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## Key Points:

- Magmatic event and microbial response
- Temporal evolution of hydrothermal fluxes and microbial communities
- Results from deep sea observatory EMSO Azores

## Supporting Information:

- Supporting Information S1
- Figure S1
- Table S1

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## Prokaryote Communities at Active Chimney and *In Situ* Colonization Devices After a Magmatic Degassing Event (37°N MAR, EMSO-Azores Deep-Sea Observatory)

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**Abstract** The mixing zone between high-temperature hydrothermal fluids and seawater produces redox gradients, promoting the development of unique ecosystems based on chemotrophy. The structure of microbial communities depends on their environment, which can vary according to space and time. Hydrothermal circulation within the oceanic crust determines the chemical composition and flow of fluids, depending on underground events (earthquakes, volcanic episodes, etc.) and impacts the development of microbial communities. This link between hydrothermal vent communities and deep geological events is the focus of the present study, the first of its kind for slow-spreading ridge. In this study, we present a unique set of multidisciplinary data collected from 2008 to 2011 on the Eiffel Tower hydrothermal site (Lucky Strike vent field, Mid-Atlantic Ridge, MAR). We benefit from continuous geophysical monitoring (temperature and seismicity) of the site, annual sampling of hydrothermal fluids (hot and diffuse) for geochemistry analyses, sampling of hydrothermal chimneys, and an in situ microbial colonization experiment over a year for microbial study. The high CO<sub>2</sub> content and concentrations of major elements (Cl, Ca, and Si) and SO<sub>4</sub> in the end-member fluids collected in 2010 indicate that a magmatic degassing occurred between 2009 and 2010 under the Lucky Strike hydrothermal field. This is supported by the large temperature variations observed in March–April 2010. These magmatic CO<sub>2</sub> inputs seem to have affected microbial communities colonizing the high-temperature chimney, as well as the basalts in the more diffuse and mixed zone, promoting the development of thermophilic/anaerobic *Archaea* and *Bacteria* (Archaeoglobales, Nautiliales, and Nitratiruptoraceae).

### 1. Introduction

Hydrothermal circulation in the global mid-ocean ridge network allows fundamental processes of material and heat transfer from the inside of the Earth to the crust (e.g., Morgan & Chen, 1993). This hydrothermal circulation originates from the percolation of seawater into the oceanic crust. Seawater chemical composition is modified during its interaction with the host rock (Sleep, 1983), undergoing an enrichment in various elements (e.g., heavy, alkali metals and reduced gases) and depletion in others (e.g., sulfate (SO<sub>4</sub>) or magnesium (Mg)) (Edmond, 1981). Therefore, the dynamic of the plumbing system (e.g., pressure and temperature) and the nature of rocks the hydrothermal fluids went through control the physicochemical properties of the hydrothermal fluid discharging in the deep seawater mass. Hydrothermal fields hosted by basaltic rocks generally give rise to fluids enriched in hydrogen sulfide (H<sub>2</sub>S) (Charlou et al., 2000; Chavagnac, Leleu, et al., 2018; Von Damm et al., 1998).

Fluxes and composition of hydrothermal fluids also vary in time and space according to subsurface processes such as (1) seafloor mineral precipitation or remobilization—biotic or abiotic—due to seawater-fluid mixing or conductive cooling (Hannington et al., 1995; Rouxel et al., 2004), (2) occurrence of earthquakes,

volcanic eruptions, dike intrusions (*i.e.*, Dziak et al., 2004; Fornari & Embley, 1995; Smith et al., 2003; Sohn et al., 1998), or (3) tidal influence (Corliss et al., 1979; Fujioka et al., 1997) and surface-generated perturbations (Adams et al., 2011).

When the superheated reduced hydrothermal fluids mix with cold oxygenated seawater beneath and above the seafloor, it creates a chemical gradient between the composition of hydrothermal fluids, enriched in reduced inorganic chemicals and gases ( $S^{2-}$ ,  $Fe^{2+}$ ,  $Mn^{2+}$ ,  $CH_4$ ,  $H_2$ ,  $CO_2$ ) and that of the oxygenated seawater, allowing the development of particular ecosystems based on chemolithoautotrophic microorganisms. In this context, the reduced elements and carbon dioxide are used as energy sources and carbon source, respectively. This gradient favors the development of a large diversity of microorganisms within the *Archaea* and *Bacteria* (Reysenbach & Shock, 2002).

In such an environment a large deep-sea vent bacterial diversity is described and the main archaeal lineages present include thermophilic Desulfurococcales, Thermococcales, Thermoproteales, Methanococcales, Archeoglobales, and Deep Hydrothermal Vent Euryarchaeota (DHVE) groups (Flores et al., 2012; Reysenbach et al., 2000; Takai et al., 2006; Takai & Horikoshi, 1999). The mesophilic to moderately thermophilic Campylobacterota (previously classified Epsilonproteobacteria, Waite et al., 2017, 2018) dominate the bacterial diversity in most niches and play a significant role in sulfur, hydrogen, nitrogen, and carbon cycles. Members of the thermophilic order Aquificales also are classically abundant in high temperature hydrothermal habitats (Flores et al., 2012; Reysenbach et al., 2000, 2002; Takai et al., 2006).

Although different niches around deep-sea vents (*i.e.*, hydrothermal sediments, active high-temperature (HT) chimney, HT fluids, diffuse fluids, seawater, basalts) share common bacterial and archaeal lineages, some are habitat specific (Edwards et al., 2011; Orcutt, Sylvan, et al., 2011). For instance, microaerophilic hyperthermophiles like *Aeropyrum* preferentially grow in basaltic hosted hydrothermal fields, while methanogens and hydrogen-oxidizing thermophiles preferentially grow in ultramafic-hosted hydrothermal fields along Mid-Atlantic Ridge (MAR) (Flores et al., 2011). Therefore, the structure of the microbial communities thriving on hydrothermal chemical fluxes deeply reflects the physicochemical conditions of the environment. Tectonic or volcanic events may disturb the physicochemical composition of fluids and thus the microbial diversity. Indeed, at the main Endeavor field (Pacific Ocean), it has been reported that the  $CO_2$ ,  $H_2$ ,  $CH_4$ , and  $H_2S$  concentrations of hydrothermal fluids significantly increased just after a volcanic event without lava flows, due to their interaction with the newly exposed or injected shallow dike (*e.g.*, Lilley et al., 2003; Seewald et al., 2003). This event induced an increase of the abundance of seafloor indigenous microorganisms such as Campylobacterota diversity (Huber et al., 2003).

Deciphering the causes and consequences of links between microbial biosphere development and hydrothermal fluid flux relies on acquiring long-term multidisciplinary data sets (Kelley & Shank, 2010). In this context, long-term *in situ* observatories have been deployed mainly on and beneath the seafloor of the Pacific Ocean (Davis et al., 2004; Orcutt et al., 2010; Orcutt, Bach, et al., 2011), where the magmatic/volcanic activity is a dominant process at the ridge axis. However, to our knowledge, limited data have been acquired so far at ultraslow to slow spreading ridge systems like the MAR, where tectonic activity is prevalent over volcanism, due to the sporadic replenishment of the magmatic chamber.

The Lucky Strike hydrothermal field (LSHF-MAR, N37°17'/W32°17') offers an excellent opportunity to examine the links between microbial biosphere development and hydrothermal fluid flux in that context. Since its discovery in 1992, LSHF has been well characterized with respect to fluid chemistry (Charlou et al., 2000; Chavagnac, Leleu, et al., 2018; Chavagnac, Saleban, et al., 2018; Langmuir et al., 1997; Pester et al., 2012; Von Damm et al., 1998), geological settings (Barreyre et al., 2012; Escartín et al., 2008; Escartín et al., 2015; Fouquet et al., 1994; Humphris et al., 2002; Ondréas et al., 2009; Singh et al., 2006), macrobiological, and microbiological communities (Crépeau et al., 2011; Cuvelier et al., 2009; Cuvelier, Sarrazin, et al., 2011; De Busslerolles et al., 2009; Desbruyères et al., 2001; Flores et al., 2011; Lee Van Dover et al., 1996; López García et al., 2003). Moreover, since 2010, the deep-sea observatory—EMSO-Azores (Colaço et al., 2011; Sarradin, Blandin, Escartín, Cannat, et al., 2010)—has been deployed allowing a continuous geophysical and biological survey, together with annual sampling of hydrothermal fluids, fauna and microbial communities. EMSO-Azores is a noncabled multidisciplinary observatory built around two sea monitoring nodes (SEAMON), and a BOREL buoy for satellite data transmission. One node deployed in the lava lake is dedicated to geophysical survey with a pressure gauge and a short-period

ocean bottom seismometer (OBS). The second node deployed at the base of Eiffel Tower active site is dedicated to ecological survey with an *in situ* iron analyzer (CHEMINI Fe), an optode oxygen sensor, a turbidity sensor, and a HD video camera (SMOOVE; Colaço et al., 2011; Sarradin, Blandin, Escartin, Cannat, et al., 2010). This setup permits a better characterization of the LSHF, both at the regional (across the segment) and local (hydrothermal vent field) scale.

For this study, we benefited from *in situ* microbial colonization experiments conducted at the base of the Aisics hydrothermal chimney (Eiffel Tower hydrothermal vent of LSHF), annual chimney walls sampling, and yearly hydrothermal fluid collection, from 2008 to 2011.

Thanks to this complete long-term data set, we were able to detect the occurrence of a magmatic degassing event in 2010, leading to large CO<sub>2</sub> emissions in the hydrothermal fluids, and strong temperature variations of the hydrothermal fluid discharges. This magmatic CO<sub>2</sub> input, in addition to the one reported in 2008 (Pester et al., 2012), seem to have affected microbial communities colonizing the high-temperature chimney, as well as the basalts in the more diffuse and mixed zone, promoting the development of thermophilic/anaerobic *Archaea* and *Bacteria* (Archaeoglobales, Nautiliales, Nitratiruptoraceae).

## 2. Geological Setting and Samples

### 2.1. The LSHF

LSHF is located on an active, 65-km-long, segment of the MAR, south of the Azores (Figure 1a). The hydrothermal activity is distributed around a depression filled with lava and hyaloclastites, surrounded by three topographic highs truncated by normal faults (Figure 1b) (Barreyre et al., 2012; Fouquet et al., 1995; Ondréas et al., 2009).

The axial magmatic chamber is imaged by seismic reflection beneath LSHF (Singh et al., 2006) at about 3 km below seafloor and measures about 3- to 4-km wide for ~7-km long, along the ridge axis. Normal faults parallel to the axial valley and those affecting the axial volcano are rooted near the roof of the axial magmatic chamber (Crawford et al., 2010; Singh et al., 2006).

The close interplay between magmatic and tectonic processes results in an intense hydrothermal circulation, evidenced at the seafloor by either (i) black smoker chimney-types associated with hydrothermal fluids whose temperature reaches up to 340 °C, or (ii) cracks and vents expelling fluids of lower temperatures (5 to 200 °C) (Barreyre et al., 2012; Chavagnac et al., 2011; Cooper et al., 2000; Langmuir et al., 1997; Rouxel et al., 2004; Von Damm et al., 1998).

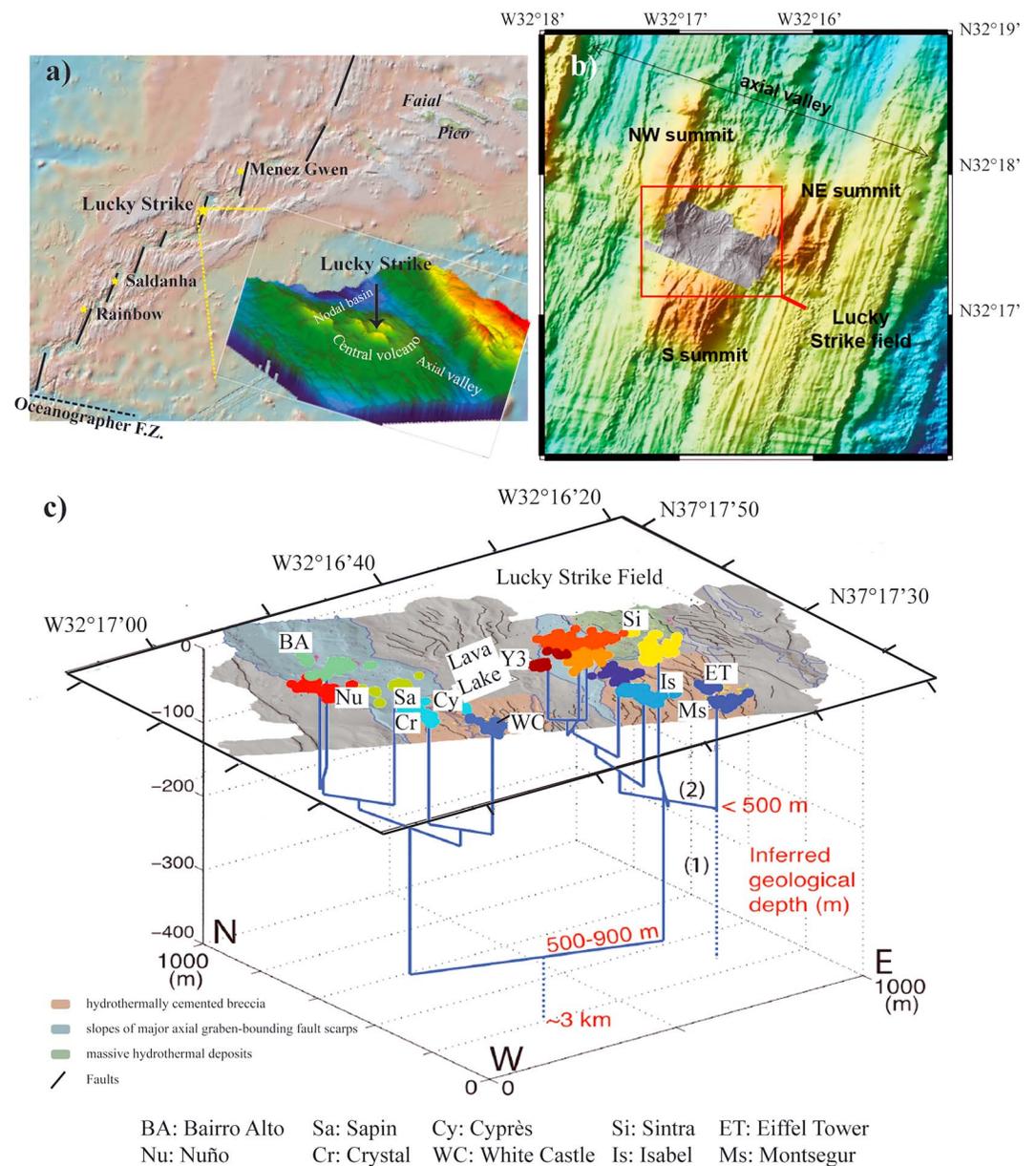
The chemical composition of HT hydrothermal fluids and gases shows variable chlorinities (higher and lower than seawater values), varying hydrogen sulfide concentrations (1.4–4.6 mM), low metal concentrations (particularly iron 0.06–0.77 mM) (Von Damm et al., 1998), and high gas concentrations (15.1–30 mM for CO<sub>2</sub>) (Charlou et al., 2000; Pester et al., 2012).

The end-member fluid chemistry is controlled by phase separation at depth as well as fluid-rock interactions taking place prior and along the upflow zone (Chavagnac et al., 2011; Chavagnac, Leleu, et al., 2018; Von Damm et al., 1998).

The composition of low-temperature (LT) fluids is interpreted as either a mixing of high temperature hydrothermal fluids with LT seawater (Leleu, 2017; Wankel et al., 2011) or a conductively heated seawater (Cooper et al., 2000).

On the seafloor, western sites and eastern ones distributed around the fossil lava lake exhibit different chemical signature, suggesting a geographical control of hydrothermal emissions to the deep ocean (Barreyre et al., 2012; Ondréas et al., 2009). This is interpreted to reflect either the occurrence of two distinct deep fluid sources or a single one which has encountered different substrates along the way up to the surface (Figure 1c; Barreyre et al., 2012; Charlou et al., 2000; Chavagnac et al., 2011; Chavagnac, Leleu, et al., 2018).

Since the discovery and monitoring of LSHF in the late 1990s, few tectonovolcanic events have been reported so far. Only one dike injection was detected in 2001 through earthquake swarm (Dziak et al., 2004), and one potential magmatic degassing in 2008 evidenced by very high CO<sub>2</sub> concentrations up to 130 mM (Pester et al., 2012).



**Figure 1.** Lucky Strike hydrothermal field location. (a) General location of the Lucky Strike segment along the MAR (Global Multi-Resolution Topography, Ryan et al., 2009) with detailed focus on the axial volcano at the center of the segment where the hydrothermal activity becomes localized (modified from Fouquet, 1997). (b) Bathymetric map of Lucky Strike segment with location of hydrothermal field around the lava lake. (c) Schematic representation of active focused and diffuse hydrothermal outflows with plumbing in depth showing the possible common origin in great depth and clustering of outflows based on chemical differences in fluids (modified from Barreyre et al., 2012).

We focus our study on the Eiffel Tower site, composed of a main towering edifice and a foothill chimney called Aisics. The Eiffel Tower site is surrounded at its base by a network of cracks, venting diffuse hydrothermal fluids up to 100 °C.

## 2.2. Samples Collection

Geomicrobiology colonization experiments together with Aisics chimney and HT hydrothermal fluids sampling at the Eiffel Tower and Aisics vents were carried out during four cruises aboard the R/V *L'Atalante* and R/V *Pourquoi Pas?* with the ROV (Remotely Operated Vehicle) *Victor 6000*, (MoMAR2008-Leg1 (Escartin, 2008), Bathyluck2009 (Escartin & Cannat, 2009), MoMARSAT2010 (Sarradin, Blandin, &

Escartín, 2010), and MoMARSAT2011 (Cannat et al., 2011)). The Aisics chimney was sampled in 2008 (Chem2008) and 2009 (Chem2009). Two *in situ* microbial colonization devices (LSTE1 and LSTE2), both containing synthetic basaltic glasses, were set up nearby the Aisics chimney for 1 year deployment in 2009 and 2010, respectively. A total of 33 HT hydrothermal fluids were collected between 2009 and 2011 at Eiffel Tower and at Aisics vents.

### 2.3. Hydrothermal Fluids Sampling

HT hydrothermal fluids were sampled at the Eiffel Tower main edifice and Aisics—the closest to the geomicrobiology modules—using 200- or 750-mL titanium syringes. They were operated and triggered one by one by the ROV hydraulic arm once the snorkel was inserted into the chimney. The 200-mL titanium syringes are gas-tight, avoiding outgassing and gas leakage during ascent to the surface. Prior to fluid sampling, the temperature of hydrothermal fluid was measured *in situ* using the high-temperature probe of the ROV *Victor* 6000. A total of 30 fluids with a temperature ranging from 295 to 325 °C, were collected among which 19 at Aisics and 11 at Eiffel Tower (detailed in Table S1 in the supporting information). In addition, three diffuse fluids, with a temperature of 56 °C, were also collected within the diffuse network cracks in 2009.

### 2.4. Hydrothermal Fluids Temperature

Temperature of HT fluids (>190 °C and up to 350 °C) from the Eiffel Tower main edifice and cracks together with shimmering fluids (<100 °C) from Eiffel Tower and Montsegur hydrothermal sites (Figure 1) were monitored during Bathyluck2009 cruise (Escartín & Cannat, 2009). For this study we used MISO HT probes deployed on vent outflow at the Eiffel Tower chimney, NKE S2T6000 probes deployed on vent outflow at the Aisics chimney, and MISO LT probes deployed at Eiffel Tower and Montsegur sites in LT discharge cracks as described in Barreyre et al. (2014b). The instrument description, data acquisition, data processing, and location of each temperature probes are reported in Barreyre et al. (2014a, 2014b). The raw temperature data are public and available at this site (doi:10.1594/PANGAEA.820343).

### 2.5. Microbial Colonization Experiment

The geomicrobiology modules consist of a ballasted plastic holder hosting up to 12 incubators, usually distributed around an autonomous temperature probe (Figure S1a). Each incubator consists in 50 mL polypropylene BD Falcon™ Conical Tubes with cap, both perforated with  $\pm 20$  holes of 1 mm in diameter. A few milligrams of synthetic basaltic glass substrates were inserted in each of them to act as microbial development substrate. The geomicrobiology colonization modules were prepared and deployed as described in Henri et al. (2016) and will not be described into details.

Synthetic basaltic glass was produced at Laboratoire Géomatériaux et Environnement (LGE, Univ. Marne La Vallée, France), using the same synthesis protocol as previously described in Henri et al. (2016). It has a typical tholeiitic basalt composition, with major element concentrations in weight % of 48.68 SiO<sub>2</sub>, 15.7 Al<sub>2</sub>O<sub>3</sub>, 11.2 CaO, 7.7 MgO, 12.5 FeO, 2.7 Na<sub>2</sub>O, 0.2 K<sub>2</sub>O, and 1.39 TiO<sub>2</sub>.

LSTE1—without temperature probe—was deployed in September 2009, in a diffuse crack covered by a microbial mat and colonized by mussels, at the base of the Aisics chimney (Figure S1b). The module was recovered after 13 months (October 2010), but was partially burned most likely by a hot fluid flux. LSTE2—equipped with an autonomous temperature sensor (NKE S2T6000, with a 3-min record frequency)—was deployed in October 2010 within a diffuse crack presenting similar microbial mat and mussels' patches as LSTE1, only 8 m further away from the Aisics chimney (Figure S1b). LSTE2 was recovered in July 2011, after 9 months deployment on the seafloor.

## 3. Methods

### 3.1. Hydrothermal Fluids Analysis

#### 3.1.1. Fluid Processing

All hydrothermal fluids were processed on board right after the ROV recovery on board the research vessel. The titanium gas-tight fluid samplers were first processed for gas extraction and storage. Gases were collected in 250-mL glass bottles prefilled with deionized water poisoned with 100- $\mu$ L HgCl<sub>2</sub>, allowing the measurement of total extracted gas volume (from 0 to 1.44 L/kg of water, see Table S1). After gas

extraction, we proceeded to fluid extraction with a small aliquot taken for on-board chemical measurements (pH at 25 °C, salinity (Table S1), density and conductivity (not reported)). The remaining fluid was filtered through a 0.45- $\mu\text{m}$  Millipore<sup>®</sup> membrane and dispatched into different plastic LDPE Nalgene<sup>®</sup> vials for specific shore-based chemical analyses.

### 3.1.2. Gases Analysis

All gases were analyzed on board or onshore at the Géosciences Environnement Toulouse Laboratory (GET, University of Toulouse, France) with a SRI 8610C gas chromatograph, fitted with a Flame Ionization Detector/Methanizer (FID-M) for the detection of methane ( $\text{CH}_4$ ), small alkanes, carbon monoxide (CO), and carbon dioxide ( $\text{CO}_2$ ), and a Helium Ionization Detector (HID) for the detection of Hydrogen ( $\text{H}_2$ ), Oxygen ( $\text{O}_2$ ), and Nitrogen ( $\text{N}_2$ ).  $\text{CH}_4$  gas is eluted together with CO and  $\text{CO}_2$  on a 3' Molecular Sieve Packed Column, using  $\text{H}_2$  as a carrier gas, while  $\text{H}_2$ ,  $\text{O}_2$ , and  $\text{N}_2$  are eluted on a 6' Silica Gel Packed Column with He as carrier gas.

### 3.1.3. Fluids Analysis

All chemical analyses of hydrothermal fluids were carried out at the GET laboratory (Toulouse, France). All the hydrothermal fluid samples were clear, void of visible particles, and not acidified during storage. DIC was measured using a Shimadzu TOC-VCSN instrument. The major and trace elements concentrations (Si, Ca, Na, K, Mg, Sr, Fe, and Mn) were measured by inductively coupled plasma optical emission spectrometry (Horiba Jobin Yvon Ultima 2), which was calibrated using seawater IAPSO standard (OISIL Limited, UK) a synthetic matrix-match standards following the measurement protocol of Besson et al. (2014). The analyses achieved a minimum precision of 2%. The full set of standards was run before and after each group of analyses to check the performance of the instrument. In addition, running one standard as a sample before, during and after each group of analyses allowed the assessment of instrumental drift during the course of the analyses. Anion concentrations (Cl and  $\text{SO}_4$ ) were measured by ion chromatography (Dionex ICS 2000), which was calibrated using seawater IAPSO standard. The analyses achieved a precision of 2% or better. All concentrations are therefore drifted and blank corrected. Standard seawater values are from the most recent literature of Reference Standard Seawater (Millero et al., 2008), as no deep background seawater samples outside the LSHF were collected.

The composition of pure hydrothermal end-member fluids was calculated from the chemical composition of hydrothermal fluids sampled (Table S2). A pure hydrothermal end-member contains no Mg, as it is totally removed from the seawater during HT water-rock interactions at reaction zone conditions (Bischoff & Dickson, 1975; Mottl & Holland, 1977; Seyfried & Bischoff, 1979). Hydrothermal fluids sampled contain a small amount of Mg corresponding to the mixing rate between pure hydrothermal fluid and seawater. A linear least squares regression fitting of the concentration of the different elements regarding the concentration of Mg through samples, allows to define the mixing line between the seawater and the pure hydrothermal fluid end-member, and extrapolate the concentration of the elements in the end-members. For redox-sensitive elements, such as Fe, only samples containing more than 70% of hydrothermal fluids (Mg < 16 mM) are considered. The 2009 diffuse fluids collected in cracks at the base of the Eiffel Tower edifice (three samples) were included with those of the HT fluids for end-member determination.

## 3.2. Sequence-Based Community Analysis

### 3.2.1. DNA Extraction

After recovery of each geomicrobiology colonization devices, each glass substrate was processed onboard under a laminar-flow hood. One fraction was stored immediately and aseptically at  $-20$  °C in 96% ethanol for molecular diversity analysis on shore at the Geomicrobiology laboratory (IPGP, Paris). The chimney samples were aseptically crushed onboard in an anaerobic chamber under  $\text{N}_2/\text{H}_2$  (90:10) atmosphere and split into several fractions stored at  $-80$  °C for molecular analysis on shore at the Laboratoire de Microbiologie des Environnements Extrêmes (LM2E, Ifremer, Brest). The total genomic DNA from synthetic basaltic glasses (LSTE1 and LSTE2) and from the Aisics chimney sampled in 2009 was extracted using the UltraClean<sup>®</sup> Soil DNA Isolation Kit (MO BIO Laboratories, Inc.) following the manufacturer protocol. The DNA of each sample was extracted in duplicate (*i.e.*, from two different subsamples), and the two extractions were pooled for later use. The total genomic DNA from the Aisics chimney sampled in 2008 was extracted using both the FastDNA<sup>®</sup> kit for soil (Obiogene, Inc, CA) with 1 g of sample following the modified protocol (Webster et al., 2003), and phenol:chloroform:isoamyl alcohol (PCI) protocol with 8 g

of sample as described in Alain et al. (2011). The DNA was extracted in duplicate (*i.e.*, from two different sub-samples) by both methods and was then mixed together.

### 3.2.2. *Archaea and Bacteria* 16S rRNA Genes Sanger Sequencing

The Chem2008 sample was processed separately from the other samples as part of an LM2E internship (Le Roy, 2009). 16S rRNA genes from this sample were amplified using the primers A8F-A1492R (Table S4) for *Archaea* and E8F-U1492R (Table S4) for *Bacteria*.

Due to the low amount of archaeal genomic DNA in the Chem2009, LSTE1, and LSTE2 samples, and the difficulty of amplifying the 16S rRNA genes, we have chosen to carry out a Sanger sequencing after amplification and cloning. The Chem2009 sample was processed at LM2E for *Archaea*, with the same protocol as Chem2008 sample. However, the very small amount of archaeal genomic DNA recovered has required nested amplification with the primers A8F-A1492R for the initial reaction, followed by A8F-915R (Table S4) primers for the nested one. The LSTE1 and LSTE2 were processed at IPGP with nested amplification with primers A21F-U1492R (Table S4) for the initial reaction, followed by primers Ar109F-A915R (Table S4) for the nested one.

The reactions and protocol of amplification are described in detail in the supporting information (Text S1).

All 16S rRNA genes clone libraries were carried out with the TOPO<sup>®</sup> XL PCR Cloning Kit, with One Shot<sup>®</sup> TOP10 Chemically Competent *E. coli* (Invitrogen<sup>™</sup>) following the manufacturer recommendations. Positive clones were processed for Sanger sequencing at GATC Biotech (Konstanz, Germany) using T7F and M13R primers for LSTE1 and LSTE2 samples, and at Ouest-Génopole platform (Roscoff, France) using M13F and M13R primers for Chem2008 and Chem2009 samples. Sequences were cleaned from vector and primers sequences (Tamura et al., 2011).

### 3.2.3. *Bacteria* 16S rRNA 454-Pyrosequencing

For LSTE1, LSTE2, and Chem2009 samples, 16S rRNA coding genes for *Bacteria* were amplified for pyrosequencing, using a direct PCR technique with primers 27F-533R targeting the V2-V3 region (Table S4). To minimize the bias of pyrosequencing, two different 10-mer multiple identifiers (MIDs) were used for each sample (Table S5). For each set of MIDs, 10 PCR were performed to decrease the bias of amplification. The reactions and protocol of amplifications are described in detail in Text S1. The 10 PCR products of each sample were pooled before purification using the QIAquick<sup>®</sup> PCR Purification Kit (QIAGEN). DNA concentration was controlled using NanoDrop (IMPMC, Paris) to ensure the equimolarity of each sample in the final mix with 4  $\mu$ g of total DNA. Pyrosequencing was performed on the Roche<sup>\*</sup> 454 GS FLX<sup>\*</sup> instrument, and demultiplexing and contigs assemblage was carried out by Beckman Coulter genomics (Denver, Massachusetts, USA).

### 3.2.4. Sequence Processing

All sequences analyses were performed using MOTHUR (v.1.22.2) (Schloss et al., 2009). For sequences obtained by pyrosequencing, only bacterial sequences whose size was comprised between 400 and 500 bp without ambiguity and a maximum homopolymer length of 8 bp were conserved as recommended (Schloss et al., 2009). A 50-bp sliding window with an average quality of 35 was used as filter parameters. Putative chimeras were eliminated using UCHIME as implemented in MOTHUR (Edgar et al., 2011).

The archaeal sequences data obtained by Sanger sequencing reported in this study have been submitted to the National Center of Biotechnology Information (NCBI) in GenBank nucleotide sequence database under accession number KX133571-KX133660 (Chem2008), KX109781-KX109819 (Chem2009), KX098393-KX098447 (LSTE1), and KX109820-KX109891 (LSTE2). The bacterial sequences data obtained by Sanger sequencing reported in this study have been submitted to the NCBI in GenBank nucleotide sequence database under accession number KX133661-KX133720 (Chem2008). The bacterial sequences data obtained by 454-pyrosequencing reported in this study have been submitted to the NCBI as part of the BioProject ID PRJNA260775, under the BioSample IDs SAMN04558175, SAMN04558168, and SAMN04546657, respectively, related to the samples Chem2009, LSTE2, and LSTE1.

Taxonomic affiliations were made using the SILVA database (v123) (Quast et al., 2013). The sequences with an affiliation bootstrap at the class level below 95% identity were considered as non-affiliated sequences. Sequence alignment, generation of the distance matrix from the aligned sequences, and calculation of the rarefaction curves and richness indicators were performed with MOTHUR v.1.22.2 (Schloss et al., 2009). We defined Operational taxonomic units (OTUs) by a 0.03 distance level (*i.e.*, sequences with  $\geq 97\%$

similarity are designated to a single OTU). Phylogenetic trees were constructed with representative sequences of significant OTU from all samples, along with closely related environmental clones and cultured species. Tree topology and branch lengths were inferred using both the neighbor-joining method with the maximum composite likelihood model (Saitou & Nei, 1987; Tamura et al., 2004) and the maximum likelihood method based on the general time reversible model (Nei & Kumar, 2000) with MEGA software version 7 (Kumar et al., 2016). Maximum likelihood bootstrapping was carried out with 1,000 replicates.

*Beta* diversity was performed with MOTHUR with a 0.03 distance level. *Beta* diversity was estimated using the Yue and Clayton theta ( $\theta_{YC}$ ) similarity coefficient (Yue & Clayton, 2006). The totality of sequences has been considered except for *Bacteria* from 454 pyrosequencing for which a random subsample of 2510 has been performed. The resultant similarity matrix  $\theta_{YC}$  was clustered and a dendrogram created using MOTHUR.

## 4. Results

### 4.1. Chemical Composition of High-Temperature Hydrothermal Fluids and Gases

#### 4.1.1. Fluids

The temporal evolution of the fluid chemistry at the Eiffel Tower/Aisics site is presented from 2008 to 2011 in Figure 2. Mean, median, minimum, maximum, 25%, and 75% of all data is presented when there are more than two end-member values (Figure 2).

Regarding 2008, the data reported by Pester et al. (2012) for Montsegur vent were considered, since it shares the same deep source as Eiffel Tower edifice (Figure 1c; Barreyre et al., 2012; Chavagnac, Leleu, et al., 2018) and in close vicinity.

Chloride (Cl) is the dominant anion of the hydrothermal fluids and will therefore control the fluid charge balance. It varies within a narrow range comprised between 401 and 438 mM, corresponding to 20–27% loss compared to the seawater value of 545 mM (Figure 2a). The mean Cl concentrations increased from about 405 mM in 2008 and 2009 to 430 mM in 2010 and 2011 (Figure 2a).

Sodium (Na) concentrations are depleted by 27 up to 34% (between 2009 and 2011) compared to the mean value of seawater that is around 320 mM in 2009 (Table S2).

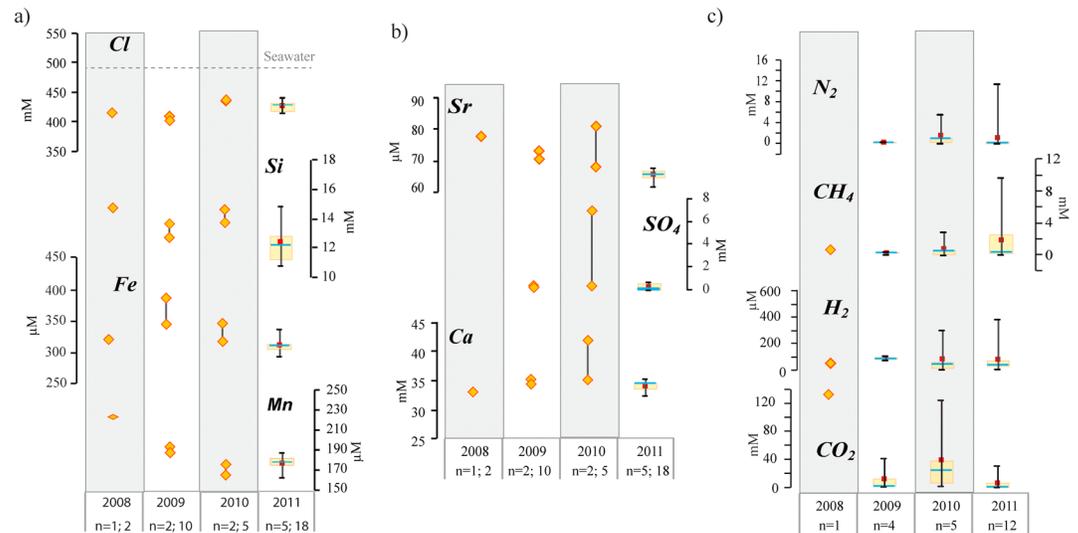
Overall, the  $\text{SO}_4$  end-members concentrations are close to nil mM except for Aisics fluids collected in 2010 (Table S2 and Figure 2b). These samples define an intercept at 7 mM (maximum value observed), with a mean value of 3.7 mM. All samples were treated similarly from year to year, indicating that additional sulfate increase due to hydrogen sulfide oxidation during sample storage is unlikely. That increase of  $\text{SO}_4$  in the 2010 fluid is link to an in situ process.

Calcium (Ca) concentrations of the end-member hydrothermal fluids are 3 times superior to the seawater value (10.3 mM) and range usually between 32.3 and 41.9 mM since 2009 (Figure 2b). The maximum concentration is observed in 2010 for the Aisics fluids, leading to a mean value of around 38.5 mM for this year, about 5 mmol higher than the previous year values (Charlou et al., 2000; Von Damm et al., 1998).

In 2010, the significant increase of Ca and  $\text{SO}_4$  concentrations of Aisics hydrothermal end-members correlates to higher strontium (Sr) concentrations reaching a maximum value of 81  $\mu\text{M}$  compared to 73  $\mu\text{M}$  for previous years (Charlou et al., 2000; Von Damm et al., 1998; Figure 2b).

Iron (Fe) and Manganese (Mn) concentrations of end-member fluids are enriched at both sites compared to seawater, with values ranging between 293–386  $\mu\text{M}$  and 161–193  $\mu\text{M}$ , respectively (Figure 2a). While Mn concentrations decreased since 2008, the Fe concentrations remained overall stable since 2008 and within the same range than the previous year values (Charlou et al., 2000; Pester et al., 2012; Von Damm et al., 1998).

The silica (Si) end-member concentrations (Si) vary between 10.8 and 14.8 mM. The maximum mean value around 14.7 mM is observed in 2008 and 2010, with a large variation of concentrations in 2011 (Figure 2a). Note in 2011 at Aisics, the presence of four outsiders' fluids (M11FLU003, M11FLU031, M11FLU032, and M11FLU072) characterized by much lower Si concentrations for similar Cl concentrations than other samples collected the same year (Table S2), leading to a specific end-member value of 2.1 mM for those four outsiders' fluids.



**Figure 2.** Variations of dissolved elements in pure hydrothermal end-members and dissolved gases concentrations of Eiffel Tower and Aisics fluids through time. (a) End-members concentrations of Chloride, Silicon, Iron, Manganese, giving information on rock/water interactions in depth. (b) End-member concentrations for Strontium, Sulfate, and Calcium giving information on anhydrite dissolution in depth. (c) Dissolved gases concentrations in the hydrothermal fluids. When the number of end-members exceeds two, the mean (red), median (light blue), maximum, and minimum values are reported. The top and bottom of yellow boxes represent the 75th and 25th percentiles, respectively. Otherwise the values are displayed by yellow diamonds. Number of end-members, followed by the total number of fluids samples, is indicated for each year. In 2008, we included pure hydrothermal end-member fluids and dissolved gases data from Montsegur (marker US4) reported by Pester et al. (2012). The gray boxes figure the occurrence of possible magmatic events

#### 4.1.2. Gases

All the fluids are enriched in various dissolved gases compared to seawater with gas volume at atmospheric pressure varying from 63 to 1,440 ml/kg at both sites (Table S1), similar to those reported for previous years (Charlou et al., 2000; Pester et al., 2012). Carbon dioxide (CO<sub>2</sub>) is the main constituent with significant variation from year to year (Figure 2c).

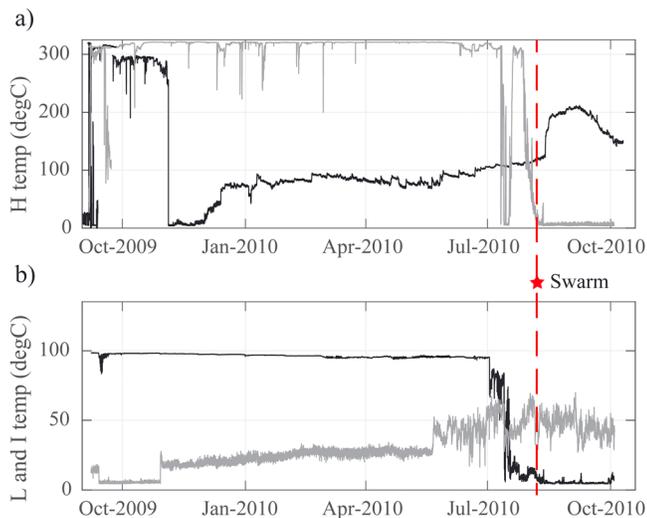
The most striking features are obtained in 2010 at the Aisics chimney. The CO<sub>2</sub> concentration vary from 1.5 to 118.8 mM between fluids sampled at 25-min intervals (Table S3 and Figure 2c). The maximum value is close to the one reported in 2008 from only one fluid sampled around Eiffel Tower and reaching to a CO<sub>2</sub> concentration of 128.8 mM (Pester et al., 2012).

Methane (CH<sub>4</sub>) and nitrogen (N<sub>2</sub>) are the most abundant gases after CO<sub>2</sub> with mean concentrations varying from 0.31 to 1.95 mM and from 0.29 to 1.58 mM, respectively. The mean CH<sub>4</sub> concentrations are twice as high than previous values (Charlou et al., 2000). Overall, from year to year we observed a large dispersion of CH<sub>4</sub> concentration especially in 2011, leading to general increase of the mean concentration through time since 2009, but with median values remaining stable (Figure 2c). The mean N<sub>2</sub> concentrations are similar to those measured in previous years (Charlou et al., 2000).

Finally, hydrogen (H<sub>2</sub>) mean concentrations vary from 51 to 88 μM (Figure 2c). In 2010 and 2011, H<sub>2</sub> concentration ranges between 2 and 380 μM (Figure 2c), same order of magnitude than worldwide values of other submarine hydrothermal vents (e.g., Charlou et al., 2000). Compared to stable H<sub>2</sub> concentrations in 2009, the large dispersion of H<sub>2</sub> concentrations, observed after, leads to a quite stable mean and median values through time.

#### 4.1.3. Hydrothermal Fluids Temperature

The HT sensor deployed at the outflow of Eiffel Tower vent (in gray Figure 3a) highlights great variations of the fluid temperature (between 300 and <10 °C), over a short period of time (July–August 2010). The HT sensor deployed at the outflow of Aisics black smoker vent (in black Figure 3a) highlights a sharp decrease of temperature from 300 to <10 °C, followed by a progressive increase up to 100 °C and a sharp one up to 200 °C around August 2010. The LT sensor at Eiffel Tower (in black Figure 3b) also recorded great short-



**Figure 3.** Temperature records for the period October 2009 to October 2010. (a) Temperature data from two high-temperature sensors deployed on black smoker chimneys at Eiffel Tower vent (TE-V02-090905-101003-HW0010B in gray) and Aisics vent (TE-AIS-090923-101010-HN29016b in black). (b) Temperature data from a low-temperature sensor deployed on a diffuse crack at Eiffel Tower vent (TE-C02-090907-101003-LW00003, black), and at Montsegur vent (MS-C02-090907-101004-LW00001, gray). The red star and dashed line represent a seismic swarm recorded through Ocean Bottom Seismometer (OBS) network deployed during the same period of time around Lucky Strike hydrothermal field (T. Barreyre, personal communication, 2016).

time variations (between 100 and  $<10$  °C), during the same period of time. In the same time, the LT sensor deployed in the Montsegur vent crack, also shows short periods of variation during a general temperature increase (in gray Figure 3b). These variations occurred at the same time as an increase in seismic events was recorded by the OBS network around LSHF (red star Figure 3).

## 4.2. Comparisons of Microbial Communities

From the 2008 Aisics chimney, we recovered 93 *Archaea*-related sequences distributed through 55 OTUs, and 62 *Bacteria*-related sequences distributed through 46 OTUs. From the 2009 Aisics chimney, we retrieved only 39 *Archaea*-related sequences distributed through 18 OTUs, and 13,103 *Bacteria*-related sequences distributed through 810 OTUs. Fifty-five *Archaea*-related sequences distributed through 19 OTUs and 2,378 *Bacteria*-related sequences distributed through 819 OTUs were retrieved from LSTE1. Finally, 76 *Archaea*-related sequences distributed through 23 OTUs and 5,233 *Bacteria*-related sequences distributed through 910 OTUs were identified from LSTE2 (see details in Tables S6–S8). We amplified very few archaeal sequences from all our samples, despite the use of different DNA extraction methods, DNA purification, nested PCR methods, and PCR product purification before cloning and sequencing. In addition, we must keep in mind that we have used different archaeal primers for chimney sample and those from colonization substrates.

In order to compare the four samples, the  $\theta_{YC}$  was used to illustrate the communities' structure and composition similarities for the bacterial and archaeal libraries.

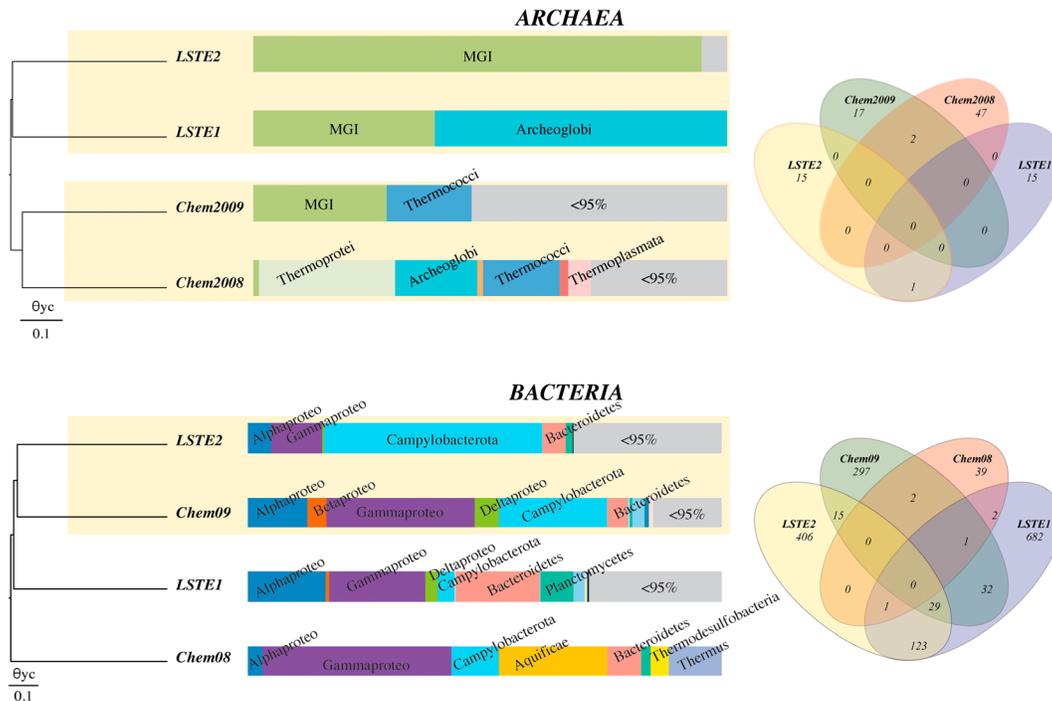
For *Archaea*, the tree constructed from the  $\theta_{YC}$  exhibits two clusters that underlined the dependency of archaeal communities on substrates (chimney vs. basalt substrate; Figure 4). There are only two shared OTUs between Chem08 and Chem09, and 1 between LSTE1 and LSTE2 (venn diagram, Figure 4).

For *Bacteria*, the tree constructed from the  $\theta_{YC}$  exhibits three clusters. Surprisingly here, samples do not cluster according to substrate. Instead, from the colonizer LSTE2 and the 2009 chimney form a cluster, whereas LSTE1 and 2008 chimney communities separate clusters. LSTE2 and the 2009 chimney indeed share 44 OTUs, while chimneys share only three OTUs (Figure 4).

## 4.3. Microbial Community Dynamics at the Eiffel Tower Over Time

### 4.3.1. The 2008 Microbial Community

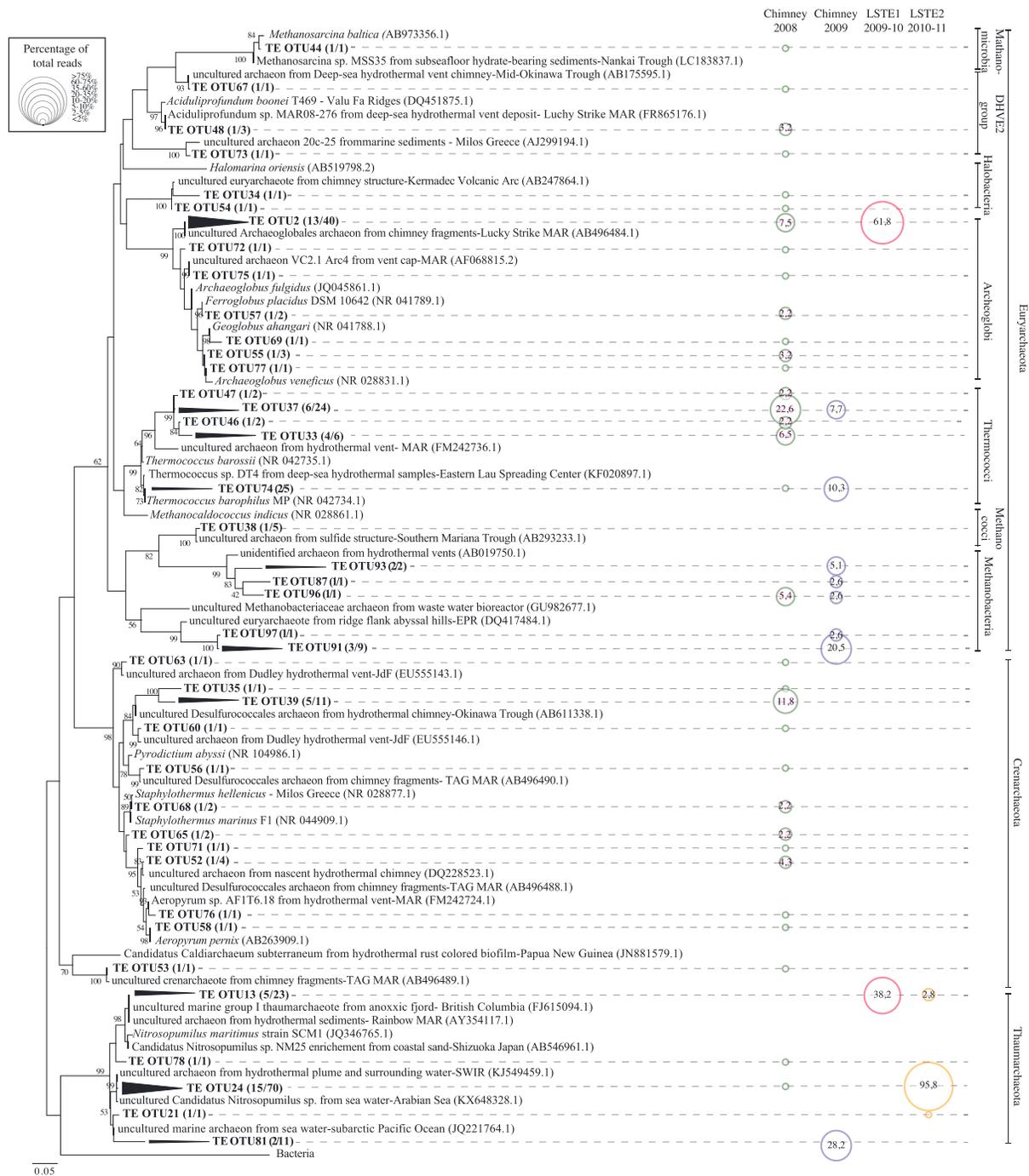
Of the 93 archaeal sequences retrieved from the 2008 chimney, 38 sequences are affiliated to the phylum Euryarchaeota, 27 to the Crenarchaeota, only one to the Thaumarchaeota (see Figure 4 and Table S6) with up to 95% similarity. The sequences of Euryarchaeota (dominating phylum) are equally distributed between the Archaeoglobi and Thermococci classes (See Figures 4 and 5) and most of the Archaeoglobi sequences are related to *Archaeoglobus veneficus* (Huber et al., 1997) or *Archaeoglobus fulgidus* (Beeder et al., 1994), with more than 96% confidence. Those two hyperthermophilic sulfate-reducers were originally isolated from a black smoker wall (MAR) and from hot oil field waters of the North Sea, respectively. Two Archaeoglobi sequences are related at 98% to the isolated strain *Ferroglobus placidus*, a hyperthermophilic iron-oxidizing archaeon retrieved from a shallow submarine hydrothermal system at Vulcano (Italy) (Hafenbradl et al., 1996), detected in hydrothermal vents (Reysenbach et al., 2000) and also shown to be able to perform anaerobic Fe reduction (Tor et al., 2001; Tor & Lovley, 2001). One Archaeoglobi sequence is also related at 94% to the isolated strain *Geoglobus ahangari*, a hyperthermophilic iron-reducing archaeon recovered from the Guaymas Basin hydrothermal system (Kashefi, 2002). All the Thermococci sequences are closely related (more than 95%) to the isolated strains *Thermococcus barophilus* (Marteinsson et al., 1999) or *Thermococcus barossii* (Duffaud et al., 1998), hyperthermophilic, chemoorganotrophic sulfur-reducing archaeon retrieved from various hydrothermal systems around the world ocean.



**Figure 4.** OTU-based analysis of the four microbial communities studied. The two dendrograms represent, for *Archaea* and *Bacteria*, the similarity of the samples based on the  $\theta_{YC}$ , a communities' structure similarity coefficient. The Venn diagrams represent the bacterial and archaeal shared OTUs between the four samples. OTUs = operational taxonomic units.

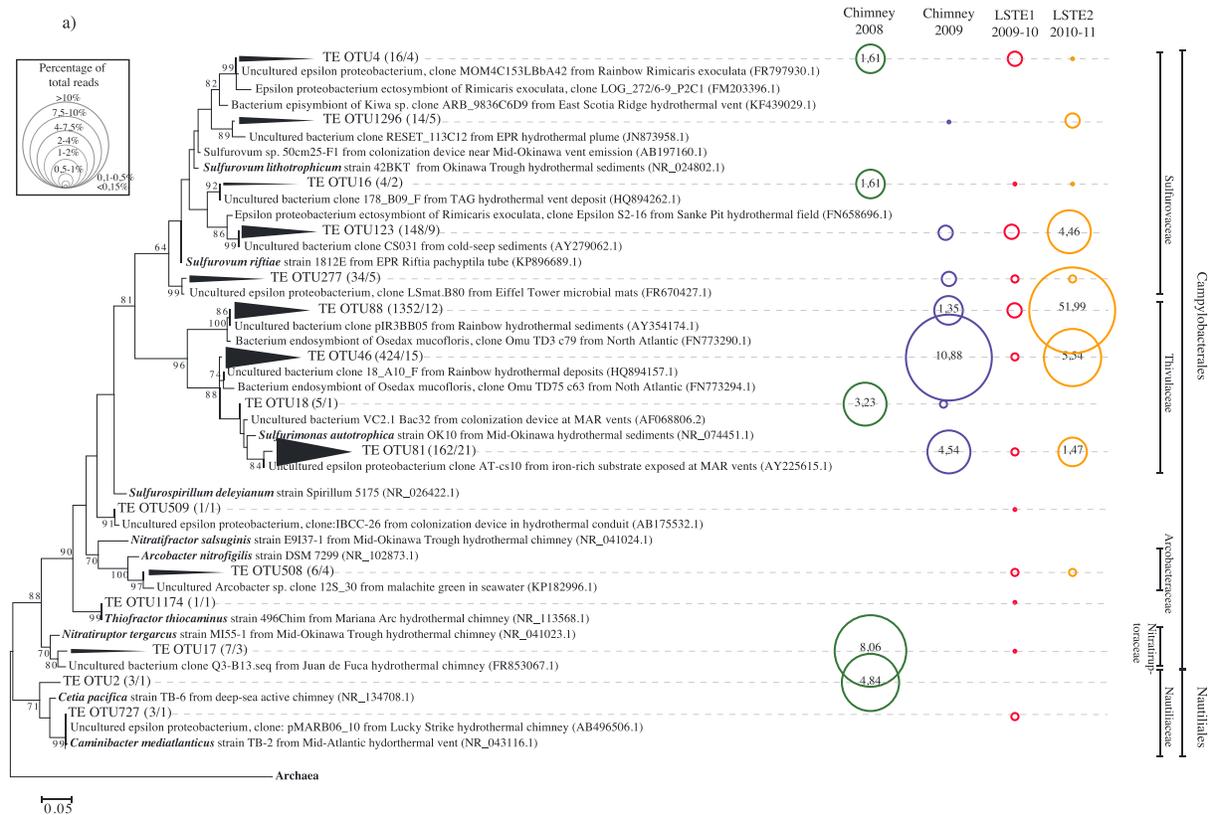
Some of the Thermoplasmata sequences belong to the Deep-Sea-Hydrothermal-Vent Group 2 (DHVE2) and are related at 98% to *Aciduliprofundum* genus isolated from Atlantic hydrothermal vents. These sequences are closely related (97%) to the isolated strain *Aciduliprofundum boonei*, retrieved from Mariner vent field in the Lau Basin and described as a thermoacidophilic, heterotrophic sulfur- and iron-reducing archaeon (Reysenbach et al., 2006). All of 27 Desulfurococcales order sequences are related at more than 98% to environmental sequences and are to either the isolated strain *Aeropyrum* sp., *Staphylothermus marinus* and *Pyrodictium abyssi* (see Figures 4 and 5). *Aeropyrum* sp. are hyperthermophilic aerobic heterotrophic archaeon retrieved from both coastal solfataric and deep-sea vent and deep sea vents (Nakagawa, 2004; Sako et al., 1996). *S. marinus* is a hyperthermophilic heterotrophic sulfur-dependent archaeon from Vulcano geothermal sediments (Italy) and hydrothermal vent at the East Pacific Rise (Fiala et al., 1986). *P. abyssi* is a hyperthermophilic heterotrophic archaeon from vents at Guaymas and Kolbeinsey ridge (Pley et al., 1991). Only two sequences belong to the phylum Thaumarchaeota and are close (up to 99%) to uncultured clones retrieved from marine samples around the world and described as autotrophic or mixotrophic ammonia oxidizers (Pester et al., 2011).

The 62 retrieved bacterial sequences of the 2008 chimney are distributed through only five phyla. Among them the Campylobacterota dominates with a total of 22 sequences (16 OTUs). Proteobacteria phylum is the second one with 20 sequences (18 OTUs). The Aquificae phylum is the third one with a total of nine sequences (six OTUs). The two other phyla are Deinococcus-Thermus with two sequences (one OTU) and Planctomycetes with one sequence (one OTU). The Campylobacterota sequences are about equally distributed between Campylobacterales (12 sequences/11 OTUs) and Nautiliales (10 sequences/five OTUs) (Figure 4 and Tables S7 and S8). Almost all Campylobacterales sequences are related (up to 96%) to uncultured *Sulfurovum* sp. or uncultured *Sulfurimonas* sp. (Figure 6). The closest isolated strains are, *Sulfurovum lithotrophicum* (Inagaki et al., 2004) and *Sulfurimonas autotrophica* (Inagaki, 2003) two mesophilic sulfur- or thiosulfate-oxidizing *Bacteria* isolated from hydrothermal system in Okinawa Trough (Japan). Among the Nautiliales sequences, the majority is closely (up to 94%) related to uncultured *Nitratiruptor* sp. isolated from Iheya hydrothermal field in Mid-Okinawa Trough (Japan) (Nakagawa, Takai, Inagaki, Hirayama, et al.,



**Figure 5.** Phylogenetic relationships among the archaeal 16S rRNA gene sequences of representative OTUs of the four samples. Sequences from this study are designed by the prefix “TE”. The corresponding number of OTU/sequences for each representative OTU is indicated in bracket. The circle size figures the percentage represented by each OTU with respect to the total number of sequences for each sample. The closest NCBI sequences are presented with their GenBank accession numbers. The tree was constructed by Maximum Likelihood method. Bootstrap values for nodes were obtained using 1,000 replicates. The scale bar represents 0.05 substitutions per nucleotide position. OTUs = operational taxonomic units; NCBI = National Center of Biotechnology Information.

2005). The closest cultivated strain is *Nitratiruptor tergaricus*, a thermophilic, autotrophic nitrate-reducing *Bacteria* isolated from the same place (Nakagawa, Takai, Inagaki, Horikoshi, et al., 2005). The others Nautiliales sequences are closely related (95%) to uncultured *Bacteria* retrieved from Snake Pit hydrothermal vent (MAR) (Reysenbach et al., 2000), and close (94%) to the cultivated strain *Cetia pacifica*, a thermophilic,



**Figure 6.** Phylogenetic relationships among the bacterial 16S rRNA gene sequences of representative OTUs of the four samples for Campylobacterota. Sequences obtained in this study are designed by the prefix “TE”. The corresponding number of OTU/sequences obtained for each representative OTU is indicated in bracket. The size of the circle in the right part figures the percentage represented by each OTU with respect to the total number of sequences for each sample. The closest National Center of Biotechnology Information sequences are presented with their GenBank accession numbers. The tree was constructed by Maximum Likelihood method. Bootstrap values for nodes were obtained using 1,000 replicates. The scale bar represents 0.05 substitutions per nucleotide position. OTUs = operational taxonomic units.

autotrophic nitrate-ammonifying bacterium isolated from an hydrothermal vent on the East Pacific Rise (9°N) (Grosche et al., 2015). The most abundant proteobacterial class is the Gammaproteobacteria with a total of 14 sequences (11 OTUs). The majority of gammaproteobacterial sequences are from the Thiotrichales order, (Figure S2) close to uncultured *Bacteria* affiliated to *Thiomicrospira* sp., or *Sulfurivarga* sp. recovered from shallow hydrothermal fields and putatively involved in the sulfur cycle. Only five sequences (five OTUs) are from the class Alphaproteobacteria and the majority are related to uncultured members of the Rhodobacterales retrieved from marine sediments or seawater and known to be organotrophs (Figure S4).

Within the Aquificae phylum, all the sequences are related to uncultured environmental representatives close (more than 95%) to *Persephonella* sp.. The closest cultivated strain is *Persephonella hydrogeniphila*, a thermophilic, autotrophic, hydrogen- and/or sulfur-oxidizing bacterium isolated from hydrothermal vent chimney from Suiyo Seamount (Izu Bonin Arc) (Nakagawa et al., 2003) (Figure S3). Finally, the two sequences (one OTU) belonging to the phylum Deinococcus-Thermus are closely related (94%) to the cultured strain *Vulcanithermus mediatlanticus*, a thermophilic, heterotrophic facultative nitrate-reducing bacterium isolated from hydrothermal chimney collected at Rainbow vent field (Miroshnichenko et al., 2003).

#### 4.3.2. The 2009 Microbial Community

Over the 39 archaeal sequences retrieved from the 2009 chimney, 11 sequences are affiliated to the Thaumarchaeota phylum, seven to the Euryarchaeota and 21 are non-affiliated (Figure 4 and Table S6). However, these 21 non-affiliated sequences are defined as unaffiliated Euryarchaeota (bootstrap over

85%), meaning that the archaeal diversity of the 2009 chimney is dominated by the Euryarchaeota phylum. The affiliated Euryarchaeota sequences belong to the Thermococci class (Figures 4 and 5) and are related at 99% to the isolated strain *Thermococcus barossii*, a hyperthermophilic heterotrophic sulfur-reducing archaeon from marine hydrothermal vents (Duffaud et al., 1998). Some non-affiliated Euryarchaeota sequences are related to the methanogen Methanobacteria class (Figure 5). In the Thaumarchaeota phylum, all the sequences are affiliated to the Nitrosopumilales (Figure 5) and are closely related to an uncultured clone isolated from sea waters in subarctic Pacific Ocean. The related cultivated strain *Nitrosopumilus maritimus* is an aerobic chemoautotrophic ammonia-oxidizer (Könneke et al., 2005).

The retrieved bacterial sequences from the 2009 chimney are distributed through 13 phyla. Among them the Proteobacteria phylum is largely dominant with 53% of the 13,103 total sequences (37% of 810 total OTUs). The other phylotypes are scattered between the Campylobacterota (23% of total sequences and 9% of total OTUs), CFB group (4% of total sequences and 9% of total OTUs), High G+C (Actinobacteria) Gram-positives (3% of total sequences and 5% of total OTUs), and Cyanobacteria, Low G+C (Firmicutes) Gram-positives, Planctomycetes and Verrucomicrobia (Figure 4 and Tables S7 and S8). Within the proteobacterial sequences, the Gammaproteobacteria class is dominant with 31% of the total bacterial sequences (18% of total OTUs) distributed between Thiotrichales (52% of gammaproteobacterial sequences), Pseudomonadales, Chromatiales, and Oceanospirillales (Figure S2). They correspond to uncultured representatives recovered from deep environments, marine sediments and symbionts-epibionts of marine invertebrates putatively involved in the sulfur cycle. The second most abundant class is Alphaproteobacteria with 13% of total sequences (8% of total OTUs). Sequences are distributed between Rhodobacterales, Rhizobiales, Sphingomonadales, and Rhodospirillales (Figure S4). Most of the sequences correspond to uncultured representatives clustering with ubiquitous phylotypes from marine sediments or seawater and known to be heterotrophic or organotrophic. A few sequences clustered with uncultured-*Sulfitobacter* sp. potentially involved in the sulfur cycle. Almost all the Campylobacterota sequences belong to Campylobacterales (Figure 6) and are related to uncultured *Sulfovorum* sp. or uncultured *Sulfurimonas* sp., known to be lithoautotrophic sulfur oxidizers using O<sub>2</sub>, or NO<sub>3</sub><sup>-</sup> as electron acceptor (Nakagawa, Takai, Inagaki, Hirayama, et al., 2005).

#### 4.3.3. The 2010 Microbial Community

Over the 55 archaeal sequences retrieved from LSTE1 in 2010, 34 sequences are affiliated to the Euryarchaeota and 21 to the Thaumarchaeota phylum (Figure 4 and Table S6). The sequences of Euryarchaeota belong to the thermophilic Archaeoglobales (Figure 5) and are closely related to *Archaeoglobus fulgidus* (96% similarity), isolated from marine hydrothermal systems in Italy or hot North Sea oil field waters (Beeder et al., 1994; Stetter et al., 1987). This species is thermophile (60–85 °C), anaerobic strict and grows in presence of H<sub>2</sub> and simple organic molecules as substrates with sulfate or thiosulfate as electron acceptors. The sequences of Thaumarchaeota belongs to the Marine Group I lineage and are closely related to *Nitrosopumilus maritimus* (98% similarity) (Könneke et al., 2005), a Nitrosopumilales isolated from rocky substratum of a tropical marine tank from Seattle Aquarium. This strain is mesophilic (optimal growth at 25–28 °C), microaerophilic, and autotrophically oxidize ammonia in the presence of CO<sub>2</sub>.

The analyses of the 2,378 bacterial sequences indicated a phylogenetically diverse bacterial population. Retrieved bacterial sequences are affiliated to 18 phyla. Among them the Proteobacteria phylum constituted the most dominant bacterial group with 40% of the total bacterial sequences (53% of 819 total OTUs), within the four subdivisions (Gamma, Alpha, Delta, and Beta). Bacteroidetes represent the second dominant bacterial group, accounting for 18% of the total bacterial sequences (12% of total OTUs). The third dominant bacterial group is Planctomycetes, accounting for 7% of the total bacterial sequences (13% of total OTU). Surprisingly, Campylobacterota represent only 4% of total sequences (5% of total OTUs). Other phylotypes appear scattered over the High G+C (Actinobacteria) and Low G+C (Firmicutes) Gram-positives, Lentisphaeria, and BD1-5 and Candidate\_division\_OD1 uncultured bacterial phyla (Figure 4 and Tables S7 and S8). Gammaproteobacteria is the most diverse and abundant class with 20% of total sequences (22% of total OTUs). The majority of gammaproteobacterial sequences are distributed between Thiotrichales, Cellvibrionales, Oceanospirillales, and Methylococcales (Figure S2) with some sequences closely affiliated to *Haliella* sp., *Colwellia* sp., *Thiothrix* sp., *Kangiella* sp., or *Methylobacter* sp. They correspond to uncultured representatives clustering with phylotypes recovered from seep environments, gas-hydrate-associated sediments, marine sediments, and symbionts-epibionts of marine invertebrates, and putatively

involved in the sulfur cycle. The second most abundant proteobacterial class is the Alphaproteobacteria with 16% of total sequences (19% of total OTUs). The majority of alphaproteobacterial sequences is distributed between Rhodobacterales, Rhizobiales, Kordiimonadales, Rhodospirillales, and Ricksettsiales (Figure S4). Most of the sequences correspond to uncultured representatives from ubiquitous phylotypes from marine sediments or seawater and known to be heterotrophic or organotrophic. A few sequences clusters with uncultured-*Roseobacter* sp. and *Sulfitobacter* sp. potentially involved in the sulfur cycle. The second most important group retrieved from LSTE1 belongs to the Bacteroidetes phylum (Figures 4 and S3). The sequences of Bacteroidetes have close relatives found in very different habitats, as Antarctic seawater, marine sediments, hydrothermal environments or symbionts-epibionts of marine invertebrates. They are generally chemoorganotroph like the closely related *Gaetbulibacter lutimaris* (Yoon et al., 2013). The majority of Campylobacterota sequences belong to the Campylobacterales and are related to uncultured *Sulfovorom* sp. or uncultured *Sulfurimonas* sp. recovered from symbionts-ectobionts from marine macrofauna (Figure 6). Their cultured representatives are known to be lithoautotrophic (i.e., using CO<sub>2</sub> as carbon source) and sulfur oxidizers using O<sub>2</sub> or NO<sub>3</sub><sup>-</sup> as electron acceptor (Nakagawa, Takai, Inagaki, Hirayama, et al., 2005). Some sequences belong to the Nautiliales and are affiliated to uncultured *Nitratifactor* sp., whose cultivated representatives are known to be lithoautotrophic growing by reducing nitrate with H<sub>2</sub> as electron donor (Nakagawa, Takai, Inagaki, Horikoshi, et al., 2005) or *Caminibacter mediatlanticus* known to be thermophilic growing by reducing nitrate with H<sub>2</sub> as electron donor and CO<sub>2</sub> as carbon source (Voordeckers et al., 2005).

#### 4.3.4. The 2011 Microbial Community

The archaeal diversity from LSTE2 is strongly dominated by the Marine Group I lineage in the Thaumarchaeota phylum, with 72 sequences over the 76 total retrieved (Figure 4 and Table S6). The majority of the Thaumarchaeota sequences are closely related to uncultured Candidatus Nitrosopumilus from Arabian Sea. Some of them are closely related to uncultured Thaumarchaeote from a British Columbia anoxic fiord. All sequences are related (93–99% similarity) to the mesophile (25–28 °C), aerophile, and autotrophic ammonia oxidizer, *Nitrosopumilus maritimus* (Könneke et al., 2005; Figure 5).

The analysis of the 5,233 bacterial sequences indicated a less diversified bacterial community than in LSTE1, Chem2008, and Chem2009 (Figure 4). Retrieved bacterial sequences are affiliated to only nine phyla. Among them Campylobacterota constitute the dominant bacterial group accounting for 46% of the total bacterial sequences (13% of the 911 total OTUs). Proteobacteria is the second dominant bacterial group, accounting for 16% of the total bacterial sequences (52% of the 911 total OTUs). Other phylotypes appear scattered over the CFB group (5% of total sequences and 21% of total OTUs), Planctomycetes, High G+C (Actinobacteria) Gram-positives and BD1-5 and Candidate\_division\_OD1 uncultured bacterial phyla (Figure 4 and Tables S7 and S8). Almost all the Campylobacterota sequences (99,9%) belong to the Campylobacterales. Majority of Campylobacterales are closely related to *Osedax mucofloris* bacterium endosymbiont (Verna et al., 2010), affiliated to *Sulfurimonas* sp. (Figure 6). The closest cultivated strain is *Sulfurimonas autotrophica* described as a mesophilic (10–40 °C) strain, growing chemolithoautotrophically with elemental sulfur, sulfide and thiosulfate as electron donors and oxygen as electron acceptor, isolated from a deep sea sediment in the Mid-Okinawa Trough hydrothermal field (Inagaki, 2003). Other Campylobacterales are closely related to *Rimicaris exoculata* ectosymbionts that are closely related to *Sulfurospirillum* sp. (Hügler et al., 2011), and to an uncultured-*Sulfurovum* isolated from deep-sea hydrothermal sediments. These sequences are closely related (more than 95%) to the cultivated strain *Sulfurovum lithotrophicum*, a mesophilic (optimum growth at 28–30 °C) chemolithoautotrophic sulfur-thiosulfate-oxidizer using oxygen or nitrate as electron acceptors and CO<sub>2</sub> as carbon source (Inagaki et al., 2004). Gammaproteobacteria represent the second most abundant class with 11% of total bacterial sequences (28% of total OTUs). Sequences of Gammaproteobacteria are scattered between the orders Oceanospirillales, Thiotrichales, Thiohalobacter, Cellvibrionales, Methylococcales, and Chromatiales (Figure S2). Some sequences are closely affiliated to *Methylobacter* sp. and to the cultivated strain *Methylobacter marinus* known to be aerobic methanotrophs, that is, using methane as sole carbon and energy source (Lidstrom, 1988). A large number of gammaproteobacterial sequences (48% of total gamma-proteobacterial sequences) are closely related to endosymbionts of different marine invertebrates, scattered between the Oceanospirillales, Chromatiales, and Thiotrichales orders. They are described to be involved in the sulfur cycle, being mainly autotrophs, (i.e., thiosulfate oxidizing using CO<sub>2</sub> as carbon source; Fujiwara et al., 2001; Duperron et al., 2005; Thiel et al., 2012).

## 5. Discussion

### 5.1. Modification of Physicochemical Environmental Features Through Time

The hydrothermal fluid composition is driven by several processes including phase separation at depth, water-rock interaction at the reaction zone, parameters including composition and state of alteration of the substratum, and pressure and temperature conditions along the up flow pathway to the seafloor (Von Damm, 1995). These conditions may change and/or vary over time due to tectonic and magmatic events within the oceanic lithosphere. Indeed, a tectonic event may lead to newly formed fractures and cracks as well as deeper fluid circulation enabling the exposure of fresh substratum to hydrothermal fluid. As a result, the hydrothermal circulation pathway may be modified and therefore the pressure-temperature (P-T) conditions at which the water-rock interaction are taking place (Wells & Ghiorso, 1991). This is evidenced by contrasted chemical composition of hydrothermal and gases prior and after a tectonic/magmatic event.

In 2008, Pester et al. (2012) detected high CO<sub>2</sub> concentrations (up to 126 mM) in hydrothermal fluids collected at several sites of LSHF. This high CO<sub>2</sub> concentration, associated with a  $\delta^{13}\text{CO}_2$  (gases) close to -4‰ is attributed to the replenishment of the magmatic heat source, that is, a potential magmatic event (Pester et al., 2012). However, the end-member fluid chemistry remained essentially identical to that of previous years.

In the present study, however, the increasing of the CO<sub>2</sub> concentration comes with chemical composition variations. Strong CO<sub>2</sub> concentrations were measured in the 2010 hydrothermal fluids at Eiffel Tower site, with concentrations reaching up to 118 mM (nearly 4 times higher than previous years (Charlou et al., 2000)) and also at other LSHF vent sites, for example, Cyprès and Crystal located south west of the fossil lava lake (C. Boulart, personal communication, 2015). Variations of CH<sub>4</sub> and CO<sub>2</sub> concentrations are correlated while they are anticorrelated with variations of hydrogen concentrations. This suggests that CH<sub>4</sub> was produced abiotically through the Sabatier reaction (reduction of CO<sub>2</sub> by hydrogen). Variations of N<sub>2</sub> concentrations follow the variations of CH<sub>4</sub> and CO<sub>2</sub>, substantiating that a magmatic degassing occurred in 2010. Meanwhile, hydrothermal fluids exhibit chemical composition variations as evidenced by increasing Cl, Ca, SO<sub>4</sub>, and Sr, indicating the beginning of the emission of the brine phase, in which these elements partitioned after a magmatic event. In addition, the increase of Si concentrations, preferentially partitioned in the vapor phase during a magmatic event, together with the decrease of Fe and Mn concentrations, indicate a deepening of the reaction zone and the occurrence of a magmatic event under LSHF between 2009 and 2010.

It is noteworthy that at the LSHF a unique deep-rooted fluid is feeding the hydrothermal circulation cell (Leleu et al., 2015). These authors also noticed that some of the Aisics and Montsegur hydrothermal fluids (outsiders) are characterized by lower Si contents compared to other sites. They propose that the Si loss may occur along the upflow zone of the deep-rooted fluid from the reaction zone to the seafloor as a result of conductive cooling. This may explain the low Si concentrations observed at Aisics and Montsegur, while the global tendency in 2010 is an increase compared to 2009 and 2011, indicating that a magmatic/tectonic event occurred under the LSHF (Table S1).

The dense fault network that truncates the three topographic highs surrounding the LSHF, allows seawater penetration within the porous basaltic crust (layer 2A). As the fossil lava lake acts as an impermeable cap, the downflowing seawater is conductively heated, leading to the formation of hydrothermally Si-cemented volcanoclastic breccias, also called "slab" (Cooper et al., 2000; Humphris et al., 2002). A well-developed slab is present at the SE area of the LSHF, where Eiffel Tower and Aisics sites are located. The discharge occurring there at the seafloor through crosscutting faults may result from the mix of the focused fluids at the base of layer 2A with conductively heated seawater (Cooper et al., 2000; Humphris et al., 2002; Ondréas et al., 2009). The potential contribution of this altered seawater is evidenced by high Ca and SO<sub>4</sub> concentration of focused fluid, attributed to anhydrite dissolution at subsurface due to retrograde solubility at temperature lower than 150 °C.

Nevertheless, the high gases contents (especially CO<sub>2</sub>) together with increasing Cl, Ca, SO<sub>4</sub>, and Si concentration lead us to propose that a magmatic/tectonic event occurred under the Lucky Strike hydrothermal field between 2009 and 2010.

These observations, in the context of a magmatic/tectonic event are consistent with changes observed after a volcanic event at fast spreading ridges. Hydrothermal circulation system evolves from a vapor-dominated to

brine-dominated fluid discharge, before reaching its preeruption chemical composition (Butterfield et al., 1997). For instance, at the EPR, the focused fluid composition changed from vapor-dominated in 1991 to brine-dominated fluids after the volcanic event of 1994, and the high-gas and low-element concentrations were consistent with vapor-dominated fluids (Von Damm et al., 1997). The authors proposed that the brine-phase that was stored in the oceanic crust vented in 1994 leading to focused fluids exhibiting high chlorinity and element concentrations (e.g., Li, Sr and Mn) as well as low concentration in CO<sub>2</sub> and H<sub>2</sub>. Similar variations of chemical composition were also observed at the Juan de Fuca Ridge, where the Main Endeavor Field underwent an intense seismic activity in June 1999 (Seewald et al., 2003). The dissolved concentrations of CO<sub>2</sub>, H<sub>2</sub>, and H<sub>2</sub>S of the focused fluids collected in September 1999 and June 2000 increased in the preearthquake samples and decreased during the following year until reaching their preeruption gas contents.

Chemical composition of high-temperature hydrothermal fluids provides a snapshot in time and space of the hydrothermal circulation dynamic. This may explain why the variations seen in the fluid chemistry do not strictly follow prior observation reported by other authors (Butterfield et al., 1997; Seewald et al., 2003; Von Damm, 1995; Von Damm et al., 1997). It depends on the timeframes at which fluid sampling was carried out, that is, just before or just after a magmatic/tectonic event.

The EMSO-Azores observatory gives us access to time series over years of hydrothermal high-, intermediate-, and low-temperatures records through a network of temperature sensors deployed in black smokers and diffuse cracks (Barreyre et al., 2014b; Barreyre & Sohn, 2016) and also to seismic events' records through the deployment of OBS network around the Lucky Strike field (Crawford et al., 2013). We report variations observed on HT and LT sensors deployed around Eiffel Tower and Montsegur sites, associated with an increase of seismic activity detected through the OBS network (T. Barreyre, personal communication, 2016). Variations of vent temperature have already been reported after tectonic/magmatic events at Juan de Fuca ridge and East Pacific Rise (Dziak et al., 2003; Johnson et al., 2000; Sohn et al., 1998). Magmatic event induces variations of gases content in fluids and then could generate fluid emission pulse, allowing more or less mixing with seawater that results in temperature variations. This also could favor precipitation or dissolution within the fluid circulation pipes, causing temperature variations, including short-time fluid emission stops. These concordant observations over different geophysical data allow us to attest to the occurrence of a magmatic event, and to date it more precisely, that is, around August 2010, just before the hydrothermal fluid sampling of MoMARSAT2010 cruise in October (Sarradin, Blandin, & Escartin, 2010).

## 5.2. Microbial Community Response to the Magmatic Degassing

Microbial communities greatly depend on habitat, with a broad coherence in community membership between geochemically and physically distinct provinces (Auguet et al., 2010; Edwards et al., 2011). For this study we have two different types of samples, hydrothermal vents chimney, on one side, and colonization substrates deployed on diffuse vents, on the other. However, we have the advantage of having sampled a magmatic degassing for each of them. In addition, we have used different archaeal primer between chimney samples and colonization substrates. Studies have shown that the use of different primers on the same microbial community might impact the abundance or the absence of specific population but not the community dynamics (Baker et al., 2003; Baker & Cowan, 2004; Wear et al., 2018). This could introduce a bias in the comparison of samples and thus partially explain the clustering observed on the dendrogram based on  $\theta_{VC}$  indices, which groups together the chimneys samples and the basaltic substrates, respectively. Shared OTUs appear only among chimneys or among basaltic substrates, as shown by the Venn diagram (Figure 3). The comparison of bacterial communities between samples is then biased by the fact that we only have a small data set on Chem2008 due to Sanger sequencing. However, it seems that if this may change the abundance of specific populations, this does not change the dynamics of communities and the relative abundance of species compared to others, as shown by a comparison on this same sample between Sanger and Miseq sequencing with different primers (D. François, personal communication, 2019). The comparison of bacterial communities across the dendrogram based on  $\theta_{VC}$  indices, apart from the Chem2008 sample, could indicate that *Bacteria* have less specificity with respect to substrate than *Archaea*.

Within deep-sea hydrothermal fields, various habitats physically connected contain distinct microbial communities, shaped by both fluid chemistry and physical characteristics (i.e., temperature, fluid flows, etc) (Dick, 2019). Thus, the chimney wall covers the entire mixing zone, from the cold seawater (outer wall) to

the end-member hot hydrothermal fluid (inner wall), hence covering a large range of temperature. The mussel bed-type habitat samples the mixing zone between diffuse hydrothermal fluids and seawater, with temperature only varying from 4 to 7 °C (Crépeau et al., 2011; Cuvelier, Sarradin, et al., 2011). However, this habitat can occasionally be exposed to high temperature, as evidenced by the burned colonization module recovered in 2010. These two habitat types sampled in this study, as well as the different colonized substrates (*i.e.*, a sulfate/sulfur edifice, chimney), regarding the tholeiitic-type basaltic glass (geomicrobiology modules) will generate differences in the microbial communities sampled for each site. Thus, hydrothermal chimneys harbor very diverse microbial communities due to the high thermal and chemical gradients, with abundances of thermophilic/hyperthermophilic anaerobic and lithoautotrophic species from Methanomicrobia, Archaeoglobi, Thermococci, Aquificae, Deltaproteobacteria, Gammaproteobacteria, and Campylobacterota (Dick, 2019), as described in our samples Chem2008 and Chem2009. Despite the difference in sequencing method and the number of microbial sequences between the two samples, there are still differences within the recovered species. Thus, even with more extensive sequencing, the absence of thermophile/hyperthermophile *Bacteria* (*e.g.*, Aquificae, Deinococci-Thermus) and *Archaea* (*e.g.*, Thermoprotei, Thermoplasmata (particularly DHVE2 family) Archaeoglobi), as well as the abundance of MGI *Archaea* mainly uncultivated species in the Chem2009 sample, compared to Chem2008 would be highlighted. In addition, Campylobacterota are more abundant in Chem2009 sample, but mainly affiliated to *Sulfurimonas* sp. and *Sulfurovum* sp. that are mesophilic sulfur- or thiosulfate-oxidizing *Bacteria*. While those from Chem2008 are mainly affiliated with *Nitratiruptor* sp. and *Cetia* sp. thermophilic, autotrophic nitrate-reducing *Bacteria*. In July 2008, vent fluids sampling at LSHF exhibited a substantial change in fluid composition, particularly with a significant increase of CO<sub>2</sub> concentrations and enrichment in dissolved chloride relative to seawater, interpreted as a replenishment of magmatic heat source, for example, a recent magmatic event (Pester et al., 2012). The 2008 chimney sample was collected just after this event.

In comparison, the diffuse flow habitat, sampled through microbial colonizers, would support microbial communities distributed between thermophilic Campylobacterota, Aquificae, and Archaeoglobi using sulfur, hydrogen, and nitrate for lithoautotrophic growth and mesophilic, aerobic and mixotrophic Campylobacterota, and Gammaproteobacteria especially filamentous sulfur-oxidizer as *Beggiatoa* or *Arcobacter* (Dick, 2019), depending on the mixing rate between seawater and hydrothermal fluid, which will define the temperatures, the oxygen concentration, and energy sources available for microbial growth. The comparison of the microbial communities from LSTE1 and LSTE2 samples faces a major problem. Indeed, the two colonizers were not deployed at exactly the same place, but were shifted by 8 m. However, both of them sampled a diffuse fluid bathing a mussel bed, at the base of the Aisics chimney. We know that the physicochemical conditions are closely linked to the pathway of the subsurface fluid, and can affect the communities' structure on small spatial scales (Akerman et al., 2012; Meier et al., 2016; Olins et al., 2017). In addition to being closer to Aisics's main chimney, we have evidenced, despite the absence of an associated temperature sensor, that LSTE1 experienced a high-temperature event during its deployment, as we have observed that its ballasted plastic base was melted, when recovered in 2010. The annual recording of temperature of hot and diffuse fluids around the Eiffel Tower and Aisics edifices shows large amplitude and large variations during a short period of time between March and April 2010, which could reflect pulses related to magmatic degassing (Figure 6). The microbial community of LSTE1 consist in both types of thermophilic/mesophilic, aerobic/anaerobic organisms, while the microbial community of LSTE2 mainly contains mesophilic and aerobic organisms. Indeed, the archaeal community of LSTE1 is dominated by hyperthermophile/anaerobic sulfate-reducing *Archaeoglobus* sp. and the bacterial community includes some representatives of thermophilic/anaerobic, nitrate/sulfur-reducing organisms such as *Nitratifactor* sp., *Thioreductor* sp. or *Caminibacter* sp., *Thermodesulfator* sp., *Desulfurobacterium* sp. (Campbell et al., 2006; Miroshnichenko, 2004; Miroshnichenko & Bonch-Osmolovskaya, 2006; Nakagawa, Inagaki, et al., 2005; Nakagawa, Takai, Inagaki, Horikoshi, et al., 2005; Sievert & Vetriani, 2012; Voordeckers et al., 2005). LSTE1 also contains mesophilic/microaerophilic, sulfur/sulfide-oxidizing Campylobacterota as *Sulfurimonas* sp., *Sulfurovum* sp., and *Arcobacter* sp. (Campbell et al., 2006; Gevertz et al., 2000; Inagaki, 2003; Inagaki et al., 2004; Wirsen et al., 2002) but in smaller quantities compared to LSTE2 where they are the dominant bacterial species. They are described as primary colonizers and primary producers sometime in symbiotic associations. They also represent one of the most important organic carbon producers in

the ecosystems through dark CO<sub>2</sub> fixation, especially at oxic-anoxic interfaces, like seawater-hydrothermal fluids interfaces (Glaubitz et al., 2010; López-García et al., 2003). Chemical composition of end-members fluids and gases argues for a magmatic degassing occurring during the deployment of LSTE1. This magmatic degassing induced sporadic temperature variations of focused and diffuse emitted fluids, as attested by temperature records from sensors deployed in high and diffuse vents around Eiffel Tower (Figure 6).

Concerning *Archaea*, all the sample contain MGI Thaumarchaeota of *Nitrosopumilus* sp. which are numerically dominant and cosmopolitan within the ocean, and described as mesophilic, aerobic ammonia-oxidizers, using inorganic or organic carbon sources (Könneke et al., 2014; Pester et al., 2011; Swan et al., 2011; Swan et al., 2014; Walker et al., 2010). They may participate to a large extent in the chemoautotrophic production in the deep ocean. Their presence, in variable quantities depending on the sample, may reflect the increasing proportion of seawater in the fluid that surrounded the various samples, that is, very low in Chem2008, middle in Chem2009 and LSTE1, and high in LSTE2. These differences in mixing rate will influence the temperature of the fluids in the different samples and partly explain the differences observed in the different microbial communities.

It has already been mentioned that the physicochemical characteristics of the microbial habitats in a hydrothermal field can control the structure of the microbial community (Nakamura & Takai, 2014; Perner et al., 2007; Reysenbach & Shock, 2002; Takai et al., 2001; Takai et al., 2004; Takai & Horikoshi, 1999; Takai & Nakamura, 2011). The variability of the gaseous components of hydrothermal fluids, in abundance and composition, particularly influences these microbial community structures (Takai et al., 2008). The CO<sub>2</sub> magmatic degassing observed at the end of 2010 and also reported in 2008 (Pester et al., 2012) may also have changed locally this oxic/anoxic interface and the mixing between hydrothermal fluids and seawater, leading to less favorable conditions for the aerobic Campylobacterota in 2008 and 2010. This may explain the low overall abundance of Campylobacterota and in particular the predominance of anaerobic/thermophilic nitrate/sulfur-reducing species in the two samples, Chem2008 and LSTE1, which represent different ecological niches. The combined effect of high temperatures and high CO<sub>2</sub> content of the fluid could have limited the microaerophilic condition required for the growth of most of Campylobacterota, especially the sulfur/sulfide oxidizers. In counterpart, these conditions would have allowed the outbreak of anaerobic sulfide reducing thermophiles, in low abundance, as observed in the bacterial community of LSTE1 colonizer and Chem2008. This enrichment in CO<sub>2</sub> together with the episodic enrichment in H<sub>2</sub> could also explain the abundance of clones related to *Archaeoglobus veneficus*, a chemolithoautotrophic sulfate/thiosulfate reducer, which requires CO<sub>2</sub> and H<sub>2</sub> to grow, in both Chem2008 and LSTE1 samples but totally absent from Chem2009 and LSTE2 samples. Members of *Archaeoglobus* genus, has been described, isolated and cultured from low temperature effluent on the CoAxial Segment of the Juan de Fuca Ridge, Gorda Ridge, or MacDonald Seamount after magmatic eruptions (Holden et al., 1998; Huber et al., 1990; Summit & Baross, 1998).

Chemical and geophysical observation leads us to believe that a tectonic/magmatic event occurred between 2009 and 2010 at Lucky Strike. A magmatic event is also reported in 2008 at Lucky Strike hydrothermal site (Pester et al., 2012). Regardless of the type of substrate and the type of habitat, these magmatic CO<sub>2</sub> inputs (in 2008 and between 2009 and 2010), associated with an increase in fluids' temperatures seems to have contributed to modifying microbial communities' colonizing the high-temperature chimney, as well as the basalts in the more diffuse and mixed zone, by promoting the development of thermophilic and anaerobic *Archaea* and *Bacteria* such as Archaeoglobales or Campylobacterota (Nautiliales and Nitratiruptoraceae). By taking advantage of the observatory status of the Lucky Strike site, more in-depth studies on different hydrothermal outlets within the Lucky Strike field and their monitoring over time, coupled with the recording of temperature data, seismicity, and geochemical data, should provide more precision on the real impact of physicochemical variations on the microbial communities inhabiting the different ecological niches.

## 6. Conclusions

The present study focuses on the detection of geological events occurring at the LSHF, through the modification of chemical and gas composition of hydrothermal fluids, and their potential impact on the microbial communities living around the hot and diffuse vents of the Eiffel Tower site. Here, we highlight a possible link between a magmatic event occurring at depth in the oceanic crust, and the development of

microorganisms living on the seafloor surface, near the hydrothermal vents. The higher CO<sub>2</sub> concentrations observed in 2010, are interpreted as a result of magmatic degassing over the 2009–2010 period which could be correlated with a change in microbial communities over the same period of time, with the emergence of hyperthermophile/anaerobic sulfur-reducing *Achaea* species and thermophile/anaerobic nitrate/sulfur-reducing *Bacteria*, all requiring CO<sub>2</sub> and H<sub>2</sub> to grow.

Fluid sampling is carried out on an annual basis when colonization devices are deployed and recovered. Conversely, the microbial communities of the colonizers display a yearlong exposure to the hydrothermal environment and its variations. We therefore have different scales of observation: chemical point data representative of a “*t*” time, and microbial community composition data resulting of a yearlong interaction with a non-homogeneous fluid. The continuous monitoring of temperature and seismic event from the observatory site, allows us a better dating the occurrence of the magmatic event and then to more confidently link the deep crust event to the seafloor microbial community changes. Our data illustrate the potential influence of magmatic event on microbial communities and emphasize the challenges associated with using molecular microbial analyses that are not appropriately matched to the geochemistry over space and time. By taking advantage of the observatory status of the Lucky Strike site, more detailed studies on different hydrothermal outlets within the Lucky Strike field and their monitoring over time, coupled with the recording of temperature data, seismicity, and geochemical data, should provide a better understanding of the direct impact of physicochemical variations on the microbial communities inhabiting the different ecological niches.

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