Salinity, microbe and carbonate mineral relationships in brackish and hypersaline lake sediments: A case study from the tropical Pacific coral atoll of Kiritimati

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Abstract
Microbiological activity can exert a substantial influence on carbonate mineral precipitation, but linking specific microbiological processes to carbonate minerals in an environmental setting is complex, as both abiotic and biotic factors ultimately influence carbonate mineral precipitation. The coral atoll of Kiritimati, Republic of Kiribati (1.9°N, 157.4°W), in the central tropical Pacific Ocean, contains hundreds of shallow water brackish to hypersaline lakes that contain a range of carbonate and evaporite minerals. Previous studies of Kiritimati lakes have investigated the microbial communities of finely laminated microbial mats and associated microbialites found in several of the more hypersaline lakes on the island. However, the microbial communities of the more brackish lakes are unknown. These brackish lakes precipitate metres of fine-grained carbonate muds, which are useful for palaeoenvironmental reconstruction. Here, the relationships between carbonate abundance, mineralogy, water chemistry, and bacterial and archaeal communities are investigated in a suite of brackish to hypersaline lakes (8.7-190 ppt) on Kiritimati. Next generation 16S rRNA gene sequencing of bacteria and archaea indicate that brackish lake sediments contain distinct microbial communities. In relation to carbonate precipitation, the relative abundance of Cyanobacteria, Chloroflexi and Deltaproteobacteria is greater in the brackish lake sediments, suggesting photosynthesis and sulphate reduction associated with these taxa may strongly influence alkalinity and carbonate precipitation in brackish lakes. The presence of dolomite in certain hypersaline lakes also coincided with the presence of a methanogenic family, indicating that methogenesis may contribute to dolomite precipitation in these lakes.

KEYWORDS
Archaea, bacteria, carbonate, dolomite, salinity
1 | INTRODUCTION

Microbial metabolic processes can play a key role in regulating the precipitation of carbonate minerals in a range of diverse environments (Dupraz et al., 2009; Dupraz & Visscher, 2005). Understanding microbial interactions responsible for forming modern organo-sedimentary carbonate deposits is essential for interpreting biosignatures on early Earth and potentially other planets, given the ubiquity of stromatolites in early Earth history and the recent discovery of finely layered sedimentary deposits on Mars (Bianciardi, Rizzo, & Cantasano, 2014; Noffke, 2014; Riding, 2011). However, the complex interplay of abiotic and biotic factors that ultimately produce calcium carbonate minerals (Fouke, 2011), creates uncertainty in assigning specific roles to microbial or abiotic processes in past carbonate precipitation.

Kiritimati, Republic of Kiribati (1.9°N, 157.4°W), an isolated carbonate platform and the world’s most extensively extensive coral atoll, is part of the group of near-equatorial Northern Line Islands in the central tropical Pacific Ocean. Studies of lacustrine microbial mats and associated carbonate minerals in select Kiritimati lakes have provided key insights into the drivers of carbonate precipitation and carbon cycling, as well as potential palaeoclimate signals archived in buried microbial mats and chemical sediments (Arp et al., 2012; Blumenberg, Thiel, & Reitner, 2015; Bühring et al., 2009; DeFarge et al., 1996; Higley, Conroy, & Schmitt, 2018; Ionescu et al., 2015; Schneider, Arp, Reimer, Reitner, & Daniel, 2013; Shen, Thiel, Duda, & Reitner, 2018; Spring et al., 2015; Trichet, Defarge, Tribble, Tribble, & Sansone, 2001). The body of research on the microbial communities found in Kiritimati lakes has mainly focused on the finely stratified microbial mats located in several of the hypersaline lakes on the island. However, the microbial communities in the sediments of brackish lakes, which do not have finely laminated microbial mats, but still precipitate copious amounts of carbonate sediment, remain unknown.

In the present study, the natural salinity gradient across a suite of Kiritimati lakes is used to investigate the relationship between water chemistry, microbial community diversity and carbonate mineralogy. The composition of microbial communities in the surficial, near-shore sediments of 25 lakes and the main lagoon of Kiritimati was analysed using next-generation high-throughput sequencing of bacterial and archaeal 16S rRNA genes. These results were then used to assess how mineral properties and microbial communities vary in the hypersaline and brackish lake systems. This spatial dataset has the potential to serve as an analogue for climate-induced temporal changes in lake salinity, and subsequently, past microbial community composition, in palaeolimnologic records from Kiritimati (Higley et al., 2018), and in geological records of interior facies from isolated carbonate atolls (Read, 1985).

2 | METHODS

2.1 | Site description and sampling

The Northern Line Islands in the central Tropical Pacific lie along a northward gradient of increasing rainfall; Kiritimati is the southernmost island and lies within the equatorial dry zone, south of the Intertropical Convergence Zone. Interannual rainfall on the island is highly variable due to the El Niño/Southern Oscillation (ENSO), with high precipitation during El Niño events and reduced precipitation during La Niña events. Approximately one quarter of the island is covered in lakes with salinities ranging from brackish (<36 ppt, local seawater) to hypersaline (>51 ppt). The largest body of water is the main lagoon (320 km²) located in the northwest of the island. Many hypersaline lakes connect to this lagoon through a series of channels. Lakes closest to the lagoon approximate lagoon salinity, and salinity increases with increasing distance from the lagoon, as more isolated lagoon waters slowly evaporate (Saenger, Miller, Smittenberg, & Sachs, 2006). Brackish lakes, with lower salinity than that of the open ocean or lagoon, are isolated from these hypersaline lakes and arise from outcropping groundwater lenses in the northeastern and southeastern parts of the island.

Water and sediment/mat samples were obtained in Kiritimati in July and August of 2014. Water was sampled from the lakes, the open ocean and the main lagoon (Figure 1; Table S1) at the depth of the sediment–water interface (20–50 cm) near shore, in the photic zone. Temperature (±0.2°C), specific conductance relative to 25°C (±0.01 mS/cm), pH (±0.2 units) and dissolved oxygen (DO) content (±0.2 mg/L; ±2%) were measured in situ using a YSI ProPlus multiparameter water quality sonde. Salinity values were calculated using specific conductance values relative to 25°C. 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). Water samples (30 ml) were taken, filtered through a 0.22 μm filter, and stored in acid washed bottles for cation analysis. The concentration of major and minor cations was measured using an ICP-OES (Perkin-Elmer, Optima 5300 DV; ±2% for major elements and ±5% for minor elements).

Nearshore sediment samples from the sediment/mat-water interface to 5 cm depth were taken from all locations at 20–50 cm water depth. The sediment was sampled in this fashion due to interest in the brackish lakes, which do not have millimetre-scale laminated microenvironments at the sediment-water interface. One sample was also taken from the main lagoon (Site 29). Site 1 was sampled at two locations (Sites 1 and 1a), separated by 145 m, to observe any small-scale spatial differences in community composition. Water salinity was the same at both locations at the time of sampling. One additional sample of beach crust and underlying sediment (Site 15b) from 5 cm below the surface was...
also taken near Site 15. Sites 25 and 25b are separate water bodies separated by 60 m. Site 25b is a small hole filled with brackish water and precipitating fine white carbonate silt (Table 1), near a larger, shallow body of open water (Site 25), with a harder underlying carbonate surface. A soil knife was cleaned and rinsed with deionized water before being submersed and washed in the lake water prior to the collection of each sediment sample. The samples were then placed in sterile Whirlpak bags, fixed with 3% glutaraldehyde in a phosphate buffer, homogenized and stored on ice. Upon return, they were placed in a 4°C cold room in the dark, with subsamples intended for DNA analysis frozen and stored at −80°C prior to DNA extraction and PCR amplification.

2.2 | Organic and inorganic carbon and mineral analysis

Prior to loss on ignition, sediment subsamples were rinsed multiple times with deionized water to remove glutaraldehyde and pore water containing sulphates, which can confound estimates of organic matter and carbonate from combustion. Rinsed sediment was dried at 120°C overnight. Dried sediment was transferred to crucibles and weighed. Samples were then burned at 550°C for 2 hr in a muffle furnace to combust organic content and 1,050°C for 4 hr to combust carbonate. Percent organic and calcium carbonate abundance were estimated based on mass measurements following each combustion (Heiri, Lotter, & Lemcke, 2001). Sediment mineralogy was assessed with powder X-ray diffraction on dried, pulverized sediment subsamples (Table S2). The samples were run on a Siemens-Bruker D5000 instrument in the Materials Research Laboratory at the University of Illinois Urbana-Champaign. Sediment mineral components were analysed with Jade+ 9.5 software. The Rietveld Refinement was applied to determine mineral phase percentages for each sample (Rietveld, 1969).

2.3 | DNA extraction from sediment

Representative sediment subsamples were removed from each Whirlpak bag under a laminar flow hood and placed in sterile conical tubes. All samples were rinsed multiple times with 3.5% phosphate buffered saline solution to remove the glutaraldehyde fixative. After rinsing, genomic

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**FIGURE 1** (A) Map of Kiritimati (Google Earth, 2017) indicating (B–D) locations of sample sites
TABLE 1 Sediment descriptions for each lake, with lake water salinity

| Site | Water salinity (ppt) | Carbonate/Evaporite sediment description                                                                 |
|------|----------------------|----------------------------------------------------------------------------------------------------------|
| 1    | 147.7                | Red pigmented microbial mat and light-brown micritic matrix with gravel-sized fragments of white carbonate sheets and knobby aggregates |
| 1a   | 147.6                | Red pigmented microbial mat and light-brown micritic matrix with gravel-sized fragments of white carbonate sheets, coarse sand-sized spherical carbonate aggregates and sand-sized white carbonate pellets |
| 2    | 148.9                | Red and green pigmented microbial mat with white-brown, coarse sand-sized carbonate sheet fragments, spherical aggregates and peloids, coarse sand to fine gravel-sized gypsum Foraminifera tests, ostracod valves and gastropod shells present |
| 3    | 64.7                 | Dark brown micritic matrix and red to brown pigmented organic matter with white, sand-sized carbonate peloids and knobby aggregates Foraminifera tests, ostracod valves and gastropod shells present |
| 4    | 65.4                 | Dark brown micritic matrix and red to dark brown pigmented organic matter with coarse-sand sized white carbonate peloids and carbonate gravel Foraminifera tests, ostracod valves and gastropod shells present |
| 6    | 28.4                 | Green pigmented organic matter and pink-light brown micritic matrix with coarse sand-sized white carbonate peloids Foraminifera tests, ostracod valves and gastropod shells present |
| 7    | 124.2                | Red pigmented microbial mat matrix with white, fine to coarse sand-sized carbonate spherules, peloids and aggregates Foraminifera tests, ostracod valves and gastropod shells present |
| 8    | 51.3                 | Gray micritic matrix and green-pigmented organic matter with white carbonate sand-sized peloids Foraminifera tests, ostracod valves and gastropod shells present |
| 10   | 58.1                 | Red pigmented microbial mat matrix with sand-sized white carbonate peloids, spherules and aggregates of carbonate spherules Ostracod valves and gastropod shells present |
| 12   | 111.5                | Green and red pigmented microbial mat matrix with sand-sized white peloids and aggregates of spherules, and gravel-sized fragments of white carbonate sheets Foraminifera tests and gastropod shells present |
| 13   | 128.3                | Green and red pigmented microbial mat matrix with sand-sized, white carbonate peloids, spherules, aggregates of spherules, irregularly shaped aggregates, and coarse sand-sized white gypsum Foraminifera tests and ostracod valves present |
| 14   | 84.8                 | White micritic matrix with white sand-sized carbonate peloids and aggregates Foraminifera tests and gastropod shells present |
| 15   | 179.8                | Red pigmented microbial mat and brown micritic matrix with sand-sized white carbonate peloids, knobby aggregates and gravel size fragments of white carbonate sheets Foraminifera tests and gastropod shells present |
| 15b  | NA                   | White micritic matrix with sand-sized white carbonate peloids and knobby aggregates Gastropod shells present |
| 16   | 139.4                | Red and green pigmented microbial mat and brown micritic matrix with sand-sized white peloids and aggregates Gastropod shells present |
| 17   | 190.0                | Red and green pigmented microbial mat and brown to white micritic matrix with coarse sand-sized white carbonate peloids and knobby aggregates Foraminifera tests, ostracod valves, and gastropod shells present |
| 18   | 174.7                | Red pigmented microbial mat and dark brown micritic matrix with coarse sand-sized white carbonate peloids and knobby aggregates Foraminifera tests, ostracod valves and gastropod shells present |

(Continues)
DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, OH) according to the manufacturer's instructions. Sterile molecular grade deionized water was used to elute DNA. The concentration of DNA in all samples was measured using a Nanodrop Spectrophotometer. Remaining samples were stored at −20°C.

2.4 | Amplicon sequencing

Sequencing libraries of 16S rRNA gene amplicons were generated using two different primer pairs, one targeting the V4 region of both bacteria and archaea (515f, 5′GTGYCAGCMGCCGCGGTAA-3′, and 806r, 5′GGACTACNVGGGTATCTAAT-3′, Caporaso et al., 2011) and the other specifically targeting archaea (Arc349f, 5′GYGCASCAGKCGMGAAW-3′, 806r, 5′GGACTACVSGGGTGATCTAAT-3′, Takai & Horikoshi, 2000). These primers were barcoded for multiplexing on a single MiSeq run and to function with the Fluidigm system. The PCR amplification for bacterial and archaeal sequencing was carried out in duplicate 25 μl volumes for each sample and then pooled. Each reaction contained 13.4 μl of sterile molecular grade water, 2.5 μl 10× PCR buffer (TaKaRa, Clontech, CA), 2 μl dNTP solution (2.5 mM each dNTP, TaKaRa), 2.5 μl of each primer at 4 μM, 0.1 μl Ex Taq polymerase (TaKaRa) and 2 μl template DNA. The PCR protocol used an initial denaturation step at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 45 s, and a final extension at 72°C for 10 min. Samples were held at 4°C in the thermocycler then stored in a freezer at −20°C. The 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 using a lower concentration of template DNA. The PCR protocol used an initial denaturation step at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 45 s, and a final extension at 72°C for 10 min. Samples were held at 4°C in the thermocycler then stored in a freezer at −20°C. The 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 using a lower concentration of template DNA. Archaeal samples that did not produce significant PCR product at 30 cycles were re-amplified with 35 cycles. Following successful amplification, PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, MD) according to the manufacturer's protocol. Purified products were checked again via agarose gel for success and stored at −20°C.

In silico PCR amplicons using 515f and 806r primers were generated using TestPrime (Klindworth et al., 2013) and found to match 87.5% of all sequences classified as domain Bacteria, 85.4% classified as Archaea and 0.0% of those

| Site | Water salinity (ppt) | Carbonate/Evaporite sediment description |
|------|----------------------|------------------------------------------|
| 19   | 135.4                | Red and green pigmented microbial mat and dark brown micritic matrix with coarse sand-sized white carbonate peloids and gravel-sized white carbonate knobby aggregates Foraminifera tests, ostracod valves and gastropod shells present |
| 20   | 131.8                | Red and green pigmented microbial mat with white gravel-sized fragments of carbonate sheets, coarse sand to gravel-sized white carbonate aggregates and gypsum sand |
| 22   | 143.6                | Red and green pigmented microbial mat with coarse sand to gravel-sized white carbonate peloids, aggregates and gypsum sand Foraminifera tests and ostracod valves present |
| 24   | 16.8                 | Brown-white micritic matrix with coarse sand-sized white carbonate peloids and knobby aggregates and gravel-sized fragments of white carbonate sheets Foraminifera tests and gastropod shells present |
| 25   | 34.6                 | Red-brown-white micritic matrix with coarse sand-sized white carbonate peloids and spherule aggregates |
| 25b  | 34.5                 | White carbonate silt with gravel-sized fragments of carbonate sheets Foraminifera tests, ostracod valves and gastropod shells present |
| 26   | 13.9                 | Light brown micritic matrix with red-pigmented organic matter and gravel-sized fragments of white carbonate sheets, coarse sand-sized peloids and knobby aggregates Foraminifera tests and gastropod shells present |
| 27   | 22.3                 | Light brown micritic matrix with green-pigmented organic matter with gravel-sized fragments of white carbonate sheets, coarse sand-sized peloids and knobby aggregates Foraminifera tests, ostracod valves and gastropod shells present |
| 28   | 26.7                 | Light red-brown micritic matrix with gravel-sized fragments of carbonate sheets, coarse sand-sized peloids, and aggregates Foraminifera tests, ostracod valves and gastropod shells present |
| 29 (lagoon) | 37.8            | White carbonate sand matrix with occasional flecks of green organic matter and sand to gravel-sized white coral and mollusc shell fragments Foraminifera tests, ostracod valves and gastropod shells present |
classified as Eukarya in the SILVA RefNR database (version 128). TestPrime analysis of the Arch349f and Arch806r primer pair found it to match 74.5% of sequences classified as Archaea and 0.0% of those classified as either Bacteria or Eukarya. Given the high degree of similarity between two reverse primers, groups of sequences are hereafter referred to by the name of the forward primer alone. V4-515f and Arc349f samples were prepared separately and submitted to the Functional Genomics Unit of the University of Illinois Urbana-Champaign Carver Biotechnology Center. Paired-end sequencing (2 × 250 base pairs) was performed on one lane of a MiSeq Nano (v2). Raw sequence data were deposited in MG-RAST (Meyer et al., 2008) and will be made publicly available to download upon publication (project number MGP82583).

2.5 Microbial community analyses

Following sequencing, amplicon libraries for each primer pair were processed separately using QIIME (Caporaso et al., 2010), USEARCH (Edgar, 2010) and phyloseq (McMurdie & Holmes, 2013). Paired-end reads generated using V4-515f primers were joined together using PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2014), and 99.02% of sequences were successfully joined. Paired-ends were not joined for amplicons generated using the Arch349f primers because the greater amplicon lengths of the amplicon (average of 457 nucleotides compared to 292 for V4-515f) and general poor quality of reverse reads prevented efficient joining. Only high-quality forward reads of Arch349f amplicons were used for downstream analyses.

Sequences were demultiplexed and quality filtered using QIIME (version 1.9.1). All sequences not meeting quality standards were discarded. Poor quality sequences were discarded based on divergence from expected amplicon length, quality scores below 25, long homopolymer runs (max = 6) and primer mismatches (max = 0). Initial quality screening for chimeric sequences, dereplicated, and the remaining sequences for Arch349f. In USEARCH, libraries were screened for chimeric sequences, dereplicated, and the remaining sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity cut-off using the cluster_otus command and the UPARSE-OTU algorithm (Edgar, 2013). Taxonomic identities were assigned to representative sequences from each OTU using the UCLUST algorithm (Edgar, 2010) and the SILVA reference database (version 128—September 2016) (Quast et al., 2013). Singleton OTUs (those represented by only a single sequence across all samples) were discarded prior to downstream analyses.

Alpha and beta diversity analyses (ANOSIM, nMDS, nonmetric multidimensional scaling; differential abundance) were conducted in either Primer (Clark & Warwick, 2001) or the R programming environment for others using the packages phyloseq (McMurdie & Holmes, 2013), vegan (Oksanen et al., 2007), and edgeR (Robinson, McCarthy, & Smyth, 2010). Analysis of similarity (ANOSIM) based on the Bray–Curtis index was used to determine if groups of samples were significantly different from each other. In this method, a statistical metric (R_{ANOSIM}) is calculated to measure the similarity of two groups of microbial communities (brackish and hypersaline). Monte Carlo simulations are then used to test a null hypothesis that there is no difference between two groups by calculating R_{ANOSIM} for 10^6 random permutations of group assignments. These random values are typically distributed normally around a mean of zero, so if the calculated R_{ANOSIM} lies far outside this distribution, one can confidently reject the null hypothesis (Clark & Warwick, 2001). The percentage of these random values that are greater than the calculated R_{ANOSIM} is reported as p. nMDS based on the Bray–Curtis index was used to visualize the degree of similarity between lake samples (Clark & Warwick, 2001).

To test how environmental parameters influence community diversity as well as to investigate associations between sediment mineralogy and community composition, nMDS plots for bacterial and archaeal 16S rRNA gene sequences were vectorized using environmental and sediment parameters. These parameters include lake water salinity, temperature, pH, DO, alkalinity, the concentrations of Ca, Mg, Na, K, Sr, Si and B, the Mg to Ca ratio (a variable of interest as it affects the mineralogy of precipitated carbonate—at similar calcification temperatures, higher Mg/Ca leads to aragonite, rather than calcite precipitation) (Morse, Wang, & Tsio, 1997), percent organic matter, percent calcium carbonate and the relative abundance of each mineral. Environmental variables that were not statistically significant were removed from the plotted environmental fitting and are not discussed (Table S3A and B). As paired water data were not obtained for 15b, a beach sample from site 15, this sample was excluded from this analysis and the remaining analyses.

The differences in the relative abundance of the most abundant phyla (at least >2% in one sample) in the brackish versus hypersaline lakes was assessed using the non-parametric Wilcoxon rank sum test, which tests the hypothesis that the relative abundance of a given phylum in the brackish and hypersaline samples comes from a continuous distribution with equal medians. This analysis also includes the most abundant classes of Proteobacteria (>2% of all identified classes in at least one sample), a diverse phylum that is the most abundant phylum in the 16S rRNA dataset. To further explore differences in the microbial communities, a pairwise comparison of families that are more abundant in the brackish versus hypersaline lakes, as well as lakes with and without dolomite was conducted using log 2-fold changes (logFC). LogFC values express the ratio between two quantities. For example, in this analysis, a logFC value of 1 indicates that a family is
twice as abundant in hypersaline lakes versus brackish lakes; a logFC value of −1 indicates a family is twice as abundant in brackish lakes.

3 | RESULTS

3.1 | Sediment and water properties

The bedrock surface of Kiritimati is covered with depressions as a result of dissolution of coral reef limestone during previous low-stands in sea level (Woodroffe & McLean, 1998). These depressions form shallow lakes several metres deep at most that range from several metres to kilometres in diameter. The current investigation focuses solely on the shallow, photic zone regions of these water bodies. The lake shorelines of the sample sites span the Kiritimati lake facies model (Saenger et al., 2006), which sequentially includes steep (0.5 m above lake surface), ‘palaeo-coral’ lake margins of the fossil reef, ‘carbonate hardpan’, a result of dissolution, re-precipitation and lithification of the fossil reef carbonate, ‘shell-rich sediment’ containing abundant mollusc shells in a carbonate silt and sand matrix, and ‘soft carbonate sand.’ Benthic macroorganisms in these water bodies include gastropods and bivalves in the brackish lakes and brine shrimp (Artemia), as well as the milkfish, Chanos chanos, in some of the saline to hypersaline lakes. Land crabs (Cardisoma carnifex), are also common along the shorelines of brackish to hypersaline lakes. Benthic eukaryotes identified microscopically in samples include halotolerant foraminifera and ostracoda.

Surface water chemistry of the sampled Kiritimati lakes varies significantly, most notably in salinity (Table S1), which ranged from 8.7 to 190.0 ppt. Many lakes are considerably more saline than either open ocean seawater (36.7 ppt) or lagoon seawater (37.7–37.9 ppt). As expected, the concentrations of the most abundant cations present in seawater, such as Mg, Na and Sr, are highly correlated with salinity (r = 0.97, 0.97, and 0.84, N = 31, p < 0.01 respectively). The pH and concentration of DO in lake water also show a wide range of values. pH values range from 7.2 to 9.2 and are negatively correlated with salinity (r = −0.53, p < 0.01, N = 31). Temperatures range from 27.3 to 36°C, and DO values in the lakes range from hypoxic (31.5%) to supersaturated (>100%), due to photosynthetic activity. The temperature or DO data is not discussed further as lakes were not sampled at the same time every day.

Sediment samples include carbonate and evaporite minerals of varying grain sizes and shapes (Table 1; Figure 2), the microbial mats on the uppermost surface of the hypersaline lakes (when present), as well as biofilms coating grains within pore spaces. Frequently observed carbonate grain shapes include spherules, aggregates of spherules, more irregular, knobby aggregates and carbonate clasts that have one flat dimension, giving the appearance of broken fragments of a carbonate sheet (‘sheet fragments’) that formed over a large surface area (Table 1). The percent organic matter in these sediment samples ranges from 3.2% to 54.4% (Table S2). The highest organic content values are found in lakes with a salinity greater than 100 ppt, although values range substantially in hypersaline lakes. All lake sediments contain CaCO3, with CaCO3 abundance ranging from 30.6 to 94.3% by mass (Figure 3A). Sediment mineralogy varies between samples, and minerals present include aragonite, calcite, magnesium calcite, dolomite, gypsum and ankerite (Figure 3B). Sediments from the brackish lakes contain mainly calcite and magnesium calcite, whereas the hypersaline lakes contain more aragonite (Figure 3C), as well as dolomite in three samples and gypsum in three samples (Figure 3; Table S2). Ankerite is found in low abundance (4%) at Site 18 only.

3.2 | Bacteria and Archaea sequencing results

The V4 region amplified 16S rRNA gene samples produced a total of 10810 OTUs (Table S4). The median relative abundance of bacterial phyla represented in the 28 samples is dominated by Proteobacteria (41.1 ± 17.6%, 1σ) and Bacteroidetes (22.9 ± 12.2%), followed by Planctomycetes (3.0 ± 4.4%), Spirochaetes (1.3 ± 3.2%), Verrucomicrobia (1.1 ± 1.4%), Chloroflexi (0.6 ± 2.2%) and Cyanobacteria (0.5 ± 0.8%). Cloacimonetes, Nitrospirae, SR1 (Absconditabacteria), Acidobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, Latecibacteria and Parcubacteria also represent at least 2% of the bacterial community in at least one sample (Figure 4A). It is possible that freezing of the sediment samples influenced the preservation of Cyanobacteria (Green, Blackford, Bucki, Jahnke, & Prufert-Bebout, 2008). Shannon diversity index values range from 2.77 to 6.18 (Table S4, Figure 5). Arch349f amplified samples produced a total of 1735 OTUs (Table S4). The difference in read quality and low sequence number in archaea is likely due in part to the greater abundance of bacteria in the samples relative to archaea. The median relative abundance of archaeal phyla is dominated by Euryarchaeota (17.9 ± 14.9%), Woesearchaeota (5.3 ± 11.9%), Lokiarchaeota (2.5 ± 16.8%) and Thaumarchaeota (2.1 ± 11.6%). Crenarchaeota, Altiarchaeales, Batharchaeota, Diapherotrites, Miscellaneous Euryarchaeotic Group and WSA2 also represent at least 1.5% of the archaeal community in at least one sample (Figure 4B). Shannon diversity index values are lower for Arch349f versus V4, and range from 1.68 to 4.67 (Table S4, Figure 5).

The OTU abundance relative to lake water salinity was visualized using nMDS (Figure 6). Both bacterial and archaeal communities within the brackish samples plot together and are distinct from the groupings of the hypersaline lake sediment samples. Analyses of similarity confirmed that the brackish-hypersaline groups were significantly different for both Bacteria and Archaea (RANOSIM = 0.66, p < 0.01, RANOSIM = 0.34,
$p < 0.01$, respectively). Samples from Sites 1 and 1a, which are from different shoreline locations in the same hypersaline lake, show similarly abundant bacterial and archaeal phyla ($r = 0.96$, $r = 0.94$ for relative abundances of phyla plotted in Figure 4 for 1 and 1a), although the archaeal community is more diverse at Site 1 ($H = 4.32$) versus 1a ($H = 3.32$). The largest differences are in Bacteroidetes, which comprises 35.0% of the bacterial phyla at Site 1 and 25.3% at Site 1a, Lokiarchaeota, which comprises 29.5% of the archaeal phyla at Site 1 and 39.0% at Site 1a, and Thaumarchaeota, which comprises 5.9% of the archaeal phyla at Site 1 and 20.0% at Site 1a.

The results of the rank-sum tests for the most abundant phyla in the brackish versus the hypersaline lakes indicate that Chloroflexi, Cyanobacteria, Latescibacteria, Gemmatimonadetes, Actinobacteria and Acidobacteria are more abundant in the brackish lake sediments, whereas Firmicutes is more abundant in the hypersaline sediments (Table 2). Within the diverse Proteobacteria phylum, which comprises the majority of the communities, Deltaproteobacteria and Alphaproteobacteria are more abundant in the brackish lake sediments, whereas Gammaproteobacteria is more abundant in the hypersaline lake sediments. For more information, complete

**FIGURE 2** Examples of Kiritimati lakes and sediment samples from this study. (A) Site 7, a hypersaline lake, (B) Site 6, a brackish lake, (C) surface mat and sediment from Site 7, (D) sediment–water interface from Site 6, (E) example of a white carbonate spherule, (F, G) aggregates of spherules, (H) a carbonate sheet fragment, (I) gypsum and (J) a knobby carbonate aggregate.
There is no difference in Shannon diversity index values for the brackish versus the hypersaline lake systems (Figure 5). The LogFC results for the brackish versus hypersaline lake sediment samples (Figure 7) indicates many families within the same phyla that are more abundant in either the brackish lakes (negative values) or hypersaline lakes (positive values). A smaller number of families were found to be more abundant in the lake sediments containing dolomite (Figure 8).

4 | DISCUSSION

4.1 | Drivers of microbial community diversity in 2014

Bacterial and archaeal communities in 2014 were found to vary between sediments of the brackish and hypersaline lakes (Figure 6). In lake environments, salinity is often considered a primary control on the composition of microbial communities of both the water column, and the underlying sediments (Jiang et al., 2007; Wu, Zwart, Schauer, Kamst-van Agterveld, & Hahn, 2006; Yang, Ma, Jiang, Wu, & Dong, 2016). As lakes transition from saline to hypersaline, the energetic cost of living in the environment also increases. Therefore, salinity acts as an environmental stress filter with fewer organisms able to tolerate hypersaline conditions (Oren, 2001, 2002). Similar to microbial diversity studies of other aquatic systems, including previously published studies from Kiritimati, this paper offers only a snapshot of microbial diversity in time and space. While gradients in physiochemical properties in individual lakes may also influence microbial diversity, here the focus is on shoreline, photic zone samples. As such, it is not assumed that these microbial communities are static in space or time. In fact, these results may serve as a baseline from which to assess future microbial communities in these lakes, for
FIGURE 4  Relative abundance of the most abundant (A) bacterial (V4) and (B) archael (Arch349F) phyla in Kiritimati sediment samples. Sampling site numbers are listed on x-axis, ranked left to right from lowest to highest salinity values. Taxa are ordered from top to bottom by median abundance across sites, excluding the gray shading which denotes other bacteria and archaia that are not listed.
Salinity is the main physiochemical factor influencing microbial community composition. Salinity has the strongest relationship with bacterial and archaeal community composition \( (r^2 = 0.84, p < 0.001 \text{ for V4}, r^2 = 0.57, p < 0.001 \text{ for Arch349f}) \), along with Na, K, Ca, Mg, Sr and B, the concentrations of which are directly tied to salinity (Figure 6; Table S3). The ratio of Mg to Ca also has a significant relationship with bacterial, but not archaeal community composition \( (r^2 = 0.34, p = 0.01 \text{ for V4}) \). pH also shows a strong relationship with bacterial and archaeal community composition \( (r^2 = 0.64, p < 0.001 \text{ for V4}, r^2 = 0.53, p < 0.001 \text{ for Arch349f}) \). pH is known to influence microbial communities in lake sediments in areas that have strong salinity gradients (Xiong et al., 2012). It may act as an integrating variable, changing in response to change in another factor like nutrient availability or salinity, or it may have a direct effect in that as it changes, it imposes new limits on the community (Lauber, Hamady, Knight, & Fierer, 2009). Microbial metabolic activities, such as sulphate reduction, iron reduction and denitrification, may also influence water pH, mediating the precipitation (or dissolution) of CaCO₃ (Dupraz & Visscher, 2005).

### 4.2 Community composition in hypersaline and brackish lakes

Previous microbial mat studies on Kiritimati investigated microbial communities found within hypersaline Lakes 21, 22 and 51 (Arp et al., 2012; Bühring et al., 2009; Shen et al., 2018; Spring et al., 2015). In the microbial communities from nine different layers of a well-stratified mat from Lake 21 (not the same as Sample 21 in this study) the dominant bacterial phyla found were Bacteroidetes (30.5%), Proteobacteria (27.3%), Spirochaetes (15.3%), Cyanobacteria (5.9%) and Chloroflexi (5.2%). The dominant archaeal phyla were Euryarchaeota (67.6%) and Thaumarchaeota (20.8%) (Schneider et al., 2013). Many of the same phyla were also found in an earlier analysis of a Lake 21 mat (Arp et al., 2012). In both studies, diversity increased with mat depth. Furthermore, at transitions between the photo-oxic, transition, and anoxic light-free zones, the relative abundance of the different phyla changed, indicating that microhabitats are both a product of and support varying microbial communities within the Lake 21 mat. In Lake 21, the overall number of

![Figure 5](image_url) **Figure 5**: Box and whisker plot of Shannon diversity index values \( (H) \) for archaea and bacteria from brackish and hypersaline lake sediments. Box boundaries are 25th and 75th percentiles, line in box indicates median value, whiskers indicate 90th and 10th percentiles.

![Figure 6](image_url) **Figure 6**: Non-metric multidimensional scaling plots of 16S rRNA (A) bacterial (V4) and (B) archaeal (Arch349f) community composition from sequencing data. Bray–Curtis similarities are plotted with significantly correlating environmental variables \( (p \leq 0.05) \) plotted as vectors. Vector length is proportional to the strength of correlation between the variable and community similarity. See Table S3A,B for environmental fitting statistics. Hypersaline samples are denoted by blue symbols, brackish by red, saline by orange, and the lagoon sample by green.
bacterial OTUs ($N = 1,409$) was also much greater than the number of archaeal OTUs ($N = 239$) (Schneider et al., 2013). This result is similar to the observations of bacteria and archaea OTU abundance reported in this paper and further suggests that bacteria overwhelm archaea in these systems. Differences in the relative abundance of phyla reported in previous studies and the new suite of samples were partly expected, due to the differences in sampling locations and the spatial resolution of the sampling. Yet overall, similar phyla are present in this 2014 spatial dataset (Figure 4). However, the overall microbial community composition across the sampled lakes is highly variable and varies with water salinity (Figure 6). Of the most abundant bacterial phyla (Figure 4), Firmicutes and Gammaproteobacteria are more abundant in the hypersaline sediments (Table 2). Members of the Firmicutes have the ability to exist in hypersaline conditions as they can either accumulate KCl or organic compatible solutes to osmotically balance their cytoplasm with hypersaline waters (Oren, 2008). Some members of Firmicutes are also spore-formers (Galperin, 2013), and are thus more capable of survival in lakes that desiccate or in lakes where salinity may change rapidly, such as with an influx of freshwater into a hypersaline lake during heavy El Niño rains. Gammaproteobacteria is also positively correlated with lake

| Phylum                  | Median relative abundance (%) brackish | Median relative abundance (%) hypersaline | p-value |
|-------------------------|----------------------------------------|------------------------------------------|---------|
| Bacteria                |                                        |                                          |         |
| Proteobacteria:         | 37.15 ± 13.73                          | 56.74 ± 19.37                           | 0.21    |
| Alphaproteobacteria     | 10.37 ± 12.40                          | 3.17 ± 6.27                             | 0.03    |
| Gammaproteobacteria     | 22.04 ± 7.84                           | 46.95 ± 25.25                           | 0.03    |
| Deltaproteobacteria     | 6.44 ± 4.17                            | 1.27 ± 3.00                             | 0.01    |
| Epsilonproteobacteria   | 0.00 ± 0.03                            | 0.01 ± 1.19                             | 0.25    |
| Bacteroidetes           | 24.05 ± 9.32                           | 20.91 ± 12.60                           | 0.37    |
| Planctomycetes          | 4.10 ± 1.26                            | 2.45 ± 5.27                             | 0.26    |
| Spirochaetes            | 1.12 ± 0.57                            | 1.4 ± 4.04                              | 0.15    |
| Verrucomicrobia         | 1.45 ± 1.65                            | 0.40 ± 1.41                             | 0.17    |
| Chloroflexi             | 2.19 ± 2.52                            | 0.34 ± 2.04                             | 0.01    |
| Cyanobacteria           | 0.90 ± 0.54                            | 0.24 ± 0.75                             | 0.01    |
| Parcubacteria           | 0.71 ± 0.51                            | 0.26 ± 2.18                             | 0.12    |
| Latiscibacteria         | 0.58 ± 1.23                            | 0.04 ± 0.44                             | 0.02    |
| Gemmatimonadetes        | 0.80 ± 0.04                            | 0.04 ± 0.61                             | 0.02    |
| Firmicutes              | 0.08 ± 0.04                            | 0.65 ± 10.13                            | 0.03    |
| Actinobacteria          | 0.27 ± 0.43                            | 0.04 ± 0.11                             | 0.02    |
| Acidobacteria           | 0.43 ± 0.51                            | 0.00 ± 0.26                             | <0.01   |
| SR1 (Absconditabacteria)| 0.05 ± 1.90                            | 0.02 ± 1.59                             | 0.53    |
| Nitrospira              | 0.00 ± 1.14                            | 0.00 ± 0.2                              | 0.78    |
| Cloacimonetes           | 0.00 ± 0.00                            | 0.00 ± 0.73                             | 0.06    |
| Archaea                 |                                        |                                          |         |
| Alticarchaeales         | 0.00 ± 0.13                            | 0.00 ± 0.01                             | 0.22    |
| Aenigmarchaeota         | 0.63 ± 0.48                            | 0.00 ± 0.30                             | <0.01   |
| Miscellaneous           |                                        |                                          |         |
| Euryarchaeotic Group (MEG) | 0.15 ± 1.48                       | 0.00 ± 0.17                             | 0.12    |
| WSA2                    | 1.07 ± 0.81                            | 0.00 ± 1.06                             | 0.05    |
| Batharchaeota           | 1.11 ± 5.83                            | 0.00 ± 11.87                            | <0.01   |
| Thaumarchaeota          | 9.87 ± 15.12                           | 0.91 ± 5.04                             | 0.03    |
| Woesearchaeota          | 4.69 ± 4.95                            | 5.63 ± 11.49                            | 0.83    |
| Lokiarchaeota           | 4.94 ± 4.79                            | 1.13 ± 17.41                            | 0.26    |
| Euryarchaeota           | 18.96 ± 17.20                          | 18.23 ± 14.57                           | 0.56    |
**FIGURE 7** LogFC pairwise comparison between (A) bacterial and (B) archaeal families within hypersaline versus brackish sediment samples. Positive LogFC values on the y-axis indicate families that are more abundant in hypersaline lake sediments. Negative LogFC values on the y-axis indicate families that are more abundant in brackish lake sediments. Colours of circles denote the phyla for each listed family, shown in the legend.
salinity in lake sediments from across the Tibetan Plateau (Yang et al., 2016). The phyla that are more abundant in the Kiritimati brackish lake sediments include Chloroflexi, Cyanobacteria, Latescibacteria, Gemmatimonadetes, Actinobacteria and Acidobacteria. Many of these phyla can tolerate a wide range of salinities and are also found in hypersaline Kiritimati lake sediments, although Acidobacteria has a negative association with salinity in soils (Canfora et al., 2014). Across a marine salinity gradient, Actinobacteria and Chloroflexi were also more abundant in low-salinity settings, with Gammaproteobacteria and Alphaproteobacteria more abundant in high-salinity settings (Dupont et al., 2014).

Phylum and class-level comparisons have limited value, as closely related groups can be both halophilic and non-halophilic (Oren, 2008). Pairwise tests of the relative abundance of specific OTUs in hypersaline and brackish lakes illustrates the above point and reveals additional associations between salinity and the families of the observed phyla (Figure 7). In Figure 7, positive LogFC values reveal specific OTUs that are significantly more abundant in sediment from hypersaline lakes relative to sediment from brackish lakes. Conversely, OTUs that are significantly more abundant in the brackish lakes are represented by negative values. While similar phyla are present in many lake sediment samples, at the family level, clear differences emerge between the different salinity groups. For example, within Proteobacteria, the family Halomonadaceae is more abundant in hypersaline samples, likely due to the halophilic nature of this family (Arahal & Ventosa, 2006). Conversely, the anoxic, sulphate-oxidizing Chromatiaceae, or phototrophic purple sulphur bacteria, is more abundant in brackish lakes. Within Bacteroidetes, the second most abundant phyla, the family Cryomorphaceae was more abundant in the brackish lakes, whereas Rhodothermaceae is more abundant in hypersaline lakes. Members of Cryomorphaceae are generally found in non-extreme habitats (Bowman, 2014), likely explaining its abundance in brackish lakes. Rhodothermaceae are often found in extreme environments and the family contains genera known for their reddish pigments (Park, Akira, & Kogure, 2014), which likely gives some of the hypersaline sediments their red-orange hue. The Halanaerobiaceae and Bacillaceae within the Firmicutes phylum are also more abundant in hypersaline lakes, and their abundance likely determines the greater overall Firmicutes abundance in hypersaline sediments. The halotolerance of Halanaerobiaceae and the ability of Bacillaceae to form endospores likely both serve as key adaptations to their hypersaline environment susceptible to large changes in lake salinity (Mandic-Mulec, Stefanić, & van Elsas, 2016; Oren, 2014).

There are more archaeal phyla (Figure 4B) found across this dataset than previously documented for Kiritimati Lake 21 (Schneider et al., 2013), although the Euryarchaeota remain the most abundant phylum represented overall. Three phyla, Aenigmarchaeota, Batharchaeota and Thaumarchaeota, were found to be more abundant in the brackish lake sediments. Considering specific archaeal families that are more abundant in brackish versus hypersaline sediments (Figure 7B), both hypersaline and brackish lakes contained Marine Benthic Group D and Deep Sea Hydrothermal Vent
Group 1 (DHVEG-1) families. Members from this group are frequently associated with anoxic environments (Liu, Yang, Zhao, & Zhang, 2014; Takai & Nakamura, 2011). This group was also found in the darker, more anoxic layers of the Lake 21 microbial mat (Schneider et al., 2013). Samples from hypersaline lakes contained a higher abundance of the halophilic Halobacteriaceae in photo-oxic layers, whereas brackish lakes had a greater abundance of Methanoregulaceae, a methanogenic family. The other most abundant archaeal phyla, Woesearchaeota and Lokiarchaeota, have uncultured members found in both hypersaline and brackish lakes, respectively (Figure 7B).

High salinity often results in low taxonomic diversity, as living at high salinity is energetically costly (Jiang et al., 2007; Oren, 2001, 2002). However, hypersaline water bodies can also have diverse, highly structured microbial mat communities, defined by a multitude of closely spaced physiicochemical niches, as is seen in the previous microbial mat studies on Kiritimati and elsewhere (Farrera et al., 1999; Ley et al., 2006; Schneider et al., 2013). To test for systematic differences in alpha diversity across the brackish and hypersaline lakes the Shannon index is compared for sediments in the brackish and hypersaline lake systems. There is a range of diversity values in both hypersaline and brackish sediments, but there is no significant difference in bacterial or archaeal alpha diversity (Figure 5). This could be partly due to the variable nature of mat stratification in individual hypersaline samples. Some samples were vertically stratified at the millimetre-level, whereas other mats and surface samples had thicker monochromatic layers in the sampled 5 cm of the sediment-water interface. Mats with finer stratification will have higher habitat heterogeneity with respect to the physical and biogeochemical gradients that are created and maintained by the microbes (Bolhuis, Cretoiu, & Stal, 2014); higher heterogeneity leads to more available niches and therefore the potential for a high diversity. Time may also play an important role in the diversity of the mats. Mats that are older may exhibit higher diversity with more developed internal environmental gradients. High resolution radiocarbon dating of mat sequences (Trichet, Hatté, & Fontugne, 2016) coupled with microbial diversity metrics may be useful to address this question.

4.3 | Microbe–mineral relationships

Microbial influences on lake water alkalinity and Ca$^{2+}$ concentration likely play a role in the precipitation of CaCO$_3$, in the water column and at the mat-sediment-water interface in Kiritimati lakes. Photosynthesis and extracellular polymeric substance (EPS) degradation were previously hypothesized to be the most important microbial processes at work in Kiritimati hypersaline lakes with microbial mats (Arp et al., 2012). Yet abiotic processes are also responsible for carbonate precipitation in lacustrine systems. For example, temperature, the mixing of water masses, and evaporative concentration of cations and anions can all lead to precipitation of CaCO$_3$. In the shallow, evaporated water bodies of Kiritimati, where mixing between seawater, groundwater and evaporated lake water are common, all of these processes are potential drivers of carbonate precipitation. Moderate to high abundance of CaCO$_3$ is present in all samples that contained diverse microbial communities and calculated saturation index values indicate that all waters were saturated with respect to calcite and aragonite (Table S1).

Although multiple metabolic and physiochemical processes likely influence carbonate precipitation in Kiritimati lakes, the differences in the abundance of taxa with specific metabolisms in brackish and hypersaline lake sediments suggest that specific metabolic processes may play important roles in precipitating carbonate in each system. The greater abundance of oxygenic photosynthesizing Cyanobacteria suggest that photosynthesis may be important in driving carbonate precipitation in these lower salinity systems. Additionally, the role of sulphate reduction in driving the alkalinity engine may also be more important for carbonate precipitation in brackish systems, given the greater abundance of Deltaproteobacteria in brackish lake sediments. However, only limited conclusions about the microbial metabolisms present can be deduced from such taxonomic identification. Future paired geological and metagenomic analyses are needed to more specifically link the presence of active metabolic processes with carbonate precipitation.

A smaller subset of sediment samples contains additional minerals beyond calcite and aragonite, including dolomite (Figure 3B). About one-third of the minerals measured in samples from Sites 1 and 1a are dolomite, with smaller quantities of dolomite found in Site 3 and Site 17. All three of these lakes are hypersaline, with high Mg/Ca values between 8.2 and 19.7 (Table S1). From an abiotic perspective, the samples taken from Site 1 are close to the location of a brackish spring (water sample 1b, 23.7 ppt) observed entering the hypersaline lake. Mixing of fresh and saline waters may play a role in dolomite precipitation by lowering SO$_4^{2-}$ concentrations, in the absence of Si (Baker & Kastner, 1981). Microbial activity is also considered necessary to drive dolomite precipitation, with sulphate reducing bacteria, iron reducers and methanogens thought to play important roles, or simply the presence of EPS or microbial biomass (Petrasch et al., 2017; Vasconcelos, McKenzie, Bernasconi, Grujic, & Tiens, 1995). Specific families are more abundant in sediments containing dolomite (Figure 8). Although most of the families identified as more abundant in the dolomite-bearing sediments have not been specifically linked to dolomite precipitation, a family of methanogenic archaea, Methanosarcinaceae, is more abundant in sediments with dolomite. Many of the other families are unknown or uncultured, indicating that the microbial metabolic processes that may drive dolomite require further
investigation. Future research focused on the active microbial metabolisms present in individual sediment samples with dolomite will be useful to gain more insight into the relationship between dolomite precipitation, abiotic factors and microbial drivers of mineral precipitation in these systems.

5 CONCLUSIONS

Utilizing a natural salinity gradient present across a suite of shallow interior lakes atop the coral atoll island of Kiritimati, a significant difference was observed in microbial community structure between the sediments of brackish and hypersaline lakes. These two types of lacustrine systems are geographically and hydrologically distinct, with the hypersaline lakes forming as a result of long-term evaporation of lagoon water, and the brackish systems a result of the surface manifestation of freshwater lenses. Kiritimati lake water salinity is variable through time, given large interannual changes in precipitation and evaporation rates associated with ENSO, as well as decadal to centennial variations in tropical Pacific climate. Therefore, microbial communities may also vary temporally, as observed for the Lake 21 mat sampled between 2002 and 2011 (Schneider et al., 2013). Thus, it is hoped that the results of this survey will serve as a baseline for subsequent analyses following large changes in hydroclimate, associated with future ENSO events.

The presence of precipitated CaCO$_3$ in all the samples and the lack of a relationship between microbial community structure and CaCO$_3$ abundance confirms that a complex combination of physicochemical and biological controls influences carbonate mineral precipitation. The greater relative abundance of Cyanobacteria in brackish lake sediments suggests that photosynthesis may have a stronger role in precipitating carbonate in brackish lakes, whereas it is one of the multiple metabolic processes likely contributing to carbonate precipitation in hypersaline lakes with more structured microbial mats and reticulate microbialites (Arp et al., 2012). Deltaproteobacteria are also more abundant in the brackish lake sediments, pointing to the possible influence of sulphate reduction on alkalinity and subsequent carbonate precipitation in these systems. The lakes precipitating dolomite appear physiochemically primed to do so, with high Mg/Ca values and in one case, mixing of fresh and hypersaline waters. A methanogenic family, Methanosarcinaceae, was also more abundant in the lakes containing dolomite, suggesting that methanogenesis may be influential in promoting dolomite formation.

Although this work adds to the body of research investigating the microbial community diversity of Kiritimati lakes and their relationship to carbonate precipitation, bulk phylogenetic studies are still of limited value. Further investigations of the brackish lake sediment microbial communities with greater spatial resolution (both downcore and within individual lakes), mat and sediment ages and growth rates, sediment crystalline growth forms and geochemistry, and the nature of sediment organic matter coupled with metagenomic analyses are all essential to more firmly link mineral precipitation to specific microbial metabolic processes in this environment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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**SUPPORTING INFORMATION**

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