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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)

Céline Rommevaux[1,2], Pauline Henri[2], Jefferson Degboe[3], Valérie Chavagnac[3], Françoise Lesongeur[4], Anne Godfroy[4], Cédric Boulart[3,5], Christine Destrigneville[3], Alain Castillo[3]

[1]Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO UM 110, 13288, Marseille, France
[2]Institut de Physique du Globe de Paris - Sorbonne Paris Cité, Université Paris Diderot, CNRS UMR 7154, Paris, France.

[3]Géosciences Environnement Toulouse (GET), UMR 5563 (CNRS/UPS/IRD/CNES), Université de Toulouse, Observatoire Midi-Pyrénées, , Toulouse, France.

[4]Univ Brest, CNRS, Ifremer, UMR 6197, Laboratoire de Microbiologie des Environnements Extrêmes, Plouzané, France.

[5]Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7144, Adaptation et Diversité en Milieu Marin, Équipe Chimie Marine, Station Biologique de Roscoff, 29680 Roscoff, France

Corresponding author: Céline Rommevaux (celine.rommevaux@ mio.osupytheas.fr)

Key Points:

Lucky Strike, Magmatic degassing, Microbial community structure, Multidisciplinary study

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....), 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). We benefit from continuous geophysical monitoring (temperature, 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 experiments over a year for microbial study.

The high CO_2 content and concentrations of major elements (Cl, Ca, Si) and SO_4 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_2 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, 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 and 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_4) 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 physico-chemical 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_2S) [*Von Damm et al.*, 1998; *Charlou et al.*, 2000; *Chavagnac et al.*, 2018b].

Fluxes and composition of hydrothermal fluids also vary in time and space according to subsurface processes such as (1) subseafloor 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. Fornari and Embley*, 1995; *Sohn et al.*, 1998; *Smith et al.*, 2003; *Dziak et al.*, 2004], 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 and 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 [*Takai and Horikoshi*, 1999; *Reysenbach et al.*, 2000; *Takai et al.*, 2006; *Flores et al.*, 2012]. 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 [*Reysenbach et al.*, 2000; 2002; *Takai et al.*, 2006; *Flores et al.*, 2012].

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 et al.*, 2011a]. 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 physico-chemical conditions of the environment. Tectonic or volcanic events may disturb the physico-chemical composition of fluids and thus the microbial diversity. Indeed, at the main Endeavour 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 subseafloor 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 datasets [*Kelley and 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; 2011b], 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 ultra-slow to slow spreading ridge systems like the Mid-Atlantic Ridge, where tectonic activity is prevalent over volcanism, due to the sporadic replenishment of the magmatic chamber.

The Lucky Strike hydrothermal field (LSHF - Mid-Atlantic Ridge, 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 [*Langmuir et al.*, 1997; *Von Damm et al.*, 1998; *Charlou et al.*, 2000; *Pester et al.*, 2012; *Chavagnac et al.*, 2018a; 2018b], geological settings [*Fouquet et al.*, 1994; *Humphris et al.*, 2002; *Singh et al.*, 2006; *Escartín et al.*, 2008; *Ondréas et al.*, 2009; *Barreyre et al.*, 2012; *Escartín et al.*, 2015], macro- and micro-biological communities [*Lee Van Dover et al.*, 1996; *Desbruyères et al.*, 2001; *López García et al.*, 2003; *Cuvelier et al.*, 2009; *De Busserolles et al.*, 2009; *Crépeau et al.*, 2011; *Flores et al.*, 2011; *Cuvelier et al.*, 2011a].

Moreover, since 2010, the deep-sea observatory - EMSO-Azores [*Sarradin et al.*, 2010a; *Colaço et al.*, 2011] – 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 non-cabled 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 (OBSC). 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) [*Sarradin et al.*, 2010a; *Colaço et al.*, 2011]. 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 dataset, we were able to detect the occurrence of a magmatic degassing event in 2010, leading to large CO_2 emissions in the hydrothermal fluids, and strong temperature variations of the hydrothermal fluid discharges. This magmatic CO_2 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 Lucky Strike hydrothermal field

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

The axial magmatic chamber (AMC) is imaged by seismic reflection beneath LSHF [*Singh et al.*, 2006] at about 3 kmbsf 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 AMC [*Singh et al.*, 2006; *Crawford et al.*, 2010].

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 340ºC, or (ii) cracks and vents expelling fluids of lower temperatures (5 to 200º C) [*Langmuir et al.*, 1997; *Von Damm et al.*, 1998; *Cooper et al.*, 2000; *Rouxel et al.*, 2004; *Chavagnac et al.*, 2011; *Barreyre et al.*, 2012].

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_2) [*Charlou et al.*, 2000; *Pester et al.*, 2012].

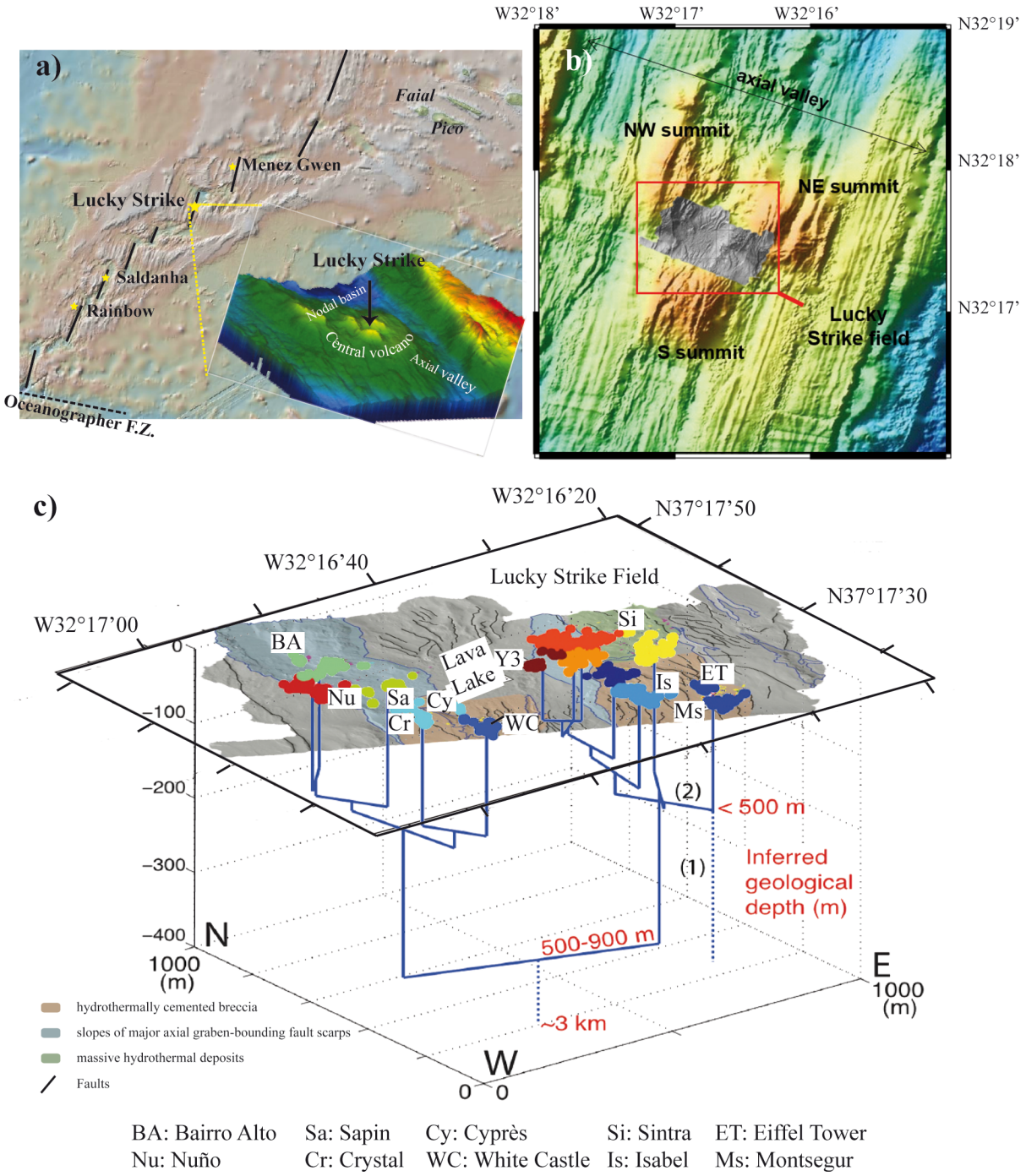


Figure 1: Lucky Strike hydrothermal field location. a) General location of the Lucky Strike segment along the MAR (Global Multi-Resolution Topography [*Ryan et al.*, 2009]) with detailed focus on the axial volcano at the center of the segment where the hydrothermal activity becomes localized (modified 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 diffuses 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]).

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 [*Von Damm et al.*, 1998; *Chavagnac et al.*, 2011; 2018b].

The composition of low-temperature (LT) fluids is interpreted as either a mixing of high temperature hydrothermal fluids with LT seawater [*Wankel et al.*, 2011; *Leleu*, 2017] 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 [*Ondréas et al.*, 2009; *Barreyre et al.*, 2012]. 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 1-c) [*Charlou et al.*, 2000; *Chavagnac et al.*, 2011; *Barreyre et al.*, 2012; *Chavagnac et al.*, 2018b].

Since the discovery and monitoring of LSHF in the late 1990`s, few tectono-volcanic 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_2 concentrations up to 130mM [*Pester 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 [*Escartín*, 2008], Bathyluck2009 [*Escartín and Cannat*, 2009], MoMARSAT2010 [*Sarradin et al.*, 2010b] 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 one 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 200mL or 750mL 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 out-gassing 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 addition, 3 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 and 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 (doi:10.1594/PANGAEA.820343).

2.5 Microbial colonization experiment

The geomicrobiology modules consist of a ballasted plastic holder hosting up to twelve incubators, usually distributed around an autonomous temperature probe (Figure S1-a). Each incubator consists in 50 mL polypropylene BD Falcon™ Conical Tubes with cap, both perforated with ± 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_2, 15.7 Al_2O_3, 11.2 CaO, 7.7 MgO, 12.5 FeO, 2.7 Na_2O, 0.2 K_2O and 1.39 TiO_2.

LSTE1 – without temperature probe - was deployed in Sept. 2009, in a diffuse crack covered by a microbial mat and colonized by mussels, at the base of the Aisics chimney (Figure S1-b). The module was recovered after 13 months (Oct. 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 Oct. 2010 within a diffuse crack presenting similar microbial mat and mussels' patches as LSTE1, only 8 m further away from the Aisics chimney (Figure S1-b). 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 pre-filled with deionized water poisoned with 100 µl $HgCl_2$, 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 µm Millipore®

membrane and dispatched into different plastic LDPE Nalgene® 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, Univ. Toulouse, France) with a SRI 8610C gas chromatograph, fitted with a Flame Ionization Detector/Methanizer (FID-M) for the detection of methane (CH_4), small alkanes, carbon monoxide (CO) and carbon dioxide (CO_2), and a Helium Ionization Detector (HID) for the detection of Hydrogen (H_2), Oxygen (O_2) and Nitrogen (N_2). CH_4 gas is eluted together with CO and CO_2 on a 3' Molecular Sieve Packed Column, using H_2 as a carrier gas, while H_2, O_2 and 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, 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. [*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 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 and Dickson*, 1975; *Mottl and Holland*, 1977; *Seyfried and 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 square 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 (3 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 on board 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 on board in an anaerobic chamber under N_2/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® 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 sub-samples) 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® kit for soil (Obiogene, Inc, CA) with 1g of sample following the modified protocol [*Webster et al.*, 2003], and phenol:chloroform:isoamyl alcohol (PCI) protocol with 8g 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 (M. Le Roy, 2019). 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 supplementary material (Text S1).

All 16S rRNA genes clone libraries were carried out with the TOPO® XL PCR Cloning Kit, with One Shot® TOP10 Chemically Competent *E. coli* (Invitrogen™) 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 supplementary materials (Text S1). The 10 PCR products of each sample were pooled before purification using the QIAquick® 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 µg of total DNA. Pyrosequencing was performed on the Roche* 454 GS FLX* instrument, and demultiplexing and contigs assemblage was carried out by Beckman Coulter genomics (Denvers, Massachussets, 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 National Center of Biotechnology Information (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 ≥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 and Nei*, 1987; *Tamura et al.*, 2004] and the Maximum Likelihood method based on the General Time Reversible model [*Nei and 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 (θ_{YC}) similarity coefficient [*Yue and 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 θ_{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 2 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 (Fig 1c; [*Barreyre et al.*, 2012; *Chavagnac et al.*, 2018b]) and is 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% lost 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 SO_4 end-members concentrations are close to nil mM except for Aisics fluids collected in 2010 (Table S2 and Figure 2-b). 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 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 2-b). 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 [*Von Damm et al.*, 1998; *Charlou et al.*, 2000].

In 2010, the significant increase of Ca and SO_4 concentrations of Aisics hydrothermal end-members correlates to higher strontium (Sr) concentrations reaching a maximum value of 81 µM compared to 73 µM for previous years [*Von Damm et al.*, 1998; *Charlou et al.*, 2000] (Figure 2-b).

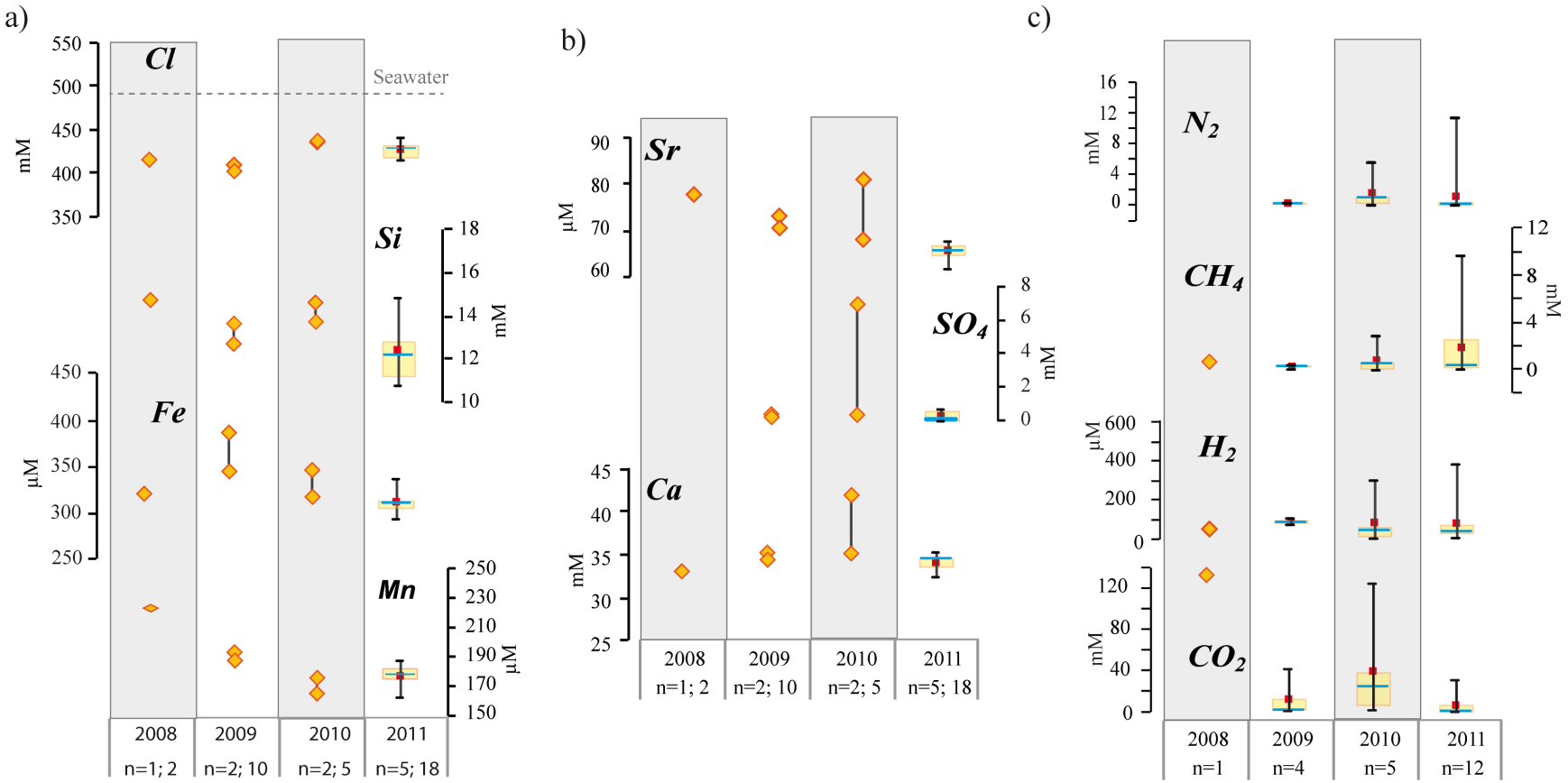


Figure 2: Variations of dissolved elements in pure hydrothermal end-members and dissolved gases concentrations of Eiffel Tower and Aisics fluids through time. a) End-members concentrations of Chloride, Silicon, Iron, Manganese, giving information on rock/water interactions in depth. b) End-member concentrations 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 grey boxes figure the occurrence of possible magmatic events.

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

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 2-a). 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 (S2 Table), leading to a specific end-member value of 2.1 mM for those four outsiders' fluids.

4.3.2 Gases.

All the fluids are enriched in various dissolved gases compared to seawater with gas volume at atmospheric pressure varying from 63 to 1440 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_2) is the main constituent with significant variation from year to year (Figure 2-c).

The most striking features are obtained in 2010 at the Aisics chimney. The CO_2 concentration vary from 1.5 mM to 118.8 mM between fluids sampled at 25 min intervals (Table S3 and Figure 2-c). The maximum value is close to the one reported in 2008 from only one fluid sampled around Eiffel Tower and reaching to a CO_2 concentration of 128.8 mM [*Pester et al.*, 2012].

Methane (CH_4) and nitrogen (N_2) are the most abundant gases after CO_2 with mean concentrations varying from 0.31 to 1.95 mM and from 0.29 to 1.58 mM, respectively. The mean CH_4 concentrations are twice as high than previous values [*Charlou et al.*, 2000]. Overall, from year to year we observed a large dispersion of CH_4 concentration especially in 2011, leading to general increase of the mean concentration through time since 2009, but with median values remaining stable (Figure 2-c). The mean N_2 concentrations are similar to those measured in previous years [*Charlou et al.*, 2000].

Finally, hydrogen (H_2) mean concentrations vary from 51 to 88 µM (Figure 2-c). In 2010 and 2011, H_2 concentration ranges between 2 and 380 µM (Figure 2-c), same order of magnitude than worldwide values of other submarine hydrothermal vents [*e.g.* [*Charlou et al.*, 2000]. Compared to stable H_2 concentrations in 2009, the large dispersion of H_2 concentrations, observed after, leads to a quite stable mean and median values through time.

4.1.3 Hydrothermal fluids temperature

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

Figure 3-b). These variations occurred at the same time as an increase in seismic events was recorded by the Ocean Bottom Seismometer (OBS) network around LSHF (red star Figure 3).

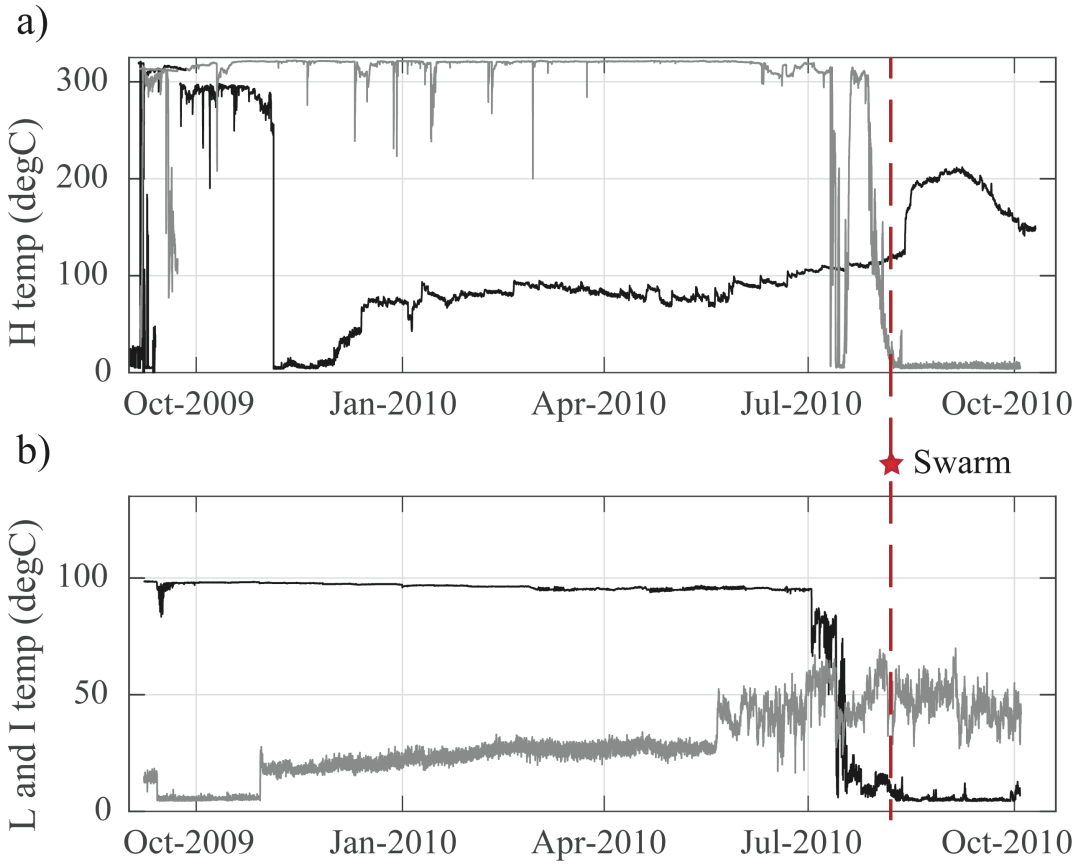


Figure 3: Temperature records for the period Oct.2009- Oct. 2010. a) Temperature data from two HT sensors deployed on black smoker chimneys at Eiffel Tower vent (TE-V02-090905-101003-HW0010B in gray) and Aisics vent (TE-AIS-090923-101010-HN29016b in black). b) Temperature data from a LT sensor deployed 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 LSHF [Crawford et al, 2013].

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 13103 *Bacteria*-related sequences distributed through 810 OTUs. 55 *Archaea*-related sequences distributed through 19 OTUs and 2378 *Bacteria*-related sequences distributed through 819 OTUs were retrieved from LSTE1. Finally, 76 *Archaea*-related sequences distributed through 23 OTUs, and 5233 *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 θ_{YC} was used to illustrate the communities' structure and composition similarities for the bacterial and archaeal libraries.

For *Archaea*, the tree constructed from the θ_{YC} exhibits 2 clusters that underlined the dependency of archaeal communities on substrates (chimney vs basalt substrate) (Figure 4). There is only 2 shared OTU's between Chem08 and Chem09, and 1 between LSTE1 and LSTE2 (venn diagram Figure 4).

For *Bacteria*, the tree constructed from the θ_{YC} exhibits 3 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 3 OTUs (Figure 4).

4.3 Microbial community dynamics at the Eiffel Tower over time.

4.3.1 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 1 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, 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 (Mid-Atlantic Ridge), 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 and Lovley*, 2001; *Tor et al.*, 2001]. One Archaeoglobi sequence is also related at 94% to the isolated strain *Geoglobus ahangari*, a hyperthermophilic iron-reducing archaeon recovered from the Guaymas Bassin 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. 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 thermo-acidophilic, 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, 5). *Aeropyrum sp.*are hyperthermophilic aerobic heterotrophic archaeon retrieved from bot coastal solfataric and deep-sea vent and deep sea vents [*Sako et al.*, 1996; *Nakagawa*, 2004]. *S. marinus* is an hyperthermophilic heterotrophic sulfur-dependent archaeon from Vulcano geothermal sediments

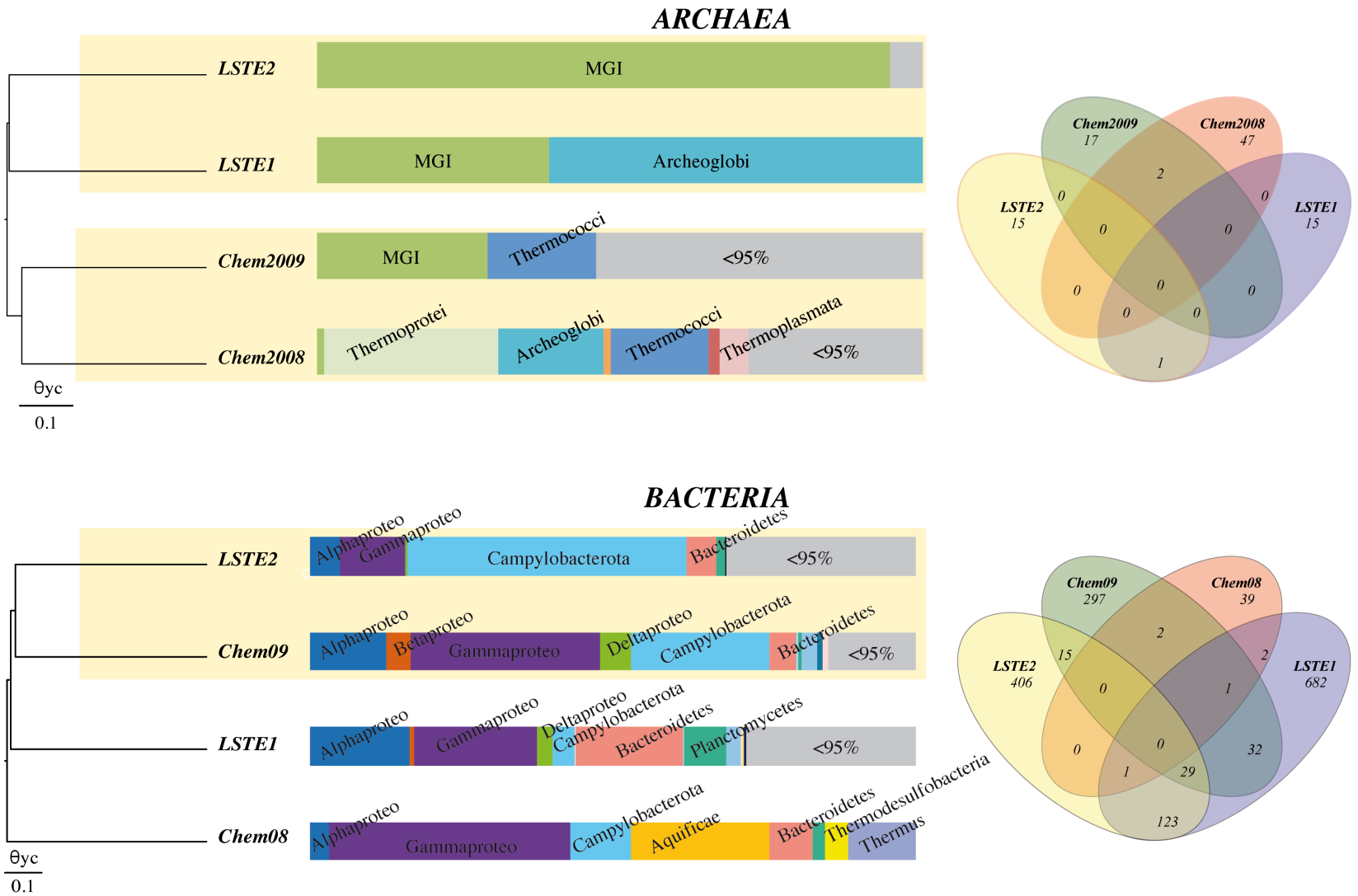


Figure 4: OTU-based analysis of the 4 microbial communities studied. The two dendrograms represent, for *Archaea* and *Bacteria*, the similarity of the samples based on the θ_{YC}, a communities' structure similarity coefficient. The Venn diagrams represent the bacterial and archaeal shared OTU's between the 4 samples.

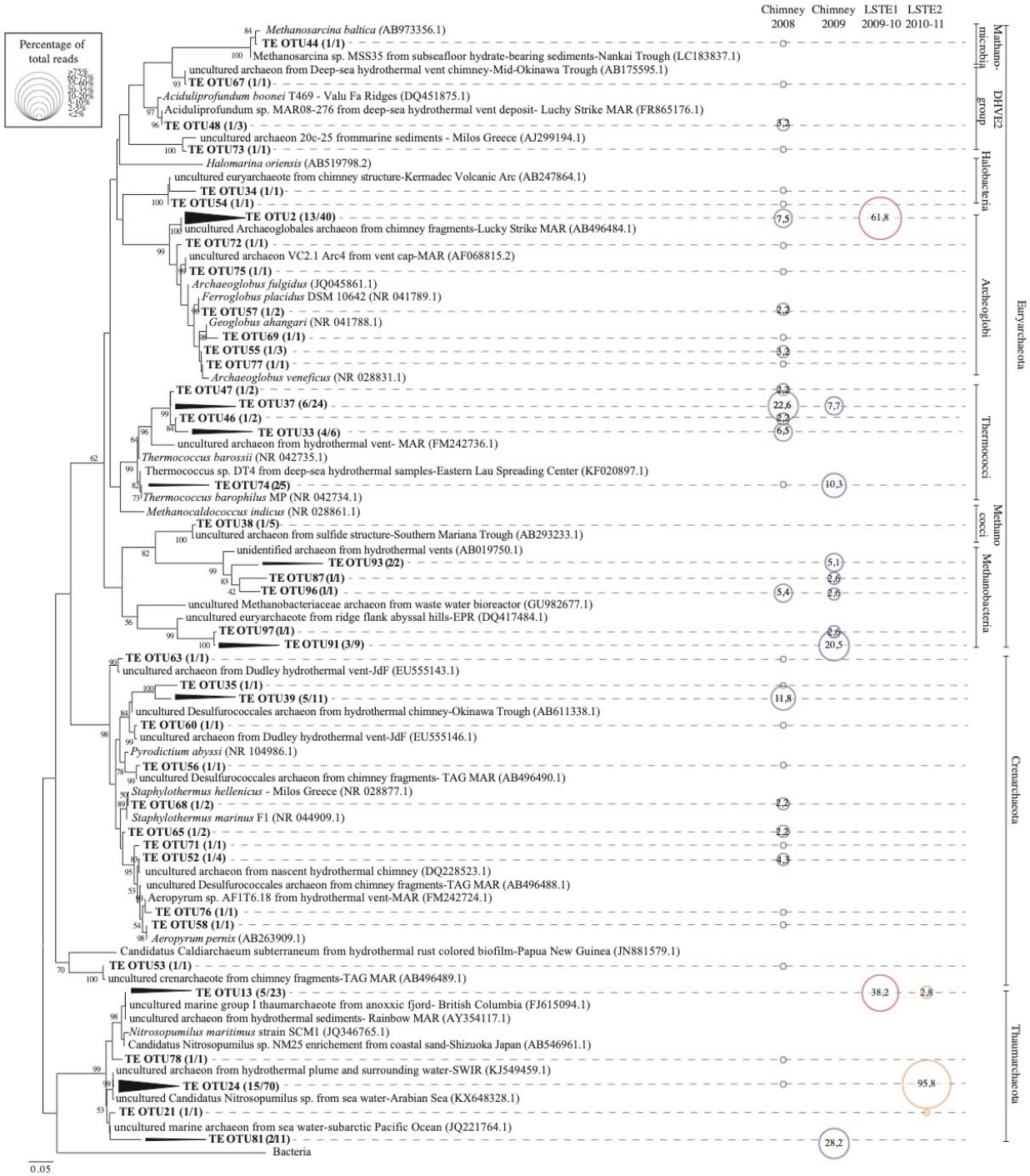


Figure 5: Phylogenetic relationships among the archaeal 16S rRNA gene sequences of representative OTUs of the four samples. Sequences from this study are designed by the prefix "TE". The corresponding number of OTU/sequences for each representative OTU is indicated in bracket. The circle size figures 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.

(Italy) and hydrothermal vent at the East Pacific Rise [*Fiala et al.*, 1986]. *P. abyssi* is an hyperthermophilic heterotrophic archaeon from vents at Guaymas and Kolbeinsey ridge [*Pley et al.*, 1991]. Only 2 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 5 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 9 sequences (6 OTUs). The two other phyla are Deinococcus-Thermus with 2 sequences (1 OTU) and Planctomycetes with 1 sequence (1 OTU). The Campylobacterota sequences are about equally distributed between Campylobacterales (12 sequences / 11 OTUs) and Nautiliales (10 sequences / 5 OTUs) (Figure 4 and Tables S7, S8). Almost all Campylobacterales sequences are related (up to 96%) to uncultured *Sulfurovum* sp. or uncultured *Sulfuromonas* sp. (Figure 6). The closest isolated stains 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 filed in Mid-Okinawa Trough (Japan) [*Nakagawa et al.*, 2005b]. The closest cultivated strain is *Nitratiruptor tergarcus,* a thermophilic, autotrophic nitrate-reducing *Bacteria* isolated from the same place [*Nakagawa et al.*, 2005c]. 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, 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 5 sequences (5 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 2 sequences (1 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].

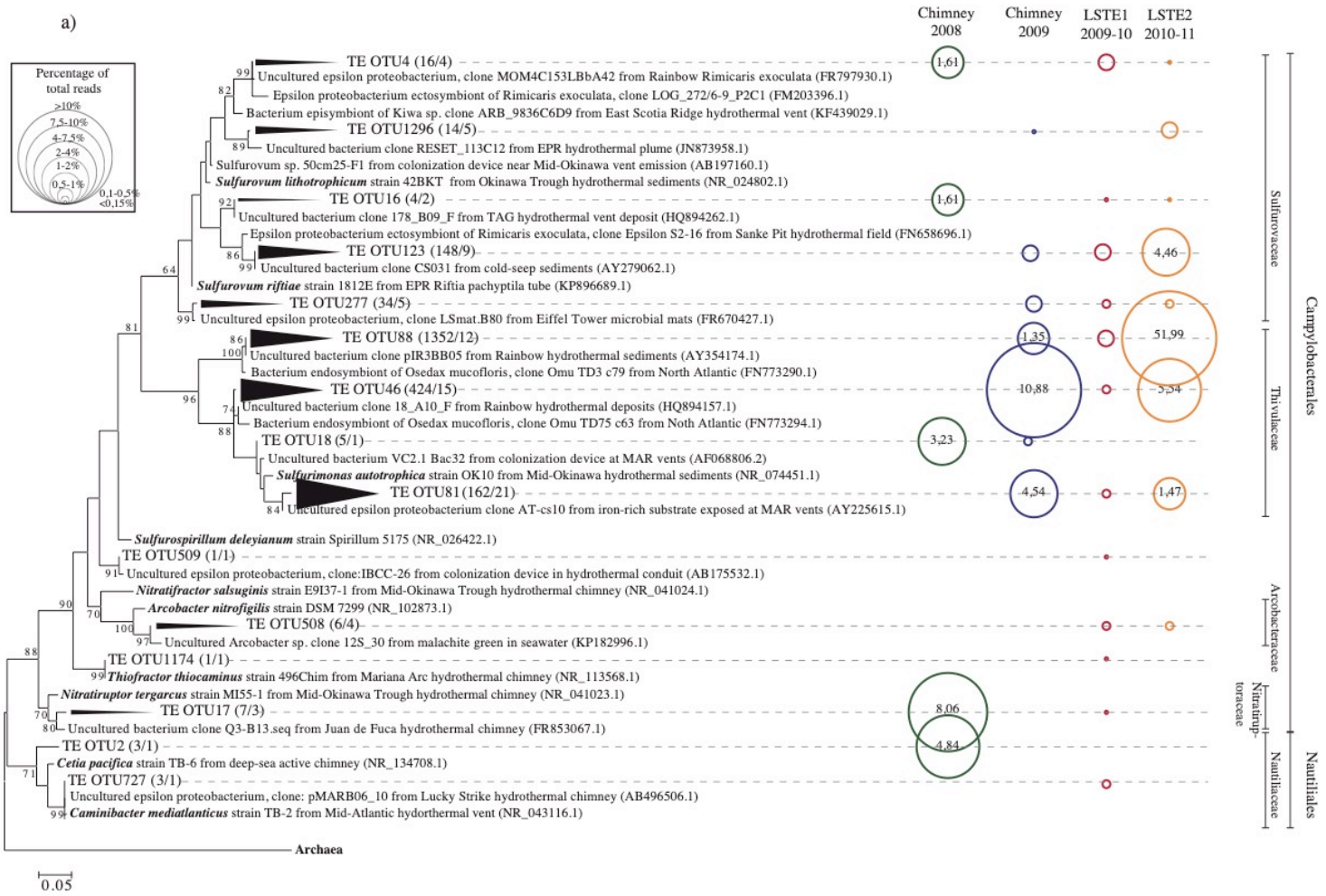


Figure 6: Phylogenetic relationships among the bacterial 16S rRNA gene sequences of representative OTUs of the four samples for Campylobacterota. Sequences obtained in this study are designed by the prefix "TE". The corresponding number of OTU/sequences obtained for each representative OTU is indicated 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 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.

4.3.2 2009 microbial community.

Over the 39 archaeal sequences retrieved from the 2009 chimney, 11 sequences are affiliated to the Thaumarchaeota phylum, 7 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, 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 13103 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, 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_2, or NO_3^- as electron acceptor [*Nakagawa et al.*, 2005b].

4.3.3 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 [*Stetter et al.*, 1987; *Beeder et al.*, 1994]. This species is thermophile (60-85°C), anaerobic strict and grows in presence of H_2 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), micro-aerophilic, and autotrophically oxidize ammonia in the presence of CO_2.

The analyses of the 2378 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, 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 *Haliea 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 (Figure 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 *Sulfovorum sp*. or uncultured *Sulfurimonas sp.* recovered from symbionts-ectobionts from marine macro-fauna (Figure 6). Their cultured representatives are known to be lithoautotrophic (*i.e.* using CO_2 as carbon source) and sulfur oxidizers using O_2 or NO_3^- as electron acceptor [*Nakagawa et al.*, 2005b]. Some sequences belong to the Nautiliales and are affiliated to uncultured *Nitratifractor sp.*, whose cultivated representatives are known to be lithoautotrophic growing by reducing nitrate with H_2 as electron donor [*Nakagawa et al.*, 2005c] or *Caminibacter mediatlanticus* known to be thermophilic growing by reducing nitrate with H_2 as electron donor and CO_2 as carbon source [*Voordeckers et al.*, 2005].

4.3.4 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 Canditatus 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 5233 bacterial sequences indicated a less diversified bacterial community than in LSTE1, Chem2008 and Chem2009 (Figure 4). Retrieved bacterial sequences are affiliated to only 9 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 appears 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, S8). Almost all the Campylobacterota sequences (99,9%) belongs 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 chemolithoautotrophicaly 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_2 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, *i.e.* using methane as sole carbon and energy source [*Lidstrom*, 1988]. A large number of gammaproteobacterial sequences (48% of total gammaproteobacterial 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_2 as carbon source) [*Fujiwara et al.*, 2001; *Duperron et al.*, 2005; *Thiel et al.*, 2012].

5 Discussion

5.1 Modification of physico-chemical 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 and 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_2 concentrations (up to 126 mM) in hydrothermal fluids collected at several sites of LSHF. This high CO_2 concentration, associated with a $\delta^{13}CO_2$ (gases) close to -4‰ is attributed to the replenishment of the magmatic heat source, *i.e.* 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_2 concentration comes with chemical composition variations. Strong CO_2 concentrations were measured in the 2010 hydrothermal fluids at Eiffel Tower site, with concentrations reaching up to 118 mM (nearly four times higher than previous years [*Charlou et al.*, 2000]) and also at other LSHF vent sites, *e.g.* Cyprès and Crystal located south west of the fossil lava lake (data not shown, C. Boulart pers. Comm.). Variations of CH_4 and CO_2 concentrations are correlated while they are anti-correlated with variations of hydrogen concentrations. This suggests that CH_4 was produced abiotically through the Sabatier reaction (reduction of CO_2 by hydrogen). Variations of N_2 concentrations follow the variations of CH_4 and CO_2, substantiating that a magmatic degassing occurred in 2010. Meanwhile, hydrothermal fluids exhibit chemical composition variations as evidenced by increasing Cl, Ca, SO_4 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 up-flow 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 Lucky Strike hydrothermal field (S1 table).

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 down-flowing 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_4 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_2) together with increasing Cl, Ca, SO_4 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 pre-eruption 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_2 and H_2. Similar variations of chemical composition were also observed at the Juan de Fuca Ridge, where the Main Endeavour Field underwent an intense seismic activity in June 1999 [*Seewald et al.*, 2003]. The dissolved concentrations of CO_2, H_2, and H_2S of the focused fluids collected in September 1999 and June 2000 increased in the pre-earthquake samples and decreased during the following year until reaching their pre-eruption 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 [*Von Damm*, 1995; *Butterfield et al.*, 1997; *Von Damm et al.*, 1997; *Seewald et al.*, 2003]. It depends on the timeframes at which fluid sampling was carried out, *i.e.* 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 and Sohn*, 2016] and also to seismic events' records through the deployment of Ocean Bottom Seismometer (OBS) network around the Lucky Strike field [*Crawford et al.*, 2013]. We report variations observed on a sub-sample of HT and LT sensors deployed around Eiffel Tower and Montsegur sites, which are not systematic on all the sensor deployed around these two sites during this same time period [*Barreyre et al.*, 2014a]. An increase of seismic activity is detected through the OBS network during the same period. Variations of vent temperature have already been reported after tectonic/magmatic events at Juan de Fuca ridge and East Pacific Rise [*Sohn et al.*, 1998; *Johnson et al.*, 2000; *Dziak et al.*, 2003]. 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. Although a lack of correlation between seismic events and temperature variations is not evidence at the LSHF over the 2009-2012 period [Barreyre et al. 2014a], these concordant observations over different geophysical data could be related to the occurrence of the magmatic degassing, which could occur during the summer of 2010, just before the hydrothermal fluid sampling of MoMARSAT2010 cruise in October [*Sarradin et al.*, 2010b].

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 and 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 θ_{YC} 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 (Fig. 3). The comparison of bacterial communities between samples is then biased by the fact that we only have a small dataset 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 (pers. comm. D. François). The comparison of bacterial communities across the dendrogram based on θ_{YC} 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…) [*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 et al.*, 2011b]. 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 tholeitic-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/hyperthermophylic anaerobic and lithoautotrophic species from Methanomicrobia, Archaeoglobi, Thermococci, Aquaficae, 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_2 concentrations and enrichment in dissolved chloride relative to seawater, interpreted as a replenishment of magmatic heat source, *e.g.* 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, 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 physico-chemical 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' 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-April 2010, which could reflect pulses related to magmatic degassing (Fig. 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 themophilic/anaerobic, nitrate/sulfur-reducing organisms such as *Nitratifactor* sp., *Thioreductor* sp. or *Caminibacter* sp., *Thermodesulfatator* sp., *Desulfurobacterium* sp. [*Miroshnichenko*, 2004; *Voordeckers et al.*, 2005; *Nakagawa et al.*, 2005a; 2005c; *Campbell et al.*, 2006; *Miroshnichenko and Bonch-Osmolovskaya*, 2006; *Sievert and Vetriani*, 2012]. LSTE1 also contains mesophilic/microaerophilic, sulfur/sulfide-oxidizing Campylobacterota as *Sulfurimonas* sp., *Sulfurovum* sp. and *Arcobacter* sp. [*Gevertz et al.*, 2000; *Wirsen et al.*, 2002; *Inagaki*, 2003; *Inagaki et al.*, 2004; *Campbell et al.*, 2006] 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 producer in the ecosystems through dark CO_2 fixation, especially at oxic-anoxic interfaces, like seawater-hydrothermal fluids interfaces [*López-García et al.*, 2003; *Glaubitz et al.*, 2010]. 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 (Fig 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 [*Walker et al.*, 2010; *Pester et al.*, 2011; *Swan et al.*, 2011; *Könneke et al.*, 2014; *Swan et al.*, 2014]. 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, *i.e.* 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 physico-chemical characteristics of the microbial habitats in a hydrothermal field can control the structure of the microbial community [*Takai and Horikoshi*, 1999; *Takai et al.*, 2001; *Reysenbach and Shock*, 2002; *Takai et al.*, 2004; *Perner et al.*, 2007; *Takai and Nakamura*, 2011; *Nakamura and Takai*, 2014]. 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_2 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_2 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_2 together with the episodic enrichment in H_2 could also explain the abundance of clones related to *Archaeoglobus veneficus*, a chemolithoautotropic sulfate/thiosulfate reducer, which requires CO_2 and H_2 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 [*Huber et al.*, 1990; *Holden et al.*, 1998; *Summit and Baross*, 1998].

Chemical and geophysical observation leads us to believe that a tectonic/magmatic event occured 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_2 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 physico-chemical 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 Lucky Strike hydrothermal field, 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_2 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_2 and H_2 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 year-long 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 year-long 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 physico-chemical variations on the microbial communities inhabiting the different ecological niches.

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The raw temperature data are publicly available (doi:10.1594/PANGAEA.820343). 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 National Center of Biotechnology Information (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.

The repartition of *Archaea* and *Bacteria* sequences and OTUs up to Order, gases and chemical composition in major and trace elements of diffuse and high temperature hydrothermal fluids are presented as supplementary data.

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