

Inter-field variability in the microbial communities of hydrothermal vent deposits from a back-arc basin

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ABSTRACT

Diverse microbial communities thrive on and in deep-sea hydrothermal vent mineral deposits. However, our understanding of the inter-field variability in these communities is poor, as limited sampling and sequencing efforts have hampered most previous studies. To explore the inter-field variability in these communities, we used barcoded pyrosequencing of the variable region 4 (V4) of the 16S rRNA gene to characterize the archaeal and bacterial communities of over 30 hydrothermal deposit samples from six vent fields located along the Eastern Lau Spreading Center. Overall, the bacterial and archaeal communities of the Eastern Lau Spreading Center are similar to other active vent deposits, with a high diversity of *Epsilonproteobacteria* and thermophilic *Archaea*. However, the archaeal and bacterial communities from the southernmost vent field, Mariner, were significantly different from the other vent fields. At Mariner, the epsilonproteobacterial genus *Nautilia* and the archaeal family *Thermococcaceae* were prevalent in most samples, while *Lebetimonas* and *Thermofilaceae* were more abundant at the other vent fields. These differences appear to be influenced in part by the unique geochemistry of the Mariner fluids resulting from active degassing of a subsurface magma chamber. These results show that microbial communities associated with hydrothermal vent deposits in back-arc basins are taxonomically similar to those from mid-ocean ridge systems, but differences in geologic processes between vent fields in a back-arc basin can influence microbial community structure.

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INTRODUCTION

Marine hydrothermal vent deposits form as acidic, metal-rich, reduced hydrothermal fluids meet cold, alkaline, and oxygenated seawater (Tivey, 2007). These porous deposits are characterized by steep physicochemical gradients that provide a range of microhabitats for microbial colonization. Numerous *Archaea* and *Bacteria* that grow over a wide range of physical and chemical conditions have been isolated and characterized from active vent deposits (e.g., Takai & Horikoshi, 2000; Takai *et al.*, 2000, 2002, 2003, 2004, 2005, 2008; Götz *et al.*, 2002; Inagaki *et al.*, 2003, 2004; Nakagawa *et al.*,

2005a,c; Voordeckers *et al.*, 2005; Reysenbach *et al.*, 2006; Imachi *et al.*, 2008). Several archaeal and bacterial communities associated with actively venting structures have also been described using cultivation-independent techniques employing 16S rRNA gene cloning (e.g., Takai & Horikoshi, 1999; Reysenbach *et al.*, 2000; Corre *et al.*, 2001; Takai *et al.*, 2001, 2008; Nercessian *et al.*, 2003; Schrenk *et al.*, 2003; Nakagawa *et al.*, 2005b; Page *et al.*, 2008; Voordeckers *et al.*, 2008; Nunoura & Takai, 2009; Zhou *et al.*, 2009; Kato *et al.*, 2010; Roussel *et al.*, 2011). Results of these studies have identified the *Epsilonproteobacteria* as being the dominant bacterial lineage associated with vent deposits, while other bacterial groups like the *Aquificales*, *Thermales*, *Thermotogales*, *Deltaproteobacteria*, and *Thermodesulfurobacteriaceae* are also often detected. Most *Archaea* associated with active

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vent deposits from cultivated lineages are thermophilic and belong to the *Archaeoglobales*, *Thermococcales*, *Aciduliprofundales* (DHVE2), *Methanococcales*, and *Desulfurococcales*. Numerous archaeal lineages with no relatives in culture have also been reported.

While we have general knowledge of archaeal and bacterial diversity associated with deep-sea hydrothermal deposits, we have a poor understanding of the inter-field variability of these communities, as most previous studies have been limited in sampling and sequencing efforts. These studies were usually limited to at most a handful of samples and less than a few hundred cloned sequences (e.g., Corre *et al.*, 2001; Takai *et al.*, 2001; Schrenk *et al.*, 2003; Kormas *et al.*, 2006; Page *et al.*, 2008; Zhou *et al.*, 2009), and therefore preclude statistical analysis of hypotheses regarding the observed patterns of biodiversity. To begin to overcome these limitations and increase the statistical rigor of analysis, we recently used barcoded pyrosequencing of the 16S rRNA gene to characterize and compare the archaeal and bacterial communities associated with many actively venting hydrothermal deposits from two vent fields located along a mid-ocean ridge (MOR) system, the Mid-Atlantic Ridge (MAR) (Flores *et al.*, 2011a). Results showed that the microbial communities associated with deposits from the basalt-hosted Lucky Strike vent field were different than those from the peridotite-hosted Rainbow vent field. These community-level differences were attributed primarily to differences in the geochemistry, particularly the hydrogen (H₂) concentration, of the hydrothermal fluids resulting from differences in host-rock composition.

Here, we extended this same barcoded pyrosequencing approach to characterize the communities of over 30 active hydrothermal deposits from six vent fields in a back-arc basin, the Eastern Lau Spreading Center (ELSC) (Fig. 1). Back-arc basins are extensional basins that form adjacent to island arcs and are geologically more diverse than MOR systems owing to the presence of both convergent and divergent plate boundaries. The spreading centers generated here support hydrothermal environments that are in many ways similar to MORs. However, the composition of hydrothermal fluids in back-arc basins are generally more variable than those from MORs owing to differences in magma composition, heterogeneity of island-arc crust versus oceanic crust, and from inputs of the subducting slab (Gamo *et al.*, 2006). Thus, the unique geologic setting of the ELSC provided a natural laboratory to further explore factors influencing inter-field variability in microbial communities associated with active, high-temperature vent deposits. In characterizing the microbial communities, we sought to address the fundamental question of whether the archaeal and bacterial communities associated with active vent deposits from several vent fields along the ELSC were different from one another. Only by collecting multiple samples from each vent field and by utilizing high-throughput sequencing techniques could we attempt to address this question.

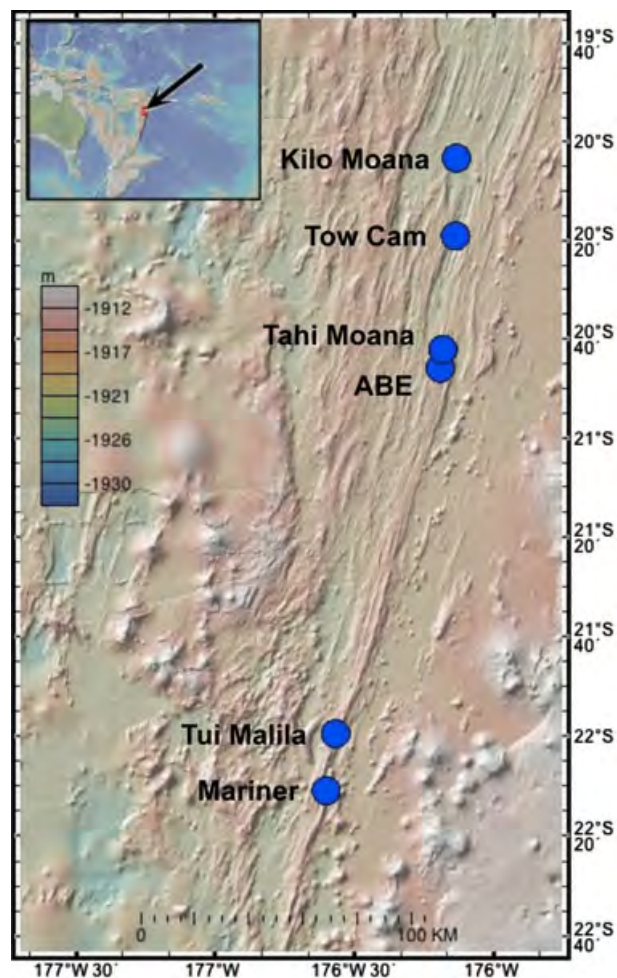


Fig. 1 Location of hydrothermal vent fields along the Eastern Lau Spreading Center in the southwestern Pacific Ocean. Map was modified from Ferrini *et al.* (2008) using the GeoMapApp (<http://www.geomapapp.org/>).

METHODS

Site description

The ELSC is a 397-km ridge segment that includes the Valu Fa Ridge (VFR), which together comprise the southernmost part of the spreading axis in the Lau back-arc basin (Ferrini *et al.*, 2008; Mottl *et al.*, 2011). The Lau back-arc basin and nearby Tofua arc formed in response to subduction of the Pacific plate beneath the Australian plate (Martinez *et al.*, 2007). Located along the ELSC are at least six vent fields: Kilo Moana, Tow Cam, Tahī Moana, ABE, Tui Malila, and Mariner (Fig. 1).

The basalt-hosted Kilo Moana vent field (176°08.03'W, 20°03.15'S) is at the northern end of the ELSC at depths between 2606 and 2640 m where the spreading rate is fast and the crust is relatively thin (Martinez *et al.*, 2006; Ferrini *et al.*, 2008). Hydrothermal activity here occurs in three areas that extends north to south over approximately 130 m (Tivey *et al.*, 2005; Ferrini *et al.*, 2008). Dominant minerals present

in Kilo Moana deposits include cubic cubanite, chalcopyrite, and wurtzite, with pyrite and marcasite more prevalent in deposit exteriors (Tivey, 2007). The Tow Cam vent field (176°08.2'W, 20°19.0'S, 2690–2728 m) is located 29 km to the south of Kilo Moana and is also hosted on basaltic substrate (Ferrini *et al.*, 2008). A newly discovered site 40 km to the south of Tow Cam, named Tahi Moana (176°10.5'W, 20°40.3'S), was also sampled. This site is basalt-hosted and lies at a depth of 2214–2260 m. Ten kilometers to the south of Tahi Moana is the ABE vent field (176°11.5'W, 20°45.8'S; 2104–2163 m). ABE is hosted in basaltic andesites (Tivey *et al.*, 2012), and hydrothermal activity here extends 600 m along the NE–SW trending fault that dominates this region (Ferrini *et al.*, 2008). Dominant minerals in ABE deposits include chalcopyrite and wurtzite with minor amounts of pyrite and barite, and trace amounts of galena (Tivey, 2007; Tivey *et al.*, 2012). Approximately 140 km south of ABE on the VFR is the Tui Malila (176°34.06'W, 21°59.35'S, 1839–1928 m) vent field (Ferrini *et al.*, 2008). Although Tui Malila is on a different spreading segment than ABE, both vent fields are hosted in basaltic andesites and are crosscut by NE–SW faults (Ferrini *et al.*, 2008). Hydrothermal activity at Tui Malila extends approximately 350 m north to south. Twenty-five kilometers south of Tui Malila is the Mariner (176°36.09'W, 22°10.82'S; 1877–1951 m) vent field. Like Tui Malila and ABE, Mariner is hosted in basaltic andesite, but dacites and rhyolites also lie close to the vents at Mariner (Tivey *et al.*, 2012). Mariner deposits are lined with chalcopyrite, and bornite, with trace amounts of tennantite observed in mid- and exterior layers. Some structures are also barite-rich (Tivey, 2007).

Hydrothermal activity along the ELSC is influenced by changes in spreading rate, magma source, subduction influence, and host-rock composition from north to south (Mottl *et al.*, 2011). In general, vent fluids of the northern fields (Kilo Moana, Tow Cam, ABE and Tui Malila) are characterized by compositions similar to those observed at typical MORs. The isotopic composition and/or abundance of numerous trace elements in these fluids, however, show systematic along-axis differences that are consistent with an increased influence of the subducted slab on crustal composition to the south. Moreover, these fields exhibit a systematic north–south decrease in temperature that is accompanied by an increase in pH and decreases in the concentrations of metabolically important compounds such as aqueous H₂, hydrogen sulfide (H₂S), and iron (Fe) (Mottl *et al.*, 2011; Table 1). Relative to the northern vent fields, fluids from Mariner are substantially different in composition. In particular, Mariner fluids have higher concentrations of carbon dioxide (CO₂), H₂S, Fe, and other transition metals and are hotter and more acidic relative to the northern vents. Many of the differences in Mariner fluid composition relative to the northern vents can be attributed to a greater input of acidic magmatic volatiles from an actively degassing magma chamber (Mottl *et al.*, 2011).

Sample collection

Hydrothermal vent deposits were obtained from six vent fields along the ELSC in June/July 2009 during the TN236 cruise aboard the RV Thomas G. Thompson. Samples were collected using the remotely operated vehicle, ROV *Jason II*. Immediately after collection at the seafloor, samples were placed in sealed, custom-made bioboxes attached to the ROV to restrict flushing of samples by surrounding seawater during the ascent. With our custom bioboxes, we were able to collect at minimum eight different samples per dive, which greatly increased our ability to collect enough samples for this study, as sampling these structures is inherently difficult. Once shipboard, samples were subsampled as described previously with the outer few millimeters (up to approximately 5 mm) kept separate from the bulk sample (Götz *et al.*, 2002; Reysenbach *et al.*, 2006). These exterior samples were homogenized and stored in cryovials at –80 °C (in 1.6 mL of aliquots, on average approximately 8 mL per sample) for subsequent DNA extraction. In total, 35 different deposit samples were examined in this study (Fig. S1, Table S1).

Hydrothermal fluid chemistry

Hydrothermal fluids were collected using isobaric gas-tight fluid samplers (Seewald *et al.*, 2002) deployed from the ROV *Jason II* and processed immediately following recovery on the ship. Vent fluid temperature was monitored continuously during fluid sampling using a thermocouple attached to the end of the sampler inlet snorkel. Geochemical features reported represent minimum and maximum values recorded for different vents within each vent field. Shipboard analysis of dissolved H₂ (2005 data only) and CH₄ concentrations was accomplished using gas chromatography following headspace extraction. Sample aliquots were archived in gas-tight serum vials for shore-based determination of total dissolved CO₂ (ΣCO₂) by gas chromatography following acidification and headspace extraction. An additional aliquot was acidified with analytical-grade Optima® HCl prior to storage for analysis of Fe and Mn by inductively coupled plasma atomic emission spectroscopy (ICPAES). Total dissolved H₂S (ΣH₂S) was quantitatively precipitated at sea as Ag₂S for subsequent gravimetric measurement in a shore-based laboratory. A Ag/AgCl combination reference electrode was used for shipboard measurement of pH (25 °C).

DNA extraction, pyrosequencing, and sequence analysis

DNA was extracted from homogenized samples (approximately 1.6–3.2 g) using the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) (Reysenbach *et al.*, 2006). Pyrosequencing of the variable region 4 (V4) of the 16S rRNA gene was conducted as previously described

Table 1 Range of physicochemical characteristics of end-member hydrothermal fluids from different vent fields along the Eastern Lau Spreading Center measured during different expeditions. Note that not all features were measured for some sampling dates and fluids were not collected from all samples collected during this study

Feature	Kilo Moana	Tow Cam	Tahi Moana	ABE	Tui Malila	Mariner	Seawater
Temperature (°C)							
2004 [*]						179–365	2
2005 [†]	293–333	287–328		269–309	229–312	240–363	
2009 [‡]	290–304	320	286–311	297–317	284–315	338–359	
pH							
2004						2.4–2.7	7.6
2005	2.9–4.0	3.7–4.1		4.3–4.6	4.2–5.7	2.5–2.7	
2009	3.9–4.1	3.6	3.3–3.9	3.9–4.0	3.8–4.2	2.2–2.4	
H ₂ (μmol/kg)							
2004						44.9–95.5	<0.001
2005	220–498	108–198		54–101	35–135	33–179	
2009							
H ₂ S (mmol/kg)							
2004						6.9–9.0	<0.0001
2005	5.4–6.4	3.7–5.2		2.6–3.6	1.2–2.5	4.2–9.3	
2009	3.5–3.9	4.8	3.1–4.1	2.8–3.9	2.8	6.1–19	
CH ₄ (μmol/kg)							
2004						7.2–8.0	<0.001
2005	28–33	44–48		42–57	31–43	5.1–7.7	
2009	33–34	44	35–39	38–42	39	9–20	
CO ₂ (mmol/kg)							
2004						41.2–71.7	2.3
2005	7.5–8.5	10.5–11.5		5.1–6.9	11.7–13.8	32.3–71.4	
2009	8.6–8.7	9.3	6.6–7.5	5.3–6.9	14	40–91	
Fe (mmol/kg)							
2004						11.1–12.8	<0.001
2005	2.49–3.84	0.27–0.37		0.16–0.37	0.14–0.26	10.5–13.0	
2009	0.53–0.59	0.21	0.23–0.33	0.08–0.15	0.15–0.18	3.8–13.1	
Mn (mmol/kg)							
2004						4.9–6.0	<0.005
2005	0.51–0.73	0.33–0.41		0.27–0.47	0.33–0.44	4.9–6.3	
2009	0.19–0.21	0.33	0.30–0.73	0.28–0.41	0.31–0.37	3.9–6.3	

^{*}From Takai *et al.* (2008).

[†]From Mottl *et al.* (2011), except H₂ values.

[‡]This study.

(Flores *et al.*, 2011a). Briefly, for amplification of the archaeal 16S rRNA gene, we used a modified 519F primer sequence (Suzuki and Giovannoni, 1996; Flores *et al.*, 2011a) fused to 6- or 8-nucleotide barcode (Cole *et al.*, 2009) and to the 454 FLX sequencing primer A (5'GCCTCCCTCGCGCCA TCAGxxxxx**CAGYMGCCRCGGKAAHACC**, where the x region represents the barcode and the SSU rRNA primer is bold). The reverse primer (5'-GCCTTGCCAGCCCGCTCA GGGACTACNSGGTMTCTAAT, where the 16S rRNA region is bold) represented a fusion of the 454 FLX sequencing primer B and a modified Arch806R primer (Takai & Horikoshi, 2000; Flores *et al.*, 2011a). Fifty microlitre PCR contained 300 nM of each primer and 20–50 ng template DNA and were performed using Platinum Taq High Fidelity polymerase (Invitrogen, Carlsbad, CA, USA) using the thermal profile 95 °C for 2 min followed by 27 cycles of denatur-

ation at 95 °C for 15 s, primer annealing at 53 °C, and extension at 68 °C for 45 s, with final extension of 68 °C for 3 min. Amplification of the bacterial 16S rRNA gene was carried out using as forward primer (560F) barcoded oligonucleotides 5'GCCTCCCTCGCGCCATCAGxxxxxx**AYTGGGYDTAAAGNG3'** and a mix of reverse primers (803R) (5'GCCTTGCCAGCCCGCTCAG:**TACCRGGGTHTCTAATCC**, :**TACCAGAGTATCTAATTC**, :**CTACDSRGGTMTCTAATC**, and :**TACNVGGGTATCTAATCC3'** in a 6:1:2:12 ratio, respectively), designed to cover most of the *Bacteria* domain (Cole *et al.*, 2009). The amplification protocol was the same as for *Archaea* except that the annealing temperature was 55 °C. Amplicons were sequenced using a 454 Life Sciences Genome Sequencer FLX (Roche Diagnostics, Indianapolis, IN, USA) at Oak Ridge National Laboratory, USA.

Raw sequences were initially processed using the RDP pyrosequencing pipeline (Cole *et al.*, 2009). We discarded sequences that were <200 bp, contained ambiguous nucleotides (n's), had mismatches to both primers and barcode, and with a quality score <20 (Flores *et al.*, 2011a). The resulting sequences were filtered using custom Perl scripts (<http://alrllab.research.pdx.edu/Aquificales/>) to include sequences <275 and 250 bp for *Archaea* and *Bacteria*, respectively. After quality filtering, archaeal sequences were, on average, 247 bp in length, while bacterial sequences were 207 bp. Samples Mariner-12, -37, -38, -39, -58, -59, Kilo Moana-36, Tow Cam-26, -27, and Tahi Moana-52 were sequenced from the 3' end and were reverse complemented before assigning them to corresponding samples based on their barcode matches. Trimmed sequences were then subjected to a pre-classification step using the RDP classifier (Wang *et al.*, 2007) in which unclassified sequences (not *Archaea* or *Bacteria*) or sequences classified to the incorrect domain (e.g., *Bacteria* in the archaeal data set) were removed. Quality sequences were aligned and clustered into operational taxonomic units (OTUs) using the SLP/PW-AL pipeline (Huse *et al.*, 2010) at a 97% similarity cutoff. The resulting cluster files were converted to a format suitable for the RDP pipeline to generate rarefaction curves (Colwell & Coddington, 1994) for each sample. Taxonomic classifications were assigned using the RDP classifier (Wang *et al.*, 2007) and manually as described previously (Flores *et al.*, 2011a) for representative sequences of each OTU. Sequence files are available for download through MG-RAST (Meyer *et al.*, 2008) or by contacting the corresponding author.

In addition to the routine denoising efforts described above, the archaeal libraries of four deposits were processed using a recently described AmpliconNoise denoising pipeline (Quince *et al.*, 2011). Comparisons of the denoising results for the four libraries with and without AmpliconNoise are shown in Table S2.

Pyrosequencing data were analyzed using a combination of OTU-based (Bray–Curtis, Sørensen) and phylogeny-based (UniFrac) (Lozupone *et al.*, 2006; Hamady *et al.*, 2010) beta-diversity metrics as previously described (Flores *et al.*, 2011a). Dissimilarity and distance matrices were visualized using non-metric multidimensional scaling (MDS) plots and tested for significance using ANOSIM with PRIMER v6 (Clarke & Gorley, 2006). Mantel tests (Spearman rank correlations) were conducted using the BEST and RELATE functions in PRIMER v6 to find correlations between community dissimilarity (Bray–Curtis), sample location, and fluid geochemistry. As geochemical data were not collected for every vent sample we analyzed, we used vent field averages of H₂S, CO₂, CH₄, Fe, and pH measured in 2009 during the same expedition (Table 1) for the correlations. SIMPER analysis was also performed in PRIMER v6 to identify discriminating OTUs between samples. Both the OTU-based and phylogeny-based analyses showed similar results, so only the OTU-based Bray–Curtis results are presented.

RESULTS

Bacterial community composition

A total of 268 542 high-quality partial 16S rRNA gene sequences were generated from 30 hydrothermal vent samples for an average of 8951 sequences per sample (4884–21 728 sequences per sample) (Table S1). From these sequences, 1699 unique OTUs (97% sequence similarity, 79–400 OTUs per sample) were identified with 42% (715) being singleton OTUs (Table S3). The most abundant OTU (20.24% of all sequences) was classified as *Sulfurovum* and was found in all 30 samples. *Nitratifactor* (4.20%), *Desulfobulbus* (3.02%), and *Hippea* (0.47%) were the only other OTUs observed in all samples. Rarefaction analysis (Fig. S2) revealed that more bacterial diversity likely exists for many of the samples surveyed.

With the exception of Kilo Moana-2 and Mariner-12, the majority (>50%) of sequences detected in all the samples were classified as *Epsilonproteobacteria* (Fig. 2A). *Caminibacter*, *Lebetimonas*, *Nautilia*, *Nitratifactor*, *Nitratiruptor*, *Sulfurimonas*, and *Sulfurovum* were associated with most samples although the dominant genera varied between samples and by vent field, as in the case of Mariner where *Nautilia* were more prevalent (Fig. 2A). Other taxa commonly observed included thermophilic and non-thermophilic lineages such as the *Alphaproteobacteria*, *Aquificales*, *Caldisericia*, *Chloroflexi*, *Deferribacteres*, *Deinococcus/Thermus*, *Deltaproteobacteria*, *Firmicutes*, *Gammaaproteobacteria*, *Thermodesulfobacteria*, and *Thermotogae* (Fig. 2B).

Inter-field bacterial community comparisons

Results of a one-way ANOSIM test comparing the bacterial communities from the different vent fields showed that the communities associated with Mariner samples were distinct, with all pairwise comparisons being statistically significant ($P < 0.05$) (Table 2). Only one other pairwise comparison (Kilo Moana vs. Tow Cam) was statistically significant ($R = 0.463$, $P = 0.029$). MDS plots support these observations as communities from Mariner clustered together to the exclusion of deposits from other vent fields, and the communities from Kilo Moana did not overlap with those from Tow Cam (Fig. 3A). Sample location and all of the geochemical characteristics tested (H₂S, CO₂, CH₄, Fe, and pH) were significantly correlated with the overall Bray–Curtis dissimilarity between vent fields (Table S4). These correlations, however, were no longer significant when communities from the Mariner vent field were removed from the tests, suggesting that the communities at Mariner drive some of these statistical differences. Results of the SIMPER analysis identified several epsilonproteobacterial genera, especially *Nautilia*, as being the largest contributors of dissimilarity between communities from Mariner and the other vent fields (Table S5). Overall,

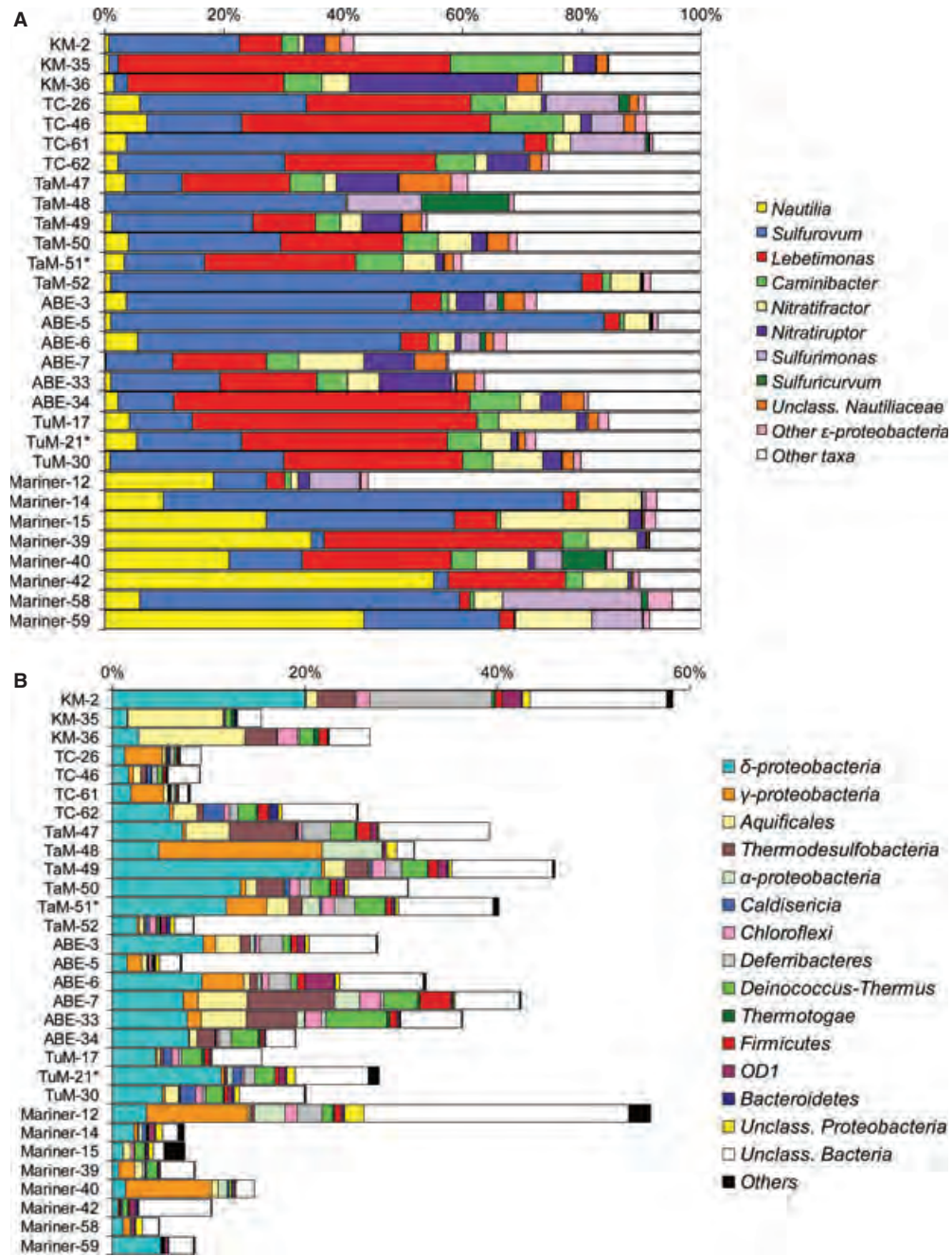


Fig. 2 Relative abundance of epsilonproteobacterial genera (A) and other bacterial taxa (B) observed in hydrothermal vent deposit samples collected from the Eastern Lau Spreading Center. Asterisks denote samples for which no corresponding archaeal data were obtained. Note that the taxa in B are represented by the white bars in A.

these results indicate that the bacterial communities from Mariner are quite different from the other vent fields along the ELSC.

Archaeal community composition

Analysis of the archaeal communities of 34 vent samples resulted in 104 277 partial 16S rRNA gene sequences (412–

6239 sequences per sample) (Table S1). A total of 656 unique OTUs (97% sequence similarity, 15–242 OTUs per sample) were identified with approximately 17% (114) containing only one sequence (Table S6). The single most abundant OTU (17.6% of all sequences) was classified as *Thermofilum* and was observed in 28 of 34 samples. The six samples that did not contain this OTU were all from the Mariner vent field. Only one OTU, which was classified as *Thermococcus* (13.2% of all

Table 2 Results of pairwise comparisons of bacterial communities from different vent fields along the Eastern Lau Spreading Center using the ANOSIM test in Primer v6. *R*- and *P*-values (parentheses) are shown for each comparison. Values in bold indicate comparisons that are statistically significant at a *P*-value < 0.05. Note that *R*-values range from -1 to 1 with values closer to 1 indicating a high degree of separation between sites

	Kilo Moana	Tow Cam	Tahi Moana	ABE	Tui Malila
Kilo Moana					
Tow Cam	0.463 (0.029)				
Tahi Moana	0.204 (0.19)	0.159 (0.148)			
ABE	0.364 (0.083)	0.194 (0.114)	-0.039 (0.645)		
Tui Malila	0.37 (0.10)	0.537 (0.057)	0.031 (0.405)	0.154 (0.226)	
Mariner	0.806 (0.006)	0.381 (0.012)	0.463 (0.001)	0.594 (0.0007)	0.411 (0.048)
Summary	6/15 pairwise comparisons are statistically significant (<i>P</i> < 0.05)				

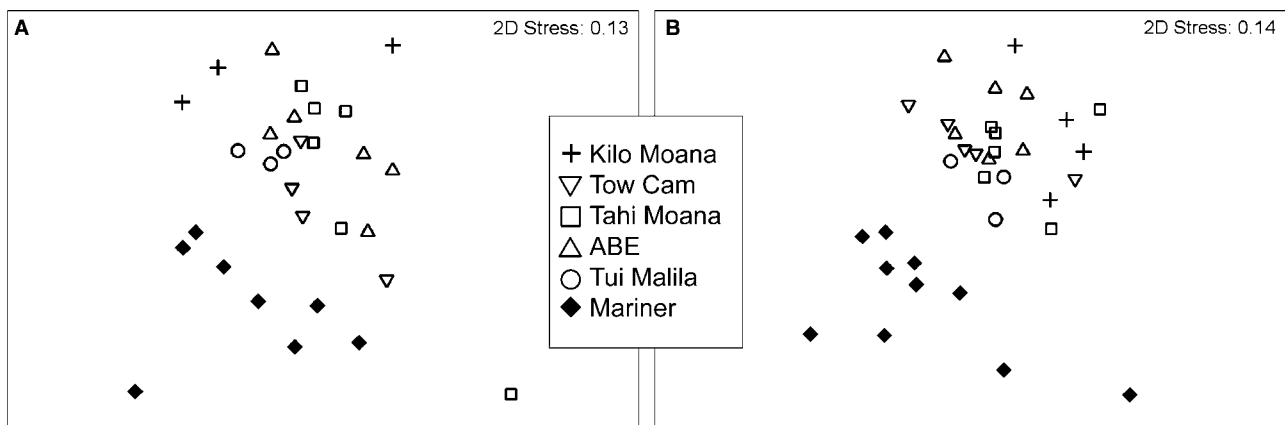


Fig. 3 Non-metric multidimensional scaling plots (MDS) based on Bray–Curtis dissimilarities of the bacterial (A) and archaeal (B) communities associated with hydrothermal vent deposits from the Eastern Lau Spreading Center. Points closer together on the ordination plot have communities that are more similar.

sequences), was observed in all samples. Archaeal diversity for some of the samples appeared to be captured as some of the rarefaction curves were asymptotic (Fig. S3).

Crenarchaeotal sequences were most abundant in 24 of the 34 samples (Fig. 4A), with members of the *Desulfurococcales*, *Thermofilaceae*, and *Thermoproteaceae* prevalent in most samples except those from Mariner. Mariner samples had, on average, a lower proportion of crenarchaeotal sequences than the other vent fields, but did harbor some unique lineages not commonly observed in marine hydrothermal environments (Fig. 4A,B). For example, one deposit from Mariner, Mariner-12, had a significant number of sequences confidently classified as *Caldisphaeraceae*. In most samples, there were many other crenarchaeotal sequences that could not be classified beyond the class (*Thermoprotei*) or order (*Desulfurococcales* and *Thermoproteales*) (Fig. 4A). Of the ten samples not dominated by *Crenarchaeota*, nine had mostly euryarchaeotal sequences (*Thermococcaceae*, DHVE2 and *Archaeoglobaceae*) and one had over 40% nanoarchaeotal sequences. The *Thermococcaceae* dominated most samples from Mariner (Fig. 4B). Methanogens (*Methanocaldococcales* and *Methanococcales*) were relatively rare in most samples, especially those from Mariner (Fig. 4B). Several unclassified lineages like the DHVE5 and the unclassified

Euryarchaeota A (Flores *et al.*, 2011a) were also detected in many samples (Fig. 4A). The *Nanoarchaeota* and *Korarchaeota* were observed in 27 and 24 of the 34 samples, respectively.

Inter-field archaeal community comparisons

As was observed with the bacterial ANOSIM test, all pairwise comparisons of Mariner with samples from the other vent fields were statistically significant (*P* < 0.05) (Table 3, Fig. 3B). Also like the bacterial communities, the overall Bray–Curtis dissimilarity values were correlated with sample location and select geochemical characteristics of the end-member fluids (Table S4). Iron was the only geochemical factor that was significantly correlated when Mariner samples were removed from the analysis, suggesting that Fe is a key determinant in shaping microbial communities along the ELSC. OTUs classified as *Thermococcus*, and several crenarchaeotal lineages (e.g., *Thermofilum*, *Thermoproteaceae* and *Staphylothermus*) were helping drive these differences (Table S7). Unlike the bacterial communities, several other pairwise comparisons including all involving samples from ABE and four of the five samples from Kilo Moana were also statistically significant (*P* < 0.05) (Table 3). These

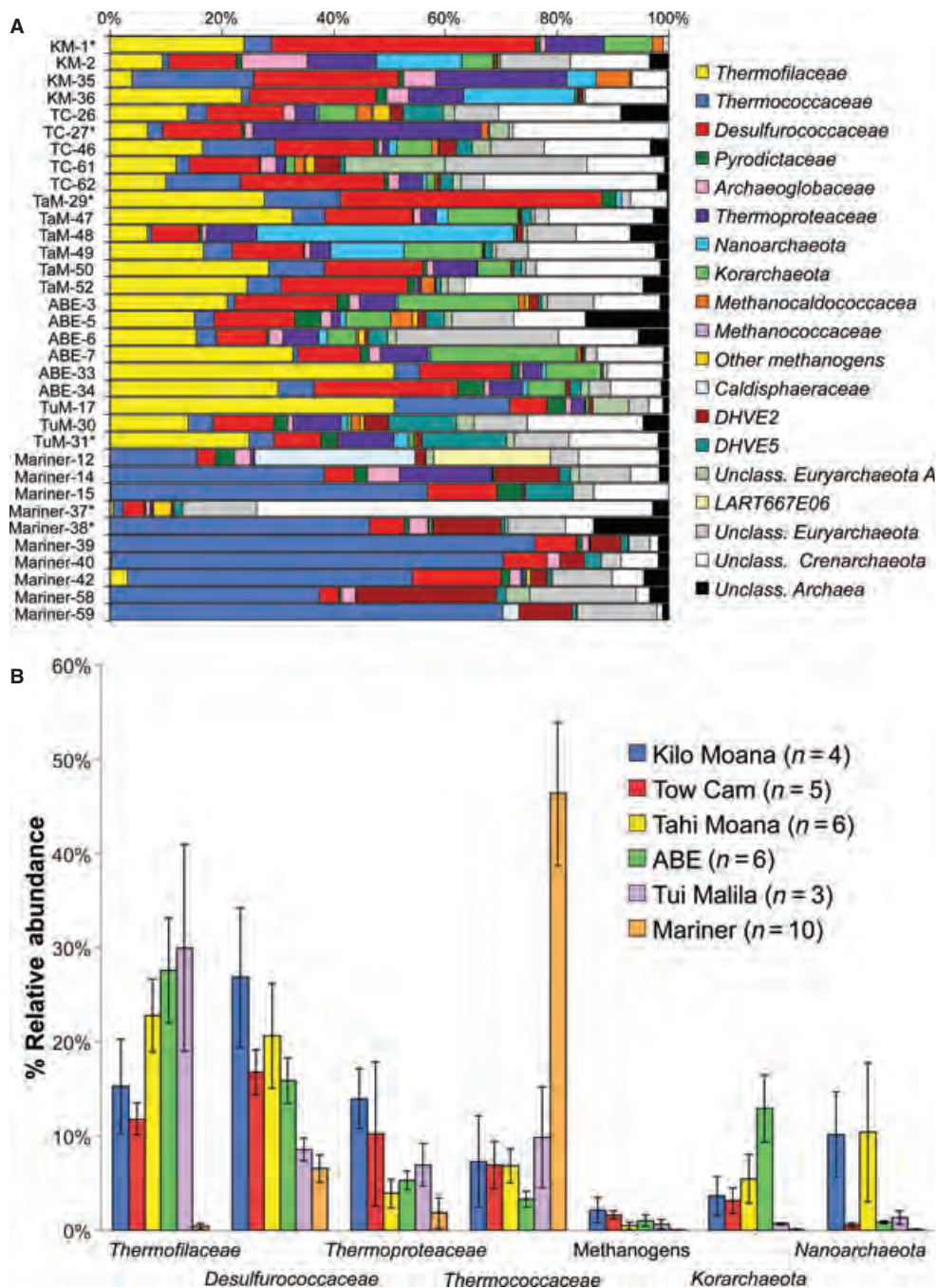


Fig. 4 Relative abundance of archaeal families (A) observed in individual deposit samples and average abundance of discriminating archaeal taxa (B) observed in deposits within individual vent fields along the Eastern Lau Spreading Center. Asterisks in A denote samples for which no corresponding bacterial data were obtained. Error bars in B indicate ± 1 standard error of the mean (SEM).

differences were in part driven by the high average abundance of *Korarchaeota* at ABE (Fig. 4B, Table S8) and *Nanoarchaeota* at Kilo Moana (Fig. 4B, Table S9). However, as little is known about the ecology of these two lineages, it would be misleading to speculate what drives these differences. Overall, these results suggest that the structure of the archaeal communities from the six vent fields, particu-

larly Kilo Moana, ABE, and Mariner, is different from each other.

DISCUSSION

In this study, we used barcoded pyrosequencing of the V4 region of the 16S rRNA gene to describe and compare the

Table 3 Results of pairwise comparisons of archaeal communities from different vent fields along the Eastern Lau Spreading Center using the ANOSIM test in Primer v6. *R*- and *P*-values (parentheses) are shown for each comparison. Values in bold indicate comparisons that are statistically significant at a *P*-value < 0.05. Note that *R*-values range from -1 to 1 with values closer to 1 indicating a high degree of separation between sites

	Kilo Moana	Tow Cam	Tahi Moana	ABE	Tui Malila
Kilo Moana					
Tow Cam	0.444 (0.032)				
Tahi Moana	0.087 (0.238)	0.077 (0.169)			
ABE	0.563 (0.01)	0.416 (0.002)	0.146 (0.041)		
Tui Malila	0.463 (0.029)	0.395 (0.071)	0.179 (0.214)	0.346 (0.048)	
Mariner	0.734 (0.002)	0.591 (0.001)	0.598 (0.0001)	0.699 (0.0001)	0.422 (0.045)
Summary	11/15 pairwise comparisons are statistically significant (<i>P</i> < 0.05)				

bacterial and archaeal communities associated with high-temperature vent deposits from six vent fields along the ELSC. Results showed that both the bacterial and archaeal communities from the southernmost Mariner vent field were distinct. The archaeal communities from Kilo Moana and ABE were also found to be different than most other vent fields. Communities were correlated with geographic location of sampling and all geochemical features of the end-member fluids tested (Table S4). These relationships, however, were driven primarily by the unique communities and geochemistry of the Mariner vent field as all but one of the correlations were not significant when Mariner samples were removed from the analysis (Table S4). As many of these geochemical features covary along the ELSC, it was also difficult to identify a single environmental factor that might be driving the structure of these communities. It is important to note that our sampling strategy may have obscured true linkages across all vent fields, as we did not collect end-member fluids from all of the samples we analyzed, forcing us to use within vent field averages for the correlations. In addition, trying to correlate end-member fluid conditions with microbial communities associated with vent deposits is tenuous as the environmental conditions experienced by the communities are typically a mixture of end-member fluids (over 300 °C) and seawater making scale-appropriate measurements impossible. These conditions can vary depending on fluid flow paths, mixing styles, porosity of deposits, and other factors. Modeling the conditions is one approach that can overcome these challenges, as we showed recently (Flores *et al.*, 2011a). With these caveats in mind, our approach in this study was to use the geochemical data as a framework to interpret the community differences we observed.

Bacterial communities

Overall, the taxonomy of the bacterial communities associated with active hydrothermal deposits from the ELSC vents resembled those from other hydrothermal vent sites elsewhere with diverse *Epsilonproteobacteria* dominating most samples. Most of the communities were a mix of mesophilic (*Sulfur-ovum* and *Nitratifactor*) and thermophilic (*Lebetimonas*,

Caminibacter, and *Nautilia*) genera, illustrating the steep thermal gradients that exist in vent deposits (Fig. 2). The prevalence of *Lebetimonas* in most deposits (59 OTUs, 24.21% of all sequences), however, was contrary to our previous observations of bacteria associated with deposits from the MAR where *Lebetimonas* were far less abundant (Flores *et al.*, 2011a). In a combined analysis of our pyrosequencing data from the MAR and ELSC, the single dominant *Lebetimonas* OTU of the ELSC (11.03% of all sequences) was not present along the MAR (data not shown). Instead, *Caminibacter* was the dominant thermophilic genus of *Epsilonproteobacteria* in most samples from the MAR. While *Caminibacter* were also widespread along the ELSC (5 OTUs, 5.41% of all sequences), they were generally lower in abundance than *Lebetimonas*. While both of these thermophilic genera belong to the same family (*Nautiliaceae*) and have moderately acidophilic members able to oxidize H₂ with elemental sulfur, known *Caminibacter* spp. are also able to use organic carbon sources and nitrate or oxygen as electron acceptors (Alain *et al.*, 2002; Miroshnichenko *et al.*, 2004; Takai *et al.*, 2005; Voordeckers *et al.*, 2005). These differences may allow them to co-occur within vents as we observed along the ELSC and MAR, but do not help to explain why one is more abundant in one vent system over the other. These biogeographical patterns may be more related to neutral processes (*i.e.*, dispersal limitation) as others have suggested for *Epsilonproteobacteria* from diffuse flow vents at underwater seamounts (Huber *et al.*, 2010).

One of the significant differences between vent field bacterial diversity as indicated by SIMPER was the notable shift in the abundance of *Nautilia* at the Mariner vent field (Fig. 2A, Table S5). Of the few cultured isolates, *Nautilia* are distinguishable from other thermophilic *Epsilonproteobacteria* like *Caminibacter* and *Lebetimonas*, by their ability to oxidize formate (Campbell *et al.*, 2006). Although the concentration of formate in fluids from the ELSC is unknown, formate concentrations in hydrothermal fluids differ as a function of total carbon, the redox state of the fluids, and temperature (Seewald *et al.*, 2006). The CO₂ concentrations at Mariner are generally three to five times higher than the other vent fields owing to active degassing of a subsurface magma chamber, which

also results in more acidic, metal-rich fluids (Table 1) (Mottl *et al.*, 2011). With higher CO₂ concentrations in the fluids, formate concentrations would also be higher relative to the other vent fields. Formate is also produced biologically by fermentative Archaea like the DHVE2 (Reysenbach *et al.*, 2006), which are also more abundant at Mariner. Thus, although speculative, we hypothesize that the prevalence of *Nautilia* in the Mariner vent field may be related to the availability of formate. Likewise, several species of *Thermococcus*, which are also abundant at Mariner (see below), have also recently been found to oxidize formate (Kim *et al.*, 2010). These observations point to the value of this approach for building new hypotheses that can be tested in the future.

In addition to *Epsilonproteobacteria*, several genera of *Deltaproteobacteria* were observed in most samples. One genus observed in all samples, albeit at relatively low abundance, was *Hippea*. Recently, several novel thermoacidophilic *Hippea* spp. were isolated from globally distributed vent fields (Flores *et al.*, 2011b). While all the end-member fluids that have been measured along the ELSC are acidic (Table 1), acidic habitats are generated in deposits by the conductive cooling of hydrothermal fluids or by diffusion of hydrothermal fluids outwards across the wall of a deposit without mixing of seawater (Tivey, 2004). Just as the presence of mesophilic and thermophilic *Epsilonproteobacteria* is consistent with the steep temperature gradients in vent deposits, the presence of thermoacidophiles in all deposits highlights the steep pH gradients that exist within these deposits.

Archaeal communities

As with the bacterial communities, archaeal taxonomy of the ELSC is generally similar to other deep-sea hydrothermal vent fields with a high diversity of thermophilic *Euryarchaeota* and *Crenarchaeota*, and more limited detection of the *Korarchaeota* and *Nanoarchaeota*. The abundance of the *Crenarchaeota* in most deposits (other than Mariner) is somewhat unusual (Fig. 4) as the majority of other 16S rRNA gene-based studies of vent deposits have typically recovered a higher proportion of euryarchaeotal sequences (e.g., Takai & Horikoshi, 1999; Takai *et al.*, 2001; Schrenk *et al.*, 2003; Kormas *et al.*, 2006; Voordeckers *et al.*, 2008; Nunoura & Takai, 2009). Generally, we know little about the physiological and metabolic capabilities of many of these *Crenarchaeota*. For example, there are very few cultivated representatives of the *Thermofilaceae* and *Desulfurococcaceae*, and thus, these samples and sites may be an important source for isolating new members of these poorly studied lineages. It is possible that because mesophilic *Bacteria* are commonly observed in these systems, many of the novel crenarchaeotal lineages could occupy lower-temperature niches. These observations highlight the need for more targeted cultivation efforts or the use of single-cell genomics techniques to help describe some of these novel lineages.

Like the shift seen in the bacterial diversity at the Mariner vent field, archaeal diversity is significantly different from the other ELSC samples studied. One of the OTUs driving these differences belongs to the *Thermococcaceae* (Figs 3 and 4, Table S7). Cultivated members of the *Thermococcaceae* are generally sulfur-reducing heterotrophs (Reysenbach *et al.*, 2002); however, recent genomic analyses of multiple *Thermococcus* isolates from different deep-sea hydrothermal environments revealed that several species have the potential to oxidize CO to CO₂ (carboxydrotrophy) (Lee *et al.*, 2008; Zivanovic *et al.*, 2009; Vannier *et al.*, 2011). Several *Thermococcus* species, including one isolated from Kilo Moana, can also conserve energy through the anaerobic oxidation of formate coupled with H₂ production (Kim *et al.*, 2010). As with the *Nautilia* discussed above, the abundance of *Thermococcaceae* at Mariner may be related to the availability and abundance of single-carbon compounds originating from the degassing magma chamber. Mariner fluids have other properties, like increased metal concentrations and lower pH, which may also be influencing the microbial diversity of these deposits. Clearly, further studies are needed to quantitatively evaluate the importance of single-carbon compounds like CO and formate, as well as other geochemical features in the ecology and diversity of deep-sea hydrothermal vent microbes.

Unlike what was observed in the bacterial communities, the archaeal communities from Kilo Moana and ABE were distinct (Table 3). As indicated by SIMPER, the abundance of *Korarchaeota* at ABE (Table S8) and the abundance of *Nanoarchaeota* at Kilo Moana (Table S9) were strong drivers of these differences (Fig. 4B). Both of these lineages have previously been detected at deep-sea hydrothermal vents (e.g., Nercessian *et al.*, 2003; Auchtung *et al.*, 2006; McCliment *et al.*, 2006; Flores *et al.*, 2011a), and, although neither has been cultivated in isolation, genomic sequences have been obtained (Elkins *et al.*, 2008; Podar *et al.*, 2008). However, we have too little information about the physiological diversity of these *Archaea* to speculate what geochemical and physical differences at the different vent fields might be driving these observations.

Recently, we reported on the archaeal diversity associated with the Lucky Strike and Rainbow vent fields along the MAR that have hydrothermal fluids with very low and very high H₂ concentrations, respectively (Lucky Strike 0.025–0.071 mM H₂ to Rainbow 12.3–16.9 mM H₂). Using multiple approaches, methanogens were not detected in samples from Lucky Strike, whereas they were abundant in samples from Rainbow (Flores *et al.*, 2011a). Although other geochemical and physical factors could have influenced these patterns, we hypothesized that H₂ concentrations were too low at Lucky Strike to support a robust methanogen community. Thus, extrapolating these observations to vents along the ELSC, we predicted that given the intermediate H₂ concentrations in ELSC fluids relative to those of Lucky Strike and Rainbow (Table 1), methanogens should be in low

abundance at all ELSC vent fields. Indeed, methanogens were low in abundance in samples from the ELSC and were essentially absent from Mariner, the site with the lowest measured H₂ (0.033–0.179 mM) along the ELSC (Fig. 4B). The relatively low abundance of methanogens (*Methanocaldococcaeae* and *Methanococcaeae*) at all ELSC vent fields further supports the importance of H₂ concentrations in structuring the archaeal communities of deep-sea hydrothermal environments (Takai *et al.*, 2006; McCollom, 2007; Perner *et al.*, 2007; Takai & Nakamura, 2010; Flores *et al.*, 2011a; Rousset *et al.*, 2011).

Clear differences in the bacterial and archaeal diversity from deposits within the same vent field were also observed. For example, archaeal communities associated with Mariner-12 were very different from any other deposit (Fig. 4A). Members of the *Caldisphaeraceae* and *Thermoplasmataceae*, previously not detected at vents, were observed in this deposit. Known *Caldisphaeraceae* are thermoacidophilic and have previously only been observed in and cultivated from terrestrial hydrothermal environments (Prokofeva *et al.*, 2000, 2009; Itoh *et al.*, 2003). Likewise, sequences related to the *Thermoplasmatales* LART667E06 (DQ451876) clone, which was first detected in 2005 from the same structure (Reysenbach *et al.*, 2006), may also represent thermoacidophiles. Multiple attempts to culture the *Caldisphaeraceae* and *Thermoplasmatales* (other than the DHVE2) from these samples (in 2005 and 2009) have thus far been unsuccessful.

In addition to Mariner, the microbial diversity in other vent fields also showed a significant degree of intra-field variability. For example, the *Nanoarchaeota* were abundant in two samples from Tahiti Moana (Tahiti Moana-48, Tahiti Moana-49), while they were in low relative abundance in the other samples from this vent field. Differences in community diversity associated with samples within a vent field have been attributed to factors such as phase separation of the fluids (Nakagawa *et al.*, 2005b; Nunoura & Takai, 2009). Fluid temperatures from vent fields along the ELSC, other than Mariner, are well below the two-phase boundary, indicating that if phase separation occurred, fluids must have cooled prior to reaching the seafloor (Mottl *et al.*, 2011). Therefore, other factors, like fluid mixing styles, deposit mineralogy, and age of the deposit, probably influenced the observed intra-field variability of archaeal communities and should be considered in future studies.

CONCLUSION

The hydrothermal vent deposit bacterial and archaeal communities from six vent fields along the ELSC shared many high-temperature vent lineages such as diverse *Epsilonproteobacteria*, *Desulfurococcaeae*, and *Thermococcaeae*. However, although the communities from the vent fields differed from one another, the most significant differences in community structure and composition were observed between deposits collected from the Mariner vent field and all other vent fields.

For example, the genus *Nautilia* and members of the *Thermococcaeae* were enriched at Mariner, while the *Thermofilaceae* were not. Differences in community composition were found to correlate with several geochemical properties of the unique end-member fluids of Mariner (Table 1). However, many of the geochemical features we measured co-vary and point to the unique geologic setting of the Mariner vent field as the driving force behind these relationships. Overall, this study builds on global patterns of biodiversity at vents and points to geochemical and geological influence on some lineages between globally distributed vent fields and within vent fields of the ELSC. Whether primarily niche or neutral processes shape the global differences remains to be determined. Using this same approach to characterize the communities of several more vent fields from geographically and geologically distinct locations as well as collecting more detailed geochemical data including fluid chemistry and deposit mineralogy, will help provide further insights into the role of these non-exclusive factors in shaping deep-sea vent microbial biogeography.

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- Figure S2.** Rarefaction curves for bacterial communities of hydrothermal vent deposits from different vent fields along the Eastern Lau Spreading Center.
- Figure S3.** Rarefaction curves for archaeal communities of hydrothermal vent deposits from different vent fields along the Eastern Lau Spreading Center.
- Table S1.** Hydrothermal deposits collected from the Eastern Lau Spreading Center used for microbial community characterization.
- Table S2.** Results of AmpliconNoise analysis of four archaeal pyrosequencing libraries from vent deposits of the Eastern Lau Spreading Center.
- Table S3.** Bacterial OTU table of communities associated with deep-sea hydrothermal vents from the Eastern Lau Spreading Center. For information about how OTUs were generated and taxonomy assignments, please refer to the main text.
- Table S4.** Correlations between Bray-Curtis dissimilarity values and average geochemical features of end-member hydrothermal fluids within each vent field along the Eastern Lau Spreading Center (Spearman rank correlations estimated using Mantel tests).
- Table S5.** Results of SIMPER analysis showing the top five discriminating OTUs driving differences between the bacterial communities of Mariner deposits and other vent fields from the Eastern Lau Spreading Center.
- Table S6.** Archaeal OTU table of communities associated with deep-sea hydrothermal vents from the Eastern Lau Spreading Center.
- Table S7.** Results of SIMPER analysis showing the top five discriminating OTUs driving differences between the archaeal communities of Mariner deposits and other vent fields from the Eastern Lau Spreading Center.
- Table S8.** Results of SIMPER analysis showing the top five discriminating OTUs driving differences between the archaeal communities of ABE deposits and other vent fields from the Eastern Lau Spreading Center.
- Table S9.** Results of SIMPER analysis showing the top five discriminating OTUs driving differences between the archaeal communities of Kilo Moana deposits and other vent fields from the Eastern Lau Spreading Center.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Location of deposits collected from each hydrothermal vent field along the Eastern Lau Spreading Center.