J Gregory Caporaso, gregcaporaso@gmail.com, www.caporasolab.us
Lewis and Clark Fund for Exploration and Field Research in Astrobiology: Microbial community characterization of the Atacama Desert soils; Award date: 31 May 2011

Hyper-aridity prohibits plant, and presumably microbial, growth in the Atacama Desert core. Receiving <1mm of rain annually this environment is among the most Mars-like on Earth. In concordance with the Lewis and Clark Fund for Exploration and Field Research in Astrobiology goals, this project is aimed at improving our understanding of microbial life in this extreme environment by collecting soils from the Atacama Desert and characterizing their microbial communities using high-throughput sequencing of environmental DNA.

To achieve these goals, a collaborative project was initiated between my laboratory at Northern Arizona University, the Raina Maier Laboratory at University of Arizona, the Jack Gilbert Laboratory at Argonne National Laboratories, and the Rob Knight Laboratory at the University of Colorado. My lab and the Knight lab are experts in the statistical and bioinformatics techniques necessary for designing studies of microbial communities and analyzing the vast quantities of DNA sequence data generated in a study of this type. The Maier lab has worked extensively in the Atacama Desert, and thus provides local knowledge about sites and logistics, and has been invaluable in tuning the aims of our study. The Gilbert lab are experts in sequencing bacterial DNA from environmental samples, and in environmental microbiology including in extreme environments.

Funding for one student's (Will Van Treuren, University of Colorado) travel to the Atacama Desert, as well as a rental truck for accessing field sites, was covered on this grant. Travel for a research technician (Audrey Copeland, University of Arizona) and research scientist (Julia Neilson, University of Arizona), as well as a second rental truck, sampling supplies, shipping costs, and sample preparation was covered by the Maier laboratory. Costs for DNA sequencing were covered by the Gilbert laboratory. I was not personally able to attend the field trip as I transitioned from a postdoc position to a faculty position between obtaining the grant and the date of the field trip, and logistically could not make a two-week trip work at that time. I therefore sent Will Van Treuren in my place. I assembled this team of researchers (some of whom had never worked together before), as a result of being awarded this travel grant. These APS funds thus attracted additional financial support, enabling a very detailed study of soil microbial communities in the Atacama.

From 10-24 March 2012, Van Treuren, Copeland and Neilson traveled to the Atacama desert to collect soil samples (Figure 1). Two parallel elevational transects were sampled extending east from the central plateau near Antofagasta above the Pacific Ocean to the Argentinian border in the Andes. The first transect ("Baqedano") passed north through Baqedano, Calama, and San Pedro de Atacama into the Andes near Paseo Jama and the second ("Yungay") was south of Antofagasta passing through Yungay, the Salar de Imilac and ending at the Paseo Socompa border crossing in the Andes. Triplicate pits were dug at each site and samples were collected at a depth of 10-20 cm and analyzed for microbial community composition and total organic carbon (TOC). TOC values ranged from 0.02% in hyperarid, unvegetated regions to 0.7% in vegetated arid zones. At each site, one of the three pits was dug to a depth of 50 cm, profile samples were collected in 10 cm increments from the surface to 50 cm and analyzed for pH and anions representing dominant mineral salt species including NO3- (nitrates), SO42-(gypsum), Cl- (halites), and PO43-. pH values ranged from 5.6 to 9.3 with the lower values found only in vegetated areas and the more alkaline soils in unvegetated regions.

Nitrate concentrations were generally highest at elevations below 2000 m while sulfates were found in unvegetated areas up to 3200 m. No salt accumulations were detected in vegetated arid zones.

Figure 1. Van Treuren and Copeland collecting soil samples from the hyper-arid (left) and arid (right) regions of the Atacama Desert. Photos by Julie Neilson.

We have now sequenced environmental 16S rRNA sequences from all samples, and are in the initial data analysis stages where we will integrate these data with the soil chemistry data described above. Initial results illustrate that aridity is correlated with community composition, as the arid sites from both transects are more similar in composition to one another than either is to the hyperarid site from the same transect (Figure 2).

The composition of these sites align with our expectations for the bacterial composition of these sites, though represent approximately 100 times greater sampling depth (i.e., number of bacterial sequences collected per sample) than has ever been achieved before, and therefore the provides the first opportunity to characterize low abundance members of Atacama Desert microbial communities. Many of the organisms observed in these samples are novel with respect to our bacterial references databases, thus representing the discovery of new species. While this is fairly common in modern DNA sequence-based studies of microbial communities, our application of these methods to the Atacama Desert does help to expand our inventory of the bacterial world.

I posed several specific questions in my proposal. The next stage of my data analysis will focus on addressing these questions.

Q1. Soil pH is strongly correlated with microbial community structure across ecosystem types. Do the soils of the Atacama Desert fit this global trend, or is extreme aridity more important?

Q2. Does the extreme environment of the Atacama Desert have a homogenizing effect across vertical transects, or do microclimates associated with soil depth produce community structures similar to layered microbial mats?

Q3. Do microbial communities in different regions of the Atacama differ mainly in community membership, or in relative abundances of ubiquitous microbes (i.e., “everything is everywhere and the environment selects”)?

Q1 will be addressed by analyzing these data in the context of the thousands of soils collected and sequenced in the Earth Microbiome Project (EMP). The EMP is an effort to systematically characterize the microbial diversity on Earth, and provides publicly available environmental bacterial DNA sequence data. In conjunction with my Atacama data, the EMP data will allow me to answer this question.

I have already partially addressed Q2, though additional statistical analysis is necessary. It does appear that the Atacama soils are phylogenetically stratified, such that different depths appear distinct from one another in their microbial composition.

Q3 will be addressed by either deep sequencing one of our samples to much greater depth, and then comparing all samples to that single sample (as outlined in my proposal), or by comparing the quantitative and qualitative differences in the phylogenetic composition of our samples.

Our team is currently in the process of moving these analyses toward publication, and I will provide further details on that publication as it comes together.

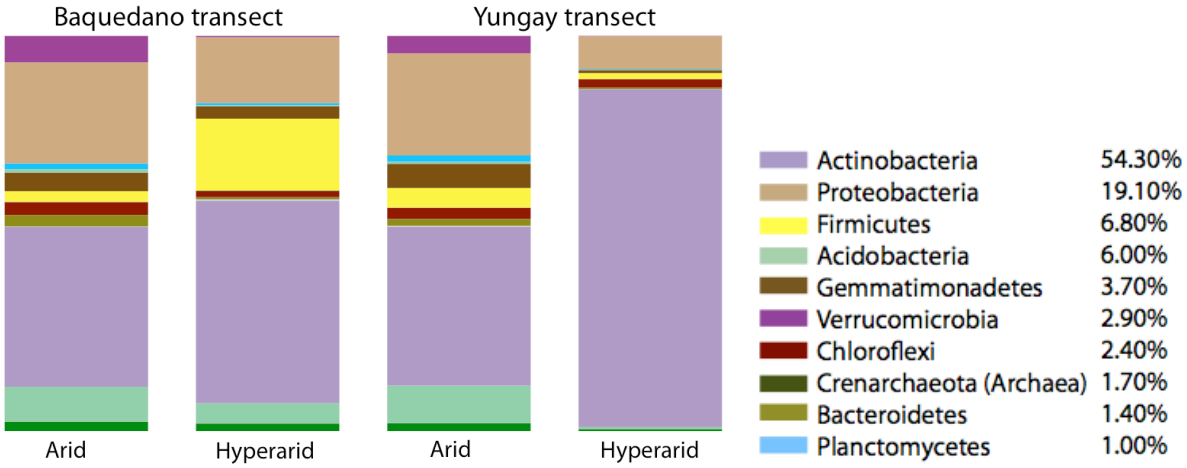

Figure 2. Average phylum-level composition of soils in the arid and hyperarid regions of the Baquedano and Yungay transects. All phyla are bacterial unless otherwise noted.

Lewis and Clark Fund for Exploration and Field Research in Astrobiology
Microbial community characterization of the Atacama Desert soils
Project Update June 2015
Prepared by Drs. Katy Califf and Greg Caporaso

In January of 2015, Dr. Julia Neilsen (Maier Laboratory, University of Arizona) and Dr. Katy Califf (Caporaso Laboratory, Northern Arizona University) traveled to the Atacama Desert in Northern Chile to retrieve soil data loggers from 22 sites along two elevational sample transects. Dr. Katy Califf's trip was funded with the remaining funds from this travel grant. These loggers had been recording environmental data variables, such as relative humidity and soil temperature, since they were deployed on the original field trip funded by this travel grant in 2012. These data were downloaded and analyzed along with 16S rRNA sequence data obtained from soil samples that were collected from these same sites on the field trip funded by this grant 2012. As previously reported, two west-east elevational transects traversing hyperarid and arid regions of the desert were sampled, and these transects were 250-300 km in length, and ranged in elevation from 895m to 4697 meters.

These data have allowed us to answer the questions posed in the original grant proposal.

Q1: Soil pH is strongly correlated with microbial community structure across ecosystem types. Do the soils of the Atacama Desert fit this global trend, or is extreme aridity more important? Our data show that the soils of the Atacama Desert in fact do not fit this global trend, and soil pH is not correlated with microbial diversity (Mantel's r = -0.04; p = 0.83). Instead, in the Atacama Desert, alternative environmental factors, particularly relative humidity, appear to be driving microbial community structure, as the number of species within a site increases with increasing relative humidity (R^2 = 0.74), and relative humidity is also strongly correlated with beta diversity (Figure 1; Mantel's r = 0.65; p < 0.001). Conversely, this means that the drier the microenvironment is within the Atacama Desert, the lower the diversity is, which leads into Q2, below. This effect of aridity has been shown on microbial diversity before, but the continuum we have documented along these transects in the Atacama Desert have not been previously shown (Figure 2).

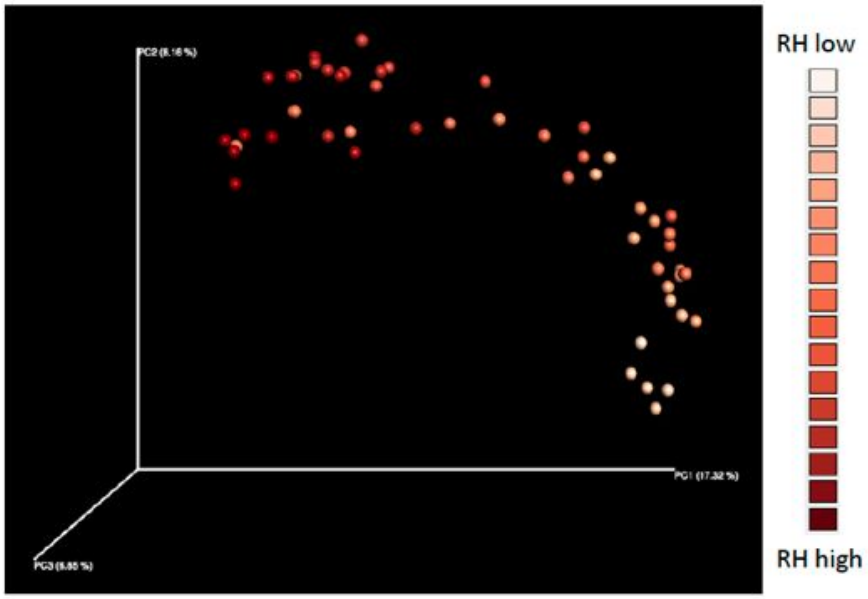

Figure 1. Principal coordinates analysis plot for individual sites. Color corresponds to relative humidity (RH). Each point represents a summary of the whole microbial community of a single sample, and points that are closer to each other in space are more similar in microbial composition. The smooth color gradient indicates that samples that are similar in microbial composition are also similar in relative humidity.

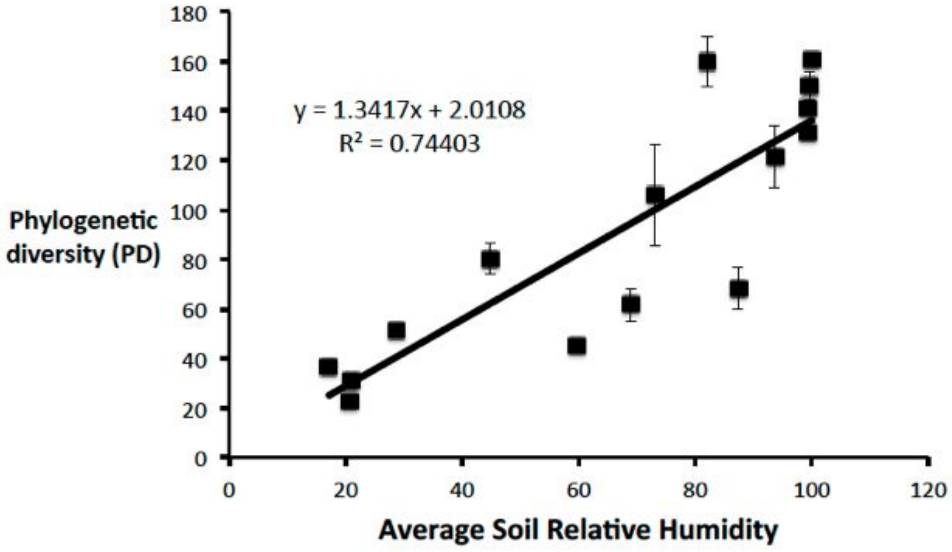

Figure 2. The average soil relative humidity is strongly correlated with phylogenetic diversity in microbial soils in the Atacama Desert.

Q2: Does the extreme environment of the Atacama Desert have a homogenizing effect across vertical transects, or do microclimates associated with soil depth produce community structures similar to layered microbial mats?
Initial data provided in the last project report showed that arid sites from both transects appeared more similar in composition to one another than either is to hyperarid sites from the same transect. Further analysis has shown a significant effect of elevation on the microbial composition and diversity of Atacama soils, such that the number of species found at any particular site increased with elevation ($R^2 = 0.58$). We found no similarity between samples that were taken from the same depth at different sites, though we only have data for multiple depths at six sites (ANOSIM R = 0.107, $p = 0.169$). Unfortunately, we did not have the data to test whether or not samples from the same depths were similar within sites due to unanticipated difficulties with collecting samples along vertical transects.

Q3: Do microbial communities in different regions of the Atacama differ mainly in community membership, or in relative abundances of ubiquitous microbes (i.e., "everything is everywhere and the environment selects")?
Our results show that microbial community membership may be changing more than compositional differences of taxa that are always present, as evidenced from a stronger effect seen in pairwise unweighted unifrac distances when compared to weighted unifrac distances (unweighted unifrac ANOSIM R = 0.79; $p = 0.001$; weighted unifrac ANOSIM R = 0.72, $p = 0.001$). We also found significant differences in community diversity between vegetated and unvegetated soils. Soils from vegetated sites were significantly more diverse ($t = 7.52$; $p = 0.001$) and more similar to each other (ANOSIM R = 0.63, $p < 0.001$) than were soils from unvegetated sampling sites.

In addition to these findings, which would stand-alone in a peer-reviewed publication, we have an additional exciting finding that is very unexpected and that will be the focus of an NSF grant that we will submit later this year. We performed a meta-analysis comparing the samples collected in this project to microbial communities from 16 biomes including polar deserts, grasslands, and temperate deciduous forests, initially published in Fierer *et al.* (2012) where the authors showed that desert soil microbial communities were clearly distinct from all of the non-desert soil microbial communities), regardless of the metric they used to measure these differences. The authors assert that these results demonstrate the need for desert ecosystems to be studied independently of other arable soils, as they are unique from communities found in other environments. In our meta-analysis, we found that all of the Feirer *et al.* desert samples overlapped in composition with the higher average soil relative humidity Atacama Desert samples along both the PC1 and PC2 axis of the principal coordinates analysis plot (Figure 3), not just the most arid of their dessert samples which was our initial expectation. These results suggest that by studying what happens to desert soil microbial communities as we move from higher to lower relative humidity in the Atacama Desert, we might learn about what to expect worldwide when other deserts become drier. These data therefore suggest that we can likely use the Atacama Desert as a model for desertification in all of the North American deserts, not only the driest ones, as was previously assumed. The Atacama Desert is truly a unique

ecosystem that covers a wide range of humidity, and should be seen as an important model to study climate change in diverse desert ecosystems.

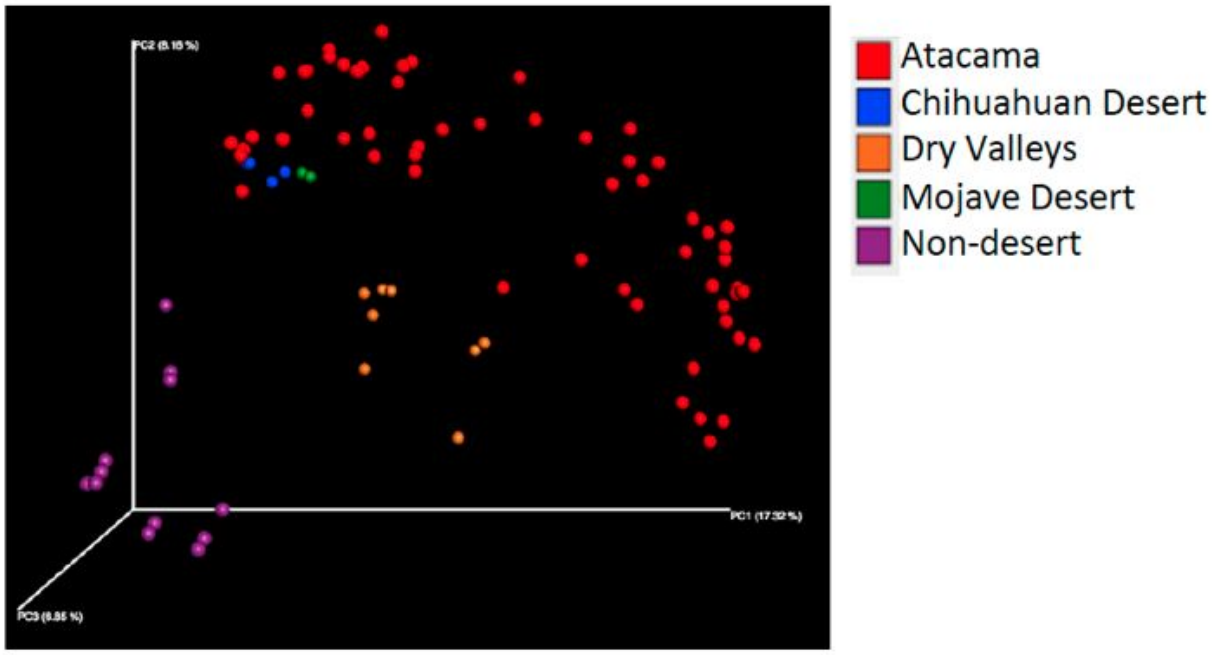

Figure 3. Principal coordinates analysis plot for individual sample sites colored by location. The X-axis corresponds to relative humidity.

These data have been presented by Dr. Katy Califf at the Arizona/Southern Nevada Branch meeting of the American Society of Microbiology in Flagstaff, Arizona on April 18th, 2015, and by Dr. Califf at the annual Astrobiology Science Conference in Chicago, Illinois on June 14th, 2015. A manuscript describing these results is currently in preparation.