical variation (Chadwick et al. 2003, Vitousek & Chadwick 2013), and we believe it is the most important driver of biological responses as well because the range of annual precipitation values (275 – 3238 mm) across sites is much more biologically relevant than the corresponding range of average annual temperatures (16 – 23°C). In addition, a recent study of Hawaiian soil bacteria along a similar temperature-elevation gradient, but where precipitation was held constant, found no effect on bacterial richness (Selmants et al. 2016). For this reason, although other factors such as temperature also change with elevation, we interpret this environmental gradient as being driven primarily by rainfall.

Field Sampling

Soil samples were collected every 300–500 meters along an East-West transect (a total distance of 14 km), with collections at 46 points (**Fig S1**). The location and elevation of each sample along the transect was recorded using GPS and the corresponding mean annual rainfall extracted from Giambelluca et al. (2013). At each point, a soil column starting in the A horizon was excavated to a depth of 30 cm using a spade, and an integrated sample was taken from 0-30 cm. In some of the drier sites, the sample also included the uppermost B horizon (Chadwick et al. 2003). Soils were homogenized and split into subsamples for measurements of water content and soil chemistry; additional soil properties and processes were evaluated from the information in

Vitousek and Chadwick (2013). In addition, a 0.25 g (wet weight) sample was placed in Lifeguard preservation buffer (MoBio, Carlsbad CA, USA) on the day of collection to preserve genetic material for later sequencing. Samples were refrigerated (4°C) on the day of collection and shipped to Stanford University, where they were stored at -80°C until DNA extraction.

Soil chemistry

To determine which environmental factors most strongly affect microbial community structure along the transect, we collected a range of soil data, including pH, exchangeable calcium, percent carbon, percent nitrogen, carbon:nitrogen ratio, resin phosphorus, initial ammonium and nitrate, and net nitrogen mineralization. Details on how each sample was processed and how the environmental data was collected are included in the Supplemental Methods.

Microbial community characterization

DNA was extracted from 0.25 g of frozen soil using the MoBio PowerSoil DNA extraction kit (MoBio, Carlsbad CA, USA) according to manufacturer's instructions. To characterize fungal community structure we used PCR to target the first internal transcribed spacer (ITS1) region of the nuclear ribosomal RNA genes using the Illumina primer constructs of Smith and Peay (2014). These primers are based on the ITS1f and ITS2 fungal specific primer pair (Gardes and Bruns 1993) with addition of the Illumina Nextera forward and reverse adapters to the 5' end of the ITS1F and ITS2 primer, respectively. A unique ten base pair golay barcode was included between the ITS2 primer and the Nextera adapter sequence, so that the final primer sequences were ITS1f (AATGATACGGCGACCACCGAGATCTACACGGCTTGGTCATTTA GAGGAAGTAA) and ITS2 (CAAGCAGAAGACGGCATACGAGATNNNNNNNNN

NCGGCTGCGTTCTTCATCGATGC). To characterize the bacterial and archaeal community structure we amplified the V2 domain of the 16S ribosomal RNA genes using the 515f and 806r-based Illumina primer constructs of Caporaso et al. (2012), which have a similar design as the fungal primer constructs (515f, AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA, 806r, CAAGCAGAAGACGGCATAACGAGATNNNNN NNNNNAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT). For both primer sets PCR was carried out in a single step using hot-start Taq and 30 amplification cycles. Barcoded PCR products were cleaned and normalized using SequelPrep plates (Invitrogen, Carlsbad CA, USA), pooled and sequenced in a single MiSeq run using 2 x 250 chemistry at the Stanford Functional Genomics Facility (Stanford University, Stanford, CA). To reduce sequencing error and uncertainty each sample was amplified in duplicate (for both ITS and 16S) using separate barcodes and sequenced independently along with extraction and PCR negative controls. Raw sequence reads for all samples are deposited in the National Center for Biotechnology Information Sequence Read Archive under Bioproject PRJNA379981 Biosamples SAMN06627646 - SAMN06627991.

Demultiplexed FASTQ files were trimmed to remove primers and low quality bases with the program CutAdapt (Martin 2011) using just forward direction reads (due to poor quality reverse reads), quality filtering, OTU (operational taxonomic unit) clustering (determined with a 97% cutoff) and chimera removal was performed using UPARSE 5.2.32 (Edgar 2013). Taxonomic identity was assigned in QIIME 1.9.0 (Caporaso et al. 2010) using top BLAST match against the UNITE reference database for Fungi (minimum e-value <0.001) and using the RDP classifier (Wang et al. 2007) with the GreenGenes reference dataset for Bacteria and Archaea (McDonald

et al. 2012). To minimize the potential effects of sequencing or contamination artifacts, we eliminated all OTUs where abundance in the negative controls was >1% of total OTU abundance, and eliminated all OTUs that were not present in both sequencing replicates of each sample. Community analyses for Fungi and Bacteria were carried out by first eliminating all OTUs in the ITS or 16S reads that could not be assigned to either Fungi or Bacteria, respectively, and then rarefying to 6000 sequences per sample. Because of the much lower total prevalence of Archaea, the 16S rRNA dataset was first rarefied to 6000 sequences per sample and then subset to include only OTUs assigned to Archaea. An alternative approach, first subsetting the full 16S dataset to include only Archaea and then rarefying to 100 sequences per sample, gave similar model results and richness estimates (linear regression $r^2 = 0.87$ between the two estimates) but eliminated a large number of samples, and so is not presented here. DNA extracts from all the sites were also used to determine the abundances of ammonia-oxidizing archaea (AOA) using quantitative PCR (qPCR) of the archaeal *amoA* gene (encoding ammonia monooxygenase subunit A), using primers Arch-amoAF (5'-STAATGGTCTGGCTTAGACG-3')/Arch-amoAR (5'-GCGGCCATCCATCTGTATGT-3') (Francis et al. 2005), as described previously (Mosier and Francis 2008) (see Supplemental Materials).

Statistical analysis

To visualize the response of fungal, bacterial, and archaeal diversity along the gradient, we plotted OTU richness for each group against mean annual rainfall (and elevation) along the gradient. Because we believe variation in rainfall, and attendant soil moisture/drought stress, is greater and more important biologically than the rather small (if consistent) variation in temperature, we have chosen to focus our analyses and discussion of the gradient in terms of

precipitation. We fit the relationship using linear regression and included a squared term (if significant) to account for unimodal relationships using R V3.2 (R Core Development Team 2016). To determine which soil variables most influenced microbial richness along the gradient, we used stepwise regressions with forward and backward selection using the stepAIC function in the MASS package (Venables and Ripley 2002). For statistical modeling, we used pairwise scatterplots to choose soil environmental variables that minimized redundancy, but maintained biologically distinct sets of conditions and resources. For example, ex-Ca, ex-Mg, ex-K and CEC were strongly correlated and represent a similar resource category. Variables ultimately included in the full model from this process were pH, resin extractable phosphorus (P), initial NH_4^+ -nitrogen ($\text{NH}_{4\text{init}}$), exchangeable calcium (ex-Ca), net nitrogen mineralization at field moisture ($\text{net-N}_{\text{min.field}}$), net nitrogen mineralization adjusted to field capacity ($\text{net-N}_{\text{min.adj}}$), percent nitrogen concentration (%N), percent carbon concentration (%C), carbon to nitrogen ratio (C:N ratio), carbon to phosphorus ratio (C:P ratio), and nitrogen to phosphorus ratio (N:P ratio). Pairwise relationships among the included predictor variables are given in **Fig S2**. We calculated the relative importance of variables in the final models as the R^2 contribution averaged over orderings among regressors (the “lmg” option in calc.relimpo from the relaimpo package; Grömping 2006). We visualized the effect of individual predictors in the final model while controlling for all other predictors using the avPlots function in the CARS library (Fox and Weisberg 2011).

Changes in community composition along the gradient were visualized with non-metric multidimensional scaling (NMDS) using the metaMDS function in the vegan package (Oksanen et al. 2008). Sequence counts were $\log x+1$ transformed to reduce the influence of dominant species and possible PCR bias. Using the ordisurf function (vegan) we then fit elevation isolines

on the NMDS ordination plots. To determine which environmental variables best predicted variation in community composition, we used the distLM function in the permANOVA+ plugin in PRIMER v6.1.13 (Clarke and Gorley 2006), using forward & backward step selection based on the Akaike information criterion (AIC). The final model was fit using the capscale function (vegan) for distance based redundancy analysis.

To understand how taxonomic composition changed across the gradient, we plotted the relative sequence abundance for major lineages within Fungi (Basidiomycota, Ascomycota), Bacteria (Acidobacteria, Actinobacteria, Firmicutes) and Archaea (Nitrososphaerales). In addition, we plotted the individual distribution of the most abundant OTUs. For both datasets (lineages and individual OTUs), relative abundance data were fit with the “super smoother” smoothing function supersmu within R, to help identify distribution patterns.

Results

The soil microbial community we observed across this Hawaiian elevation-climate transect was highly diverse, with 5428 bacterial OTUs from 1.46×10^6 sequences, 1715 fungal OTUs from 1.3×10^6 sequences, and 57 archaeal OTUs from 2.8×10^4 sequences in the final dataset (**Table S1**). Per sample richness after rarefaction to 6000 sequences was greatest for Bacteria (mean \pm sd = 527 ± 142), then Fungi (98 ± 45) and Archaea (9 ± 5). Richness of Fungi, Bacteria and Archaea changed significantly across the gradient, and the shape of the richness responses differed between taxonomic groups (**Fig 1; Table S2**), with fungal richness increasing monotonically with increasing rainfall (and elevation), bacterial richness highest at an intermediate position along the rainfall-elevation gradient, and archaeal richness generally highest at low rainfall and elevation. Richness was uncorrelated between fungi and other groups

(Pearson correlation $P > 0.05$), but there was an overall positive correlation between bacterial and archaeal richness (Pearson's $r = 0.37$, $P = 0.005$). The best univariate predictors (excluding rainfall and elevation) varied between taxa (Table S2), with %C for fungi ($r^2 = 0.33$), pH for bacteria ($r^2 = 0.11$), and %N for Archaea ($r^2 = 0.36$). The soil variables selected for multivariate models also varied between groups (**Fig 2**). In order of relative importance, fungal richness was most strongly affected by the amount of carbon (%C), pH, % N, C:P ratio, N:P ratio, and available phosphorus (resin P) of soils (Multiple $R^2 = 0.53$, $P < 0.001$; **Table S2**). For Bacteria, % C, C:P ratio, pH, and NH_4_{mit} were the best predictors of richness (Multiple $R^2 = 0.42$, $P < 0.001$, Table S2). For Archaea, the final model included net N mineralization at both field ($N_{\text{min.field}}$) and adjusted moisture ($N_{\text{min.adj}}$), %C, %N, and C:N ratio (Model $\text{adj-}R^2 = 0.61$, $P < 0.001$; **Table S2**). The direction of these relationships differed in important ways between taxonomic groups. For example, bacterial richness increased and fungal richness decreased with pH, and fungal and bacterial richness increased and archaeal richness decreased with %C (**Fig 2**, **Table S2**). A number of variables showed different responses in univariate and multivariate models. For example, %C was not a significant univariate predictor of bacterial richness but had a strong positive effect in multivariate models, while %N was positively correlated in a univariate model of fungal richness but negatively correlated in the best multivariate model (**Table S2**).

By contrast, Fungi, Bacteria and Archaea showed generally convergent patterns of community similarity across the gradient. For all three taxa, both constrained and unconstrained ordinations had a unimodal shape, with intermediate sites along the transect at the apex (**Fig 3**). Distance based redundancy (dbRDA) models explained 37% of community variation for Fungi, 55% for Bacteria, and 63% for Archaea. For both Fungi and Bacteria, the model with the lowest

AIC included pH, net N mineralization (both $\text{net-N}_{\text{min. field}}$ and $\text{net-N}_{\text{min. adj}}$), exchangeable Ca and N:P ratio. For Archaea, the best model included pH, $\text{net-N}_{\text{min. field}}$, %C, ex-Ca, C:P ratio and N:P ratio.

Individual taxonomic groups showed very different response patterns across the gradient. For Fungi, Ascomycota (53.5% of fungal sequences) decreased linearly and Basidiomycota (29%) increased linearly as precipitation increased (**Fig 4**). For Bacteria, Proteobacteria (52%) rapidly increased and then plateaued around 1000 mm of rainfall, Acidobacteria (14% of bacterial sequences) were most abundant at both the driest and wettest ends of the gradient, while both Actinobacteria (9%) and Firmicutes (6%) decreased with increasing precipitation (**Fig 5**). For Archaea, the Nitrososphaerales (89% of archaeal sequences) showed a unimodal distribution, with peak abundance between 500-800 mm of annual rainfall and then tapering off >2000mm (**Fig 6a**). The archaeal *amoA* qPCR data (**Fig 6b**) generally supports the 16S rRNA-based archaeal abundance trends along the rainfall gradient between 0 and 1250 mm, particularly the major peak between ~500-1250m. Overall, most individual OTUs within Phyla occupied a restricted range of the gradient with single distinct abundance peaks (**Fig 4-6**).

Discussion

Diversity and composition of taxa spanning the three domains of microbial life changed dramatically along this tropical environmental gradient. These changes were evident despite occurring over a relatively small spatial scale (~14 km; ~1000m elevation), on soils derived from a single parent material, and under a simplified plant community composed primarily of introduced pasture grasses. While natural environmental gradients are inherently complex, the

differential rates and patterns of change among soil variables (e.g. Vitousek and Chadwick 2013) enables us to at least partly disentangle some of the major factors thought to influence microbial community structure. While Fungi, Bacteria, and Archaea responded strongly to these coupled changes in climate and soil processes across the gradient analyzed, there were also important differences in the direction of change between taxonomic groups and the importance of specific soil variables. While a number of functional traits appear to be distributed broadly among microbial taxa (Martiny et al. 2015), the results we see suggest that there are important ecological features (e.g. pH optimum, carbon sources) that consistently differ between these major taxonomic groups.

Controls over microbial richness

Prior studies of microbial diversity over elevational/climate gradients have reported mixed diversity trends. Fierer et al. (2011) found no significant changes in soil bacterial richness over a 3200m elevation change in the Peruvian Andes, despite strong changes in plant and animal richness over the same gradient. Bryant et al. (2008) found that Acidobacteria decreased in richness over a change of 1000 m elevation in the Rocky Mountains. Similarly mixed results have been found for Fungi, with evidence for greatest richness at mid-elevations due to range overlap (Bahram et al. 2012, Counce et al. 2014, Miyamoto et al. 2014), lack of change with elevation (Zimmerman and Vitousek 2012, Counce et al. 2014, Jarvis et al. 2015), decreased richness at higher elevations (Looby et al. 2016), or increased richness at higher elevations (Pellissier et al. 2014). Fewer studies have examined Archaea, but work on two Japanese mountains found a mid-elevation peak in OTU richness (Singh et al. 2012, Singh et al. 2016).

Overall, we observed large changes in the richness of all microbial groups along the environmental transect studied. These differences in richness were ~2-5-fold depending on the group; they are comparable or even larger than changes seen for Bacteria (Ladau et al. 2013) and Fungi (Tedersoo et al. 2014) across large latitudinal ranges. The primary microbial groups we examined showed somewhat different trends, either decreasing (Archaea), increasing (Fungi) or unimodal (Bacteria) along the gradient. The inconsistency of microbial richness responses across studies and taxonomic groups contrasts with the consistently strong patterns of monotonically declining or mid-elevation peaks for macrobial taxonomic groups (Rahbek 1995, Gaston 2000). However, the different patterns we observe for Bacteria, Fungi and Archaea is consistent with overall variability in the microbial literature. Other studies along elevation gradients have generally viewed changes in temperature, the factor that changes most consistently with elevation (and latitude) as the driving variable (Rahbek 1995, Gaston 2000, Fierer et al. 2011). While we cannot entirely disentangle temperature and precipitation in this system due to their high correlation, the biogeochemistry along this soil climosequence is probably most strongly influenced by precipitation (Chadwick et al. 2003). While there is certainly evidence that temperature alone can influence microbial diversity (Zhou et al. 2016), the inconsistent responses to elevation in the microbial literature and across taxonomic groups in our study suggests that other environmental factors likely play important roles in controlling the diversity of soil microbial communities. For example, a recently published examination of bacterial richness on the island of Hawaii along a similar elevation-temperature gradient, but where precipitation was constant, found no effect of temperature on bacterial richness (Selmants et al. 2016). Based on these results, we instead hypothesize that taxon-specific soil resources (or constraints) appear to most directly influence microbial diversity. The diversity of microbial diversity responses

observed in the literature likely reflects the unique ways in which changes in precipitation and temperature influence soil resource availability in specific systems and the particular taxonomic groups being studied.

There has been much debate in ecology about what controls richness of local communities in general (Ricklefs 1987, Hubbell 2001), and specifically along elevation gradients (Rahbek 1995). For Fungi, previous studies have examined how the distribution and diversity at the level of all Fungi (Zhang et al. 2013), at the functional group level (Bahram et al. 2012) and at the phylum level (Looby et al. 2016) change over an elevational gradient, and found that changes across the gradient became more apparent as lower taxonomic levels and/or ecological trait differences were accounted. We see similar patterns in our system, and changes in richness across this soil climosequence were clearly linked to resources for each group. For example, Fungi are the predominant terrestrial decomposers of plant cell tissues (de Boer et al. 2005), and the increase in fungal richness at higher soil carbon concentration may be linked with a greater diversity of soil carbon niche axes. While we did not measure the presence of specific carbon compounds, the increase in Basidiomycete fungi at high elevation/carbon concentration is consistent with their primary role in the production of oxidative enzymes capable of breaking down recalcitrant carbon compounds, such as lignin (Baldrian 2006, Floudas et al. 2012). Alternatively, C:N, C:P and N:P ratios also peaked at high elevation, and fungi are thought to outcompete bacteria in these conditions (Strickland and Rousk 2010). Overall high ratios of these elements tended to have positive influences on fungal richness and negative influences on bacterial richness (Table S2), although elemental ratios were not the strongest predictors of fungal richness.

The most abundant Archaea in our study belonged to a lineage (Nitrososphaerales) known for their role in ammonia oxidation (Stahl and de la Torre 2012). This is a critical process within

the terrestrial nitrogen cycle, and may explain the positive relationship between archaeal diversity and nitrogen mineralization observed. Previous studies have found that the abundance of AOA is controlled by pH (Gubry-Rangin et al. 2011, Gubry-Rangin et al. 2015) and C:N ratio (Bates et al. 2011), both of which influenced archaeal richness in our study as well. For Bacteria, richness was most consistently positively affected by pH (**Table S1**), consistent with other studies (Fierer and Jackson 2006, Lauber et al. 2009). While pH was one of the best univariate soil variable predicting bacterial richness, in multivariate models carbon (which was a non-significant univariate predictor) had a strong positive effect. This makes sense as more carbon provides more energy to fuel heterotrophic bacterial growth, however bacterial richness does not peak with soil %C due to the negative effect of other variables, such as decreasing pH and increasing carbon to nutrient stoichiometry. For example, C:P ratios had consistently negative effects on bacterial richness in both uni- and multivariate models. For Fungi, our results are consistent with the view that fungi are less sensitive to pH changes than bacteria (Rousk et al. 2010, Peay et al. 2016).

Controls over microbial community composition

While Fungi, Bacteria, and Archaea exhibited contrasting richness patterns with elevation-climate, patterns of change in community composition were largely convergent, as evidenced by an inverted u-shaped distribution in both constrained and unconstrained ordinations (**Figure 4**). While peaks in richness may indicate conditions that promote species coexistence (i.e. greater niche packing), changes in community composition more strongly reflect the environmental niche breadth of taxa. For instance, our results suggest that for Fungi a greater number of available niches exist in in wet, high carbon, environments, but also that most of the fungi found

in those sites are successful over only a limited portion of the environmental conditions spanned by our study. Thus, while Fungi, Bacteria and Archaea vary in the resource combinations that maximize their diversity, species in all groups tended to have narrow environmental niches along the elevation-climate gradient.

While microbial community composition can sometimes be strongly influenced by host plant identity, within the pasture grass communities we sampled, the magnitude of this effect was likely small. All the dominant plants are perennial C4 pasture grasses, the two most abundant (*Pennisetum clandestinum* at higher rainfall, and *Cenchrus ciliare*) are closely related (Chemisquy et al. 2010), and the two grass species dominate sites receiving above and below 1200 mm/yr of precipitation (where there is no distinct break in microbial communities). Moreover, Schlatter et al. (2015) and Peay et al. (2015) both found negligible effects of plant species identity on bacteria and fungi, respectively. Similarly, Zimmerman and Vitousek (2012) found strong changes in fungal endophyte composition along an elevation gradient in Hawai'i, despite sampling from a single host. In contrast with richness, redundancy analysis showed that the factors affecting community composition were highly overlapping across groups. Axis 1 of the ordinations was most strongly aligned with changes in pH, which changes monotonically with rainfall. Axis 2 was most strongly associated with indicators of resource quality (N mineralization, exchangeable Ca, resin P) that peaked at intermediate elevations. Consistent with this, the abundance of oligotrophic lineages of Bacteria (e.g. Acidobacteria; **Fig 6**) and Fungi (Basidiomycota; **Fig 5**) decreased at intermediate portions of the gradient, and reached their peak in abundance at low-fertility, high-rainfall, low-pH sites. Overall, these results illustrate that the primary controls on microbial community composition can be separated into two major axes, environmental-physiological constraints (Axis 1) and nutrient availability (Axis 2). These axes

likely influence the microbial community somewhat independently, as evidenced by the ordinations, even if they are not independent of each other.

Overall, our results with regard to community composition are consistent with recent work suggesting that patterns of phylogenetic distribution of microbes across habitats result from evolutionary conservation of ecological strategies (Treseder et al. 2014, O'Dwyer et al. 2015). Many major taxonomic groups showed clear patterns of distribution; for example, Proteobacteria contain many copiotrophs (Fierer et al. 2007) and peaked in relative abundance at intermediate elevation where N-mineralization and other measures of soil fertility were highest. Ascomycota were most abundant at the dry end of the gradient, which is consistent with some work showing that Ascomycota diversity tends to be relatively higher in xeric environments (Smith et al. 2007). However, some recent studies have suggested that Ascomycota are generally less drought-tolerant than Basidiomycota (Lennon et al. 2012, Treseder et al. 2014), suggesting that there is also a possibility that their abundance in this section of the gradient is related to other environmental factors. While the most parsimonious explanation for these taxonomic patterns likely arise from shared environmental preferences, given the strong gradient here, they may also be driven by positive interactions, such as niche-complementarity or facilitation (Toju et al. 2016). Better functional characterization of soil fungi and bacteria (Martiny et al. 2015, Treseder and Lennon 2015) would aid in distinguishing between these different ecological mechanisms.

Individual OTUs generally showed unimodal peaks in abundance and occupied only a small portion of the total gradient (**Fig 5-7**). This type of distributional pattern is consistent with environmental filtering observed along other gradients (Whittaker 1956). The continuous turnover of individual OTUs we observe in the field confirms results from experimental studies showing significant variance in physiological optima and tolerances among microbial species

(Lennon et al. 2012). Such niche partitioning is likely a large contributor to the high beta-diversity of microbial communities in this and other systems. In addition, while there were clear trends across phylogenetic lineage (i.e. overall decreasing abundance of Ascomycota, Actinobacteria and Thaumarchaeota with rainfall), at the OTU level there were also many clear exceptions in each domain. For example, while Basidiomycota were most abundant at high rainfall, Basidiomycota OTU15 was abundant and restricted to low elevation. Also, Acidobacteria were generally most abundant at high rainfall but, upon closer inspection of the distribution at the OTU level, it is evident certain OTUs (e.g., OTU 2357, OTU 43, OTU 3943) are actually most abundant at mid-elevation (**Figure 5**). This is also true for the distribution of some Thaumarchaeota sequences; while 9 of the 10 most abundant archaeal 16S OTUs show greatest relative abundance between 300 and 1200 mm annual rainfall (**Figure 6c**), a single unimodal, mid-elevation relative abundance peak exists for archaeal OTU 78 at 700 m. The lack of ecological coherence for all OTUs within a higher taxonomic group seen here provides evidence that phylogenetic generalizations about ecological strategies can be useful but must be interpreted with caution (Philippot et al. 2010).

Despite the existence of clearly limited environmental ranges for most OTUs, within any portion of the gradient there also were many OTUs with overlapping peaks in distribution. While this is not a surprising outcome, given the high alpha-diversity of microbial communities, it suggests two possible scenarios for coexistence. First, soils are highly heterogeneous at small scales, and there are likely many additional niche axes that species may partition. For example, microbial communities are often vertically stratified along the soil profile (Lindahl et al. 2007, Baldrian et al. 2012). Alternatively competitive exclusion may play only a weak role in structuring microbial communities within a given portion of the gradient. Neutral dynamics have

been suggested to be important in other high diversity systems, where the number of species outstrips the apparent dimensionality of niche axes (Hubbell 2001, 2005). Experiments to tease apart the relative importance of equalizing and stabilizing mechanisms (Chesson 2000, Levine and HilleRisLambers 2009) in maintaining microbial alpha-diversity would be a promising area for future research.

The microbial role in nutrient cycling

The changes in microbial community composition that we observe occur in tandem with large changes in the availability of nitrogen, phosphorus and carbon, among other elements. While it is clear that microbial metabolic activity drives major geochemical cycles (Falkowski et al. 2008), there has been debate about the relative extent to which microbes versus environmental conditions control observed variation in these processes (Schimel 1995). Experimental manipulations have shown that microbial community composition can directly influence ecosystem processes (Strickland et al. 2009, Allison et al. 2013). In this system, it is clear that rainfall exerts primary control over the cycling of rock-derived elements (Vitousek and Chadwick 2013), and we interpret most of our results from this perspective. While precipitation may set the stage, and resulting differences in nutrient availability may proximately control the importance of many microbial OTUs, the unique physiological traits of microbial communities along the gradient may still constrain nutrient cycles along the gradient (Diaz and Cabido 2001, Treseder and Lennon 2015), particularly for dynamic elements like nitrogen. Indeed, both 16S rRNA and functional gene data revealed that AOA (Thaumarchaeota) are present to varying extents (with pronounced maxima and minima) in soils across the entire rainfall/elevation/pH gradient; furthermore, detection of nitrification activity in these same soils (von Sperber 2017)

suggests that these diverse AOA are not only present but are biogeochemically-active members of the microbial community. In addition, microbial composition may also contribute to variability in ecosystem functions within sections of the gradient (Allison et al. 2013).

Conclusion

Climate-environment altitudinal gradients have long provided natural laboratories for the development of ecological theory and for predictions of future climatological changes. The contrarian nature of microbial diversity along these gradients has sometimes challenged existing ecological theories. Here we show that there are strong changes in diversity and composition of microbes in three domains of microbial life along a tropical environmental gradient, and that climate change likely will impact microbial communities primarily through its effects on soil properties and biogeochemistry. Where trends within Fungi, Bacteria, and Archaea do not match each other they are understandable in terms of the physiological constraints and ecological strategies evolved by each group. This suggests that theories of biodiversity can be generalized by rooting them in the specific evolutionary history or ecological strategy of the taxonomic groups under study. Determining the appropriate phylogenetic scale at which these generalizations can be made is an important research endeavor and will also have practical applications, such as predicting how climate change could affect soil microbial community development.

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Figure Legends

Figure 1. Changes in microbial richness along a tropical elevation-climate gradient on the Island of Hawai'i. OTU richness for Fungi, Bacteria, and Archaea were estimated after rarefying to 6000 sequences (see text for details). Linear models show different response patterns with respect to elevation and rainfall for Fungi (increasing), Bacteria (unimodal) and Archaea (unimodal / decreasing).

Figure 2. Drivers of microbial richness along a tropical elevation-climate gradient on the Island of Hawai'i. OTU richness for Fungi (green), Bacteria (yellow), and Archaea (red) were estimated after rarefying to 6000 sequences. Partial regression plots show the relationship between the best predictors based on AIC selection and richness (model residuals) for each taxonomic group after controlling for the effect of other significant predictor variables in the model. Panels are arranged left to right in order of decreasing explanatory power, measured as partial r^2 from multivariate models.

Figure 3. Drivers of microbial composition along a tropical elevation-climate gradient on the Island of Hawai'i. Points represent the set of species characterized from individual soil samples for Fungi (green), Bacteria (yellow) and Archaea (red). In unconstrained ordinations (NMDS, left panels) the colors change from light (dry) to dark (wet), with elevation (m) isoclines to illustrate community change along the gradient. Constrained ordinations (db-RDA, right panels) show vectors for significant predictors based on AIC model selection, where length and

orientation of arrows indicate the strength and direction of greatest community change, respectively, for each variable.

Figure 4: Distribution of fungal lineages and OTUs across a tropical elevation-climate gradient on the Island of Hawai'i. Left panels show contrasting patterns for the two most abundant phyla (Basidiomycota, Ascomycota). Right panels show smoothed lines fit to the distribution of the top 100 OTUs for each phyla (grey), with colored lines overlain for the 10 most common OTUs. The y-axis shows abundance relative to each OTUs maximum. Most taxa show clear preferences for particular zones along the gradient.

Figure 5. Distribution of bacterial lineages and OTUs across a tropical elevation-climate gradient on the Island of Hawai'i. Left panels show contrasting patterns for three of the most abundant phyla (Proteobacteria, Acidobacteria, Actinobacteria). Corresponding panels on the right show smoothed lines fit to the distribution of the top 100 OTUs for each phyla (grey), with colored lines overlain for the 10 most common OTUs. The y-axis shows abundance relative to each OTUs maximum. Most taxa show clear preferences for particular zones along the gradient.

Figure 6. Distribution of the archaeal lineage, Nitrososphaerales, functional genes, and individual OTUs across a tropical elevation-climate gradient on the Island of Hawai'i. The top panel shows the overall pattern for Nitrososphaerales (which contains known ammonia-oxidizing archaeon). The middle panel shows abundance of thaumarchaeal *amoA* genes (encoding the key enzyme in the ammonia oxidation pathway) quantified in the same samples. The bottom panel shows smoothed lines fit for all Nitrososphaerales OTUs (grey), with colored lines overlain for the 10 most common OTUs. The y-axis shows abundance relative to each OTUs maximum. Most taxa show clear preferences for particular zones along the gradient.

Conflict of interest

The authors declare no conflict of interest

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Figure 1. Changes in microbial richness along a tropical elevation-climate gradient on the Island of Hawai'i. OTU richness for Fungi, Bacteria, and Archaea were estimated after rarefying to 6000 sequences (see text for details). Linear models show different response patterns with respect to elevation and rainfall for Fungi (increasing), Bacteria (unimodal) and Archaea (unimodal / decreasing).

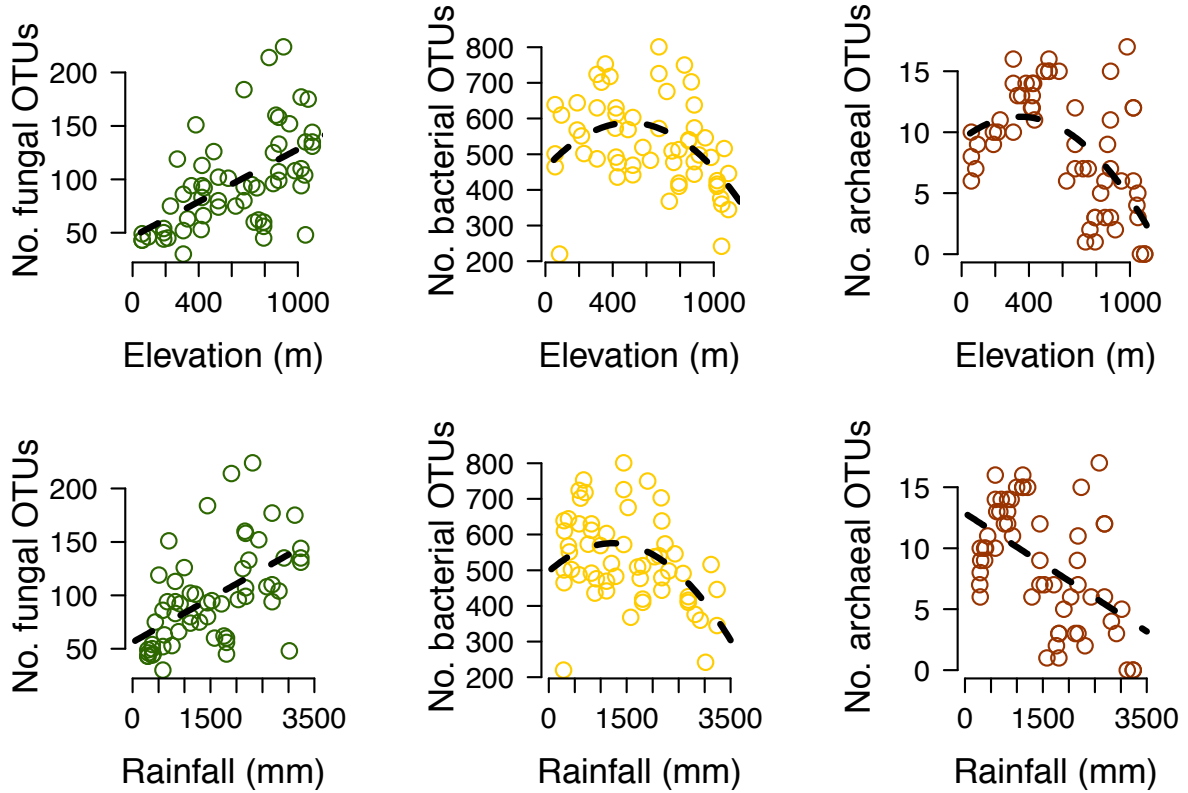


Figure 2. Drivers of microbial richness along a tropical elevation-climate gradient on the Island of Hawai'i. OTU richness for Fungi (green), Bacteria (yellow), and Archaea (red) were estimated after rarefying to 6000 sequences. Partial regression plots show the relationship between the best predictors based on AIC selection and richness (model residuals) for each taxonomic group after controlling for the effect of other significant predictor variables in the model. Panels are arranged left to right in order of decreasing explanatory power, measured as partial r^2 from multivariate models.

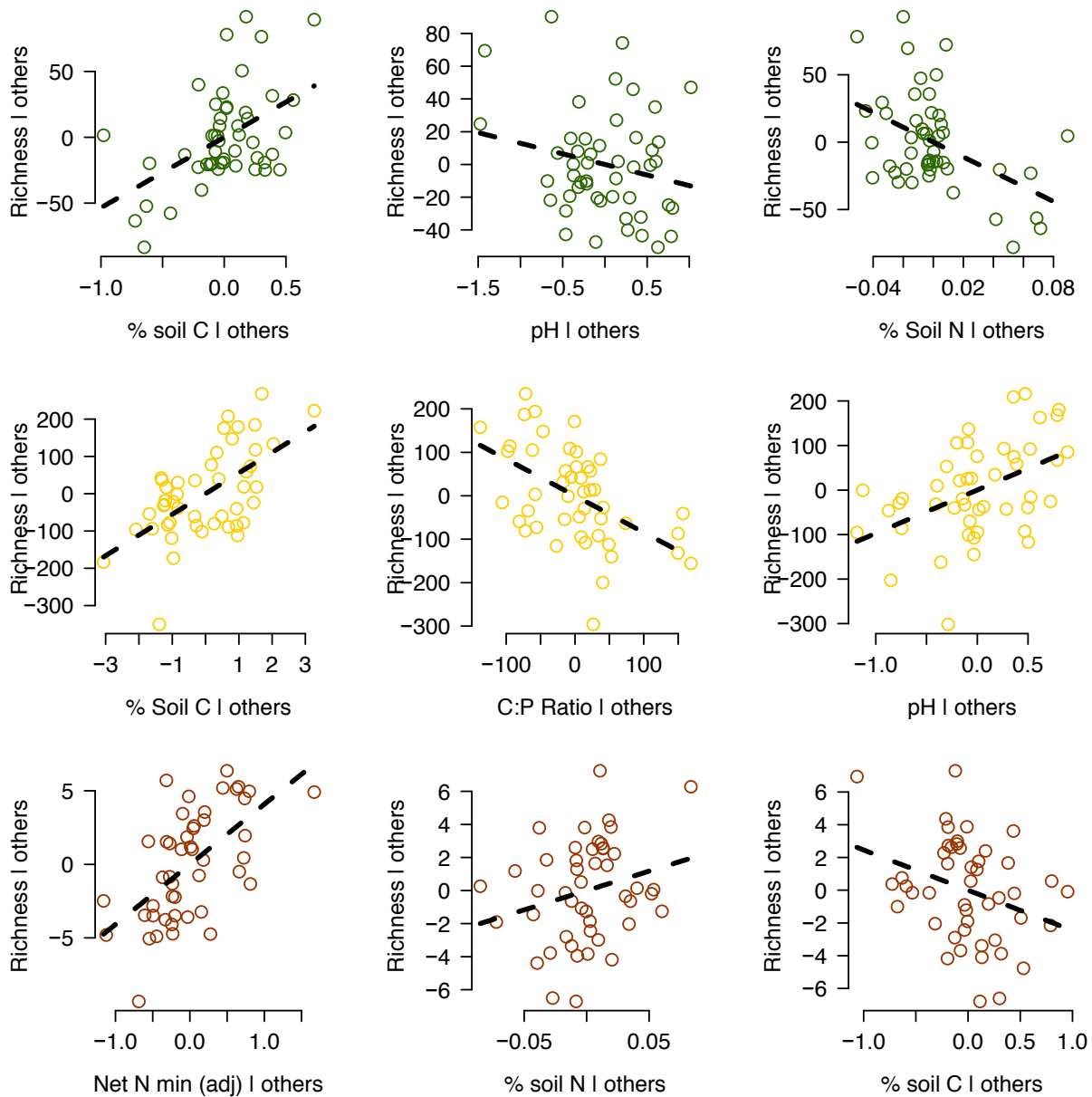


Figure 3. Drivers of microbial community composition along a tropical elevation-climate gradient on the Island of Hawai'i. Points represent the set of species characterized from individual soil samples for Fungi (green), Bacteria (yellow) and Archaea (red). In unconstrained ordinations (NMDS, left panels) the colors change from light (dry) to dark (wet), with elevation (m) isoclines to illustrate community change along the gradient. Constrained ordinations (db-RDA, right panels) show vectors for significant predictors based on AIC model selection, where length and orientation of arrows indicate the strength and direction of greatest community change, respectively, for each variable.

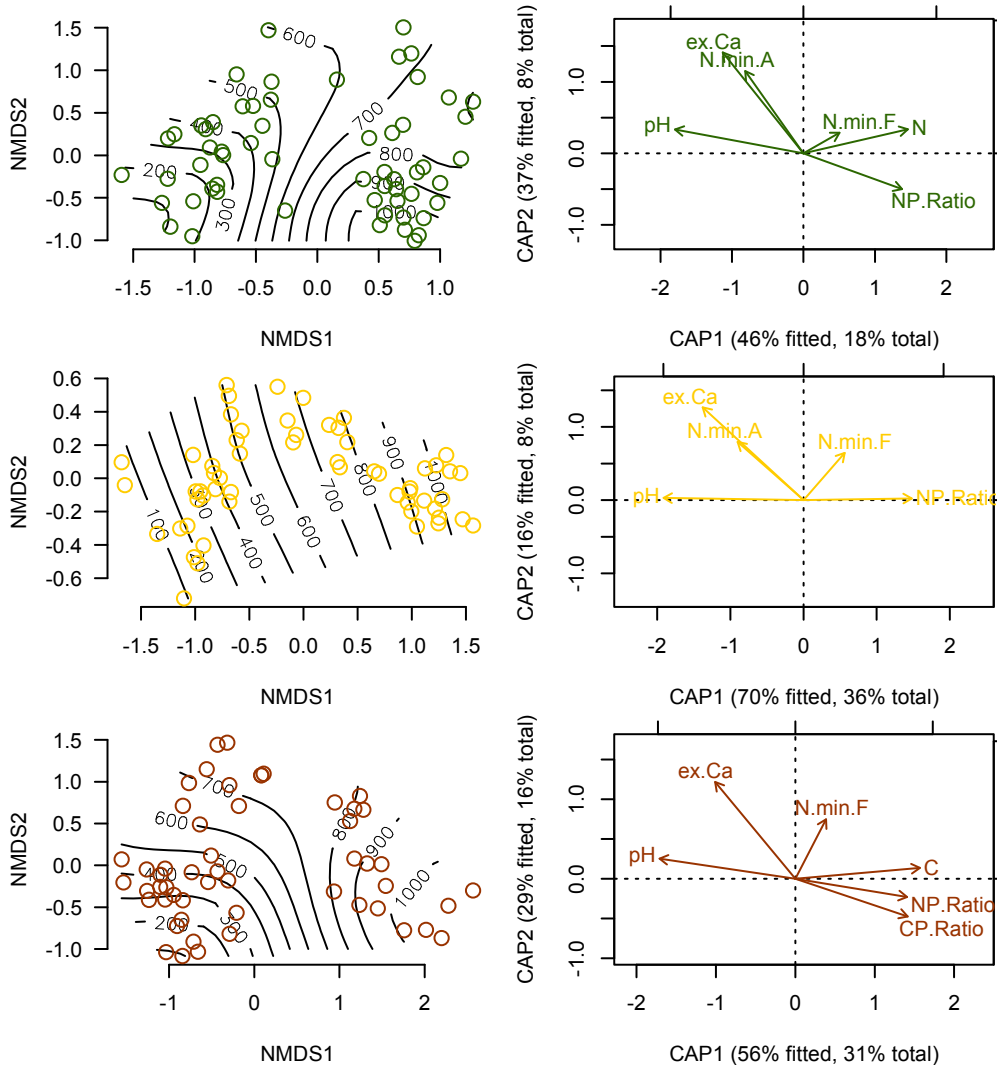


Figure 4: Distribution of fungal lineages and OTUs across a tropical elevation-climate gradient on the island of Hawai'i. Left panels show contrasting patterns for the two most abundant phyla (Basidiomycota, Ascomycota). Right panels show smoothed lines fit to the distribution of the top 100 OTUs for each phyla (grey), with colored lines overlain for the 10 most common OTUs. The y-axis shows abundance relative to each OTUs maximum. Most taxa show clear preferences for particular zones along the gradient.

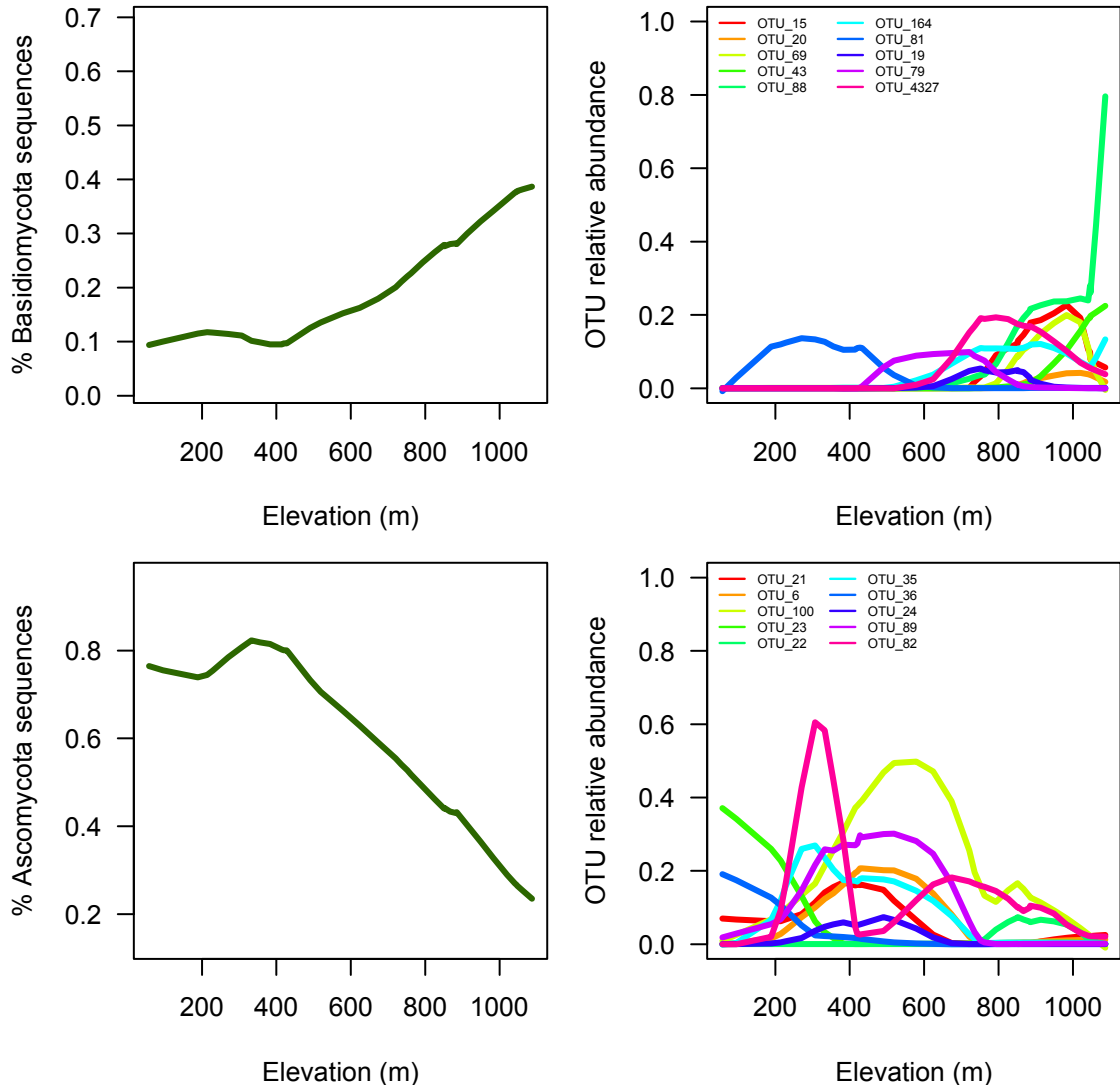


Figure 5. Distribution of bacterial lineages and OTUs across a tropical elevation-climate gradient on the Island of Hawai'i. Left panels show contrasting patterns for three of the most abundant phyla (Proteobacteria, Acidobacteria, Actinobacteria). Corresponding panels on the right show smoothed lines fit to the distribution of the top 100 OTUs for each phyla (grey), with colored lines overlain for the 10 most common OTUs. The y-axis shows abundance relative to each OTUs maximum. Most taxa show clear preferences for particular zones along the gradient.

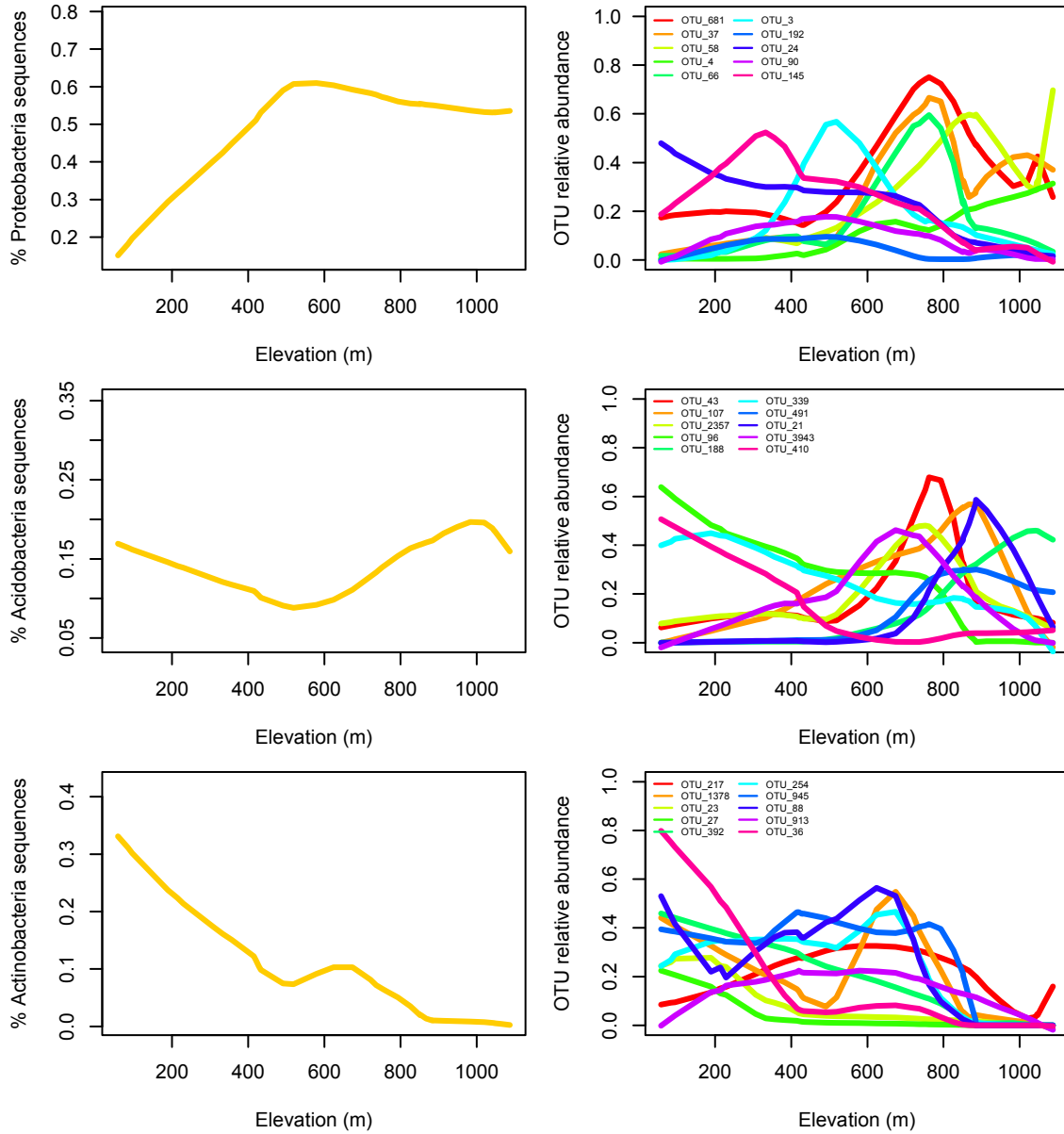


Figure 6. Distribution of the archaeal lineage, Nitrososphaerales, functional genes, and individual OTUs across a tropical elevation-climate gradient on the Island of Hawai'i. The top panel shows the overall pattern for Nitrososphaerales (which contains known ammonia-oxidizing archaeon). The middle panel shows abundance of thaumarchaeal *amoA* genes (encoding the key enzyme in the ammonia-oxidation pathway) quantified in the same samples. The bottom panel shows smoothed lines fit for all Nitrososphaerales OTUs (grey), with colored lines overlain for the 10 most common OTUs. The y-axis shows abundance relative to each OTUs maximum. Most taxa show clear preferences for particular zones along the gradient.

