BACTERIAL AND ARCHAEAL COMMUNITY STRUCTURE ACROSS A GRADIENT OF SALINE LAKES IN KIRITIMATI, REPUBLIC OF KIRIBATI

BY

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Abstract

Microbial mats, multilayered sheets of microorganisms often found in extreme environments, are increasingly gaining attention for their utility and influence on the global carbon cycle. However, our understanding of the organisms that define microbial mats and how they vary across environmental gradients remains limited, given the sparse sampling of these systems worldwide. Here we investigate a series of distinct microbial communities across a gradient of natural saline lakes on Kiritimati to define how mat communities in hypersaline lakes, where microbial mats have been previously assessed, differ from microbial communities in fresher lakes. Preliminary terminal restriction fragment length polymorphism analysis indicated that samples from the least saline lakes were statistically distinct from the most saline lake microbial communities. Results from Illumina sequencing of 16S rRNA gene amplicons support this finding and also pointed to both salinity and pH as major drivers of community variability. Alpha diversity measurements show no apparent link between salinity and microbial diversity. Extremely saline samples had both higher and lower Shannon index values, whereas lower salinity groups showed a range of Shannon index values. Our findings suggest pH may interact with salinity to influence microbial community structure and that diversity at high salinities may be controlled by both environmental and temporal factors. Greater insight into the drivers of community structure and diversity requires a deeper understanding of functional groups within brackish and brine lakes.

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Introduction

Microbial mats are multilayered sheets of functional groups composed of bacteria, archaea, and eukarya, as well as silicate and carbonate minerals, and are often found within extreme environments such as cold seeps, hydrothermal vents, and hypersaline lakes (Reitner, 2011; Bolhuis et al., 2014). These complex systems of microorganisms are increasingly being studied for many reasons: Cyanobacteria are a large component of these mats and are considered earth's first oxygen producing phototrophs, hypothesized to be responsible for the oxygenation of earth's atmosphere. Living microbial mats can thus be used as modern analogs of early cyanobacterial stromatolites to examine the processes that led to early earth oxygenation (Bühring et al., 2009). Mats may also offer insights into the nature of potential past extraterrestrial life, given the recent discovery of sedimentary structures resembling microbialites on Mars (Bianciardi et al., 2014; Noffke, 2015). Furthermore, microbial mats and their cultures are increasingly useful as bioremediation tools for a wide range of contaminants (Bender et al., 2000; Bender & Phillips, 2004; Writer et al., 2011). Lastly, microbial mats can play a large role in the global carbon cycle through their facilitation of carbonate mineral precipitation. The precipitation of carbonate is governed by the concentration of calcium and dissolved inorganic carbon, pH, and the availability of nucleation sites (De Muynck et al., 2010). However, calcium carbonate precipitation can be driven by three main groups of microbes – cyanobacteria, sulfate reducing bacteria, and species of organisms involved in the nitrogen cycle, specifically in relation to the ammonification of amino-acids, degradation of urea, and the dissimilatory reduction of nitrates (Castanier et al., 1999), which influence the above properties (Ariyanti et al., 2012).

Many studies have focused on the role of microbial mats in carbonate precipitation, particularly in terms of microbe-mineral interactions, with emphasis on metabolic facilitation of carbonate precipitation (Dupraz et al., 2004; Casillas-Martinez et al., 2005; Kandianis et al., 2008; Glunk et al., 2011; Schneider et al., 2013; Spring et al., 2015). For example, some studies have focused on specific communities within mats, like methanogens (e.g., Smith et al. (2008)) to determine their effect on carbon cycling and remineralization within the mat. Such modern

day analog studies are essential for understanding fossil microbialites. However, these studies often focus on a small number of sites where microbial mats are present and do not consider microbial mats across a range of environments (Ley et al., 2006; Arp et al., 2012). Studies that assess microbial distribution across environmental gradients are enormously helpful for evaluating potential relationships between a given environmental variable and microbial composition (Řeháková et al., 2009; Bolhuis et al., 2013; Liu et al., 2015; Bryanskaya et al., 2016). This can give further insight into why microbial mats communities develop relative to surrounding areas that do not contain mats, and potentially permit development of microbial facies models (Fouke et al., 2000; Fouke et al., 2003; Meyer-Dombard et al., 2005; Osburn et al. 2011).

The central tropical Pacific island of Kiritimati, Republic of Kiribati (2°N, 157°W) is a prime site to study lacustrine bacterial and archaeal communities. Roughly 25% of the island's surface area is covered in lakes, ranging in salinity from brackish (0.5-30ppt) to hypersaline, (>50 ppt) (Saenger et al. 2006). Many hypersaline lakes on Kiritimati contain microbial mats that exhibit well-defined layers that allow for high spatial resolution profiling of bacterial and archaeal communities (Arp et al., 2012; Schneider et al., 2013). However, previous bacterial and archaeal studies on Kiritimati microbial mats have only focused on a small number of the many lakes on the island, usually focusing on two of the most hypersaline lakes with well-defined mat stratigraphy, lake 2 and 21 (Bühring et al., 2009; Arp et al., 2012; Schneider et al., 2013; Spring et al., 2015). Thus, the biotic and abiotic profiles of most Kiritimati lakes remain unknown.

In this study, we investigated microbial communities for surface sediments from 25 Kiritimati lakes and the Kiritimati lagoon, where salinities range from nearly fresh to hypersaline. Using a combination of terminal restriction fragment (T-RFLP) analysis and Illumina sequencing of 16S rRNA genes allowed us to determine differences in the microbial communities across different salinity ranges. The sequencing enabled us to further confirm significant differences in microbial and archaeal communities found in the initial T-RFLP analysis, investigate microbial diversity and identify specific bacteria and archaea, and more

deeply investigate environmental variables that may have caused variability in microbial composition.

The importance of studying the effects of a natural salinity gradient on these communities is twofold. First, it will simply test the hypothesis that taxonomic diversity decreases with increasing salinity, as living in extreme environments is energetically costly (Jiang et al., 2007). This would lead to a low number of organisms capable of maintaining that cost (Oren, 2001; Oren, 2002). However, microbial mats in hypersaline lakes can offer an alternative where the formation of microhabitats can potentially allow for higher diversity. Second, it will provide information on heretofore unknown microbial communities of Kiritimati's brackish lake systems, which have not been investigated. This in turn will allow for comparison between lake sediment microbial communities across a range of salinities therefore providing information on how environments that maintain well-stratified mats differ from those in the same region that do not contain mats.

Methods

Site Description and Sampling

Kiritimati, Republic of Kiribati (2°N, 157°E) is the world's largest coral atoll and is part of the group of near-equatorial Line Islands in the central tropical Pacific. These islands lie along a northward gradient of increasing rainfall. Kiritimati, the southernmost island, lies in the equatorial dry zone, south of the Intertropical Convergence Zone. Rainfall on the island is highly variable due to its high sensitivity to the El Niño-Southern Oscillation (Morrison & Woodroffe, 2009). Approximately a quarter of the island is covered in lakes that range from brackish to hypersaline. The largest body of water is a lagoon (320km^2) in the northwestern part of the island. Lakes are connected to this lagoon through a series of channels with the closest connections being the least saline (Saenger et al., 2006). Other, less saline lakes are linked to groundwater sources in the northeastern and southeastern parts of the island.

Water chemistry data was obtained from 33 sites, including samples from both the lagoon and the ocean, while on the island in July-August of 2014 (Figure 1). The estimated depth at which samples were taken ranged from a few centimeters to approximately two meters. Shorelines of many of the lakes were composed of bedrock or coral. In situ, temperature (±0.2°C), conductivity (±0.001 mS/cm), pH (±0.2 units) and dissolved oxygen content (±0.2 mg/L; ±2%) were measured for each lake using a YSI ProPlus multiparameter water quality sonde and a Hach test kit was used to measure in situ alkalinity (±1% for readings over 100 digits and ±1 digit for those less than 100). 30 mL water samples were filtered through a 22 micron filter and stored in acid washed bottles for cation analysis. The concentration of major and minor cations was measured using ICP-OES (Perkin-Elmer, Optima 5300 DV) at the University of Arizona (±2% for major elements and ±5% for minor elements).

Surface sediment samples were taken from 28 location including the lagoon. A soil knife was rinsed with deionized water and subsequently with lake water before each sediment sample was taken at each site. The samples were then placed in sterile whirlpak bags and fixed with 3% glutaraldehyde in phosphate buffer and stored on ice. Upon return, they were placed

in a 4°C cold room in the dark until use, with subsamples intended for DNA analysis frozen and stored at -80°C.

Loss on Ignition

All samples were rinsed multiple times with deionized water to remove glutaraldehyde and pore water containing sulfates, which can confound estimates of organic matter and carbonate. Sediment was dried at 120°C overnight. After desiccation, dry sediment was transferred to clean dry crucibles and weighed. Samples were burned at 550°C for 2 hours in a muffle furnace to remove organic content. Lids were placed on the crucibles beforehand to prevent the flying of ash. The crucibles were transferred to a desiccator until cool enough to weigh accurately. Samples were then burned at 950°C for 4 hours to remove carbonate. They were left in the furnace for a few hours to cool before being transferred to the desiccator for complete cooling.

Equations 1-3 were used to calculate the percent abundance of organic carbon, calcium carbonates, and residuals.

Equation 1: %Organic = [(Dry weight – 550°Cweight)/(Dry weight)] x 100

Equation 2: $\%CaCO_3$ = [(550°C weight – 950°C weight)/(Dry weight)] x 2.274 x 100

Equation 3: %Residual = 100 – (%Organic + $\%CaCO_3$)

The value of 2.274 in Equation 2 comes from dividing the molecular mass of calcium carbonate by the molecular mass of carbon dioxide. To be able to calculate the quantity of calcium carbonate, the mass of carbon dioxide lost must be multiplied by some factor to account for the calcium oxide that remains in the residual.

DNA Extraction

Sediment samples were extracted from whirlpak bags and placed in sterile Falcon tubes. All samples were rinsed multiple times with 3.5% phosphate buffered saline to remove the glutaraldehyde fixative. After rinsing, genomic DNA was extracted using the FastDNA SPIN Kit

for Soil (MP Biomedicals, OH, USA) according to the manufacturer's protocol. DNA was eluted using sterile molecular grade deionized water. The concentrations of all samples were measured using a Nanodrop Spectrophotometer. After measurement, DNA samples were stored in a -20°C freezer until further use.

Terminal Restriction Fragment Length Polymorphism (TRFLP) & Data Analysis

To prepare for TRFLP, DNA extracts were first amplified using a 16S rRNA primer pair. The bacterial specific 16S forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') was modified to have a 5' fluorescent tag on the end - 6-carboxyfluorescein (6-FAM). The bacterial 16S reverse primer (5'-ACGGCTACCTTGTTACGACTT-3') remained unmodified. The PCR mix for each sample (25µL) contained 13.4µL of sterile molecular grade water, 2.5µL 10x PCR buffer (TaKaRa, Clontech, CA, USA), 2µL dNTP solution (2.5mM each dNTP, TaKaRa, Clontech, CA, USA), 2.5µL of each primer at 4µM, 0.1µL Ex Taq polymerase (TaKaRa, Clontech, CA, USA), and 2µL template DNA. The reagents were amplified in the thermocycler in the following way: an initial denaturation at 95°C for 3 mins, then 30 cycles of denaturation at 95°C for 30s, annealing at 55°C for 45s, and extension at 72°C for 45s, last was a final extension at 72°C for 10 minutes. Samples were held at 4°C in the thermocycler until they were placed in a -20°C freezer for storage. PCR products were visualized via electrophoresis on a 0.8% agarose gel to check for successful amplification. Samples that showed no bands were re-amplified with the template DNA at a lower concentration. PCR was repeated to obtain approximately 50µL of each sample.

Following successful amplification, PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, MD, USA) according to the manufacturer's protocol. Purified products were checked again via agarose gel for success and stored at -20°C. Lastly, purified products were separated into two sets and then digested using different restriction enzymes, Rsa1 and Msp1 (New England Biolabs, MA, USA). Samples were digested according to the manufacturer's protocol. The digested samples were sent to the High-Throughput Sequencing and Genotyping Unit of the University of Illinois Biotechnology Center for TRFLP fragment analysis per their sample preparation instructions.

Fragment analysis files were uploaded to GeneMapper Software (release 5.0). Peak size and height were used to determine the relative abundance for each fragment size in each lake sample. The abundance data was then imported into Primer 6 software and the Bray-Curtis similarity was calculated for all samples. An analysis of similarity (ANOSIM) was conducted to determine if samples were significantly different from each other and results were visualized using nonmetric multidimensional scaling plots (Clarke and Warwick, 2001). This was done for both restriction enzyme digests.

Illumina Sequencing

PCR amplification for bacterial and archaeal sequencing follows the same procedure as for the TRFLP except for the primer pairs. Two different primer pairs which were linked to a Fluidigm system specific sequence were used to amplify 16S rRNA gene sequences from microbes in the saline lakes of Kiritimati. The first was designed to primarily target archaea and paired the forward primer Arch349f (5'-GYGCASCAGKCGMGAAW-3') with the reverse primer Arch806r (5'-GGACTACVSGGGTATCTAAT-3'). The second targeted a broader range of both bacteria and archaea using the forward primer V4-515f (5'-GTGYCAGCMGCCGCGGTAA-3') and the reverse primer V4-806r (5'-GGACTACNVGGGTWTCTAAT-3'). Given the high degree of similarity between these reverse primers, these groups of sequences will hence be described by the forward primer used. PCR products were visualized via 0.8% agarose gel electrophoresis. Archaeal samples that displayed no products were re-amplified with an increased number of PCR cycles (35 rather than 30). Archaeal and bacterial samples were prepared on separate plates and submitted to the Function Genomics Unit of the University of Illinois Biotechnology Center. The sequencing was performed on one lane of a v2 2x250nt MiSeq Nano. This initial processing resulted in four sets of data: unsorted data, demultiplexed data, primer sorted data, and primer sorted, demultiplexed data.

Sequencing Analysis

For the 28 samples, a total of 143,686 paired-end sequences were generated using Arch349f while 576,993 sequences were generated for V4-515f. Paired ends were joined

together for V4-515f sequences (99.02% of sequences successfully joined) using PEAR (Zhang et al. 2014), but not for Arch349f because the length of the amplicon (457 nucleotides compared to 292 for V4-515f) prevented efficient joining. Thus only the forward read was used for Arch349f. Sequences were processed separately from raw FASTQ files using QIIME (Caporaso et al., 2010). Singletons were removed, de novo OTU picking with uclust was used to cluster sequences at 97% similarity, and Greengenes (4feb2011) was used to assign taxonomies. Sequences that were unable to be classified at the domain level were discarded. Analysis of similarity (ANOSIM) calculations and nonmetric multidimensional scaling plots were conducted in Primer-7 (Clarke and Warwick, 2001) and additional alpha and beta diversity comparisons were calculated in R using the packages phyloseq (McMurdie and Holmes, 2013) and edgeR (Robinson et al., 2010).

Results and Discussion

Water Chemistry and Carbon Content

Water chemistry data exhibited wide ranges for many variables, most notably salinity (Table 1a-e), which ranged from 7.9ppt to 159.9ppt. Independently measured cation values were highly correlated with salinity (e.g., magnesium, sodium, and strontium had correlations of 0.97, 0.97, and 0.85, ($p<0.001$) respectively, with salinity. The wide range of salinity and cation values can be attributed to the degree of connectivity to the main lagoon (salinity of lagoon in ppt) as lakes more closely connected to the main lagoon exhibit lower salinities. As the distance from the lagoon increased, so did the salinity due to evaporation and solute concentration (Trichet et al., 2001; Schoonmaker et al., 2005; Saenger et al., 2006). However, some lakes that are isolated from the lagoon were found to have lower salinities due to mixing of groundwater and seawater.

Other environmental variables, including pH, dissolved oxygen, and the concentrations of many of the dissolved ions, also showed a wide range of values. Of these, pH and dissolved oxygen showed a negative correlation to salinity. The pH of the lakes ranged from neutral to slightly basic (7.2-9.2) and was significantly correlated with salinity ($r= -0.56$, $p<0.001$). The dissolved oxygen (in %) of the lakes ranged from hypoxic (1-30%) to supersaturated (>100%). The correlation between dissolved oxygen and salinity was slightly weaker than that of pH and salinity ($r= -0.47$, $p<0.01$). However, it is important to note that dissolved oxygen values may be affected by differences in time of day the measurement was taken, due to temperature effects on the dissolution of oxygen.

Loss on ignition results show percent organic carbon, carbonate, and the residual for 27 surface sediment samples (Table 2). Percent organic carbon ranged from 3.2 to 54.4%, with the highest values found on more saline lakes (Figure 2a). Lakes with microbial mats had the highest organic carbon content. All lake sediment samples contained abundant calcium carbonate, with values ranging from 30.6 to 94.3% (Figure 2b). Most lakes seem to be actively producing carbonate. A weak negative correlation between salinity and the amount of calcium

carbonate does exist (r = -0.34), but it is slightly past the 5% confidence interval (p=0.08). The residual percent abundances from loss on ignition range from 2.4 to 42.8%, and likely reflect the presence of gypsum.

Preliminary Testing of Microbial Communities

TRFLP of bacterial 16S rRNA using two different enzymes (MspI and RsaI) was performed to profile the communities of the different lakes and test for differences between microbial communities across lakes of varying salinities. The lakes were divided into four categories: fresh (<0.5 ppt), brackish (0.5-30 ppt), saline (31-50 ppt), and hypersaline (>50 ppt). A lagoon sample (36 ppt) was also used as a comparative reference for the different salinity groupings. Bray-Curtis similarity for both TRFLP enzyme sets was used to create nonmetric multidimensional scaling plots (nMDS) (Figure 3) for visualization of the different lake salinity categories. In both sets, brackish lakes have community profiles that are distinct relative to community profiles from the more saline lakes, indicating a significant difference in community composition.

While the visualization gave similar results for both enzymes, analyses of similarity gave slightly different results. Pairwise tests comparing the groups for MspI digested samples showed both brine-brackish and saline-brackish groups as being significantly different at the 5% confidence level (4.5% and 2.2% respectively). However, RsaI digested samples only showed the brine-brackish test as being significant (0.9%). This difference may be caused by differences in the communities themselves and how they react to the restriction enzyme. While certain populations may have different sites for one of the enzymes, they may have the same restriction site for the other enzyme. In the latter situation, the population would appear as the same terminal restriction fragment (TRF) peak, therefore causing a reduction in diversity (Marsh et al., 2000; Schütte et al., 2008). Despite the differences shown by the ANOSIMs, both restriction enzyme digests confirm that the sediment samples from the most saline lakes have communities that are distinctly different from the brackish lake sediment samples.

Archaeal and Bacterial Sequencing Results

Bacterial samples produced a combined total of 1,213,504 forward and reverse reads from primer sorted and demultiplexed data resulting in a total of 16,231 OTUs. Archaeal samples produced a combined total of 294,096 forward and reverse reads. Only the forward reads were used to determine archaeal OTUs to prevent the filtering of good reads. This led to a total of 3,445 OTUs. This difference in read and sequence quality can clearly be seen in the relative abundances for the two domains (Figure 4). The difference in read quality and low sequence number in archaea is likely due in part to the abundance of bacteria in the samples relative to archaea. While the primer used was designed for archaea PCR, it was still capable of amplifying bacteria. If the amount of bacteria in the samples is much richer compared to archaea, it can possibly overwhelm the archaeal signal.

OTU abundance was analyzed in a similar fashion to TRFLP and was visualized using nMDS (Figure 5). Sequencing data confirmed TRF results: communities within the brackish samples plot together and are distinct from the groupings of the more saline lake sediment samples. Analyses of similarity confirmed that the brackish-brine groups were different with significance levels for both bacteria and archaea, despite the lower quality, below 5% (0.1% and 1.8% respectively). However, due to better overall quality, only bacterial sequences were used in further analyses.

Drivers of Community Diversity

In hypersaline environments, salinity is often the primary driver of microbial composition (Jiang et al. 2007; Wu et al. 2009). As lakes become more saline, the energetic cost of living in the environment also increases. Therefore salinity acts as a filter as fewer organisms will be able tolerate increasing salinity (Oren 2001, 2002). Due to its extreme variability across Kiritimati lakes, salinity has been the main physiochemical factor investigated thus far as a driver of microbial community composition. To test whether other environmental parameters influence community diversity, the nMDS plot for bacterial sequences was vectorized using the environmental parameters of salinity, temperature, pH, dissolved oxygen content, alkalinity,

percent organic matter, percent calcium carbonate, magnesium to calcium ratio, and the concentrations of calcium, magnesium, sodium, potassium, strontium, silicon, and boron (Figure 6). Environmental variables that were not statistically significant were removed from the environmental fitting; any vectors that were $P > 0.05$ were discarded, leaving salinity, pH, dissolved oxygen, alkalinity, percent organic matter, magnesium to calcium ratio, and the concentrations of calcium, magnesium, sodium, potassium, strontium, and boron as statistically significant variables (Table 3).

Figure 6 shows salinity has the strongest effect on microbial community composition, along with Na, K, Mg, Sr, and B, the concentrations of which are directly tied to salinity (see Table 3 for r^2 values). The vector associated with pH also shows a strong influence on microbial community composition. pH has been known to influence microbial communities in soils (Rousk et al. 2010), as well as in lake sediments in areas that have strong salinity gradients (Xiong et al. 2012). pH may have both direct and indirect effects on microbial communities. It may have an indirect effect on the community by acting as an integrating variable that changes in response to a change in another factor like nutrient availability, soil moisture, or even salinity. A change in one of these other variables may lead to a change in pH and that may drive the change in community composition (Liu et al. 2015). pH may have a direct effect in that as it changes, it will impose new limits on the community. This would lead to a reduction or complete disappearance of the microbes that are not tolerant to the new pH level (Lauber et al. 2009).

Microbial metabolic activities may also mediate water pH. Heterotrophic metabolisms of microbes, like those associated with the sulfur and nitrogen cycles, may lead to changes in pH and also carbonate precipitation. For instance, sulfate-reducing bacteria are known to affect pH through their reduction of sulfate and production of sulfide leading to an increase in pH. Additionally, this process can have effects on calcium carbonate precipitation since it also results in an increase in carbonate alkalinity and a change in the saturation index (Baumgartner et al. 2006; Braissant et al. 2007). Sulfate reduction has been estimated to account for more than 50% of the mineralization of organic carbon in marine sediments (Jørgensen 1982; Muyzer and Stams 2008) thus indicating how important the SRBs can be to carbonate precipitation. In

our samples, the lakes with the highest pH level were the brackish lakes. The relative abundance of taxa known for sulfate reduction (e.g. *Desulfobacterales* and *Desulfovibrionales*) were compared between the brackish lakes with high pH and the lower pH hypersaline lakes. Brackish lakes did have a slightly higher overall abundance of taxa known for sulfate reduction; sulfate reducers were 2.76% of the overall abundance in brackish lakes compared to 1.45% in hypersaline lakes. However, further investigation into the bacterial metabolisms present in the individual samples and their measured abundance is necessary to gain more insight into this potential mechanism for carbonate precipitation.

The nitrogen cycle can also increase pH and carbonate precipitation via three different pathways: ammonification of amino acids, reduction of nitrate, and degradation of urea. These pathways cause an increase in pH and shift the bicarbonate equilibrium to the formation of carbonate ions. These ions then can bond with any available calcium ions leading to formation of layers of calcium carbonate (Monty et al., 1995; Castanier et al., 1999; Hammes et al., 2003; Zhu & Dittrich,, 2016). However, other steps within the nitrogen cycle are known to decrease pH and create acidic conditions. Nitrification involves the oxidation of ammonium or ammonia to nitrite via ammonia oxidizing bacteria, primarily found in the phyla *β-proteobacteria* and *gammaproteobacteria* (Purkhold et al., 2000), and ammonia oxidizing archaea, found in *Thaumarchaeota* (Hatzenpichler, 2012). Nitrite is then oxidized to nitrate via nitrite oxidizing bacteria. The oxidation of these compounds releases hydrogen ions as products and will create a more acidic environment. In sum, the above examples illustrate the complex linkages between environmental variables, microbial communities' geochemical cycles, and carbonate precipitation.

Comparison of Families and Diversity between Brine and Brackish Lakes

Previous microbial mat studies on Kiritimati investigated bacterial and archaeal phyla found within the hypersaline Lakes 21 and 2a (Bühring et al. 2009; Arp et al. 2012; Schneider et al. 2013; Spring et al. 2015). Schneider et al. (2013) analyzed the microbial communities from 9 different layers of a well stratified mat from Lake 21. In their study, the dominant phyla from the overall bacterial community included *Bacteriodetes* (30.5%), *Proteobacteria* (27.3%),

Spirochaetes (15.3%), *Cyanobacteria* (5.9%), and *Chloroflexi* (5.2%). In their earlier study, Arp et al. (2012) found many of the same phyla in their analysis of the Lake 21 mat. In both studies, they found that diversity increased with mat depth. Furthermore, at transitions between the photo-oxic, transition, and anoxic light-free zones, the abundance of the different phyla changed, indicating that microhabitats are both a product of and support varying microbial communities within the mat sample. In Lake 21, the number of bacterial OTUs (N=1409) was much greater than the number of Archaea (N=239) (Schneider et al. 2013). This supports our hypothesis that the archaeal amplification in our samples may have been overwhelmed by better quality bacteria DNA. In the following sections, we investigate whether these same phyla and families are present in brackish lake systems and in other hypersaline lakes on Kiritimati.

As in previous studies, based on relative abundance, we find tha*t Proteobacteria* and *Bacteriodetes* are the dominant phyla across the suite of lake sediment samples, followed by *Planctomycetes*, *Firmicutes*, and *Spirochaetes*. Preliminary normalization analyses using percent organic from LOI as a proxy for total biomass give similar results. *Proteobacteria* and *Bacteriodetes* were dominate followed by *Planctomycetes* and *Firmicutes*. *Chloroflexi* replaced *Spirochaetes* as the fifth most dominant. Overall, the preliminary normalization was similar to the relative abundance, however, the less abundant phyla begin to differ in their abundance. While the major phyla seem to be similar to those found by Arp et al. (2012) and Schneider et al. (2013), the moderately abundant phyla differ. In the previous studies, *Cyanobacteria* was more abundant, especially in the upper layers of the stratified mats. Additionally, Schneider et al. (2013) found a greater abundance of *Deltaproteobacteria* in the lower light-free mat layers. It is likely that differences in phyla between the previous data and our new dataset are due to the spatial resolution of the sampling (e.g., individual layers versus bulk surface samples across many lakes). Additionally, by chance, more of a certain type of bacteria may have been sampled. Ultimately, we find that the microbial composition across the sampled lakes is highly variable.

As was found with TRFLP, brackish lakes and hypersaline lakes have distinctive communities. Pairwise tests using the phyla and families of the two groups illustrate these

differences (Figure 7). Positive log2-fold changes in Figure 7 reveal OTUs that are more abundant in hypersaline lakes, while the negative values indicate those that are less abundant. More simply put, positive values correlate to OTUs that are more representative of the hypersaline lakes and negative values correlate to the brackish lakes. While similar phyla are present in many lake sediment samples, at the family level, differences emerge between the different salinity groups: within *Proteobacteria,* the family *Halomonadaceae* is the most abundant in hypersaline samples (as well as overall abundance). This is likely due to the halophilic nature of this family (Arahal and Ventosa 2006). Conversely, the family *Kiloniellaceae*, which comprises marine organisms known to be halotolerant, is the most abundant family in brackish lakes (Wiese et al. 2009). Within the *Proteobacteria*, the family *Idiomarinaceae*, is the second most abundant overall, and is abundant in both hypersaline and brackish lakes. This family is known for being able to exist in a wide range of salinities, including coastal waters and inland hypersaline wetlands (Albuquerque and da Costa 2014). As noted previously, the second most abundant phylum was *Bacteriodetes*. Within this phylum, the most abundant family within the brackish lakes, and fourth most abundant family overall, was found to be *Cryomorphaceae*. Members within this family are generally found in non-extreme habitats (Bowman 2014); this likely explains its abundance in brackish lakes. In both hypersaline and brackish lakes, we identified two families that could potentially degrade extracellular polymeric substance (EPS): *Cytophagaceae* in the brackish lakes and *Saprospiraceae* in both brackish and hypersaline lakes. Degradation of EPS can facilitate the precipitation of calcium carbonate through the release of calcium ions into the environment (Dupraz et al., 2004; Glunk et al., 2011). Thus, members of these families may play a part in the breakdown of EPS polysaccharides and facilitate carbonate precipitation within these lake systems (McBride et al., 2014; McIlroy & Nielsen, 2014). Furthermore, previous studies on the island have also found that exopolymer degradation is a key factor in carbonate precipitation within well stratified mats (Arp et al., 2012)

Our limited archaeal data show two major phyla– *Crenarchaeota* and *Euryarchaeota*. Schneider et al. (2013) also found that *Euryarchaeota* was highly abundant, with *Thaumarchaeota* as the second most abundant phylum. However, they did note that members

of *Crenarchaeota* had been reordered to *Thaumarchaeota*. This is based on the findings that some former crenarchaeal members had phylogenies that branched deeper than previously assumed (Brochier-Armanet et al. 2008). Therefore some of the members of the *Crenarchaeota* found may actually belong to *Thaumarchaeota*, and our results may align more with those previously found on the island. Both hypersaline and brackish lakes had Deep Sea Hydrothermal Vent Group 1 (DHVEG-1) as their most abundant group. Members from this group are frequently associated with anoxic environments, like in hydrothermal vents and in sediments (Takai and Nakamura 2011; Liu et al. 2014). Schneider et al. (2013) also found this group in the darker, more anoxic layers of their microbial mats. Also, our hypersaline samples contained a higher abundance of the halophilic *Halobacteriaceae*, found by Schneider et al. (2013) in photo-oxic layers. This further indicates that our bulk samples combine families from both oxic and anoxic microenvironments.

High salinity can result in low taxonomic diversity, as living at high salinity is energetically costly (Oren 2001, 2002; Jiang et al. 2007). However, hypersaline water bodies can also have dense, highly structured microbial mat communities, defined by many different types of microbes, as is seen in the previous studies on Kiritimati (Bühring et al. 2009; Arp et al. 2012; Schneider et al. 2013; Spring et al. 2015). To test for systematic differences in diversity across the brackish and hypersaline lakes, we calculated the Shannon index for each sample (Figure 8). Our results found a range of diversity in hypersaline lakes. The brackish and saline samples also had high and low diversity. Thus, no real trend in diversity with increasing salinity was detected. The cause for this lack of relationship could be due to the nature of the mats themselves. Some of the mats observed were the highly vertically stratified communities observed in previous investigations, while other “mats” were single, monochromatic layers at the sediment-water interface. Lakes with well-stratified mats will have higher habitat heterogeneity with respect to the physical and biogeochemical gradients that are created and maintained by the microbes (Bolhuis et al. 2014); higher heterogeneity leads to more available niches and therefore the potential for a high diversity. Another factor to consider is the resolution with which diversity is measured. While higher taxonomic levels may indicate low diversity, there is the potential for hidden microdiversity at a finer scale (Wu et al. 2006; Melendrez et al. 2011). A third factor

influencing diversity metrics may be predation. Organisms that feed on the microbes may not be able to tolerate higher salinities (Garrett, 1970; Ley et al., 2006). The lower predation rate may thus allow for greater microbial diversity. Lastly, time may play an important role in the diversity of the mats. Mats that are older may exhibit a higher diversity since there is more time to have become established and create environmental gradients within themselves. High resolution radiocarbon dating of mat sequences coupled with diversity metrics may be useful to address this question.

Conclusion

Investigation of microbial communities across environmental gradients is helpful for evaluating relationships between the given environmental variable of interest and community structure. Utilizing a natural salinity gradient across a suite of lakes on the island of Kiritimati, we found a significant difference in the community structure between brackish and hypersaline lakes. A strong relationship between microbial communities and pH, which has the potential to drive community structure as well as change in response to the microbes present, was also detected. This finding suggests future studies should investigate the relationship between specific microbial metabolisms, environmental properties of the lakes, and calcium carbonate abundance. Alpha diversity metrics measured across the salinity gradient showed no apparent correlation between the diversity of bacterial communities and salinity. Further investigations into the degree of mat stratification, amount of predation, and age of the mats will improve understanding of what drives diversity in these systems. Overall, findings from this study illustrate that microbial community composition is highly variable across the lakes of Kiritimati, even in systems of similar salinities.

Figures

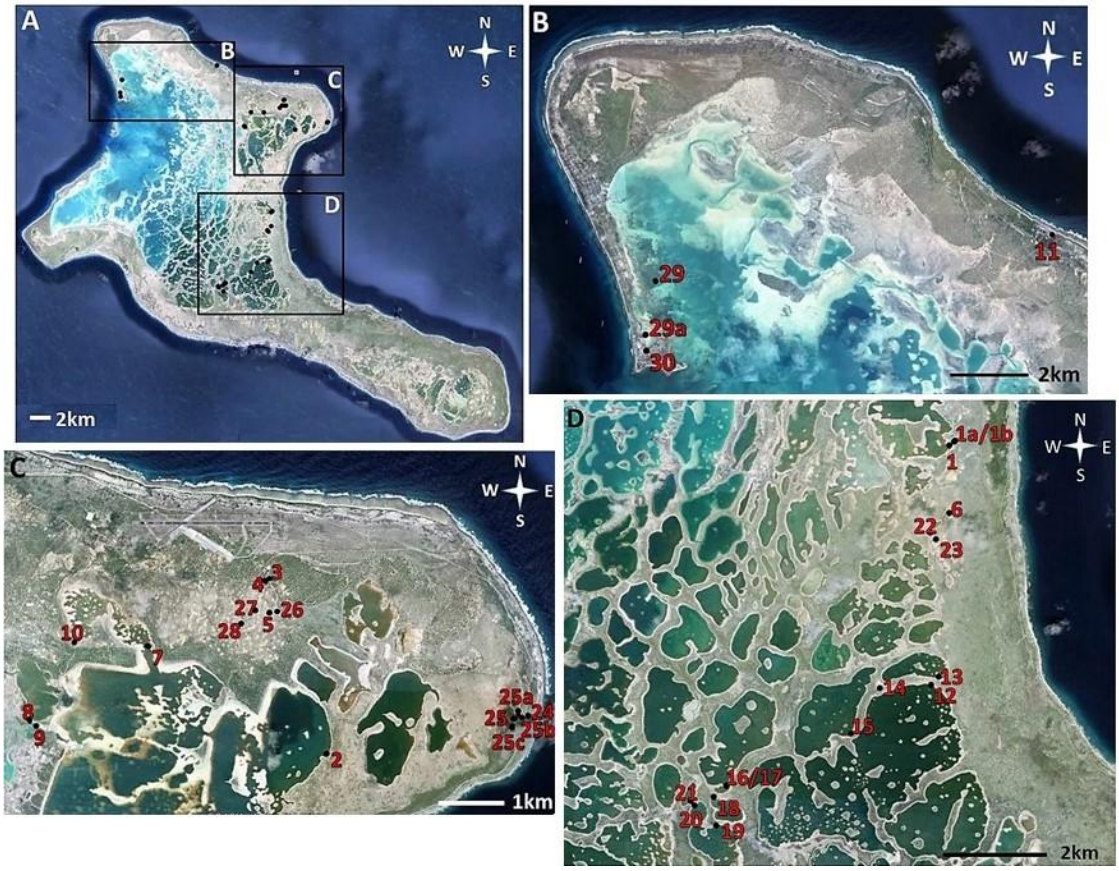

Figure 1: Map of Kiritimati from Google Earth indicating locations (black dots) of sampled lakes. (A) is a site overview. (B),(C), and (D) are site close ups ("Kiritimati, Line Islands, Kiribati").

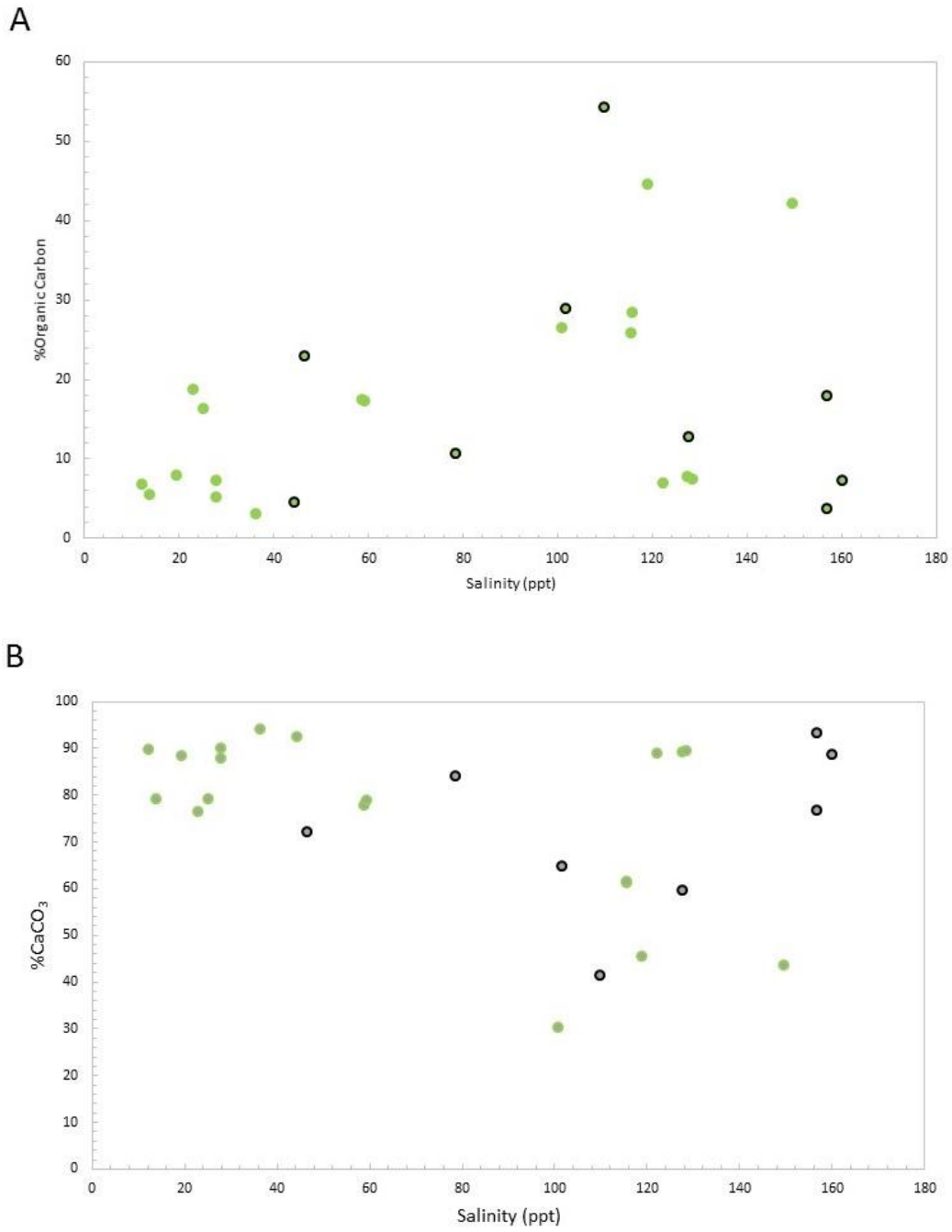

Figure 2: Loss on ignition plots for organic content (A) and carbonate content (B) of collected surface samples, plotted versus salinity. Samples with black borders are locations that had microbial mats.

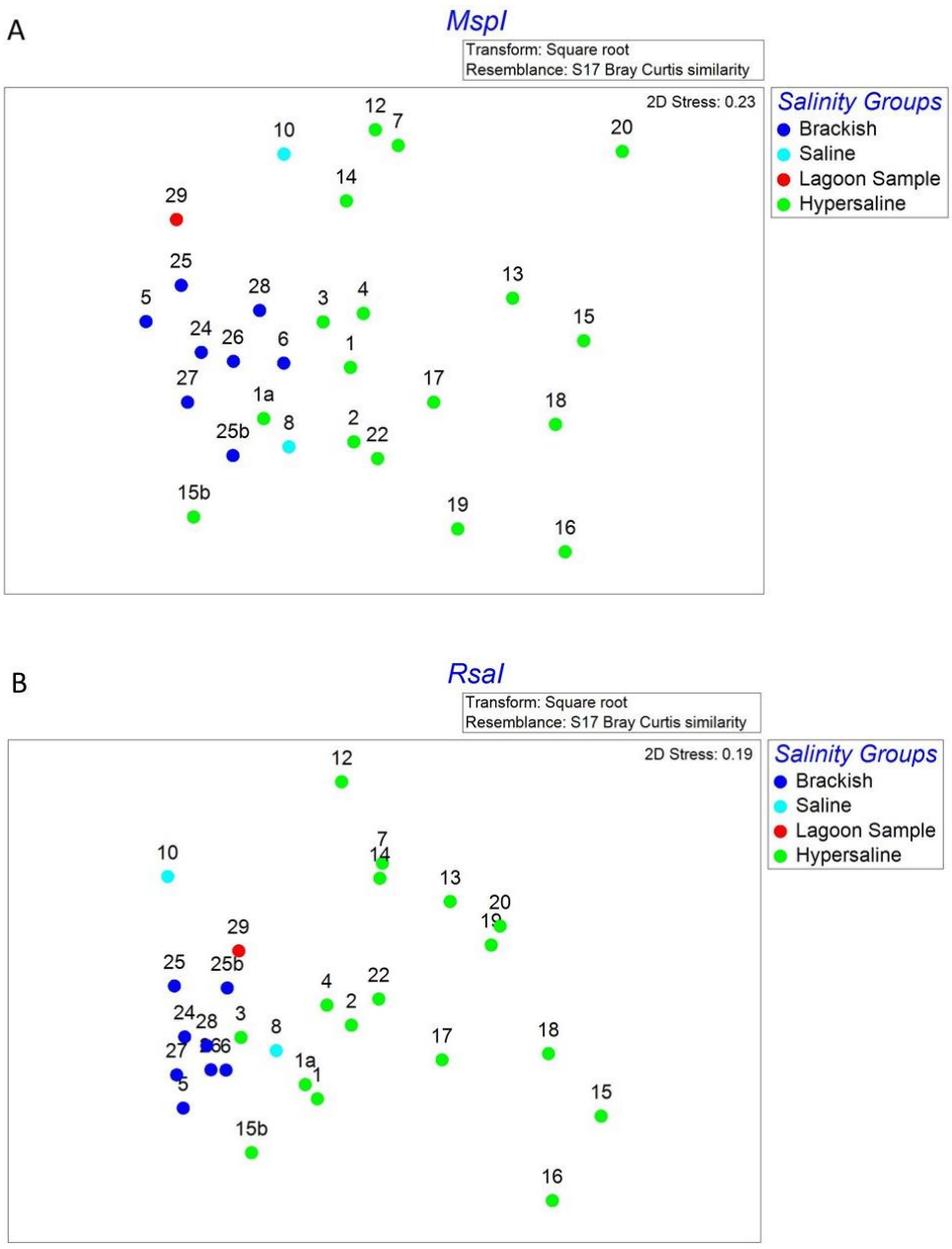

Figure 3: Non-metric multidimensional scaling (MDS) plot displaying the results of bacterial 16s rRNA terminal restriction fragment length polymorphism analysis with two different restriction enzyme digests, MspI (A) and RsaI (B). Hypersaline samples are denoted by green symbols, brackish by dark blue, saline by light blue, and the lagoon sample is red. Numbers above the sample correspond to the sample locations. TRF data were compared among samples using Bray-Curtis similarity.

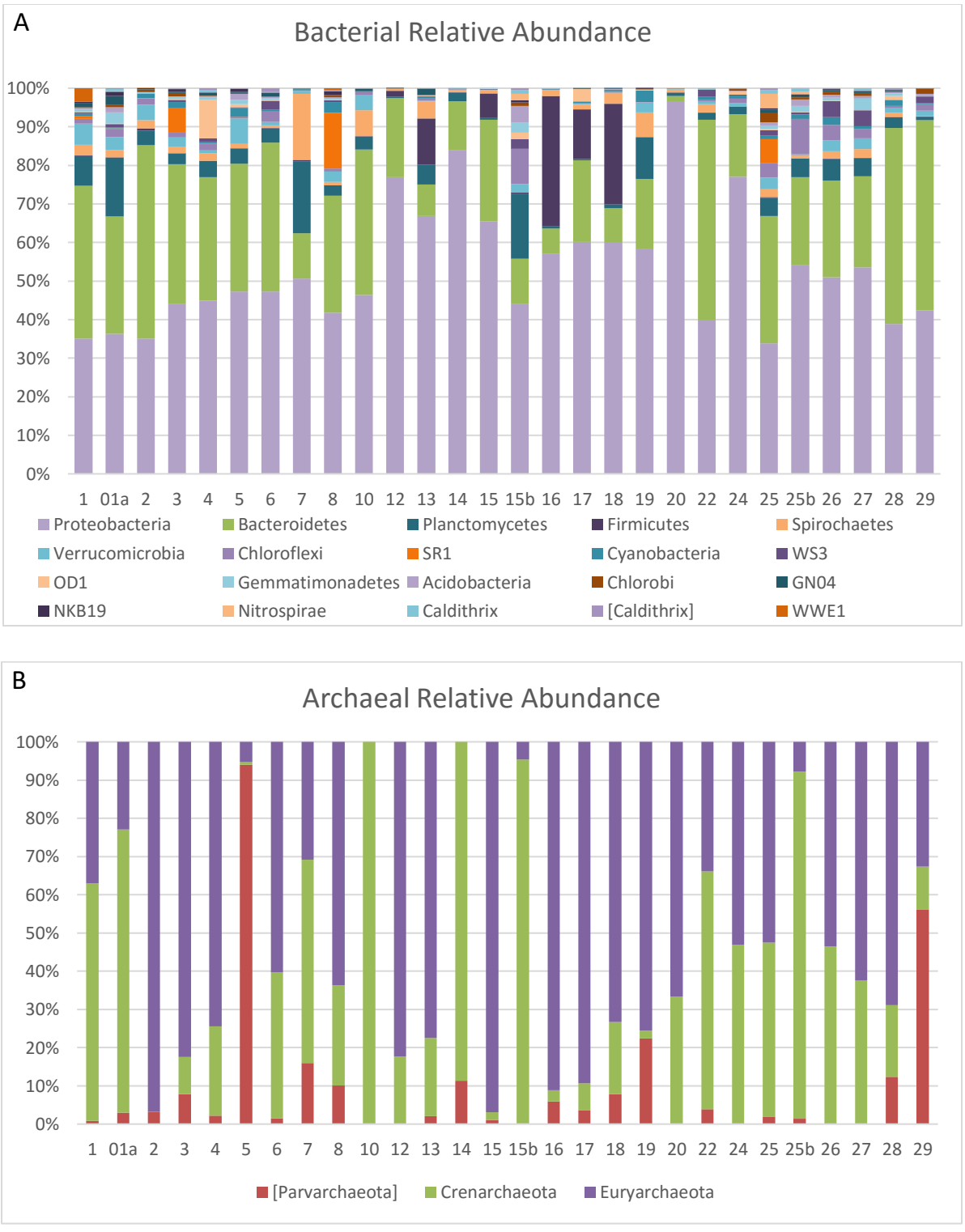

Figure 4: Relative abundance for bacterial (A) and archaeal (B) phyla. Bacterial plot shows only OTUs for the twenty most abundant taxa. Unclassified sequences were not included.

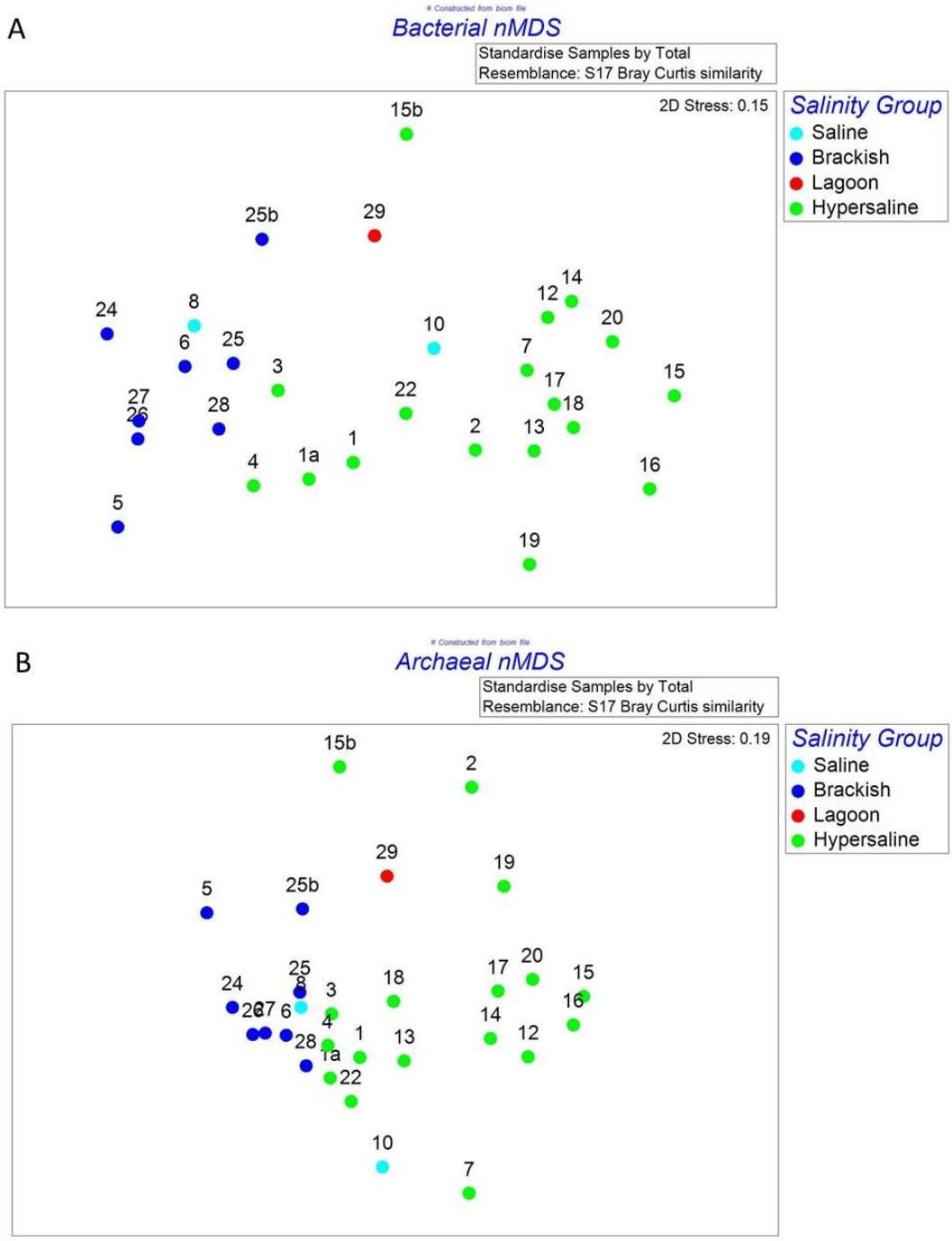

Figure 5: Non-metric multidimensional scaling (MDS) plot displaying 16S rRNA bacterial (A) and archaeal (B) community composition from sequencing data. Hypersaline samples are denoted by green symbols, brackish by dark blue, saline by light blue, and the lagoon sample is red. Numbers above the sample correspond to the sample locations. Sequence data were compared among samples using Bray-Curtis similarity.

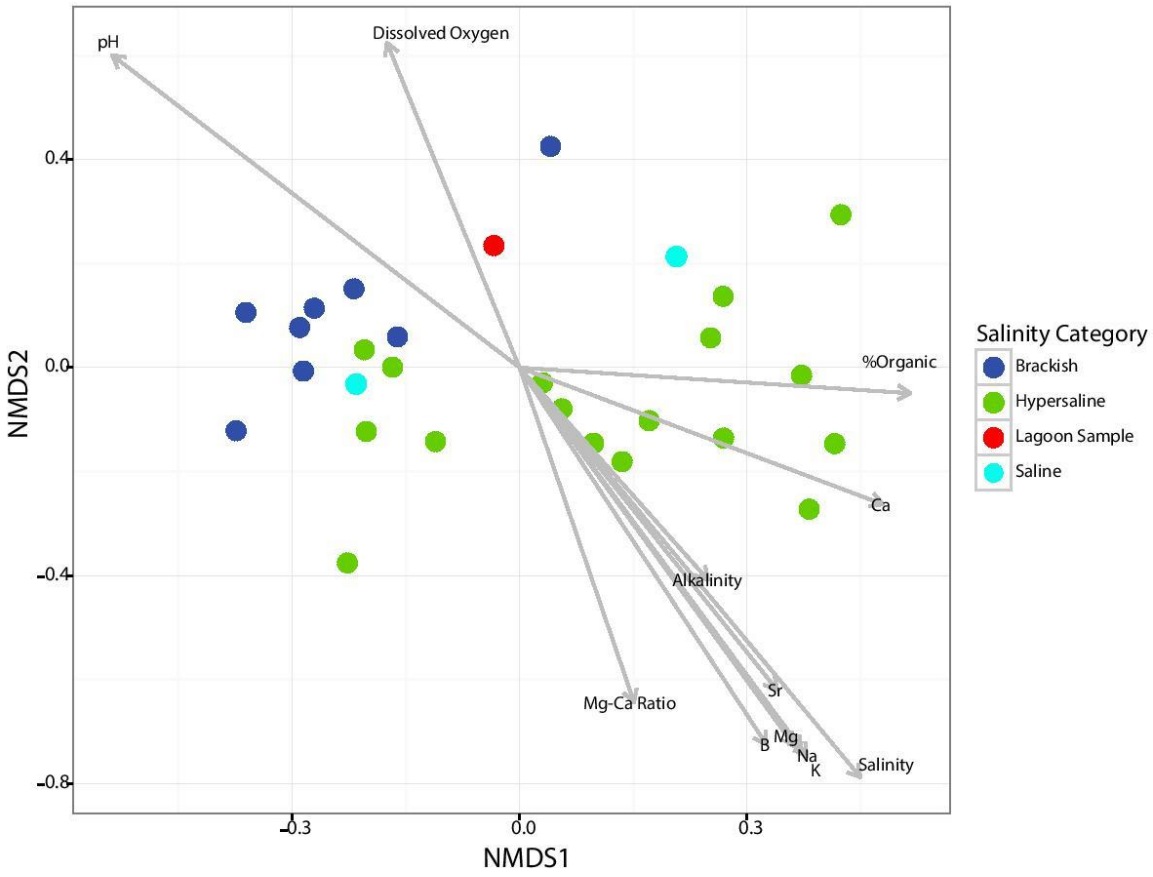

Figure 6: Nonmetric multidimensional scaling plot of bacterial 16s rRNA Bray-Curtis similarities with best correlating environmental variables ($P \leq 0.05$) plotted as vectors. Hypersaline samples are denoted by green symbols, brackish by dark blue, saline by light blue, and the lagoon sample is red The length of the arrow is proportional to the strength of correlation between the variable and community similarity. See Table 3 for environmental fitting statistics.

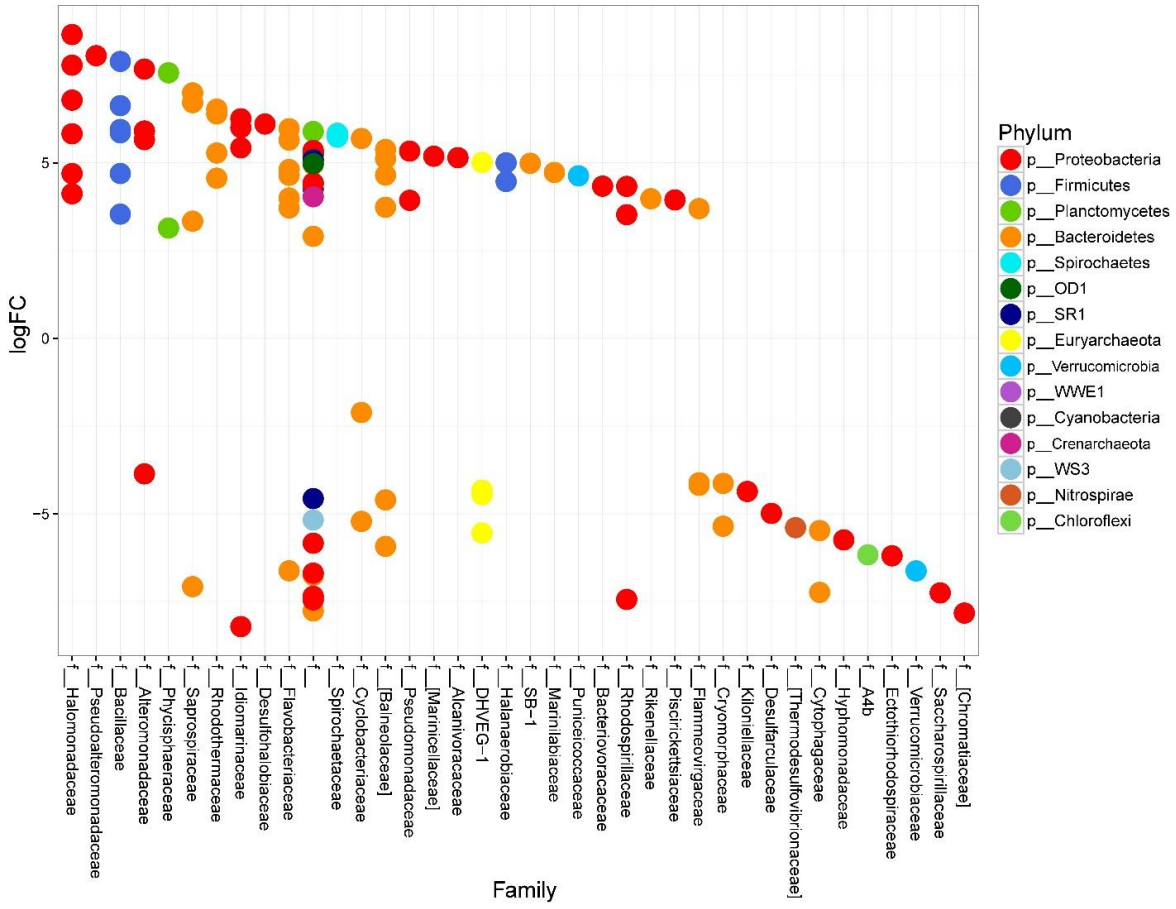

Figure 7: Pairwise comparison between brine and brackish samples. Log2-fold (LogFC) change is for the hypersaline samples relative to brackish samples. Positive logFC values indicate OTUs that are more relatively abundant in the hypersaline than brackish lakes. Negative logFC values are those that are less relatively abundant in hypersaline lakes compared to brackish.

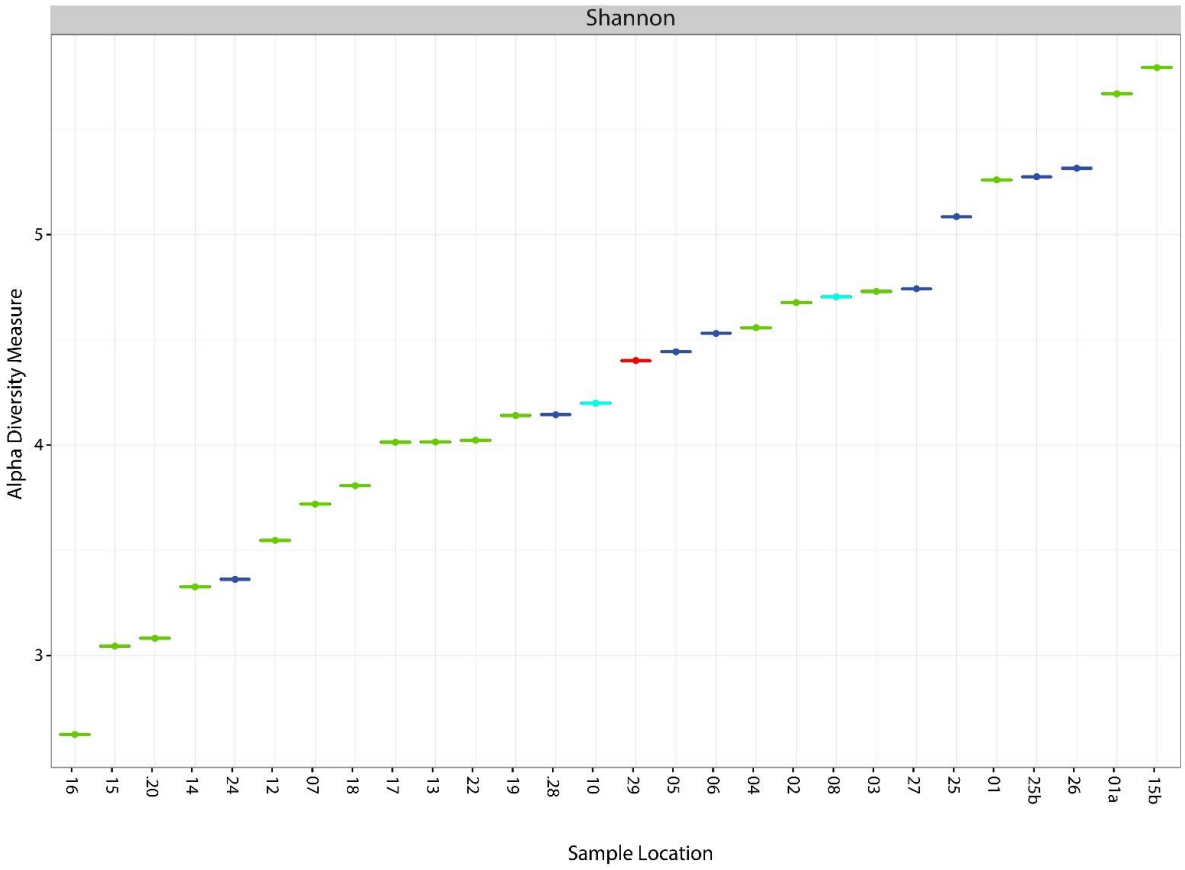

Figure 8: Alpha diversity of rarefied OTUs measured via the Shannon diversity index across all sampling locations. Hypersaline samples are denoted by green symbols, brackish by dark blue, saline by light blue, and the lagoon sample is red High values are representative of more diverse communities.

Tables

Table 1a: Hydrochemical data from lakes, seawater, and well water on Kiritimati

Sample ID	Lake ID (Sanger et al. 2006)	Lake ID (Arp et al. 2012)	Latitude	Longitude	Sampling Date
1	NA	NA	1.88526	-157.35507	7/24/2014
1a	NA	NA	1.88605	-157.35402	7/24/2014
1b	NA	NA	1.88605	-157.35402	7/24/2014
2	137	Lake 51	1.95273	-157.33267	7/24/2014
3	NA	NA	1.97785	-152.3409	7/25/2014
4	NA	NA	1.97743	-157.34135	7/25/2014
5	NA	NA	1.97294	-157.34081	7/25/2014
6	30	NA	1.87364	-157.35541	7/26/2014
7	NA	NA	1.96785	-157.35822	7/30/2014
8	NA	NA	1.95721	-157.37489	7/30/2014
9	157	NA	1.95648	-157.37399	7/30/2014
10	NA	NA	1.96856	-157.36888	7/30/2014
11 (Ocean)	NA	NA	2.00835	-157.39568	7/30/2014
12	1	Lake 2	1.84421	-157.35936	7/31/2014
13	2	NA	1.84555	-157.35803	7/31/2014
14	2a	NA	1.84392	-157.36821	7/31/2014
15	122	NA	1.83626	-157.37338	7/31/2014
15b	122	NA	1.83626	-157.37338	7/31/2014
16	14	NA	1.82788	-157.39503	7/31/2014
17	XP2	NA	1.82798	-157.39502	7/31/2014
18	3	NA	1.82611	-157.39726	7/31/2014
19	123	NA	1.82119	-157.39699	7/31/2014
20	5a	NA	1.82474	-157.40063	7/31/2014
21	4	NA	1.82553	-157.40126	7/31/2014
22	101	Lake 30	1.87037	-157.35876	8/1/2014
23	103	NA	1.86917	-157.35782	8/1/2014
24	NA	NA	1.958	-157.3	8/1/2014
25	NA	NA	1.95769	-157.30597	8/1/2014
25a	NA	NA	1.9582	-157.30547	8/1/2014
25b	NA	NA	1.95754	157.305455	8/1/2014
25c	NA	NA	1.956467	157.306681	8/1/2014
26	NA	NA	1.97293	-157.33971	8/2/2014
27	NA	NA	1.97322	-157.3429	8/2/2014
28	NA	NA	1.97128	-157.34489	8/2/2014
29 (Lagoon)	NA	NA	1.9991	-157.47478	8/3/2014
29a (Lagoon)	NA	NA	1.98848	-157.47667	8/3/2014
30 (Well)	NA	NA	1.98528	-157.47649	8/4/2014

Table 1b: Hydrochemical data from lakes, seawater, and well water on Kiritimati

Sample ID	Observed Microbial Mat Sample	Temperature (°C)	Conductivity (µS/cm)	Salinity (ppt)
1	NA	31.2	176,384	127.3
1a	NA	30.8	176,238	128.3
1b	NA	28.8	37,473	21.9
2	Stratified	31.4	177,395	127.7
3	NA	29.6	90,694	58.5
4	NA	29.6	91,445	59.1
5	NA	33.5	14,946	7.3
6	NA	31.5	44,087	24.8
7	NA	30.3	154,483	109.7
8	NA	32	74,257	44.2
9	NA	29.6	61,350	37.3
10	Non-stratified	35.6	82,671	46.5
11 (Ocean)	NA	29.32	55,340	33.4
12	Stratified	29	141,920	101.6
13	Non-stratified	29.5	158,376	115.3
14	Stratified	28.4	113,674	78.4
15	Non-stratified	30.5	203,925	156.7
15b	NA	30.5	203,925	156.7
16	Non-stratified	30.6	168,833	122.0
17	Non-stratified	31.9	212,210	159.9
18	Non-stratified	31.3	199,682	149.5
19	Stratified	30.5	165,052	118.8
20	Stratified	30.6	161,730	115.5
21	NA	32	172,651	121.6
22	NA	30.6	144,951	100.6
23	NA	29.4	140,160	99.1
24	NA	35.5	27,413	13.6
25	NA	35.9	52,492	27.7
25a	NA	35.4	36,084	18.4
25b	NA	36	52,387	27.5
25c	NA	33.6	27,350	14.1
26	NA	32.3	22973	11.9
27	NA	32.1	35429	19.2
28	NA	32.6	41663	22.8
29 (Lagoon)	NA	27.3	56893	36.0
29a (Lagoon)	NA	27.7	56683	35.6
30 (Well)	NA	27.9	3761	1.9

Table 1c: Hydrochemical data from lakes, seawater, and well water on Kiritimati						
Sample ID	pH	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	$\delta^{18}O$ (%VSMOW)	δD
1	8.0	61.6	2.06	221	1.81	3.75
1a	8.0	50.80	1.67	NA	NA	NA
1b	7.5	44.6	2.96	NA	NA	NA
2	7.8	54.7	1.77	238	2.76	9.01
3	8.4	44.4	2.36	323	3.47	13.22
4	8.5	56.1	2.65	255	3.52	13.36
5	9.2	112	7.42	68	3.92	21.6
6	8.9	114.6	7.24	238	3.75	19.01
7	8.0	88.5	3.1	272	2.5	10.33
8	7.9	54.8	3.02	272	4.05	14.77
9	8.3	82.2	5.04	153	1.33	8.47
10	8.4	148.1	6.59	255	4.6	19.79
11 (Ocean)	8.3	111.4	7.01	119	0.32	3.16
12	8.0	100	3.81	221	2.11	8.65
13	8.0	86.3	2.79	255	2.18	7.07
14	8.1	72.3	3.42	153	3.31	15.87
15	7.8	63.1	1.67	272	1.9	2.32
15b	7.8	63.1	1.67	272	1.9	2.32
16	7.6	31.5	1.07	255	2.24	6.69
17	7.8	51.3	1.34	238	1.89	0.73
18	7.8	64.3	1.57	221	1.8	1.71
19	8.1	86.7	3.08	221	1.92	5.99
20	8.0	78.4	2.88	255	2.12	6.25
21	8.0	84.7	2.71	255	1.91	3.95
22	8.2	88.4	3.42	204	3.64	13.37
23	8.1	70.3	2.94	187	3.61	15.37
24	8.8	156.8	9.94	170	-0.77	-6.59
25	9.2	163.4	9.24	68	2.63	12.75
25a	8.7	95.1	5.72	NA	1.16	3.32
25b	8.8	64.8	3.69	NA	NA	NA
25c	8.3	154	9.9	NA	NA	NA
26	9.2	153.9	10.39	170	4.93	24.92
27	8.6	122.2	7.91	221	4.94	24.14
28	8.5	144.1	9.45	204	4.27	21.78
29 (Lagoon)	8.0	69.2	4.45		1.04	5.02
29a (Lagoon)	8.0	73.5	4.6	119	NA	NA
30 (Well)	7.2	24.6	1.84	0	-3.58	-19.27

Table 1d: Hydrochemical data from lakes, seawater, and well water on Kiritimati					
Sample ID	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	Sr (μg/L)
1	1241	6170	50890	1879	31678
1a	NA	NA	NA	NA	NA
1b	NA	NA	NA	NA	NA
2	835	4437	36002	1448	20731
3	433	3018	23687	898	10335
4	454	3079	23701	918	11433
5	110	351	2503	109	5596
6	260	1127	8706	348	11047
7	804	3488	28948	1133	17355
8	510	1565	13059	520	10863
9	455	1477	12269	475	8373
10	342	1577	12941	518	6395
11 (Ocean)	358	1139	9494	376	6671
12	1504	4776	38409	1463	26684
13	1027	3987	32233	1262	21170
14	1252	2540	22595	829	25225
15	656	5470	45992	1790	26160
15b	656	5470	45992	1790	26160
16	539	4250	36675	1434	18748
17	483	5765	47734	1910	22885
18	601	5687	47072	1864	24796
19	960	4054	32834	1288	21879
20	1043	4123	34117	1304	22654
21	581	3809	31013	1199	20391
22	1015	3701	29075	1154	21874
23	942	3158	25776	1037	25530
24	241	611	5052	195	4829
25	386	1182	9588	378	7721
25a	308	840	7105	269	6175
25b	NA	NA	NA	NA	NA
25c	NA	NA	NA	NA	NA
26	148	542	4355	182	7712
27	264	905	6938	282	12723
28	649	1199	8705	345	20957
29 (Lagoon)	429	1392	11597	448	7937
29a (Lagoon)	NA	NA	NA	NA	NA
30 (Well)	106	100	482	29	2179

Table 1e: Hydrochemical data from lakes, seawater, and well water on Kiritimati

Sample ID	Si (mg/L)	B (µg/L)	Mg / Ca (mg/L)	Cl (mg/L)	SO_4 (mg/L)
1	1.87	24432	4.97	NA	NA
1a	NA	NA	NA	NA	NA
1b	NA	NA	NA	NA	NA
2	1.60	18221	5.31	NA	NA
3	1.54	15560	6.98	NA	NA
4	1.21	15510	6.78	NA	NA
5	0.59	1922	3.20	3963	817
6	0.89	4588	4.34	11130	1723
7	0.92	13017	4.34	NA	NA
8	6.27	6275	3.07	NA	NA
9	0.33	5419	3.25	NA	NA
10	1.17	8327	4.61	NA	NA
11 (Ocean)	0.52	4748	3.18	17221	2317
12	2.26	17556	3.18	NA	NA
13	1.81	15986	3.88	NA	NA
14	1.53	10419	2.03	NA	NA
15	1.89	21345	8.33	NA	NA
15b	1.89	21345	8.33	NA	NA
16	2.09	16370	7.88	NA	NA
17	1.92	23521	11.94	NA	NA
18	bmdl	22140	9.46	NA	NA
19	1.66	15952	4.22	NA	NA
20	1.79	15968	3.95	NA	NA
21	1.39	15088	6.55	NA	NA
22	1.00	14856	3.65	NA	NA
23	1.03	12327	3.35	NA	NA
24	0.18	2248	2.54	NA	NA
25	0.63	5073	3.07	NA	NA
25a	0.28	2996	2.73	NA	NA
25b	NA	NA	NA	NA	NA
25c	NA	NA	NA	NA	NA
26	0.81	2806	3.67	NA	NA
27	0.25	4669	3.43	NA	NA
28	1.13	5395	1.85	NA	NA
29 (Lagoon)	0.24	5135	3.24	16629	2246
29a (Lagoon)	NA	NA	NA	NA	NA
30 (Well)	1.06	422	0.95	NA	NA

Table 2: Surface Sediment loss on ignition data			
Sample	%Organic	%CaCO3	%Residual
K14-01	8.0	89.4	2.6
K14-01a	7.6	89.8	2.6
K14-02	12.8	59.8	27.3
K14-03	17.6	78.1	4.3
K14-04	17.4	79.1	3.5
K14-06	16.4	79.4	4.2
K14-07	54.4	41.4	4.2
K14-08	4.7	92.6	2.7
K14-10	23.1	72.3	4.7
K14-12	28.9	64.8	6.4
K14-13	26.0	61.6	12.5
K14-14	10.8	84.2	5.0
K14-15	17.9	76.8	5.3
K14-15b	3.8	93.3	2.9
K14-16	7.1	89.2	3.7
K14-17	7.3	88.7	4.0
K14-18	42.2	43.7	14.1
K14-19	44.7	45.8	9.5
K14-20	28.5	61.7	9.7
K14-22	26.6	30.6	42.8
K14-24	5.7	79.5	14.9
K14-25	7.3	90.3	2.4
K14-25b	5.3	88.0	6.8
K14-26	6.9	90.1	3.1
K14-27	8.1	88.6	3.4
K14-28	18.9	76.6	4.5
K14-29	3.2	94.3	2.5

Table 3: Environmental Fitting Statistics

Variable	NMDS1	NMDS2	r^2	Pr(>r)	Significance
Temperature	-0.87906	0.4767	0.1515	0.13009	
Salinity	0.49568	-0.86851	0.8223	1.00E-05	***
pH	-0.66657	0.74544	0.6489	1.00E-05	***
Dissolved O_2	-0.2696	0.96297	0.4205	0.00115	**
Alkalinity	0.52016	-0.85407	0.2282	0.03844	*
Ca	0.87681	-0.48083	0.2984	0.01178	*
Mg	0.44588	-0.89509	0.6582	2.00E-05	***
Na	0.4515	-0.89227	0.6763	1.00E-05	***
K	0.45232	-0.89186	0.7011	1.00E-05	***
Sr	0.48034	-0.87708	0.4989	2.40E-04	***
Si	0.29722	-0.95481	0.0949	2.90E-01	
B	0.40974	-0.9122	0.6305	3.00E-05	***
Mg-Ca Ratio	0.22852	-0.97354	0.4367	7.60E-04	***
Percent OM	0.99545	-0.09528	0.2679	2.00E-02	*
Percent $CaCO_3$	-0.86465	-0.50237	0.0802	3.53E-01	

Significance Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '

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