

Microbial succession during the transition from active to inactive stages of deep-sea hydrothermal vent sulfide chimneys

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Abstract

Background Deep-sea hydrothermal vents are highly productive, yet ephemeral biodiversity hotspots in the deep ocean supported by chemosynthetic microorganisms that play critical roles in the maintenance and development of these extreme ecosystems. While several studies have investigated the microbial diversity in both active and inactive sulfide chimneys that have been extinct for a long time, little is known about chimneys that ceased activity more recently as well as the microbial succession occurring during the transition from active to inactive chimneys.

Results Genome-resolved metagenomics was applied to an active and a recently ceased (~7 years) sulfide chimney from the 9°50'N hydrothermal vent field on the East Pacific Rise. Full-length 16S rRNA gene and a total of 173 high quality metagenome assembled genomes (MAGs) were retrieved for comparative analysis. In the active chimney (EPR-L), sulfide- and/or hydrogen-oxidizing Campylobacteria and Aquificae with the potential for denitrification were identified as the dominant community members and primary producers, fixing carbon through the reductive tricarboxylic acid (rTCA) cycle. In contrast, the microbiome of the recently extinct chimney (EPR-M) was largely composed of heterotrophs from various bacterial phyla, including Delta -/ Beta -/ Alphaproteobacteria and Bacteroidetes . Gammaproteobacteria were identified as the main primary producers, using the oxidation of metal sulfides and/or iron oxidation coupled to nitrate reduction to fix carbon through the Calvin-Benson-Bassham (CBB) cycle. Further analysis revealed a phylogenetically distinct Nitrospirae cluster that has the potential to oxidize sulfide minerals coupled to oxygen and/or nitrite reduction, as well as for sulfate reduction, and that might serve as an indicator for the early stages of chimneys after venting has ceased.

Conclusions This study shed light on the composition, metabolic functions, and succession of microbial communities in deep-sea hydrothermal vent chimneys. Collectively, microbial succession during the life span of a chimney could be described to go from a "fluid-shaped" microbial community in newly formed and actively venting chimneys to a "mineral-shaped" community after hydrothermal activity has ceased. Remarkably, the transition appears to happen early on, after which the communities stay stable for thousands of years.

Introduction

Since the discovery of the first deep-sea hydrothermal vent (DSHV) near the Galapagos Islands in 1977 [1], more than 700 DSHV fields have been discovered and in many cases investigated along mid-oceanic ridges, at back-arc spreading centers and volcano arcs [2]. Water-rock reactions at high temperatures generated by subsurface magmatic heating transforms the seawater percolating into the ocean crust into hot, reduced, metal-rich hydrothermal fluids venting from the seafloor. Therefore, DSHV systems are considered as a critical conduit for the exchange of energy and matter between the Earth's interior and the ocean [3, 4]. The hydrothermal vent field located on East Pacific Rise (EPR) at 9°-10°N is an archetypical fast-spreading mid ocean ridge system (550 mm year^{-1}) and as a result of a range of multi- and interdisciplinary studies over the last two and half decades represents one of the best studied

hydrothermal systems [5]. More importantly, two massive volcanic eruptions have been documented at this location in 1991 and 2006, respectively [6, 7], providing unparalleled opportunities to study the evolution of a hydrothermal vent system with dynamic volcanic activity as well as the corresponding microbial succession during this process.

Hydrothermal sulfide chimneys are typical vent structures, which are formed over short spatial and temporal scales by the precipitation of metal sulfides following the mixing of venting hydrothermal fluids with the surrounding cool, oxygenated seawater [8, 9]. The resulting thermodynamic and redox disequilibria provide conditions conducive for the growth of chemoautotrophic microorganisms, which colonize the interior and exterior parts of chimney walls according to their growth preference and contribute to the overall biomass production at DSHV [10, 11]. Previous microbiological investigations have shown that the microbial communities inhabiting active sulfide chimneys are diverse, including Campylobacteria (previously known as Epsilonproteobacteria [12]), Aquificae, Gammaproteobacteria and some archaeal taxa [13–16]. Among them, Campylobacteria are frequently found as the dominant chemoautotrophic microorganisms of active hydrothermal vent chimneys [14, 16–22], often forming microbial mats covering the exterior of venting chimneys [18, 20, 23, 24]. Most Campylobacteria identified at DSHV are uncultured, but information on available isolates suggests that they are either thermophiles or mesophiles with the capability of chemoautotrophy driven by oxidation of H_2 and/or H_2S dissolved in the vent fluids [12, 25, 26]. However, once hydrothermal activity ceases, the disappearing of the previously available energy sources and thermal gradients results in a pronounced shift of the microbial communities inhabiting the inactive chimneys to a community dominated by Gamma-, Delta-, Alpha-, Betaproteobacteria and Bacteroidetes [27–31]. Most of the studies on inactive chimneys have focused on revealing the microbial diversity and community structure using 16S rRNA-based analyses [27–30]. Only recently, meta-'omic' approaches have been applied to study microbial metabolic potentials in extinct chimneys, which indicated that, among other findings, the oxidation of metal sulfides serves as important energy source mediated mainly by sulfur-oxidizing Gammaproteobacteria [31]. However, most of the inactive chimneys studied so far have been extinct for a long time (up to more than thousand years) [28–30]. Although the study by Meier et al [31] included a recently extinct chimney, dating put the age range of the chimney between 0-160 years, and thus the exact time when it became extinct is not known. Consequently, at present less is known about the changes and succession in the microbial communities that occur in the immediate aftermath of an actively venting chimney that becomes inactive.

Here, an actively venting sulfide chimney and another one that ceased venting 7 years before sampling were collected from the same hydrothermal field at EPR 9°50'N to analyze the composition and the metabolic capabilities of the resident microbial communities using genome-resolved metagenomics and to elucidate the changes occurring during the transition from an active to an inactive chimney. Based on our data and available information from previous studies, we are proposing a conceptual model of microbial succession from the initiation to maturation, post-maturation, and finally extinction of a DSHV sulfide chimney.

Material And Methods

Chimney and fluid samples collection

Sulfide chimney samples were collected by ROV Jason at the 9°50'N deep-sea hydrothermal vent field on the East Pacific Rise (EPR) during research cruise AT26-10 (December 2013 to January 2014). One sample (named as EPR-L) was collected from the flange of an active chimney at the L-vent (104.2789° W, 9.7712° N), venting hydrothermal fluid with temperature of 231 °C; the other one (EPR-M) was collected from a chimney at the M-vent (104.2931° W, 9.8466° N) that became inactive as a result of the volcanic eruption in 2006 [32]. At the time of sampling in January 2014, the highly weathered EPR-M chimney was found to emit warm fluid (35 °C) at a low flow rate. Chimney pieces were placed in sealed bioboxes to prevent mixing with ambient seawater during the recovery. Both samples were kept at -70 °C immediately after sample retrieval on board, and were then transported to the laboratory with dry ice and stored at -80 °C before analysis.

Fluid samples from both vents were taken with isobaric gastight samplers (IGT) to maintain fluids at seafloor pressure [33]. During sampling, the IGT snorkel with a thermocouple at its tip was positioned directly into the chimney orifices. When the temperature achieved a stable reading, the inlet valve of the sampler was opened for two minutes and then closed to maintain *in situ* pressure during recovery.

Geochemistry measurements

Fluid samples were extracted from the retrieved IGT sampler for geochemical analysis either directly on board the ship or upon return back in the laboratory. The pH was measured on board at 25 °C with an Ag/AgCl combination reference electrode. Dissolved methane and hydrogen concentrations were determined on board using a gas chromatograph equipped with a 5 Å molecular sieve packed column and serially connected thermal conductivity and flame ionization detectors following quantitative headspace extraction. Total dissolved sulfide ($\Sigma\text{H}_2\text{S} = \text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$) was determined potentiometrically using a sulfide-selective electrode on board the ship. Dissolved Mg^{2+} , K^+ , Ca^{2+} , Na^+ and Cl^- concentrations were analyzed by ion chromatography with suppressed conductivity detection back in the shore lab.

DNA extraction, sequencing, assembly and mapping

A modified SDS-based DNA extraction method was used to recover sufficient high-quality DNA from two chimney samples [34]. The paired-end sequencing were performed using a 2×100bp illumina HiSeq 2000 platform (TruSeq SBS KIT-HS V3, Illumina, at BGI-Shenzhen, China). Metagenome raw reads were trimmed with Sickle (v1.33) (<https://github.com/najoshi/sickle>) using the '-pe' option with default parameters. Clean reads were merged and assembled using IDBA-UD (v1.1.3) with parameters: pre_correction, mink 52, maxk 92, step 8 and seed kmer 52 [35]. Clean reads were mapped onto their assembled contigs respectively using bowtie2 (v2.2.8) with -very sensitive mode [36]. The resulting sam

file was sorted and converted to bam using samtools (v1.3.1), and depth of each contig was generated by using the *cytoscapeviz* script of multi-metagenome project [37].

16S rRNA gene reconstruction

Full-length 16S rRNA genes were reconstructed from clean reads of two metagenomes respectively by EMIRGE (v0.60.4) with default parameters and BLASTed against SILVA 123 SSURef_NR99 database with e-values of $< 1 \times 10^{-10}$ for taxonomic information [38, 39]. Relative abundance in phylum level (class level for Proteobacteria) is summarized based on the average sequencing depth of taxonomically assigned 16S rRNA genes from two samples, respectively.

Annotation and statistic comparison of functional genes

For contigs larger than 1 Kb, open reading frames (ORFs) were predicted and translated by using prodigal (v2.6.3) with -p meta parameters [40], and the resulting amino acid sequences were uploaded to webserver GhostKOALA (KEGG Orthology And Links Annotation) in genus_prokaryotes + family_eukaryotes database with default parameters for KO annotation [41]. For cross checking, potential key genes involved in further analysis were also annotated in eggNOG database through emapper-eggnoG (v0.0.1) as well as in Pfam 31.0, TIGRFAM 15.0 and custom databases via hmmsearch with cutoff e-value $< 1 \times 10^{-10}$ [42-45]. Specific key genes and their accession in different databases are listed in Additional file 2: Table S4.

To quantitatively compare key genes between the two chimney samples, reads mapped to each gene were recruited by using featureCounts (v.1.5.0) [46], which was normalized by gene length, and the normalized relative abundance of key genes was determined for two samples:

[Please see the supplementary files section to access the equation.]

Statistical tests of key genes involved in carbon, nitrogen and sulfur metabolism between the two metagenomes were performed by pairwise comparisons of their abundance by using two-sided Fisher's exact test with confidence intervals at 95% significance using the Newcombe-Wilson method and Benjamini-Hochberg FDR multiple test correction in STAMP [47]. For those important catalytic genes, their taxonomy was assigned based on BLAST results in the NCBI NR database (updated in October 2018) with coverage $> 50\%$ and e-value $< 1 \times 10^{-10}$. Taxonomic assignment and relative abundance were summarized at the phylum level (class for Proteobacteria).

Phylogenetic analysis of functional genes

Dissimilatory sulfite reductase (DSR) catalyzes either the reduction of sulfate in sulfate-reducing microorganisms or the reverse reaction in sulfide-oxidizing bacteria [48]. Here, the phylogeny of *dsrA* was

used to distinguish between the two types[49]. The retrieved *dsrA* sequences from the current study were aligned with all high-quality *dsrA* amino acid sequences downloaded from NCBI NR database on July 2018 using MAFFT (v7.313) [50]. Gaps in the alignment were trimmed by the trimalAI (v1.4) with -automated1 and checked manually [51]. A phylogenetic tree was generated by using IQ-tree (v1.6.6) using parameters: iqtree -m LG+C60+F+G -alrt 1000 -bb 1000[52]. A similar approach was taken to phylogenetically characterize the marker gene *soxB* of the multienzyme sulfur-oxidizing (Sox) system and the *cyc2* gene.

Metagenomic binning

The binning method used here is modified from Wang et al [53]. Contigs larger than 3 Kb from the two metagenomes were included to independently recover MAGs using MetaBAT2 (v2.12.1) and Maxbin (v2.2.1) with default parameters [54, 55]. The completeness and contamination of MAGs were estimated via CheckM (v1.0.9) with lineage-specific marker genes [56]. Because different independent automated binning methods reconstructed multiple similar MAGs from the same microbial taxa, we here used a modified method described in Donovan *et al.* to refine MAGs [57]. First, a pair of MAGs with identical taxonomic classification were combined into one single bin using the “merge” function of CheckM if integrated completeness and contamination would increase $\geq 10\%$ and $\leq 1\%$ respectively. Next, the contigs with divergent genomic properties (GC content, tetranucleotide and sequencing depth) and incongruent taxonomic classifications were filtered from their belonged MAGs by using the “outliers” method of RefineM (v0.0.22) [57]. Then, average amino acid identity (AAI) was calculated between each refined bin with CompareM (v0.0.23) (<https://github.com/dparks1134/CompareM>). We kept the one with higher completeness if two MAGs share AAI $\geq 99\%$. After filtered the reduplicate contigs in multiple MAGs, we kept qualified MAGs with completeness $\geq 70\%$ and contamination $\leq 9\%$ based on the evaluation from CheckM with parameter lineage-specific.

Taxonomic assignment of MAGs

Qualified MAGs from two samples were phylogenetically assigned to appropriate taxonomic classifications based on a set of 37 concatenated universal single-copy protein sequences [58]. The reference genome dataset was downloaded from NCBI genome database in February 2018, including all available archaeal genomes and selected bacterial genomes with at least 10 from each order. Then, 37 marker genes were predicted in every reference genome and MAG by using reciprocal BLAST in the COG database, which was aligned separately by MAFFT (v7.313) with auto parameter and trimmed using trimalAI (v1.4) with automated1[50, 51]. Phylogenetic tree was generated using RAxML (v8.2.8) with PROTGAMMALG model and 1000 bootstraps replicates [59]. The resulting phylogenetic tree as visualized using the Interactive Tree Of Life (iTOL) webtool[60]. In addition, further phylogenetic analyses were carried out for the MAGs assigned to *Nitrospirae*, *Gammaproteobacteria* and *Campylobacteria*, respectively.

Coverage, relative abundance and replication rate of MAGs

The coverage of recovered MAGs in the communities were estimated through a method based on the unique marker gene *RpS3* [61], which were retrieved from these two chimney metagenomes by using *hmmsearch* (cutoff e-value 1×10^{-10}) in the *pfam* 31.0 database [45]. Relative abundance for each MAG is determined by the proportion of length normalized depth of their binned contigs in all of contigs larger than 3 Kb. Index of replication (iRep) value is a quantitative measurement of the *in situ* replicate rate of bacteria based on its sequencing coverage trend [62]. Here, we determined the iRep value for those bacterial MAGs with ≤ 175 scaffolds per Mb by using the official script (<https://github.com/christophertbrown/iRep>).

Metabolic analysis of MAGs

All retrieved MAGs were annotated by using *eggnog-mapper-1.03* in the *EggNOG* database with e-value 10^{-10} , which were further cross checked in the *pfam* 31.0, *TIGRfam* 15.0 and custom *hmmer* databases with e-value 10^{-10} . Hydrogenases were predicted and classified based on hydrogenase classifier *HydDB* by using *hmmsearch* (e-value cut-off of $1e^{-10}$) [63]. After assignments of key genes, MAGs were assessed for the completeness of specific pathways and functions based on the canonical pathways available in *KEGG Pathway Database* (www.kegg.jp). Besides, aerobic CO dehydrogenase (CODH) shared a highly similarity with another molybdenum hydroxylase enzyme like xanthine dehydrogenase [64], which probably cause overestimating potential of CO oxidation, so we performed similar phylogenetic analysis for its catalyzed subunit *coxL* to confirm their performance in each MAG, reference sequences are from Gary's work [64].

Results

Geochemical features of hydrothermal fluids

The temperature of the hydrothermal fluid discharged from the EPR-L chimney was 231 °C, and the concentrations of sulfide and hydrogen were 1,970 and 268 μM , respectively. For EPR-M, the temperature was measured at 35 °C with low concentrations of sulfide (0.42 μM) and hydrogen (below detection). Methane was also detected in both the EPR-L and -M fluid at a concentration of 25.8 and 21.7 μM , respectively. Despite the stark difference in temperature, dissolved Mg^{2+} , K^+ , Ca^{2+} , Na^+ and Cl^- concentrations and pH of the two fluid samples were largely identical (Table 1).

Microbial taxonomic diversity based on full-length 16S rRNA genes

After removing low-quality metagenomic reads, a total of 319,138,012 and 352,707,814 reads were obtained from EPR-L and -M, respectively. Subsequently, these reads were *de novo* assembled to 644,620 and 545,008 contigs for EPR-L and -M, respectively (Additional file 2: Table S1). In particular, 162 and 372

full-length 16S rRNA genes were retrieved from the EPR-L and -M metagenomes, respectively. The phylogenetic analyses of the 16S rRNA gene showed that the active EPR-L chimney was dominated by *Campylobacteria* (phylum *Campylobacterota*) (55.4%), including the genera *Sulfurovum* (20.2%), *Nitratifactor* (8.6%), *Sulfurimonas* (8.6%) and *Caminibacter* (6.3%) (Additional file 2: Table S3). Bacteria belonging to the phylum *Aquificae* had the second highest relative abundance (14.7%), followed by members of the phylum *Chlorobi* (4.7%), *Thermodesulfobacteria* (3.2%) and *Deinococcus-Thermus* (2.4%) (Fig.1a). In sharp contrast, the bacterial community of the inactive EPR-M chimney was mainly composed of *Gammaproteobacteria* (22.9%) and *Nitrospirae* (17.3%), as well as *Alpha*- and *Deltaproteobacteria* (7.4% and 5.6%, respectively) (Fig.1b). The detailed taxonomic information and relative abundance of all reconstructed 16S rRNA genes are listed in Additional file 2: Table S3 and S4.

Distribution of key metabolic genes

Key genes for microbial carbon, nitrogen and sulfur metabolisms were searched in the metagenomes of the two chimneys, and striking differences were revealed regarding gene inventories and the pathways utilized by these two communities (Fig. 2).

Carbon fixation: Genes encoding for the ATP-citrate lyase (*acIA/B*), the key enzyme of reductive tricarboxylic acid (rTCA) cycle, were identified in significant higher abundance (P-value < 0.05) in the active EPR-L sample compared to EPR-M (Fig.2a), and more than 99% of them share high similarities with those from *Campylobacteria* and *Aquificae* (Additional file 1: Figure S1a). In contrast, genes encoding enzymes of the Calvin-Benson-Bassham (CBB) cycle ribulose-bisphosphate carboxylase and phosphoribulokinase (*rbcL/S* and *PRK*) are significantly enriched in the inactive EPR-M chimney, and the majority (42.6% of *rbcL*; 86.2% of *rbcS* and 74.9% of *PRK*) are assigned with *Gammaproteobacteria* (Additional file 1: Figure S1b). For the Wood–Ljungdahl (WL) pathway, genes encoding for the delta subunit of the archaeal acetyl-CoA decarboxylase/synthase complex (*cdhD*) and for the bacterial acetyl-CoA synthase (*acsB*) were more prevalent in the EPR-M community, while the genes encoding for the alpha, beta, and epsilon subunits of the archaeal acetyl-CoA decarboxylase/synthase complex (*chdA*, *cdhC* and *cdhB*, respectively) were present in higher abundances in the EPR-L community (Fig.2a).

Nitrogen metabolism: Genes encoding the periplasmic nitrate reductase (*napA/B*) and membrane-bound nitrate reductase (*narG/H*) were identified in both EPR-L and EPR-M samples, but with distinctly different abundances (Fig. 2b). In the EPR-M chimney, *narG/H* were significantly enriched, with 42.8% of *narG* assigned to *Alphaproteobacteria* (Additional file 1: Figure S1b), while *napA/B* were more enriched in the active EPR-L chimney, with 97.8% of *napA* assigned to *Campylobacteria* and *Aquificae* (Additional file 1: Figure S1a). Genes of the dissimilatory nitrate reduction to ammonia (DNRA) pathway were more abundant in the inactive EPR-M chimney, with 73.9% of nitrite reductase large subunit (*nirB*) assigned to the *Gammaproteobacteria* (Additional file 1: Figure S1b). For the denitrification pathway, the gene encoding for the beta subunit of the nitric oxide reductase (*norB*) and for the nitrous-oxide reductase (*nosZ*) were identified in significantly higher abundance in the active EPR-L chimney compared

to EPR-M (Fig.2b), with the majority of them assigned to *Campylobacteria* and *Aquificae* (Additional file 1: Figure S1a; 80.1% of *norB* and 75.4% of *nosZ*). On the other hand, the EPR-M community was more enriched in genes encoding for subunits of the nitrogenase (*nifD/K/H*), which is involved in N₂-fixation, compared to EPR-L (Fig.2b), with 43.3% of *nifH* being assigned to *Nitrospirae* (Additional file 1: Figure S1b).

Sulfur metabolism: A significantly higher abundance of genes encoding for adenylylsulfate reductase (*aprA/B*) and sulfite reductase (*dsrA/B*) were identified in the EPR-M sample (Fig.2c). Particularly, most *aprA/B* were taxonomically assigned to *Gamma*- and *Deltaproteobacteria* (Additional file 1: Figure S1b; 59.6% of *aprA* and 68.4% of *aprB*). Since the majority of *dsrA/B* were assigned to unclassified species, we inferred the taxonomy and catalytic type of *dsrA* based on their phylogenies. The results suggest that 13 of 14 *dsrA* presented in the EPR-L were of the reductive type, including *Deltaproteobacteria*, *Archaeoglobus* and *Acidobacteria*, while 36 of 72 *dsrA* genes from EPR-M were of the oxidative type belonging to sulfur-oxidizing *Alpha*- and *Gammaproteobacteria*, with the remainder being of the reductive type belonging to *Deltaproteobacteria* (10), *Nitrospirae* (12) and *Acidobacteria* (14) (Additional file 1: Figure S2). For the Sox sulfur oxidation system, similar abundances were found for *soxB* from EPR-L and EPR-M, however the majority of *soxB* from EPR-L were assigned to *Aquificae* and *Campylobacteria*, while those from EPR-M were largely assigned to *Gamma*- and *Alphaproteobacteria* (Additional file 1: Figure S3). On the other hand, *soxA/C/Y/Z* were found highly enriched in the active EPR-L chimney, most of which (>95%) were assigned to the *Aquificae* and *Campylobacteria* (Additional file 1: Figure S1a). Additionally, genes encoding for the sulfide-quinone oxidoreductase (*sqr*) were present in higher abundance in the EPR-L community, with a similar taxonomic profile as the *sox* genes (Additional file 1: Figure S1a).

Phylogeny of metagenome-assembled genomes (MAGs)

After filtration of low-quality MAGs, 71 and 102 high-quality MAGs with a completeness $\geq 70\%$ and potential contamination $\leq 10\%$ were obtained for further analysis from EPR-L and -M metagenomes, respectively. A total of 66.4% of these MAGs have a completeness $\geq 80\%$ and a contamination $\leq 5\%$ (Additional file 2: Table S2). For EPR-L and -M, 42.3% and 48.3% reads were retrieved to their respective MAGs. Among these MAGs, 20 and 34 genomes of the top 50 most abundant microbial species were identified from EPR-L and -M, respectively, including the top three taxa of the EPR-L community and the most and third most abundant taxa of EPR-M (Additional file 1: Figure S4). Therefore, the retrieved MAGs are representative of the majority of microbial taxa of both communities. Overall, the 173 retrieved MAGs could be taxonomically assigned to more than 20 phyla, including several novel candidate bacterial phyla without cultivated representatives (Fig.3). Particularly, for the 71 MAGs from the EPR-L chimney, 11 MAGs were taxonomically assigned to the phylum *Aquificae*, which was identified as the dominant taxon (14.5%) based on their reads mapped to the whole EPR-L metagenome (Fig.4). *Thermodesulfobacteria* was the second most abundant bacterial MAG (8.0%), and 1 in 4 recovered MAGs belonging to this phylum had the highest relative abundance among all MAGs from EPR-L sample (L-MaxBin-1, 6.6%,

Additional file 2: Table S9). 17 MAGs belonged to *Camplyobacteria* representing only 3.6% of the whole microbial community, this discrepancy to the 16S rRNA gene-based results probably due to their high interspecies diversity and similar genomic features making it difficult to retrieve more MAGs. *Chloroflexi* (5 MAGs), Chlorobi (7 MAGs), *Gammaproteobacteria* (4 MAGs) and *Thermotogae* (2 MAGs) accounted for 2% ,1.7%, 1.5%, and 1.1%, respectively. For Archaea, 4 and 8 MAGs were assigned to the phyla *Euryarchaeota* and *Crenarchaeota*, representing 4.6% and 2.8%, respectively (Additional file 2: Table S6). Phylogenetic analysis indicated that 3 out of 4 euryarchaeotal MAGs belonged to the methanogenic classes *Methanococci* and *Methanopyri* and most of the *Crenarchaeota* were distantly related to *Ignicoccus* (Additional file 1: Figure S8). Moreover, 4 MAGs were classified as DPANN groups, including *Micrarchaeota* (2), *Diapherotrites* (1) and *Nanohalarchaeota* phyla (1). L-Maxbin-88 was not close to any other taxa in the phylogenetic tree.

For the EPR-M sample, 16 *Gammaproteobacteria* MAGs accounted for 11.4% of the whole community, most of which were assigned to *Ca. Tenderia electrophaga* and also closely related to those recovered from previously analyzed inactive chimneys (Additional file 1: Figure S7) [31]. 11 *Deltaproteobacterial* MAGs were recovered with a total relative abundance of 4.9%. In addition, *Chlorobi* (11 MAGs; 4.99%), *Calditrichaeota* (6 MAGs; 3.72%), *Alphaproteobacteria* (7 MAGs; 2.77%), *Nitrospirae* (6 MAGs; 2.94%), *PVC* group (9 MAGs; 4.35%) and *Acidobacteria* (4 MAGs; 2.57%) were represented as major microbial groups among the MAGs of the EPR-M microbial community. Phylogenetic analysis showed that the 6 *Nitrospirae* MAGs were assigned into two distinct clades (Fig.3): one is named “sulfide mineral” clade comprised of 4 *Nitrospirae* MAGs from EPR-M and other 5 MAGs either from inactive sulfide chimneys or subseafloor massive sulfides (SMS) [31, 65], the other 2 *Nitrospirae* MAGs are away from the “sulfide mineral” clade (Additional file 1: Figure S6). Besides, 3 MAGs assigned to novel taxa in the candidate phyla radiation (CPR), including candidate TM6, SR1 and *Campbellbacteria*, were retrieved from the EPR-M chimney. 2 MAGs were assigned to the phylum *Micrarchaeota* in DPANN group, and 1 MAG (M-MaxBin-034) was not close to any other microbial taxa.

Index of replication value (iRep) of bacterial MAGs

Most of the retrieved bacterial MAGs in the chimneys represent active replicating bacterial taxa as indicated by iRep values calculated from 52 and 91 high-quality bacterial MAGs from the EPR-L and -M samples, respectively. The average iRep value of bacterial MAGs from the recently inactive EPR-M is 1.51, which is higher than that from the active EPR-L (1.42) (Additional file 2: Table S6). In the EPR-L, *Camplyobacteria* had the highest average iRep value (1.52), followed by *Chloroflexi* (1.5), *Gammaproteobacteria* (1.47), *Thermodesulfobacteria* (1.43) and *Aquificae* (1.40). In EPR-M, *Calditrichaeota* had the highest iRep value (1.8), followed by *Chlorobi* (1.74), *Chloroflexi* (1.59), *Nitrospirae* (1.49), *Alpha-/Deltaproteobacteria* (1.48 and 1.42, respectively) and *Gammaproteobacteria* (1.4). iRep value for each MAGs and the average iRep value of other major microbial groups (>1% in each sample) are shown in the Fig.4 and Additional file 2: Table S6, respectively.

Metabolic reconstruction of MAGs

The metabolic potential of the main microbial groups from both chimneys were revealed by respective MAG analysis (Fig.4):

The active EPR-L chimney

The dominating *Campylobacteria* (17 MAGs) and *Aquificae* (11 MAGs) are potential sulfur/hydrogen-oxidizing bacteria with capabilities of denitrification and carbon fixation through the rTCA cycle. All 28 MAGs encode at least one *sqr* gene, 45% and 24% of them also encode complete or near complete Sox system (Fig.4; Additional file 2: Table S6). The rTCA cycle is the sole carbon fixation pathway and was prevalently identified in the *Aquificae* and *Campylobacteria* MAGs (55% and 47%, respectively). In addition, the majority of *Aquificae* and *Campylobacteria* MAGs encode hydrogenase group 1 and 2 for hydrogen uptake. Further, *napA/B* genes were identified in every MAG assigned to *Campylobacteria* and 45% of *Aquificae* MAGs. Genes encoding for the enzymes catalyzing the subsequent steps of denitrification (*nirS/K*, *norB/C*, *nosZ*; Additional file 2: Table S5) were identified in 73% and 47% of the MAGs belonging to the *Aquificae* and *Campylobacteria*, respectively (Fig.4; Additional file 2: Table S6).

Besides *Aquificae* and *Campylobacteria*, 4 MAGs (8.2%) belonging to the *Thermodesulfobacteria* were identified in the EPR-L chimney that have the capacity of reducing multiple sulfur species. They contained not only the key genes encoding the complete sulfate reducing pathway (i.e., *sat*, *aprA/B* and *dsrA/B*), but also other essential marker genes like *dsrD*, the sulfite reductase-associated electron transfer complex (*dsrM/K/J/O/P*), and the electron transfer complex (*QmoA/B/C*) (Additional file 2: Table S7). Moreover, genes encoding for the thiosulfate reductase (*phsA/B*) and tetrathionate reductase (*ttrA*) were also identified in 2 of them. Thermodesulfobacteria MAGs from EPR-L chimney share highly similar metabolic potential with their sulfur-disproportioning isolates [66].

We also identified 2 MAGs belonging to the phylum *Euryarchaeota* that contained the complete gene cluster encoding for the methyl coenzyme M reductase *mcrABG* and also genes encoding for the Group 3/4 hydrogenase, indicating a methanogenic metabolism (Fig.4; Additional file 2: Table S6). The other major microbial groups, such as *Chloroflexi* and *Chlorobi*, are potential organotrophic bacteria, either using fermentation or respiration, as indicated by the absence of genes indicating autotrophic pathways and having considerable number of genes related to carbohydrate degradation (Fig.4).

The recently extinct EPR-M chimney

The dominating *Gammaproteobacteria* (16 MAGs) in the EPR-M chimney are potential chemoautotrophic sulfur-oxidizing bacteria using the CBB cycle, for carbon fixation and reducing nitrate via the DNRA pathway (Fig.4). For sulfur oxidation, most of the *Gammaproteobacterial* MAGs contained the genes encoding for the Sox system (69%) and *sqr* gene (63%) (Fig.4; Additional file 2: Table S6). In addition, 50% of the *Gammaproteobacterial* MAGs also contain the gene encoding for the reverse DSR as

evidenced by the phylogenetic assignment of the *dsrA* gene (Additional file 1: Figure S3), indicating the potential for the oxidative DSR pathway for sulfur oxidation. Moreover, 5 of these MAGs also contain the *cyc2* gene encoding for an outer membrane *c*-type cytochrome, which is closely related with their expressed homologs in the electroautotrophic *Ca. Tenderia electrophaga* (Additional file 1: Figure S9).

Deltaproteobacteria (11 MAGs) were identified as one of the major microbial taxa in the EPR-M chimney. Based on their gene content, they are putative sulfate-reducing bacteria (SRB) having the potential to oxidize organic matter through the WL pathway, with 64% of the MAGs encoding the reductive DSR pathway and WL pathway. Specifically, genes involved in carbohydrate degradation are significantly enriched in the *Deltaproteobacteria* (19.2 CAZyme genes per MAG on average; Additional file 2: Table S6). Based on the identification of genes encoding for *napAB/narGH* and the subsequent DNRA pathway in most of their MAGs, nitrate appears to be a potential alternative electron acceptor for *Deltaproteobacteria*.

The 4 *Nitrospirae* MAGs recovered belonging to the “sulfide mineral” clade encode essential genes of DSR, WL pathway, nitrite reduction and nitrogen fixation, same as the other 5 MAGs in this clade (Fig.4; Additional file 2: Table S8). 3 of them encode the key enzyme of *cbb3*-type cytochrome *c* oxidase (Additional file 2: Table S9). Furthermore, 5 of all 9 *Nitrospirae* from the “sulfide mineral” clade (including the 2 most abundant recovered from the EPR-M and the other 3 derived from recently extinct sulfide chimneys and SMS, respectively [31, 65]) encode the *cyc2* gene (Additional file 2: Table S8). Their *cyc2* genes are phylogenetically closely related to each other and distantly related to their counterparts identified in the genomes of *Zeta-/Betaproteobacteria* Fe-oxidizing bacteria (FeOB) [67] (Additional file 1: Figure S9). Moreover, these “sulfide mineral” *Nitrospirae* MAGs have relatively small genomes (<2Mb) and fewer genes involved in sulfur oxidation pathways (*sqr* and *sox*) compared with other *Nitrospirae* species (Additional file 2: Table S8). Besides the 4 MAGs assigned to the “sulfide mineral” clade, the other two *Nitrospirae* MAGs recovered from the EPR-M have distinct metabolic features: one doesn't have any genes involved in sulfate reduction, the other one encodes the rTCA pathway instead of the WL pathway and is closely related with another metabolically-similar *Nitrospirae* MAG recovered from a long-time inactive sulfide chimney [31] (Additional file 2: Table S8).

Based on the prevalence of genes encoding for *narGH*, *napAB*, and the subsequent DNRA pathway (*nrfA/H*, *nirB* and *nirD*; Additional file 2: Table S5) in their genomes, other microorganisms in the EPR-M chimney including the *Chlorobi*, *PVC*, *Calditrichaeota*, *Alphaproteobacteria*, *Chloroflexi* and *Actinobacteria* are likely to be nitrate-respiring heterotrophs (Fig.4). That's also supported by the enrichment of genes for carbohydrate degradation identified in their MAGs, especially for the *Calditrichaeota*, *Chlorobi*, and *PVC* (Fig.4). Interestingly, some *Calditrichaeota* (1 in 6 MAGs), *Alphaproteobacteria* (2 in 7 MAGs) and *Actinobacteria* (2 in 3 MAGs) encode carbon monoxide dehydrogenase (*coxM/L/S*) which catalyzes CO oxidation. The phylogenetic analysis of *coxL* from EPR-M suggests that they are largely assigned to the putative FormII/BMS clade (Additional file 1: Figure S5). The *Euryarchaeota* MAGs recovered from EPR-M are potential sulfate reducing archaea that are phylogenetically closely related to the *Archaeoglobi* lineage, which is supported by the retrieved 16S rRNA gene of the genus *Geoglobus* (Additional file 2:

Table S4). 3 of the 4 MAGs encode the complete reductive DSR pathway, the archaeal WL pathway as well as group 1 hydrogenase (Fig.4; Additional file 2: Table S6).

Discussion

Microbial communities inhabiting hydrothermal sulfide chimneys are largely shaped by the local geochemical, physical, and geological conditions, and consequently the taxonomy and metabolic capabilities vary with the development of sulfide chimneys [27-31]. Due to the unpredictable nature of volcanic eruptions combined with sampling challenges, it has proven difficult to follow and investigate the succession of microbial communities of a sulfide chimney through its lifetime all the way to extinction. Here, we present the first reported metagenome from an inactive sulfide chimney that went extinct at a precisely known time after a volcanic eruption in 2006, making it significant to understand the microbial succession taking place during the initial transition from an active chimney to an inactive one. The observation that EPR-M emitted warm fluids (35°C) with a similar ionic composition compared to the hot (231°C) EPR-L fluids at the time of sampling suggests that these fluids might represent conductively cooled high-temperature fluids that might originate from a reservoir below the extinct EPR-M structure (Table 1), which was not venting any fluids 6 months after the eruption [5]. Moreover, we also retrieved and deciphered 173 high quality MAGs from both chimneys, which provide genomic insights into the metabolic functions and ecological roles of these microbes. Combining data obtained in the present study with those published previously (including studies from Guaymas Basin, East Pacific Rise 9°/13°N, Mid-Atlantic Ridge, Main Endeavor Field, Loki's Castle vent field; as well as some *in situ* incubations and lab enrichments, details see Additional file 2: Table S10), we are proposing a conceptual model to understand the pattern of microbial succession: microbes in the sulfide chimney shift from a “fluid-shaped” community into a “sulfide-mineral shaped” one during the lifetime of a vent from initiation to extinction (Fig.5).

The “Fluid Shaped” Microbial Community

A few studies on the first colonizers of newly formed chimneys suggested that microbes colonized the sterile sulfide structures quickly, and got established within a short period of time (<14 days) [68-73]. Hyperthermophiles, including *Methanocaldococcus* and *Ignicoccus* species together with its symbiont *Nanoarchaeum*, were identified as the dominant pioneer microorganisms of freshly formed chimneys [71, 72, 74]. In the present study, these putative archaeal pioneers were also identified in the active EPR-L chimney, including one *Methanocaldococcus*-like MAG in considerable abundance (3.8%; Fig.4). Metabolic reconstruction suggests that these uncultured organisms have the ability to utilize hydrogen as an electron donor for methanogenesis and sulfur reduction, respectively, in line with the metabolism of their cultured hyperthermophilic deep-sea vent relatives *Methanocaldococcus* and *Ignicoccus* (Fig.4) [75, 76]. In view of their requirement for hydrogen, high temperature and anoxic conditions, these hyperthermophilic archaea are likely present in the interior layers of the chimney, where these pioneers are expected to consistently contribute to the primary production throughout the lifetime of an active chimney [77, 78] (Fig.5a).

During the development of a sulfide chimney, available spatial, redox and thermal gradients for microorganisms are expanding with accumulating mineral deposits along with the oxidative weathering from seawater. Colonization is followed by the thermophilic *Aquificae* and thermophilic and mesophilic *Campylobacteria*, which colonize the cooler exterior layers and eventually become the dominating microbial taxa during the growth of the chimney. For example, the *Campylobacteria* quickly became the dominant community members after 5 days inside the growth chamber of an *in situ* incubation device [73], sharing high similarity with the taxonomic profile of most investigated mature sulfide chimneys, including EPR-L [14-16, 18-20, 79]. Undoubtedly, hydrogen from venting fluids is the most important electron donor during the early microbial succession stage, not only for archaeal pioneers, but also for the following *Aquificae* and *Campylobacteria* in the mature stage (Fig.4). Subsequently, oxidation of H₂S and other reduced sulfide compounds contained in the fluid become important energy sources, as evidenced by the prevalence of genes involved in sulfur oxidation, like Sqr and the Sox system, identified in most MAGs assigned to these and other bacterial chemolithotrophs (Fig.2 and 4), which is also in line with the observation that the *Campylobacteria* dominate active chimneys with low H₂ concentrations [24, 80]. In addition, the potentials for aerobic respiration and denitrification indicate that the *Aquificae* and *Campylobacteria* are able to utilize oxygen and nitrate from seawater percolating through the chimney as electron acceptors, further implying that they inhabit the relative outer layers and exterior of the chimney wall. In short, hydrothermal fluid chemistry largely controls the primary microbial colonization in the newly formed pro-chimneys and shapes the microbial community within sulfide chimneys from the initiation to the mature stage (Fig.5a, b).

The “Mineral Shaped” Microbial Community

During the transition phase from a mature to an inactive sulfide chimney, a hot “fluid shaped” microbiome as described above is expected to shift to a “mineral shaped” community (Fig.5c, d). First, the decrease in temperature plays a key role in shaping the microbial community, changing from one dominated by thermophiles to one dominated by mesophiles and finally psychrophiles. This is well supported by the distinct differences between active and inactive sulfide chimneys, i.e., a community dominated by the *Aquificae* and *Campylobacteria*; and an assemblage of the *Gamma-/Delta-/Alphaproteobacteria* and *Bacteroidetes*, respectively [4, 27-29, 78]. Surprisingly, the overall microbial composition of the EPR-M chimney that only recently became inactive was highly similar to chimneys that had been extinct for much longer time periods [27, 29-31], suggesting that the microbial succession took place mainly during the early stages after venting ceases and that the microbial community stayed relatively stable afterwards over long temporal scales.

Along with the diminishing hydrothermal fluids, available energy sources for chemoautotrophs supporting the DSHV ecosystem gradually shift from reduced chemicals contained in the vent fluids (mainly H₂ and H₂S) to the sulfide minerals making up the chimneys. This is another critical factor in driving microbial succession at taxonomic and metabolic level after chimney stop venting. First of all, the *Gammaproteobacteria* are inferred to replace the *Campylobacteria* and *Aquificae* as the major primary producers during this process, fixing CO₂ via CBB cycle and retrieving energy from mineral sulfides

through multiple sulfur oxidation pathways (reverse DSR, Sox system and *sqr*). Besides sulfides, Fe²⁺ from minerals (like pyrite and pyrrhotite) serves likely an alternative electron donor for these autotrophic *Gammaproteobacteria* in view of the prevalent *cyc2* gene identified in their MAGs. This gene encodes an outer membrane cytochrome c as a candidate genetic marker for FeOB, which was demonstrated to mediate iron oxidation in the acidophilic FeOB *Acidithiobacillus ferrooxidans* and also found in all available genomes of neutrophilic FeOB [67, 81, 82]. Moreover, highly expressed *cyc2* gene identified in the electroautotrophic *Ca. Tenderia electrophaga* is inferred to be involved in extracellular electron transfer (EET) [83], which also phylogenetically close to the *cyc2* genes identified in these *Gammaproteobacteria* MAGs (Additional file 2: Table S9). Therefore, this suggests that the *Gammaproteobacteria* identified in EPR-M have the potential to oxidize the Fe²⁺ in sulfide minerals via EET, like pyrite and pyrrhotite composed the chimney structure, meanwhile this may also facilitate the access of released mineral sulfides in view of the disappearing hydrothermal fluids. In support of this hypothesis, *Thiomicrospira* sp. SC-1, a gammaproteobacterial FeOB recently isolated from an *in situ* incubation with pyrrhotite, and was capable of autotrophically growing with iron oxides and sulfur intermediates [84]. Thus, these putative chemolithoautotrophic *Gammaproteobacteria* very likely play essential roles in the ecosystems of recently and long-time inactive sulfide chimneys, just like the *Aquificae* and *Campylobacteria* do for active chimneys. They utilize the metal sulfides from chimney minerals as available energy source to support themselves and the relatively stable community for thousands of years after cessation of venting [27-30]. Besides the *Gammaproteobacteria*, *cyc2* gene was also identified among many other bacterial taxa in the inactive EPR-M chimney, including the *Alpha-/Deltaproteobacteria*, *Nitrospirae*, and *Chlorobi* (Fig.4; Additional file 1: Figure S9). Previously, *cyc2*-containing chemolithotrophic *Alpha-/Betaproteobacteria* had been shown to accelerate aerobic pyrite oxidation in freshwater sediments and the *Alphaproteobacteria* was also identified in pyrrhotite incubation experiments as dominant members [84, 85]. Thus, our results imply that the capability to oxidize iron sulfides for chemolithotrophic growth may be widespread among microbes living in the inactive chimneys.

Generally, putative heterotrophic bacteria dominating the EPR-M and other inactive chimneys, like the *Chlorobi*, *Alpha-/Deltaproteobacteria*, *Chloroflexi* and *PVC*, also have been widely identified in active chimneys generally with relative limited abundances [14, 16, 79, 86-88]. This suggests that these heterotrophs probably already colonized the exterior of actively venting chimneys, and then gradually become more prevalent towards the inner layer with diminishing hydrothermal activity, implying they are either thermophiles or mesophiles with wide temperature tolerance range could survive in the hydrothermal transition stage and flourish afterwards or replaced with more mesotrophic or psychrophilic relatives within the same phylum/class taxonomic level in this process. However, for those strict hyper-/thermophiles, like the *Deinococcus-Thermus* and *Thermotogae* [79, 80, 87, 89], they are common in the active chimneys but rarely found in those inactive ones, including the recently inactive EPR-M, suggesting temperature is a critical factor controlling the survival of heterotrophs during the hydrothermal succession (Fig.1). Prevalent cytochrome c oxidases genes identified in most of MAGs from the EPR-M suggest that oxygen is very likely a widespread electron acceptor for microbial communities inhabiting

inactive sulfide chimneys (Additional file 2: Table S9). Interestingly, compared with the active EPR-L chimney, our results further revealed that the metabolic potential for sulfate reduction and *nar*-mediated DNRA were more prevalent in the EPR-M chimney, both at the gene and genomic level (Fig. 2 and 4). This suggests that sulfate and nitrate from seawater are universal electron acceptors for the majority of microorganisms in the EPR-M chimney. In view of the fact that membrane-bound nitrate reductase encoded by *nar* is more efficient than the *nap*-encoded periplasmic nitrate reductase at high nitrate concentrations [90], the higher frequency of *nar* gene observed in the EPR-M possibly reflects the microbial adaptation to the increased accessibility of nitrate, implying that the intrusion of seawater plays an important role in the microbial succession after venting ceases. In addition, comparable or higher iRep values of the bacterial MAGs from EPR-M chimney compared to the active EPR-L chimney indicates that these heterotrophic bacteria are actively replicating *in situ*, which is consistent with the previous observation of significant enzymatic activity in the inactive chimneys [28].

Based on 16S rRNA gene analysis, the *Nitrospirae* was one of the major taxa (17.3%) identified in the recently extinct EPR-M chimney (Fig.1). The genome tree showed that *Nitrospirae* has two distinct lineages with long phylogenetic distance (Fig.2), which is in line with the polyphyletic feature of *Nitrospirae* reported before [57]. That's implying that the *Nitrospirae* phylum probably needs to be reclassified in the further study. *Nitrospirae* was rarely reported as one of the dominant taxa in any active or extinct sulfide chimneys [14, 19, 70, 79, 87, 91]. The only exceptions besides the present study were two recently described inactive chimneys, one of them being a relatively young inactive chimney (0 ± 160 years) with the *Nitrospirae* making up 83% of the community, the other one much older inactive chimney (~2,093 years) with a *Nitrospirae* abundance of 38% [31]. However, the young inactive chimney described by Meier's study and the 7-year old inactive EPR-M chimney shared similar dominant *Nitrospirae* phylotypes within a unique "sulfide mineral" clade, which was distant from the *Nitrospirae*-1 phylotype dominating another older inactive chimney in Meier's study [31] (Additional file 1: Figure S6). This suggests that the *Nitrospirae* "sulfide mineral" clade probably only flourishes in the early stage of inactive sulfide chimneys, making them as a marker microorganism for young, recently extinct sulfide chimneys. Mineral sulfate, like anhydrite and/or barite, has been proposed as the potential electron acceptors for *Nitrospirae* inhabiting at the young inactive chimneys [31]. Here, the finding of prevalence of *cyc2* gene in the "sulfide mineral" clade further suggests that iron oxidizing capability might play a critical role for these unique *Nitrospirae* to survive and succeed in the recently inactive chimneys. While *cyc2* has been described to be involved in the oxidation of external electron donors, such as iron [67, 81, 82], which are very likely coming from the reduced metals making up the recently inactive sulfide chimneys, as well as other relatively reducing anoxic hydrothermal environments like buried subsurface massive sulfides (SMS). In supporting this hypothesis, a *Nitrospirae* MAG recovered from SMS in the Southern Mariana Trough was retrieved belonging to the "sulfide mineral" clade [65] (Additional file 1: Figure S6), suggesting that there are similar environmental features between the recently inactive chimneys and SMS, the conservation of metal sulfide minerals that are still not fully oxidatively weathered by permeating seawater probably is one of them. Genes encoding *cbb3*-type cytochrome *c* and nitrite reductase were identified in most *Nitrospirae* MAGs belonging to the "sulfide mineral" clade (Additional file 2: Table S8 and S9), implying that oxygen

and nitrite might be their potential electron acceptors when they conserve energy via chemolithotrophic iron oxidation, besides the sulfate reducing capability implied previously (Fig.5). Therefore, we propose that the presence of abundant *Nitrospirae*, especially those belonging to the “sulfide mineral” clade, in a deep-sea hydrothermal sulfide chimney likely indicates that the hydrothermal activity thereof is waning. Furthermore, sulfide mineral utilization might be the key factor for these *Nitrospirae* to thrive in the recently inactive sulfide chimneys, their decay is supposed to depend on the oxidative weathering degree of sulfide chimneys, that is largely proportional to the age of the inactive chimneys but also controlled by the particular mineralogical and geological properties. However, more sampling and further experiments are needed to test this hypothesis and to elucidate their specific ecological significance during the succession from active to inactive chimneys as well as the mechanism involved in sulfide mineral oxidation and using sulfate minerals as an electron acceptor.

Conclusions

Here, the metagenome of a sulfide chimney that became recently inactive (~7 years) is being compared with an actively venting chimney from the same hydrothermal vent field located on the East Pacific Rise at 9°50' N. Their microbial communities have distinct compositional structures and energy-yielding metabolic potentials, indicating that the shifting of the microbial community is highly controlled/influenced by the availability of energy sources provided by the hydrothermal activity. Microbial succession during the life span of a hydrothermal vent chimney could be described shifting from a “fluid-shaped” microbial community in newly formed and actively venting chimneys to a “mineral-shaped” community after hydrothermal activity has ceased. The transition appears to happen early on, after which the communities stay stable for thousands of years. This study provides new insights into microbial succession during the transition from active to inactive sulfide chimneys, driven by the available energy sources that shift from venting fluids to sulfide minerals as the hydrothermal activity diminishes.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Metagenomic assembled sequences are available in the Integrated Microbial Genomes and Microbiomes (IMG/M) database with IMG Object ID 3300005095/3300005096. All MAGs from the current study have been deposited in the NCBI GenBank under the project ID PRJNA557557.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JLH analyzed the metagenomic data and wrote the first draft. SMS, FPW, and XX conducted fieldwork and sampling. YZW contributed to the data analysis and conceived the study. VPN performed laboratory experiments. JSS measured and provided the chemical data. FPW and XX designed the experiment and conceived the study. All authors edited the manuscript and approved the final draft.

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Table

Please see the supplementary files section to access the table.

Additional File Information

Additional file 1: Figure 1 Taxonomic classification of key functional genes retrieved from the EPR-L and EPR-M chimney. (a) The key genes enriched in the active EPR-L chimney. (b) The key genes enriched in the recently inactive ERP-M chimney. **Figure S2** Maximum-likelihood phylogeny of *dsrA* genes retrieved from EPR-L and EPR-M chimney. The red branches represent the *dsrA* genes recovered from active the

EPR-L chimney, while the blue ones are those from recently inactive EPR-M chimney. Numbers of *dsrA* gene for each sample are displayed in the parenthesis after the clade name. **Figure S3** Maximum-likelihood phylogeny of *soxB* genes retrieved from EPR-L and EPR-M chimney. The red nodes represent the *soxB* genes recovered from the active EPR-L chimney, while the blue ones are those from the recently inactive EPR-M chimney. Four *soxB* genes from EPR-M chimney form a distinct clade (marked with red star) that different with other *soxB*. **Figure S4** Distribution and quantity of top 50 *RpS3* genes retrieved in the MAGs (metagenomic assembled genomes) for two chimney samples. (a) in the active EPR-L chimney. (b) in the recently inactive EPR-M chimney. The x-axis represents each *RpS3* gene identified in two samples, the y-axis is the sequencing depth for each *RpS3* gene. The red bars indicate the *RpS3* genes identified in the MAGs in corresponding samples, while the blue ones are those are not included in the MAGs. **Figure S5** Maximum-likelihood phylogeny of *coxL* genes retrieved from EPR-L and EPR-M chimney. The red branches represent the *coxL* genes recovered from active the EPR-L chimney, while the blue ones are those from recently inactive EPR-M chimney. Support for internal nodes was constructed from 1000 bootstrap replicates, black dots represent those nodes with bootstrap value > 75%, which is direct proportional to their diameter. **Figure S6** Phylogeny of 6 *Nitrospirae* MAGs recovered from EPR-M chimney. The phylogenetic tree is based on 37 concatenated ribosomal proteins and collapsed at the genus level. The red nodes represent those MAGs recovered from EPR-M chimney, the green ones are those from subseafloor massive sulfide deposits [65] and the organe ones are those retrieved from inactive chimneys [31]. The green-shded branches are proposed as “Sulfide mineral” clade of *Nitrospirae* in this study. **Figure S7** Phylogeny of 20 *Gammaproteobacteria* MAGs recovered from EPR-M and EPR-L chimney. The phylogenetic tree is based on 37 concatenated ribosomal proteins and collapsed at the genus level. The red nodes represent those MAGs recovered from EPR-L chimney, and blue for EPR-M chimney. The organe ones are those retrieved from inactive chimneys [31]. **Figure S8** Phylogeny of 8 *Crenarchaeota* MAGs recovered from EPR-L chimney. The phylogenetic tree is based on 37 concatenated ribosomal proteins and collapsed at the family level. The red nodes represent those MAGs recovered from EPR-L chimney. **Figure S9** Maximum-likelihood phylogeny of *cyc2* genes retrieved from EPR-L and EPR-M chimney. The red branches represent the *cyc2* genes recovered from the active EPR-L chimney, while the blue ones are those from recently inactive EPR-M chimney. the green ones are those from subseafloor massive sulfide deposits [65] and the organe ones are those retrieved from inactive chimneys [31]. The other reference sequences are come from Kato et al., 2015 [67]. Support for internal nodes was constructed from 1000 bootstrap replicates, black dots represent those nodes with bootstrap value > 70%, which is direct proportional to their diameter.

Additional file 2: Table S1 General metagenomic and assembly characteristics of EPR-M and EPR-L chimney sample. **Table S2** Completeness and contamination of 173 MAGs recovered from EPR-L and EPR-L chimney samples. **Table S3** Taxonomic classification and relative abundance of full-length 16S rRNA genes recovered from EPR-L sulfide chimney sample. **Table S4** Taxonomic classification and relative abundance of full-length 16S rRNA genes recovered from EPR-M sulfide chimney sample. **Table S5** Key genes of metabolic pathways for MAG annotation. The completeness of each metabolic pathway is classified into 3 level: 1- complete, 2 - uncomplete; 3- partial. The particular completeness classification

standard for each pathway are listed in the following: Sulfide oxidation: complete-*sqr* or *fccB*; Sox pathway (thiosulfate oxidation): complete - *sox A/B/X/Y/Z/C/D*, incomplete - *soxB*, partial: other Sox genes expect *soxB/C/D*; Dissimilatory sulfate reduction/oxidation: complete-*sat/dsrAB/aprAB*, incomplete - two of *sat/dsrAB/aprAB*, partial - one of *sat/dsrAB/aprAB*. Subsequent dissimilatory nitrate reduction: complete-*nirB* or *nrfA/H*, incomplete-one of *nrfA/H*. Subsequent denitrification: complete-*nirS/K* and *norB/C* and *nosZ*; incomplete - two of *nirS/K* and *norB/C* and *nosZ*; partial - one of *nirS/K* and *norB/C* and *nosZ*. Nitrogen fixation: complete-*nifK/D/H*, incomplete - two of *nifD/H/K*; partial - one of *nifD/K/H*. WL pathway: complete-*cdhC* or *acsB* and *cdhE* and *cdhD* and *cooS*, *cdhC* or *acsB* and one of *cdhE/D* and *cooS*, partial - two of *cdhC/B* and *cdhE/D* and *cooS*. CO oxidation: complete - *coxM/L/S*, incomplete - *coxL* and one of *coxS/M*; partial - one of *coxM/L/S*. Acetate utilization/production: complete - *ACSS* or *pta/ack*. rTCA: complete - *aclA/B*, incomplete - *aclA* or *aclB*. CBB pathway: complete - *rbcL/S*, incomplete - *rbcL* or *rbcS*. Methanogenesis/aerobic methane oxidation: complete - *mcrA/G/B*, incomplete: two of *mcrA/B/G*; partial - one of *mcrA/G/B*. Cytochrome c oxidase: complete - two *Cox/Cyd/Qox* genes or three *cco/Cyo* genes; incomplete- one of *Cox/Cyd/Qox* genes or two of *cco/Cyo* genes; partial- one of *cco/Cyo* genes. **Table S6:** Metabolic potential of MAGs for the major microbial groups (>1%) recovered from the EPR-L and EPR-M chimney. The specific method for estimating relative abundance/iRep are listed in the "Method and Materials" in the main text. The average iRep value of bacterial MAGs recovered from EPR-L and EPR-M sample are 1.42 and 1.51, respectively. The numbers in the metabolic pathway columns represent the percentage of the microbial taxa encoded the complete pathway (based on quantity of MAGs, e.g. 0.33 means 1 in 3 of the MAGs have the pathway). **Table S7** Sulfur metabolic pathways of Thermodesulfobacteria MAGs recovered. **Table S8** Metabolic potential of Nitrospirae MAGs retrieved from this study and phylogenetically closed species. The red ID represented those *Nitrospirae* MAGs reconstructed from EPR-M, the green ones represent those recovered from subseafloor massive sulfides [65] and the blue ones refer to those *Nitrospirae* retrieved from inactive sulfide chimneys [31]. Numbers in the form represent the completeness of specific pathways for each *Nitrospirae* genome/MAG, 1 indicates that complete key genes of the pathway were identified in the genomes/MAGs, other else the specific key genes were listed if the pathway not complete. For *sqr*, cytochrome C oxidase, multi-heme *cyc* and *cyc2*, numbers represent the quantity of specific genes identified in the genomes/MAGs. **Table S9** Distribution and quantity of cytochrome c oxidase genes in 173 MAGs retrieved from EPR-M and EPR-L. Specific definition rules and types of cytochrome c oxidase genes could find in Table S5. **Table S10** Summary of the activity, study method and microbial composition of deep-sea hydrothermal sulfide chimneys or *in situ* incubation experiments cited in this study.

Figures

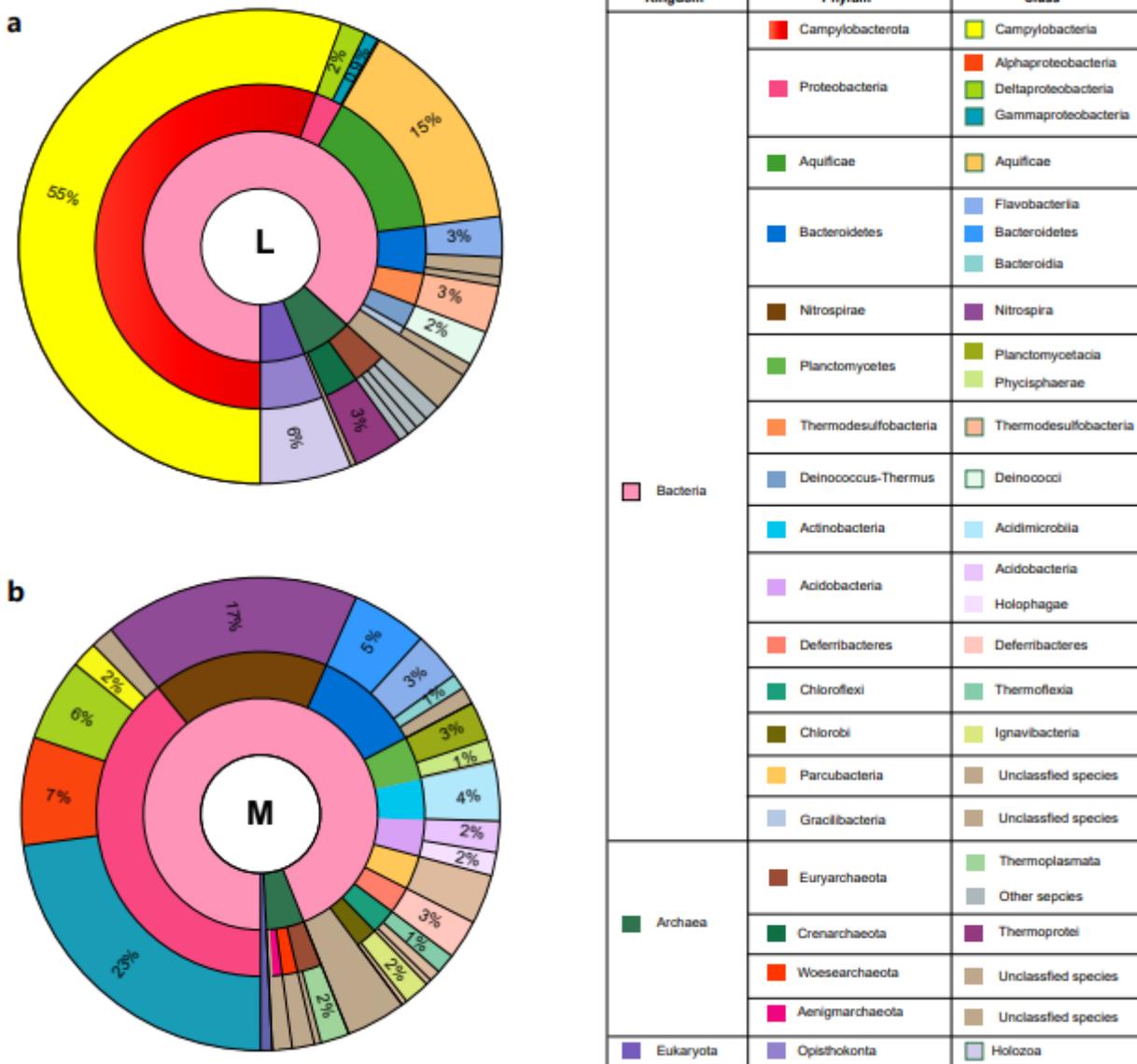


Figure 1

Taxonomic composition of microbial communities from the active EPR-L chimney (a) and recently inactive EPR-M chimney (b). Numbers in the pie charts represent percentages of each taxonomic unit in class level, which are estimated based on the full-length 16S rRNA genes retrieved from the two metagenomes. Detailed information is displayed in the Additional file 2: Table S1 and S2.

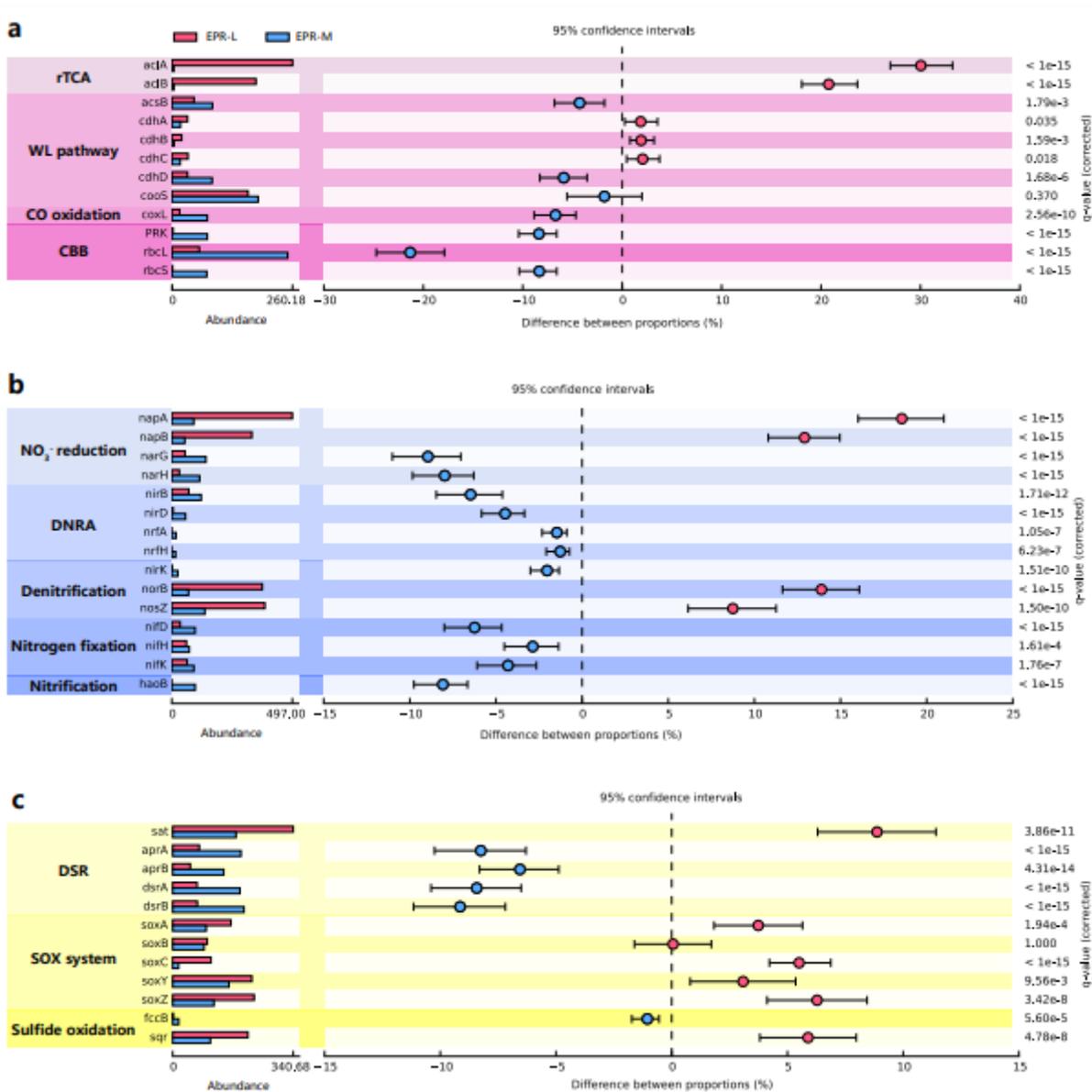


Figure 2

Abundance comparison of key genes involved in carbon, nitrogen and sulfur metabolisms between active EPR-L chimney and recently inactive EPR-M chimney. (a) Key genes of rTCA, WL pathway, CBB and CO oxidation. (b) Key genes of nitrate reduction, DNRA, denitrification, nitrogen fixation and nitrification. (c) Key genes of DSR, Sox system and sulfide oxidation. Abbreviation: rTCA (reverse citric acid) cycle; CBB (Calvin-Benson-Bassham) pathway; WL (Wood-Ljungdahl) Pathway; DNRA (Dissimilatory nitrate reduction to ammonia) pathway; DSR (Dissimilatory sulfate reduction) pathway. The napA/B and narG/H genes encoding nitrate reductase are tested individually and not included in the DNRA and Denitrification pathway. The p values are based on Fisher's exact test and corrected by Benjamini-Hochberg FDR.

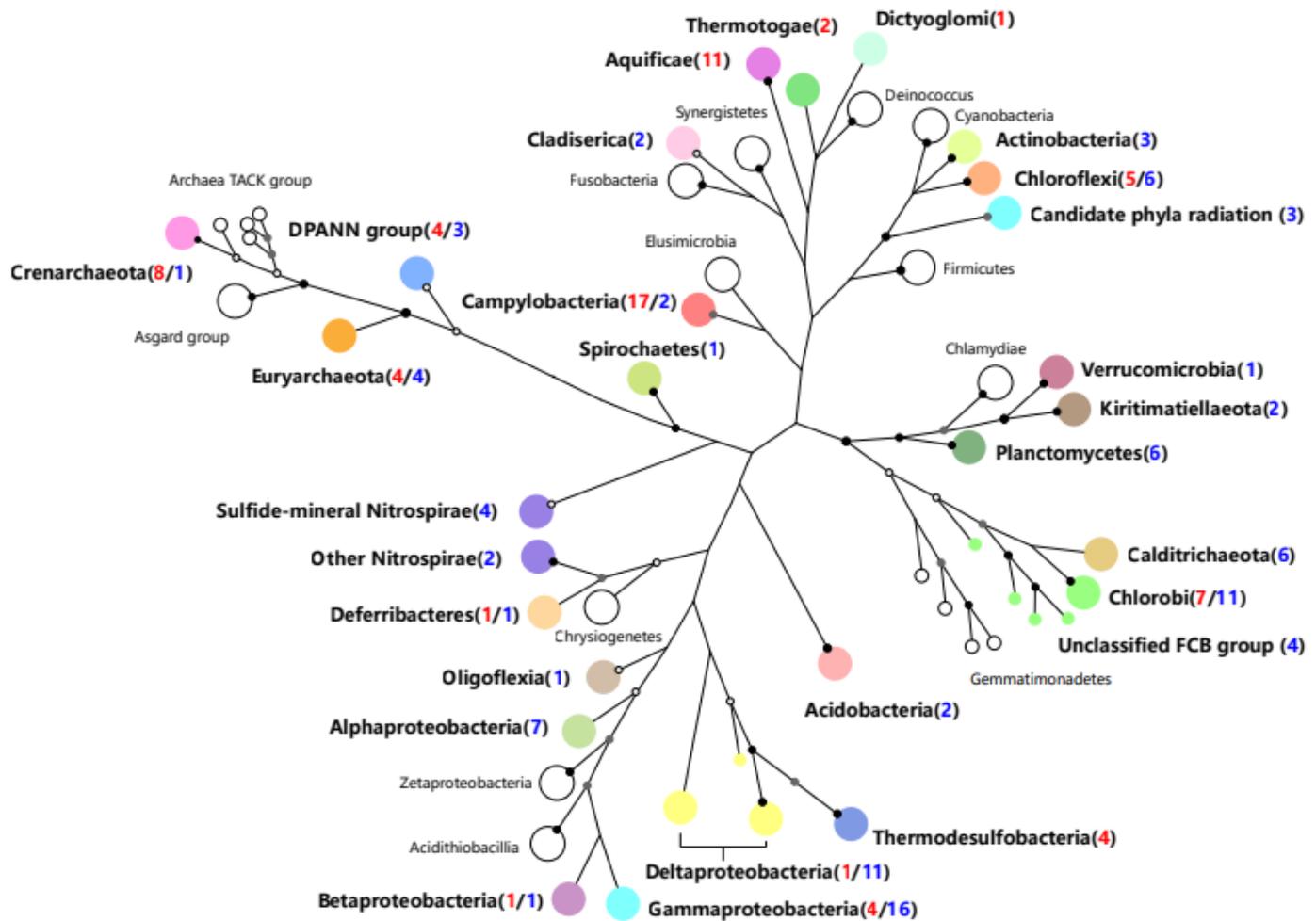


Figure 3

Phylogeny of 173 high-quality MAGs (metagenomic assembled genomes) recovered from active EPR-L chimney and recently inactive EPR-M chimney. The phylogenetic tree is based on 37 concatenated ribosomal proteins and collapsed at the phylum level (class for Proteobacteria and Campylobacteria). Color nodes represent the MAGs assigned within this clade were retrieved from at least one chimney sample. The red and blue numbers in the parenthesis denote the number of MAGs recovered from EPR-L and -M, respectively. Support for internal nodes was constructed from 1000 bootstrap replicates (white $\geq 50\%$, gray $\geq 75\%$, black $\geq 95\%$ confidence, no shading $\leq 50\%$).

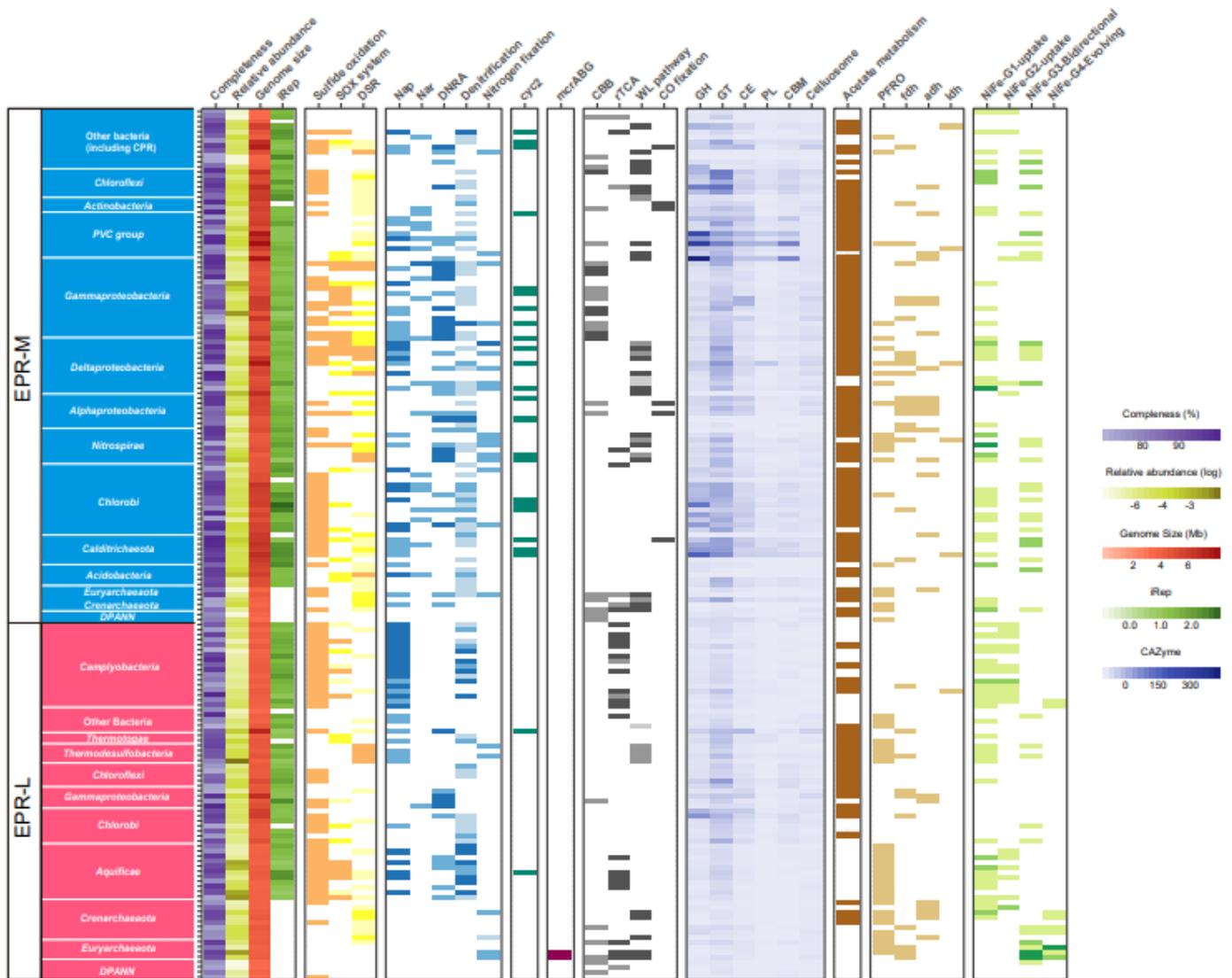


Figure 4

Genomic features and metabolic potential of 173 MAGs (metagenomic assembled genomes) retrieved from the active EPR-L and recently inactive EPR-M chimney. Different color gradients in completeness, relative abundance (logged), genomic size, iRep value and CAzyme represent their quantities among MAGs. Differentially shaded tiles represent the completeness of displayed metabolic pathways, including none, partial, uncomplete and complete four levels. There are only two levels (encode or not) for cyc2 and genes involved in fermentation. The specific key genes and completeness definition involved in each metabolic pathway could find in Additional file 2: Table S4.

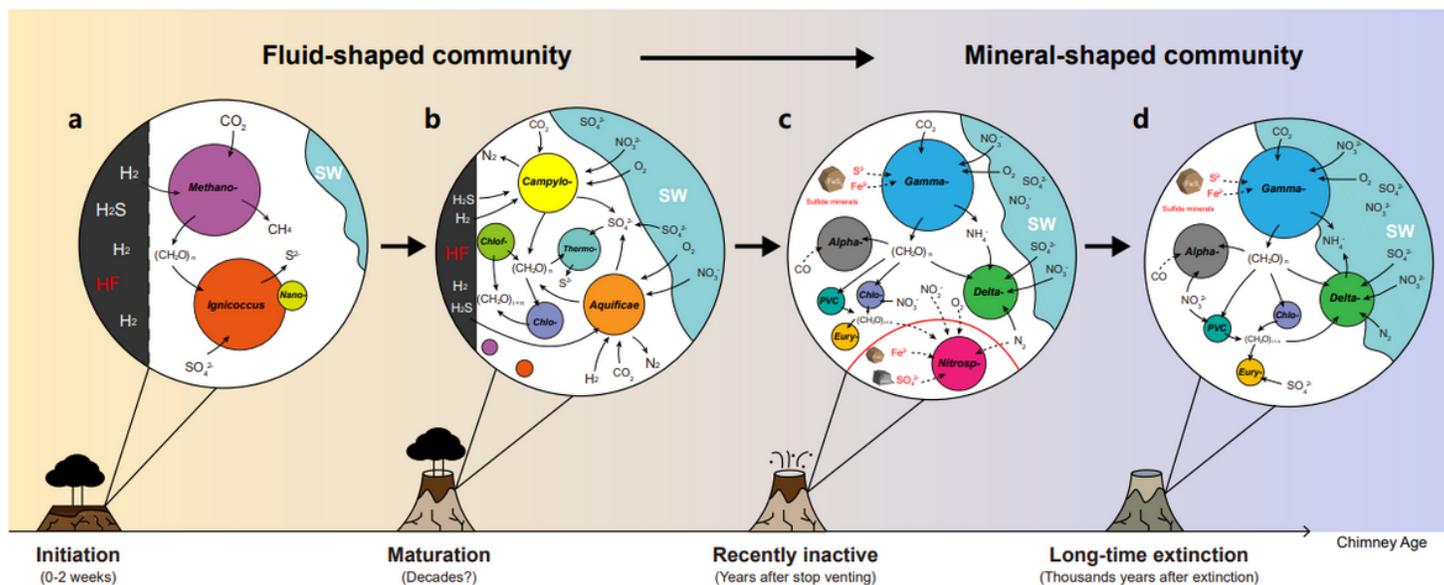


Figure 5

Genome-resolved conceptual models of microbial succession within the sulfide chimney from initiation, maturation, recently inactive and long-time extinction. The models of maturation and recently inactive stage are based on EPR-L and EPR-M in this study, data of initiation and long-time extinction stage are summarized from previous studies. Abbreviation: HF, hydrothermal fluids; SW, seawater; Methano-, Methanocaldococcus; Nano-, Nanoarchaeum; Campylo-, Campylobacteria; Chlof-, Chloroflexi; Thermo-, Thermodesulfobacteria; Chlo-, Chlorobi; Alpha-, Alphaproteobacteria; Gamma-, Gammaproteobacteria; Delta-, Deltaproteobacteria, Nitrosp-, Nitrospirae; PVC, PVC group; Eury-, Euryarchaeota; FeS₂, pyrite-like sulfide minerals; CaSO₄, anhydrite-like sulfate minerals.

Supplementary Files

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