

# Microbial lipids in artificial and natural hydrothermal chimneys of the Iheya North field, Okinawa Trough

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**Microbial lipids in artificial and natural hydrothermal chimneys  
of the Iheya North field, Okinawa Trough**

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A Thesis for the Degree of Doctor of Science

March 2023

沖縄トラフの伊平屋北フィールドにおける  
人工および天然の熱水チムニー中の微生物膜脂質

**The Dissertation for a Degree of Doctor of Science**  
**Kyushu University**

Title:

**Microbial lipids in artificial and natural hydrothermal chimneys of the  
Iheya North field, Okinawa Trough**

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## Abstract

Hydrothermal chimney structures characteristic of seafloor hydrothermal vents are the physiologically diverse and high productive microbial habitats at the light energy-limited deep-sea environments. As primary producers of the ecosystem, microorganisms support almost all life living in deep-sea hydrothermal vent environments. According to the recent study, an annual global biomass production at active hydrothermal vent fields is estimated to reach 1.4 Tg of carbon, significantly contributing to its geochemical cycling at the deep-sea environment. While the diversity of microbial communities associated with hydrothermal chimney and their biogeochemical functions have been well documented in recent years, the processes of formation and development of microbial communities are not well understood for a hydrothermal chimney from its deposition to the end of hydrothermal activity. The environmental factors controlling microbial activity are also poorly understood due to the unpredicted new hydrothermal fluid discharge and chimney formation, and the difficulty of temporal sampling.

Therefore, the present study focused on the microbial communities developed in artificially formed hydrothermal chimney structures by deep-sea drillings (artificial chimneys) at an active hydrothermal vent field in the Iheya North Knoll of the mid-Okinawa Trough. In September 2010, deep-sea drilling was conducted at total five sites (Site C0013-C0017) along the slope of the Iheya North knoll, including the summit of an active hydrothermal mound (Hole C0016A) and its flank part (Hole C0016B) by the Integrated Ocean Drilling Program (IODP) Expedition 331. Among the those drilling site, the rapid growth of sulfide mineral-rich chimney structures was observed at Hole C0016A and Hole C0016B. Because their formation ages are known (i.e., time periods after the drilling) and the mineralogical and physicochemical properties of chimney bodies and vent fluids have been followed and investigated during the post-drilling surveys, those artificial chimneys were considered to be good candidates for the study of microbial colonization and succession process in the newly formed hydrothermal chimneys. However, the previous analysis of the artificial chimneys 11 and 18 months after the drilling indicated that detectable microbial communities were not developed despite several studies dedicated to the colonization processes in hydrothermal chimneys based on *in situ* culture experiments that microorganisms quickly colonize the initially sterile chimney structures and get established within a short period of time (< 14 days). To date, it remains unknown microbial community structures, biomass and activities associated with those artificial chimneys.

The objectives of this study are characterization of microbial community assemblages, biomass inhabiting in those artificial chimneys formed after deep-sea drilling, and to clarify the microbial succession process in the hydrothermal chimneys. To accomplish the objectives, microbe-derived intact polar membrane lipids (IPLs) and core lipids (CLs) composition, as indicator for active and fossil microorganisms, were investigated in two artificial chimneys, two natural chimneys, a sulfide ore deposited on the sedimentary seafloor and a hydrothermally altered surface sediment, which were collected in 2018, 8 years after the drilling by IODP Expedition 331. Total organic carbon (TOC) and total nitrogen (TN) contents, and stable carbon isotope ratios of TOC ( $\delta^{13}\text{C}_{\text{TOC}}$ ) of those samples were measured to evaluate the major sources of organic matters and to estimate microbial biomass. In addition, mineralogical analysis was also performed to constrain on each chimney habitat.

This work began with the development of a new method for simultaneously analysis of CLs and IPLs in the total lipid extracts from various biological and natural samples using normal-phase high-performance liquid chromatography coupled with high resolution mass spectrometry equipped heated electrospray ionization (NP-HPLC/HESI-HRMS). In this analytical approach, chromatographic separation patterns can be compared to conventional NP-HPLC methods for the separation of each form of lipid, and by estimating chemical composition using accurate mass of HRMS, a wide range of core and polar lipids in a sample can be separated and detected in a single analysis.

This simultaneous analytical method was used for the study of microbial communities developed in the artificial chimneys of the Iheya North field. In Hole C0016A artificial chimney, IPLs derived from *Archaea* were dominant, in good agreement with their distribution in natural chimney sample from Hidarite-ha-Soerudake Chimney (HSC) of Aki site. In these chimneys, HGDGTs-IPLs, a typical biomarker of thermophilic *Archaea*, is major, being consistent with a mineral assemblage dominated by high temperature sulfide minerals. On the other hand, in Hole C0016B artificial chimney, non-archaeal IPLs were dominant and archaeal-IPLs were minor. Their IPL distribution was similar to natural chimney sample from High Radioactive Vent (HRV) of Original site, with a predominance of bacteria and minor methanogenic archaeal activity. These chimneys contained carbonate and sulfate minerals suggesting mixing or alteration by seawater under relatively low temperatures. On the other hand, the reference samples had abundant non-archaeal IPLs with simple distribution compared to chimney samples. Regardless of the high TOC concentrations, the abundance of archaeal-IPLs and -CLs as well as non-archaeal CLs were trace or undetectable. The  $\delta^{13}\text{C}_{\text{TOC}}$  values in the reference samples was lower than that of normal marine sedimentary organic matter, suggesting active

decomposition of organic matter by bacteria. The low TOC and TN concentrations, small TOC/TN ratios, and relatively higher TOC carbon isotopic composition of the artificial and natural chimneys clearly distinguish the bulk organic matter composition from the reference samples, with little or negligible organic matter contribution from the sedimentary environment. Furthermore, the positive correlation between TOC and TN concentrations in the artificial and natural chimneys and the TOC/TN ratios ( $\sim 5.5$  by atomic) suggest that the organic matter in these chimneys is derived primarily from microbial cells.

Based on the carbon and nitrogen content of cells estimated from natural thermophilic microbial communities, the TOC and TN concentrations of these artificial and natural chimneys were used to calculate biomass, suggesting that there is a nearly equivalent microbial biomass ( $\sim 10^8$  cells/g and  $\sim 10^7$ - $10^8$  cells/g, respectively). Therefore, it is likely that thermophilic archaeal communities in the high temperature region and diverse bacterial communities in the mixed seawater-thermal water region developed in the two artificial chimneys at 8 years after the drilling. The estimated biomass of artificial chimneys is similar to that of the surrounding natural chimneys and with a common IPL composition in each. Although these results are rough estimates based on several assumptions and a limited number of samples, this study has provided a timescale for the development of mature microbial communities similar to natural chimneys in newly formed chimneys by deep-sea drilling.

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## **Chapter 1. General introduction**

### **1.1. Overview of submarine hydrothermal systems**

A deep-seafloor hydrothermal circulation is an active water-rock reaction system, in which hydrothermal fluids circulate at the interface between the lithosphere and hydrosphere, originating from magmatic activity beneath the seafloor (e.g., Humphris et al., 1995). The submarine hydrothermal system is divided into three zones: i) recharge zone, ii) reaction zone, and iii) upwelling zone (or discharge zone). i) The recharge zone is where seawater percolates down to the seafloor along fractures of the oceanic crust. ii) The reaction zone is where seawater that has penetrated deep beneath the seafloor is heated by magma and reacts with surrounding rocks. In this zone, cold and oxidizing seawater is transformed into a hot, reduced, and metal-rich hydrothermal fluid through processes such as water-rock reactions at high temperatures and pressures, and the supply of volatiles from magma (e.g., Gamo et al., 1997; Tivey, 2007). Finally, the heated and buoyant hydrothermal fluid rises and erupts from the seafloor. This area is iii) upwelling zone (or discharge zone). When the metal-rich and reduced hydrothermal fluid is discharged into cold and oxidizing seawater, metal ions (e.g., iron, lead, copper, zinc) precipitate primarily as sulfide and sulfate minerals, forming the “chimney” structures on the seafloor (e.g., Tivey, 1998; Haymon, 1983).

To date, more than 700 marine hydrothermal vent fields have been discovered on the Earth, and most of them are distributed at water depths below 200 m (Beaulieu et al., 2020). In general, submarine hydrothermal activity is unevenly distributed in areas of magmatic activity associated with plate tectonics such as the mid-ocean ridge, island- and back-arc systems (Hannington et al., 2011). The mid-ocean ridge system is most active site of submarine hydrothermal activity on the Earth. About 60% of the Earth's volcanic activity occurs at the mid-ocean ridge, most of which is submarine volcanism (Fisher and Schmincke, 1984). At the mid-ocean ridge system, the mantle rises to fill the rift created by the pull of the oceanic plate, and its partial decompression and melting generates basaltic magma, leading to volcanism and the formation of new oceanic crust. Submarine hydrothermal activity at the mid-ocean ridge is generally based on rocks of relatively homogeneous chemical composition, called mid-ocean ridge basalt (MORB) but also involves hydrothermal systems where mantle peridotite rises to the seafloor surface and is exposed (e.g., Charlou et al., 2002). The island-arc and back-arc basin system is also the most volcanically active region. In the island-arc-back-arc basin system, dehydrated water from the oceanic crust deeply subducted beneath the continental crust lowers the melting point of the mantle, resulting in partial melting of the mantle and lower crust, and magmatic activity (Hannington et al., 2011). Hydrothermal active areas in the island-arc and

back-arc basins are often located at depths shallower than 2,000 m. The composition of host rocks in the hydrothermal active areas of the island-arc and back-arc basins is more diverse than that of the mid-ocean ridge, which is mainly the MORBs. In this study, the Iheya North hydrothermal field, located in the central part of the Okinawa Trough, a back-arc basin in the East China Sea, was selected as the study area.

## **1.2. Biological systems at deep-sea hydrothermal vents**

Despite the extreme conditions of darkness, high hydrostatic pressure and steep gradient of cold ( $\sim 2^{\circ}\text{C}$ ) to extremely high temperatures ( $\sim 300\text{--}400^{\circ}\text{C}$ ) in hydrothermal active areas of deep seafloor, it has known that high density and productive ecosystems unique to there exist (e.g., Van Dover, 1995; Van Dover, 2000). This submarine hydrothermal ecosystem isolate from solar energy is supported by the active primary production of free-living and symbiotic chemolithoautotrophic microorganisms that utilize inorganic substrates such as  $\text{CO}_2$ ,  $\text{H}_2$ ,  $\text{H}_2\text{S}$ ,  $\text{CH}_4$  and metal ions (e.g.,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ) derived from hydrothermal vent fluids (e.g., Takai et al., 2006; Schrenk et al., 2008). In this environment, the rapid mixing of reducing hydrothermal fluids with oxidizing seawater creates a chemically non-equilibrium state that allows chemolithoautotrophic microorganisms to obtain energy through various redox reactions (McCollom and Shock, 1997; Takai and Nakamura, 2010; Amend et al., 2011). Microorganisms inhabit almost every niche associated with active hydrothermal environments, including chimney structures, hydrothermal plumes, vent fauna communities and the subsurface hydrothermal circulation zones (e.g., Schrenk et al., 2003; Nakagawa et al., 2005; Pagé et al., 2005; Yanagawa et al., 2015; Yanagawa et al., 2017). The hydrothermal chemosynthetic ecosystems have attracted much attention since their discovery because of their unique biological activities that strongly rely on the Earth's internal energy, and because of their relevance to early life on the primitive Earth and to the evolution of life on Earth and also as model ecosystems for extraterrestrial life (e.g., Baross and Hoffman, 1985; Martin et al., 2008; McCollum, 1999).

Submarine hydrothermal activity is a global process that plays an important role in the exchange of heat and various chemicals between the Earth's interior and the oceans. Therefore, it has a significant impact on global marine biogeochemical cycles from the deep-sea to the ocean surface. The potential for primary production by chemosynthesis in deep-sea bottom hydrothermal vents is estimated to be  $10^{13}$  g of biomass/year, which corresponds to approximately 0.02% of the primary production by photosynthesis in the ocean surface layer (McCollom and Shock, 1997). According to the recent study, an annual global biomass

production at active hydrothermal vent fields including the sub-vent biosphere is estimated to reach 1.4 Tg of carbon, significantly contributing to its geochemical cycling at the deep-sea environment (McNicol et al., 2018). Furthermore, it has been suggested that microbial activities are also involved in the processes of precipitation, oxidation, and dissolution of sulfide minerals that constitute submarine hydrothermal deposits. For example, Nozaki et al. (2020) discovered pyrite grains with extremely low sulfur isotopic compositions in core samples collected from a submarine hydrothermal active zone and suggested that the sulfur source was sulfate ions in seawater reduced by microbial metabolism, indicating that the initial formation process of submarine hydrothermal deposits is partly induced and promoted by microbial activity. Thus, it has been revealed that microbial activities in the seafloor hydrothermal active zone contribute significantly to the cycling of various organic and inorganic elements in the boundary region between the hydrosphere and the lithosphere. Furthermore, the thermostable enzymes produced by thermophilic microorganisms living in hydrothermal environments have excellent industrial versatility and are useful as genetic resources (e.g., Christopher et al., 2010).

Studies on the diversity and physiological functions of microbial communities inhabiting submarine hydrothermal vent environments (mainly, chimney structures) around the world have been intensively conducted, mainly using culture and molecular biological techniques. In a well-established hydrothermal chimney, various microbial activities ranging from mesophilic to hyperthermophilic archaea and bacteria are identified in cultural or non-cultural studies using phylogenetic techniques (e.g., Flores et al., 2011; Kato et al., 2010; Schrenk et al., 2003; Takai and Horikoshi, 1999; Takai et al., 2001). Distinct microbial communities are also observed between inter-chimneys in a single hydrothermal field (e.g., Flores et al., 2012; Nakagawa et al., 2005; Takai et al., 2009). This variation of microbial communities should depend on the physical and chemical characteristics of the chimneys at the hydrothermal fields (e.g., Flores et al., 2011; Flores et al., 2012; Nakagawa et al., 2005; Roussel et al., 2011). On the other hand, the temporal changes in the distribution, abundance, diversity, and physiological functions of microbial communities in chimneys from the onset of hydrothermal discharging to their cessation, and the factors controlling them, remain largely unknown. Several *in situ* cultures and experimental studies in the early stages (< a few months) of natural chimney formation have reported the emergence of hyperthermophilic archaea within a few days of chimney formation and changes in microbial community composition with changes in temperature and mineral composition (e.g., McCliment et al., 2006; Pagé et al., 2008; Reysenbach et al., 2000; Wang et al., 2009). Recently, Hou et al. (2020) performed metagenomic analyses of active and inactive chimneys and showed that the primary energy source for microbial activity may shift

to hydrothermal fluids and to chimney ore bodies after cessation of hydrothermal discharging within timescales of a few years, and that microbial communities may remain stable for the following thousands of years.

### **1.3. Deep-sea drillings and artificial chimney formation at an active hydrothermal vent field**

To date, anthropogenic hydrothermal discharges have been observed during several field seafloor operations (Cowen et al., 2003; Gieskes et al., 2002; Marumo et al., 2008; Kato et al., 2012; Takai et al., 2012). Artificial chimney structures formed by artificial hydrothermal vents and boreholes in hydrothermally active areas have a known formation age (i.e., the period from the completion of drilling), and their temporal changes can be traced from the beginning of hydrothermal discharges. Therefore, artificial chimneys are the best target to study the temporal changes of microbial communities in chimneys from the beginning to the cessation of hydrothermal venting and the factors controlling them, which have not been clarified so far.

During IODP Expedition 331 in 2010 in the Iheya North Knoll of the mid-Okinawa Trough, artificial hydrothermal vents were intentionally created as a platform to study post-drilling changes in hydrothermal activity and the effects of drilling on the chemosynthetic ecosystem, and chimney structures were formed directly above them (Takai et al., 2012). Therefore, post-drilling observations of the artificial hydrothermal vents have been continuously carried out, and many research results on the temperature and chemical composition of those hydrothermal vents, mineralogical characteristics, and variations in the distribution of chemosynthetic invertebrates have been reported (Kawagucci et al., 2013; Nakajima et al., 2015; Nozaki et al., 2016; Nozaki et al., 2021). Although experimental studies of microbial colonization in the early stages of natural chimney formation have observed the emergence of microorganisms within a few days, in the artificial chimneys, detectable microbial communities had not developed in the artificial chimneys produced by IODP Expedition 331 even 11, 18 months after the end of drilling (Kawagucci et al., 2013). To date, no information on the microbial community of the artificial chimney has been available.

Artificial chimneys generated by IODP Expedition 331 have a rapid growth rate in a short period of time and are rich in polymetallic sulfide minerals except for the outermost part, which is an unusual mineralogical feature for a new chimney (Nozaki et al., 2016). Such rapid growth of sulfide mineral-rich chimney structures has been observed in events in which hydrothermal flow rates were suddenly and rapidly increased by swarm earthquakes or submarine volcanic eruptions (McClain et al., 1993; Kelly et al., 2012). Therefore, the unique

mineralogy of artificial chimneys may be attributed to the formation of extremely large hydrothermal channels, which are rarely found in nature, by drilling, and the enhancement of hydrothermal flow rate and the mixing process between hydrothermal fluids and seawater (Nozaki et al., 2016). In addition, the artificial chimneys have numerous flange structures, and the trapping of hydrothermal fluids in them may also have contributed to the enhanced precipitation of sulfide minerals (Nozaki et al., 2016). Furthermore, the hydrothermal fluids are considered to be trapped in the lower part of hydrothermal reservoir at the seafloor due to gas-liquid phase separation (boiling) and density partitioning under the seafloor, because of their higher Cl concentration and chemical composition depleted in gas components compared to seawater (Kawagucci et al., 2013). Nakagawa et al. (2005) showed that the number of viable microorganisms, especially chemosynthetic microorganisms, was relatively lower in the natural chimney structure of the Iheya North hydrothermal field where hydrothermal fluids with low gas composition were depleted than in the surrounding chimney structures, and this was attributed to the lack of gas composition to support the activities of chemosynthetic microorganisms. This was attributed to the lack of gaseous components that support the activity of chemosynthetic microorganisms. These facts suggest that the specificity of the formation process, structural features, and chemical composition of the hydrothermal effluent of artificial chimneys may be a rate-limiting factor for the development of microbial communities in them. Therefore, it is appropriate to regard artificial chimneys as a special submarine hydrothermal environment formed by anthropogenic operations, which is different from natural chimneys.

#### **1.4. Lipid biomarkers**

Recent studies of microbial communities in deep-sea hydrothermal vent ecosystems have widely applied culture experiments and gene-based approaches. Membrane lipids, the essential components of cellular membranes of microorganisms, have also been used as a powerful molecular tool to investigate modern and ancient microbial ecosystems in various environments.

The plasma membrane of prokaryotic cells is a semipermeable membrane that protects the cellular contents from the external environment and consists of bilayers and/or monolayers of amphiphilic membrane lipids (intact polar membrane lipids; IPLs). In addition, the membrane incorporates proteins, which play roles in selective material transport into and out of the cell, signal transduction, and energy transduction. The major membrane lipids are glycerol-based lipids, which consist of 1) a hydrophobic portion consisting of two fatty acids and/or alcohols bonded via esters or ether bonds to two hydroxyl groups of glycerol (core lipid;

CL) and 2) a hydrophilic polar head group (e.g., derivatives of phosphates, glycosides, amino acids or sulfates) attached to the remaining hydroxyl group of glycerol. In general, the glycerol-based membrane lipids constitute ~2-6% of a procaryotic cell in dry weight (Langworthy et al., 1983).

The glycerol backbones of glycerol-based membrane lipids in *Archaea* and other biological domains (i.e., *Bacteria* and *Eukarya*) are stereoisomeric with respect to each other, and this is widely accepted as one of the general concepts distinguishing these biological domains (Kates, 1993; Koga and Morii, 2007). Archaeal membrane lipids consist of ether-type lipids with isoprenoid alcohols ether-linked to the *sn*-2, *sn*-3 positions and a polar head group attached to the *sn*-1 position of a glycerol backbone. On the other hand, the typical membrane lipids of *Bacteria* and *Eukarya* consist of an ester-type lipid with fatty acids ester-linked to the *sn*-1, *sn*-2 positions of a glycerol backbone and a polar headgroup attached to the *sn*-3 position.

Despite the lack of phylogenetic specificity of 16S rRNA genes, the distribution of core lipids and polar heads has been successfully used to correlate lipid structure with source organism, biological processes, and environmental conditions. IPLs are suitable biomarkers for viable microorganisms because the polar head groups are hydrolyzed and removed from core lipids (CLs) soon after the cell death (White et al., 1979; Harvey et al., 1986; Logemann et al., 2011). Thus, lipid biomarkers have been used as another indicator to study microbial communities in submarine hydrothermal environments (e.g., Hedrick et al., 1992; Blumenberg et al., 2007, Blumenberg et al., 2012; Bradley et al., 2009; Gibson et al., 2013; Reeves et al., 2014). Gibson et al. (2013) showed that IPLs, although less taxonomically specific than 16S rRNA genes, are useful for differentiating microbial communities in chimney structures in submarine hydrothermal active areas at the domain or kingdom level (possibly even family level) and have the potential to quantify microbial communities. On the other hand, CLs structure is relatively inert and preserved for a long time to record past signals (e.g., Shouten et al., 2002; Shouten et al., 2007). Therefore, the occurrences of IPLs and CLs are regarded as active and dead signals, respectively. Furthermore, the membrane lipid compositions of each microorganism will change depending on environmental factors such as temperature, pH, salinity and availability of nutrients (e.g., Meador et al., 2014; Shimada et al., 2008; Uda et al., 2001). Therefore, the variation of IPLs and CLs in hydrothermal chimneys can be used to evaluate the present and past activity for each microorganism.

### **1.5. The objectives of this thesis**

In this study, we compared the lipid biomarker composition, organic element composition, and mineral composition of artificial chimneys and surrounding natural chimneys

eight years after drilling by IODP Expedition 331 to characterize the biomass and community composition of microbial communities that develop in artificially formed submarine hydrothermal vent environments. We attempted to characterize the biomass and composition of microbial communities that develop in artificial submarine hydrothermal vent environments and to reconstruct the developmental history of these microbial communities by comparing the compositions of lipid biomarker, organic elements and stable carbon isotope ratios, and mineral assemblages of natural chimneys around the artificial chimneys.

## **Chapter 2. Simultaneous analysis of core and polar lipid biomarkers by high-performance liquid chromatography/high resolution mass spectrometry**

This section was originally published as; Horai, S., Yamauchi, N., Naraoka, H. (2019) Simultaneous total analysis of core and polar membrane lipids in archaea by high-performance liquid chromatography/high-resolution mass spectrometry coupled with heated electrospray ionization. *Rapid Communications in Mass Spectrometry*, **33**, 1571–1577.

### **2.1. Introduction**

The membrane lipids of archaea have characteristic structures that distinguish them from bacteria and eukaryotes. Archaeal membrane lipids are composed of glycerol di- and/or tetraethers, with isoprenoid alcohols attached to the *sn*-2 and *sn*-3 positions of the glycerol backbone (i.e., core lipids; CL) and a polar head group attached to the *sn*-1 position (Kates, 1993; Koga and Morii, 2007). The stereochemistry of the glycerol backbone in archaeal lipids is enantiomeric to that of bacterial and eukaryotic lipids. Therefore, the presence of archaea in natural samples has generally been evaluated by the occurrence of membrane lipids as well as DNA/RNA sequences. Because polar head groups are rapidly hydrolyzed after cell death (White et al., 1979; Harvey et al., 1986; Logemann et al., 2011), intact membrane lipids (IPLs) can provide information on *in situ* archaeal and bacterial activity in various natural ecosystems (e.g., Lipp et al., 2008; Rossel et al., 2008; Schubotz et al., 2009; Schubotz et al., 2013; Gibson et al., 2013; Reeves et al., 2014). The polar head groups, including sugars and phosphates, are often specific to archaeal species due to their molecular strategies to cope with the surrounding extreme environments (Zeng et al., 2018). On the other hand, the residual archaeal CLs such as glycerol dibiphytanyl glycerol tetraether (GDGT) have been used as proxies for paleoenvironmental studies (Schouten et al., 2007). A detection of IPLs and CLs in natural samples is regarded as an active and ceased indicator of microorganisms, respectively.

In general, archaeal membrane lipids have been analyzed by high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) using atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). APCI and ESI are the most common ionization modes for MS. The APCI method is applicable to many types of lipids (archaeal CL, non-archaeal phospholipids, free fatty acids, sterols, etc.), and its mass spectrum is relatively simple. For example, the spectrum of GDGT measured by APCI-MS is dominated by protonated ions (Schouten et al., 2007). However, some studies have reported that some archaeal lipids, including hydroxyalkyls, become unstable during sample preparation

(hydrolysis) and ionization (Sprott et al., 1990; Liu et al., 2012a; Zhu et al., 2013). In some cases, the APCI method may degrade such unstable lipids, producing degradation products (Liu et al., 2012a; Zhu et al., 2013). This degradation results in the loss of information such as the relationship between the isoprenoid chain and the polar head group. In addition, the ionization principle of APCI is not suitable for the analysis of polar molecules of high molecular weight (>1000 Da) molecules such as polar GDGTs. Therefore, archaeal IPLs cannot be analyzed using APCI due to their presence of polar head groups and are generally analyzed using ESI.

Eventually, archaeal CLs and IPLs in natural samples have generally been analyzed separately by HPLC/MS using APCI (Hopmans et al., 2000; Schuten et al., 2007) and ESI (Sturt et al., 2004; Yoshinaga et al., 2011), respectively. To analyze both CLs and IPLs in one sample, at least two HPLC/MS analyses are required after sample preparation. The total lipid extract (TLE) of samples is pre-separated into polar (containing IPLs) and non-polar (containing CLs) fractions, and the polar fraction is acid hydrolyzed for analysis. Alternatively, TLE is separated by preparative silica gel chromatography before HPLC/MS analysis (Oba et al., 2006; Pitcher et al., 2009). Such analytical procedures require a relatively large amount of sample and significant time for the sample preparation.

A separation of CLs in HPLC by APCI has been achieved using a diol, cyano, or NH<sub>2</sub> column as stationary phases under normal phase conditions using a mixture of hexane-propanol as eluents (Hopmans et al., 2000; Schuten et al., 2007). Chromatographic methods using alternative columns for various IPLs by ESI-MS have been investigated to demonstrate new separation modes for polar lipid classes, in which the reversed-phase separation using C18 columns were applied for the simultaneous analysis of CL and IPL (Wömer et al., 2013; Zhu et al., 2013). However, the simultaneous analysis of CLs and IPLs by normal phase separation with ESI-MS has not been attempted. In this study, a new separation mode using normal-phase chromatography and high-resolution mass spectrometry (HRMS) with heated electrospray ionization (HESI) was investigated for the simultaneous analysis of CL and IPL. The HESI method can convert ions in solution to the gas phase by using electrospray at high temperatures.

## **2.2. Experimental**

### **2.2.1. Samples**

Two culture samples of *Sulfolobus acidcaldarius* (strain JCM 8929) and *Methanosarcina barkeri* (strain DSM 800) were used in this study. Total lipid extracts (TLE) from these culture samples were used to optimize the measurement conditions for the simultaneous analysis of core and polar lipids described below. In addition, core GDGTs

fraction samples from *S. acidcarudarius* were also used to compare the ionization and sensitivity of core GDGTs by different ionization methods, HESI and APCI.

Lyophilized cultures of *S. acidcaldarius* (84 mg) and *M. barkeri* (203 mg) were sonicated using a modified Bligh and Dyer method to obtain TLE (Sturt et al., 2004). Briefly, a mixture of methanol (MeOH)/dichloromethane (DCM)/water solution (2/1/0.8, v/v/v) was used to perform four extraction steps every 15 minutes. In the first two steps, the aqueous portion of the extraction solvent was 50 mM phosphate buffer at pH 7.4, and the last two steps were 5% (w/v) trichloroacetic acid (TCA) solution at pH 2.0. The solution after each extraction was centrifuged (3000 rpm  $\times$  15 min) and the supernatants from the first neutral extract and the next TCA acidic extract were collected separately in a separatory funnel to prevent degradation of lipids that are sensitive to acid hydrolysis. The DCM and ultrapure water were added to each supernatant to give a final MeOH/DCM/water ratio of 1:1:0.8 (v/v/v) and the organic phase was obtained. The remaining aqueous phase was re-extracted three times with DCM, and the DCM extract was combined with the organic phase. The organic phase was then washed three times with ultrapure water to remove residual salts, buffer, and acid. Finally, each organic phase was combined and evaporated under a gentle stream of N<sub>2</sub> gas to obtain TLEs. The yield of TLEs from *S. acidcaldarius* and *M. barkeri* cultures was about 790  $\mu$ g and 1370  $\mu$ g, respectively. The TLEs were dissolved in hexane/2-propanol (7/3, v/v) and stored at -20°C until HPLC/MS analysis.

### 2.2.2. Analytical conditions

The simultaneous analysis of archaeal CLs and IPLs was performed using HPLC (LC-20A Prominence HPLC system, Shimadzu Corporation) equipped with HRMS (Q-Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer, ThermoFisher Scientific) using a HESI-II ion source (ThermoFisher Scientific). A diol column (Inertsil diol; 1.5 mm i.d.  $\times$  250 mm, 3  $\mu$ m particle size; GL Sciences) was used with a guard column (1.5 mm i.d.  $\times$  10 mm) of the same packing material at 30°C. The eluent was a mixture of A (hexane) and B (2-propanol/formic acid/14.8 M ammonium hydroxide, 99.8/0.1/0.1, v/v) at 0.1 mL/min and the gradient program was: 99.4% A/0.6% B for 5 min, then linear to 98.2% A/1.1% B. The gradient was. 45 min to 8% B, 5 min to 82% A/18% B, 5 min maintenance, 30 min to 35% A/65% B, 10 min maintenance, 1 h to 99.4% A/0.6% B, and a linear gradient to hold for 1 h to re-equilibrate the column for the next run. The optimized HESI-HRMS settings were as follows: spray voltage; 4.5 kV, ion transfer capillary temperature; 275°C, sheath and auxiliary N<sub>2</sub> gas flow rates for desolvation; 35 and 10 (both arbitrary units), respectively, and HESI heater

temperature; 200°C. To compare the quantitative accuracy and sensitivity of core lipid analysis, the HPLC/APCI-HRMS analysis was performed using a diol column (LiChrosphere Diol-100; 2.1 mm i.d. × 250 mm, 5 µm in particle size; Merck) with the same gradient program at 0.2 mL/min.

## 2.3. Results and discussion

### 2.3.1. Ionization and sensitivity

Both CLs and IPLs were well ionized by HESI and their chromatographic peaks were detected with high sensitivity (**Figure 2.1**). The typical structures are shown in **Figure 2.2**. Most ions were observed as  $[M+H]^+$ ,  $[M+NH_4]^+$ ,  $[M+Na]^+$  and/or  $[M+K]^+$ , in which  $[M+H]^+$  or  $[M+NH_4]^+$  was the most strongest, followed by  $[M+Na]^+$ . The CLs can be analyzed well by HESI using a non-polar solvent (hexane) containing a small amount of aqueous soluble component (2-propanol containing HCOOH and  $NH_4OH$ ). Therefore, in addition to the ESI mode using polar solvents (2-propanol/methanol) by reversed-phase chromatography (Wömer et al., 2013; Zhu et al., 2013), the HESI ionization by normal-phase chromatography is another mode of detection for CLs. The APCI method usually requires a higher eluent flow rate for glow discharge ionization ( $> 0.2$  mL/min) is required for glow discharge ionization, while a lower flow rate ( $< 0.1$  mL/min) can be used for ESI and HESI. The lower flow rate with a narrow column for HPLC can save solvent and generally improve detection sensitivity. Ionization efficiency is dependent on analytical conditions such as sample volume, column size, solvent flow rate, and spray voltage (and current), so sensitivity cannot be directly compared between APCI and HESI. However, using the sample volume in this study (sub-ng to 100 ng), both ionization methods can measure CL with comparable sensitivity using a reasonable calibration curve (**Figure 2.3**).

Simultaneous analysis of both CL and IPL allows direct comparison of lipid compositions with and without polar head groups. However, since the composition of CL is generally determined using APCI, the composition of CL by HESI in this study must be compared with that by APCI using the same analytical conditions except for the difference between HESI and APCI. The relative abundance of CL (GDGT) was identical between the HESI and APCI methods (**Figure 2.4, Table 2.1**), where a ring index (Pearson et al., 2004) of 3.4 was obtained for the HESI analysis and 3.5 for the APCI analysis. Therefore, the different ionization methods gave the same result.

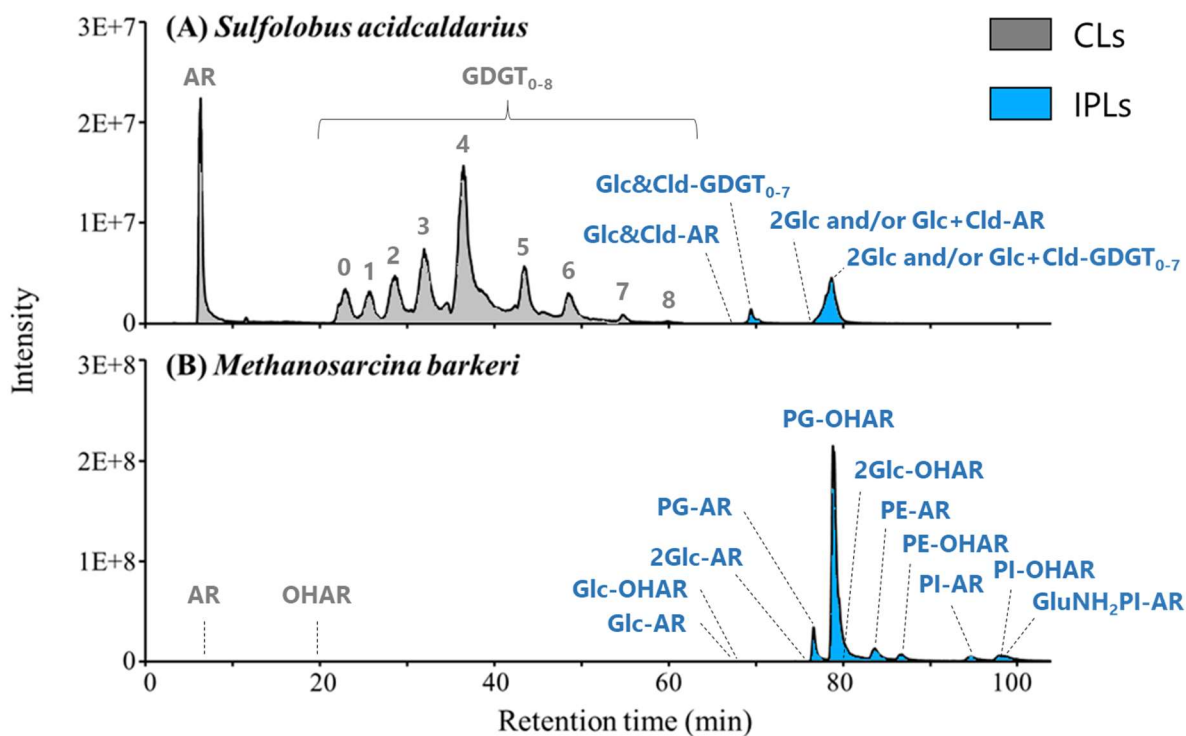
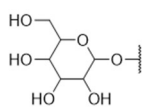
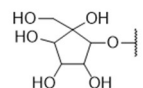


**Figure 2.1.** Combined extracted ion chromatograms of CLs and IPLs from the TLEs of (A) *Sulfolobus acidocaldarius* and (B) *Methanosarcina barkeri*. Lipids were scanned using  $[M+H]^+$ ,  $[M+NH_4]^+$ ,  $[M+Na]^+$  and/or  $[M+K]^+$  with mass accuracy  $< 2$  ppm integrated. Peaks of IPLs and CLs are showing in blue and gray, respectively. The structures are summarized in **Figure 2.2**.

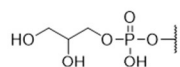
## Polar head groups



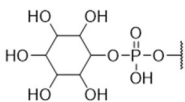
Mono-glucose **Glc**



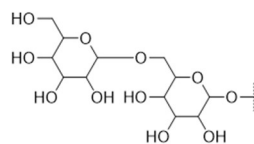
Calditol **Cld**



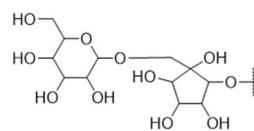
Phosphoglycerol **PG**



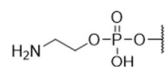
Phosphoinositol **PI**



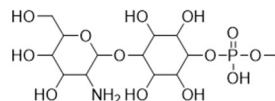
Di-glucose **2Glc**



Glucose-calditol **Glc+Cld**

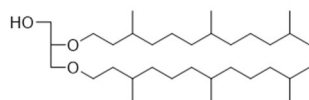


Phosphoethanolamine **PE**

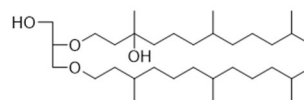


Glucosaminyl phosphoinositol **GluNH<sub>2</sub>PI**

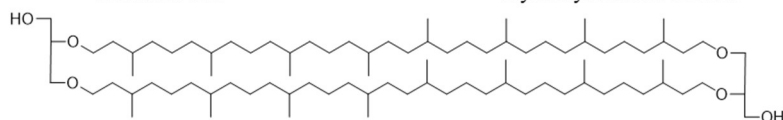
## Core lipids



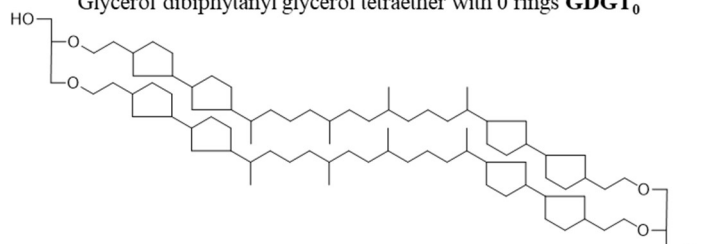
Archaeol **AR**



Hydroxyarchaeol **OHAR**



Glycerol dibiphytanyl glycerol tetraether with 0 rings **GDGT<sub>0</sub>**



Glycerol dibiphytanyl glycerol tetraether with 8 rings **GDGT<sub>8</sub>**

**Figure 2.2.** Representative chemical structures for polar head groups and core lipids described in Chapter 2.

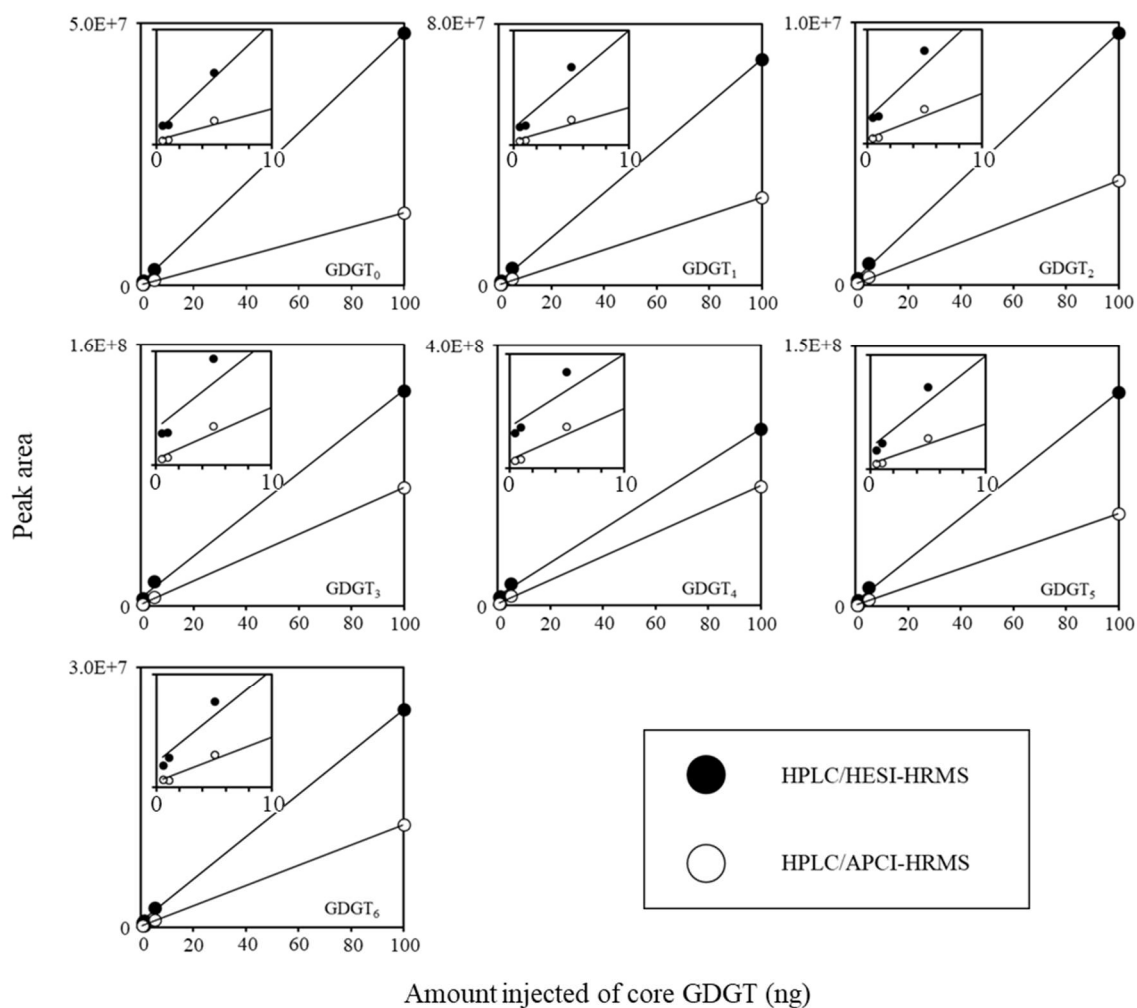


**Figure 2.3.** Calibration curves for a series of core GDGTs measured with HESI (filled symbols) and APCI (open symbols) method. The injected amounts of CLs were ~5-100 ng. Insert: the calibration curves in the lower range.

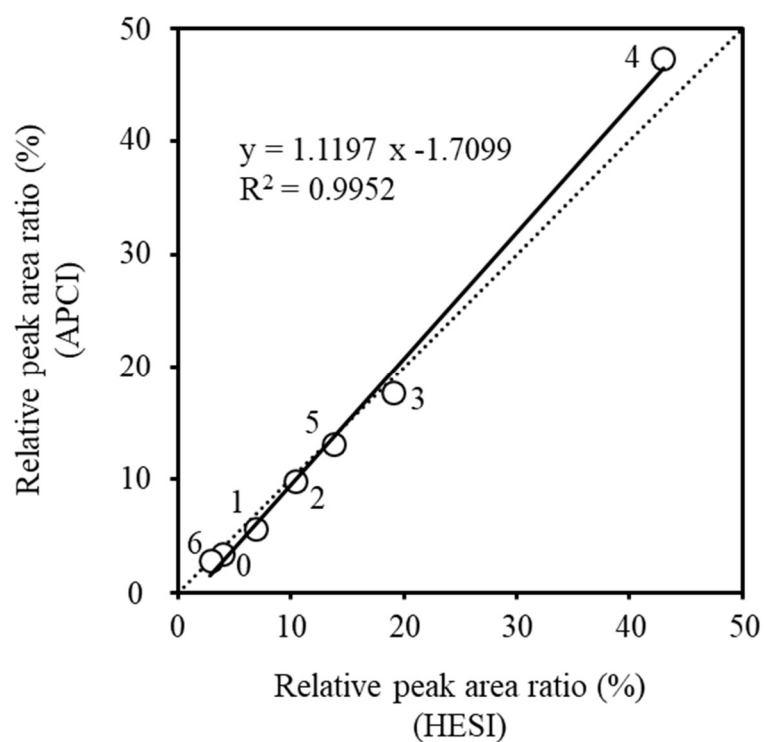


**Figure 2.4.** Comparison of core GDGTs distribution patterns between HESI and APCI method. The injected amounts of core GDGTs were 5 ng.

**Table 2.1** Comparison of relative amounts of peak areas and ring indexes of core GDGTs by HESI and APCI methods.

| Ionization | Relative distributions (%) |        |        |        |        |        |        | Ring index |
|------------|----------------------------|--------|--------|--------|--------|--------|--------|------------|
|            | GDGT-0                     | GDGT-1 | GDGT-2 | GDGT-3 | GDGT-4 | GDGT-5 | GDGT-6 |            |
| HESI       | 4.3                        | 6.5    | 10.6   | 21.9   | 42.4   | 10.8   | 3.5    | 3.4        |
| APCI       | 3.4                        | 5.7    | 9.9    | 17.7   | 47.4   | 13.1   | 2.8    | 3.5        |

The ring index was calculated by the following equation (adapted to Pearson et al., 2004): (%GDGT-1+ %GDGT-2+ %GDGT-3+ %GDGT-4 + %GDGT-5 +%)/100.

### 2.3.2. Chromatographic separation and mass resolution

Chromatograms of the extracted ions of CLs and IPLs from the TLEs of *S. acidcardarius* and *M. barkeri* are shown in **Figure 2.1**. The diphytanyl diether CLs containing archaeol (AR) and hydroxyarchaeol (OHAR) eluted first, followed by tetraether GDGTs containing up to eight cyclopentane rings (**Figure 2.5**), which was consistent with previous studies (De Rosa and Gambacorta, 1988; Zeng et al., 2018). The HRMS (mass resolving power of  $\sim 100,000$  at  $m/z \sim 1600$ ) using monoisotopic mass is useful for determining the intensity or area for each peak. A mass accuracy of most compounds is  $< 2$  ppm, defined as  $[(\text{measured } m/z) - (\text{calculated } m/z)] / (\text{calculated } m/z) \times 10^6$  (ppm).

After elution of CLs, the monosaccharide glycerol diphytanyl IPL was found to be eluted with glucose-AR (Glc-AR) followed by calditol-AR (Cld-AR) (**Figure 2.6**). *S. acidcardarius* contained more Cld-AR and less Glc-AR. the 2-sugar IPLs was identified in *S. acidcardarius* (Glc + Cld-AR) and in *M. barkeri* (2Glc-AR), but 2Glc'-ARs were detected little for both samples, suggesting that they may be structural isomers with different glycosidic linkages. Two structural isomers of monoglycosyl GDGT IPLs were identified up to GDGT-7, the former being Glc-GDGTs and the latter probably being Cld-GDGTs (**Figure 2.7A**). Monoglycoside lipids, especially monoglycoside GDGTs, were detected a little or not detected by the conventional methods using a diol column due to interference from coexisting non-retained compounds (Wömer et al., 2013; Zhu et al., 2013). The same chromatographic peaks were also obtained using the exact mass of the  $^{13}\text{C}$ -substituted compounds (**Figure 2.8**), suggesting that the two structural isomer peaks are not mixtures of unknown compounds. Calditol was abundant in GDGT-0 and the Glc/Cld ratio increased with increasing number of cyclopentane rings (**Figure 2.7A**). This indicates that the formation of calditol from glucose proceeds in association with the degree of cyclization in the membrane lipid of *S. acidcardarius*. In the case of the disaccharide GDGT, the chromatographic resolution was not as high as that of the monosaccharide GDGT, but a similar peak shift was observed (**Figure 2.7B**), possibly due to the change in sugar composition caused by the increased cyclopentane ring in the GDGTs. The relative amounts of GDGT were revealed to be correlated positively between CLs and IPLs (**Figure 2.9, Table 2.2**), which is an advantage of this study because both CLs and IPLs were simultaneously analyzed. Simultaneous measurement of lipid compositions may be useful in identifying endogenous or exogenous sources for biological activity.

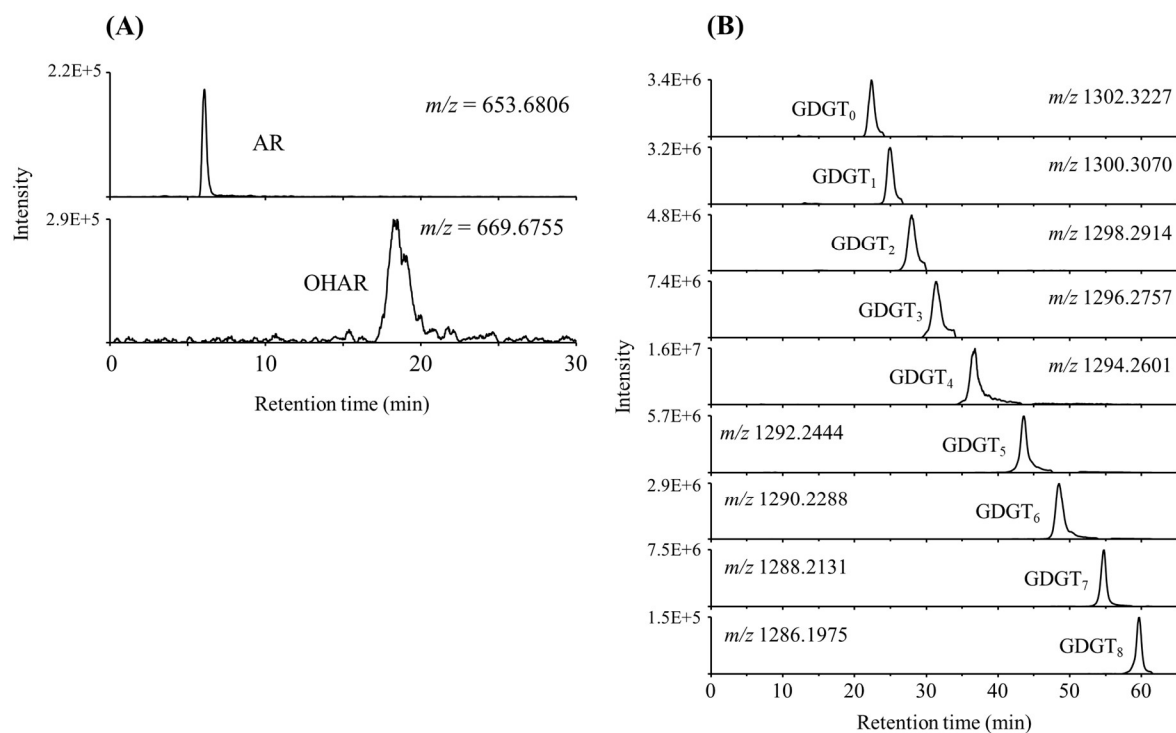


**Figure 2.5.** Extracted ion chromatograms of (A) archaeol (AR) and hydroxyarchaeol (OHAR) of *M. barkeri* and (B) GDGT-0 to -8 of *S. acidocaldarius*. CLs were scanned using  $[M+H]^+$  with mass accuracy < 2ppm integrated.

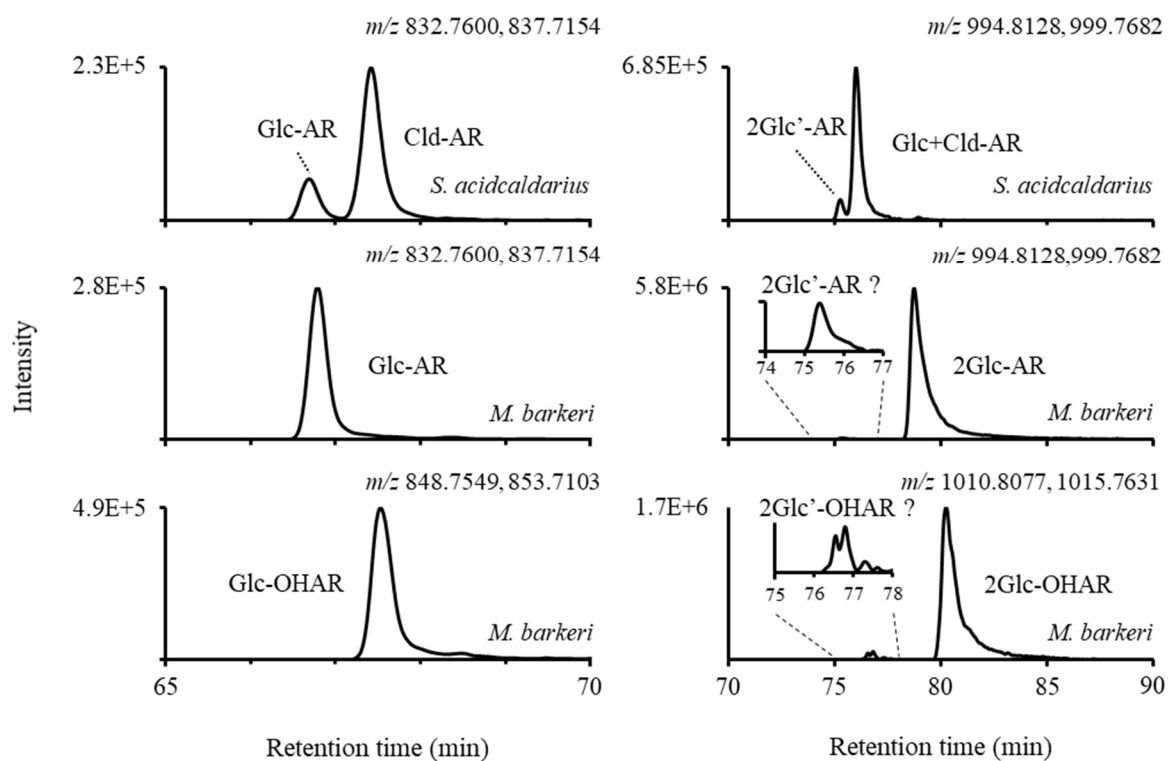


**Figure 2.6.** Extracted ion chromatograms of glycosyl diphypitanyl lipids of *S. acidocaldarius* and *M. barkeri*. Lipids were scanned using  $[M+NH_4]^+$  and  $[M+Na]^+$  with mass accuracy  $< 2$  ppm integrated.

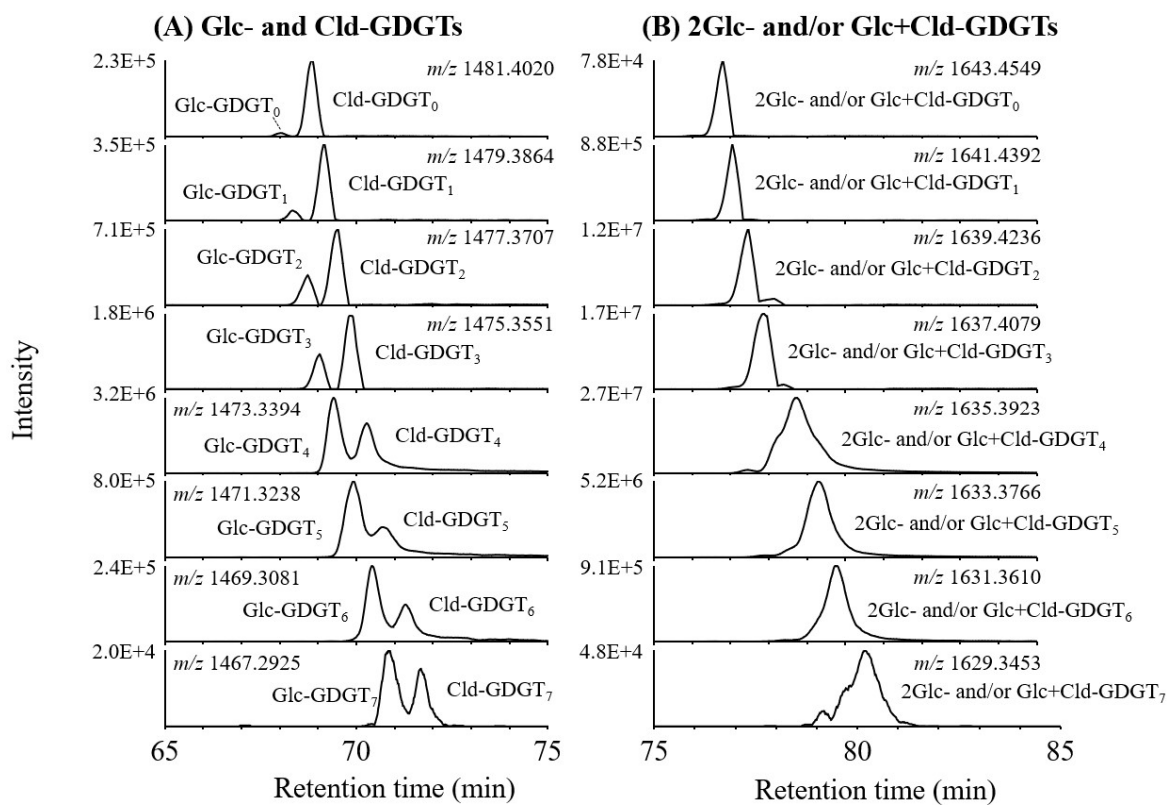


**Figure 2.7.** Extracted ion chromatograms of (A) Glc- and Cld-GDGTs and (B) 2Glc- and/or Glc+Cld-GDGTs of *S. acidcardarius*. Lipids were scanned using  $[M+NH_4]^+$  with mass accuracy < 2ppm integrated.

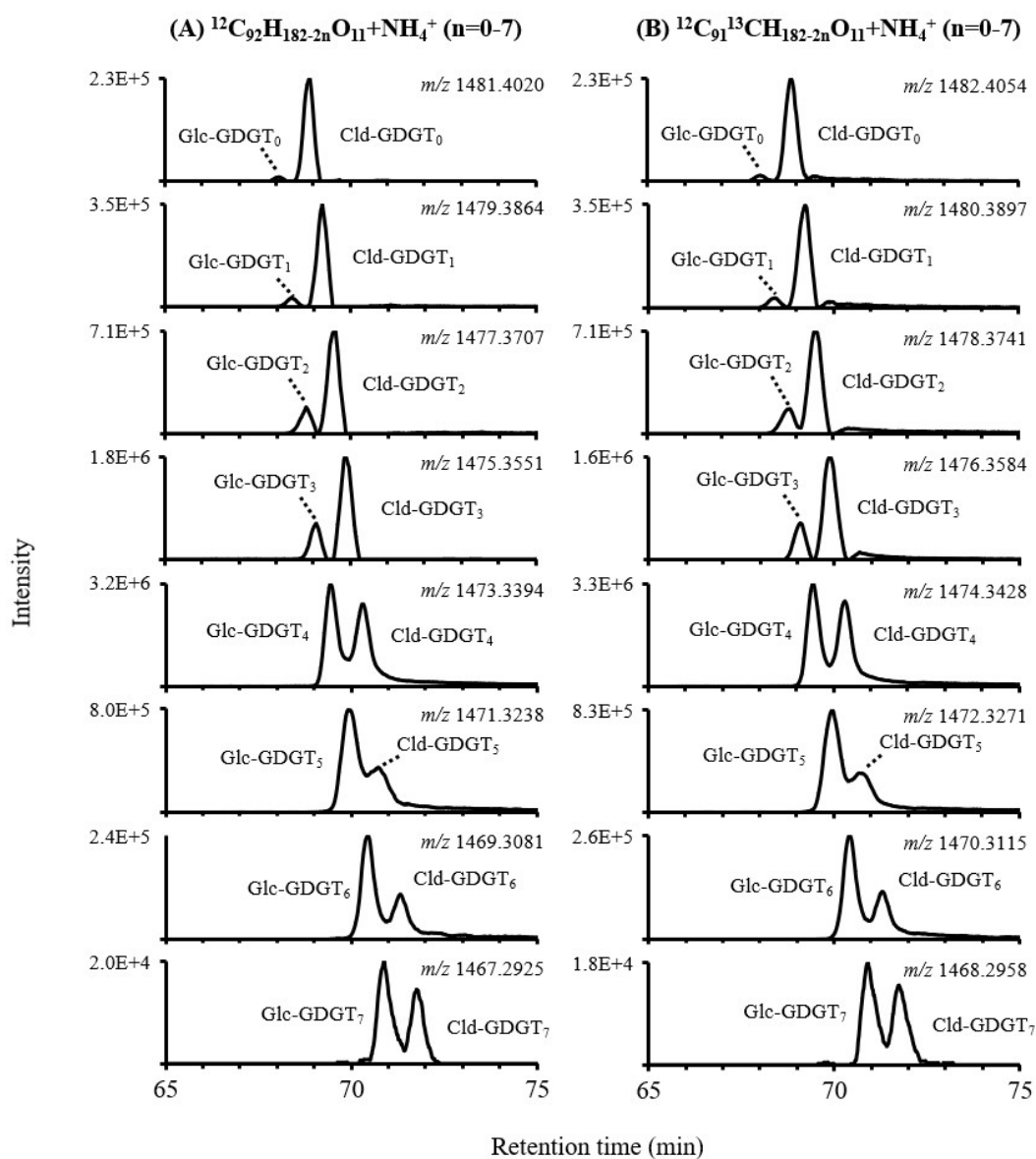


**Figure 2.8.** Extracted ion chromatograms of (A) Glc- and Cld-GDGTs and (B) 2Glc- and/or Glc+Cld-GDGTs of *S. acidcardarius*. Mass accuracy < 2 ppm.

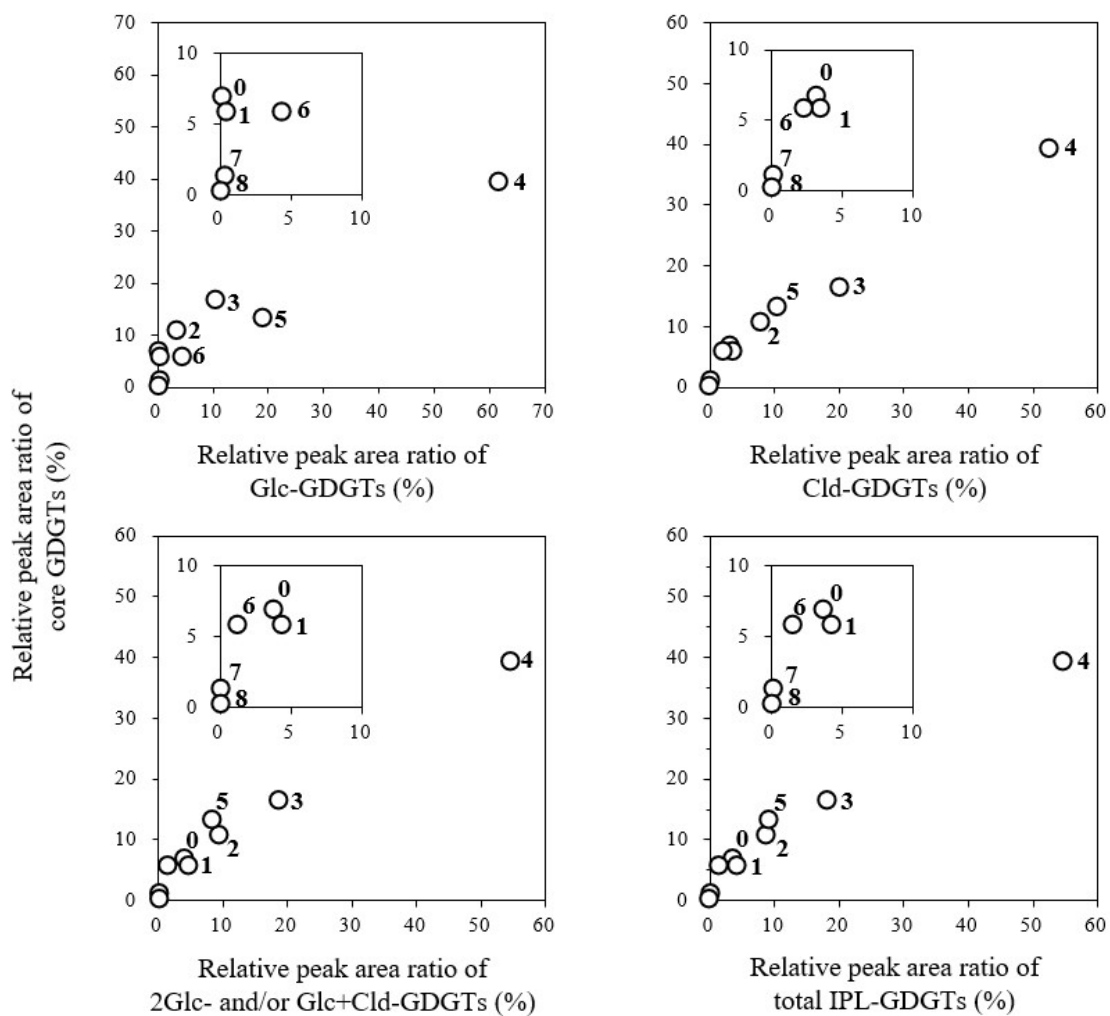


**Figure 2.9.** The cross plots of relative peak area ratio of core and intact polar GDGTs of *S. acidocaldarius*. Details are summarized in **Table 2.2**.

**Table 2.2.** Comparison of relative amounts of peak areas and ring indexes of core and intact polar GDGTs of *S. acidocrudarius*.

| GDGT type            | Relative distributions (%) |        |        |        |        |        |        |        |        | Ring index |
|----------------------|----------------------------|--------|--------|--------|--------|--------|--------|--------|--------|------------|
|                      | GDGT-0                     | GDGT-1 | GDGT-2 | GDGT-3 | GDGT-4 | GDGT-5 | GDGT-6 | GDGT-7 | GDGT-8 |            |
| Core                 | 6.9                        | 5.9    | 10.9   | 16.4   | 39.4   | 13.2   | 5.9    | 1.3    | 0.3    | 3.5        |
| Glc-                 | 0.2                        | 0.5    | 3.5    | 10.6   | 61.7   | 19.0   | 4.3    | 0.3    | n.d.   | 4.3        |
| Cld-                 | 3.1                        | 3.5    | 7.7    | 20.1   | 52.8   | 10.4   | 2.3    | 0.2    | n.d.   | 3.5        |
| 2Glc and/or Glc-Cld- | 3.8                        | 4.5    | 9.2    | 18.5   | 54.5   | 8.2    | 1.3    | 0.1    | n.d.   | 3.4        |
| total IPL            | 3.6                        | 4.2    | 8.8    | 18.2   | 54.8   | 9.0    | 1.5    | 0.1    | n.d.   | 3.4        |

The ring index was calculated by the following equation (adapted to Pearson et al., 2004): (%GDGT-1+ %GDGT-2+ %GDGT-3+ %GDGT-4 + %GDGT-5 + %GDGT-6+ %GDGT-7 + %GDGT-8)/100.

Various diphytanyl IPLs were identified from *M. barkeri*, with sugar IPLs such as Glc-AR and 2Glc-AR eluting first, followed by abundant phospholipids such as PG-AR and PG-OH (**Figures 2.1, Figure 2.6, Figure 2.10**). In addition to glucosaminylphosphoinositol (GluNH<sub>2</sub>PI) AR, there are a total of seven AR and OHAR derivatives with phosphoglycerol (PG), phosphoethanolamine (PE) and phosphoinositol (PI) groups, with PG-AR and PG-OHAR being the most abundant. The separation pattern of the phospholipid classes was similar to that from previous studies using the conventional diol column method (e.g., Sturt et al., 2004). The AR-CLs were predominant over OHAR-CLs (**Figure 2.5A**), but the AR/OHAR ratio varied depending on the lipid classes (**Figure 2.6; Figure 2.10**). The GluNH<sub>2</sub>PI group may be a characteristic head group of ARs, since GluNH<sub>2</sub>PI-OHARs were not detected in this and previous studies (Koga and Morii, 2005).

The chromatographic separation of this study gave an elution sequence of compound classes according to the polarity of the analyte (**Figure 2.11**). The separation of this sequence by HPLC will be used to fractionate the lipid classes further studies, such as stable isotope measurements of individual lipids and sugars, even though the separation of compound classes has been performed by silica gel chromatography (Oba et al., 2006; Pitcher et al., 2009) to investigate natural microbial activities related to archaeal metabolism.

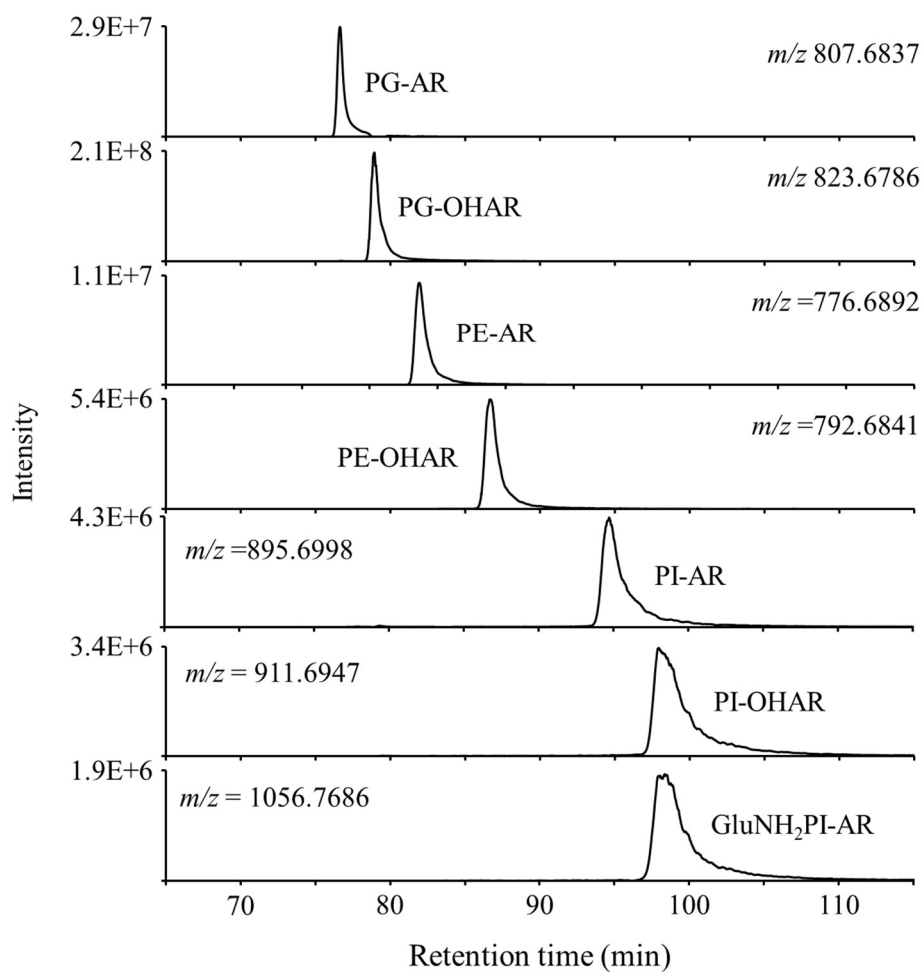


**Figure 2.10.** Extracted ion chromatograms of phospho diphytanyl lipids of *M. barkeri*. Lipids were scanned using  $[M+H]^+$  with mass accuracy  $< 2$  ppm integrated.

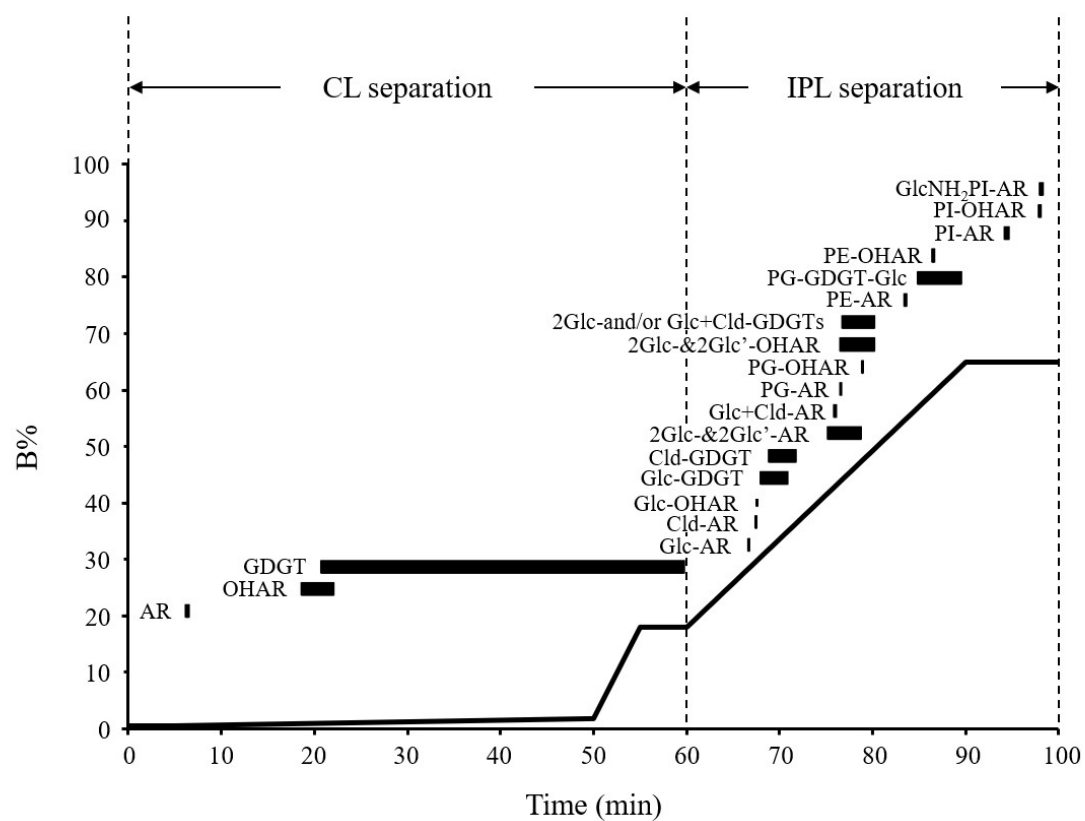


**Figure 2.11.** The HPLC gradient profile and the elution time ranges of core and polar lipids by the simultaneous analytical method.

## 2.4. Summary

An analytical method has been developed to simultaneously characterize the CLs and IPLs of archaeal membranes using HPLC/HESI-HRMS. The HESI allows simultaneous sensitive analysis of both core and intact polar lipids (sub ng to 100 ng) and can be separated by normal-phase chromatography using a semi-bore diol column. Core lipids eluted first, followed by archaeol, then glycerol dibiphytanyl glycerol tetraether (GDGT), and polar lipids with glycosides and glycoposphates were separated. The relative GDGT composition is identical for the HESI and APCI methods.

This method not only can achieve the different separation modes of CLs and IPLs to study biomarkers of microorganisms, but also has advantage of saving a small amount of sample, solvents, and time of sample preparation. The developed method can be applied to the total analysis of live and dead archaeal cells in various samples. In addition, the chromatographic analysis allows for the fractionation of compound classes according to the polarity of the head groups for application of compound-specific isotope analysis for each lipid class.

## **Chapter 3. Microbial community developments in artificial hydrothermal chimneys**

### **3.1. Introduction**

The IODP Exp. 331, the Deep Hot biosphere project, aimed to investigate the existence of a functionally active, metabolically diverse seafloor microbial ecosystems and their relationship to physical and chemical setting at the hydrothermally active seafloor (Takai et al., 2011). A total four artificial hydrothermal vents were created at the Iheya North Knoll in the mid-Okinawa Trough after the deep-sea drillings by IODP Expedition 331 in September 2010 (Takai et al., 2011). New artificial chimney structures were formed on the two drilling holes at an active hydrothermal mound: Hole C0016A and Hole C0016B. Several previous studies on microbial community formation in hydrothermal vents have detected microbial genes within a few days after the artifact installation or chimney deposition (McCliment et al., 2006; Nercessian et al., 2003; Pagé et al., 2008; Reysenbach et al., 2000; Wang et al., 2009). However, in those artificial chimneys of the Iheya North field, microbial community analysis was conducted using molecular biology techniques, but microbial DNA could not be amplified and no detectable community developed within 11 and 18 months after the drilling (Kawagucci et al., 2013). There have been no reports of microbiological studies in these artificial chimneys since then. It remains unclear what kind and how many microorganisms are living in these chimneys by the time of this study.

Intact polar lipids (IPLs) are major components of cell membrane, which are regarded as indicators of currently living or recently living cells because their polar head groups are rapidly removed after the death of cell (e.g., Harvey et al., 1986; White et al., 1979). In contrast, core lipids (CLs) that have lost their polar head groups have also been used as chemical fossils of past-living microorganisms, partly because CLs are relatively resistant to degradation and preserved for a long time. In this study, what kind of microorganisms live and how microbial community have been developed at artificial chimneys are studied using both IPLs and CLs biomarkers.

### **3.2. Materials and Methods**

#### **3.2.1. Study field**

The Okinawa Trough is a 1,200 km long and 200 km wide back-arc basin in the Eurasian continental margin area of the eastern East China Sea formed by the subduction of the Philippine Sea Plate beneath the Eurasian Plate (**Figure 3.1**). The Okinawa Trough is in a rifting stage prior to the expansion of the back arc (e.g., Letouzey and Kimura, 1986; Sibuet et al.,

1987), and is a geological field where extensional tectonics is prone to the development of submarine hydrothermal circulation systems. The fracture systems and magma intrusion occur frequently. The Okinawa Trough is characterized by bimodal igneous activity in which felsic rhyolite and mafic basalt coexist (Shinjo and Kato, 2000). The Okinawa Trough is divided into three areas: northern, middle, and southern, according to water depth and location of the rift. The mid-Okinawa Trough has areas of concentrated volcanic activity along the rift corresponding to the axis of extension (Sibuet et al., 1987), and is known as an area of active submarine hydrothermal activity (e.g., Ishibashi and Urabe, 1995; Ishibashi et al., 2015).

The Iheya North Knoll (27°47'5N, 126°53'8E; water depth: ~1,000 m) is a submarine volcanic complex in the mid-Okinawa Trough, located about 150 km northeast of Okinawa Island (**Figure 3.1**). In the Iheya North Knoll, three active hydrothermal sites, called as the Original Site, Natsu Site and Aki Site, have been discovered (Momma et al., 1996; Nakamura et al., 2015; Kasaya et al., 2015). They are distributed along the eastern slope of the knoll on the east side of the ridge in a longitudinal N-S direction (**Figure 3.2A**). Since the discovery of hydrothermal activity at the Iheya North Knoll in 1995 (Momma et al., 1996), Iheya North field is one of the most active deep-sea hydrothermal vent sites in the Okinawa Trough, which have been studied well (e.g., Nakagawa et al., 2005; Kumagai et al., 2010; Kawagucci et al., 2011). The Original Site is located along the eastern slope of the knoll in the northwestern part of the Iheya North Knoll. About 10 active hydrothermal mounds are distributed in a southwesterly direction, where North Big Chimney (NBC) mound (~30 m height) is located as a center region (Kawagucci et al., 2011). The NBC mound has numerous flange structures and the main vent at the top has hottest (~311°C) hydrothermal plumes of any natural hydrothermal vent at the site over 10 years (Kawagucci et al., 2011). The Natsu Site is located in a depression about 1.2 km south-southwest of the Original Site. The hydrothermal mounds of Natsu Site emit hydrothermal fluids at temperatures in excess of 300°C and widespread chemosynthetic communities have been observed (Takai et al., 2014). The Aki Site is located in a depression approximately 2.6 km south-southwest of the Original Site. Numerous hydrothermal mounds are also distributed at this site, and the highest hydrothermal vent of ~317 °C was observed in the Iheya North Knoll (Miyazaki et al., 2017a).

The central depression and the outer margin of the mountain body of the trough are covered by thick sedimentary layers of terrestrial source sediments mainly supplied by the Yangtze River and by porous pumice-rich volcanoclastic debris (Narita et al., 1990; Hun and Su, 1999; Tsuji et al., 2012). The sedimentary layers influence the chemical composition of hydrothermal fluids and submarine hydrogeological structures. The observation of heat flow

rate during IODP Expedition 311 indicated that extensive fluid movement on a scale of several kilometers beneath the seafloor occurred around a typical hydrothermal vent area at the Original Site (Masaki et al., 2011). The three hydrothermal zones should form a vast hydrothermal pool that extends over the entire Iheya North Knoll using logging while drilling (LWD) during the CK14-04 and CK16-01 cruises by the deep-sea drilling vessel *Chikyu* and seismic reflection data acquired during the preliminary survey of IODP Expedition 311 (Tsuji et al., 2012).

The hydrothermal fluids from the Iheya North hydrothermal field have a pH of ~5, and are rich in magmatic volatiles (e.g., CO<sub>2</sub>, H<sub>2</sub>S) as well as sedimentary organic-derived gases (e.g., CH<sub>4</sub>, NH<sub>4</sub><sup>+</sup>). Such chemical characteristics are common features in hydrothermally active areas of the Okinawa Trough (Kawagucci et al., 2011; Kawagucci et al., 2015; Miyazaki et al., 2017a). The end-member chemical composition of hydrothermal fluids at the Okinawa Trough is characterized by phase separation due to phase separation processes, indicating mixing of gas and liquid phases at different ratios (Nakagawa et al., 2005; Kawagucci et al., 2011). The common fluid source under the common underground structure of the three hydrothermal vents are also supported by the same end-member chemical composition and biological similarity of chemoautotrophs (Miyazaki et al., 2017a).

In September 2010, scientific drilling was performed by IODP Expedition 311 at five locations (Sites C0013-C0017) on the slope of the mountain body where the NBC mound is located (**Figure 3.2B**). Site C0016 (active hydrothermal vent site) was located at the crest of the NBC mound (Hole C0016A) and the western flank of the NBC mound (Hole C0016B) ~20 m horizontal to and west of Hole C0016A. Site C0014 was located at the eastern region ~450 m away from the NBC mound at Site C0016, where *Calymene* calm colonizing soft brownish sediment and no hydrothermal discharge was observed prior to the drilling (Kawagucci et al., 2013; Nakajima et al., 2015). The boreholes (i.e., hydrothermal channels) of the artificial chimneys are much larger (~ 50 cm in diameter) than the normal natural chimney boreholes (5-10 cm in diameter, e.g., Tivey, 1998), resulting in a larger flow rate for the artificial chimneys. The heavy, triangular, gimbaled guide bases were deployed at Hole C0016B and Hole C0014G, which were cased in open-ended stain-less pipes (Takai et al., 2011). At the Hole C0016B, 3 m long pipe (14 cm in diameter) inserted to a depth of 0.4 m below the seafloor and has a large hydrothermal flow rate. The hydrothermal fluids originate from several meters below the seafloor in Hole C0016A and ~ 38 meters below the seafloor in Hole C0016B (Kawagucci et al., 2013).

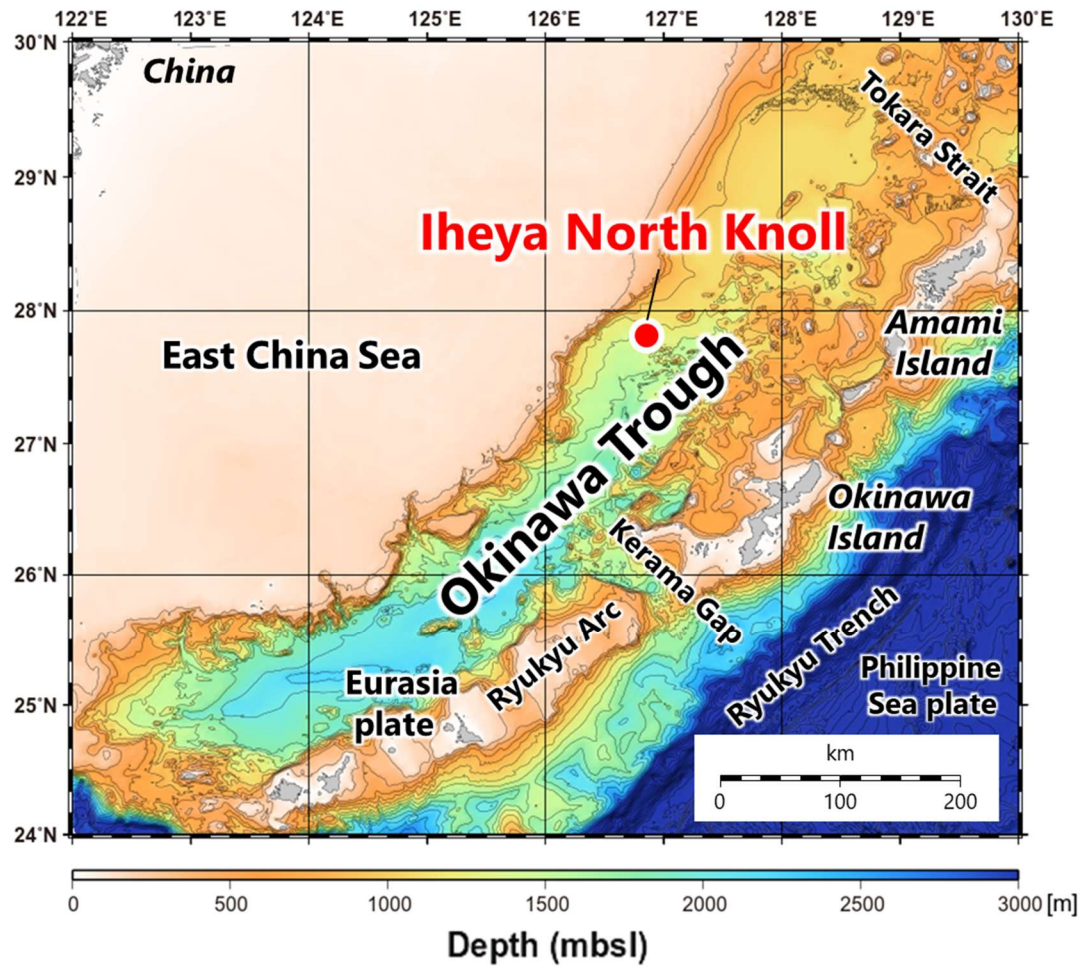


**Figure 3.1.** Location map of the Iheya North hydrothermal field of the mid-Okinawa Trough, the eastern East China Sea (modified after Totsuka et al., 2019).

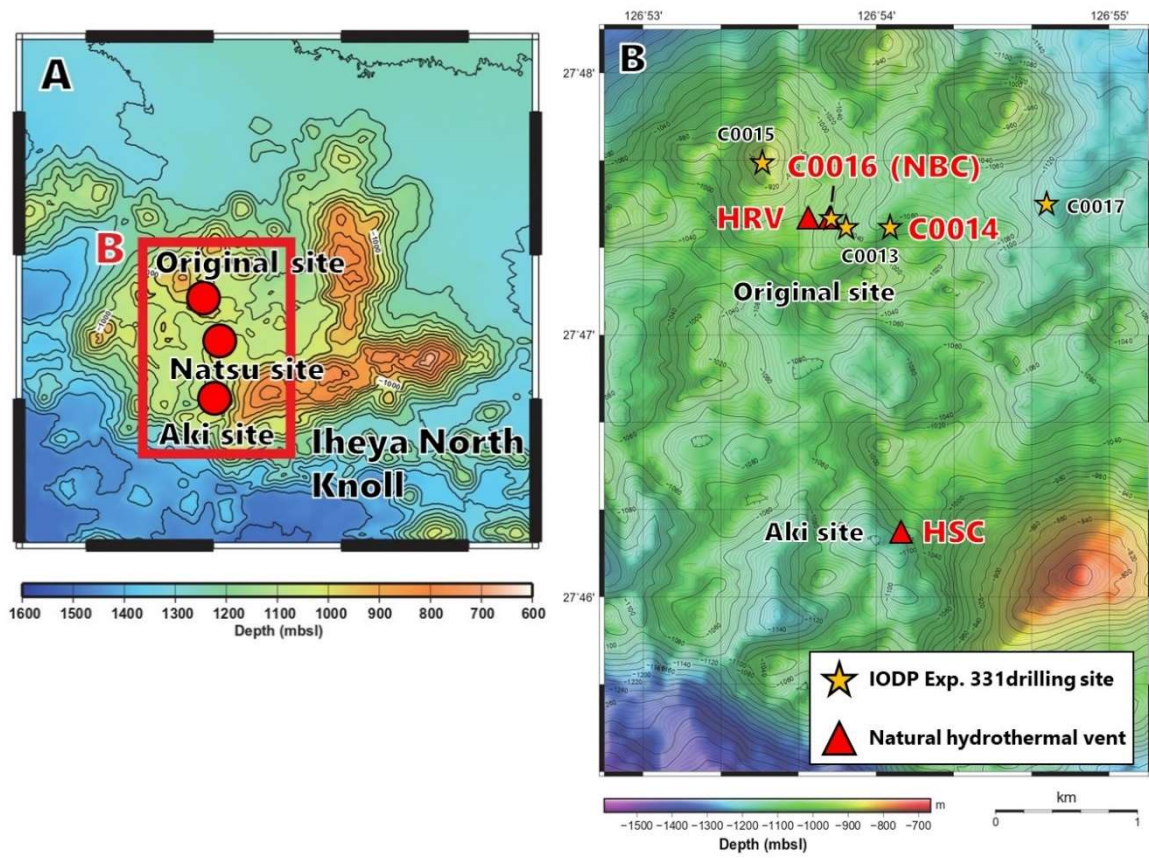


**Figure 3.2.** Maps of the study area (modified after Totsuka et al., 2019). (A) Bathymetric map of active hydrothermal vent sites in the Iheya North Knoll and (B) Locations of the IODP Expedition 331 drill sites and typical natural hydrothermal vents. Names of the sampling sites of this study are represented in red.

### 3.2.2. Sampling and sample description

A total of six samples were used in this study: two artificial chimney samples, two natural chimney samples, and two reference samples (**Table 3.1**). The two artificial chimney samples included AC-C0016A from Hole C0016A and AC-C0016B from Hole C0016B. The two natural chimney samples were NC-HSC from the Hidariteha-Soerudake-Chimney (HSC) mound at the Aki Site, and NC-HRV from the High-Radioactivity Vent (HRV) mound at Site C0014. The two reference samples were a sulfide ore sample (RF-SO) and a hydrothermal altered surface sediment sample (RF-AS) at Site C0014. All samples for this study were collected during JAMSTEC scientific cruises KR 18-14 Leg. 1 and Leg. 2 in October to November 2018 using a research vehicle (R/V) *Kairei* and a remote operated vehicle (ROV) *Kaiko Mk-IV*.

#### 1) Artificial chimney samples

##### i) AC-C0016A sample:

Hole C0016A was drilled at the North Big Chimney (NBC) hydrothermal mound (27°47.4611'N, 126°53.8004'E; 978 m in water depth) in September 2010 during the IODP 331 Expedition. An intense hydrothermal venting was observed immediately after the drilling. A ~4 m high chimney was formed after 5 months of drilling (Kawagucci et al., 2013; Nozaki et al., 2016). The C0016A chimney grew to a height of about 15 m within 25 months of excavation, despite the fact that the top was broken down several times for sample collection (Kawagucci et al., 2013). The the C0016A chimney was collapsed during the expeditions KY14-01 by R/V *Kaiyo* in 2014 and YK16-11 Leg. 2 by R/V *Yokosuka* in 2016. In expedition YK16-11 Leg. 2, hydrothermal fluid was venting from the tip of the remaining base of the chimney (Takai et al., 2016). When samples were collected on Expedition KR18-14 Leg. in October 2018, the morphology of the base of Hole C0016A chimney had not changed since 2016, but a ~1 m high hydrothermal vent with a black smoker had formed at the top. The temperature of hydrothermal fluids showed approximately 280°C during the expedition KY14-01 in 2014 (Takai et al., 2014). The sample of this study was collected from the flange (JAMSTEC sample ID KK#796-R01) at the basement of the artificial chimney developed at Hole C0016A during KR18-14 Leg. 1 in October 2018. The chimney was about 3 m high and 1.5 m wide at the time of sample collection (**Figure 3.3A**). The hydrothermal fluid was a white-grayish smoker from the top and a clear hydrothermal venting from the basement (the temperature was not measured). White fibrous microbial colonies were observed at the base of the chimney, and the entire surface was inhabited by *Shinkaicaris* shrimps.

**Table 3.1.** Summary of sample information.

| Sample                     | Location                  | Depth (m) | Latitude     | Longitude     | Description                                                                                                                                                  |
|----------------------------|---------------------------|-----------|--------------|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Artificial chimneys</b> |                           |           |              |               |                                                                                                                                                              |
| AC-C0016A                  | Original site, Site C0016 | 978       | 27°47.4611'N | 26°53.8004'E  | A flange structure sample collected from the bottom part of Hole C0016A artificial chimney. Black hard material.                                             |
| AC-C0016B                  | Original site, Site C0016 | 976       | 27°47.4569'N | 126°53.7944'E | A flange structure developed on the guide base of Hole C0016B. The outermost parts were covered by polychaetas colonies. Dark gray soft material.            |
| <b>Natural chimneys</b>    |                           |           |              |               |                                                                                                                                                              |
| NC-HSC                     | Aki site,                 | 1,052     | 27°46.0629'N | 126°54.166'E  | An interior part of chimney wall collected from top of the HSC mound of Aki site. Max fluid temperature during the sampling was ~299°C. Black soft material. |
| NC-HRV                     | Original site             | 952       | 27°47.4585'N | 126°53.6812'E | A chimney wall collected from the middle of HRV mound of the Original site. The gathered crabs were colonized. Gray hard material.                           |
| <b>Reference samples</b>   |                           |           |              |               |                                                                                                                                                              |
| RF-SO                      | Original site, Site C0014 | 1,053     | 27°47.4154'N | 126°54.0443'E | Black sulfidic ore deposited on the hydrothermally altered sedimentary seafloor at Site C0014 collected close to ~155°C vent. Black hard material.           |
| RF-AS                      | Original site, Site C0014 | 1,053     | 27°47.4154'N | 126°54.0443'E | Hydrothermally altered surface sediment collected close to ~155°C vent. Blown soft material.                                                                 |

## ii) AC-C0016B sample:

Hole C0016B (27°47.4586'N, 126°53.7778'E; ~986.5 m in water depth) was drilled ~20 m to the southwest from Hole C0016A (Takai et al., 2011). The borehole was cased to a depth of ~0.4 mbsf with a 14 cm diameter stainless steel pipe and an engineered vent platform at the guide basement (~10 m high and 1.26 m diameter) (Takai et al., 2011; Kawagucci et al., 2013; Nozaki et al., 2016). The maximum temperature of the hydrothermal fluid from the Hole C0016B vent was measured 308°C in 2012 (Kawagucci et al., 2013). Within 5 months of drilling, a ~20-30 cm high chimney had formed at the top of the pipe. The majority of the guide basement was covered by numerous chimneys on expedition KY14-01 in 2014. On YK16-11 Leg. 2 in 2016, a ~1.5 m high chimney had developed in the center of the guide basement with flange structures (Takai et al., 2016). The sample of this study was collected from a clear hydrothermal gushing flange extending above the guide basement at Hole C0016B during dive #796 of KR18-14 Leg 1 (JAMSTEC sample ID KK#796-R05). When collecting samples, the 126 cm guide basement was completely covered by a flange structure with numerous small chimneys having clear hydrothermal gushing (**Figure 3.3B**). The flange structures were also developed below the guide basement, and the roots were buried in hydrothermal sediments. The surface of the chimney structure above the guide base was entirely covered with polychaetes (*Paralvinella* sp.) colonies and *Shinkaicaris* shrimps were also observed. *Shinkaia* crabs were found on the surrounding seafloor, but not inside the guide framework or the guide basement. The outermost part covered with polychaetas colony were removed from the sample surface on board the vessel using tweezers cleaned with ethanol.

## 2) Natural chimney sample

### i) NC-HSC sample:

Hidariteha-Soerudake-Chimney (HSC) (27°46.063'N, 126°54.166'E; ~1052 m in water depth) is a natural chimney at the top of the central hydrothermal mound (~10 m high) of the Aki site, which is located ~2.8 km from Site C0016 (**Figure 3.2B**). The hottest hydrothermal plume of ~316°C was observed in the Iheya North hydrothermal field when it was discovered during KY14-01 cruise in 2014 (Takai et al., 2014; Miyazaki et al., 2017a). The sample of this study was collected from the bulk chimney structure of the HSC at dive#798 on KR18-14 Leg. 1 in 2018 (JAMSTEC sample ID KK#798-R03). At the time of sample collection, the HSC was a mushroom-like structure (~40 cm high and ~50 cm wide, **Figure 3.3C**). Clear hydrothermal fluid was gushing from the entire surface of the chimney, and white-grayish smokers were emanating from the outer rim. The maximum temperature of

~299°C was measured for the fluid at the time of sample collection. White microbial colonies were present on the surface of the chimney and on the surrounding mound pile. *Shinkaicaris* sp. were sparsely observed on the chimney, and *Shinkaia crabs* were present on the bottom of the chimney. Polychaete colonies were also found on the surrounding mound. The sample used for analysis was the inner black fragment obtained when the specimen was split on board the vessel.

## ii) NC-HRV sample

High Radioactive Vent (HRV) (27°47.4585'N, 126°53.6812'E; 952 m in water depth) is a ~20 m high hydrothermal mound with many flange structures located ~ 200 m from the Site C0016. The maximum temperature of the fluid is reported to be 238°C (Kawagucci et al., 2011). The sample of this study was collected from the base of a ~1 m high chimney within a colony of *Shinkaia crabs* in the middle of the HRV mound at dive#804 of KR18-14 Leg. 2 (JAMSTEC sample ID KK#804-R03) (**Figure 3.3D**). No visible plumes were observed prior the sample collection, but white-grayish hydrothermal plumes were observed immediately after the sampling (the temperature was not measured).

## 3) Reference samples

### RF-AS and RF-SO samples:

Reference samples were collected at the Site C0014 (Hole C0014G: 27°47.4182'N, 126°54.0508'E; ~1,050 m water depth), which is located ~450 m east from the Site C0016 (**Figure 3.2B**). No apparent hydrothermal venting was observed at Site C0014 area before drilling, where it was covered by silty sediments and interspersed with colonies of *Calypogena calms* (Kawagucci et al., 2013). Numerous hydrothermal shimmering and small chimneys were observed at the seafloor around the borehole after 11 months of drilling. The bottom sediment was hardened by hydrothermal alteration and extensive colonies of microbial mats as well as chemosynthetic communities were formed (Kawagucci et al., 2013; Nakajima et al., 2015). The samples of this study were collected around northern region of Site C0014 at dive#795 on KR18-14 Leg. 1. The RF-AS sample was collected from an altered surface sediment (~15 cm below the seafloor, JAMSTEC sample ID KK#795-R01, **Figure 3.3E**). The RF-SO sample was obtained from a ~20 cm tall chimney and its basement as a massive sulfide ore deposited at its base (JAMSTEC sample ID KK#795-R05, **Figure 3.3E**). Clear hydrothermal plumes of ~155°C were observed from the seafloor around the chimney where

samples were collected. Polychaetes, *Shinkaikaris* shrimps, and *Shinkaia* crabs were observed around the field.

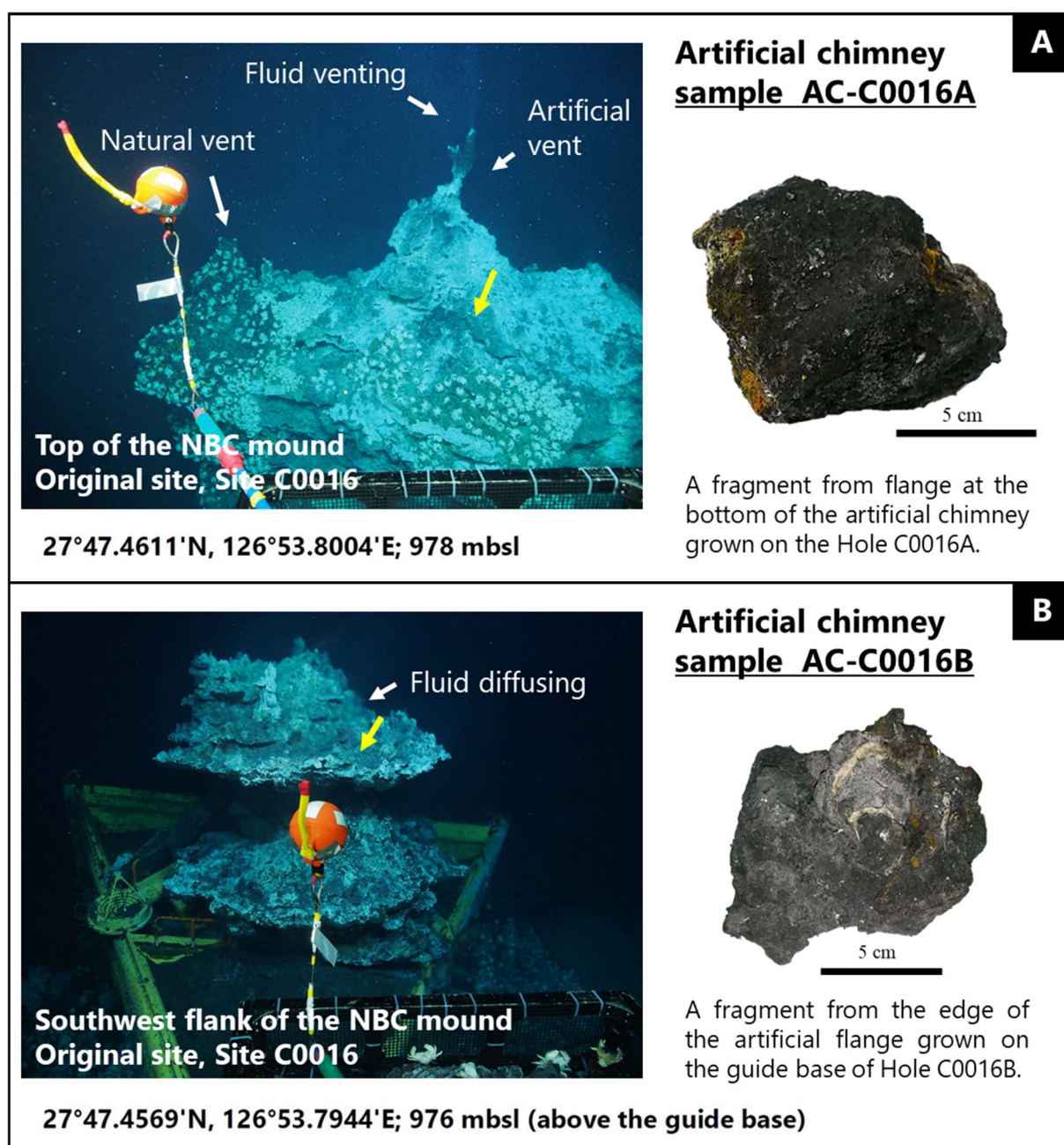


**Figure 3.3.** Photographs of the sampling sites and sample fragments analyzed in this study. (A) Hole C0016A artificial chimney (sample AC-C0016A), (B) Hole C0016A artificial chimney (sample AC-C0016B).

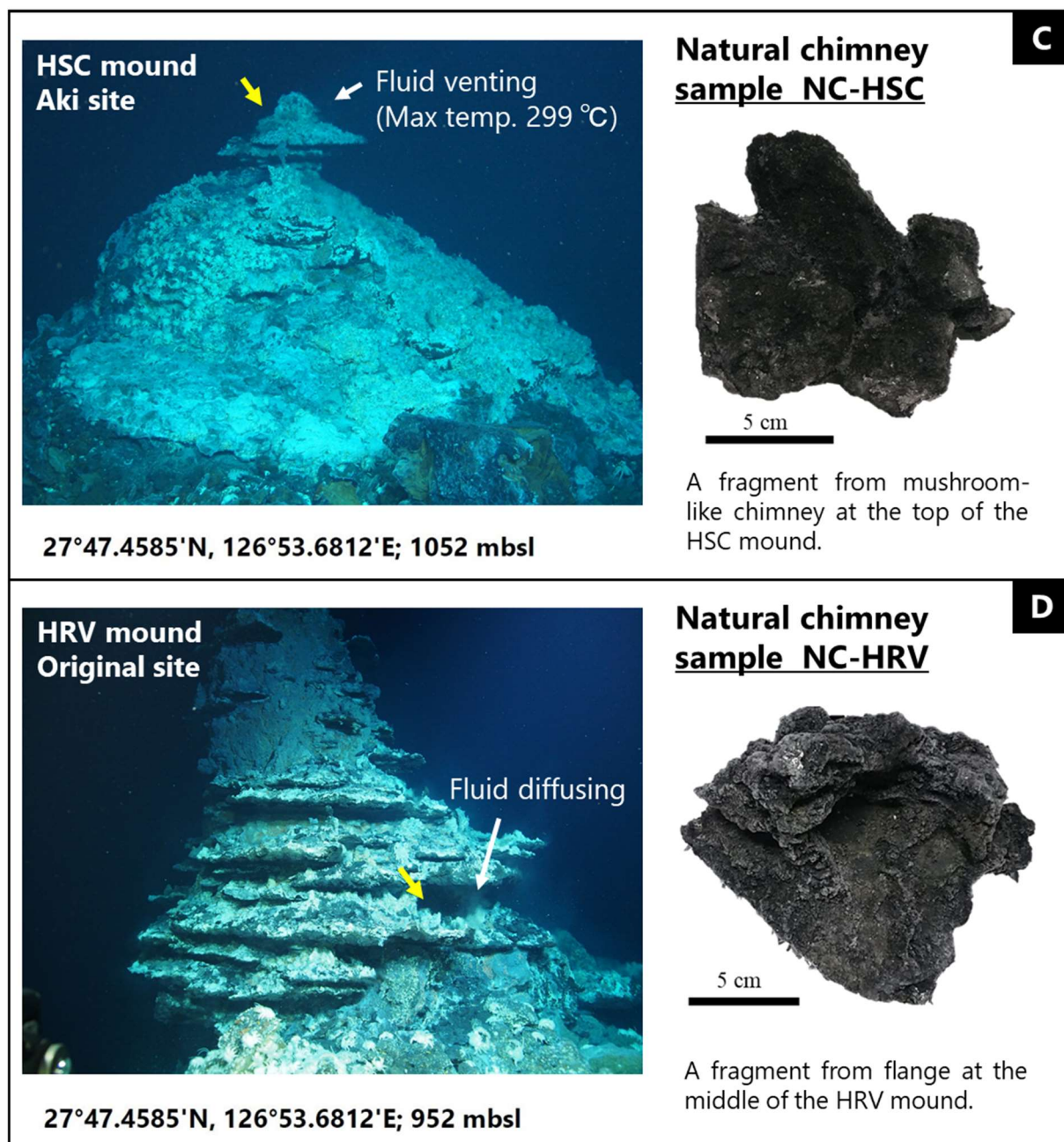


**Figure 3.3.** (Continued) Photographs of the sampling sites and sample fragments analyzed in this study. (C) Natural chimney HSC (sample NC-HSC), (D) Natural chimney HRV (sample NC-HRV).

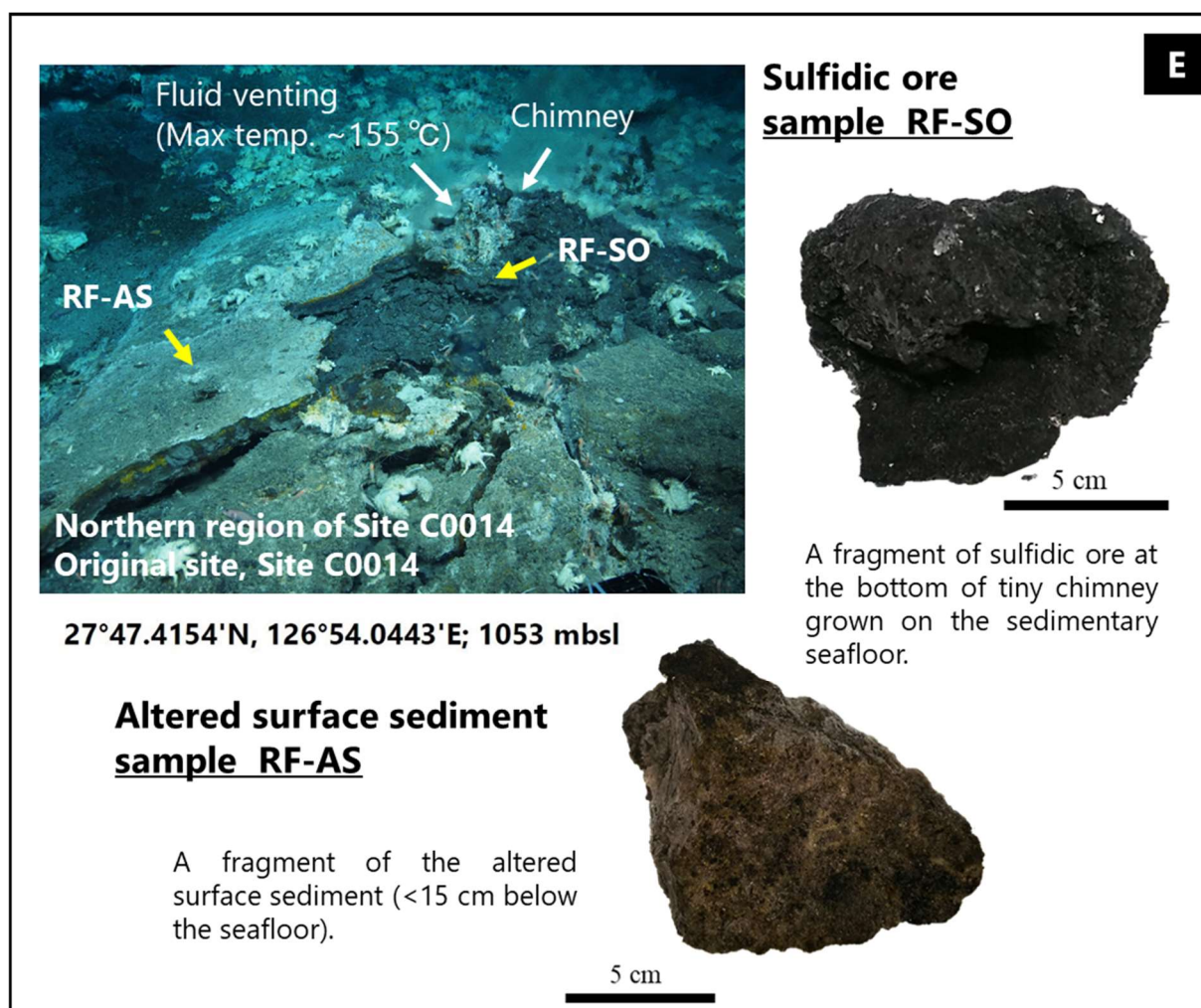


**Figure 3.3.** (Continued) Photographs of the sampling sites and sample fragments analyzed in this study. (E) Sedimentary seafloor at Site C0014 (sample RF-SO and RF-AS).

Immediately after the recovery onboard, these samples were fragmented to sufficient sizes for different analysis using a clean chisel and hammer. The sample fragments were vacuum-packed and stored at -80°C onboard. After returning to the laboratory, a portion of the sample was freeze-dried and ground to a fine powder. The powdered samples were split for lipid analysis, organic elemental analysis, and mineralogical analysis. The samples for organic geochemical analysis were stored at -20 °C until analysis.

### **3.2.3. X-ray diffraction (XRD) analysis**

An aliquot of powdered samples was analyzed by X-ray diffraction (Rigaku Ultima IV) to determine the mineralogical composition. CuK $\alpha$ 1 radiation was operated at an applied voltage of 40 kV, with a 20mA beam current. The  $2\theta$  incidence angles spanned from 4° to 60°, with a scan speed of 10°/min using the continuous scan mode.

### **3.2.4. Organic element and stable carbon isotope ratio analysis**

An aliquot of powdered sample (~0.6-3.2 g) was placed in 12M HCl bath at room temperature for overnight three times, and the residue was dried under a vacuum. After drying, each sample was weighted into Sn capsules (~6-44 mg) and analyzed for total organic carbon (TOC) concentration, total nitrogen (TN) concentration, and stable carbon isotope ratio of TOC ( $\delta^{13}\text{C}_{\text{TOC}}$ ) using an elemental analyzer-isotope ratio mass spectrometer (Carlo-Erba NA 1500 Elemental Analyzer-Thermo Finnigan Delta<sup>plus</sup> XL Isotope Ratio Mass Spectrometer). Acetanilide ( $\delta^{13}\text{C} = -25.66 \pm 0.03\text{‰}$ , vs. Pee Dee Belemnite (PDB) standard) was used as working standard to calibrate TOC and TN concentrations and  $\delta^{13}\text{C}_{\text{TOC}}$  values. The  $\delta^{13}\text{C}_{\text{TOC}}$  values relative to PDB standard were calculated using the follow equation:  $\delta^{13}\text{C}_{\text{TOC}} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}} - 1] \times 1000$ . The analytical error based on triplicate analysis for TOC is < 0.02%, TN is < 0.004% and  $\delta^{13}\text{C}_{\text{TOC}}$  is < 0.4‰.

### **3.2.5 Lipid extraction and simultaneous analysis of core and intact polar lipids**

The total lipids were extracted from an aliquot of freeze-dried and powdered samples according to the modified Bligh and Dyer methods (Gibson et al., 2013). Briefly, a mixture of methanol (MeOH; FUJIFILM-Wako, Japan), dichloromethane (DCM; FUJIFILM-Wako, Japan), and buffer solution (2/1/0.8, v/v/v) was added to the sample powder (~54-107 g) into a polytetrafluoroethylene (PFTE, Teflon) tube, and sonicated in ice water for 15 min for a total of 6 extractions. Phosphate buffer (50 mM, pH 7.4) was used for the first three extractions, and trichloroacetate buffer (TCA, 50 g/L, pH 2.0) for the remaining three extractions. After each

extraction, the extract was centrifuged (3000 rpm  $\times$  15 min) and the supernatant was collected in a PFTE separatory funnel. To prevent degradation of acid-sensitive lipids, the initial neutral extract and the TCA-acidified extract were collected separately. Finally, DCM and ultrapure water were added to each extract to give a MeOH/DCM/water ratio of 1/1/0.8 (v/v/v), and the organic layer was separated. The remaining aqueous layer was extracted with DCM three times, and the DCM extract was added to the organic layer. The organic layer was then washed three times with ultrapure water to remove any remaining salt, buffer, and acid. Each washed organic layer was mixed, desulfurized using reduced copper, and then dried using a rotary evaporator. Additionally, a procedural blank was prepared from sea sand (FUJIFILM-Wako, Japan), which was preheated at 500°C for 3 hours, using the same analytical procedure described above. The total lipid extract (TLE) obtained was dissolved in a solvent mixture of hexane/2-propanol (7/3, v/v), followed by filtered through a 0.45- $\mu$ m cellulose filter (Merck, Germany), and stored in a Teflon vial at -20°C until analysis.

A simultaneously analysis of microbial CLs and IPLs in the TLEs from each sample was performed by high performance liquid chromatography/high-resolution mass spectrometry (HPLC/HRMS) using heated electrospray ionization (HESI) in a normal-phase system with several modifications of the instrumental conditions described in Horai et al. (2019) and Chapter 2. Prior to analyze the TLE samples, commercially available L- $\alpha$ -phosphatidyl glycerol, distearoyl, sodium salt (C<sub>36:0</sub> DAG-PG; FUJIFILM-Wako, Japan) and L- $\alpha$ -phosphatidyl choline, dipalmitoyl (C<sub>32:0</sub> DAG-PC; FUJIFILM-Wako, Japan) were analyzed as standards. After their separation and detection characteristics (ionization and sensitivity) were examined, the measurement conditions were determined, in which C<sub>36:0</sub> DAG-PG was detected at 80.8 min and C<sub>32:0</sub> DAG-PC at 119.1 min. Therefore, the data acquisition time of the mass spectrometer was set to 120 min, and the mobile phase was kept at the same composition from the time it reached the final composition until the end of the measurement. Other measurement conditions used the same settings as described in Horai et al. (2019) and Chapter 2. Briefly, a diol column (Inertsil diol; 1.5 mm i.d.  $\times$  250 mm, 3  $\mu$ m particle size; GL Sciences) was used with a guard column (1.5 mm i.d.  $\times$  10 mm) of the same packing material at 30°C. The eluent was a mixture of A (hexane) and B (2-propanol/formic acid/14.8 M ammonium hydroxide, 99.8/0.1/0.1, v/v) at 0.1 mL/min. The HESI-HRMS settings were as follows: spray voltage; 4.5 kV, ion transfer capillary temperature; 275°C, sheath and auxiliary N<sub>2</sub> gas flow rates; 35 and 10 (both arbitrary units), respectively, and heater temperature; 200°C. Before the measurement of each sample, an external mass calibration was performed using the Ultramark 1621 standard, and the protonated ions of the Ultramark 1621 were used for internal mass calibration (lock mass)

during the measurement. The mass accuracy in this study was set to  $< 5$  ppm based on measurements of two phospholipid standards.

Lipid molecules were identified based on retention times and chemical compositional formulas determined from exact mass spectra. Unknown compounds were tentatively identified based on their chemical compositions determined by accurate mass spectra and elution patterns from previously reported normal-phase HPLC/MS (e.g., Roussel et al., 2008; Schouten et al., 2007; Sturt et al., 2004; Yoshinaga et al., 2011). Due to the lack of suitable standards for quantifying individual lipid compounds, each compound detected in this study was presented semi-quantitatively by normalizing their peak areas to the TOC concentration of each sample. Note, therefore, that the composition of each lipid compound reported in this study does not represent their absolute concentrations.

### 3.3. Results

#### 3.3.1. Mineral compositions

The major constituent minerals of the artificial and natural chimneys were sulfide minerals, mainly sphalerite (**Figures 3.4A-D**). The AC-C0016A sample was composed of sulfide minerals (sphalerite, galena, chalcopyrite and wurtzite; **Figure 3.4A**). On the other hand, the AC-C0016B sample also consisted mainly of sulfide minerals (sphalerite, galena, chalcopyrite, pyrite and wurtzite), but contained carbonate (cerussite) as a characteristic mineral (**Figure 3.4B**). Similarly, the natural NC-HSC sample was composed mainly of sulfide minerals (sphalerite, galena, chalcopyrite, pyrite and marcasite, **Figure 3.4C**), while the NC-HRV sample contained sulfide minerals (sphalerite, galena, chalcopyrite, pyrite, wurtzite and marcasite) and trace amounts of sulfate minerals (anhydrite and gypsum) and halite (**Figure 3.4D**).

The RF-SO sample was composed of sulfide minerals (sphalerite, galena, chalcopyrite, pyrite and wurtzite) and trace amounts of quartz and halite (**Figure 3.4E**), while the XRD pattern of the RF-AS sample, which was obscured by weak peak intensity, was composed of silicate minerals and altered minerals (quartz, phlogopite, albite, clinocllore) with trace amounts of halite and sphalerite. A broad peak observed at  $2\theta = 20-30^\circ$  is considered as amorphous silica (**Figure 3.4F**).

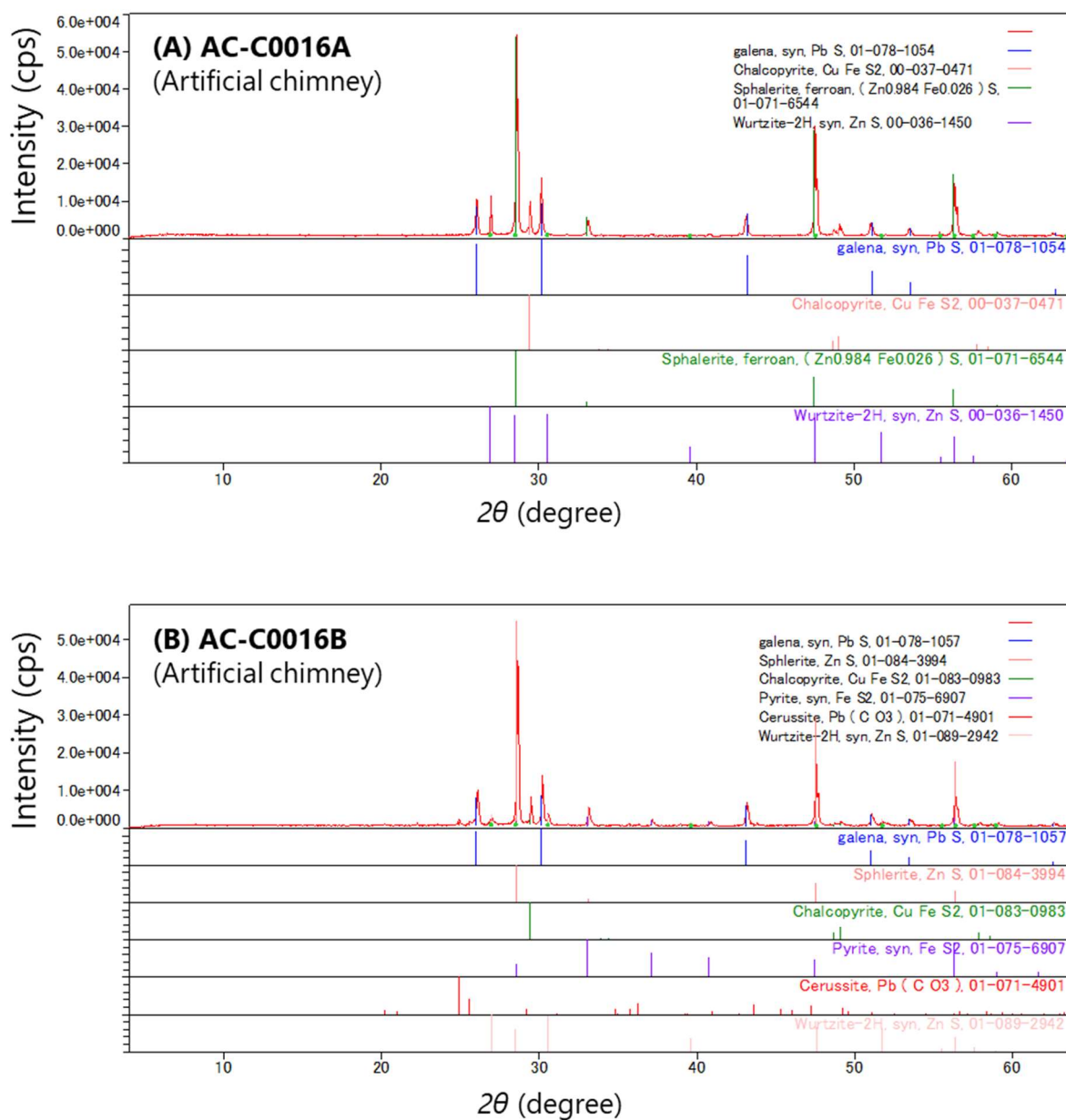


**Figure 3.4.** The powdered X-ray diffraction (XRD) patterns showing mineral assemblages of (A) sample AC-C0016A, (B) sample AC-C0016B.

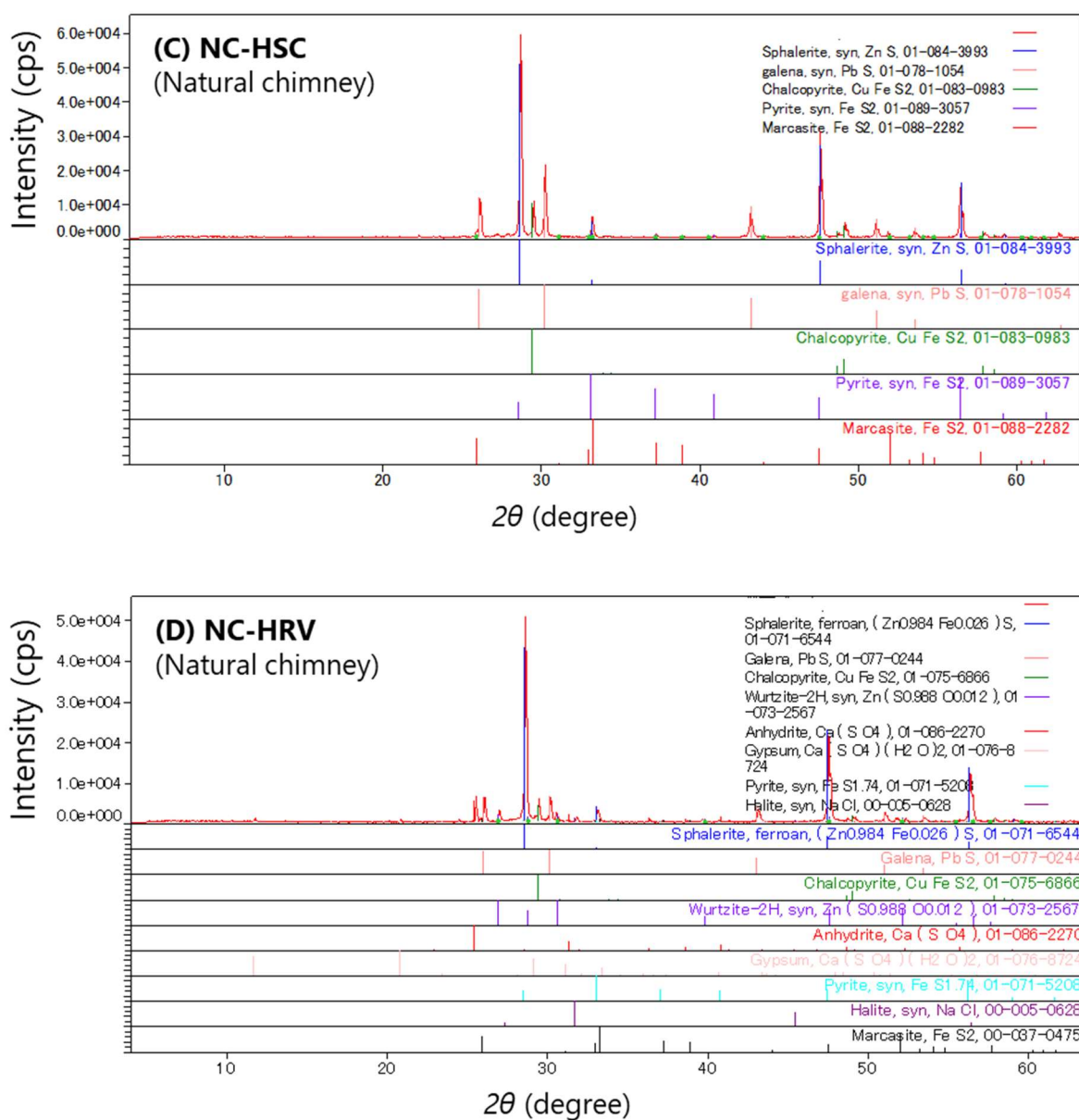


**Figure 3.4.** (Continued) The powdered X-ray diffraction (XRD) patterns showing mineral assemblages of (C) sample NC-HSC, (D) sample NC-HRV.

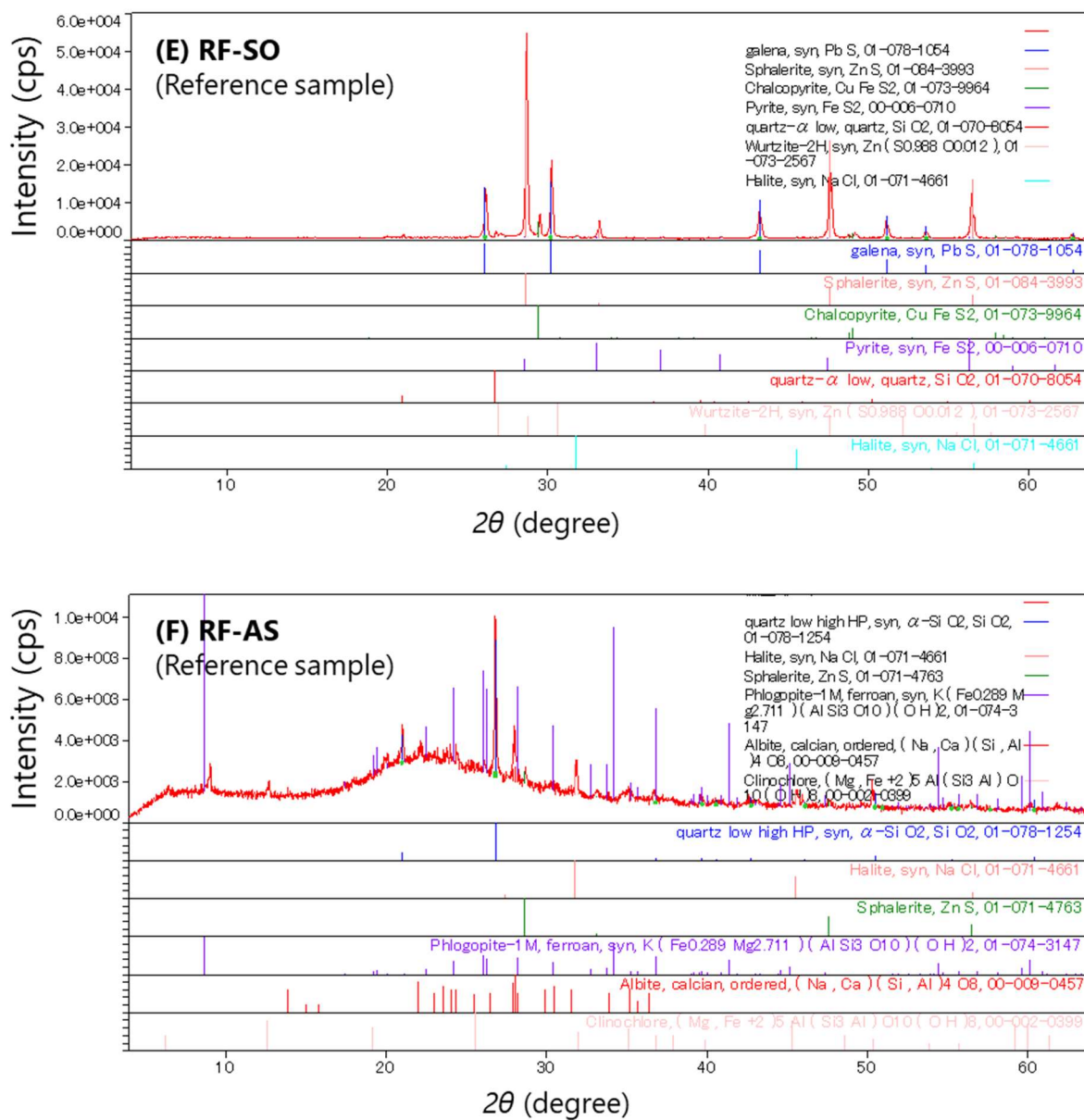


**Figure 3.4.** (Continued) The powdered X-ray diffraction (XRD) patterns showing mineral assemblages (E) sample RF-SO, (F) sample RF-AS.

### 3.3.2. TOC and TN contents, TOC/TN atomic ratios and $\delta^{13}\text{C}_{\text{TOC}}$

Total organic carbon (TOC) concentrations, total nitrogen (TN) concentrations, TOC/TN atomic ratios and carbon isotope ratios of total organic carbon ( $\delta^{13}\text{C}_{\text{TOC}}$ ) are shown in **Table 3.2**. The TOC and TN concentrations of the artificial chimney samples were equal to or a little higher than those of natural chimneys, ranging from 0.005-0.052 wt.% and 0.001-0.012 wt.%, respectively. On the other hand, both TOC and TN concentrations in the reference samples were up to one order of magnitude higher than those values in the artificial and natural chimneys. The TOC and TN concentrations in the artificial and natural chimneys showed a strong positive correlation (correlation coefficient,  $r^2 = 0.983$ , **Figure 3.5A**). The TOC/TN atomic ratios of the artificial and natural chimneys were around 5.5, clearly smaller than the TOC/TN ratio of the reference samples (8-11). The  $\delta^{13}\text{C}_{\text{TOC}}$  values of the two artificial chimneys (AC-C0016A, -25.9‰; AC-C0016B, -26.0‰) were similar to the natural chimney values (NC-HSC, -21.8‰; NC-HRV, -26.8‰). These chimney samples were more enriched in  $^{13}\text{C}$  than the reference samples (RF-SO, -34.0‰; RF-AS, -31.2‰). The carbon isotopic compositions are clearly distinct between chimney and reference samples (**Table 3.2**; **Figure 3.5B**).

**Table 3.2.** Total organic carbon (TOC) and total nitrogen (TN) contents, TOC/TN atomic ratios and stable carbon isotope ratios of TOC ( $\delta^{13}\text{C}_{\text{TOC}}$ ).

| Elemental and isotopic compositions             | Artificial chimneys |                   | Natural chimneys  |                   | Reference samples |                   |
|-------------------------------------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                                 | AC-C0016A           | AC-C0016B         | NC-HSC            | NC-HRV            | RF-SO             | RF-AS             |
| TOC (wt.%)                                      | $0.017 \pm 0.001$   | $0.052 \pm 0.001$ | $0.005 \pm 0.000$ | $0.049 \pm 0.001$ | $0.151 \pm 0.009$ | $0.205 \pm 0.024$ |
| TN (wt.%)                                       | $0.004 \pm 0.000$   | $0.012 \pm 0.001$ | $0.001 \pm 0.000$ | $0.017 \pm 0.005$ | $0.017 \pm 0.000$ | $0.027 \pm 0.004$ |
| TOC/TN (by atomic)                              | $5.1 \pm 0.1$       | $5.2 \pm 0.2$     | $5.4 \pm 0.5$     | $5.8 \pm 0.1$     | $8.9 \pm 0.4$     | $10.6 \pm 0.5$    |
| $\delta^{13}\text{C}_{\text{TOC}}$ (‰, vs. PDB) | $-25.9 \pm 0.2$     | $-26.0 \pm 0.2$   | $-21.8 \pm 0.4$   | $-27.2 \pm 0.1$   | $-34.1 \pm 0.2$   | $-31.4 \pm 0.1$   |

These values and errors are reported as the means and standard deviations by measurements in triplicate.

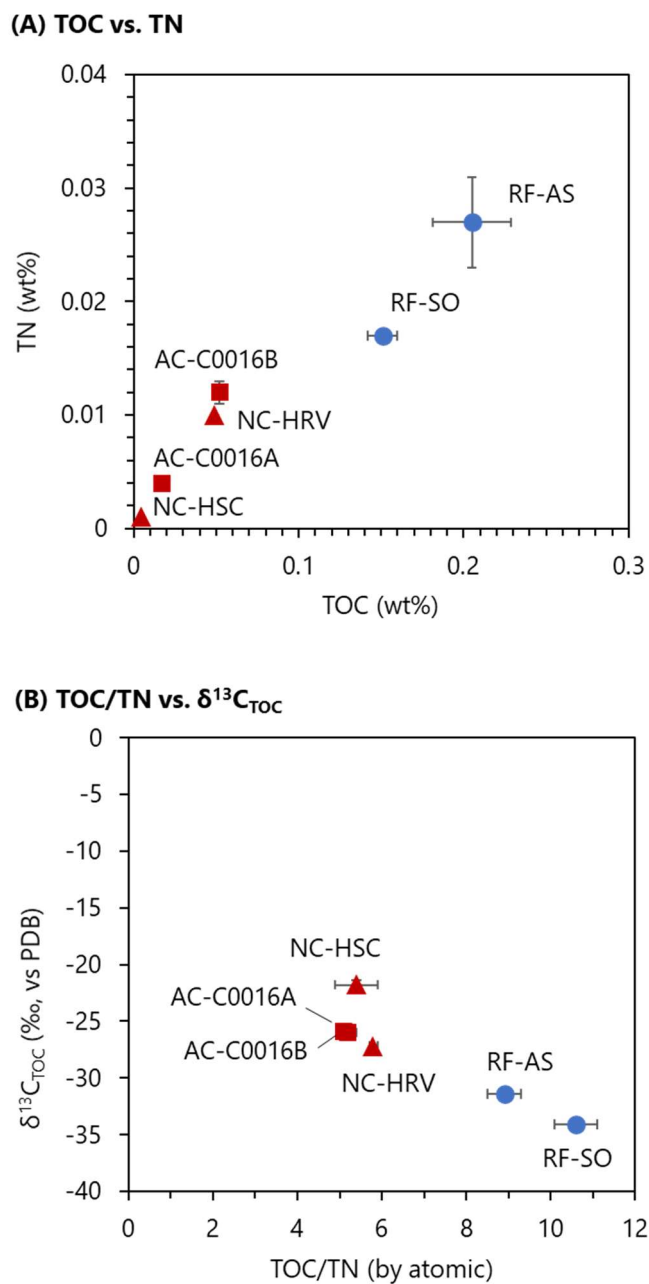


**Figure 3.5.** Plots of organic elemental compositions of artificial chimneys, natural chimneys and reference samples. (A) TOC contents versus TN contents. (B) TOC/TN ratios versus  $\delta^{13}\text{C}_{\text{TOC}}$ . Positive correlation ( $r^2 = 0.983$ ) between TOC contents and TN contents observed in artificial and natural chimneys. Details are summarized in **Table 3.2**.

### 3.3.3. Lipid biomarker distributions

**Figure 3.6** and **Figure 3.7** show the partial positive ion base peak chromatograms of the representative IPLs and CLs observed in the TLEs from artificial chimneys, natural chimneys and reference samples. The numerous numbers of archaeal and non-archaeal polar and core lipids were observed in those samples. However, due to the high ion intensity of the Ultramark 1621 used for Lock mass, the peaks of lipids could not be adequately shown on these chromatograms. Therefore, please refer to the **Appendix Tables** at the end of this dissertation for more information on all lipid biomarkers detected and discussed in this chapter. The typical chemical structures of lipid biomarkers described in this chapter are shown in **Figure 3.8**.

#### 1) AC-C0016A

##### i) Archaeal IPLs

The archaeal IPLs of the AC-C0016A sample included 15 compounds possessing the mono-glycosidic-group (1G-) as a polar group. In combination with the lipid core, these are classified into 5 classes: 1G-archaeol (1G-AR), 1G-macrocylicarchaeol (1G-MAR), 1G-glycerol-dialkyl-glycerol-tetrathers (1G-GDGTs), 1G-H-shaped GDGTs (1G-HGDGTs), and 1G-methylated HGDGTs (1G-nMeHGDGTs; n = number of additional methyl groups). The total concentration of Archaeal-IPLs was estimated to be  $\sim 1.1 \times 10^6$  ng/g organic carbon (OC), equivalent to  $\sim 1.8 \times 10^2$  ng/g sample (**Figure 3.9A**). 1G-AR was the most dominant IPL, which was estimated to be  $\sim 3.8 \times 10^6$  ng/g of OC, representing 35.5% of total archaeal IPL (48.2% of total archaeal-IPL peak area, **Table 3.3**). 1G-MAR is estimated to be  $\sim 5.1 \times 10^4$  ng/g, representing 4.8% of the total archaeal IPL (6.5% of the total archaeal-IPL peak area, **Table 3.3**).

Within the same lipid class, the RT difference caused by unsaturated bonds (e.g., cyclopentane ring) in the alkyl side chains is very small ( $\sim 0.5$  min), while the RT differences in more polar lipid structures, including polar functional groups (e.g., OH groups) or macrocyclic structures, is as large as  $> 3$  min (Horai et al., 2019). Therefore, 1G-macrocylic archaeol (MAR, RT: 70.4 min; **Appendix Table A3.1**) is not a mono-unsaturated-archaeol (1unsAR) whose lipid core contains one unsaturated bond in the alkyl side chain, but having macrocyclic structures based on the RT difference compared to 1G-AR. 1G-GDGTs contained 0-5 ring structures were detected between 69.0-70.6 min, being estimated to be  $\sim 6.3 \times 10^4$  ng/g (5.8 % of total archaeal IPL or 4.4% of total archaeal-IPL peak area, **Table 3.3**). The molecular distribution of cyclized compounds of 1GDGTs is the following: 1G-GDGT-0 (86.8%,  $5.5 \times 10^4$  ng/g)  $\gg$  1G-GDGT-4 (7.5%,  $4.7 \times 10^3$  ng/g)  $>$  1G-GDGT-1 (2.4%,  $1.5 \times 10^3$  ng/g)  $>$  1G-

GDGT-3 (1.5%,  $9.3 \times 10^2$  ng/g) > 1G-GDGT-2 (1.1%,  $7.1 \times 10^2$  ng/g) > 1G-GDGT-5 (0.7%,  $4.3 \times 10^2$  ng/g) (**Table 3.5**). The number of ring structure in GDGTs (Ring Index, RI) was calculated to be 0.43 (**Table 3.6**) using the following equation (Pearson et al., 2004).

$$RI = 0 \times (\text{GDGT-0}) + 1 \times (\text{GDGT-1}) + 2 \times (\text{GDGT-2}) + 3 \times (\text{GDGT-3}) + 4 \times (\text{GDGT-4}) + 5 \times (\text{GDGT-5}) / \Sigma \text{GDGTs}$$

1G-HGDGTs were tentatively identified as isomers of the 1G-GDGT-1 to -5 between 71.4-72.6 min in RT (**Appendix Table A3.1**), possessing as a lipid core HGDGTs that cross-link the two alkyl chains of the GDGTs in an H-shaped manner. 1G-HGDGTs possess 0-4 cyclic structures, with the abundance of  $5.4 \times 10^5$  ng/g of OC, representing 49.6 % of total archaeal IPL (37.6% of total archaeal-IPL peak area, **Table 3.3**). The distribution of cyclized 1G-HGDGTs compounds was in the order: 1G-HGDGT-0 (65.6%,  $3.5 \times 10^5$  ng/g) >> 1G-HGDGT-1 (13.5%,  $7.2 \times 10^4$  ng/g) > 1G-HGDGT-2 (9.6%,  $5.1 \times 10^4$  ng/g) >> 1G-GDGT-3 (6.4%,  $3.4 \times 10^4$  ng/g) > 1G-HGDGT-4 (4.9%,  $2.6 \times 10^2$  ng/g). The ring index (RI) of 1G-HGDGTs was calculated to be 0.72 (**Table 3.5**).

1G-nMe-HGDGTs containing 1-2 methyl (Me) groups and 4 ring structures, (1G-1Me-HGDGT-4 and 1G-2Me-HGDGT-4) were detected as co-eluting components of 1G-HGDGT-4 at 72.5 min in RT (**Appendix Table A3.1**). 1G-nMe-HGDGTs were estimated to be  $4.7 \times 10^4$  ng/g of OC, accounting for 4.3% of the total archaeal IPL (3.2% of the total archaeal-IPL peak area). 1G-1Me-HGDGT-4 and 1G-2Me-HGDGT-4 were estimated at  $4.2 \times 10^4$  ng/g OC and  $1.5 \times 10^4$  ng/g OC, respectively (**Table 3.3**). The methylation index (MIX) was calculated to be 1.32 (**Table 3.6**) using the following equation (Sollich et al., 2017);

$$MIX = 1 \times (1\text{MeHGDGT}) + 2 \times (2\text{MeHGDGT}) + 3 \times (3\text{MeHGDGT-3}) + 4 \times (4\text{MeHGDGT}) / \Sigma n\text{MeHGDGT}$$

## ii) Archaeal core lipids

The total amount of archaeal core lipids (Archaeal-CLs) of the AC-C0016A sample was estimated to be  $\sim 2.1 \times 10^6$  ng/g OC (**Figure 3.9B**). Among the Archaeal-CLs, two types of diether lipids were identified; AR and MAR (RT: 10.7 min; **Appendix Table A3.2**), accounting for 20.4% and 5.4% of the total abundance, respectively (**Table 3.4**). On the other hand, four types of tetraether lipids were detected: glycerol trialkyl glycerol tetraether (GTGT)-0, GDGTs, HGDGTs, and nMe-HGDGTs. GDGTs (RT: 33.8-42.6 min) contained 0 to 5 ring structures, accounting for 16.6% of the total abundance (**Table 3.6; Figure 3.10**). HGDGTs have a structure in which the two biphytanyl chains of GDGTs are crosslinked, which were separated in normal phase HPLC to elute after GDGTs using another hexane/2-propanol eluent (e.g.,

Schouten et al., 2008). Based on this separation, the series of HGDGTs compounds (HGDGT-0, -1, -2, -3, and -4) were identified as isomeric compounds of GDGT-1, -2, -3, -4, and -5 with retention times of 44.8-57.9 min. The two isomers of HGDGT-0 have been reported (Baumann et al., 2018). In this study, two peaks within  $\sim 0.6$  ppm of the calculated exact mass ( $m/z$  1300.3070) corresponding to HGDGT-0 were observed at 44.8 min and 47.3 min (**Appendix Table A3.1**), which were deduced to be the two isomers of HGDGT-0 (HGDGT-0 and HGDGT-0'). The EICs for HGDGT-1, -2, -3, and -4 could not be determined due to two to three peaks mixing (unknown isomers) or peak splitting. The HGDGTs and their isomers accounted for 37.1% of the total abundance (**Table 3.6, Figure 3.10**).

The GDGTs and HGDGTs classes have a group of analogous compounds containing 1 to 4 Me groups that co-elute with them (nMe-GDGTs and nMe-HGDGTs; Knappy et al., 2014; Reeves et al., 2014). They also contain 0-4 ring structures (Sollich et al., 2017). Therefore, a series of compound groups attributed to masses within 1.1 ppm of the calculated exact mass of  $[\text{HGDGTs} + \text{C}_n\text{H}_{2n} + \text{H}]^+$  at RT 46.6-58.1 min indicating 1 to 4 Me-containing groups were identified as 1Me-, 2Me-, 3Me- and 4Me-HGDGTs (**Appendix Table A3.2**). 1Me-HGDGTs and 2Me-HGDGTs contained 0-4 ring structures and accounted for 4.7% and 8.5% of the total abundance, respectively (**Table 3.4**). 3Me- and 4Me-HGDGTs contained 3 and 4 ring structures and accounted for 5.4% and 1.6% of the total abundance, respectively (**Table 3.5**).

The glycerol dialkanol diethers (GDDs) and glycerol monoalkanol diethers (GMDs) compounds were identified as a group of related compounds of GDGTs, HGDGTs, and nMe-HGDGTs. The GDDs and GMDs compounds are isoprenoid diether-alcohol compounds having an OH group at the end of isoprenoid chain, which are equivalent to GDGTs and HGDGTs structures without one glycerol (Liu et al., 2012; Bauersachs and Schwark, 2015). The GDDs contained 0-4 ring structures and accounted for 0.04% of the total (**Table 3.4**), and the GMDs contained 0-4 ring structures and accounted for 0.1% (**Table 3.4**). GMDs also contained a group of co-eluting compounds attributed to the compositional formula  $[\text{GMDs} + \text{CH}_2 + \text{H}]^+$ , which were inferred to be monomethylated GMDs (1Me-GMDs) by analogy to 1Me-HGDGTs (**Appendix Table A3.2**). The 1Me-GMDs contained 0-4 ring structures and accounted for 0.01% of the total (**Table 3.4**).

The distribution of cyclic and methylated compounds of tetraether lipids and their related compounds is the followings (**Table 3.5, Table 3.6**). GDGTs were distributed as 0 (80.2%) >> 4 (7.4%) > 1 (4.8%) > 2 (3.8%) > 3 (3.5%) >> 5 (0.3%) with an RI of 0.54. GDDs were distributed as 0 (84.0%) >> 4 (6.9%) > 1 (3.1%) > 2 (3.0%), 3 (3.0%) >> 5 (0.3%) with an RI of 0.46. HGDGTs were distributed as 0 (55.4%) >> 1 (18.4%) >> 2 (13.7%) > 3 (7.7%)

> 3 (4.8%), with an RI of 0.88. The distribution of GMDs was 0 (75.5%) >> 1 (9.7%) > 2 (5.8%) > 4 (4.9%) > 3 (4.3%) with an RI of 0.53. 1Me-HGDGT was 4 (40.8%) > 2 (22.1%) > 3 (18.3%) > 1 (10.8%) > 0 (8.0%) with an RI of 2.73. 1Me-GMD was 4 (64.0%) > 2 (15.4%) > 3 (9.8%) > 0 (8.8%) > 1 (2.0%) and the RI was 3.18. 2Me-HGDGT was 4 (70.4%) > 3 (15.4%) > 2 (10.0%) > 1 (2.4%) > 0 (1.7%) and the RI was 3.51. 3Me-HGDGT was 4 (90.7%) > 3 (8.7%) > 2 (0.7%) and the RI was 3.90. 4Me-HGDGT was 4 (95.8%) > 3 (4.2%) and the RI was 3.96. The MIX (Sollich et al., 2017) for nMe-GDGTs was 2.19.

### iii) Non-archaeal IPLs

Non-archaeal IPLs were not detected in the AC-C0016A sample.

### iv) Non-archaeal core lipids

The non-archaeal core lipids were classified into three classes, diether glycerol lipids (DEGs), acyl/ether-glycerol lipids (AEGs), and diacylglycerol lipids (DAGs), according to the bonding state between glycerol and alkyl carbon chains, with the abundance of 93.7%, 1.7%, and 4.7%, respectively (**Figure 3.12, Table 3.8**). The 30 types of DEGs were detected, ranging from C<sub>30:0</sub> to C<sub>39:2</sub> of which C<sub>37:1</sub> DEG and C<sub>34:0</sub> DEG were the most dominant. The average carbon number and degree of unsaturation of DEGs were 35.1 and 0.7, respectively. The 14 AEGs compounds were detected, ranging from C<sub>31:0</sub> to C<sub>35:2</sub> of which C<sub>34:1</sub>AEG and C<sup>33:1</sup>AEG were dominant. The average carbon number and degree of unsaturation of AEGs were 33.3 and 0.9, respectively. 28 DAGs were detected, ranging from C<sub>30:0</sub> to C<sub>38:2</sub> of which C<sub>32:2</sub> DAG and C<sub>34:0</sub> DAG were dominant. The average carbon number and degree of unsaturation of DAGs were 33.4 and 1.2, respectively.

## 2) AC-C0016B

### i) Archaeal IPLs

For archaeal IPLs of the AC-C0016B sample, 13 compounds possessing mono- and di-glycosidic groups (1G- and 2G-) as polar groups were detected and classified into seven classes according to their combination with the lipid core: 1G-AR, 1G-MAR, 1G-OHAR, 1G-GDGTs, 1G-HGDGTs, 1G-nMe-HGDGTs and 2 G-GDGT. The total amount of Archaeal-IPLs was estimated to be  $\sim 4.6 \times 10^4$  ng/g OC ( $\sim 24.2$  ng/g sample) (**Figure 3.9A**).

1G-AR was estimated to be  $\sim 1.4 \times 10^4$  ng/g OC, accounting for 29.8% of the total archaeal IPL (34.5% of total archaeal-IPL peak area). 1G-MAR (RT: 70.6 min) was estimated to be  $\sim 1.5 \times 10^4$  ng/g OC, accounting for 31.4% of the total archaeal IPL (36.4% of total

archaeal-IPL peak area). 1G-OHAR (RT: 69.9 min) was estimated to be  $\sim 3.9 \times 10^3$  ng/g OC and accounted for 8.4% of the total archaeal IPL (9.5% of total archaeal-IPL peak area) (**Table 3.3**). 1G-GDGTs were detected between 69.0-70.6 min, containing 0, 1, 2, and 5 ring structures, which were estimated to be  $\sim 7.7 \times 10^3$  ng /g OC, accounting for 16.5 % of total archaeal IPL (10.7% of total archaeal-IPL peak area) (**Table 3.3**). The distribution of cyclized compounds of 1GDGTs was estimated to be 1G-GDGT-0 (60.1%,  $4.3 \times 10^3$  ng/g OC) > 1G-GDGT-5 (26.0%,  $2.2 \times 10^3$  ng/g OC) >> 1G-GDGT-2 (7.3%,  $6.2 \times 10^2$  ng/g OC) > 1G-GDGT-1 (6.6%,  $5.6 \times 10^2$  ng/g OC) (**Table 3.5**). The RI of 1G-GDGTs was calculated to be 0.43. The 1G-HGDGTs compounds were detected between 71.4-72.7 min and included 0, 1 and 4 ring structures, totaling  $\sim 4.4 \times 10^3$  ng/g OC and accounted for 9.5 % of the total archaeal IPL (6.1% of total archaeal-IPL peak area). The distribution of cyclized compounds of 1G-HGDGTs was 1G-HGDGT-0 (85.5%,  $3.8 \times 10^3$  ng/g OC) >> 1G-HGDGT-1 (8.5%,  $3.8 \times 10^2$  ng/g OC) > 1GHGDGT-4 (6.0%,  $2.6 \times 10^2$  ng/g OC). The RI of 1G-HGDGTs was calculated to be 0.32 (**Table 3.5**). As the 1G-nMe-HGDGTs group including 1- 2 methyl groups and 4 ring structures, 1G-1Me-HGDGT-4 and 1G-2Me-HGDGT-4 were detected at RT 72.6 min as co-elution components with 1G-HGDGT-4 (**Appendix Table A3.1**). The 1G-nMe-HGDGTs accounted for  $1.3 \times 10^3$  ng/g OC, or 2.7% of the total archaeal IPL (**Table 3.3**). 1G-1Me-HGDGT-4 and 1G-2Me-HGDGT-4 were estimated to be  $8.2 \times 10^2$  ng/g OC and  $4.5 \times 10^2$  ng/g of OC, respectively, and their MIX was calculated to be 1.35. Only 2G-GDGT-0 (no ring structure) of 2G-GDGTs was detected at 80.8 min (**Appendix Table A3.1**). The 2G-GDGT-0 compound accounted for  $8.2 \times 10^2$  ng/g OC, or 1.8% of the total archaeal IPL (1.0% of total archaeal-IPL peak area) (**Table 3.3**). The RI of IPL-GDGTs (1G-GDGTs + 2G-GDGT-0) was calculated to be 1.51 (**Table 3.6**), and the abundance ratio of diether to tetraether IPLs (IPL-diether/IPL-tetraether) was 69.5/30.5.

## ii) Archaeal CLs

Archaeal CLs of the AC-C0016B sample were estimated at  $\sim 7.1 \times 10^5$  ng/g OC (**Figure 3.9B**). Three types of diether lipids were detected as AR, MAR, and OHAR, which accounted for 14.0%, 6.0%, and 2.1% of the total archaeal CLs, respectively (**Table 3.4**). Four types of tetraether lipids were detected as GTGT-0, GDGTs, HGDGTs, and nMe-HGDGTs. GTGT-0 contained no ring structures and accounted for 0.1% of the total (**Table 3.4**). GDGTs contained 0 to 5 ring structures and accounted for 53.5% of the total (**Table 3.4**). GDGT-3 and GDGT-4 were detected as two structural isomers, respectively (**Appendix Table A3.2**). HGDGTs contained from 0 to 4 ring structures and accounted for 13.1% of the total (**Table 3.4**).

Each of HGDGT-0, HGDGT-1, HGDGT-2, and HGDGT-3 contained two structural isomers (**Appendix Table A3.2**). nMe-HGDGTs contained additional one to four methyl groups (**Appendix Table A3.2**). 1Me-HGDGTs, 2Me-HGDGTs, and 3Me-HGDGTs contained zero to four ring structures, while only 4Me-HGDGTs containing four ring structures were detected. 1Me-HGDGTs, 2Me-HGDGTs, 3Me-HGDGTs, and 4Me-HGDGTs accounted for 4.9%, 5.3%, 0.8%, and 0.1% of the total, respectively (**Table 3.4**). GDD-0, GDD-5, GMD-0 and 1Me-GMD-0 were also detected, accounting for 0.03%, 0.1% and 0.01%, respectively (**Table 3.4**).

The distribution of cyclic compounds of tetraether lipids and related compounds is described in the following (**Table 3.5**, **Table 3.6**). The distribution of GDGTs was 0 (86.0%) >> 5 (5.1%) > 1 (3.3%) > 2 (2.7%) > 4 (1.7%) > 3 (1.2%) with an RI of 0.45. The distribution of HGDGTs was 0 (78.8%) >> 1 (8.6%) > 4 (5.9%) > 2 (4.0%) > 3 (2.9%) with a RI of 0.48. 1Me-HGDGTs were 0 (31.8%) > 4 (35.3%) > 3 (12.8%) > 2 (10.6%) > 1 (9.6%) with a RI of 1.96. 2Me-HGDGT was 4 (36.3%) > 0 (27.0%) > 2 (13.2%) > 1 (12.1%) > 3 (11.5%) with a RI was 2.18. 3Me-HGDGT was 4 (73.6%) > 3 (12.0%) > 0 (7.2%) > 2 (4.6%) > 1 (2.6%) with a RI of 3.42. GDD-0 and GDD-5 were 78.1% and 21.9%, respectively, with a RI was 1.1. Me-HGDGT was the only one containing four ring structures. GMD and 1Me-GMD were not detected containing any ring structures. The nMe-HGDGTs compounds were detected with a MIX of 1.66.

### iii) Non-archaeal IPLs

For non-archaeal IPLs of the AC-C0016B sample, 227 compounds were detected and classified into 16 classes based on the combination of polar groups and core structures (**Appendix Table A3.3**): acyl/ether-glycerol (AEG) with PG, PE, PME (phospho-*N*-methyl-ethanolamine), PDME (phospho-*N, N*-dimethyl-ethanolamine), PC, DGTS (*N, N, N*-trimethyl-homoserine) as polar head groups, diacyl-glycerol (DAG) with 1G as a polar head group, diether-glycerol (DEG) with PE, PME, PDME, PC as polar head groups, and sphingolipid (Sph) with 1G as a polar head group. The percentages of DAG-IPL, AEG-IPL, DEG-IPL and Sph-IPL were 73.1%, 7%, 7% and 7%, respectively (**Table 3.7**; **Figure 3.12**). The total amount of non-archaeal IPLs was estimated to be  $\sim 7.8 \times 10^5$  ng/g of OC (**Figure 3.9A**).

a) Diacylglycerol-phospho-glycerol (DAG-PG) was identified as  $[M+NH_4]^+$  ions within 2 ppm of mass precision between 81.8-83.1 min in RT. DAG-PG accounted for 0.1% of the total non-archaeal IPL peak area (**Table 3.7**). DAG-PG contained C<sub>32:1</sub>-C<sub>36:3</sub> DAG as a core lipid, with C<sub>32:2</sub> DAG, C<sub>32:1</sub> DAG, and C<sub>34:2</sub> DAG being the major ones. The average carbon number was

calculated to be 33.0 and the average unsaturation to be 1.7 (**Table 3.7**).

b) Diacylglycerol-phospho-ethanolamine (DAG-PE) was identified as  $[M+H]^+$  ions within 2.5 ppm of mass precision. Four series groups with different retention times were detected: DAG-PE (86.3-91.2 min) distinguished from DAG-PE and its N-methylated homologues of DAG-PME (90.5-94.3 min), DAG-PDME (92.5-95.8 min), and DAG-PC (114.1-120.0 min) (**Appendix Table A3.3**). DAG-PE contained C<sub>30:0</sub>-C<sub>40:6</sub> DAG as a core lipid, accounted for 4.8% of the total peak area of the non-archaeal IPL (**Table 3.7**) with C<sub>32:2</sub> DAG, C<sub>32:1</sub> DAG and C<sub>34:2</sub> DAG being the major ones. The average carbon number was calculated to be 33.8 and the average unsaturation to be 2.3 (**Table 3.7**).

c) Diacylglycerol-phospho-*N*-methyl-ethanolamine (DAG-PME) was tentatively identified as a series that was retained for about 3-4 min relative to DAG-PE of the same carbon number and degree of unsaturation DAG-PME (**Appendix Table A3.3**), accounting for 7.6% of the total peak area of the non-archaeal IPL (**Table 3.7**). DAG-PME contained C<sub>30:0</sub>-C<sub>37:3</sub> DAG as core lipids, with C<sub>34:2</sub>, C<sub>32:1</sub>, C<sub>32:2</sub> and C<sub>36:2</sub> being the major ones. The average carbon number was calculated to be 33.4 and the average unsaturation to be 1.7.

d) Diacylglycerol-phospho-*N*-methyl-ethanolamine (DAG-PDME) was tentatively identified as a series retained for about 3 min relative to DAG-PME of the same carbon number and degree of unsaturation (**Appendix Table A3.3**), accounting for 4.8% of the total peak area of the non-archaeal IPL (**Table 3.7**). DAG-PME contained C<sub>30:0</sub>-C<sub>36:4</sub> DAG as core lipids, with C<sub>34:2</sub> being the major one. The average carbon content was calculated to be 33.8 and the average unsaturation to be 1.0 (**Table 3.7**).

e) Diacylglycerol-phospho-choline (DAG-PC) was identified as  $[M+H]^+$  ion at mass precision of < 2.5 ppm, accounting for 38.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). DAG-PC contained C<sub>32:0</sub>-C<sub>40:4</sub> DAG as core lipids, with C<sub>34:1</sub> and C<sub>36:2</sub> DAG being major ones. The average carbon number was calculated to be 35.8 and the average unsaturation to be 1.0 (**Table 3.7**).

f) Diacylglycerol-(*N,N,N*)-trimethylhomoserine (DGTS) was tentatively identified at mass precision of < 2 ppm as  $[M+H]^+$  ions during 82.4-85.3 min (**Appendix Table A3.3**). DGTS accounted for 16.0% of the total peak area of the non-archaeal IPL (**Table 3.7**). DGTS contained C<sub>30:0</sub>-C<sub>37:3</sub> DAG as core lipids with C<sub>36:2</sub>, C<sub>32:2</sub>, and C<sub>36:3</sub> being major ones. The average carbon number was calculated to be 34.3 and the average unsaturation to be 1.8.

g) Monoglycosyl-diacylglycerols (DAG-1G) was tentatively identified as  $[M+NH_4]^+$  between retention times of 72.3-73.2 min (**Appendix Table A3.3**), accounted for 1.7% of the total peak area of the non-archaeal IPL (**Table 3.7**). DAG-1G contained C<sub>30:0</sub>-C<sub>38:2</sub> as core lipids, with

C<sub>36:2</sub> being a major component. The average carbon number was calculated to be 34.7 and the average degree of unsaturation was 1.7.

h) Acyl/ether-glycerol-phosphoethanolamine (AEG-PE) and its N-methylated homologues (AEG-PME, AEG-PDME, AEG-PC) were distinguished as four series with different retention time ranges (AEG-PE: 85.4-88.2 min, AEG-PME: 87.3-89.5 min, AEG-PDME: 88.9-91.3 min, AEG-PC: 104.8-113.4 min, **Appendix Table A3.3**), accounting for 1.8% of the total peak area of the non-archaeal IPL (**Table 3.7**). AEG-PE contained C<sub>31:0</sub>-C<sub>36:6</sub> as core lipids, with C<sup>33:1</sup> and C<sub>34:2</sub> being major ones. The average carbon number was calculated to be 33.5 and the average degree of unsaturation 1.7.

i) Acyl/ether-glycerol-phospho-*N*-methyl-ethanolamine (AEG-PME) accounted for 1.4% of the total peak area of the non-archaeal IPLs (**Table 3.7**) and contained C<sub>31:0</sub>-C<sub>36:2</sub> as core lipids, with C<sub>31:1</sub> being major one. The average carbon number was calculated to be 32.4 and the average degree of unsaturation 1.1.

j) Acyl/ether-glycerol-phospho-*N,N*-dimethyl-ethanolamine (AEG-PDME) accounted for 1.7% of the total peak area of the non-archaeal IPL (**Table 3.7**), and contained C<sub>31:0</sub>-C<sub>34:2</sub> as core lipids with C<sub>34:4</sub> being major one. The average carbon number was calculated to be 32.4 and the average degree of unsaturation 1.1.

k) Acyl/ether-glycerol-phosphatidyl choline (AEG-PC) accounted for 2.8% of the total peak area of the non-archaeal IPL (**Table 3.7**) and contained C<sub>36:1</sub>-C<sub>36:4</sub> as core lipids with C<sub>36:2</sub> being major one. The average carbon number was calculated to be 36.0 with the average degree of unsaturation of 1.0.

l) Diether-glycerol-phosphoethanolamine (DEG-PE) and its *N*-methyl homologues (DEG-PME, DEG-PDME) were distinguished as three series with different retention time ranges (DEG-PE: 84.0-86.3 min, DEG-PME: 86.2-88.5 min, DEG-PDME: 86.8-89.7 min) (**Appendix Table A3.3**). DEG-PE accounted for 2.7% of the total peak area of the non-archaeal IPLs (**Table 3.7**) and contained C<sub>36:1</sub>-C<sub>36:4</sub> as core lipids with C<sub>34:2</sub> being major one. The average carbon number was calculated to be 33.0 with the average degree of unsaturation of 1.7.

m) Diether-glycerol-phospho-*N*-methyl-ethanolamine (DEG-PME) accounted for 2.7% of the total peak area of the non-archaeal IPLs (**Table 3.7**) and contained C<sub>29:0</sub>-C<sub>34:2</sub> as core lipids with C<sub>30:0</sub>, C<sub>31:1</sub>, C<sub>34:2</sub> and C<sub>32:2</sub> being major ones. The average carbon number was calculated to be 31.6 with the average degree of unsaturation of 1.0 (**Table 3.7**).

n) Diether-glycerol-phospho-*N,N*-dimethyl-ethanolamine (DEG-PDME) accounted for 2.7% of the total peak area of the non-archaeal IPLs (**Table 3.7**), and contained C<sub>30:0</sub>-C<sub>34:2</sub> as core lipids

with C<sub>34:2</sub> and C<sub>32:2</sub> being major ones. The average carbon number was calculated to be 33.3 with the average degree of unsaturation of 1.5.

o) 1G-sphingolipid (1G-Sph) was tentatively detected between 73.8-75.8 min as [M+H]<sup>+</sup> ions within -2.7 ppm of mass precision (**Appendix Table A3.3**). Sphingolipid contains a backbone sphingoid bases including sphingosine instead of glycerol (**Figure 3.8**). The total carbon number of sphingosine and fatty acids ranged from C<sub>33:0</sub>-C<sub>40:3</sub> with the major of C<sub>38:2</sub>. The average carbon number and degree of unsaturation were calculated to be 36.6 and 1.8, respectively (**Table 3.7**).

#### iv) Non-archaeal core lipids

Non-archaeal core lipids of the AC-C0016B sample were classified into three classes: Diether glycerol lipids (DEGs), Acyl/ether-glycerol lipids (AEGs), and Diacylglycerol lipids (DAGs), accounted for 72.8%, 10.9%, and 16.3%, respectively (**Figure 3.8**). As shown in **Appendix Table A3.4**, 34 DEG compounds were detected ranging from C<sub>30:0</sub> to C<sub>39:2</sub> as the core lipids with the average carbon number of 33.5 and degree of unsaturation of 1.1. For AEGs, 28 compounds were detected, ranging from C<sub>30:0</sub> to C<sub>38:3</sub> as the core lipids with the average carbon number of 33.1 and degree of unsaturation of 1.3. For DAGs, 33 compounds were detected ranging from C<sub>30:0</sub> to C<sub>38:4</sub> with the average carbon number of 33.9 and degree of unsaturation of 2.2.

### 3) NC-HSC

#### i) Archaeal IPLs

As archaeal-IPLs of the NC-HSC sample, 10 classes were detected, including of 20 compounds: ARs and MARs bearing PE as a polar head group (1G-AR, 2G-AR, PE-AR, 1G-MAR, 2G-MAR, PE-MAR) as well as 1G- GDGTs, 1G-HGDGTs, 1G-nMeHGDGTs and 2GHGDGTs. The total concentration of the detected archaeal-IPLs was estimated at  $\sim 3.3 \times 10^6$  ng/g of OC ( $\sim 1.7 \times 10^2$  ng/g of sample) (**Figure 3.9A**).

1G-AR (RT: 66.4 min) was estimated to be  $\sim 5.5 \times 10^5$  ng/g of OC, accounting for 16.6% of the total archaeal IPL (23.2% of the total archaeal-IPL peak area, **Table 3.3**). 2G-AR (RT: 80.8 min) was estimated to be  $\sim 1.7 \times 10^5$  ng/g of OC, accounting for 5.1% of the total archaeal IPL (5.9% of the total archaeal-IPL peak area, **Table 3.3**). PE-AR (RT: 81.2 min) was estimated to be  $\sim 6.4 \times 10^2$  ng/g of OC, accounting for 0.02% of the total archaeal IPL (0.3% of the total archaeal-IPL peak area, **Table 3.3**).

1G-MAR (RT: 70.2 min) was estimated to be  $\sim 5.1 \times 10^4$  ng/g of OC and accounted for 1.5% of total archaeal IPL (2.1% of total archaeal-IPL peak area, **Table 3.3**). 2G-MAR (RT: 84.8 min) was estimated to be  $\sim 6.0 \times 10^3$  ng/g of OC, accounting for 0.2% of total archaeal IPL (0.2% of total archaeal-IPL peak area, **Table 3.3**). PE-MAR (RT: 86.6 min) was estimated to be  $\sim 1.7 \times 10^2$  ng/g of OC, accounting for 0.01% of total archaeal IPL (0.1% of total archaeal-IPL peak area, **Table 3.3**).

1G-GDGTs with cyclic structures were detected between 68.8-70.3 min in RT, containing 0-4 ring structures. The total concentration was estimated to be  $\sim 1.4 \times 10^5$  ng/g of OC, accounting for 4.2 % of the total archaeal IPL (3.3% of the total archaeal-IPL peak area, **Table 3.3**). The distribution of cyclic structure of 1G-GDGTs was 1G-GDGT-0 (56.2%) > 1G-GDGT-4 (27.8%) > 1G-GDGT-3 (8.1%) > 1G-GDGT-2 (4.0%) > 1G-GDGT-1 (3.9%) (**Table 3.5**). The ring index (RI) was calculated to be 1.47 (**Table 3.6**).

1G-HGDGTs with cyclic structures were detected between 71.2-72.5 min in RT, containing 0-4 ring structures. The total concentration was estimated to be  $\sim 2.1 \times 10^6$  ng/g of OC, accounting for 63.2 % of the total archaeal IPL (49.4% of the total archaeal-IPL peak area, **Table 3.3**). The distribution of cyclic structure of 1G-HGDGTs was 1G-HGDGT-0 (55.7%) > 1G-HGDGT-1 (23.9%) > 1G-HGDGT-2 (13.0%) > 1G-HGDGT-3 (4.0%) > 1G-HGDGT-4 (3.9%). The RI of 1G-HGDGTs was calculated to be 0.72 (**Table 3.5**, **Table 3.6**).

1G-nMe-HGDGTs included 1G-1Me-HGDGT-3 and 1G-1Me-HGDGT-4, which contained one methyl group and three or four ring structures, and 1G-2Me-HGDGT-4, which contained two methyl groups and four ring structures. They were detected at retention times of 72.0-72.4 min, as co-eluting components of 1G-HGDGT-3 and 1G-HGDGT-4 (**Appendix Table A3.1**). The concentration of 1G-nMe-HGDGTs was estimated to be  $\sim 2.2 \times 10^5$  ng/g of OC, accounting for 5.9% of the total archaeal IPL (13.0% of total archaeal-IPL peak area, **Table 3.3**). The estimated amounts of 1G-1Me-HGDGT-3, 1G-1Me-HGDGT-4, and 1G-2Me-HGDGT-4 were  $\sim 2.7 \times 10^4$  ng/g of OC,  $\sim 1.3 \times 10^5$  ng/g of OC, and  $\sim 4.1 \times 10^4$  ng/g of OC, respectively. Methylation index (MIX) was calculated to be 1.21 (**Table 3.5**, **Table 3.6**). 2G-HGDGT was detected at 83.2 min, as only 2GHGDGT-0 without any ring structure. The concentration of 2G-HGDGT-0 was estimated to be  $\sim 1.1 \times 10^5$  ng/g of OC, accounting for 3.4% of total archaeal IPL (2.4% of total archaeal-IPL peak area). The ring index of IPL-HGDGTs (1G-HGDGTs + 2G-HGDGT-0) was calculated to be 0.72 (**Table 3.5**, **Table 3.6**). The IPL-diether/IPL-tetraether ratio was 23.4/76.6, and the phospholipid/glycolipid ratio was 0.03/99.97.

## ii) Archaeal CLs

The total concentration of archaeal CLs of the NC-HSC sample was estimated to  $\sim 6.9 \times 10^6$  ng/g of OC (**Figure 3.9B**). Two types of diether lipids were detected, AR and MAR, accounting for 30.0% and 2.2% of the total archaeal CLs, respectively (**Table 3.4**). Four types of tetraether lipids were detected: GTGT-0, GDGTs, HGDGTs, and nMe-HGDGTs. The GTGT-0 contained no ring structure and accounted for 0.1% of the total (**Table 3.4**). GDGTs contained 0 to 5 ring structures and accounted for 15.7% of the total; HGDGTs contained 0-4 ring structures and accounted for 31.0% of the total (**Table 3.4**). Possible two structural isomers were found in each HGDGT-0, HGDGT-1 and HGDGT-3 (**Appendix Table A3.2**). nMe-HGDGTs contained 1-4 additional methyl groups. 1Me-HGDGTs, 2Me-HGDGTs and 3Me-HGDGTs contained 0-4 ring structures, while only 4Me-HGDGTs containing 4-ring structures were detected. 1Me-HGDGTs, 2Me-HGDGTs, 3Me-HGDGTs and 4Me-HGDGTs accounted for 7.0%, 11.1%, 0.9% and 0.3 % of the total. GDDs, GMDs, 1Me-GMDs, and 2Me-GMDs were also detected, accounting for 0.1%, 1.2%, 0.2%, and 0.2%, respectively (**Table 3.4**).

The distribution of tetraether lipids and related cyclic compounds were detected as follows (**Table 3.5, Table 3.6**). The distribution of GDGTs was GDGTs-0 (84.6%) >> GDGTs-4 (7.1%) > GDGTs-1 (3.1%) > GDGTs-2 (2.4%) > GDGTs-3 (1.2%) > GDGTs-5 (0.8%) with a ring index (RI) of 0.46. The distribution of HGDGTs was HGDGTs-0 (61.9%) >> HGDGTs-1 (20.0%) > HGDGTs-2 (10.9%) > HGDGTs-4 (4.0%) > HGDGTs-3 (3.4%) with an RI of 0.68. 1Me-HGDGTs distributed as 1Me-HGDGTs-0 (39.2%) > 1Me-HGDGTs-4 (25.0%) > 1Me-HGDGTs-1 (15.5%) > 1Me-HGDGTs-2 (12.3%) > 1Me-HGDGTs-3 (8.0%), with an RI of 1.64. 2Me-HGDGT distributed as 2Me-HGDGT-0 (30.8%) > 2Me-HGDGT-1 (23.2%) > 2Me-HGDGT-2 (21.7%) > 2Me-HGDGT-4 (18.8%) > 2Me-HGDGT-3 (5.6%), with an RI of 1.59. 3Me-HGDGT distributed as 3Me-HGDGT-4 (88.7%) >> 3Me-HGDGT-3 (6.8%) > 3Me-HGDGT-2 (3.1%) > 3Me-HGDGT-1 (1.0%) > 3Me-HGDGT-0 (0.5%), with an RI of 3.82. Only 4Me-HGDGT was detected with the four ring structures. GDDs distributed as GDDs-0 (72.8%) > GDDs-4 (13.7%) > GDDs-3 (5.4%) > GDDs-1 (4.7%) > GDDs-2 (3.4%) with an RI of 0.83. GMDs distributed as GMDs-0 (72.8%) > GMDs-1 (13.2%) > GMDs-2 (8.3%) > GMDs-4 (3.8%) > GMDs-3 (2.8%) with an RI of 0.53. 1Me-GMDs distributed as 1Me-GMDs-4 (38.2%) > 1Me-GMDs-0 (36.0%) > 1Me-GMDs-3 (9.5%) > 1Me-GMDs-1 (8.7%) > 1Me-GMDs-2 (7.6%) with an RI of 2.05. 2Me-GMDs distributed as 2Me-GMDs-0 (29.8%) > 2Me-GMDs-4 (23.9%) > 2Me-GMDs-2 (20.5%) > 2Me-GMDs-1 (18.9%) > 2Me-GMDs-3 (6.8%), with an RI of 1.76. The MIX of nMe-HGDGTs was calculated to be 1.71.

### iii) Non-archaeal IPLs

As non-archaeal IPLs of the NC-HSC sample, 86 compounds were detected and classified into 11 classes based on the combination of polar head groups and core moieties (**Table 3.4**). They are classified into diacylglycerol (DAG) with PG, PE, PME, PC, APT and 1G as polar head groups; acyl/etherglycerol (AEG) with PE, PME and APT as polar head groups; and dietherglycerol (DEG) with PE and PME as polar head groups. The percentages of DAG-IPLs, AEG-IPLs, and DEG-IPLs are 95.1%, 1.4%, and 2.7%, respectively (**Table 3.7**). The total amount of non-archaeal IPLs was estimated to be  $\sim 4.2 \times 10^6$  ng/g of OC (**Figure 3.9A**).

a) Diacylglycerol-phospho-glycerol (DAG-PG) accounted for 17.5% of the total peak area of the non-archaeal IPLs (**Table 3.7**), which contained C<sub>32:0</sub>-C<sub>36:2</sub> as the core lipid with the major abundance of C<sub>36:2</sub>. The average carbon number was calculated to be 35.2 with the average degree of unsaturation of 1.3.

b) Diacylglycerol-phospho-ethanolamine (DAG-PE) accounted for 17.5% of the total peak area of the non-archaeal IPL (**Table 3.7**). DAG-PE contained C<sub>32:0</sub>-C<sub>36:2</sub> as the core lipid with the most abundance of C<sub>36:2</sub>. The average carbon number was calculated to be 35.2 with the average degree of unsaturation of 1.3.

c) Diacylglycerol-phospho-*N*-methyl-ethanolamine (DAG-PME) accounted for 0.7% of the total peak area of the non-archaeal IPLs (**Table 3.7**). The DAG-PE contained C<sub>32:1</sub>-C<sub>35:4</sub> as the core lipids with the major abundance of C<sub>32:1</sub> and C<sub>34:2</sub>. The average carbon number was calculated to be 33.1 with the average unsaturation of 1.9 (**Table 3.7**).

d) Diacylglycerol-phospho-choline (DAG-PC) accounted for 15.9% of the total peak area of the non-archaeal IPLs (**Table 3.7**). The DAG-PC contained C<sub>34:1</sub>-C<sub>37:2</sub> as the core lipid with the most abundance of C<sub>36:2</sub>. The average carbon number was calculated to be 35.9 with the degree of unsaturation of 1.9 (**Table 3.7**).

e) Diacylglycerol-phospho- (DAG-APT) accounted for 0.5% of the total peak area of the non-archaeal IPLs (**Table 3.7**). The DAG-APT contained C<sub>32:0</sub>-C<sub>34:2</sub> as the core lipid with the major abundance of C<sub>32:1</sub>, C<sub>34:1</sub> and C<sub>34:2</sub>. The average carbon number was calculated to be 33.1 with the degree of 1.2.

f) DAG-1G accounted for 0.3% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-1G contained C<sub>30:0</sub>-C<sub>32:1</sub> as the core lipid with the major abundance of C<sub>32:1</sub>, C<sub>34:1</sub> and C<sub>34:2</sub>. The average carbon number and the degree of unsaturation were calculated to be 30.3 and 1.1, respectively.

g) AEG-PE accounted for 1.0% of the total peak area of the non-archaeal IPL (**Table 3.7**). The

AEG-PE contained C31:1-C34:2 as the core lipid with the major abundance of C<sub>34:2</sub>, C<sub>33:1</sub>, and C<sub>32:1</sub>. The average carbon number was calculated to be 33.0 with the degree of unsaturation of 1.3 (**Table 3.7**).

h) AEG-PME accounted for 0.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PME possessed C<sub>31:0</sub> and C<sub>33:1</sub> as the core lipid. The average carbon number was calculated to be 31.0 with the degree of unsaturation of 0.7.

i) AEG-APT accounted for 0.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-APT possessed C<sub>37:1</sub>, C<sub>37:2</sub>, and C<sub>37:3</sub> as the core lipid. The average carbon number was calculated to be 37.0 with the degree of unsaturation of 1.8.

j) DEG-PE accounted for 2.4% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PE possessed C<sub>30:0</sub>-C<sub>36:2</sub> as the core lipid with the most abundance of C<sub>34:2</sub>. The average carbon number was calculated to be 33.5 with the degree of unsaturation of 1.0.

k) DEG-PME accounted for 0.2% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PME possessed C<sub>30:0</sub>-C<sub>36:2</sub> as the core lipid with the most abundance of C<sub>34:2</sub>. The average carbon number was calculated to be 30.6 with the degree of unsaturation of 0.3.

#### iv) Non-archaeal CLs

Non-archaeal core lipids of the NC-HSC sample were classified into three classes: Diether glycerol lipids (DEGs), Acyl/ether-glycerol lipids (AEGs), and Diacylglycerol lipids (DAGs), accounting for 97.7%, 2.1%, and 0.2%, respectively (**Table 3.8; Figure 3.13**).

### 4) NC-HRV

#### i) Archaeal IPLs

Archaeal-IPLs of the NC-HRV sample were detected as 21 compounds with 15 classes. They include ARs that possess 1G- (1G-AR), 2G- (2G-AR), PE- (PE-AR), phosphoglycerol (PG-AR), and phosphoinositol (PI-AR) as polar head groups and MARs that possess 1G- (1G-AR) and 2G (2G-AR) as polar head groups. OHARs were classified into 1G-OHAR, 2G-OHAR, PG-OHAR and PE-OHAR. As tetraether lipids, 1G-GDGTs, 1G-HGDGTs, 1G-nMeHGDGTs and 2G-GDGTs were detected. The total concentration of archaeal-IPLs was estimated to be  $\sim 1.3 \times 10^5$  ng/g of OC ( $\sim 65.0$  ng/g of sample) (**Figure 3.9A**).

In detail, the concentration of 1G-AR (RT: 67.4 min) was estimated to be  $\sim 1.2 \times 10^4$  ng/g of OC, accounting for 8.8% of the total archaeal IPL (8.0% of the total archaeal-IPL peak area, **Table 3.3**). The concentration of 2G-AR (RT: 77.3 min) was estimated to be  $\sim 3.7 \times 10^4$  ng/g of OC, accounting for 27.7% of the total archaeal IPL (20.9% of total archaeal-IPL peak

area **Table 3.3**). The concentration of PG-AR (RT: 76.5 min) was estimated to be  $\sim 9.8 \times 10^3$  ng/g of OC, accounting for 7.4% of the total archaeal IPL (6.7% of total archaeal-IPL peak area **Table 3.3**). The concentration of PE-AR (RT: 81.2 min) was estimated to be  $\sim 2.2 \times 10^3$  ng/g of OC, accounting for 1.7% of the total archaeal IPL (14.1% of the total archaeal-IPL peak area **Table 3.3**). The concentration of PI-AR (RT: 94.4 min) was estimated to be  $\sim 5.1 \times 10^3$  ng/g of OC, accounting for 3.8% of the total archaeal IPL (3.2% of the total archaeal-IPL peak area, **Table 3.3**).

The concentration of 1G-MAR (RT: 70.5 min) was estimated to be  $\sim 1.1 \times 10^4$  ng/g of OC, accounting for 8.6% of the total archaeal IPL (7.8% of the total archaeal-IPL peak area, **Table 3.3**). The concentration of 2G-MAR (RT: 83.2 min) was estimated to be  $\sim 2.2 \times 10^3$  ng/g of OC, accounting for 1.6% of the total archaeal IPL (1.2% of the total archaeal-IPL peak area, **Table 3.3**). The concentration of 1G-OHAR (RT: 69.8 min) was estimated to be  $\sim 2.3 \times 10^4$  ng/g of OC, accounting for 17.1% of the total archaeal IPL (15.2% of the total archaeal-IPL peak area, **Table 3.3**). The concentration of 2G-OHAR (RT: 78.8 min) was estimated to be  $\sim 2.7 \times 10^3$  ng/g of OC, accounting for 2.0% of the total archaeal IPL (1.5% of the total archaeal-IPL peak area, **Table 3.3**). The concentration of PG-OHAR (RT: 78.6 min) was estimated to be  $\sim 1.5 \times 10^4$  ng/g of OC, accounting for 11.2% of total archaeal IPL (10.1% of the total -IPL peak area, **Table 3.3**). The concentration of PE-OHAR (RT: 84.3 min) was estimated to be  $\sim 1.0 \times 10^3$  ng/g of OC, accounting for 0.8% of the total archaeal IPL (6.4% of the total archaeal-IPL peak area, **Table 3.3**).

As tetraether lipids, 1G-GDGTs were detected between 69.0-70.6 min and contained 0-5 ring structures. The concentration of 1G-GDGTs were estimated to be  $\sim 6.7 \times 10^3$  ng/g of OC, accounting for 5.1% of the total archaeal IPL (3.3% of the total archaeal-IPL peak, **Table 3.3**). The distribution of cyclization numbers in 1G-GDGTs was 1G-GDGT-0 (43.5%) > 1G-GDGT-2 (23.2%,) > 1G-GDGT-1 (14.8%) > 1GGDGT-4 (9.0%) > 1G-GDGT-3 (7.5%) > 1G-GDGT-5 (2.0%) with an RI of 1.16 (**Table 3.5**, **Table 3.6**). The 1G-HGDGTs compounds were detected between 71.4 and 72.7 min and contained 0 and 4 ring structures. The concentration of 1G-HGDGTs was estimated to be  $\sim 4.5 \times 10^3$  ng/g of OC, accounting for 1.7% of the total archaeal IPL (49.4% of the total archaeal-IPL, **Table 3.3**). 1G-HGDGT-0 ( $4.2 \times 10^3$  ng/g of OC) was more abundant than 1G-HGDGT-4 ( $3.3 \times 10^2$  ng/g of OC). For 1G-nMe-HGDGTs, only 1G-2Me-HGDGT-4 containing two methyl groups and four ring structures was detected as a co-eluting component of 1G-HGDGT-4 at a retention time of 72.4 min (**Appendix Table A3.1**). For 2G-GDGTs, only 2G-GDGT-0 without any ring structures was detected at 80.8 min. The concentration of 2G-GDGT-0 was estimated to be  $\sim 9.1 \times 10^2$  ng/g of OC, accounting for

0.7% of the total archaeal IPL (0.3% of the total archaeal-IPL peak area, **Table 3.3**). The ring index of IPL-GDGTs (1G-GDGTs+2G-GDGT-0) was calculated to be 1.16 (**Table 3.6**). The IPL diether/tetraether ratio was 90.7/9.3, and the phospholipid/glycolipid ratio was 24.8/73.2.

## ii) Archaeal CLs

The concentration of archaeal CLs of the NC-HRV sample was estimated to be  $\sim 1.1 \times 10^6$  ng/g of OC (**Figure 3.9B**). Three types of diether lipids, AR, MAR and OHAR, were detected, accounting for 28.4%, 5.8% and 6.3% of the total archaeal CLs, respectively (**Table 3.4**). Four types of tetraether lipids were detected as GTGT-0, GDGTs, HGDGTs, and nMe-HGDGTs. GDGTs contained 0-5 ring structures and accounted for 49.5% of the total. HGDGTs contained 0-4 ring structures and accounted for 4.2% of the total (**Table 3.4**). Two possible structural isomer peaks were detected in each HGDGT-0, HGDGT-1 and HGDGT-2 (**Appendix Table A3.2**). GDD-0 and GMD-0 were also detected, accounting for 0.1% and 0.1%, respectively (**Table 3.4**). nMe-HGDGTs contained 1-3 additional methyl groups and from 0-4 ring structures. The abundances of 1Me-HGDGTs, 2Me-HGDGTs, and 3Me-HGDGTs were 3.6%, 3.1%, and 0.3% of the total archaeal CLs, respectively (**Table 3.4**).

The cyclic distributions of tetraether lipids and related compounds are described in **Table 3.5** and **Table 3.6**. The distribution of GDGTs was GDGT-0 (89.8%) >> GDGT-1 (4.1%) > GDGT-2 (3.9%) > GDGT-4 (0.9%) > GDGT-5 (0.8%) > GDGT-3 (0.7%) with an RI of 0.21. The distribution of HGDGTs was HGDGT-0 (64.3%) >> HGDGT-4 (14.4%) > HGDGT-1 (7.4%) > HGDGT-3 (7.0%) > HGDGT-2 (6.8%) with an RI of 1.00. The distribution of 1Me-HGDGTs was 1Me-HGDGT-4 (34.3%) > 1Me-HGDGT-0 (25.6%) > 1Me-HGDGT-3 (16.5%) > 1Me-HGDGT-2 (14.0%) > 1Me-HGDGT-1 (9.6%) with an RI of 2.24. The distribution of 2Me-HGDGTs was 2Me-HGDGT-4 (31.9%) > 2Me-HGDGT-0 (20.5%) > 2Me-HGDGT-2 (19.1%) > 2Me-HGDGT-3 (16.2%) > 2Me-HGDGT-1 (12.5%) with an RI of 2.26. The distribution of 3Me-HGDGTs was 3Me-HGDGT-0 (53.9%) > 3Me-HGDGT-2 (17.7%) > 3Me-HGDGT-4 (12.9%) > 3Me-HGDGT-1 (12.2%) > 3Me-HGDGT-3 (3.3%) with an RI of 1.09. GDD and GMD were detected without the ring structure.

## iii) Non-archaeal IPLs

For non-archaeal IPLs of the NC-HRV sample, 335 compounds were detected (**Appendix Table A3.3**) and classified into 19 classes based on the combination of polar head groups and core structures (**Table 3.7**). The total amount of non-archaeal IPLs was estimated to be  $\sim 3.2 \times 10^6$  ng/g of OC (**Figure 3.9A**). They were the most diverse in molecular structures

among the 6 samples of this study: a) DAG-PG, b) DAG-PE, c) DAG-PME, d) DAG-PDME, e) DAG-PC, f) DAG-APT, g) DGTS, h) DAG-1G, i) AEG-PG, j) AEG-PE, k) AEG-PME, l) AEG-PDME, m) AEG-PC, n) DEG-PG, o) DEG-PE, p) DEG-PME, q) DEG-PME, r) DPG, s) Sph-1G. The abundance ratio of DAG-IPL, AEG-IPL, DEG-IPL, DPG-IPL, and Sph-IPL was 66.1%, 14.0%, 17.3%, 0.4%, and 1.9%, respectively (**Table 3.7; Figure 3.12**).

a) DAG-PG accounted for 2.5% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PG possessed  $C_{30:0}$ - $C_{36:4}$  as the core lipid with the major abundance of  $C_{34:2}$ ,  $C_{32:0}$ , and  $C_{32:1}$ . The average carbon number was calculated to be 33.4 with the degree of unsaturation of 1.8.

b) DAG-PE accounted for 15.7% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PE possessed  $C_{30:0}$ - $C_{40:6}$  as the core lipid with the major abundance of  $C_{32:1}$ ,  $C_{32:2}$ , and  $C_{34:2}$ . The average carbon number was calculated to be 32.6 with the degree of unsaturation of 1.6.

c) DAG-PME accounted for 17.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PME possessed  $C_{30:0}$ - $C_{37:3}$  as the core lipid with the most abundance of  $C_{32:1}$ . The average carbon number was calculated to be 32.5 with the degree of unsaturation of 1.2.

d) DAG-PDME accounted for 8.8% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PDME possessed  $C_{30:0}$ - $C_{36:5}$  as the core lipid with the major abundance of  $C_{32:1}$ ,  $C_{34:2}$ , and  $C_{32:2}$ . The average carbon number was calculated to be 32.8 with the degree of unsaturation of 1.6 (**Table 3.7**).

e) DAG-PC accounted for 21.0% of the total peak area of the non-archaeal IPL. The DAG-PC possessed  $C_{33:0}$ - $C_{40:4}$  as the core lipid with the major abundance of  $C_{34:1}$  and  $C_{36:2}$ . The average carbon number was calculated to be 35.5 with the degree of unsaturation of 1.8 (**Table 3.7**).

f) DAG-APT accounted for 0.03% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-APT possessed  $C_{30:1}$ - $C_{32:2}$  as the core lipid with the major abundance of  $C_{30:1}$ . The average carbon number was calculated to be 30.9 with the degree of unsaturation of 1.5.

g) DGTS accounted for 0.7% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DGTS possessed  $C_{32:0}$ - $C_{36:3}$  as the core lipid with  $C_{30:1}$  DAG as the major. The average carbon number was calculated to be 34.5 with the degree of unsaturation of 1.7.

h) DAG-1G accounted for 0.7% of the total peak area of the non-archaeal IPL. The DAG-1G contained total even-carbon number of  $C_{30:0}$ - $C_{38:2}$  as the core lipid with the major abundance of  $C_{32:2}$  and  $C_{32:1}$ . The average carbon number was calculated to be 32.5 with the degree of unsaturation of 1.2 (**Table 3.7**).

- i) AEG-PG accounted for 0.3% of the total peak area of the non-archaeal IPL. The AEG-PG possessed C<sub>30:0</sub>-C<sub>34:2</sub> as the core lipid with the most abundance of C<sub>31:0</sub>. The average carbon number was calculated to be 31.8 with the degree of unsaturation of 0.8.
- j) AEG-PE accounted for 6.0% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PE possessed C<sub>30:1</sub>-C<sub>36:5</sub> as the core lipid with the major abundance of C<sub>33:1</sub>. The average carbon number was calculated to be 31.8 with the degree of unsaturation of 0.8.
- k) AEG-PME accounted for 1.5% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PME possessed C<sub>31:0</sub>-C<sub>35:1</sub> as the core lipid with the most abundance of C<sub>31:1</sub>. The average carbon number was calculated to be 31.6 with the degree of unsaturation of 0.8.
- l) AEG-PDME accounted for 0.3% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PDME possessed C<sub>32:0</sub>, C<sub>33:1</sub>, and C<sub>34:2</sub> as the core lipid with the most abundance of C<sub>33:1</sub>. The average carbon number was calculated to be 33.2 with the degree of unsaturation of 1.2.
- m) AEG-PC accounted for 5.9% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PC possessed C<sub>30:0</sub>-C<sub>40:4</sub> as the core lipid with the major abundance of C<sub>34:1</sub> and C<sub>34:2</sub>. The average carbon number was calculated to be 34.1 with the degree of unsaturation of 1.6.
- n) DEG-PG accounted for 1.0% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PG possessed C<sub>30:0</sub>-C<sub>35:2</sub> as the core lipid with the major abundance of C<sub>34:2</sub>. The average carbon number was calculated to be 32.6 with the degree of unsaturation of 1.2.
- o) DEG-PE accounted for 10.3% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PE possessed C<sub>30:0</sub>-C<sub>36:3</sub> as the core lipid with the major abundance of C<sub>34:2</sub>. The average carbon number was calculated to be 32.8 with the degree of unsaturation of 1.5.
- p) DEG-PME accounted for 5.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PME possessed C<sub>29:0</sub>-C<sub>35:2</sub> as the core lipid with the major abundance of C<sub>30:0</sub> and C<sub>31:1</sub>. The average carbon number was calculated to be 31.3 with the degree of unsaturation of 0.8 (**Table 3.7**).
- q) DEG-PDME accounted for 0.9% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PDME possessed C<sub>30:0</sub>-C<sub>34:2</sub> as the core lipid with the major abundance of C<sub>31:1</sub> and C<sub>34:2</sub>. The average carbon number was calculated to be 33.2 with the degree of unsaturation of 1.4.
- r) DPG accounted for 0.4% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DPG possessed C<sub>60:1</sub>-C<sub>72:6</sub> as the core lipid with C<sub>64:3</sub> being the major. The average carbon number was calculated to be 65.1 with the degree of unsaturation of 3.1.
- s) 1G-Sph accounted for 1.9% of the total peak area of the non-archaeal IPL (**Table 3.7**). 1G-

Sph possessed C<sub>32:0</sub>-C<sub>40:2</sub> as the core lipid with C<sub>38:2</sub> being the major one. The average carbon number was calculated to be 36.7 with the degree of unsaturation of 1.5.

#### iv) Non-archaeal CLs

Non-archaeal core lipids of the NC-HRV sample were classified into three classes. Diether glycerol lipids (DEGs), acyl/ether-glycerol lipids (AEGs), and diacylglycerol lipids (DAGs) accounted for 55.1%, 24.6%, and 20.3%, respectively (**Table 3.8, Figure 3.13**). As shown in **Appendix Table A3.4**, 28 DEG compounds were detected, ranging from C<sub>30:0</sub> to C<sub>37:3</sub> with the major of C<sub>34:2</sub>. The average carbon number and degree of unsaturation of the DEGs were 32.5 and 1.3, respectively. Twenty compounds of AEGs were detected, ranging from C<sub>30:0</sub> to C<sub>36:2</sub> with the major of C<sub>31:0</sub>. The average carbon number and average degree of unsaturation of the AEGs were 32.2 and 1.0, respectively. 36 compounds of DAGs were detected, ranging from C<sub>30:0</sub> to C<sub>40:3</sub> with the major of C<sub>34:2</sub>. The average carbon number and degree of unsaturation of the DAGs were 34.3 and 1.9, respectively.

### 5) RF-SO

#### i) Archaeal IPLs

For archaeal-IPLs of the RF-SO sample, 11 compounds bearing 1G as a polar head group were detected and classified into 3 classes: 1G-AR, 1G-GDGTs, and 1G-HGDGTs (**Table 3.3**). The total concentration of the archaeal-IPLs was estimated to be  $\sim 2.6 \times 10^3$  ng/g of OC ( $\sim 4.0$  ng/g of sample) (**Figure 3.9A**).

The concentration of 1G-ARs (RT: 66.6 min) were estimated to be  $\sim 1.1 \times 10^3$  ng/g of OC, accounting for 42.1% of the total archaeal IPL (8.0% of the total archaeal-IPL peak area, **Table 3.3**). 1G-GDGTs with 0, 2, 3, 4, and 5 ring structures were detected between 69.0-70.6 min, of which the total concentration was estimated to be  $\sim 4.3 \times 10^2$  ng/g of OC, accounting for 16.2 % of the total archaeal IPL (3.3% of the total archaeal-IPL peak area, **Table 3.3**). The distribution of cyclic compounds of 1G-GDGTs was 1G-GDGT-4 (43.9%) > 1G-GDGT-0 (42.3%) >> 1G-GDGT-3 (6.2%) > 1G-GDGT-2 (5.2%) > 1G-GDGT-5 (2.4%) with an RI of 2.17 (**Table 3.5, Table 3.6**). 1G-HGDGTs with 0-4 ring structures were detected between 71.3-72.6 min, of which the total concentration was estimated to be  $\sim 1.1 \times 10^3$  ng/g of OC, accounting for 41.7% of the total archaeal IPL (49.4% of the total archaeal-IPL peak area, **Table 3.3**). The distribution of cyclic compounds of 1G-HGDGTs was 1G-HGDGT-0 (58.3%) > 1G-HGDGT-2 (13.4%) > 1G-HGDGT-1 (10.1%) > 1G-HGDGT-4 (1.7%) >> HGDGT-3 (8.5%)

with an RI of 1.01 (Table 3.5, Table 3.6). The IPL-diether/IPL-tetraether ratio was 42.1%/57.9%.

## ii) Archaeal CLs

The concentration of archaeal CLs of the RF-SO sample was estimated to be  $\sim 1.7 \times 10^3$  ng/g of OC (Figure 3.9B). Only ARs were detected for diether lipids, accounting for 11.4% of the total archaeal CLs (Table 3.4). Tetraether lipids were detected as GDGT-0, GDGT-1 and HGDGT-0, accounting for 79.2%, 2.1%, and 7.3%, respectively of the the total archaeal CLs with an RI of 0.03 (Table 3.4, Table 3.6).

## iii) Non-archaeal IPLs

For non-archaeal IPLs of the RF-SO sample, 265 compounds were detected (Appendix Table A3.3) and classified into 13 classes based on the combination of polar head groups and core lipids; a) DAG-PG, b) DAG-PE, c) DAG-PME, d) DAG-PDME, e) DAG-PC, f) AEG-PG, g) AEG-PE h) AEG-PC, i) DEG-PG, j) DEG-PE, k) DEG-PME, l) DPG, and m) Sph-1G (Table 3.7). The abundance ratio of DAG-IPL, AEG-IPL, DEG-IPL, DPG-IPL, and Sph-IPL was 86.9%, 5.5%, 6.6%, 0.5%, and 0.5%, respectively (Table 3.7, Figure 3.13). The concentration of non-archaeal IPLs was estimated to be  $\sim 4.1 \times 10^5$  ng/g of OC (Figure 3.9A).

a) DAG-PG accounted for 5.4% of the total peak area of the non-archaeal IPL (Table 3.7). The DAG-PG possessed  $C_{30:0}$ - $C_{36:2}$  as the core lipid with the major abundance of  $C_{34:1}$  and  $C_{34:2}$ . The average carbon number was calculated to be 33.8 with the degree of unsaturation of 1.3.

b) DAG-PE accounted for 7.4% of the total peak area of the non-archaeal IPL (Table 3.7). The DAG-PE possessed  $C_{30:0}$ - $C_{42:4}$  as the core lipid with the most abundance of  $C_{32:1}$ . The average carbon number was calculated to be 32.9 with the degree of unsaturation of 1.4.

c) DAG-PME accounted for 1.4% of the total peak area of the non-archaeal IPL (Table 3.7). The DAG-PME possessed  $C_{32:0}$ - $C_{35:1}$  as the core lipid with the major abundance of  $C_{32:1}$  and  $C_{34:2}$ . The average carbon number was calculated to be 33.1 with the degree of unsaturation of 1.3.

d) DAG-PDME accounted for 0.4% of the total peak area of the non-archaeal IPL (Table 3.7). The DAG-PDME possessed  $C_{32:0}$ - $C_{34:2}$  as the core lipid with the major abundance of  $C_{34:2}$ . The average carbon number was calculated to be 33.2 with the degree of unsaturation of 1.5.

e) DAG-PC accounted for 72.3% of the total peak area of the non-archaeal IPL (Table 3.7). The DAG-PC possessed  $C_{30:0}$ - $C_{40:4}$  as the core lipid with the most abundance of  $C_{34:1}$ . The average carbon number was calculated to be 33.7 with the degree of unsaturation of 1.2.

- f) AEG-PG accounted for 0.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PG possessed  $C_{30:0}$ - $C_{36:2}$  as the core lipid with the major abundance of  $C_{31:0}$ . The average carbon number was calculated to be 32.3 with the degree of unsaturation of 0.5.
- g) AEG-PE accounted for 2.8% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PE possessed  $C_{32:0}$ - $C_{40:4}$  as the core lipid with the major abundance of  $C_{38:3}$  and  $C_{36:2}$ . The average carbon number was calculated to be 36.4 with the degree of unsaturation of 2.3.
- h) AEG-PC accounted for 2.6% of the total peak area of the non-archaeal IPL (**Table 3.7**). The AEG-PC possessed  $C_{32:0}$ - $C_{36:4}$  as the core lipid with the major abundance of  $C_{32:0}$ . The average carbon number was calculated to be 33.4 with the degree of unsaturation of 1.2.
- i) DEG-PE accounted for 1.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PE possessed  $C_{30:0}$ - $C_{34:2}$  as the core lipid with the major abundance of  $C_{30:0}$ ,  $C_{34:2}$ , and  $C_{34:0}$ . The average carbon number was calculated to be 32.4 with the degree of unsaturation of 0.8.
- j) DEG-PME accounted for 0.1% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PME possessed  $C_{30:0}$ ,  $C_{31:0}$ , and  $C_{32:0}$  as the core lipid with  $C_{30:0}$  being the major one. The average carbon number was 30.7 with the degree of unsaturation of 0.0.
- k) DEG-PG accounted for 5.4% of the total peak area of the non-archaeal IPL. The DEG-PG possessed  $C_{30:0}$ - $C_{35:2}$  as the core lipid with the most abundance of  $C_{30:0}$ . The average carbon number was calculated to be 31.6 with the degree of unsaturation of 0.4 (**Table 3.7**).
- l) DPG accounted for 0.5% of the total peak area of the non-archaeal IPL. The DPG possessed  $C_{60:0}$ - $C_{72:6}$  as the core lipid with  $C_{64:4}$  and  $C_{66:3}$  as the major ones. The average carbon number was calculated to be 66.0 with the degree of unsaturation of 2.7.
- m) 1G-Sph accounted for 0.5% of the total peak area of the non-archaeal IPL (**Table 3.7**). 1G-Sph possessed  $C_{34:0}$ - $C_{38:2}$  as the core lipid with  $C_{34:1}$  and  $C_{36:0}$  being the major ones. The average carbon number was calculated to be 35.7 with the degree of unsaturation of 0.9.

#### **iv) Non-archaeal CLs**

Non-archaeal CLs were not detected in the RF-SO sample.

### **6) RF-AS**

#### **i) Archaeal IPLs**

Archaeal IPLs were not detected in the RF-AS sample.

#### **ii) Archaeal CLs**

The total concentration of archaeal CLs of the RF-AS sample was estimated to be  $\sim 3.3 \times 10^3$  ng/g of OC (**Figure 3.9B**). Only ARs were detected as diether lipids, accounting for 18.2% of the total archaeal CLs (**Table 3.4**). Tetraether lipids were detected as GDGTs and HGDGTs. GDGTs contained 0, 2, 4, and 5 ring structures and accounted for 54.3% of the total (**Table 3.4**). HGDGTs contained 0 and 4 ring structures and accounted for 12.2% of the total (**Table 3.4**). The cyclic distribution of GDGTs was GDGT-0 (75.4%) > GDGT-2 (15.3%) > GDGT-5 (6.0%) > GDGT-4 (3.3%) with an RI of 0.74 (**Table 3.5, Table 3.6**). The cyclic distribution of HGDGTs was HGDGT-0 (81.4%) > HGDGT-4 (18.7%) with an RI of 0.74 (**Table 3.5, Table 3.6**).

### iii) Non-archaeal IPLs

For non-archaeal IPLs of the RF-AS sample, 85 compounds were detected (**Appendix Table A3.3**) and classified into four classes; a) DAG-PG, b) DAG-PE, c) DAG-PC, d) DEG-PE, based on the combination of polar head groups and core lipids. The non-archaeal IPLs of the RF-AS sample had the least molecular diversity among the six samples (**Table 3.7; Figure 3.12**). The total amount of non-archaeal IPLs was estimated to be  $\sim 1.6 \times 10^6$  ng/g of OC, and the abundances of DAG-IPL and DEG-IPL were 99.7% and 0.3%, respectively (**Table 3.7; Figure 3.13**).

a) DAG-PG accounted for 3.5% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PG possessed  $C_{30:0}$ - $C_{36:2}$  as the core lipid with the most abundance of  $C_{34:2}$ . The average carbon number was calculated to be 33.4 with the degree of unsaturation of 1.8.

b) DAG-PE accounted for 11.9% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PE possessed  $C_{30:0}$ - $C_{38:4}$  as the core lipid with the most abundance of  $C_{32:2}$ . The average carbon number was calculated to be 32.6 with the degree of unsaturation of 1.7.

c) DAG-PC accounted for 84.3% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DAG-PC possessed  $C_{30:0}$ - $C_{36:2}$  as the core lipid with the major abundance of  $C_{34:1}$ . The average carbon number was calculated to be 33.4 with the degree of unsaturation of 1.8.

d) DEG-PE accounted for 0.3% of the total peak area of the non-archaeal IPL (**Table 3.7**). The DEG-PE possessed  $C_{30:0}$ - $C_{36:0}$  as its lipid core with the major abundance of  $C_{36:0}$ . The average carbon number was calculated to be 33.5 with the degree of unsaturation of 0.5.

### iv) Non-archaeal CLs

Non-archaeal CLs were not detected in the RF-AS sample.

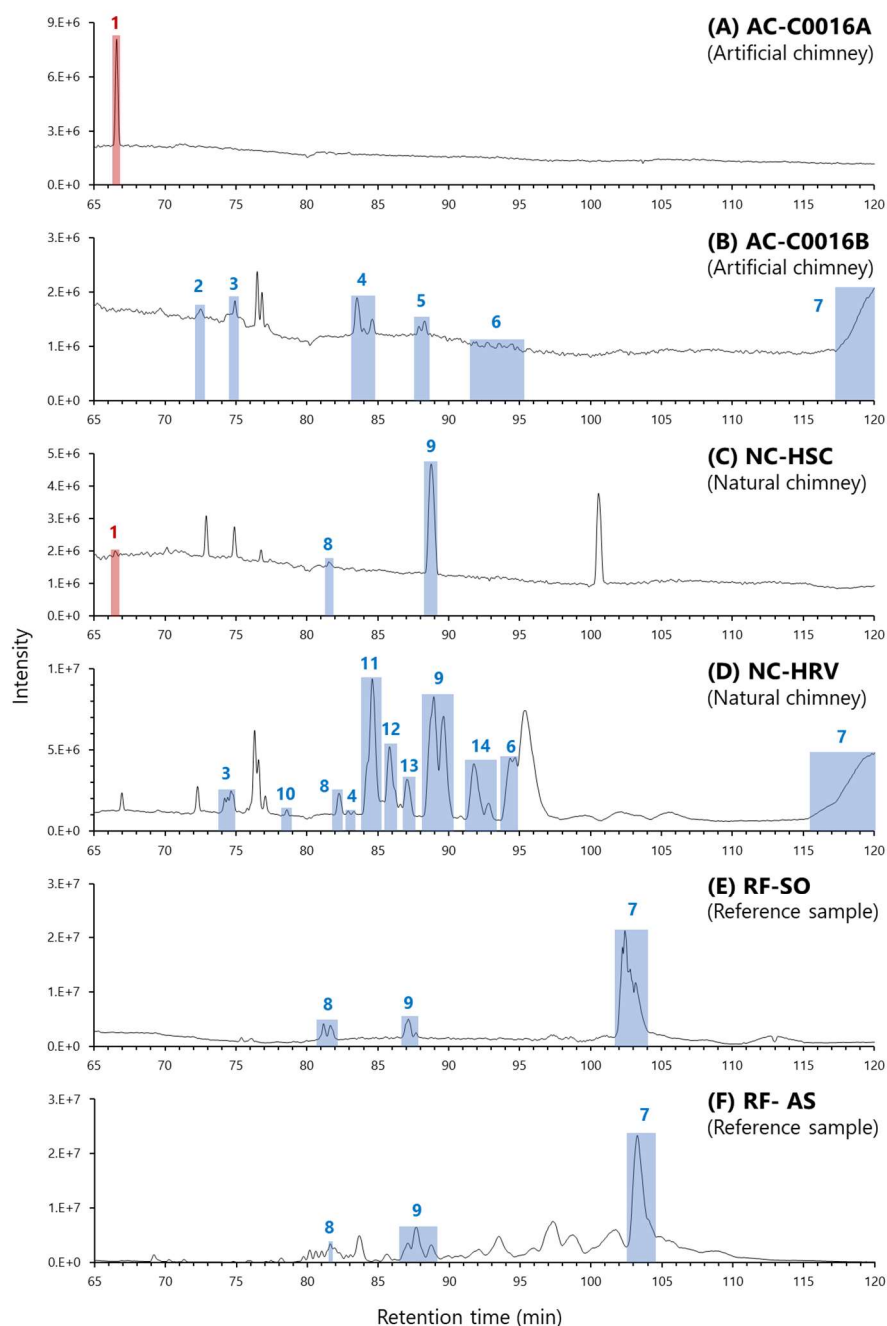


**Figure 3.6.** Fractional positive ion base peak chromatograms ( $m/z$  600-2000; 65-120 min) showing representative IPLs observed in (A) sample AC-C0016A, (B) sample AC-C0016B, (C) sample NC-HSC, (D) sample NC-HRV, (E) sample RF-SO, (F) sample RF-AS. 1: 1G-AR; 2: 1G-DAGs; 3: 1G-Sphs; 4: DGTSs; 5: PDME-DEGs; 6: PDME-DAGs; 7: PC-DAGs; 8: PG-DAGs; 9: PE-DAGs; 10: PG-DEGs; 11: PE-DAGs; 12: PE-AEGs; 13: PME-DEGs. The detail information of all archaeal and non-archaeal IPLs observed in this study are summarized in **Appendix table** in the end of the thesis. For structures see **Figure 3.8**.

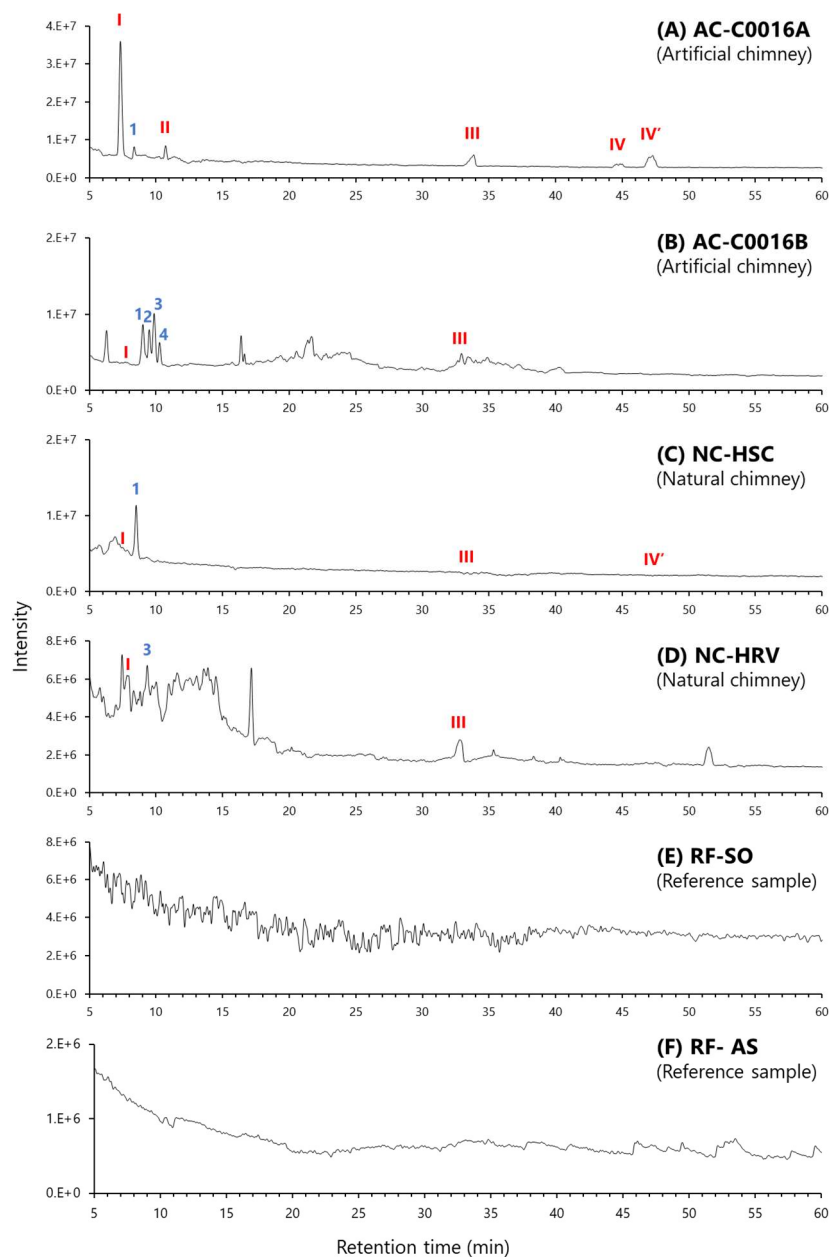


**Figure 3.7.** Fractional positive ion base peak chromatograms ( $m/z$  500-1400; 5-65 min) showing representative CLs observed in (A) sample AC-C0016A, (B) sample AC-C0016B, (C) sample NC-HSC, (D) sample NC-HRV, (E) sample RF-SO, (F) sample RF-AS. I: Archaeol (AR); II: Macrocyclic-archaeol (MAR); III: GDGT-0; IV: HGDGT-0; IV': tentative HGDGT-0 isomer; 1: C<sub>34:0</sub> Diether-glycerol (DEG); 2: C<sub>33:1</sub> DEG; 3: C<sub>34:2</sub> DEG; 4: C<sub>32:2</sub> DEG. The detail information of all archaeal and non-archaeal CLs observed in this study are summarized in **Appendix Tables** in the end of the thesis. For structures see **Figure 3.8**.

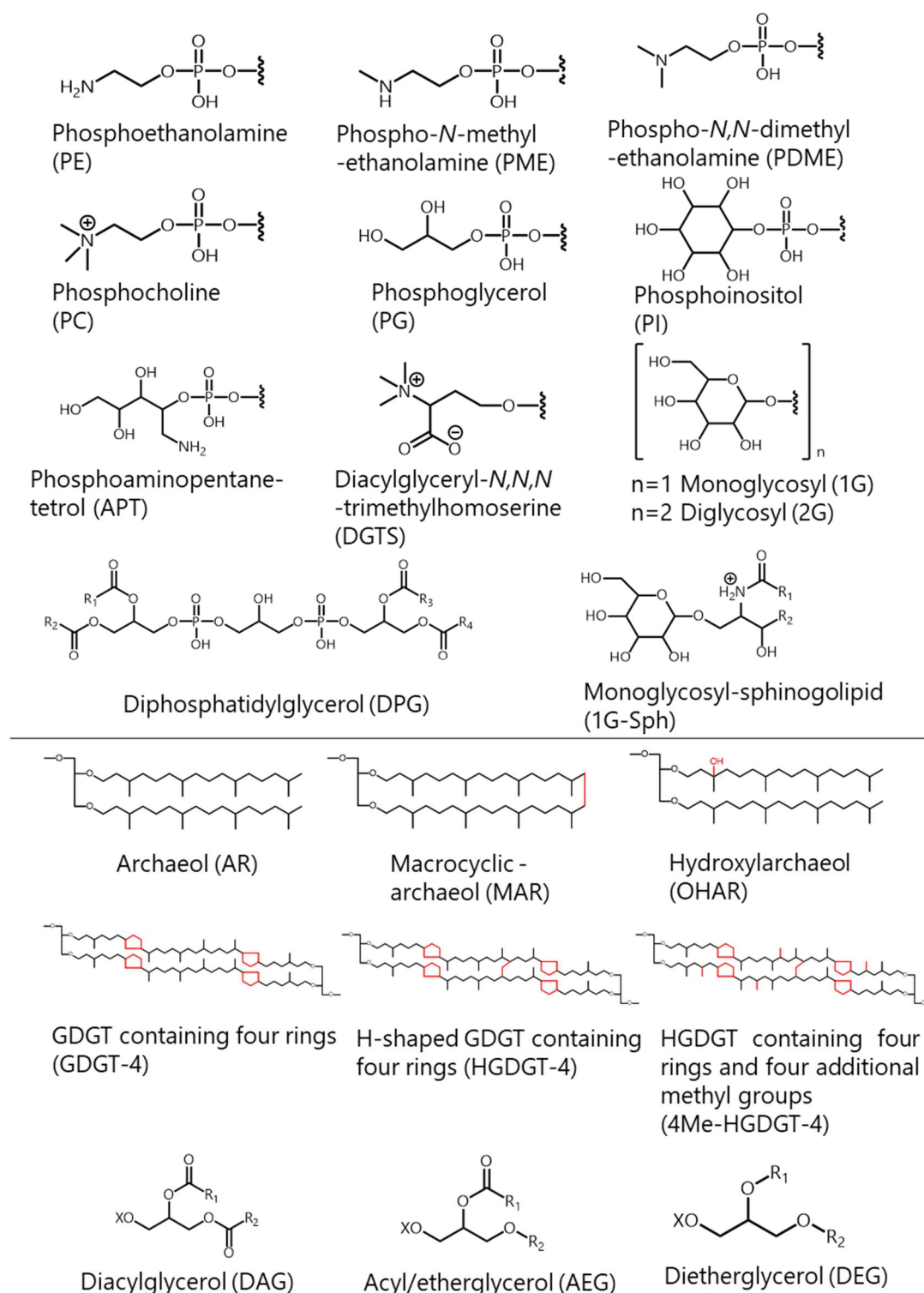


**Figure 3.8.** Representative chemical structures for polar head groups and core lipids described in Chapter 3.

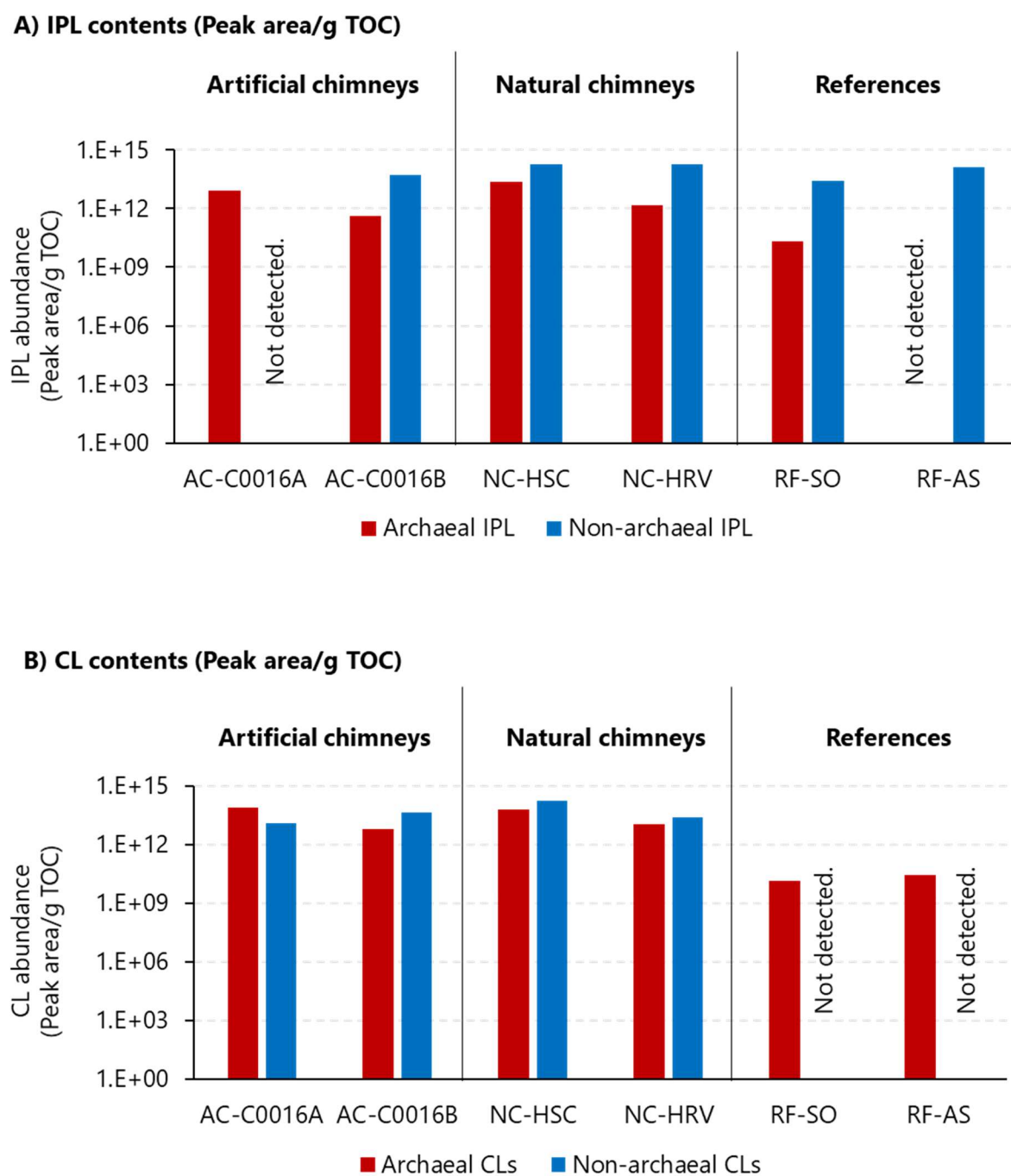


**Figure 3.9.** Total peak area amounts normalized by TOC contents of archaeal and non-archaeal IPLs, (B) archaeal and non-archaeal CLs observed in each sample. Because ionization efficiencies of individual compounds and matrix effect are not known, these should not be interpreted as absolute abundance.

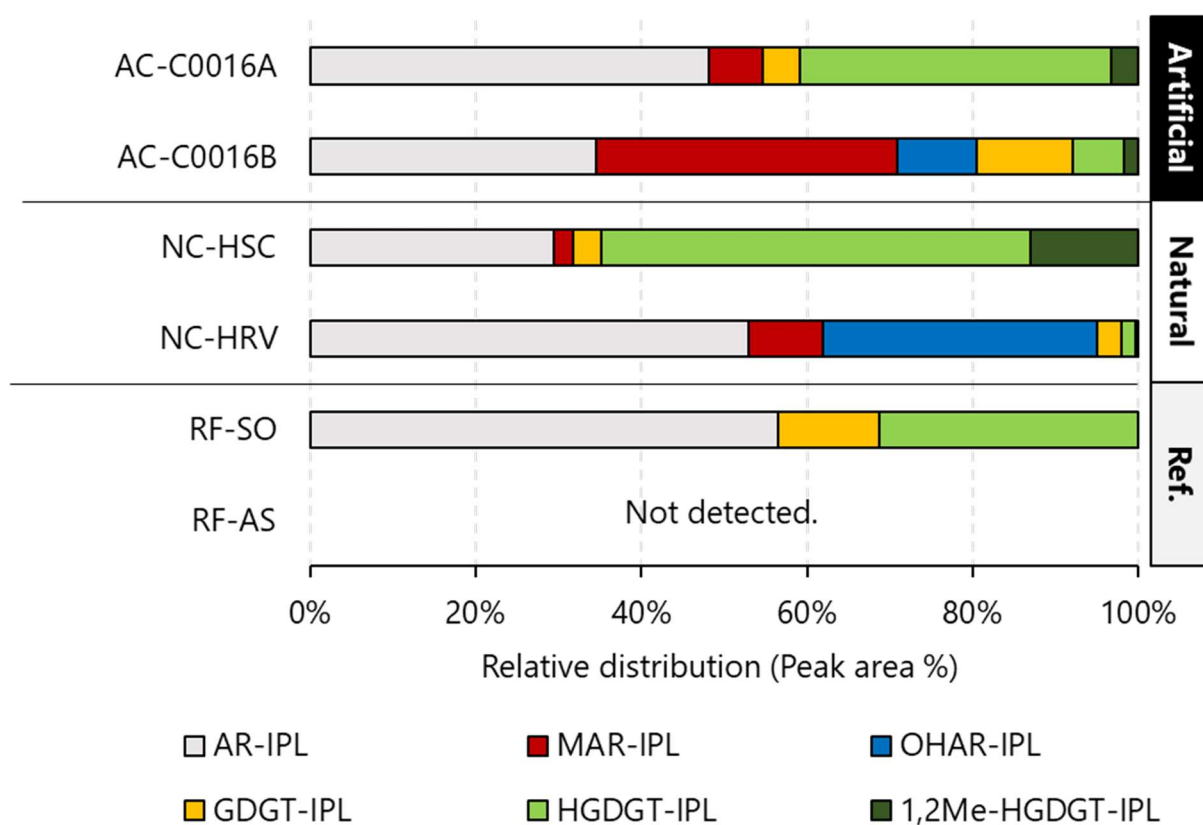


**Figure 3.10.** Distributions of the relative amounts of archaeal IPL peak areas observed in each sample. Details are summarized in **Table 3.3**. For structures see **Figure 3.8**.

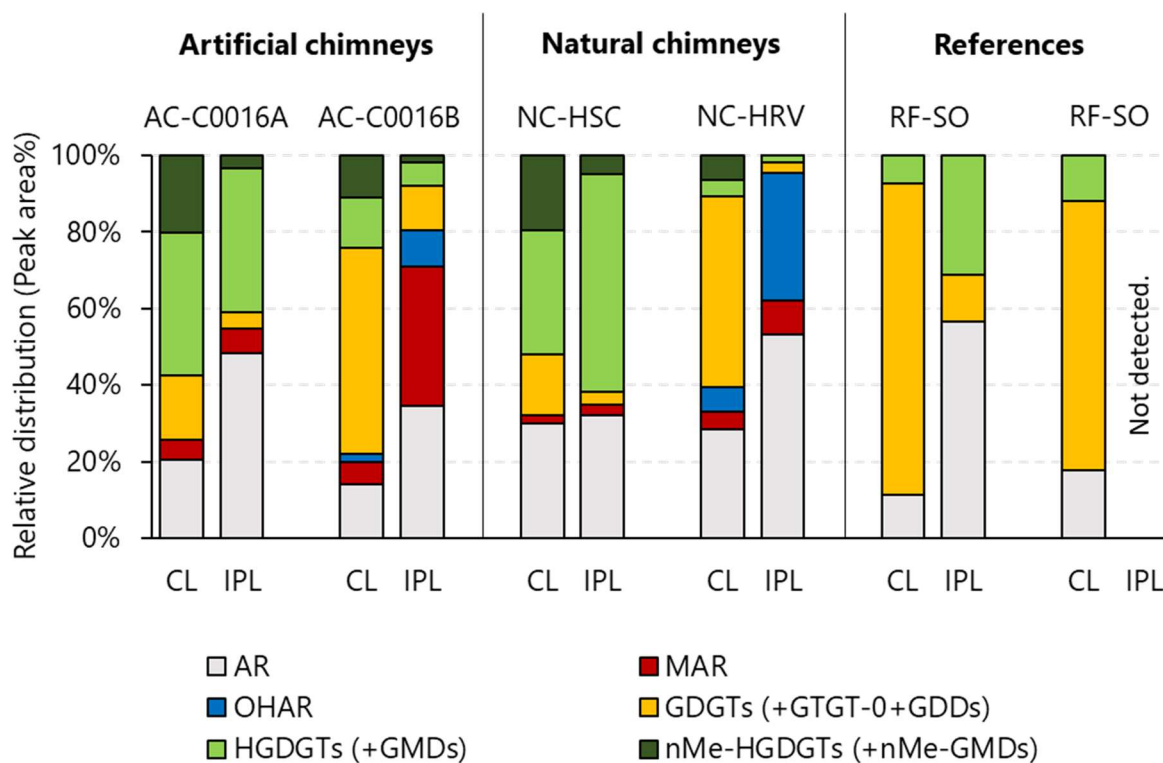


**Figure 3.11.** Comparison of the relative distributions of archaeal IPL and CLs observed in each sample. Details are summarized in **Table 3.3** and **Table 3.4**. For structures see **Figure 3.8**.

**Table 3.3.** Relative abundance of peak areas of archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples.

| Compounds        | Artificial chimneys |           | Natural chimneys |        | Reference samples |       |
|------------------|---------------------|-----------|------------------|--------|-------------------|-------|
|                  | AC-C0016A           | AC-C0016B | NC-HSC           | NC-HRV | RF-SO             | RF-AS |
| 1G-AR            | 48                  | 35        | 25               | 8      | 57                | -     |
| 2G-AR            | -                   | -         | 6                | 21     | -                 | -     |
| PG-AR            | -                   | -         | -                | 7      | -                 | -     |
| PE-AR            | -                   | -         | tr               | 14     | -                 | -     |
| PI-AR            | -                   | -         | -                | 3      | -                 | -     |
| 1G-MAR           | 6                   | 36        | 2                | 8      | -                 | -     |
| 2G-MAR           | -                   | -         | -                | 1      | -                 | -     |
| PE-MAR           | -                   | -         | tr               | -      | -                 | -     |
| 1G-OHAR          | -                   | 10        | -                | 15     | -                 | -     |
| 2G-OHAR          | -                   | -         | -                | 2      | -                 | -     |
| PG-OHAR          | -                   | -         | -                | 10     | -                 | -     |
| PE-OHAR          | -                   | -         | -                | 6      | -                 | -     |
| 1G-GDGT-0        | 4                   | 6         | 2                | 1      | 5                 | -     |
| 2G-GDGT-0        | -                   | 1         | -                | tr     | -                 | -     |
| 1G-GDGT-1        | tr                  | 1         | tr               | tr     | -                 | -     |
| 1G-GDGT-2        | tr                  | 1         | tr               | 1      | 1                 | -     |
| 1G-GDGT-3        | tr                  | -         | tr               | tr     | 1                 | -     |
| 1G-GDGT-4        | tr                  | -         | 1                | tr     | 5                 | -     |
| 1G-GDGT-5        | tr                  | 3         | -                | tr     | tr                | -     |
| 1G-HGDGT-0       | 25                  | 5         | 30               | 2      | 18                | -     |
| 2G-HGDGT-0       | -                   | -         | 3                | -      | -                 | -     |
| 1G-HGDGT-1       | 5                   | 1         | 13               | -      | 3                 | -     |
| 1G-HGDGT-2       | 4                   | -         | 7                | -      | 4                 | -     |
| 1G-HGDGT-3       | 2                   | -         | 2                | -      | 3                 | -     |
| 1G-HGDGT-4       | 2                   | tr        | 2                | tr     | 3                 | -     |
| 1G-1Me-HGDGT-3   | -                   | -         | 1                | -      | -                 | -     |
| 1G-1Me-HGDGT-4   | 2                   | 1         | 3                | -      | -                 | -     |
| 1G-2Me-HGDGT-4   | 1                   | 1         | 1                | tr     | -                 | -     |
| % IPL-GDGTs      | 4                   | 12        | 4                | 3      | 12                | -     |
| % IPL-HGDGTs     | 38                  | 6         | 57               | 2      | 31                | -     |
| % IPL-1Me-HGDGTs | 2                   | 1         | 4                | -      | -                 | -     |
| % IPL-2Me-HGDGTs | 1                   | 1         | 1                | tr     | -                 | -     |

Because ionization efficiencies of individual compounds and matrix effect are not known, these should not be interpreted as absolute abundance percentages. ‘-’, Not detected. ‘tr’: trace (< 1%). For structures see **Figure 3.8**. Details of all archaeal IPLs observed in this study are summarized **Appendix Tables** in end of this thesis.

**Table 3.4.** Relative abundance of peak areas of archaeal CLs observed in artificial chimneys, natural chimneys and reference samples.

| Compounds     | Artificial chimneys |           | Natural chimneys |        | Reference samples |       |
|---------------|---------------------|-----------|------------------|--------|-------------------|-------|
|               | AC-C0016A           | AC-C0016B | NC-HSC           | NC-HRV | RF-SO             | RF-AS |
| AR            | 20                  | 14        | 30               | 28     | 11                | 18    |
| MAR           | 5                   | 6         | 2                | 5      | -                 | -     |
| OHAR          | -                   | 2         | -                | 6      | -                 | -     |
| GTGT-0        | tr                  | tr        | tr               | tr     | -                 | -     |
| GDGT-0        | 13                  | 46        | 13               | 44     | 79                | 53    |
| GDGT-1 (+1')  | 1                   | 2         | tr               | 2      | 2                 | -     |
| GDGT-2 (+2')  | 1                   | 1         | tr               | 2      | -                 | 11    |
| GDGT-3 (+3')  | 1                   | 1         | tr               | tr     | -                 | -     |
| GDGT-4 (+4')  | 1                   | 1         | 1                | 1      | -                 | 2     |
| GDGT-5 (+5')  | tr                  | 3         | tr               | tr     | -                 | 4     |
| HGDGT-0 (+0') | 21                  | 10        | 19               | 3      | 7                 | 10    |
| HGDGT-1 (+1') | 7                   | 1         | 6                | tr     | -                 | -     |
| HGDGT-2 (+2') | 5                   | tr        | 3                | tr     | -                 | -     |
| HGDGT-3 (+3') | 3                   | tr        | 1                | tr     | -                 | -     |
| HGDGT-4 (+4') | 2                   | 1         | 1                | tr     | -                 | -     |
| 1Me-HGDGT-0   | tr                  | 2         | 3                | 1      | -                 | -     |
| 1Me-HGDGT-1   | 1                   | tr        | 1                | tr     | -                 | -     |
| 1Me-HGDGT-2   | 1                   | 1         | 1                | tr     | -                 | -     |
| 1Me-HGDGT-3   | 1                   | 1         | 1                | 1      | -                 | -     |
| 1Me-HGDGT-4   | 2                   | 2         | 2                | 1      | -                 | -     |
| 2Me-HGDGT-0   | tr                  | 1         | 3                | 1      | -                 | -     |
| 2Me-HGDGT-1   | tr                  | 1         | 3                | tr     | -                 | -     |
| 2Me-HGDGT-2   | 1                   | 1         | 2                | 1      | -                 | -     |
| 2Me-HGDGT-3   | 1                   | 1         | 1                | tr     | -                 | -     |
| 2Me-HGDGT-4   | 6                   | 2         | 2                | 1      | -                 | -     |
| 3Me-HGDGT-0   | -                   | tr        | tr               | tr     | -                 | -     |
| 3Me-HGDGT-1   | -                   | tr        | tr               | tr     | -                 | -     |
| 3Me-HGDGT-2   | tr                  | tr        | tr               | tr     | -                 | -     |
| 3Me-HGDGT-3   | tr                  | tr        | tr               | tr     | -                 | -     |
| 3Me-HGDGT-4   | 5                   | 1         | 1                | 1      | -                 | -     |
| 4Me-HGDGT-3   | tr                  | -         | -                | -      | -                 | -     |
| 4Me-HGDGT-4   | 2                   | tr        | tr               | -      | -                 | -     |
| GDD-0         | tr                  | tr        | tr               | tr     | -                 | -     |
| GDD-1         | tr                  | -         | tr               | -      | -                 | -     |
| GDD-2         | tr                  | -         | tr               | -      | -                 | -     |

**Table 3.4.** (Continued) Relative abundance of peak areas of archaeal CLs observed in artificial chimneys, natural chimneys and reference samples.

|              |           |           |          |          |    |    |
|--------------|-----------|-----------|----------|----------|----|----|
| GDD-3        | tr        | -         | tr       | -        | -  | -  |
| GDD-4        | tr        | -         | tr       | -        | -  | -  |
| GDD-5        | -         | tr        | -        | -        | -  | -  |
| GMD-0        | tr        | tr        | 1        | tr       | -  | -  |
| GMD-1        | tr        | -         | tr       | -        | -  | -  |
| GMD-2        | tr        | -         | tr       | -        | -  | -  |
| GMD-3        | tr        | -         | tr       | -        | -  | -  |
| GMD-4        | tr        | -         | tr       | -        | -  | -  |
| 1Me-GMD-0    | tr        | tr        | tr       | -        | -  | -  |
| 1Me-GMD-1    | tr        | -         | tr       | -        | -  | -  |
| 1Me-GMD-2    | tr        | -         | tr       | -        | -  | -  |
| 1Me-GMD-3    | tr        | -         | tr       | -        | -  | -  |
| 1Me-GMD-4    | tr        | -         | tr       | -        | -  | -  |
| 2Me-GMD-0    | -         | -         | tr       | -        | -  | -  |
| 2Me-GMD-1    | -         | -         | tr       | -        | -  | -  |
| 2Me-GMD-2    | -         | -         | tr       | -        | -  | -  |
| 2Me-GMD-3    | -         | -         | tr       | -        | -  | -  |
| 2Me-GMD-4    | -         | -         | tr       | -        | -  | -  |
| % GDGTs      | 17        | 53        | 16       | 49       | 81 | 70 |
| % HGDGTs     | 37        | 13        | 31       | 4        | 7  | 12 |
| % 1Me-HGDGTs | 5         | 5         | 7        | 3        | -  | -  |
| % 2Me-HGDGTs | 9         | 5         | 11       | 3        | -  | -  |
| % 3Me-HGDGTs | 5         | 1         | 1        | tr (0.1) | -  | -  |
| % 4Me-HGDGTs | 2         | tr (0.1)  | tr (0.3) | -        | -  | -  |
| % GDDs       | tr (0.04) | tr (0.1)  | tr (0.1) | tr (0.1) | -  | -  |
| % GMDs       | tr (0.1)  | tr (0.1)  | 1        | tr (0.1) | -  | -  |
| % 1Me-GMDs   | tr (0.01) | tr (0.01) | tr (0.2) | -        | -  | -  |
| % 2Me-GMDs   | -         | -         | tr (0.2) | -        | -  | -  |

Because ionization efficiencies of individual compounds and matrix effect are not known, these should not be interpreted as absolute abundance percentages. ‘-’, Not detected. ‘tr’: trace (< 1%). For structures see **Figure 3.8**. Details of all archaeal CLs observed in this study are summarized **Appendix Tables** in end of this thesis.

**Table 3.5.** Fractional relative abundance peak areas of archaeal polar and core tetraether lipids and their derivatives.

|                          | Artificial chimneys |    |           |    | Natural chimneys |    |        |    | Reference samples |     |       |    |
|--------------------------|---------------------|----|-----------|----|------------------|----|--------|----|-------------------|-----|-------|----|
|                          | AC-C0016A           |    | AC-C0016B |    | NC-HSC           |    | NC-HRV |    | RF-SO             |     | RF-AS |    |
|                          | IPL                 | CL | IPL       | CL | IPL              | CL | IPL    | CL | IPL               | CL  | IPL   | CL |
| <b><i>GDGTs</i></b>      |                     |    |           |    |                  |    |        |    |                   |     |       |    |
| GDGT-0                   | 87                  | 80 | 60        | 85 | 54               | 85 | 49     | 89 | 42                | 97  | -     | 75 |
| GDGT-1                   | 2                   | 5  | 7         | 3  | 4                | 3  | 13     | 4  | -                 | 3   | -     | -  |
| GDGT-2                   | 1                   | 4  | 7         | 3  | 4                | 2  | 21     | 4  | 5                 | -   | -     | 15 |
| GDGT-3                   | 1                   | 4  | -         | 1  | 9                | 2  | 7      | 1  | 6                 | -   | -     | -  |
| GDGT-4                   | 7                   | 7  | -         | 3  | 30               | 7  | 8      | 1  | 44                | -   | -     | 3  |
| GDGT-5                   | 1                   | tr | 26        | 5  | -                | 1  | 2      | 1  | 2                 | -   | -     | 6  |
| <b><i>HGDGTs</i></b>     |                     |    |           |    |                  |    |        |    |                   |     |       |    |
| HGDGT-0                  | 66                  | 55 | 85        | 77 | 58               | 62 | 93     | 64 | 58                | 100 | -     | 81 |
| HGDGT-1                  | 14                  | 18 | 9         | 9  | 23               | 20 | -      | 7  | 10                | -   | -     | -  |
| HGDGT-2                  | 10                  | 14 | -         | 4  | 12               | 11 | -      | 7  | 13                | -   | -     | -  |
| HGDGT-3                  | 6                   | 8  | -         | 3  | 4                | 3  | -      | 7  | 8                 | -   | -     | -  |
| HGDGT-4                  | 5                   | 5  | 6         | 6  | 3                | 4  | 7      | 14 | 10                | -   | -     | 19 |
| <b><i>1Me-HGDGTs</i></b> |                     |    |           |    |                  |    |        |    |                   |     |       |    |
| 1Me-HGDGT-0              | -                   | 8  | -         | 35 | -                | 39 | -      | 26 | -                 | -   | -     | -  |
| 1Me-HGDGT-1              | -                   | 11 | -         | 10 | -                | 15 | -      | 10 | -                 | -   | -     | -  |
| 1Me-HGDGT-2              | -                   | 22 | -         | 11 | -                | 12 | -      | 14 | -                 | -   | -     | -  |
| 1Me-HGDGT-3              | -                   | 18 | -         | 13 | 17               | 8  | -      | 16 | -                 | -   | -     | -  |
| 1Me-HGDGT-4              | 100                 | 41 | 100       | 32 | 83               | 25 | -      | 34 | -                 | -   | -     | -  |
| <b><i>2Me-HGDGTs</i></b> |                     |    |           |    |                  |    |        |    |                   |     |       |    |
| 2Me-HGDGT-0              | -                   | 2  | -         | 27 | -                | 31 | -      | 20 | -                 | -   | -     | -  |

**Table 3.5.** (Continued) Fractional relative abundance peak areas of archaeal polar and core tetraether lipids and their derivatives.

|                          |     |    |     |     |     |     |     |     |   |   |   |   |
|--------------------------|-----|----|-----|-----|-----|-----|-----|-----|---|---|---|---|
| 2Me-HGDGT-1              | -   | 2  | -   | 12  | -   | 23  | -   | 12  | - | - | - | - |
| 2Me-HGDGT-2              | -   | 10 | -   | 13  | -   | 22  | -   | 19  | - | - | - | - |
| 2Me-HGDGT-3              | -   | 15 | -   | 11  | -   | 6   | -   | 16  | - | - | - | - |
| 2Me-HGDGT-4              | 100 | 70 | 100 | 36  | 100 | 19  | 100 | 32  | - | - | - | - |
| <b><i>3Me-HGDGTs</i></b> |     |    |     |     |     |     |     |     |   |   |   |   |
| 3Me-HGDGT-0              | -   | -  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 3Me-HGDGT-1              | -   | -  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 3Me-HGDGT-2              | -   | -  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 3Me-HGDGT-3              | -   | 4  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 3Me-HGDGT-4              | -   | 96 | -   | 100 | -   | 100 | -   | -   | - | - | - | - |
| <b><i>4Me-HGDGTs</i></b> |     |    |     |     |     |     |     |     |   |   |   |   |
| 4Me-HGDGT-0              | -   | -  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 4Me-HGDGT-1              | -   | -  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 4Me-HGDGT-2              | -   | -  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 4Me-HGDGT-3              | -   | 4  | -   | -   | -   | -   | -   | -   | - | - | - | - |
| 4Me-HGDGT-4              | -   | 96 | -   | 100 | -   | 100 | -   | -   | - | - | - | - |
| <b><i>GDDs</i></b>       |     |    |     |     |     |     |     |     |   |   |   |   |
| GDD-0                    | -   | 84 | -   | 78  | -   | 73  | -   | 100 | - | - | - | - |
| GDD-1                    | -   | 3  | -   | -   | -   | 5   | -   | -   | - | - | - | - |
| GDD-2                    | -   | 3  | -   | -   | -   | 3   | -   | -   | - | - | - | - |
| GDD-3                    | -   | 3  | -   | -   | -   | 5   | -   | -   | - | - | - | - |
| GDD-4                    | -   | 7  | -   | -   | -   | 14  | -   | -   | - | - | - | - |
| GDD-5                    | -   | -  | -   | 22  | -   | -   | -   | -   | - | - | - | - |

**Table 3.5.** (Continued) Fractional relative abundance peak areas of archaeal polar and core tetraether lipids and their derivatives.

|                        |   |    |   |     |   |    |   |     |   |   |   |   |
|------------------------|---|----|---|-----|---|----|---|-----|---|---|---|---|
| <b><i>GMDs</i></b>     |   |    |   |     |   |    |   |     |   |   |   |   |
| GMD-0                  | - | 75 | - | 100 | - | 72 | - | 100 | - | - | - | - |
| GMD-1                  | - | 10 | - | -   | - | 13 | - | -   | - | - | - | - |
| GMD-2                  | - | 6  | - | -   | - | 8  | - | -   | - | - | - | - |
| GMD-3                  | - | 4  | - | -   | - | 3  | - | -   | - | - | - | - |
| GMD-4                  | - | 5  | - | -   | - | 4  | - | -   | - | - | - | - |
| <b><i>1Me-GMDs</i></b> |   |    |   |     |   |    |   |     |   |   |   |   |
| 1Me-GMD-0              | - | 9  | - | 100 | - | 36 | - | -   | - | - | - | - |
| 1Me-GMD-1              | - | 2  | - | -   | - | 9  | - | -   | - | - | - | - |
| 1Me-GMD-2              | - | 15 | - | -   | - | 8  | - | -   | - | - | - | - |
| 1Me-GMD-3              | - | 10 | - | -   | - | 10 | - | -   | - | - | - | - |
| 1Me-GMD-4              | - | 64 | - | -   | - | 38 | - | -   | - | - | - | - |
| <b><i>2Me-GMDs</i></b> |   |    |   |     |   |    |   |     |   |   |   |   |
| 2Me-GMD-0              | - | -  | - | -   | - | 30 | - | -   | - | - | - | - |
| 2Me-GMD-1              | - | -  | - | -   | - | 19 | - | -   | - | - | - | - |
| 2Me-GMD-2              | - | -  | - | -   | - | 20 | - | -   | - | - | - | - |
| 2Me-GMD-3              | - | -  | - | -   | - | 7  | - | -   | - | - | - | - |
| 2Me-GMD-4              | - | -  | - | -   | - | 24 | - | -   | - | - | - | - |

‘-’, Not detected.

**Table 3.6** Proxies of based on archaeal tetraether lipids and their derivatives.

| Proxies                             | Artificial chimneys |      |           |      | Natural chimneys |      |        |      | Reference samples |      |       |      |
|-------------------------------------|---------------------|------|-----------|------|------------------|------|--------|------|-------------------|------|-------|------|
|                                     | AC-C0016A           |      | AC-C0016B |      | NC-HSC           |      | NC-HRV |      | RF-SO             |      | RF-AS |      |
|                                     | IPL                 | CL   | IPL       | CL   | IPL              | CL   | IPL    | CL   | IPL               | CL   | IPL   | CL   |
| <b>(i) Ring index (RI)</b>          |                     |      |           |      |                  |      |        |      |                   |      |       |      |
| GDGTs                               | 0.43                | 0.54 | 1.51      | 0.48 | 1.57             | 0.46 | 1.16   | 0.23 | 2.17              | 0.03 | n.a.  | 0.74 |
| GDDs                                | n.a.                | 0.46 | n.a.      | 1.09 | n.a.             | 0.83 | n.a.   | 0    | n.a.              | n.a. | n.a.  | n.a. |
| HGDGTs                              | 0.72                | 0.88 | 0.32      | 0.52 | 0.72             | 0.68 | 0.29   | 1.00 | 1.01              | 0    | n.a.  | 0.74 |
| GMDs                                | n.a.                | 0.53 | n.a.      | 0    | n.a.             | 0.53 | n.a.   | 0    | n.a.              | n.a. | n.a.  | n.a. |
| 1Me-HGDGTs                          | 4                   | 2.73 | 4         | 1.96 | 3.83             | 1.64 | n.a.   | n.a. | n.a.              | n.a. | n.a.  | n.a. |
| 2Me-HGDGTs                          | 4                   | 3.51 | 4         | 2.18 | 4                | 1.59 | 4      | 2.26 | n.a.              | n.a. | n.a.  | n.a. |
| 3Me-HGDGTs                          | n.a.                | 3.90 | n.a.      | 3.42 | n.a.             | 3.82 | n.a.   | 1.09 | n.a.              | n.a. | n.a.  | n.a. |
| 4Me-HGDGTs                          | n.a.                | 3.96 | n.a.      | 4    | n.a.             | 4    | n.a.   | n.a. | n.a.              | n.a. | n.a.  | n.a. |
| 1Me-GMDs                            | n.a.                | 3.18 | n.a.      | 0    | n.a.             | 2.05 | n.a.   | n.a. | n.a.              | n.a. | n.a.  | n.a. |
| 2Me-GMDs                            | n.a.                | n.a. | n.a.      | n.a. | n.a.             | 2.51 | n.a.   | n.a. | n.a.              | n.a. | n.a.  | n.a. |
| <b>(ii) Methylation index (MIX)</b> |                     |      |           |      |                  |      |        |      |                   |      |       |      |
| (nMe)-HGDGTs                        | 0.11                | 0.77 | 0.30      | 0.77 | 0.10             | 0.66 | 0.10   | 0.91 | 0                 | 0    | n.a.  | 0    |
| (nMe)-GMDs                          | n.a.                | 0.20 | n.a.      | 0.07 | n.a.             | 1.14 | n.a.   | 0    | n.a.              | n.a. | n.a.  | n.a. |

‘n.a.’, Not applicable.

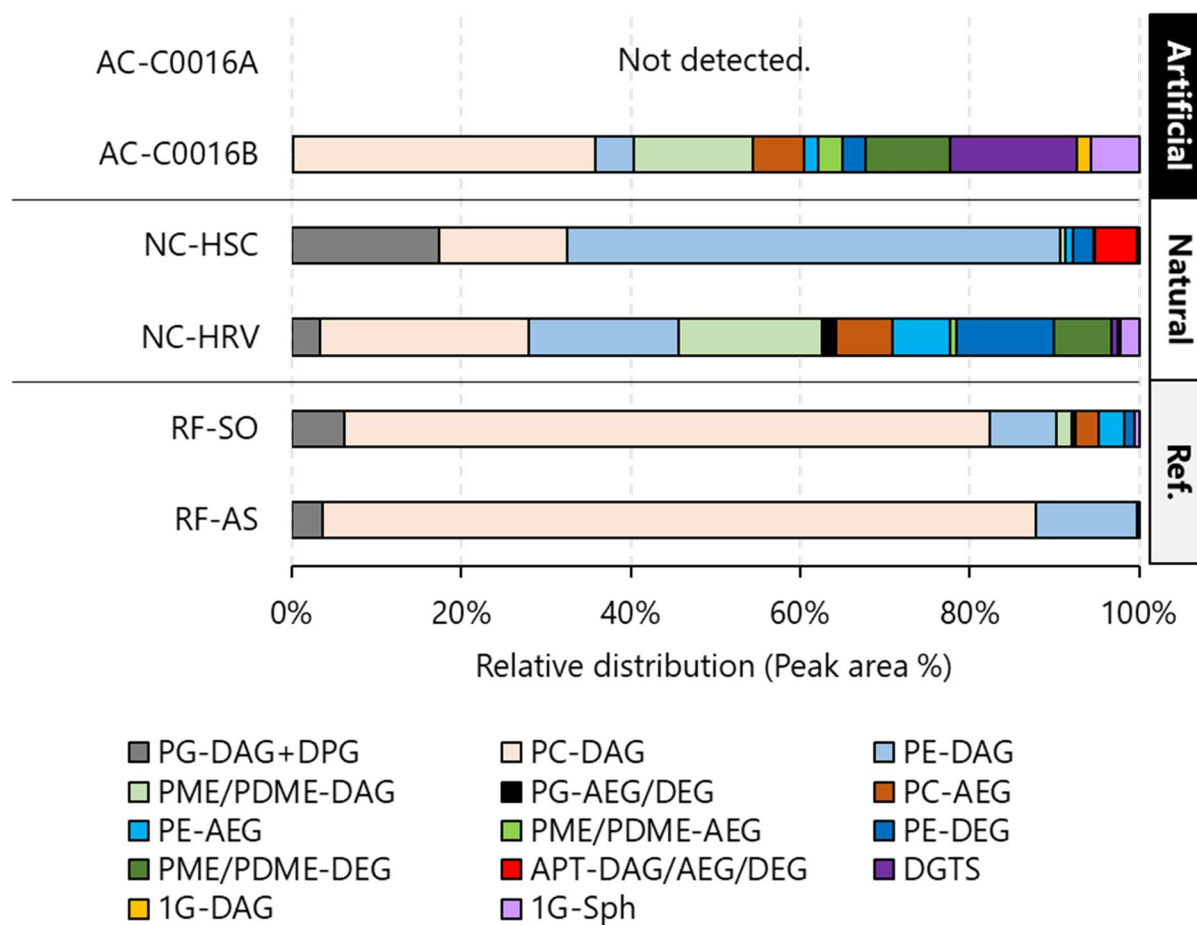


**Figure 3.12.** Distributions of the relative amounts of non-archaeal IPL peak areas observed in each sample. Details are summarized in **Table 3.7**. For structures see **Figure 3.8**.

**Table 3.7.** Relative abundance of peak areas of Non-archaeal IPL class observed in artificial chimneys, natural chimneys and reference samples.

| IPL-Class     | Artificial chimneys |           | Natural chimneys |        | Reference samples |       |
|---------------|---------------------|-----------|------------------|--------|-------------------|-------|
|               | AC-C0016A           | AC-C0016B | NC-HSC           | NC-HRV | RF-SO             | RF-AS |
| PG-DAG        | -                   | tr        | 17               | 3      | 6                 | 4     |
| PC-DAG        | -                   | 36        | 15               | 25     | 76                | 84    |
| PE-DAG        | -                   | 5         | 58               | 18     | 8                 | 12    |
| PME-DAG       | -                   | 7         | 1                | 7      | 1                 | -     |
| PDME-DAG      | -                   | 7         | -                | 10     | tr                | -     |
| APT-DAG       | -                   | -         | tr               | tr     | -                 | -     |
| DGTS          | -                   | 15        | -                | 1      | -                 | -     |
| 1G-DAG        | -                   | 2         | tr               | tr     | -                 | -     |
| PG-AEG        | -                   | -         | -                | tr     | tr                | -     |
| PC-AEG        | -                   | 6         | -                | 7      | 3                 | -     |
| PE-AEG        | -                   | 2         | 1                | 7      | 3                 | -     |
| PME-AEG       | -                   | 1         | tr               | tr     | -                 | -     |
| PDME-AEG      | -                   | 2         | -                | tr     | -                 | -     |
| APT-AEG       | -                   | -         | tr               | -      | -                 | -     |
| PG-DEG        | -                   | -         | -                | 1      | tr                | tr    |
| PE-DEG        | -                   | 3         | 2                | 12     | 2                 | -     |
| PME-DEG       | -                   | 3         | tr               | 6      | -                 | -     |
| PDME-DEG      | -                   | 7         | -                | 1      | -                 | -     |
| APT-DEG       | -                   | -         | 5                | -      | -                 | -     |
| DPG           | -                   | -         | 5                | tr     | 1                 | -     |
| 1G-Sph        | -                   | 6         | -                | 2      | 1                 | -     |
| % of IPL-DAGs | -                   | 71        | 92               | 63     | 92                | 99.7  |
| % of IPL-AEGs | -                   | 11        | 1                | 14     | 6                 | -     |
| % of IPL-DEGs | -                   | 13        | 7                | 18     | 2                 | tr    |

Because ionization efficiencies of individual compounds and matrix effect are not known, these should not be interpreted as absolute abundance percentages. ‘-’, Not detected. ‘tr’: trace (< 1%). For structures see **Figure 3.8**. Details of all non-archaeal IPLs observed in this study are summarized **Appendix Tables** in end of this thesis.

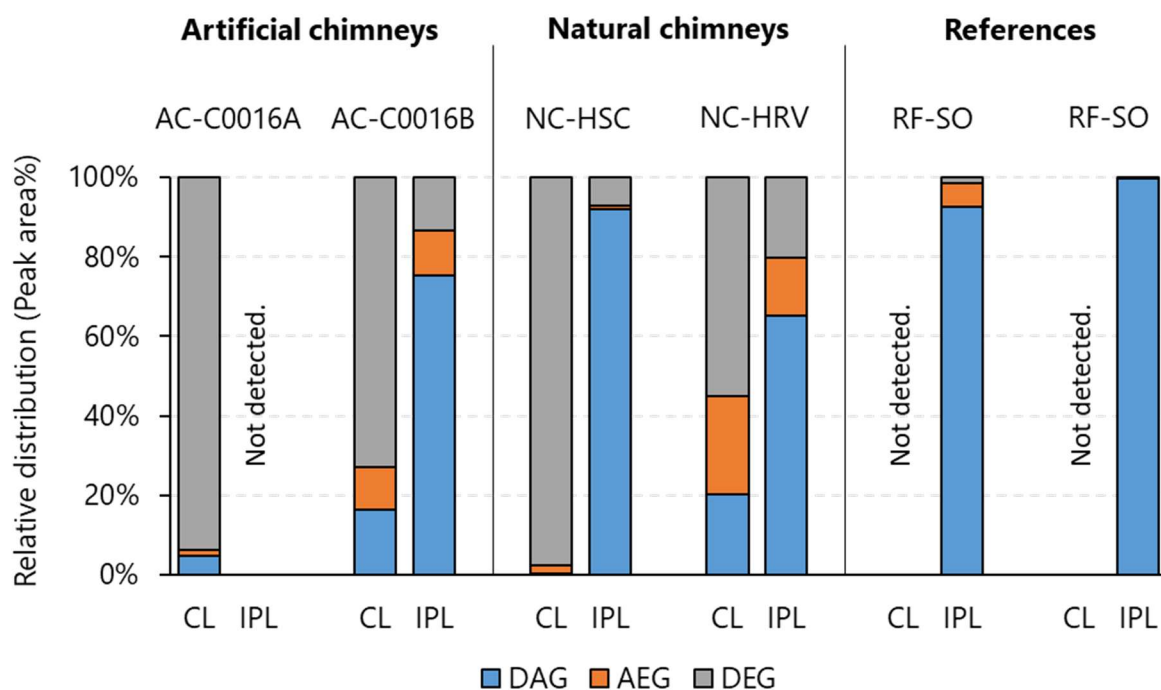


**Figure 3.13.** Comparison of the relative distributions of non-archaeal IPLs and CLs observed in each sample. Details are summarized in **Table 3.7** and **Table 3.8**

**Table 3.8.** Relative abundance of peak areas of Non-archaeal CL class observed in artificial chimneys, natural chimneys and reference samples.

|           | Artificial chimneys |           | Natural chimneys |        | Reference samples |       |
|-----------|---------------------|-----------|------------------|--------|-------------------|-------|
|           | AC-C0016A           | AC-C0016B | NC-HSC           | NC-HRV | RF-SO             | RF-AS |
| % CL-DAGs | 5                   | 16        | tr               | 20     | -                 | -     |
| % CL-AEGs | 2                   | 11        | 2                | 25     | -                 | -     |
| % CL-DEGs | 94                  | 73        | 98               | 55     | -                 | -     |

Because ionization efficiencies of individual compounds and matrix effect are not known, these should not be interpreted as absolute abundance percentages. ‘-’, Not detected. ‘tr’: trace (< 1%). For structures see **Figure 3.8**. Details of all non-archaeal CLs observed in this study are summarized **Appendix Tables** in end of this thesis.

### 3.4. Discussion

#### 3.4.1. Mineralogical constraints on chimney habitat

The AC-C0016A and NC-HSC samples are composed entirely of sulfide minerals (**Figure 3.4A, C**), which may reflect the high temperature and reducing conditions inside the chimney with little contact with seawater. On the other hand, the AC-C0016B sample contains cerussite, and the NC-HRV sample contains sulfate minerals (**Figure 3.4B, D**), indicating a mixing with seawater. The AC-C0016B sample exhibits a distinctive mineral composition, consisting primarily of sulfide minerals, without accompanying sulfate minerals, and including oxidized minerals (**Figure 3.4B**).

In general, hydrothermal fluid and seawater mix rapidly during the initial stage of chimney formation, and anhydrite precipitates first followed by forming the external framework of the chimney (Haymon et al., 1983). Subsequently, sulfide minerals precipitate toward the inside of the anhydrite barrier. At this stage, some of the anhydrite is redissolved and replaced by sulfide minerals. The precipitation of Cu-Zn-Pb sulfide minerals from hydrothermal fluids depends mainly on the relationship between the solubility of each metal and temperature, with Cu (chalcopyrite) precipitating between 250-350°C and Zn (sphalerite) and Pb (galena) precipitating from 150-250°C (e.g., Hannington et al., 1995). Thus, the mineral precipitation between 150-250°C was likely to be predominant in the chimney sample from hydrothermal fluids above 300°C in this study. The mineral assemblage of Zn-rich sulfides (sphalerite, and sometimes wurtzite), galena (PbS) and chalcopyrite ( $\text{CuFeS}_2$ ) of the artificial and natural hydrothermal chimney samples other than the RF-AS samples is characteristic of mineral precipitation at temperatures lower than about 250°C from high-temperature thermal fluid of about 250-350°C (Hannington et al., 1995). In addition, marcasite and pyrite displace pyrrhotite at pH 5 (Murowchick and Barnes, 1986; Qian et al., 2011) indicating intermediate pH conditions.

In addition, the chimney samples other than the AC-C0016B sample did not contain oxide minerals. The mineral occurrence indicates that the chimney samples were formed under microaerobic to anaerobic conditions with little exposure to seawater or oxidizing agents. Pb is highly reactive with carbonate ions in seawater and precipitates as cerussite ( $\text{PbCO}_3$ ) or hydrocerussite ( $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ ) (Angel et al., 2016; Fuchida et al., 2018). Cerussite formation occurs at neutral pH (Hatsukawa et al., 2019; Nozaki et al., 2021).

Dense colonies of *Paralvinella hessleri* were found on the external surface of the AC-C0016B specimen. Vascular polychaetes of the family *Alvinellidae*, to which *Paralvinella hessleri* belongs, form heat- and chemical-resistant tubes composed of mucus secretions and

mineral particles (Tunnicliffe, 1993). The tubes act as a seal layer that buffers the mixing of seawater and hydrothermal fluids at the chimney surface and facilitates the precipitation of sulfide minerals and replacement of sulfate minerals with sulfide minerals in the underlying mineral base (Juniper et al., 1992). Therefore, it is likely that the artificial chimneys, which consisted primarily of sulfide minerals produced by drilling, were covered with tubes of *Paralvinella hessleri*, thereby promoting the formation of sulfide minerals and limiting contact between seawater and oxidizers.

However, as the inside of the casing pipe began to be blocked by hydrothermal precipitates, leaking of hydrothermal fluids was observed between the borehole and the pipe insert (Kawagucci et al., 2013). Eight years after drilling, the basement of the guide framework of Hole C0016B was buried in a transparent fluid-shimmering flange structure (**Figure 3.3B**). In addition, the top of the guide base is completely covered by hydrothermal deposits (**Figure 3.3B**), which may indicate that the fluid flow was dispersed due to blockage of the inside of the casing pipe by hydrothermal deposits. It is likely that the blockage and dispersion of the fluid flow have reduced the amount of fluid outflow from the guide framework. Therefore, the mineral composition of the AC-C0016B sample, most of which is protected from contact with seawater by the tubes of *Paralvinella hessleri*, may indicate that seawater intrusion is partially dominant with decreasing the rate of hydrothermal outflow.

Nakajima et al. (2015) pointed out a possibility of either gypsum/barite precipitation or silicification of the sediments as a hardening process of marine sediments at Site C0014. In the hardened sediment (RF-AS) down to a depth of about 15 mbsf, silicates and their alteration products were the major constituent minerals (**Figure 3.4F**). The amorphous silica in the RF-AS sample is likely derived from small pieces of pumice observed in the sample before it was crushed. The presence of trace amounts of chlorite (Clinochlore) suggests that it has experienced hydrothermal alteration above 220°C (e.g., Fulignati, 2020), but the coexisting amorphous silica (probably derived from pumice) is stable at temperatures below 150°C (Henley and Ellis, 1982). Neither gypsum nor barite sulfate minerals were detected in sample RF-AS, although they could be formed in this temperature range. Therefore, silicification of the sediments, rather than precipitation of gypsum/barite, appears to be the primary hardening process in the surface layer of the marine sediment at Site C0014.

As described above, the temperature and pH at the time of formation and alteration of the minerals in each sample are not necessarily indicative of the conditions that existed when the microorganisms were present, but the environmental conditions experienced by each sample could be inferred from the mineral composition.

### 3.4.2. Sources of organic matters

Primary sources of organic matter in the artificial chimney, natural chimney, and reference samples are discussed using bulk elemental and isotopic compositions. TOC concentrations (0.005-0.052 wt.%) of the artificial and natural chimneys are small but in the same range as reported values (0.005-0.035 wt.%) of the active chimneys of other hydrothermal systems in the back-arc basin (Kato et al., 2010; Reeves et al., 2014). The bulk organic matter compositions (TOC and TN concentrations, TOC/TN ratio and  $\delta^{13}\text{C}_{\text{TOC}}$ ) of the artificial chimneys were in good agreement with the natural chimneys (**Table 3.2; Figure 3.5A, B**). On the other hand, the TOC concentrations of the reference samples (0.15-0.2 wt.%) were much higher than those of the artificial and natural chimneys (**Table 3.2**). Therefore, the main origins of the organic matter in the artificial and natural chimneys are identical, and the organic matter in the reference samples may have different origins than those in the artificial and natural chimneys.

The Iheya North Knoll located in the continental margin region is covered by organic-rich terrestrial source sediments mainly supplied from the Yangtze and Yellow rivers (e.g., Narita et al., 1990; Huh and Su, 1999). The sediments also influence the chemical composition of hydrothermal fluids (Kawagucci et al., 2011). In such an environment, pyrolyzed sedimentary organic matter could accumulate in the chimney and altered the TOC composition (Simoneit, 1990). The TOC and TN in the core samples from Site C0016 were extremely low; 0.007-0.022 wt.% and below detection limit ( $< 0.001$  wt.%), respectively (Takai et al., 2012). In addition, only one of the core samples recovered from Site C0016 (Section 331-C0016B-3L-1) was amenable to analysis for volatile hydrocarbons ( $\text{C}_1$ - $\text{C}_3$ ) of the headspace gas, but all samples except  $\text{CH}_4$  were below detection limits (Takai et al., 2012). Kawagucci et al. (2010) considered that the pyrolysis of sedimentary organic matter alone cannot sustain the current  $\text{CH}_4$  flux based on the geology of the Iheya North field as well as the stable isotopic composition of  $\text{CH}_4$  and the mass balance of carbon and concluded that the archaeal  $\text{H}_2$ -consuming methanogenesis at the recharge zone could be the primary source of  $\text{CH}_4$  dissolved in the hydrothermal fluids in the field. Therefore, the organic matter in the two artificial chimney samples of Site C0016 (sample AC-C0016A and AC-C0016B) is unlikely to contain a pyrolysis product of sedimentary organic matter.

On the other hand, the drilling core samples recovered from Site C0014 had higher TOC and TN contents ( $< 0.001$  wt.% to 1.20 wt.%) than the cores from Site C0016 ( $< 0.001$  wt.% to 0.6 wt.%). Their TOC/TN ratios are higher than the Redfield ratio of 6.6 in most sections, suggesting an input of sedimentary organic origin (Takai et al., 2012). In addition,

Zhang et al. (2015) reported that the TOC and TN contents of seafloor sediments at distance of 0.4-6.3 km from the hydrothermal vent site of the Iheya North Knoll and the southern Iheya Minor Ridge were 0.10-0.38 wt.% and 0.01-0.13 wt.%, respectively, with the TOC/TN ratio of 7.4-11.7. Therefore, the TOC/TN ratios of the two natural chimney samples (NC-HSC and NC-HRV) from the Original Site and Aki Site are consistent with those of the two artificial chimney samples (AC-C0016A and AC-C0016B) from Site C0016 (**Table 3.2**), indicating no contribution or negligible of pyrolytic organic matter to the samples. This is supported by the fact that the TOC content in these samples is closer to the values reported from the chimney structures in the hydrothermal field without sediment related (Kato et al., 2010; Reeves et al., 2014) than in other hydrothermal fields with sediment hosted (Jaeschke et al., 2012; Jaeschke et al., 2014).

Furthermore, compared to normal marine sediments, the TOC and TN contents of these samples are extremely small and their TOC/TN ratios are clearly low (**Table 3.2**). It is likely no contribution from the marine sediments, even though it cannot be ruled out that the TOC in the artificial and natural chimneys contains the remains of the past microorganisms. On the other hand, the TOC and TN contents as well as the TOC/TN ratios of the reference samples (RF-SO) and hardened sediment (RS-AS) from Site C0014 are similar to those of the seafloor sediment (**Table 3.2**), suggesting that the organic matter of these samples could be contributed by the seafloor sediments.

Kato et al. (2010) reported a strong positive correlation between TN and TOC content in active and inactive chimney structures of the southern Mariana Trough, which have little contribution from the surrounding sediments. Although it is not clear an activity of thermophilic archaea at active chimneys using acid phosphatase activity under the temperature conditions of measurement (37°C), the TN and TOC contents in inactive chimney structures had a similar relationship to those of this study. The positive correlation between TN and TOC was considered evidence that the organic matter in the samples is mainly derived from microbial cells. Therefore, the organic matter in the two artificial chimney samples from Site C0016 (AC-C0016A and AC-C0016B) and the two natural chimney samples from the Hara and Aki sites (NC-HSC and NC-HRV) is considered to be mainly derived from microbial cells.

Although the detailed TOC/TN ratios of microbial cells is not known in submarine hydrothermal environments, the TOC/TN atomic ratios of active chimney structures in the Southern Mariana Trough are 5.3 for the exterior and 5.8 for the interior (Kato et al., 2010), which is in good agreement with the values from two artificial chimney samples at Site C0016 (AC-C0016A and AC-C0016B) and the two natural chimney samples from Original Site and

Aki Site (NC-HSC and NC-HRV) of this study (**Table 3.2**). Meador et al. (2014) reported that *Thermococcus kodakarensis* (KOD1), a hyperthermophilic Archaea belonging to the order *Thermococcales* that frequently occurs in seafloor hydrothermal environments, had the TOC/TN ratio that varied between approximately 4-8 depending on the growing stage and the availability of organic substrates and phosphorus sources. On the other hand, the average TOC/TN ratio of marine bacteria is estimated to be 5-7. The TOC/TN ratio of marine bacteria is known to be largely unaffected by the TOC/TN ratio of the substrate (Bratbak, 1985; Goldman et al., 1987; Nagata and Watanabe, 1990), and does not differ significantly between different oceanic regions (Fukuda et al., 1998), suggesting that it is relatively less variable in marine environments. The two artificial chimney samples from Site C0016 (AC-C0016A and AC-C0016B) and two natural chimney samples from Original Site and Aki Site (NC-HSC and NC-HRV) also have a similar value within these reported values (**Table 3.2**), supporting that the organic matter in these samples is derived from microbial cells. Although the comparative information is limited, the TOC/TN ratio of the microbial community in the seafloor hydrothermal environment is around 5.5, which, like other marine environments, may be relatively less variable.

In addition to bulk elemental compositions of organic matter, the carbon isotopic ratio of organic matter ( $\delta^{13}\text{C}_{\text{TOC}}$ ) also provides information about the origin and metabolic processes of organic matter. The  $\delta^{13}\text{C}_{\text{TOC}}$  values of the artificial chimney samples were nearly identical at -26‰, whereas the  $\delta^{13}\text{C}_{\text{TOC}}$  values of the natural chimney samples (-27.2 and -21.8‰) were a little wider (**Table 3.2**). However, they were clearly distinguishable from the lower  $\delta^{13}\text{C}_{\text{TOC}}$  values of the reference samples (-34.1‰ and -31.4‰). The  $\delta^{13}\text{C}$  values of the artificial and natural chimneys (-27.2 to -21.8‰) are within the range of the active chimney of the sediment-engaged hydrothermal system (-39.3 to -14.2‰, Jaeschke et al., 2012; Jaeschke et al., 2014; Li et al., 2017). The  $\delta^{13}\text{C}_{\text{TOC}}$  values of the active chimney of the less sediment-associated back arc basin hydrothermal system (-23.0 to -15.5‰, Kato et al., 2010; Reeves et al., 2014), which is similar to or slightly enriched in  $^{13}\text{C}$  compared to the hydrothermal chimney samples of this study.

The  $\delta^{13}\text{C}_{\text{TOC}}$  values of seafloor sediments in the mid-Okinawa Trough ranges from -20.5‰ to -21.5‰ (Shao et al., 2016). In general, the bulk organic matter of marine sediments has a higher TOC/TN ratio with lower  $\delta^{13}\text{C}$  if the contribution from terrestrial source organic matter is larger (Mayer, 1993). The TOC/TN ratios of the reference samples (RF-SO:  $10.6 \pm 0.5$ , RF-AS:  $8.9 \pm 0.4$ ; **Table 3.2**) may indicate a contribution from terrestrial source organic matter (mainly, terrestrial higher plants). However, the  $\delta^{13}\text{C}_{\text{TOC}}$  values of RF-SO ( $-34.0 \pm 0.2\text{‰}$ )

and RF-AS ( $-31.2 \pm 0.2\text{‰}$ ) may be too low to be explained by the contribution from terrestrial organic matter alone. Of the reference samples, the RF-SO sample is a sulfide ore formed on the sediment-covered seafloor surface, probably produced by post-drilling hydrothermal activity in altered seafloor sediments ( $< 15$  cmbsf). The contribution of drilling (drilling mud and oil) and post-drilling hydrothermal activity (organisms associated with hydrothermal activity and accumulation of isotopically lighter organic matter resulting from pyrolysis of sedimentary organic matter) may be responsible for their low  $\delta^{13}\text{C}_{\text{TOC}}$  values.

### 3.4.3. Source organisms of lipid biomarkers

The numerous numbers of IPLs with various polar head groups and CLs without polar head groups were detected as lipid biomarkers for archaea and bacteria (**Figure 3.6, Figure 3.7 and Appendix Tables**). Because of the rapid degradation of IPLs in cells after microbial death (White et al., 1979; Harvey et al., 1986; Logemann et al., 2011; Schouten et al., 2010), the presence of IPLs in samples has been considered as evidence of microbial live or recently dead cells (e.g., Lipp et al., 2008; Gibson et al., 2013; Reeves et al., 2014). Only archaeal IPLs were detected in the artificial chimney (AC-C0016A), and bacterial IPLs were below the detection limit (**Figure 3.9A**). This lipid occurrence indicates that archaea activity has recently been developed in AC-C0016A. In contrast, no archaeal IPLs were detected in the reference sample RF-AS, and only non-archaeal IPLs and archaeal CLs without polar groups were detected (**Figure 3.9A, B**). This indicates that archaeal activity was present in RF-AS in the past, but non-archaeal biological activity had occupied at the time of the sample collection. Despite the higher organic carbon content in the two reference samples than in the chimney samples (**Table 3.2**), non-archaeal CLs were not detected in the two reference samples (**Figure 3.8B**), suggesting a long-term lipid degradation by bacterial activity. For the remaining three chimney samples, both IPLs and CLs were present for both archaea and non-archaea (**Figure 3.9A, B**). The observation indicates the involvement of various biological activities in the current and past of chimney formation.

#### 1) Intact polar lipids (IPLs)

Following the classification using 16S rRNA genes, biomarkers of IPL types have been used to classify microbial species in natural samples. For an example, in sulfide chimneys of the Mid-Atlantic Ridge and Manus Basin, the structural diversity of IPLs was found to reflect the relative distribution of *Archaea* and *Bacteria* activity, which was consistent with the gene-based classification approach (Gibson et al., 2013; Reeves et al., 2014). Furthermore, as the

combination of polar head groups and lipid cores that comprise the IPLs modulates the physicochemical properties of cell membranes, the IPLs biomarker is expected to provide insight into the physiological adaptation of microbial communities in hydrothermal environments (e.g., Sollich et al., 2017).

### i) Archaeal IPLs

Glycolipids were commonly identified in all samples, except for the RF-AS sample that no IPLs were detected (**Table 3.3**). Because glycosidic head groups are distributed widely in archaea (e.g., Kates, 1993; Koga et al., 1993), so taxonomic specificity is poor, but the structure and relative distribution of the core lipids bound to them can be used to reconstruct the sources or origins of major organisms. In this study, archaeal IPLs composed of three diether core lipids (AR, MAR and OHAR) and three tetraether core lipids (GDGTs, HGDGTs and nMe-HGDGTs) were detected (**Table 3.3; Figure 3.10**).

MAR is known as the major core lipid of (hyper-)thermophilic methanogens belonging to the order *Methanococcales* (e.g., Comita et al., 1984; Sturt et al., 2004; Baumann et al., 2018). OHAR is widely found in methanogens (e.g., Koga and Morii, 2005), but is also reported as the major lipid of ANME-2 and -3 in anaerobic methane-oxidizing archaea (e.g., Blumenberg et al., 2004; Rossel et al., 2008; Wegener et al., 2016). HGDGTs are synthesized by (hyper-)thermophilic methanogens or non-methanogenic archaea and are found in (hyper-)thermophilic methanogens occurred in submarine hydrothermal environments. Therefore, they are used as biomarkers for (hyper-)thermophilic archaea in submarine hydrothermal environments (e.g., Jaeschke et al., 2012; Gibson et al., 2013; Jaeschke et al., 2014; Reeves et al., 2014; Sollich et al., 2017). To date, the HGDGT core lipids bearing up to four ring structures are known from *Acidulprofundum boonei* (DHVEG), *Ignisphaera aggregans* (*Desulfurococcales*), and *Pyrococcus furiosus* (*Thermococcales*) (Schouten et al., 2008; Knappy et al., 2011; Tourte et al., 2020a). nMe-HGDGTs are the only reported lipids with the maximum two methyl groups (i.e., 1 and 2Me-HGDGT-0) without ring structures of HGDGT core lipids from a culture of hyper-thermophilic methanogen *Methanopyrus kandleri* (Liu et al., 2012b), but analogous species containing up to four methyl groups and four rings have been found in other deep-sea hydrothermal vents and shallow-sea hydrothermal deposits (e.g., Reeves et al., 2014; Sollich et al., 2017).

The archaeal IPL composition of AC-C0016A is in good agreement with that of the geographically most distant NC-HSC sample and differs from that of AC-C0016B and NC-HRV (**Figure 3.10**). The most common features of the archaeal IPL compositions between AC-

C0016A and NC-HSC are the relatively high percentages of HGDGT-IPLs (37.6% and 51.9%, respectively), and the predominance of 0 ring with the smaller the number of rings being the smaller percentage (**Figure 3.10, Table 3.5**). The cyclization patterns of these HGDGTs were similar to observed in *A. booni* (DHVE-2) and *P. furiosus* (*Thermococcales*, *Pyrococcus*) (Schouten et al., 2008; Tourte et al., 2020a). Therefore, the HGDGT-IPLs in AC-C0016A and NC-HSC are mainly derived from DHVE-2 and *Thermococcales*, with a possible contribution from *Desulfurococcales*. DHVE-2, *Thermococcales* and *Desulfurococcales* also synthesize GDGTs containing up to four ring structures (Schouten et al., 2008; Tourte et al., 2020a). In addition, *Thermococcales* are also known to contain a high percentage of ARs (< ~80%, Tourte et al., 2020a), and considered to be one of the major AR source organisms in these samples. Because these three species are likely heterotrophic archaea, other autotrophic microorganisms should coexist symbiotically. MAR-IPLs in AC-C0016A and NC-HSC are relatively small (6.5% and 2.4%, respectively, **Table 3.3; Figure 3.10**). Probably MAR-IPLs are derived from (hyper-) thermophilic methanogens belonging to the order *Methanococcales*, which are one of the autotrophic microorganisms that support the microbial ecosystem.

In contrast to AC-C0016A and NC-HSC, the AC-C0016B and NC-HRV samples have relatively small percentages of HGDGTs-IPLs (6.1% and 1.7%, respectively), but higher percentages of MAR-IPLs and OHAR-IPLs (**Table 3.3; Figure 3.10**). In the AC-C0016B sample, 1G-AR (34.5%) and 1G-MAR (36.4%) accounted for the majority of archaeal IPLs detected, implying the predominant (hyper-) thermophilic methanogens belonging to the order *Methanococcales*. *Methanococcales* can also synthesize GDGT-0 and HGDGT-0 as a CLs (Baumann et al., 2018). In contrast, OHAR-IPLs and AR-IPLs comprise the majority of archaeal IPLs in NC-HRV (**Table 3.3; Figure 3.10**). Prior to drilling by IODP Expedition 331, chimneys (including NBC) and *in-situ* incubation at the Iheya North field yielded *Thermococcales*, *Methanococcales*, and *Archaeoglobales*, and the 16S rRNA gene sequences of DHVEG, *Desulfurococcales*, Marine Group 1 and *Korarchaeota* were also detected (Nakagawa et al., 2005).

## ii) Non-archaeal IPLs

A total of 20 classes of non-archaeal IPLs were identified in all samples except AC-C0016A (**Table 3.7**). Non-archaeal IPLs accounted for the major IPL of these samples (> 70 % of total IPL peak area, **Figure 3.9A**). This indicates that the relative proportion of bacteria in the total biomass is greater than in archaea with an exception of the AC-C0016A sample. The distribution of non-archaeal IPLs does not necessarily indicate the relative abundance of

individual IPL classes but reflects the diversity of bacterial communities in each environment (**Figure 3.12; Table 3.7**).

PE-DAG, PG-DAG, and PC-DAG were detected in all samples except for the AC-C0016A sample (**Figure 3.12, Table 3.7**). These IPL classes may originate from both bacterial and eukaryotic origins (e.g., Sturt et al., 2004), but they were present in active hydrothermal chimneys associated with bacterial activity at the Lucky Strike and the Rainbow hydrothermal fields in the central Atlantic Ridge and the PAMCMANUS hydrothermal field in the Manus Basin, as well as in bacteria-associated seafloor hydrothermal sediments at the Spathi Bay in the Mediterranean (Gibson et al., 2013; Reeves et al., 2014; Sollich et al., 2017). PE-DAG, PG-DAG and small amounts of DPG are common phospholipids in bacterial membranes (e.g., Goldfine, 1984; Dowhan, 1997; Sohlenkamp et al., 2003). PC-DAG, on the other hand, has been thought to be present only in specific inner membrane structures of photosynthetic bacteria or some bacteria that are symbiotically or pathogenic related to eukaryotes (Goldfine, 1982; Lopeza -Lara and Geiger, 2001). However, genomic data analysis indicates that at least 10% of all bacteria contain sequences encoding homologs of enzymes involved in PC biosynthesis (Sohlenkamp et al., 2003; Geiger et al., 2013). Indeed, PC-DAGs are found in bacteria with phylogenetically diverse lifestyles (e.g., Sohlenkamp et al., 2016). Therefore, these IPL lipids lack taxonomic clarification and may be difficult to assign a specific origin.

### **iii) Characteristic IPLs: PME/PDME-(DAG/AEG/DEG)**

In general, bacterial PC biosynthetic pathways include the phospholipid N-methyltransferases (Pmt) pathway (Sohlenkamp et al., 2003; Hacker et al., 2008), which methylates phosphoethanolamine (PE) three times, and the PC synthase (Pcs) pathway (de Ruber et al., 1999; Sohlenkamp et al., 2000), which directly condenses chlorine to cytidine diphosphate (CDP). The polar head groups of PME and PDME, which are methylated derivatives of PE, are intermediates in PC biosynthesis in the Pmt pathway, and some bacteria are known to use them as end products (e.g., Goldfine and Ellins, 1964; Tornabene, 1973; Moser et al., 2014). PME- and PDME-DAG are common phospho-lipids of *Alpha-proteobacteria*, *Gamma-proteobacteria* and some *Betaproteobacteria* (e.g., Goldfine, 1984; Sohlenkamp and Geiger, 2016), of which they may be derived from methane-consuming bacteria (Makula, 1978; Fang et al., 2000) and/or sulfur-oxidizing bacteria (Barridge and Shively, 1968; Borin et al., 2010).

The 16S rRNA sequences of *Gammaproteobacteria* phylotypes have been detected at relatively high frequencies in chimneys and *in situ* incubators at the Iheya North field prior to

drilling (Nakagawa et al., 2005). The biomass of the sulfur oxidizing bacterial SUP05 group of *Gammaproteobacteria* is reported to be the most abundant near the vent fauna habitat (Yanagawa et al., 2015). Theoretical thermodynamic calculations by Nakamura and Takai (2014) showed that sulfur oxidation and methane oxidation were the most energetic metabolisms available in the mixed hydrothermal and seawater region of the Iheya North field. It is consistent with the predominance of a free-living group of methane-oxidizing bacteria (*Methyloprofundus*) belonging to *Gammaproteobacteria* in the Iheya North field (Hirayama et al., 2022).

AEGs and DEGs possessing the polar head groups of PME and PDME have been reported from marine environments (e.g., Schubotz et al., 2009; Flores et al., 2021), but not detected from cultured bacteria. It is unclear whether they are derived from uncultured bacteria or whether both PE methylation and ether lipid synthesis do not occur simultaneously under laboratory culture conditions. Recently, key enzymes for bacterial ether lipid biosynthesis have been identified, and genomic analysis has shown that diverse strains of bacteria have an ability to synthesize ether lipids (Sahonero-Canaviesi et al., 2022). Non-isoprenoid glycerol-ether lipids are thought to be specific to *Bacteria* with an exception of plasmalogens (e.g., Vincon-Laugier et al., 2017), which are also found in *Eukaryotes*. To date, dietherglycerol lipids (DEG) have been found in *Cystobacteriaceae* (Caillon et al., 1983; Asselineau and Asselineau, 1990), *Desulfobacteraceae* (Grossi et al., 2015; Vincon-Laugier et al., 2016), *Planctomycetaceae* (Sinninghe Damsté et al., 2005), *Thermodesulfobacteriaceae* (Langworthy et al., 1983; Sturt et al., 2004; Hamilton-Brehm et al., 2013), *Thermoanaerobacteraceae* (Huber et al., 1996), and *Aquificaceae* (Huber et al., 1992; Janke et al., 2001). Among these lineages, *Desulfobacteraceae* (Grossi et al., 2015; Vincon-Laugier et al., 2016) and *Planctomycetaceae* (Sinninghe Damsté et al., 2005) are capable of ether lipid synthesis and PE methylation.

#### **iv) Phosphorous non-containing IPLs: Phosphorous depletion**

DGTS has been described in a very few species of *Alphaproteobacteria* and *Planctomycetes* (e.g., Benning et al., 1995; Geiger et al., 1999; Sohlenkap and Geiger, 2016). Bacteria synthesize DGTS using the enzymes BtaA, which is involved in the formation of diacylglycerol homoserine (DGHS) being the precursor of DGTS, and BtaB, which catalyzes methylation of DGHS (Riekhof et al., 2005). The genes encoding of the BtaA homologs are also found in the genomes of *Gammaproteobacteria*, *Deltaproteobacteria*, *Firmicutes*, *Deinococcus*, *Acidobacterium* and *Cyanobacteria* (Geiger et al., 2010; Sohlenkap and Geiger, 2016). Bacteria are known to replace phospholipids with phosphorus-free membrane lipids such

as aminolipids and glycolipids like DGTS under the phosphate-limited conditions, allowing phosphate to be used for other cellular processes such as nucleic acid synthesis (e.g., Benning et al., 1995; Sebastian et al., 2016). The phospholipase activity in *Sinorhizobium meliloti* of *Alphaproteobacteria* and the expression of enzymes required for the synthesis of DGTS and glycolipids are induced under phosphate-limiting conditions (Zavaleta-Pastor et al., 2010). Marine heterotrophic bacteria can replace PE-DAG with either 1G-DAG or DGTS (Carini et al., 2015; Sebastian et al., 2016; Yao et al., 2015). The culture experiments have reported that the sulfate-reducing bacterium *Desulfovibrio alaskensis* synthesizes alternative lipids even at phosphate concentrations on the order of  $\mu\text{M}$  (Bosak et al., 2016).

1G-DAGs were detected in AC-C0016B, NC-HSC and NC-HRV, but not in the reference samples (**Table 3.7**). 1G-DAGs and genes involved in glycolipid synthesis are found in Gram-positive bacteria (e.g., Jorasch et al., 1998), and some bacteria are known to synthesize them when phosphate is depleted in the environment (e.g., Benning et al., 1995; Geske et al., 2013; Diercks et al., 2015). 1G-DAG is also known to be present in thermophilic bacteria such as *Thermus*/*Deinococcus* groups (e.g., Pask-Hughes and Shaw, 1982; Anderson and Hansen, 1985; Lu et al., 2004) and *Thermotogae* (Huber et al., 1986; Manca et al., 1992; Sinningh-Damsté et al., 2007). A member of *Thermus* was cultured in the Iheya North field (Nakagawa et al., 2005), and is considered as a major source organism of 1G-DAG.

## 2) Core lipids

Biomarkers of membrane lipids of possible archaea or bacteria origin were detected in artificial chimneys, natural chimneys, and reference samples in the form of either polar membrane lipids (IPLs) possessing polar groups or core lipids (CLs) not possessing polar groups, or both (**Figures 3.6, Figure 3.7**). More stable CLs were transformed from the IPLs after the death of the microorganism (White et al., 1979; Harvey et al., 1986). Some of the more stable CLs have been preserved over geologic timescales and have been widely used to reconstruct past environmental conditions (Schouten et al., 2002; Schouten et al., 2007; Schouten et al., 2013). However, CLs in environmental samples are mixtures derived from both older generations of microbial communities and recently dead microbial cells. The CLs without polar head groups have less taxonomic information than IPLs (e.g., Sturt et al., 2004). Furthermore, their compositions may be complicated by processes such as 1) time-series variation in microbial community structure, 2) changes in membrane lipid composition for physiological adaptation, and 3) biological and thermochemical degradation.

### i) Archaeal CLs

The lipid core structure of archaea IPLs detected in each sample is generally consistent with the type of CLs detected in the same sample, the compositions of some CLs groups differ from IPLs (**Figure. 3.11**). In general, common features of CL composition relative to IPL composition in archaea include (i) a larger overall proportion of CLs-GDGTs and CL-nMe-HGDGTs and (ii) a smaller overall proportion of diether lipids. Overall, the differences in the distribution of IPL and CL appear to be related to the abundance of CL-ARs, CL-GDGTs, and CL-nMe-HGDGTs. The CL-ARs and CLs-GDGTs are the most universal core lipids within archaea. They are produced more frequently than the other relatively specific archaeal diether core lipids detected (CL-MAR, CL-OHAR) and tetraether core lipids (CL-HGDGTs, CL-nMe-HGDGTs), which are more likely to be produced by fluctuations in a community structure, changes in membrane lipid composition for environmental adaptation, biological and physicochemical degradation, or other processes. As consequence, the specific lipid biomarkers would result in relatively large fluctuations (larger proportions appear to be relatively more variable).

In general, in microbial degradation of compounds with ether bonds, enzymatic oxidation and subsequent hydrolysis of the ether bond is prior to the degradation of aliphatic chain (White et al., 1996). Furthermore, at least one GDGT degradation pathway involves a stepwise process that result in glycerol loss and glycerodialkanol (GDD) formation during acid hydrolysis of GDGTs for 72 hours (Liu et al., 2012). Based on these findings on the biological and thermochemical degradation processes of ether core lipids, it appears that the diether core lipids are degraded much faster than the tetraether core lipids, resulting in a shorter residence time of diether lipids compared to tetraether lipids. Therefore, differences in the diether lipid/tetraether lipid ratio between IPL and CL in the samples may not be indicative of time series changes in archaeal community or changes in membrane lipid composition with environmental changes. Abundance of CL-MAR and IPL-MAR in the artificial chimney AC-C0016A and the natural chimney NC-HSC was almost equal as a percentage of the total (**Figure. 3.11**). which did not record variation in the order *Methanococcales*, their likely origin. In the artificial chimney AC-C0016B and natural chimney HRV, IPL-MAR and IPL-OHAR were predominant, respectively, but their CLs accounted for a smaller proportion of the total. In contrast, the relative ratios of CL-MAR and CL-OHAR are similar to those of IPL (**Figure. 3.11**).

Assuming that the differences in the proportions of GDGTs and nMe-HGDGTs in IPLs and CLs are due to time-series changes in archaeal community structure, the contribution of

some member of the archaeal community that synthesizes GDGTs as the most major membrane lipid core may have become smaller (or disappeared) in response to the environmental changes. For example, the thermophilic and acidophilic archaeal group of DHVE-2 closely related to *Thermoplasmatales*; *A. booni*, the only archaeon cultured in DHVE-2, has been shown to synthesize from 0 to 4 GDGTs depending on environmental changes, which account for >80% of total membrane lipids (Tourte et al., 2020a). Biogeographic studies of thermophilic and acidophilic DHVE-2 suggest that their distribution and abundance are controlled by end-component hydrothermal pH and the degree of mixing of hydrothermal and seawater within each hydrothermal vent (Flores et al., 2012). The pH of the hydrothermal fluid in the Iheya North field is around 4.5-5.0 (Kawagucci et al., 2011; Kawagucci et al., 2013; Miyazaki et al., 2017a) and easily approaches neutral pH when mixed with seawater. The mineralogy of the artificial chimney and natural chimney samples in this study indicates an unsuitable environmental history for the growth of DHVE-2, which requires thermophilic and acidophilic conditions. The Fe sulfide minerals, marcasite and pyrite displace pyrrhotite at around pH 5, where pyrite is favored than marcasite at pH > 5 (Murowchick and Barnes, 1986). Cerussite precipitation detected in artificial chimney AC0016B also indicates near neutral pH.

## 2) Sources of non-archaeal CLs

Archaeal IPLs were abundantly detected in the AC-C0016A sample, but non-archaeal IPLs were not (**Figure 3.9A**). The current microbial activity is dominated by archaea, and although there are traces of bacterial activity being present in the past, the bacterial biomass is currently too small or not established for those IPLs to be detected. The major non-archaeal CLs in the AC-C0016A sample are of the relatively stable diether type (**Figure 3.9B**), suggesting that the ester lipids may have been degraded or that the environment is too hot for bacteria to grow. This is consistent with the fact that the ether core lipids are more stable against degradation during diagenesis and are better preserved than core lipids containing ester bonds (Schouten et al., 2010; Vinço-Laugier et al., 2018).

Non-archaeal CLs were not detected in either of the two reference samples (**Figures 3.7, Figure 3.8B**). The non-archaeal IPLs were mostly composed of the diacyl form (**Figures 3.13**), which is less stable (Vinço-Laugier et al., 2018). These indicate that diacyl-type lipids are degraded promptly after death, even in the presence of non-archaeal IPLs, which are active indicators of microbial activity. The relative abundances of the core lipid portion in the non-archaeal IPLs and non-archaeal CLs of the AC-C0016B and NC-HRV samples were in good agreement (**Figure 3.13**). This indicates that the developmental stages of non-archaeal

microbial activity are similar between artificial and natural chimneys.

#### **3.4.4. Estimation of microbial biomass developed in artificial chimney structures**

Total organic carbon (TOC) content and its stable carbon isotopic composition ( $\delta^{13}\text{C}_{\text{TOC}}$ ) have been used as indicators to estimate microbial biomass and carbon metabolism within hydrothermal vents (e.g., Jaescke et al., 2012; Olins et al., 2013; Li et al., 2017). However, it is sometimes of problematic in environments where exogenous organic matter, such as sediments and settled particles in the surrounding seawater, contributes significantly to the bulk organic matter composition within hydrothermal vents (e.g., Simoneit et al., 1990; Reeves et al., 2014). Therefore, in this study, the TOC and TN contents, TOC/TN ratios and  $\delta^{13}\text{C}_{\text{TOC}}$  of samples from artificial and natural chimneys were compared to reference samples (RF-AS and RF-SO) of surface sediments undergoing hydrothermal alteration and hydrothermal sulfide precipitates deposited on the sediments to discriminate the organic matter. The bulk organic matter composition of the artificial and natural chimneys was clearly distinguished from the reference samples by their low TOC and TN contents, small TOC/TN ratios, and relatively high  $\delta^{13}\text{C}_{\text{TOC}}$  values (**Table. 3.2; Figure 3.5A, B**).

Although the carbon and nitrogen compositions of microbial communities in submarine hydrothermal vent environments are unknown, a relatively stable range of atomic TOC/TN ratios of 4-7 has been reported for various marine and coastal planktonic bacterial communities (e.g., Fukuda et al., 1998), thermophilic microbial communities in terrestrial hot springs (Kimura et al., 2010), and cultured bacteria and archaea (e.g., Nagata et al., 1999; Meador et al., 2014). The results of these natural and culture studies support the hypothesis that microbial TOC/TN ratios are largely unaffected by substrate TOC/TN ratios and remain nearly constant in the marine environment (Bratbak, 1985; Goldman et al., 1987; Nagata et al., 1999; Fukuda et al., 1998). Therefore, the TOC/TN ratios of microbial communities in submarine hydrothermal vent environments are assumed to be in a similar range. Therefore, the organic matter in the artificial and natural chimney samples in this study could be derived primarily from microbial cells. However, while the TOC/TN ratio of microorganisms is relatively less variable, the carbon and nitrogen content per microbial cell has been reported to vary widely in the order of a few ng to a few pg, depending on different environments and culture conditions (e.g., Fukuda et al., 1998; Kimura et al., 2010; Meador et al., 2014). It is considered that this variability is related to differences in microbial community composition and physiological conditions (e.g., Fukuda et al., 1998; Meador et al., 2014). In general, microbial cells that grow rapidly under nutrient-rich conditions tend to be larger than those under starvation conditions

(e.g., Nagata and Watanabe, 1990). Indeed, the carbon content of marine bacteria has been reported to vary about 10-fold with geography and season (e.g., Fukuda et al., 1998). The cultured thermophilic archaea *Thermococcus kodakarensis* has been reported to grow at about the same rate as in normal medium under limited organic substrates and phosphorus sources in the medium, but resulting in smaller cell size and lower carbon, nitrogen, and phosphorus contents (Meador et al., 2014). On the other hand, Meador et al. (2014) found that the cell volume power function (an index of the relationship between cell volume and carbon content, Simon and Azam, 1989) of natural and cultured thermophiles was greater than that measured for planktonic bacteria and estimated for archaea from deep-sea sediments (i.e., higher cell carbon density). This may imply physiological adaptations of thermophiles that differ from other bacterial and archaeal communities with respect to relative cellular carbon content. Therefore, in this study, the carbon and nitrogen contents for thermophilic microbial communities (1.35 pg C/cell, 0.19 pg N/cell) determined by Kimura et al. (2010) were used to estimate microbial biomass using the TOC and TN contents of artificial and natural chimneys. In addition, similar calculations were performed using the TOC and TN contents reported by Kato et al. (2010) from active and inactive chimneys in the southern Mariana Trough to examine the validity of the estimated values by comparing them to the total bacterial counts estimated from the 16S rRNA gene copy numbers in the same study.

**Table 3.9** shows the microbial biomass estimated from TOC and TN amounts for artificial chimneys and natural chimneys of this study, and chimney samples from the southern Mariana Trough, as well as the microbial biomass estimated by Kato et al. (2010) using 16S rRNA gene copy numbers for the southern Mariana Trough chimney samples. Both estimates were nearly equivalent; biomass estimated from TOC and TN amounts was  $\sim 10^8$  cells/g for the artificial chimney samples and  $\sim 10^7$ – $10^8$  cells/g for the natural chimney samples. The estimated values of  $\sim 1.2 \times 10^8$ – $3.2 \times 10^8$  cells/g and  $\sim 1.3 \times 10^8$ – $1.1 \times 10^9$  cells/g were obtained for active and inactive chimneys in the Southern Mariana Trough, respectively. These results are roughly consistent with the range of values estimated from the copy number of 16S rRNA genes (i.e.,  $1.0 \times 10^8$ – $3.8 \times 10^9$  cells/g vs.  $8.2 \times 10^7$ – $9.2 \times 10^8$  cells/g; Kato et al. 2010). Furthermore, it is also consistent with the range of values reported from natural chimneys in various hydrothermally active areas of the Okinawa Trough ( $10^5$ – $10^8$  cells/g; Nakagawa et al., 2005; Nunoura and Takai, 2009; Takai et al., 2015; Miyazaki et al., 2017b). Therefore, although the comparable information is limited, the estimated values of microbial biomass in artificial and natural chimneys of this study are generally considered reasonable. The results suggest that microbial production in the two artificial chimneys in this study was comparable to that in the

surrounding natural chimneys, although the organic carbon and nitrogen in these samples did not necessarily originate entirely from active microbial cells. It was reported that microbial DNA was not amplified from the C0016A and C0016B samples collected 11 and 18 months, respectively, after the drilling and that no detectable microbial communities were developed (Kawagucci et al., 2013). Therefore, within approximately 6 years thereafter, the microbial biomass in these artificial chimneys would have reached approximately the same level as the surrounding mature natural chimneys. Even a rough estimate based on several assumptions, the microbial biomass was quantified for the first time that developed in artificial hydrothermal vents formed by drilling in submarine hydrothermal active areas, giving a framework for the time of period required for the development of microbial communities.

**Table 3.9.** Estimated microbial biomass in artificial and natural chimneys of Iheya North field and compared to the published data from the hydrothermal fields of the Southern Mariana Trough reported by Kato et al. (2010).

| Estimated biomass<br>(cell/g) | Iheya North field, Okinawa Trough     |                                       |                                       |                                       | Southern Mariana Trough               |                                       |
|-------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
|                               | Artificial chimneys                   |                                       | Natural chimneys                      |                                       |                                       |                                       |
|                               | AC-C0016A                             | AC-C0016B                             | NC-HSC                                | NC-HRV                                | Active chimneys                       | Inactive chimneys                     |
| from TOC                      | $1.3 \times 10^8 \pm 3.9 \times 10^6$ | $3.8 \times 10^8 \pm 1.7 \times 10^7$ | $3.4 \times 10^7 \pm 1.2 \times 10^6$ | $3.7 \times 10^8 \pm 8.9 \times 10^6$ | $1.2 \times 10^8$ - $1.3 \times 10^8$ | $1.3 \times 10^8$ - $7.3 \times 10^8$ |
| from TN                       | $2.1 \times 10^8 \pm 5.9 \times 10^6$ | $6.1 \times 10^8 \pm 3.8 \times 10^7$ | $3.5 \times 10^7 \pm 1.3 \times 10^6$ | $5.4 \times 10^8 \pm 1.3 \times 10^7$ | $2.1 \times 10^8$ - $3.7 \times 10^8$ | $2.6 \times 10^8$ - $1.1 \times 10^9$ |
| from 16S rRNA copies          | -                                     | -                                     | -                                     | -                                     | $1.0 \times 10^8$ - $3.8 \times 10^9$ | $8.2 \times 10^7$ - $9.2 \times 10^8$ |
| Reference                     | This study                            | This study                            | This study                            | This study                            | Kato et al. (2010)                    | Kato et al. (2010)                    |

Estimated values of microbial biomass were calculated by conversion from TOC and TN contents in each sample to cell numbers using the averaged carbon and nitrogen contents of natural thermophilic communities (1.35 pg/cells and 0.19 pg/cells, respectively) determined by Kimura et al. (2010). The values and errors for the samples analyzed in this study are presented as means and standard deviations obtained by triplicated measurements of TOC and TN contents.

### 3.4.5. Microbial communities developed in the artificial chimneys

Studies of the microbial colonization process in newly formed chimneys have been based on field experiments and have shown that microorganisms rapidly develop and establish themselves in a short period of time, usually within < 15 days (Reysenbach et al., 2000; Nercessian et al., 2003; McCliment et al., 2006; Pagé et al., 2008; Wang et al., 2009). However, this study of the Hole C0016A and Hole C0016B artificial chimneys revealed that microbial DNA was not amplified from samples collected 11 and 18 months after drilling, respectively, and that detectable microbial communities were not well developed (Kawagucci et al., 2013). In this study, lipid biomarkers of archaea and bacteria were detected in chimney samples (ACC0016A and AC-C0016B) collected from these artificial chimneys 8 years after drilling. The distribution of lipid biomarkers detected indicated that the microbial community structure developed in these two artificial chimneys was distinctly different at the domain level (**Figure 3.9A**). In the AC-C0016A sample, archaea were predominant, and even though there were traces of bacteria present, bacterial biomass does not appear to be sufficient for detection of the IPLs (**Figure 3.9A, B**). In contrast, in the AC-C0016B sample, bacteria are predominant and archaea are minor (~ 1% of total IPL detected) (**Figure 3.9A**).

Previous studies have shown that during chimney development, hyperthermophilic chemosynthetic autotrophic microorganisms (e.g., *Methanocaldococcales*) rapidly colonize and accumulate organic carbon. Then, there is a continuous transition to a heterotrophic community structure (e.g., *Thermococcales*), as the internal environment gets cooling (Reysenbach et al., 2000; McCliment et al., 2006; Pagé et al., 2008). The artificial chimneys of this study have hydrothermal channels (i.e., borehole diameter) of approximately 50 cm in diameter (Kawagucci et al., 2013; Nozaki et al., 2016), which have never been observed in nature. The enormous amount of hydrothermal fluid discharged from the channels may have caused a prolonged period of time before the thermal conditions were favorable for microbial growth. No detectable microbial communities developed in artificial chimneys C0016A and C0016B within at least 18 months of drilling (Kawagucci et al., 2013), even though previous studies have shown that hyperthermophilic microbes begin to colonize at natural chimneys within several days of their deposition (Reysenbach et al., 2000; McCliment et al., 2006; Page et al., 2008).

On the other hand, it is possible that IPLs from older generations of microorganisms that escaped degradation may accumulate in natural chimneys. Harvey et al. (1986) showed that sugar diether lipids of archaea were degraded much more slowly than phosphor-diester lipids of bacteria in culture experiments with organic-rich sediments under aerobic and anaerobic

conditions. In addition, theoretical studies of the degradation rates of archaeal IPLs in deep subsurface sediments (Schouten et al., 2010) and experimental studies using isotopic labeling techniques (Xie et al., 2013) indicate that archaeal glycolipids may be preserved over geologic timescales. The archaeal IPLs detected in the artificial and natural chimneys were indeed predominantly glycolipids (**Table 3.3**). However, it is questionable whether IPLs can be stably preserved for long periods of time in the extreme and dynamic conditions of submarine hydrothermal vent environments, as opposed to the low-temperature and less variable environments of deep-seafloor sediments. The hydrothermal pyrolysis experiments (160-330°C, 72 h) of sediments containing solvent-extracted GDGTs revealed that GDGTs are readily liberated from kerogen between 160-220°C and that GDGTs decreased rapidly between 240-300 °C due to cleavage of ether bonds. Above 300°C, the residual GDGTs were below the detection limit (Schouten et al., 2004).

Furthermore, Bentley et al. (2022) reported a loss of archaeal membrane lipids in hydrothermally active sediments of the Guaymas Basin in the Gulf of California with a magnitude that cannot be explained by diagenesis and thermochemical degradation alone. The loss of archaeal lipids may be due to extreme lipid metabolic turnover by biodegradation and recycling metabolism by heterotrophic organisms. These studies may indicate that even inside of active chimneys, IPLs released from dead cells and CLs of their degradation products do not easily escape thermal degradation or biodegradation. In addition, the polar head sugars have stronger hydrogen-bonding between membrane lipid molecules than those of phosphate esters (Curatolo, 1987; Baba et al., 2001), which are thought to play an important role in maintaining membrane stability and mass transport functions even under extreme conditions of high temperature and low pH (e.g., Wang et al., 2012; Sollich et al., 2017). Culture experiments have shown that *Thermoplasma acidophilum*, a thermophilic- and acidophilic archaea, increases the percentage of glycolipids with increasing growth temperature (Shimada et al., 2008). Glycolipids have been reported to be predominant in archaeal IPLs in both terrestrial and marine hydrothermal environments around the world (e.g., Gibson et al., 2013; Schubotz et al., 2013; Reeves et al., 2014; Sollich et al., 2017). Some microorganisms are also known to synthesize glycolipids and aminolipids instead of phospholipids when phosphorus is depleted, and the phosphate concentrations in the hydrothermal plume of the Iheya North field have been reported to be lower than in the surrounding seawater (Okamura et al., 2013).

**Figure 3.14.** shows a schematic image of microbial community development in artificial chimneys. Microbial community development in deep-sea hydrothermal chimneys is undoubtedly influenced by physical conditions such as temperature and hydrothermal flow rate,

as well as chemical conditions such as nutrients used by the microbes. It was an achievement to show that microbial community development can take as long as 18 months during the formation of a new artificial chimney in this study. As shown in **Figure 3.14**, the microbial community developed in the artificial chimney is considered to have colonized and established *via* (1) the hydrothermal plume, (2) the surrounding seawater, and (3) the vent faunal community under the upper temperature limit for existence of life and biomolecules ( $\sim 150^{\circ}\text{C}$ , White, 1984; Merino et al., 2019).

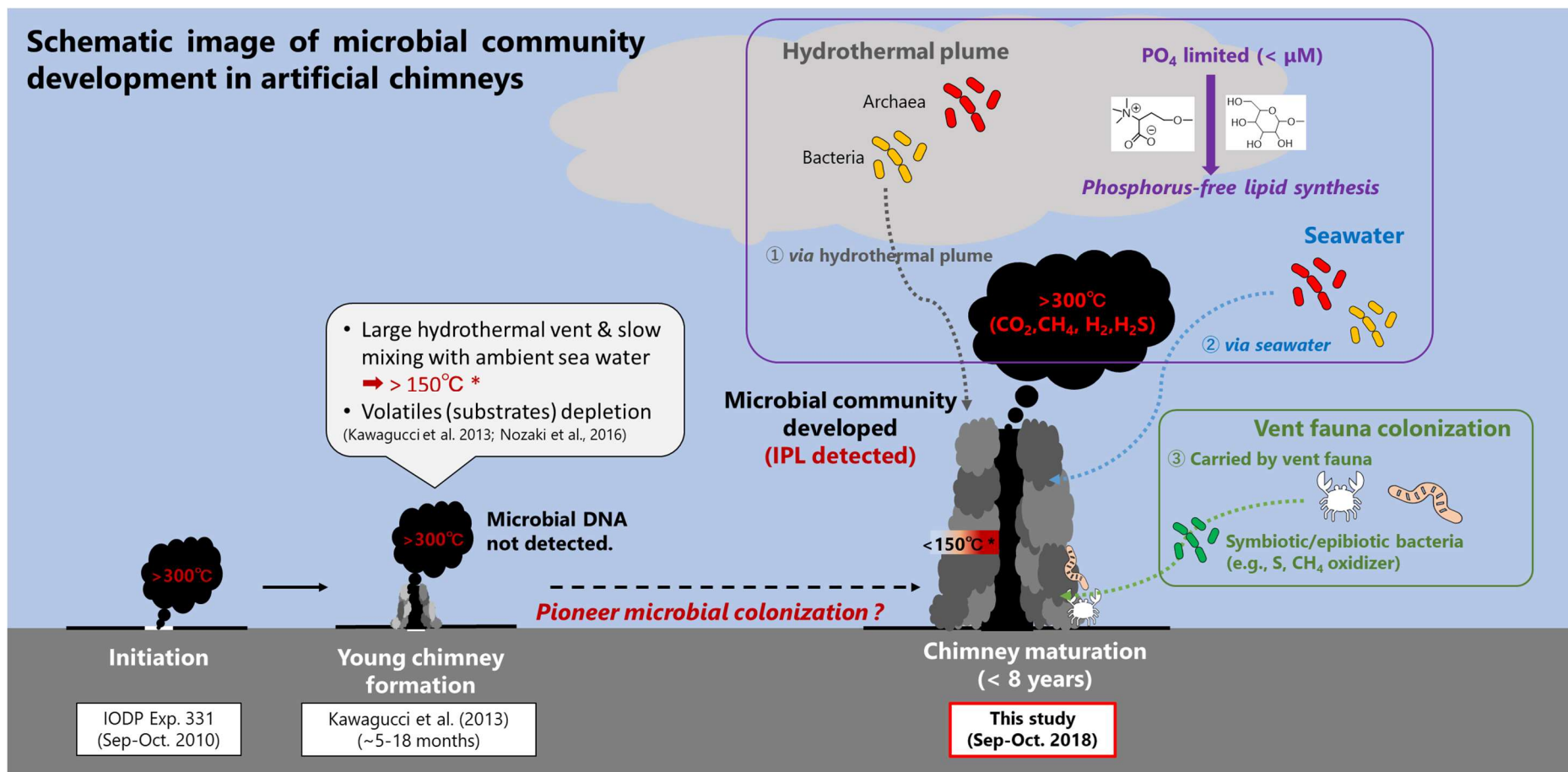


**Figure 3.14.** A schematic image of microbial community development in artificial chimneys.

\* Theoretical upper limit temperature for existence of life and biomolecules (White, 1984; Merino et al., 2019).

## Chapter 4. General conclusion

This dissertation focused on the study of microbial communities developed in two artificial hydrothermal chimney structures formed by deep-sea drilling operations in the Iheya North hydrothermal field of the mid-Okinawa Trough. Although previous studies dedicated to the colonization processes in hydrothermal chimneys based on *in situ* culture experiments at the several hydrothermal vent fields have indicated that microorganisms start colonization at the initially sterile chimney structures quickly, and get established within a short period of time (< 14 days, Reysenbach et al., 2000; McCliment et al., 2006; Páge et al., 2008; Wang et al., 2009), the previous gene analysis of the artificial chimneys 11 and 18 months after drillings indicated that the detectable microbial communities were not well developed (Kawagucci et al., 2013). To date, it remains unknown microbial community structures, biomass and activities associated with those artificial chimneys. In this study, the simultaneous analysis of intact polar and core lipid biomarkers, organic elements and stable carbon isotope analyses as well as mineralogical analysis were integrated to characterize microbial communities developed in the artificial chimney structures 8 years after the drilling and to clarify the microbial development process in the chimney structures.

At first, a new analytical method of the simultaneous analysis for intact polar and core lipids in total lipid extracts was developed for various biological and natural samples using the NP-HPLC/HESI-HRMS during this work (Chapter 2). The simultaneous analysis enables to the detection of a wide range of lipid biomarkers in complex mixtures of samples because the chromatographic separation pattern of core and polar lipids is comparable to the previous normal-phase HPLC methods for each form of lipids (Schouten et al., 2007; Sturt et al., 2004). Using chemical formula determined by the exact mass (Horai et al., 2019), the complex sample preparation other than lipid extraction is not required. Therefore, the sample loss and fractionation bias are minimized during the pretreatment. In addition, the chromatographic separation can be applied to fractionation for compound-specific isotopic analysis of lipid biomarkers.

Lipid biomarkers of microbial communities developed in artificial chimney structures of the Iheya North field were studied by using the simultaneous analytical method (Chapter 3). The numerous numbers of archaeal and non-archaeal polar and core lipids were detected in artificial chimneys, natural chimneys and reference samples collected from the Iheya North field. The IPL composition showed that *Archaea* were dominant in the Hole C0016A artificial chimney, whereas *Bacteria* were predominant and *Archaea* minor in the Hole C0016B artificial chimney. The distribution of IPLs derived from *Archaea* in Hole C0016A artificial chimney

was in good agreement with natural chimney sample NC-HSC, which was mainly enriched in HGDGTs-IPLs, a biomarker for thermophilic archaea. On the other hand, the biomarker composition of Hole C0016B artificial chimney was relatively similar to that of natural chimney sample NC-HRV, with AR-IPLs and MAR-IPLs and OHAR-IPLs as major archaeal IPLs, suggesting an activity of methanogenic archaea. In the reference samples, IPLs and CLs derived from *Archaea* were detected in trace amounts compared to artificial and natural chimneys, despite their higher TOC concentrations. The observation suggests that archaeal lipids in artificial and natural chimneys were primarily biosynthesized in the *in situ* environment. The distribution of non-archaeal IPLs was also relatively similar between artificial chimney AC-C0016B and natural chimney NC-HRV samples and being more diverse than natural chimney sample NC-HSC and reference samples.

The bulk organic matter composition of the artificial and natural chimneys was clearly distinguishable from the reference samples due to their low TOC and TN contents, low TOC/TN ratio, and relatively high  $\delta^{13}\text{C}_{\text{TOC}}$  values, suggesting that the contribution from sedimentary organic matter in the bulk organic matter of the artificial and natural chimneys is negligible. Non archaeal-IPLs were detected in the reference samples, but non-archaeal CLs were not detected, suggesting active bacterial degradation and/or alteration of organic matter in association with a lower TOC carbon isotopic composition than normal marine sedimentary organic matters. In addition, the positive correlation between TOC and TN contents and TOC/TN atomic ratios ( $\sim 5.5$ ) of artificial and natural chimneys indicates that organic matters in those chimney samples are mainly originated from microbial cell materials. Therefore, the TOC and TN contents of the artificial and natural chimneys were used to calculate microbial biomass using the cellular carbon and nitrogen contents from the natural thermophilic microbial communities (Kimura et al., 2010). The estimated biomass was approximately equal between the artificial and natural chimneys ( $\sim 10^8$  cells/g and  $\sim 10^7$ - $10^8$  cells/g, respectively). Taken together, these results suggest that the two artificial chimneys, eight years after drilling, have microbial biomass that is nearly equal to that of the surrounding natural chimneys, and that archaea and/or bacteria communities have developed well to share similar IPLs compositions. Although these results are rough estimate based on several assumptions and a limited number of samples, they provided one indication of the timescale required for microbial community maturation in artificially formed chimney structures resulting from large-scale deep-sea drilling.

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## **Appendix**

The appendix summarizes the details of all lipid biomarkers observed in artificial chimneys, natural chimneys and reference samples collected from the Iheya North field. These compounds have been identified and discussed in Chapter 3. For their typical chemical structures see **Figure 3.8**. The appendix consists of four tables as follow:

**Table A3.1.** Archaeal IPLs

**Table A3.2.** Archaeal CLs

**Table A3.3.** Non-archaeal IPLs

**Table A3.4.** Non-archaeal CLs

**Appendix Table A3.1.** Detail information of all archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field.

**(1) Artificial chimney sample: AC-C0016A**

| Compounds  | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1G-AR      | 66.6        | C49H99O8          | M+H     | 815.7355        | 815.7334         | 2.6            | 4.E+12             |
|            |             | C49H102NO8        | M+NH4   | 832.7624        | 832.7600         | 2.9            |                    |
|            |             | C49H98O8Na        | M+Na    | 837.7152        | 837.7154         | -0.2           |                    |
|            |             | C49H98O8K         | M+K     | 853.6895        | 853.6893         | 0.2            |                    |
| 1G-MAR     | 70.3        | C49H97O8          | M+H     | 813.7193        | 813.7178         | 1.8            | 5.E+11             |
|            |             | C49H100NO8        | M+NH4   | 830.7461        | 830.7443         | 2.1            |                    |
|            |             | C49H96O8Na        | M+Na    | 835.7008        | 835.6997         | 1.3            |                    |
|            |             | C49H96O8K         | M+K     | 851.6742        | 851.6737         | 0.6            |                    |
| 1G-GDGT-0  | 69.0        | C92H183O11        | M+H     | 1464.3762       | 1464.3755        | 0.5            | 3.E+11             |
|            |             | C92H186NO11       | M+NH4   | 1481.4027       | 1481.4020        | 0.5            |                    |
|            |             | C92H182O11Na      | M+Na    | 1486.3579       | 1486.3574        | 0.3            |                    |
|            |             | C92H182O11K       | M+K     | 1502.3308       | 1502.3308        | -0.4           |                    |
| 1G-GDGT-1  | 69.3        | C92H184ON11       | M+NH4   | 1479.3845       | 1479.3864        | -1.3           | 9.E+09             |
|            |             | C92H180O11Na      | M+Na    | 1484.3396       | 1484.3418        | -1.5           |                    |
| 1G-GDGT-2  | 69.6        | C92H182NO11       | M+NH4   | 1477.3685       | 1477.3707        | -1.5           | 4.E+09             |
|            |             | C92H178O11Na      | M+Na    | 1482.3268       | 1482.3261        | 0.4            |                    |
| 1G-GDGT-3  | 70.0        | C92H180NO11       | M+NH4   | 1475.3552       | 1475.3551        | 0.1            | 5.E+09             |
|            |             | C92H176O11Na      | M+Na    | 1480.3102       | 1480.3105        | -0.2           |                    |
| 1G-GDGT-4  | 70.5        | C92H175O11        | M+H     | 1456.3135       | 1456.3129        | 0.4            | 3.E+10             |
|            |             | C92H178NO11       | M+NH4   | 1473.3392       | 1473.3394        | -0.1           |                    |
|            |             | C92H174O11Na      | M+Na    | 1478.2944       | 1478.2948        | -0.3           |                    |
|            |             | C92H174O11K       | M+K     | 1494.2676       | 1494.2688        | -0.8           |                    |
| 1G-GDGT-5  | 70.7        | C92H176NO11       | M+NH4   | 1471.3228       | 1471.3238        | -0.7           | 2.E+09             |
|            |             | C92H172O11Na      | M+Na    | 1476.2787       | 1476.2792        | -0.3           |                    |
| 1G-HGDGT-0 | 71.3        | C92H181O11        | M+H     | 1460.3600       | 1462.3598        | 0.1            | 2.E+12             |
|            |             | C92H184ON11       | M+NH4   | 1479.3875       | 1479.3864        | 0.7            |                    |
|            |             | C92H180O11Na      | M+Na    | 1484.3406       | 1484.3418        | -0.8           |                    |
|            |             | C92H180O11K       | M+K     | 1500.3157       | 1500.3157        | -0.3           |                    |
| 1G-HGDGT-1 | 71.5        | C92H179O11        | M+H     | 1460.3385       | 1460.3442        | -3.9           | 4.E+11             |
|            |             | C92H182NO11       | M+NH4   | 1477.3716       | 1477.3707        | 0.6            |                    |
|            |             | C92H178O11Na      | M+Na    | 1482.3250       | 1482.3261        | -0.8           |                    |
|            |             | C92H178O11K       | M+K     | 1498.3011       | 1498.3001        | 0.7            |                    |
| 1G-HGDGT-2 | 71.7        | C92H177O11        | M+H     | 1458.3298       | 1458.3285        | 0.9            | 3.E+11             |
|            |             | C92H180NO11       | M+NH4   | 1475.3561       | 1475.3551        | 0.7            |                    |
|            |             | C92H176O11Na      | M+Na    | 1480.3087       | 1480.3105        | -1.2           |                    |
|            |             | C92H176O11K       | M+K     | 1496.2839       | 1496.2844        | -0.3           |                    |
| 1G-HGDGT-3 | 72.0        | C92H175O11        | M+H     | 1456.3115       | 1456.3129        | 0.7            | 2.E+11             |
|            |             | C92H178NO11       | M+NH4   | 1473.3405       | 1473.3394        | 0.7            |                    |
|            |             | C92H174O11Na      | M+Na    | 1478.2952       | 1478.2948        | 0.2            |                    |
|            |             | C92H174O11K       | M+K     | 1494.2683       | 1494.2688        | -0.3           |                    |
| 1G-HGDGT-4 | 72.5        | C92H173O11        | M+H     | 1454.2949       | 1454.2972        | -1.6           | 2.E+11             |
|            |             | C92H176NO11       | M+NH4   | 1471.3252       | 1471.3238        | 1.0            |                    |
|            |             | C92H172O11Na      | M+Na    | 1476.2798       | 1476.2792        | 0.4            |                    |
|            |             | C92H172O11K       | M+K     | 1492.2538       | 1492.2531        | 0.4            |                    |

(Continued to next page)

**Appendix Table A3.1.** Detail information of all archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (Continued).

**(1) Artificial chimney sample: AC-C0016A**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1G-1Me-HGDGT-4 | 72.5        | C93H175O11        | M+H     | 1468.3129       | 1468.3129        | 0.0            | 2.E+11             |
|                |             | C93H178NO11       | M+NH4   | 1485.3405       | 1485.3394        | 0.7            |                    |
|                |             | C93H174O11Na      | M+Na    | 1490.2955       | 1490.2948        | 0.4            |                    |
|                |             | C93H174O11K       | M+K     | 1506.2677       | 1458.3196        | -0.7           |                    |
| 1G-2Me-HGDGT-4 | 72.4        | C94H177O11        | M+H     | 1482.3251       | 1482.3285        | -2.3           | 8.E+10             |
|                |             | C94H180NO11       | M+NH4   | 1499.3558       | 1499.3551        | 0.5            |                    |
|                |             | C94H176O11Na      | M+Na    | 1504.3107       | 1504.3105        | 0.1            |                    |
|                |             | C94H176O11K       | M+K     | 1520.2842       | 1520.3844        | -0.2           |                    |

**(2) Artificial chimney sample: AC-C0016B**

|                |      |               |       |           |           |      |        |
|----------------|------|---------------|-------|-----------|-----------|------|--------|
| 1G-AR          | 67.5 | C49H102NO8    | M+NH4 | 832.7610  | 832.7600  | 1.3  | 1.E+11 |
|                |      | C49H98O8Na    | M+Na  | 837.7157  | 837.7154  | 0.4  |        |
|                |      | C49H98O8K     | M+K   | 853.6886  | 853.6893  | -0.9 |        |
| 1G-OHAR        | 69.9 | C49H102NO9    | M+NH4 | 848.7552  | 848.7549  | 0.3  | 2.E+11 |
|                |      | C49H98O9Na    | M+Na  | 853.7105  | 853.7103  | 0.2  |        |
|                |      | C49H98O9K     | M+K   | 869.6840  | 869.6842  | -0.3 |        |
| 1G-MAR         | 70.6 | C49H100NO8    | M+NH4 | 830.7454  | 830.7443  | 1.3  | 4.E+10 |
|                |      | C49H96O8Na    | M+Na  | 835.7007  | 835.6997  | 1.1  |        |
|                |      | C49H96O8K     | M+K   | 851.6730  | 851.6737  | -0.8 |        |
| 1G-GDGT-0      | 69.0 | C92H183O11    | M+H   | 1464.3770 | 1464.3755 | 1.0  | 2.E+10 |
|                |      | C92H186NO11   | M+NH4 | 1481.4016 | 1481.4020 | -0.3 |        |
|                |      | C92H182O11Na  | M+Na  | 1486.3562 | 1486.3574 | -0.8 |        |
|                |      | C92H182O11K   | M+K   | 1502.3329 | 1502.3308 | 1.0  |        |
| 1G-GDGT-1      | 69.4 | C92H184ON11   | M+NH4 | 1479.3845 | 1479.3864 | -1.3 | 4.E+09 |
|                |      | C92H180O11Na  | M+Na  | 1484.3391 | 1484.3418 | -1.8 |        |
| 1G-GDGT-2      | 69.5 | C92H182NO11   | M+NH4 | 1477.3707 | 1477.3707 | 0.0  | 3.E+09 |
|                |      | C92H178O11Na  | M+Na  | 1482.3226 | 1482.3261 | 1.6  |        |
| 1G-GDGT-5      | 70.7 | C92H176NO11   | M+NH4 | 1471.3243 | 1471.3238 | 0.4  | 4.E+09 |
|                |      | C92H172O11Na  | M+Na  | 1476.2798 | 1476.2792 | 0.4  |        |
| 1G-HGDGT-0     | 71.4 | C92H184ON11   | M+NH4 | 1479.3843 | 1479.3864 | -1.4 | 1.E+10 |
|                |      | C92H180O11Na  | M+Na  | 1484.3400 | 1484.3418 | -1.2 |        |
| 1G-HGDGT-1     | 71.7 | C92H182NO11   | M+NH4 | 1477.3689 | 1477.3707 | -1.2 | 2.E+10 |
|                |      | C92H178O11Na  | M+Na  | 1482.3217 | 1482.3261 | -3.0 |        |
| 1G-HGDGT-4     | 72.7 | C92H176NO11   | M+NH4 | 1471.3195 | 1471.3238 | -2.9 | 2.E+09 |
|                |      | C92H172O11Na  | M+Na  | 1476.2782 | 1476.2792 | -0.7 |        |
| 1G-1Me-HGDGT-4 | 72.6 | C93H178NO11   | M+NH4 | 1485.3422 | 1485.3394 | 1.8  | 2.E+09 |
|                |      | C93H174O11Na  | M+Na  | 1490.2909 | 1490.2948 | -2.6 |        |
| 1G-2Me-HGDGT-4 | 72.6 | C94H180NO11   | M+NH4 | 1499.3530 | 1499.3551 | -1.4 | 5.E+09 |
|                |      | C94H176O11Na  | M+Na  | 1504.3104 | 1504.3105 | -1.1 |        |
| 2G-GDGT-0      | 80.8 | C98H196NO16   | M+NH4 | 1643.4552 | 1643.4549 | 0.2  | 3.E+09 |
|                |      | C98H192NO16Na | M+Na  | 1648.4070 | 1648.4103 | -2.0 |        |

(Continued to next page)

**Appendix Table A3.1.** Detail information of all archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (Continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1G-AR          | 66.4        | C49H99O8          | M+H     | 815.7350        | 815.7334         | 2.0            | 6.E+12             |
|                |             | C49H102NO8        | M+NH4   | 832.7618        | 832.7600         | 2.2            |                    |
|                |             | C49H98O8Na        | M+Na    | 837.7158        | 837.7154         | 0.4            |                    |
|                |             | C49H98O8K         | M+K     | 853.6898        | 853.6893         | 0.5            |                    |
| 2G-AR          | 80.8        | C55H112NO13       | M+NH4   | 994.8133        | 994.8128         | 0.5            | 1.E+12             |
|                |             | C55H108O13Na      | M+Na    | 999.7681        | 999.7682         | -0.1           |                    |
| PE-AR          | 81.2        | C45H95NO6P        | M+H     | 776.6896        | 776.6892         | 0.6            | 6.E+10             |
| 1G-MAR         | 70.2        | C49H100NO8        | M+NH4   | 830.7455        | 830.7443         | 1.4            | 5.E+11             |
|                |             | C49H96O8Na        | M+Na    | 835.7006        | 835.6997         | 1.0            |                    |
|                |             | C49H96O8K         | M+K     | 851.6735        | 851.6737         | -0.3           |                    |
| 2G-MAR         | 84.8        | C55H110NO13       | M+NH4   | 992.7975        | 992.7972         | 0.4            | 5.E+10             |
|                |             | C55H106O13Na      | M+Na    | 997.7534        | 997.7526         | 0.8            |                    |
| PE-MAR         | 86.6        | C45H93NO6P        | M+H     | 774.6747        | 774.6735         | 1.5            | 2.E+10             |
| 1G-GDGT-0      | 68.8        | C92H186NO11       | M+NH4   | 1481.4020       | 1481.4020        | 0.0            | 4.E+11             |
|                |             | C92H182O11Na      | M+Na    | 1486.3583       | 1486.3574        | 0.6            |                    |
|                |             | C92H182O11K       | M+K     | 1502.3308       | 1502.3308        | -0.4           |                    |
| 1G-GDGT-1      | 69.2        | C92H184ON11       | M+NH4   | 1479.3877       | 1479.3864        | 0.9            | 3.E+10             |
|                |             | C92H180O11Na      | M+Na    | 1484.3411       | 1484.3418        | -0.5           |                    |
| 1G-GDGT-2      | 69.5        | C92H182NO11       | M+NH4   | 1477.3707       | 1477.3707        | 0.0            | 3.E+10             |
|                |             | C92H178O11Na      | M+Na    | 1482.3197       | 1482.3261        | -4.3           |                    |
| 1G-GDGT-3      | 69.8        | C92H180NO11       | M+NH4   | 1475.3542       | 1475.3551        | -0.6           | 7.E+10             |
|                |             | C92H176O11Na      | M+Na    | 1480.3118       | 1480.3105        | 0.9            |                    |
| 1G-GDGT-4      | 70.3        | C92H175O11        | M+H     | 1456.3125       | 1456.3129        | -0.3           | 2.E+11             |
|                |             | C92H178NO11       | M+NH4   | 1473.3395       | 1473.3394        | 0.0            | 0.E+00             |
|                |             | C92H174O11Na      | M+Na    | 1478.2938       | 1478.2948        | -0.7           | 0.E+00             |
|                |             | C92H174O11K       | M+K     | 1494.2700       | 1494.2688        | 0.8            |                    |
| 1G-HGDGT-0     | 71.2        | C92H181O11        | M+H     | 1462.3572       | 1462.3598        | -1.8           | 7.E+12             |
|                |             | C92H184ON11       | M+NH4   | 1479.3873       | 1479.3864        | 0.6            |                    |
|                |             | C92H180O11Na      | M+Na    | 1484.3413       | 1484.3418        | -0.3           |                    |
|                |             | C92H180O11K       | M+K     | 1500.3143       | 1500.3157        | -0.9           |                    |
| 1G-HGDGT-1     | 71.4        | C92H179O11        | M+H     | 1460.3440       | 1460.3442        | -0.1           | 6.E+11             |
|                |             | C92H182NO11       | M+NH4   | 1477.3715       | 1477.3707        | 0.5            |                    |
|                |             | C92H178O11Na      | M+Na    | 1482.3260       | 1482.3261        | -0.1           |                    |
|                |             | C92H178O11K       | M+K     | 1498.2985       | 1498.3001        | -1.1           |                    |
| 1G-HGDGT-2     | 71.8        | C92H177O11        | M+H     | 1458.3261       | 1458.3285        | -0.9           | 3.E+12             |
|                |             | C92H180NO11       | M+NH4   | 1475.3557       | 1475.3551        | 0.4            |                    |
|                |             | C92H176O11Na      | M+Na    | 1480.3107       | 1480.3105        | 0.1            |                    |
|                |             | C92H176O11K       | M+K     | 1496.2855       | 1496.2844        | 0.7            |                    |
| 1G-HGDGT-3     | 72.0        | C92H178NO11       | M+NH4   | 1473.3392       | 1473.3394        | -0.1           | 2.E+12             |
|                |             | C92H174O11Na      | M+Na    | 1478.2947       | 1478.2948        | -0.1           |                    |
|                |             | C92H174O11K       | M+K     | 1494.2708       | 1494.2688        | 1.3            |                    |
| 1G-HGDGT-4     | 72.4        | C92H173O11        | M+H     | 1454.2941       | 1454.2972        | -2.2           | 5.E+11             |
|                |             | C92H176NO11       | M+NH4   | 1471.3240       | 1471.3238        | 0.1            |                    |
|                |             | C92H172O11Na      | M+Na    | 1476.2783       | 1476.2792        | -0.6           |                    |
|                |             | C92H172O11K       | M+K     | 1492.2531       | 1492.2531        | 0.0            |                    |
| 1G-1Me-HGDGT-3 | 72.0        | C93H180NO11       | M+NH4   | 1487.3566       | 1487.3551        | 1.0            | 4.E+11             |
|                |             | C93H176O11Na      | M+Na    | 1492.3109       | 1492.3105        | 0.3            |                    |

(Continued to next page)

**Appendix Table A3.1.** Detail information of all archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (Continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1G-1Me-HGDGT-4 | 72.4        | C93H175O11        | M+H     | 1468.3135       | 1468.3129        | 0.4            | 2.E+11             |
|                |             | C93H178NO11       | M+NH4   | 1485.3402       | 1485.3394        | 0.5            |                    |
|                |             | C93H174O11Na      | M+Na    | 1490.2953       | 1490.2948        | 0.3            |                    |
|                |             | C93H174O11K       | M+K     | 1506.2679       | 1458.3196        | -0.6           |                    |
| 1G-2Me-HGDGT-4 | 72.4        | C94H180NO11       | M+NH4   | 1499.3569       | 1499.3551        | 1.2            | 7.E+11             |
|                |             | C94H176O11Na      | M+Na    | 1504.3102       | 1504.3105        | -0.2           |                    |
| 2G-HGDGT-0     | 83.2        | C98H194NO16       | M+NH4   | 1641.4381       | 1641.4392        | -0.7           | 2.E+11             |
|                |             | C98H190O16Na      | M+Na    | 1646.3899       | 1646.3946        | -2.9           |                    |

**(4) Natural chimney sample: NC-HRV**

|           |      |              |       |           |           |      |        |
|-----------|------|--------------|-------|-----------|-----------|------|--------|
| 1G-AR     | 67.4 | C49H102NO8   | M+NH4 | 832.7612  | 832.7600  | 1.5  | 1.E+11 |
|           |      | C49H98O8Na   | M+Na  | 837.7155  | 837.7154  | 0.1  |        |
|           |      | C49H98O8K    | M+K   | 853.6888  | 853.6893  | -0.6 |        |
| 2G-AR     | 77.3 | C55H109O13   | M+H   | 977.7863  | 977.7863  | 0.0  | 3.E+11 |
|           |      | C55H112NO13  | M+NH4 | 994.8135  | 994.8128  | 0.7  |        |
|           |      | C55H108O13Na | M+Na  | 999.7681  | 999.7682  | -0.1 |        |
|           |      | C55H108O13K  | M+K   | 1015.7410 | 1015.7422 | -1.1 |        |
| PG-AR     | 76.5 | C46H96O8P    | M+H   | 807.6848  | 807.6837  | 1.3  | 1.E+11 |
| PE-AR     | 81.2 | C45H95NO6P   | M+H   | 776.6899  | 776.6892  | 0.9  | 2.E+11 |
|           |      | C45H94NO6PNa | M+Na  | 798.6732  | 798.6711  | 2.6  |        |
| PI-AR     | 94.4 | C49H100O11P  | M+H   | 895.7006  | 895.6998  | 0.9  | 5.E+10 |
| 1G-OHAR   | 69.7 | C49H99O9     | M+H   | 831.7283  | 831.7284  | -0.1 | 1.E+11 |
|           |      | C49H102NO9   | M+NH4 | 848.7556  | 848.7549  | 0.8  |        |
|           |      | C49H98O9Na   | M+Na  | 853.7104  | 853.7103  | 0.2  |        |
|           |      | C49H98O9K    | M+K   | 869.6839  | 869.6842  | -0.4 |        |
| 2G-OHAR   | 78.8 | C55H109O14   | M+H   | 993.7808  | 993.7812  | -0.4 | 2.E+10 |
|           |      | C55H108O14Na | M+Na  | 1015.7672 | 1015.7631 | -0.4 |        |
| PG-OHAR   | 78.6 | C46H96O9P    | M+H   | 823.6793  | 823.6786  | 0.7  | 2.E+11 |
|           |      | C46H95O9PNa  | M+Na  | 845.6599  | 845.6606  | -0.9 |        |
| PE-OHAR   | 84.3 | C45H95NO7P   | M+H   | 792.6851  | 792.6841  | 1.3  | 2.E+10 |
| 1G-MAR    | 70.5 | C49H100NO8   | M+NH4 | 830.7452  | 830.7443  | 1.1  | 2.E+11 |
|           |      | C49H96O8Na   | M+Na  | 835.7000  | 835.6997  | 0.3  |        |
|           |      | C49H96O8K    | M+K   | 851.6722  | 851.6737  | -1.7 |        |
| 2G-MAR    | 83.2 | C55H110NO13  | M+NH4 | 992.7974  | 992.7972  | 0.2  | 1.E+11 |
|           |      | C55H106O13Na | M+Na  | 997.7505  | 997.7526  | -2.0 |        |
| 1G-GDGT-0 | 68.9 | C92H183O11   | M+H   | 1464.3755 | 1464.3755 | -4.0 | 2.E+10 |
|           |      | C92H186NO11  | M+NH4 | 1481.3992 | 1481.4020 | -1.9 |        |
|           |      | C92H182O11Na | M+Na  | 1486.3508 | 1486.3574 | -4.4 |        |
|           |      | C92H182O11K  | M+K   | 1502.3260 | 1502.3308 | -3.5 |        |
| 1G-GDGT-1 | 69.2 | C92H184ON11  | M+NH4 | 1479.3794 | 1479.3864 | -4.7 | 5.E+09 |
|           |      | C92H180O11Na | M+Na  | 1484.3384 | 1484.3418 | -2.3 |        |
| 1G-GDGT-2 | 69.6 | C92H182NO11  | M+NH4 | 1477.3693 | 1477.3707 | -1.0 | 6.E+09 |
|           |      | C92H178O11Na | M+Na  | 1482.3218 | 1482.3261 | -2.9 |        |
| 1G-GDGT-3 | 69.9 | C92H180NO11  | M+NH4 | 1475.3553 | 1475.3551 | 0.2  | 9.E+09 |
|           |      | C92H176O11Na | M+Na  | 1480.3063 | 1480.3105 | -2.8 |        |

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**Appendix Table A3.1.** Detail information of all archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (Continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1G-GDGT-4      | 70.4        | C92H178NO11       | M+NH4   | 1456.3134       | 1473.3394        | 0.3            | 3.E+09             |
|                |             | C92H174O11Na      | M+Na    | 1473.3339       | 1478.2948        | -3.8           |                    |
|                |             | C92H174O11K       | M+K     | 1478.2888       | 1494.2688        | -4.1           |                    |
| 1G-GDGT-5      | 70.5        | C92H176NO11       | M+NH4   | 1471.3198       | 1471.3238        | -2.7           | 3.E+09             |
|                |             | C92H172O11Na      | M+Na    | 1476.2743       | 1476.2792        | -3.3           |                    |
| 1G-HGDGT-0     | 71.4        | C92H184ON11       | M+NH4   | 1479.3843       | 1479.3864        | -1.4           | 8.E+08             |
|                |             | C92H180O11Na      | M+Na    | 1484.3400       | 1484.3418        | -1.2           |                    |
| 1G-HGDGT-4     | 71.4        | C92H176NO11       | M+NH4   | 1471.3213       | 1471.3238        | -1.7           | 2.E+10             |
|                |             | C92H172O11Na      | M+Na    | 1476.2761       | 1476.2792        | -2.1           |                    |
| 1G-2Me-HGDGT-4 | 72.4        | C94H180NO11       | M+NH4   | 1499.3522       | 1499.3551        | -1.9           | 2.E+09             |
|                |             | C94H176O11Na      | M+Na    | 1504.3105       | 1504.3105        | 0.0            |                    |
| 2G-GDGT-0      | 80.8        | C98H196NO16       | M+NH4   | 1643.4552       | 1643.4549        | 0.2            | 1.E+09             |
|                |             | C98H192NO16Na     | M+Na    | 1648.4070       | 1648.4103        | -2.0           |                    |

**(5) Reference sample:RF-SO**

|            |      |              |       |           |           |      |        |
|------------|------|--------------|-------|-----------|-----------|------|--------|
| 1G-AR      | 66.6 | C49H102NO8   | M+NH4 | 832.7590  | 832.7600  | -1.2 | 1.E+10 |
|            |      | C49H98O8Na   | M+Na  | 837.7141  | 837.7154  | -1.5 |        |
|            |      | C49H98O8K    | M+K   | 853.6874  | 853.6893  | -2.3 |        |
| 1G-GDGT-0  | 69.0 | C92H186NO11  | M+NH4 | 1481.4003 | 1481.4020 | -1.2 | 1.E+09 |
|            |      | C92H182O11Na | M+Na  | 1486.3556 | 1486.3574 | -1.2 |        |
| 1G-GDGT-2  | 69.6 | C92H182NO11  | M+NH4 | 1477.3708 | 1477.3707 | 0.1  | 1.E+08 |
| 1G-GDGT-3  | 70.1 | C92H180NO11  | M+NH4 | 1475.3527 | 1475.3551 | -1.6 | 2.E+08 |
| 1G-GDGT-4  | 70.6 | C92H175O11   | M+H   | 1456.3101 | 1456.3129 | -1.9 | 1.E+09 |
|            |      | C92H178NO11  | M+NH4 | 1473.3381 | 1473.3394 | -0.9 |        |
|            |      | C92H174O11Na | M+Na  | 1478.2919 | 1478.2948 | -2.0 |        |
| 1G-GDGT-5  | 70.6 | C92H176NO11  | M+NH4 | 1471.3175 | 1471.3238 | -4.3 | 6.E+07 |
| 1G-HGDGT-0 | 71.3 | C92H184NO11  | M+NH4 | 1479.3850 | 1479.3864 | -0.9 | 4.E+09 |
|            |      | C92H180O11Na | M+Na  | 1484.3367 | 1484.3418 | -3.4 |        |
| 1G-HGDGT-1 | 71.4 | C92H182NO11  | M+NH4 | 1477.3700 | 1477.3707 | -0.5 | 6.E+08 |
| 1G-HGDGT-2 | 71.8 | C92H180NO11  | M+NH4 | 1475.3505 | 1475.3551 | -3.1 | 8.E+08 |
| 1G-HGDGT-3 | 72.0 | C92H178NO11  | M+NH4 | 1473.3374 | 1473.3394 | -1.4 | 5.E+08 |
| 1G-HGDGT-4 | 72.6 | C92H176NO11  | M+NH4 | 1471.3180 | 1471.3238 | -3.9 | 6.E+08 |

**(6) Reference sample:RF-AS**

Not detected.

**Appendix Table A3.2.** Detail information of all archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field.

**(1) Artificial chimney sample: AC-C0016A**

| Compounds   | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| AR          | 7.3         | C43H89O3          | M+H     | 653.6829        | 653.6806         | 3.5            | 2.E+13             |
| MAR         | 10.7        | C43H87O3          | M+H     | 651.6671        | 651.6650         | 3.3            | 4.E+12             |
| GTGT-0      | 16.5        | C86H175O6         | M+H     | 1304.3390       | 1304.3383        | 0.5            | 2.E+11             |
| GDGT-0      | 33.8        | C86H173O6         | M+H     | 1302.3235       | 1302.3227        | 0.6            | 1.E+13             |
| GDGT-1      | 35.5        | C86H171O6         | M+H     | 1300.3076       | 1300.3070        | 0.5            | 6.E+11             |
| GDGT-2      | 37.4        | C86H169O6         | M+H     | 1298.2921       | 1298.2914        | 0.6            | 5.E+11             |
| GDGT-3      | 39.5        | C86H167O6         | M+H     | 1296.2765       | 1296.2757        | 0.6            | 4.E+11             |
| GDGT-4      | 42.0        | C86H165O6         | M+H     | 1294.2607       | 1294.2601        | 0.5            | 9.E+11             |
| GDGT-5      | 42.6        | C86H163O6         | M+H     | 1292.2451       | 1292.2444        | 0.5            | 4.E+10             |
| HGDGT-0     | 44.8        | C86H171O6         | M+H     | 1300.3077       | 1300.3070        | 0.6            | 2.E+13             |
| HGDGT-0'    | 47.3        | C86H171O6         | M+H     | 1300.3076       | 1300.3070        | 0.5            |                    |
| HGDGT-1     | 46.3        | C86H169O6         | M+H     | 1298.2626       | 1298.2914        | 0.9            | 5.E+12             |
| HGDGT-1     | 48.5        | C86H169O6         | M+H     | 1298.2914       | 1298.2914        | 0.0            |                    |
| HGDGT-2     | 50.0        | C86H167O6         | M+H     | 1296.2766       | 1296.2757        | 0.7            | 4.E+12             |
| HGDGT-2     | 51.0        | C86H167O6         | M+H     | 1296.2761       | 1296.2757        | 0.3            |                    |
| HGDGT-3     | 51.4        | C86H165O6         | M+H     | 1294.2612       | 1294.2601        | 0.9            | 2.E+12             |
| HGDGT-3     | 52.9        | C86H165O6         | M+H     | 1294.2609       | 1294.2601        | 0.6            |                    |
| HGDGT-4     | 55.2        | C86H163O6         | M+H     | 1292.2454       | 1292.2444        | 0.7            | 1.E+12             |
| HGDGT-4     | 57.9        | C86H163O6         | M+H     | 1292.2452       | 1292.2444        | 0.6            |                    |
| 1Me-HGDGT-0 | 46.6        | C87H173O6         | M+H     | 1314.3225       | 1314.3227        | -0.1           | 3.E+11             |
| 1Me-HGDGT-1 | 49.3        | C87H171O6         | M+H     | 1312.3071       | 1312.3070        | 0.1            | 4.E+11             |
| 1Me-HGDGT-2 | 51.7        | C87H169O6         | M+H     | 1310.2920       | 1310.2914        | 0.5            | 8.E+11             |
| 1Me-HGDGT-3 | 54.3        | C87H167O6         | M+H     | 1308.2765       | 1308.2757        | 0.6            | 6.E+11             |
| 1Me-HGDGT-4 | 57.1        | C87H165O6         | M+H     | 1306.2617       | 1306.2601        | 1.3            | 1.E+12             |
| 2Me-HGDGT-0 | 47.0        | C88H175O6         | M+H     | 1328.3368       | 1328.3383        | -1.1           | 1.E+11             |
| 2Me-HGDGT-1 | 49.5        | C88H173O6         | M+H     | 1326.3218       | 1326.3227        | -0.7           | 2.E+11             |
| 2Me-HGDGT-2 | 51.5        | C88H171O6         | M+H     | 1324.3064       | 1324.3070        | -0.5           | 6.E+11             |
| 2Me-HGDGT-3 | 53.9        | C88H169O6         | M+H     | 1322.2909       | 1322.2914        | -0.4           | 1.E+12             |
| 2Me-HGDGT-4 | 57.1        | C88H167O6         | M+H     | 1320.2765       | 1320.2757        | 0.6            | 4.E+12             |
| 3Me-HGDGT-2 | 51.6        | C89H173O6         | M+H     | 1338.3223       | 1338.3223        | -0.3           | 3.E+10             |
| 3Me-HGDGT-3 | 54.3        | C89H171O6         | M+H     | 1336.3064       | 1336.3070        | -0.5           | 3.E+11             |
| 3Me-HGDGT-4 | 56.6        | C89H169O6         | M+H     | 1334.2909       | 1334.2914        | -0.4           | 4.E+12             |
| 4Me-HGDGT-3 | 55.4        | C90H173O6         | M+H     | 1350.3229       | 1350.3227        | 0.2            | 5.E+10             |
| 4Me-HGDGT-4 | 58.1        | C90H171O6         | M+H     | 1348.3066       | 1348.3070        | -0.3           | 1.E+12             |
| GDD-0       | 62.9        | C83H169O5         | M+H     | 1246.2960       | 1246.2965        | -0.3           | 3.E+10             |
| GDD-1       | 63.0        | C83H167O5         | M+H     | 1244.2775       | 1244.2808        | -2.7           | 1.E+09             |
| GDD-2       | 63.1        | C83H165O5         | M+H     | 1242.2665       | 1242.2652        | 1.1            | 1.E+09             |
| GDD-3       | 63.2        | C83H163O5         | M+H     | 1240.2474       | 1240.2495        | -1.7           | 1.E+09             |
| GDD-4       | 63.3        | C83H161O5         | M+H     | 1238.2322       | 1238.2339        | -1.4           | 2.E+09             |
| GMD-0       | 63.9        | C83H167O5         | M+H     | 1244.2804       | 1244.2808        | -0.3           | 7.E+10             |
| GMD-1       | 64.0        | C83H165O5         | M+H     | 1242.2635       | 1242.2652        | -1.3           | 9.E+09             |
| GMD-2       | 64.1        | C83H163O5         | M+H     | 1240.2483       | 1240.2495        | -1.0           | 5.E+09             |
| GMD-3       | 64.3        | C83H161O5         | M+H     | 1238.2300       | 1238.2339        | -3.1           | 4.E+09             |
| GMD-4       | 64.5        | C83H159O5         | M+H     | 1236.2183       | 1236.2182        | 0.0            | 4.E+09             |
| 1Me-GMD-0   | 63.9        | C84H169O5         | M+H     | 1258.2970       | 1258.2965        | 0.4            | 7.E+08             |
| 1Me-GMD-1   | 64.1        | C84H167O5         | M+H     | 1256.2794       | 1256.2808        | -1.1           | 2.E+08             |
| 1Me-GMD-2   | 64.2        | C84H165O5         | M+H     | 1254.2633       | 1254.2652        | -1.5           | 1.E+09             |

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**Appendix Table A3.2.** Detail information of all archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(1) Artificial chimney sample: AC-C0016A**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1Me-GMD-3 | 64.4        | C84H163O5         | M+H     | 1252.2507       | 1252.2495        | 1.0            | 8.E+08             |
| 1Me-GMD-4 | 64.6        | C84H161O5         | M+H     | 1250.2329       | 1250.2339        | -0.8           | 5.E+09             |

**(2) Artificial chimney sample: AC-C0016B**

|             |      |           |     |           |           |      |        |
|-------------|------|-----------|-----|-----------|-----------|------|--------|
| AR          | 7.8  | C43H89O3  | M+H | 653.6823  | 653.6806  | 2.6  | 9.E+11 |
| MAR         | 11.5 | C43H87O3  | M+H | 651.6666  | 651.6650  | 2.5  | 4.E+11 |
| OHAR        | 33.4 | C43H89O4  | M+H | 669.6768  | 669.6755  | 1.9  | 1.E+11 |
| GTGT        | 16.7 | C86H175O6 | M+H | 1304.3369 | 1304.3383 | -1.1 | 5.E+09 |
| GDGT-0      | 32.6 | C86H173O6 | M+H | 1302.3231 | 1302.3227 | 0.3  | 3.E+12 |
| GDGT-1      | 34.8 | C86H171O6 | M+H | 1300.3075 | 1300.3070 | 0.4  | 1.E+11 |
| GDGT-2      | 36.9 | C86H169O6 | M+H | 1298.2919 | 1298.2914 | 0.4  | 9.E+10 |
| GDGT-3      | 38.3 | C86H167O6 | M+H | 1296.2759 | 1296.2757 | 0.1  | 4.E+10 |
| GDGT-3'     | 39.6 | C86H167O6 | M+H | 1296.2762 | 1296.2757 | 0.4  |        |
| GDGT-4      | 39.5 | C86H165O6 | M+H | 1294.2605 | 1294.2601 | 0.3  | 8.E+10 |
| GDGT-4'     | 41.9 | C86H165O6 | M+H | 1294.2606 | 1294.2601 | 0.4  |        |
| GDGT-5      | 42.5 | C86H163O6 | M+H | 1292.2451 | 1292.2444 | 0.5  | 2.E+11 |
| HGDGT-0     | 44.9 | C86H171O6 | M+H | 1300.3077 | 1300.3070 | 0.6  | 6.E+11 |
| HGDGT-0'    | 47.0 | C86H171O6 | M+H | 1300.3077 | 1300.3070 | 0.6  |        |
| HGDGT-1     | 46.3 | C86H169O6 | M+H | 1298.2927 | 1298.2914 | 1.0  | 7.E+10 |
| HGDGT-1'    | 48.3 | C86H169O6 | M+H | 1298.2921 | 1298.2914 | 0.6  |        |
| HGDGT-2     | 49.8 | C86H167O6 | M+H | 1296.2765 | 1296.2757 | 0.6  | 4.E+10 |
| HGDGT-2'    | 51.2 | C86H167O6 | M+H | 1296.2781 | 1296.2757 | 1.8  |        |
| HGDGT-3     | 53.7 | C86H165O6 | M+H | 1294.2600 | 1294.2601 | 0.0  | 3.E+10 |
| HGDGT-3'    | 55.1 | C86H165O6 | M+H | 1294.2593 | 1294.2601 | -0.6 |        |
| HGDGT-4     | 58.1 | C86H163O6 | M+H | 1292.2449 | 1292.2444 | 0.4  | 5.E+10 |
| 1Me-HGDGT-0 | 46.5 | C87H173O6 | M+H | 1314.3230 | 1314.3227 | 0.3  | 1.E+11 |
| 1Me-HGDGT-1 | 49.0 | C87H171O6 | M+H | 1312.3082 | 1312.3070 | 0.9  | 3.E+10 |
| 1Me-HGDGT-2 | 51.7 | C87H169O6 | M+H | 1310.2921 | 1310.2914 | 0.6  | 3.E+10 |
| 1Me-HGDGT-3 | 54.1 | C87H167O6 | M+H | 1308.2760 | 1308.2757 | 0.2  | 4.E+10 |
| 1Me-HGDGT-4 | 57.5 | C87H165O6 | M+H | 1306.2601 | 1306.2601 | 0.3  | 1.E+11 |
| 2Me-HGDGT-0 | 46.4 | C88H175O6 | M+H | 1328.3381 | 1328.3383 | -0.1 | 9.E+10 |
| 2Me-HGDGT-1 | 48.8 | C88H173O6 | M+H | 1326.3217 | 1326.3227 | -0.8 | 4.E+10 |
| 2Me-HGDGT-2 | 51.5 | C88H171O6 | M+H | 1324.3062 | 1324.3070 | -0.7 | 4.E+10 |
| 2Me-HGDGT-3 | 54.0 | C88H169O6 | M+H | 1322.2903 | 1322.2914 | -0.8 | 4.E+10 |
| 2Me-HGDGT-4 | 57.5 | C88H167O6 | M+H | 1320.2761 | 1320.2757 | 0.3  | 1.E+11 |
| 3Me-HGDGT-0 | 47.1 | C89H177O6 | M+H | 1342.3530 | 1342.3540 | -0.7 | 4.E+09 |
| 3Me-HGDGT-1 | 49.1 | C89H175O6 | M+H | 1340.3374 | 1340.3383 | -0.7 | 1.E+09 |
| 3Me-HGDGT-2 | 51.8 | C89H173O6 | M+H | 1338.3225 | 1338.3223 | -0.1 | 2.E+09 |
| 3Me-HGDGT-3 | 54.4 | C89H171O6 | M+H | 1336.3075 | 1336.3070 | 0.4  | 6.E+09 |
| 3Me-HGDGT-4 | 57.0 | C89H169O6 | M+H | 1334.2917 | 1334.2914 | 0.3  | 4.E+10 |
| 4Me-HGDGT-4 | 58.3 | C90H171O6 | M+H | 1348.3080 | 1348.3070 | 0.7  | 9.E+09 |
| GDD-0       | 62.9 | C83H169O5 | M+H | 1246.2970 | 1246.2965 | 0.4  | 6.E+09 |
| GDD-5       | 63.5 | C83H159O5 | M+H | 1236.2174 | 1236.2182 | -0.6 | 2.E+09 |
| GMD-0       | 64.0 | C83H167O5 | M+H | 1244.2792 | 1244.2808 | -1.3 | 8.E+09 |
| 1Me-GMD-0   | 64.0 | C84H169O5 | M+H | 1258.2948 | 1258.2965 | -1.3 | 6.E+08 |

(Continued to next page)

**Appendix Table A3.2.** Detail information of all archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds   | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| AR          | 7.4         | C43H89O3          | M+H     | 653.6826        | 653.6806         | 3.0            | 2.E+13             |
| MAR         | 10.7        | C43H87O3          | M+H     | 651.6668        | 651.6650         | 2.8            | 1.E+12             |
| GTGT        | 15.9        | C86H175O6         | M+H     | 1304.3405       | 1304.3383        | 1.6            | 3.E+10             |
| GDGT-0      | 32.3        | C86H173O6         | M+H     | 1302.3227       | 1302.3227        | 0.7            | 8.E+12             |
| GDGT-1      | 34.7        | C86H171O6         | M+H     | 1300.3077       | 1300.3070        | 0.6            | 3.E+11             |
| GDGT-2      | 36.8        | C86H169O6         | M+H     | 1298.2906       | 1298.2914        | -0.6           | 2.E+11             |
| GDGT-3      | 38.8        | C86H167O6         | M+H     | 1296.2766       | 1296.2757        | 0.7            | 2.E+11             |
| GDGT-4      | 41.6        | C86H165O6         | M+H     | 1294.2610       | 1294.2601        | 0.7            | 7.E+11             |
| GDGT-5      | 42.1        | C86H163O6         | M+H     | 1292.2444       | 1292.2444        | 0.0            | 8.E+10             |
| HGDGT-0     | 44.5        | C86H171O6         | M+H     | 1300.3080       | 1300.3070        | 0.7            | 1.E+13             |
| HGDGT-0'    | 46.7        | C86H171O6         | M+H     | 1300.3076       | 1300.3070        | 0.5            |                    |
| HGDGT-1     | 46.7        | C86H169O6         | M+H     | 1298.2910       | 1298.2914        | -0.3           | 4.E+12             |
| HGDGT-1'    | 48.0        | C86H169O6         | M+H     | 1298.2920       | 1298.2914        | 0.5            |                    |
| HGDGT-2     | 49.7        | C86H167O6         | M+H     | 1296.2757       | 1296.2757        | 0.4            | 2.E+12             |
| HGDGT-3     | 52.2        | C86H165O6         | M+H     | 1294.2607       | 1294.2601        | 0.5            |                    |
| HGDGT-3     | 54.6        | C86H165O6         | M+H     | 1294.2599       | 1294.2601        | -0.1           | 7.E+11             |
| HGDGT-4     | 57.3        | C86H163O6         | M+H     | 1292.2450       | 1292.2444        | 0.4            | 8.E+11             |
| 1Me-HGDGT-0 | 46.1        | C87H173O6         | M+H     | 1314.3235       | 1314.3227        | 0.6            | 2.E+12             |
| 1Me-HGDGT-1 | 48.7        | C87H171O6         | M+H     | 1312.3077       | 1312.3070        | 0.5            | 7.E+11             |
| 1Me-HGDGT-2 | 51.2        | C87H169O6         | M+H     | 1310.2922       | 1310.2914        | 0.7            | 6.E+11             |
| 1Me-HGDGT-3 | 54.2        | C87H167O6         | M+H     | 1308.2759       | 1308.2757        | 0.1            | 4.E+11             |
| 1Me-HGDGT-4 | 57.1        | C87H165O6         | M+H     | 1306.2607       | 1306.2601        | 0.5            | 1.E+12             |
| 2Me-HGDGT-0 | 46.7        | C88H175O6         | M+H     | 1328.3378       | 1328.3383        | -0.4           | 2.E+12             |
| 2Me-HGDGT-1 | 48.7        | C88H173O6         | M+H     | 1326.3224       | 1326.3227        | -0.2           | 2.E+12             |
| 2Me-HGDGT-2 | 51.0        | C88H171O6         | M+H     | 1324.3065       | 1324.3070        | -0.4           | 2.E+12             |
| 2Me-HGDGT-3 | 53.6        | C88H169O6         | M+H     | 1322.2909       | 1322.2914        | -0.4           | 4.E+11             |
| 2Me-HGDGT-4 | 56.9        | C88H167O6         | M+H     | 1320.2761       | 1320.2757        | 0.3            | 1.E+12             |
| 3Me-HGDGT-0 | 46.7        | C89H177O6         | M+H     | 1342.3521       | 1342.3540        | -1.4           | 3.E+09             |
| 3Me-HGDGT-1 | 48.9        | C89H175O6         | M+H     | 1340.3380       | 1340.3383        | -0.2           | 6.E+09             |
| 3Me-HGDGT-2 | 51.2        | C89H173O6         | M+H     | 1338.3242       | 1338.3223        | 1.2            | 2.E+10             |
| 3Me-HGDGT-3 | 54.2        | C89H171O6         | M+H     | 1336.3060       | 1336.3070        | -0.7           | 4.E+10             |
| 3Me-HGDGT-4 | 56.5        | C89H169O6         | M+H     | 1334.2913       | 1334.2914        | -0.1           | 5.E+11             |
| 4Me-HGDGT-4 | 58.0        | C90H171O6         | M+H     | 1348.3075       | 1348.3070        | -0.4           | 2.E+11             |
| GDD-0       | 63.0        | C83H169O5         | M+H     | 1246.2946       | 1246.2965        | -1.5           | 6.E+10             |
| GDD-1       | 63.1        | C83H167O5         | M+H     | 1244.2795       | 1244.2808        | -1.0           | 4.E+09             |
| GDD-2       | 63.3        | C83H165O5         | M+H     | 1242.2654       | 1242.2652        | 0.2            | 3.E+09             |
| GDD-3       | 63.8        | C83H163O5         | M+H     | 1240.2485       | 1240.2495        | -0.8           | 5.E+09             |
| GDD-4       | 63.9        | C83H161O5         | M+H     | 1238.2310       | 1238.2339        | -2.3           | 1.E+10             |
| GMD-0       | 64.1        | C83H167O5         | M+H     | 1244.2805       | 1244.2808        | -0.2           | 6.E+11             |
| GMD-1       | 64.3        | C83H165O5         | M+H     | 1242.2651       | 1242.2652        | 0.0            | 1.E+11             |
| GMD-2       | 64.5        | C83H163O5         | M+H     | 1240.2485       | 1240.2495        | -0.8           | 6.E+10             |
| GMD-3       | 63.8        | C83H161O5         | M+H     | 1238.2317       | 1238.2339        | -1.7           | 2.E+10             |
| GMD-4       | 64.0        | C83H159O5         | M+H     | 1236.2183       | 1236.2182        | 0.0            | 3.E+10             |
| 1Me-GMD-0   | 64.2        | C84H169O5         | M+H     | 1258.2952       | 1258.2965        | -1.0           | 5.E+10             |
| 1Me-GMD-1   | 64.3        | C84H167O5         | M+H     | 1256.2809       | 1256.2808        | 0.1            | 1.E+10             |
| 1Me-GMD-2   | 64.5        | C84H165O5         | M+H     | 1254.2621       | 1254.2652        | -2.4           | 1.E+10             |
| 1Me-GMD-3   | 63.8        | C84H163O5         | M+H     | 1252.2482       | 1252.2495        | -1.1           | 1.E+10             |

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**Appendix Table A3.2.** Detail information of all archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| 1Me-GMD-4 | 64.0        | C84H161O5         | M+H     | 1250.2322       | 1250.2339        | -1.3           | 5.E+10             |
| 2Me-GMD-0 | 64.2        | C85H171O5         | M+H     | 1272.3109       | 1272.3121        | -0.9           | 4.E+10             |
| 2Me-GMD-1 | 64.3        | C85H169O5         | M+H     | 1270.2959       | 1270.2965        | -0.4           | 3.E+10             |
| 2Me-GMD-2 | 64.4        | C85H167O5         | M+H     | 1268.2814       | 1268.2808        | 0.4            | 3.E+10             |
| 2Me-GMD-3 | 64.1        | C85H165O5         | M+H     | 1266.2629       | 1266.2652        | -1.7           | 1.E+10             |
| 2Me-GMD-4 | 64.2        | C85H163O5         | M+H     | 1264.2498       | 1264.2495        | 0.2            | 3.E+10             |

**(4) Natural chimney sample: NC-HRV**

|             |      |           |     |           |           |      |        |
|-------------|------|-----------|-----|-----------|-----------|------|--------|
| AR          | 7.4  | C43H89O3  | M+H | 653.6827  | 653.6806  | 3.1  | 3.E+12 |
| MAR         | 10.9 | C43H87O3  | M+H | 651.6667  | 651.6650  | 2.6  | 5.E+11 |
| OHAR        | 32.3 | C43H89O4  | M+H | 669.6772  | 669.6755  | 2.5  | 7.E+11 |
| GTGT        | 17.4 | C86H175O6 | M+H | 1304.3385 | 1304.3383 | 0.1  | 1.E+10 |
| GDGT-0      | 32.8 | C86H173O6 | M+H | 1302.3231 | 1302.3227 | 0.3  | 5.E+12 |
| GDGT-1      | 35.2 | C86H171O6 | M+H | 1300.3074 | 1300.3070 | 0.3  | 2.E+11 |
| GDGT-2      | 36.9 | C86H169O6 | M+H | 1298.2920 | 1298.2914 | 0.5  | 2.E+11 |
| GDGT-3      | 38.3 | C86H167O6 | M+H | 1296.2757 | 1296.2757 | 0.4  | 5.E+10 |
| GDGT-3'     | 39.8 | C86H167O6 | M+H | 1296.2767 | 1296.2757 | 0.8  |        |
| GDGT-4      | 39.6 | C86H165O6 | M+H | 1294.2603 | 1294.2601 | 0.1  | 5.E+10 |
| GDGT-4'     | 42.4 | C86H165O6 | M+H | 1294.2603 | 1294.2601 | 0.1  |        |
| GDGT-5      | 41.3 | C86H163O6 | M+H | 1292.2440 | 1292.2444 | -0.3 | 4.E+10 |
| GDGT-5'     | 43.0 | C86H163O6 | M+H | 1292.2449 | 1292.2444 | 0.4  |        |
| HGDGT-0     | 45.2 | C86H171O6 | M+H | 1300.3074 | 1300.3070 | 0.3  | 3.E+11 |
| HGDGT-0'    | 47.3 | C86H171O6 | M+H | 1300.3077 | 1300.3070 | 0.6  |        |
| HGDGT-1     | 48.9 | C86H169O6 | M+H | 1298.2915 | 1298.2914 | 0.1  | 3.E+10 |
| HGDGT-1'    | 49.8 | C86H169O6 | M+H | 1298.2920 | 1298.2914 | 0.5  |        |
| HGDGT-2     | 50.4 | C86H167O6 | M+H | 1296.2754 | 1296.2757 | -0.3 | 3.E+10 |
| HGDGT-2'    | 52.6 | C86H167O6 | M+H | 1296.2766 | 1296.2757 | 0.7  |        |
| HGDGT-3     | 55.2 | C86H165O6 | M+H | 1294.2599 | 1294.2601 | -0.1 | 3.E+10 |
| HGDGT-4     | 58.4 | C86H163O6 | M+H | 1292.2448 | 1292.2444 | 0.3  | 6.E+10 |
| 1Me-HGDGT-0 | 47.1 | C87H173O6 | M+H | 1314.3226 | 1314.3227 | 0.0  | 9.E+10 |
| 1Me-HGDGT-1 | 49.4 | C87H171O6 | M+H | 1312.3075 | 1312.3070 | 0.4  | 3.E+10 |
| 1Me-HGDGT-2 | 51.9 | C87H169O6 | M+H | 1310.2917 | 1310.2914 | 0.3  | 5.E+10 |
| 1Me-HGDGT-3 | 54.5 | C87H167O6 | M+H | 1308.2759 | 1308.2757 | 0.1  | 6.E+10 |
| 1Me-HGDGT-4 | 57.7 | C87H165O6 | M+H | 1306.2609 | 1306.2601 | 0.6  | 1.E+11 |
| 2Me-HGDGT-0 | 47.3 | C88H175O6 | M+H | 1328.3378 | 1328.3383 | -0.4 | 7.E+10 |
| 2Me-HGDGT-1 | 49.2 | C88H173O6 | M+H | 1326.3218 | 1326.3227 | -0.7 | 4.E+10 |
| 2Me-HGDGT-2 | 51.8 | C88H171O6 | M+H | 1324.3070 | 1324.3070 | 0.0  | 6.E+10 |
| 2Me-HGDGT-3 | 54.5 | C88H169O6 | M+H | 1322.2904 | 1322.2914 | -0.7 | 5.E+10 |
| 2Me-HGDGT-4 | 56.9 | C88H167O6 | M+H | 1320.2761 | 1320.2757 | 0.3  | 1.E+11 |
| 3Me-HGDGT-0 | 47.2 | C89H177O6 | M+H | 1342.3538 | 1342.3540 | -0.2 | 5.E+09 |
| 3Me-HGDGT-1 | 49.8 | C89H175O6 | M+H | 1340.3378 | 1340.3383 | -0.4 | 1.E+09 |
| 3Me-HGDGT-2 | 52.2 | C89H173O6 | M+H | 1338.3229 | 1338.3223 | 0.2  | 2.E+09 |
| 3Me-HGDGT-3 | 55.0 | C89H171O6 | M+H | 1336.3062 | 1336.3070 | -0.6 | 3.E+08 |
| 3Me-HGDGT-4 | 57.8 | C89H169O6 | M+H | 1334.2915 | 1334.2914 | 0.1  | 1.E+09 |
| GDD-0       | 62.8 | C83H169O5 | M+H | 1246.2952 | 1246.2965 | -1.0 | 1.E+10 |
| GMD-0       | 63.8 | C83H167O5 | M+H | 1244.2803 | 1244.2808 | -0.4 | 7.E+09 |

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**Appendix Table A3.2.** Detail information of all archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| AR        | 7.3         | C43H89O3          | M+H     | 653.6797        | 653.6806         | -1.4           | 2.E+09             |
| GDGT-0    | 32.4        | C86H173O6         | M+H     | 1302.3229       | 1302.3227        | 0.2            | 1.E+10             |
| GDGT-1    | 36.6        | C86H171O6         | M+H     | 1300.3083       | 1300.3070        | 1.0            | 3.E+08             |
| HGDGT-0   | 47.2        | C86H171O6         | M+H     | 1300.3079       | 1300.3070        | 0.6            | 1.E+09             |

**(6) Reference sample:RF-AS**

|         |      |           |     |           |           |      |        |
|---------|------|-----------|-----|-----------|-----------|------|--------|
| AR      | 8.87 | C43H89O3  | M+H | 653.6787  | 653.6806  | -2.9 | 5.E+09 |
| GDGT-0  | 31.5 | C86H173O6 | M+H | 1302.3224 | 1302.3227 | -0.2 | 1.E+10 |
| GDGT-2  | 36.7 | C86H169O6 | M+H | 1298.2885 | 1298.2914 | -2.2 | 3.E+09 |
| GDGT-4  | 42.0 | C86H165O6 | M+H | 1294.2563 | 1294.2601 | -2.9 | 6.E+08 |
| GDGT-5  | 42.6 | C86H163O6 | M+H | 1292.2401 | 1292.2444 | -3.3 | 1.E+09 |
| HGDGT-0 | 44.5 |           | M+H | 1300.3009 | 1300.3070 | -4.7 | 3.E+09 |
| HGDGT-4 | 57.6 |           | M+H | 1292.2413 | 1292.2444 | -2.4 | 6.E+08 |

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field.

**(1) Artificial chimney sample: AC-C0016A**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|

Not detected.

**(2) Artificial chimney sample: AC-C0016B**

**PG-DAG**

|              |      |              |       |          |          |      |        |
|--------------|------|--------------|-------|----------|----------|------|--------|
| C31:1 DAG-PG | 83.0 | C37H75NO10P+ | M+NH4 | 724.5138 | 724.5123 | 2.0  | 4.E+09 |
| C32:1 DAG-PG | 82.5 | C38H77NO10P+ | M+NH4 | 738.5291 | 738.528  | 1.6  | 2.E+10 |
| C32:2 DAG-PG | 83.1 | C38H75NO10P+ | M+NH4 | 736.5132 | 736.5123 | 1.2  | 1.E+10 |
| C33:1 DAG-PG | 82.2 | C39H79NO10P+ | M+NH4 | 752.5442 | 752.5436 | 0.8  | 4.E+09 |
| C34:1 DAG-PG | 81.9 | C40H81NO10P+ | M+NH4 | 766.5592 | 766.5593 | -0.1 | 1.E+10 |
| C34:2 DAG-PG | 82.4 | C40H79NO10P+ | M+NH4 | 764.5438 | 764.5436 | 0.3  | 3.E+10 |
| C36:2 DAG-PG | 81.8 | C42H83NO10P+ | M+NH4 | 792.5759 | 792.5749 | 1.2  | 3.E+10 |

**PC-DAG**

|              |       |             |     |          |          |     |        |
|--------------|-------|-------------|-----|----------|----------|-----|--------|
| C32:0 DAG-PC | 120.0 | C40H81NO8P+ | M+H | 734.5707 | 734.5694 | 1.8 | 1.E+11 |
| C32:1 DAG-PC | 120.0 | C40H79NO8P+ | M+H | 732.5552 | 732.5538 | 2.0 | 2.E+10 |
| C33:0 DAG-PC | 119.8 | C41H83NO8P+ | M+H | 748.5869 | 748.5851 | 2.4 | 3.E+10 |
| C33:1 DAG-PC | 120.0 | C41H81NO8P+ | M+H | 746.571  | 746.5694 | 2.1 | 7.E+10 |
| C34:1 DAG-PC | 120.0 | C42H83NO8P+ | M+H | 760.5851 | 760.5851 | 1.5 | 6.E+12 |
| C34:2 DAG-PC | 120.0 | C42H81NO8P+ | M+H | 758.5712 | 758.5694 | 2.3 | 4.E+11 |
| C35:1 DAG-PC | 118.5 | C43H85NO8P+ | M+H | 774.6021 | 774.6007 | 1.7 | 3.E+11 |
| C35:2 DAG-PC | 119.9 | C43H83NO8P+ | M+H | 772.5862 | 772.5851 | 1.4 | 1.E+11 |
| C36:1 DAG-PC | 116.2 | C44H87NO8P+ | M+H | 788.6177 | 788.6164 | 1.6 | 7.E+11 |
| C36:2 DAG-PC | 119.3 | C44H85NO8P+ | M+H | 786.6017 | 786.6007 | 1.2 | 6.E+12 |
| C36:3 DAG-PC | 120.0 | C44H83NO8P+ | M+H | 784.5851 | 784.5864 | 1.6 | 7.E+11 |
| C37:2 DAG-PC | 116.3 | C45H87NO8P+ | M+H | 800.6173 | 800.6164 | 1.2 | 1.E+11 |
| C38:2 DAG-PC | 115.3 | C46H89NO8P+ | M+H | 814.6331 | 814.632  | 1.3 | 1.E+12 |
| C38:3 DAG-PC | 118.4 | C46H87NO8P+ | M+H | 812.6173 | 812.6164 | 1.1 | 2.E+12 |
| C38:4 DAG-PC | 119.9 | C46H85NO8P+ | M+H | 810.6017 | 810.6007 | 1.2 | 5.E+11 |
| C38:5 DAG-PC | 119.9 | C46H83NO8P+ | M+H | 808.5858 | 808.5851 | 0.9 | 7.E+10 |
| C40:2 DAG-PC | 112.1 | C48H93NO8P+ | M+H | 842.6636 | 842.6633 | 0.3 | 1.E+11 |
| C40:3 DAG-PC | 114.1 | C48H91NO8P+ | M+H | 840.6487 | 840.6477 | 1.2 | 2.E+11 |
| C40:4 DAG-PC | 116.6 | C48H89NO8P+ | M+H | 838.6329 | 838.632  | 1.1 | 2.E+11 |

**PE-DAG**

|              |      |             |     |          |          |     |        |
|--------------|------|-------------|-----|----------|----------|-----|--------|
| C30:0 DAG-PE | 89.5 | C35H71NO8P+ | M+H | 664.4925 | 664.4912 | 2.0 | 3.E+10 |
| C30:1 DAG-PE | 89.9 | C35H69NO8P+ | M+H | 662.4771 | 662.4755 | 2.3 | 1.E+11 |
| C30:2 DAG-PE | 90.5 | C35H67NO8P+ | M+H | 660.4612 | 660.4599 | 2.1 | 2.E+10 |
| C31:0 DAG-PE | 89.0 | C36H73NO8P+ | M+H | 678.5079 | 678.5068 | 1.6 | 8.E+09 |
| C31:1 DAG-PE | 89.5 | C36H71NO8P+ | M+H | 676.4921 | 676.4912 | 1.3 | 8.E+10 |
| C31:2 DAG-PE | 90.2 | C36H69NO8P+ | M+H | 674.4774 | 674.4755 | 2.8 | 6.E+09 |
| C32:0 DAG-PE | 88.4 | C37H75NO8P+ | M+H | 692.5229 | 692.5225 | 0.6 | 5.E+09 |
| C32:1 DAG-PE | 89.0 | C37H73NO8P+ | M+H | 690.5081 | 690.5068 | 0.0 | 3.E+11 |
| C32:2 DAG-PE | 89.6 | C37H71NO8P+ | M+H | 688.4925 | 688.4912 | 1.9 | 5.E+11 |
| C32:3 DAG-PE | 90.5 | C37H69NO8P+ | M+H | 686.4767 | 686.4755 | 1.7 | 4.E+10 |
| C33:0 DAG-PE | 88.0 | C38H77NO8P+ | M+H | 706.5396 | 706.5381 | 2.1 | 1.E+10 |
| C33:1 DAG-PE | 88.7 | C38H75NO8P+ | M+H | 704.5237 | 704.5225 | 1.8 | 8.E+10 |
| C33:2 DAG-PE | 89.4 | C38H73NO8P+ | M+H | 702.5079 | 702.5068 | 1.6 | 7.E+10 |
| C34:0 DAG-PE | 87.8 | C39H79NO8P+ | M+H | 720.5542 | 720.5538 | 0.6 | 4.E+09 |
| C34:1 DAG-PE | 88.2 | C39H77NO8P+ | M+H | 718.5386 | 718.5381 | 0.7 | 4.E+10 |
| C34:2 DAG-PE | 88.8 | C39H75NO8P+ | M+H | 716.5961 | 716.5225 | 1.2 | 3.E+11 |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds       | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:3 DAG-PE    | 89.6        | C39H73NO8P+       | M+H     | 714.5079        | 714.5068         | 1.5            | 1.E+11             |
| C34:4 DAG-PE    | 90.2        | C39H71NO8P+       | M+H     | 712.4924        | 712.4912         | 1.8            | 4.E+10             |
| C36:1 DAG-PE    | 87.4        | C41H81NO8P+       | M+H     | 746.5706        | 746.5694         | 1.6            | 1.E+10             |
| C36:2 DAG-PE    | 88.0        | C41H79NO8P+       | M+H     | 744.5549        | 744.5538         | 1.5            | 1.E+11             |
| C36:3 DAG-PE    | 88.7        | C41H77NO8P+       | M+H     | 742.5394        | 742.5381         | 1.7            | 1.E+11             |
| C36:4 DAG-PE    | 89.4        | C41H75NO8P+       | M+H     | 740.5234        | 740.5225         | 1.2            | 1.E+11             |
| C38:2 DAG-PE    | 87.1        | C43H83NO8P+       | M+H     | 772.5858        | 772.5851         | 1.0            | 2.E+10             |
| C38:3 DAG-PE    | 87.8        | C43H81NO8P+       | M+H     | 770.5704        | 770.5694         | 1.2            | 3.E+10             |
| C38:4 DAG-PE    | 88.5        | C43H79NO8P+       | M+H     | 768.5547        | 768.5538         | 1.3            | 6.E+10             |
| C38:5 DAG-PE    | 88.9        | C43H77NO8P+       | M+H     | 766.5392        | 766.5381         | 1.5            | 5.E+10             |
| C38:6 DAG-PE    | 89.6        | C43H75NO8P+       | M+H     | 764.5231        | 764.5225         | 0.8            | 4.E+09             |
| C40:2 DAG-PE    | 86.3        | C45H87NO8P+       | M+H     | 800.6164        | 800.6164         | 0.0            | 8.E+09             |
| C40:3 DAG-PE    | 87.0        | C45H85NO8P+       | M+H     | 798.6005        | 798.6007         | 0.1            | 7.E+09             |
| C40:4 DAG-PE    | 87.6        | C45H83NO8P+       | M+H     | 796.5862        | 796.5851         | 1.5            | 2.E+10             |
| C40:5 DAG-PE    | 88.0        | C45H81NO8P+       | M+H     | 794.5704        | 794.5694         | 1.3            | 9.E+10             |
| C40:6 DAG-PE    | 88.7        | C45H79NO8P+       | M+H     | 792.5549        | 792.5538         | 1.4            |                    |
| <b>PME-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C29:0 DAG-PME   | 93.2        | C35H71NO8P+       | M+H     | 664.4926        | 664.4912         | 2.1            | 2.E+10             |
| C30:0 DAG-PME   | 92.9        | C36H73NO8P+       | M+H     | 678.5079        | 678.5068         | 1.6            | 1.E+11             |
| C30:1 DAG-PME   | 93.2        | C36H71NO8P+       | M+H     | 676.4924        | 676.4912         | 1.8            | 1.E+11             |
| C30:2 DAG-PME   | 94.3        | C36H69NO8P+       | M+H     | 674.4765        | 674.4755         | 1.4            | 9.E+09             |
| C31:0 DAG-PME   | 92.0        | C37H75NO8P+       | M+H     | 692.5237        | 692.5225         | 1.7            | 4.E+10             |
| C31:1 DAG-PME   | 92.8        | C37H73NO8P+       | M+H     | 690.5068        | 690.5068         | 1.6            | 2.E+11             |
| C31:2 DAG-PME   | 93.4        | C37H71NO8P+       | M+H     | 688.4921        | 688.4912         | 1.3            | 2.E+10             |
| C32:0 DAG-PME   | 91.7        | C38H77NO8P+       | M+H     | 706.5386        | 706.5381         | 0.7            | 2.E+10             |
| C32:1 DAG-PME   | 92.0        | C38H75NO8P+       | M+H     | 704.5241        | 704.5225         | 2.3            | 5.E+11             |
| C32:2 DAG-PME   | 93.0        | C38H73NO8P+       | M+H     | 702.5081        | 702.5068         | 1.8            | 5.E+11             |
| C33:0 DAG-PME   | 91.0        | C39H79NO8P+       | M+H     | 720.5547        | 720.5538         | 2.7            | 2.E+10             |
| C33:1 DAG-PME   | 91.7        | C39H77NO8P+       | M+H     | 718.5394        | 718.5381         | 1.8            | 9.E+10             |
| C33:2 DAG-PME   | 92.4        | C39H75NO8P+       | M+H     | 716.5234        | 716.5225         | 1.3            | 7.E+10             |
| C34:1 DAG-PME   | 91.1        | C40H79NO8P+       | M+H     | 732.5551        | 732.5538         | 1.8            | 1.E+11             |
| C34:2 DAG-PME   | 91.8        | C40H77NO8P+       | M+H     | 730.5395        | 730.5381         | 1.9            | 8.E+11             |
| C34:3 DAG-PME   | 92.6        | C40H75NO8P+       | M+H     | 728.5239        | 728.5225         | 1.9            | 8.E+10             |
| C35:1 DAG-PME   | 90.8        | C41H81NO8P+       | M+H     | 746.5713        | 746.5694         | 2.5            | 2.E+10             |
| C35:2 DAG-PME   | 91.4        | C41H79NO8P+       | M+H     | 744.555         | 744.5538         | 1.6            | 1.E+11             |
| C36:2 DAG-PME   | 91.0        | C42H81NO8P+       | M+H     | 758.5704        | 758.5694         | 1.2            | 5.E+11             |
| C36:3 DAG-PME   | 91.3        | C42H79NO8P+       | M+H     | 756.5548        | 756.5538         | 1.4            | 3.E+11             |
| C37:2 DAG-PME   | 90.5        | C43H83NO8P+       | M+H     | 772.5497        | 772.5851         | 1.3            | 2.E+10             |
| C37:3 DAG-PME   | 91.0        | C43H81NO8P+       | M+H     | 770.5704        | 770.5694         | 1.3            | 8.E+10             |
| <b>PDME-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PDME  | 95.2        | C37H75NO8P+       | M+H     | 692.5234        | 692.5225         | 1.4            | 4.E+10             |
| C30:1 DAG-PDME  | 95.8        | C37H73NO8P+       | M+H     | 690.5084        | 690.5068         | 2.2            | 2.E+11             |
| C31:0 DAG-PDME  | 94.7        | C38H77NO8P+       | M+H     | 706.5395        | 706.5381         | 1.9            | 2.E+10             |
| C31:1 DAG-PDME  | 95.5        | C38H75NO8P+       | M+H     | 704.5242        | 704.5225         | 2.4            | 1.E+11             |
| C31:2 DAG-PDME  | 96.2        | C38H73NO8P+       | M+H     | 702.5082        | 702.5068         | 2.0            | 7.E+09             |
| C32:0 DAG-PDME  | 94.0        | C39H79NO8P+       | M+H     | 720.5553        | 720.5538         | 2.1            | 2.E+10             |
| C32:1 DAG-PDME  | 94.6        | C39H77NO8P+       | M+H     | 718.5397        | 718.5381         | 2.1            | 7.E+11             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C32:2 DAG-PDME | 95.5        | C39H75NO8P+       | M+H     | 716.524         | 716.5225         | 2.1            | 4.E+11             |
| C33:1 DAG-PDME | 94.1        | C40H79NO8P+       | M+H     | 732.5551        | 732.5538         | 1.7            | 1.E+11             |
| C34:1 DAG-PDME | 93.5        | C41H81NO8P+       | M+H     | 746.5708        | 746.5694         | 1.8            | 2.E+11             |
| C34:2 DAG-PDME | 94.3        | C41H79NO8P+       | M+H     | 744.5553        | 744.5538         | 2.0            | 8.E+11             |
| C34:3 DAG-PDME | 95.2        | C41H77NO8P+       | M+H     | 742.5397        | 742.5381         | 2.1            | 1.E+11             |
| C36:1 DAG-PDME | 92.4        | C43H85NO8P+       | M+H     | 774.6013        | 774.6007         | 0.7            | 1.E+10             |
| C36:2 DAG-PDME | 93.2        | C43H83NO8P+       | M+H     | 772.5862        | 772.5851         | 1.5            | 3.E+11             |
| C36:3 DAG-PDME | 93.6        | C43H81NO8P+       | M+H     | 770.5707        | 770.5694         | 1.6            | 3.E+11             |
| C36:4 DAG-PDME | 94.1        | C43H79NO8P+       | M+H     | 768.5551        | 768.5538         | 1.7            | 6.E+10             |
| <b>DGTS</b>    |             |                   |         |                 |                  |                |                    |
| C30:0-DGTS     | 85.1        | C40H78NO7+        | M+H     | 684.578         | 684.5773         | 1.1            | 9.E+10             |
| C30:1-DGTS     | 85.5        | C40H76NO7+        | M+H     | 682.5625        | 682.5616         | 1.3            | 1.E+11             |
| C31:0-DGTS     | 84.5        | C41H80NO7+        | M+H     | 698.5941        | 698.5929         | 1.7            | 4.E+10             |
| C31:1-DGTS     | 85.2        | C41H78NO7+        | M+H     | 696.5784        | 696.5773         | 1.6            | 8.E+10             |
| C32:0-DGTS     | 84.0        | C42H82NO7+        | M+H     | 712.6094        | 712.6086         | 1.2            | 1.E+11             |
| C32:1-DGTS     | 84.6        | C42H80NO7+        | M+H     | 710.5939        | 710.5929         | 1.4            | 1.E+12             |
| C32:2-DGTS     | 85.3        | C42H78NO7+        | M+H     | 708.5782        | 708.5773         | 1.3            | 3.E+11             |
| C33:0-DGTS     | 83.6        | C43H84NO7+        | M+H     | 726.625         | 726.6242         | 1.1            | 3.E+10             |
| C33:1-DGTS     | 84.2        | C43H82NO7+        | M+H     | 724.6096        | 724.6086         | 1.3            | 3.E+11             |
| C33:2-DGTS     | 84.7        | C43H80NO7+        | M+H     | 722.5938        | 722.5903         | 1.2            | 8.E+10             |
| C34:0-DGTS     | 83.3        | C44H86NO7+        | M+H     | 740.6404        | 740.6399         | 0.7            | 1.E+10             |
| C34:1-DGTS     | 83.7        | C44H84NO7+        | M+H     | 738.6251        | 738.6242         | 1.2            | 7.E+11             |
| C34:2-DGTS     | 84.2        | C44H82NO7+        | M+H     | 736.6096        | 736.6086         | 1.4            | 6.E+11             |
| C34:3-DGTS     | 85.3        | C44H80NO7+        | M+H     | 734.5937        | 734.5929         | 1.0            | 1.E+10             |
| C35:1-DGTS     | 83.4        | C45H86NO7+        | M+H     | 752.6406        | 752.6399         | 0.9            | 1.E+11             |
| C35:2-DGTS     | 83.9        | C45H84NO7+        | M+H     | 750.6251        | 750.6242         | 1.1            | 1.E+11             |
| C36:0-DGTS     | 82.4        | C46H90NO7+        | M+H     | 768.6714        | 768.6712         | 0.3            | 3.E+09             |
| C36:1-DGTS     | 82.9        | C46H88NO7+        | M+H     | 766.6558        | 766.6555         | 0.4            | 7.E+10             |
| C36:2-DGTS     | 83.6        | C46H86NO7+        | M+H     | 764.6402        | 764.6399         | 0.4            | 2.E+12             |
| C36:3-DGTS     | 84.0        | C46H84NO7+        | M+H     | 762.6216        | 762.6242         | 0.7            | 1.E+12             |
| C36:4-DGTS     | 84.7        | C46H82NO7+        | M+H     | 760.6088        | 760.6086         | 0.3            | 6.E+10             |
| C37:2-DGTS     | 83.2        | C47H88NO7+        | M+H     | 778.6559        | 778.6555         | 0.5            | 2.E+11             |
| C37:3-DGTS     | 83.5        | C47H86NO7+        | M+H     | 776.6401        | 776.6399         | 0.3            | 1.E+11             |
| <b>1G-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-1G   | 73.2        | C39H78NO10+       | M+NH4   | 720.5629        | 720.562          | 1.3            | 4.E+10             |
| C30:1 DAG-1G   | 72.6        | C39H76NO10+       | M+NH4   | 718.5474        | 718.5464         | 1.5            | 4.E+10             |
| C32:2 DAG-1G   | 72.6        | C41H78NO10+       | M+NH4   | 744.5632        | 744.562          | 1.6            | 8.E+10             |
| C33:0 DAG-1G   | 73.1        | C42H84NO10+       | M+NH4   | 762.5649        | 762.609          | 0.7            | 1.E+10             |
| C34:1 DAG-1G   | 72.7        | C43H84NO10+       | M+NH4   | 774.6099        | 774.609          | 1.1            | 3.E+10             |
| C34:2 DAG-1G   | 73.0        | C43H82NO10+       | M+NH4   | 772.5939        | 772.5933         | 0.8            | 8.E+10             |
| C35:1 DAG-1G   | 72.5        | C44H86NO10+       | M+NH4   | 788.6254        | 788.6246         | 1.0            | 3.E+10             |
| C35:2 DAG-1G   | 72.7        | C44H84NO10+       | M+NH4   | 786.6099        | 786.609          | 1.2            | 1.E+11             |
| C36:2 DAG-1G   | 72.6        | C45H86NO10+       | M+NH4   | 800.6258        | 800.6246         | 1.5            | 3.E+11             |
| C38:2 DAG-1G   | 72.3        | C47H90NO10+       | M+NH4   | 828.6568        | 828.6559         | 1.1            | 1.E+11             |
| <b>PE-AEG</b>  |             |                   |         |                 |                  |                |                    |
| C31:0 AEG-PE   | 86.2        | C36H75NO7P+       | M+H     | 664.5286        | 664.5276         | 1.5            | 2.E+10             |
| C31:1 AEG-PE   | 86.7        | C36H73NO7P+       | M+H     | 662.5131        | 662.5119         | 1.8            | 3.E+10             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds       | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C31:2 AEG-PE    | 87.4        | C36H71NO7P+       | M+H     | 660.4977        | 660.4963         | 2.2            | 4.E+09             |
| C32:0 AEG-PE    | 85.8        | C37H77NO7P+       | M+H     | 678.5446        | 678.5432         | 2.1            | 9.E+09             |
| C32:1 AEG-PE    | 86.2        | C37H75NO7P+       | M+H     | 676.5286        | 676.5276         | 1.5            | 6.E+10             |
| C32:2 AEG-PE    | 86.9        | C37H73NO7P+       | M+H     | 674.513         | 674.5119         | 1.6            | 3.E+10             |
| C33:1 AEG-PE    | 85.9        | C38H77NO7P+       | M+H     | 690.5432        | 690.5432         | 1.8            | 3.E+11             |
| C33:2 AEG-PE    | 86.6        | C38H75NO7P+       | M+H     | 688.5286        | 688.5276         | 1.5            | 5.E+10             |
| C34:1 AEG-PE    | 85.6        | C38H79NO7P+       | M+H     | 704.5596        | 704.5589         | 1.1            | 8.E+09             |
| C34:2 AEG-PE    | 86.2        | C39H77NO7P+       | M+H     | 702.5443        | 702.5432         | 1.5            | 2.E+11             |
| C34:3 AEG-PE    | 87.4        | C39H75NO7P+       | M+H     | 700.5291        | 700.5276         | 2.1            | 7.E+09             |
| C34:4 AEG-PE    | 88.1        | C39H73NO7P+       | M+H     | 698.5136        | 698.5119         | 2.4            | 4.E+09             |
| C35:1 AEG-PE    | 85.2        | C40H81NO7P+       | M+H     | 718.5745        | 718.5745         | -0.1           | 6.E+09             |
| C35:2 AEG-PE    | 85.8        | C40H79NO7P+       | M+H     | 716.5596        | 716.5589         | 1.0            | 3.E+10             |
| C36:2 AEG-PE    | 85.5        | C41H81NO7P+       | M+H     | 730.5755        | 730.5745         | 1.3            | 6.E+10             |
| C36:3 AEG-PE    | 86.6        | C41H79NO7P+       | M+H     | 728.5589        | 728.5589         | 0.6            | 2.E+10             |
| C36:4 AEG-PE    | 87.3        | C41H77NO7P+       | M+H     | 726.5444        | 726.5432         | 1.6            | 9.E+09             |
| C36:5 AEG-PE    | 87.6        | C41H75NO7P+       | M+H     | 724.5288        | 724.5276         | 1.7            | 1.E+10             |
| C36:6 AEG-PE    | 88.2        | C41H73NO7P+       | M+H     | 722.5127        | 722.5119         | 1.1            | 2.E+10             |
| <b>PME-AEG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0 AEG-PME   | 89.1        | C36H75NO7P+       | M+H     | 664.529         | 664.5276         | 2.1            | 2.E+10             |
| C30:1 AEG-PME   | 89.5        | C36H73NO7P+       | M+H     | 662.514         | 662.5119         | 3.1            | 1.E+10             |
| C31:0 AEG-PME   | 88.4        | C37H77NO7P+       | M+H     | 678.5442        | 678.5432         | 1.4            | 1.E+11             |
| C31:1 AEG-PME   | 89.0        | C37H75NO7P+       | M+H     | 676.529         | 676.5276         | 2.1            | 2.E+11             |
| C31:2 AEG-PME   | 89.3        | C37H73NO7P+       | M+H     | 674.512         | 674.5119         | 0.2            | 3.E+09             |
| C32:0 AEG-PME   | 88.0        | C38H79NO7P+       | M+H     | 692.5601        | 692.5589         | 1.8            | 2.E+10             |
| C32:1 AEG-PME   | 88.6        | C38H77NO7P+       | M+H     | 690.5432        | 690.5432         | 1.9            | 6.E+10             |
| C32:2 AEG-PME   | 89.3        | C38H75NO7P+       | M+H     | 688.5284        | 688.5276         | 1.3            | 3.E+10             |
| C33:0 AEG-PME   | 87.6        | C38H81NO7P+       | M+H     | 706.5767        | 706.5745         | 3.0            | 2.E+09             |
| C33:1 AEG-PME   | 88.1        | C38H79NO7P+       | M+H     | 704.5608        | 704.5589         | 2.7            | 8.E+10             |
| C33:2 AEG-PME   | 88.8        | C39H77NO7P+       | M+H     | 702.5446        | 702.5432         | 1.9            | 2.E+10             |
| C34:1 AEG-PME   | 87.6        | C40H81NO7P+       | M+H     | 718.5754        | 718.5745         | 1.2            | 1.E+10             |
| C34:2 AEG-PME   | 88.4        | C40H79NO7P+       | M+H     | 716.561         | 716.5589         | 3.0            | 7.E+10             |
| C35:1 AEG-PME   | 87.3        | C41H83NO7P+       | M+H     | 732.5909        | 732.5902         | 1.1            | 9.E+09             |
| C35:2 AEG-PME   | 88.0        | C41H81NO7P+       | M+H     | 730.5751        | 730.5745         | 0.8            | 3.E+10             |
| <b>PDME-AEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 AEG-PDME  | 91.1        | C37H77NO7P+       | M+H     | 678.5447        | 678.5432         | 2.2            | 2.E+10             |
| C30:1 AEG-PDME  | 91.7        | C37H75NO7P+       | M+H     | 676.5281        | 676.5276         | 0.8            | 2.E+10             |
| C31:0 AEG-PDME  | 90.5        | C38H79NO7P+       | M+H     | 692.5611        | 692.5589         | 3.2            | 8.E+09             |
| C31:1 AEG-PDME  | 91.1        | C38H77NO7P+       | M+H     | 690.5432        | 690.5432         | 1.1            | 5.E+10             |
| C31:2 AEG-PDME  | 91.8        | C38H75NO7P+       | M+H     | 688.5293        | 688.5276         | 2.5            | 3.E+09             |
| C32:0 AEG-PDME  | 90.0        | C38H81NO7P+       | M+H     | 706.5757        | 706.5745         | 1.7            | 2.E+10             |
| C32:1 AEG-PDME  | 90.5        | C38H79NO7P+       | M+H     | 704.5601        | 704.5589         | 1.8            | 5.E+10             |
| C32:2 AEG-PDME  | 91.2        | C39H77NO7P+       | M+H     | 702.5447        | 702.5432         | 2.2            | 4.E+10             |
| C33:0 AEG-PDME  | 89.4        | C40H83NO7P+       | M+H     | 720.5914        | 720.5902         | 1.8            | 3.E+09             |
| C33:1 AEG-PDME  | 90.1        | C40H81NO7P+       | M+H     | 718.5759        | 718.5745         | 1.9            | 4.E+11             |
| C33:2 AEG-PDME  | 90.8        | C40H79NO7P+       | M+H     | 716.5599        | 716.5589         | 1.5            | 2.E+10             |
| C34:0 AEG-PDME  | 89.0        | C41H85NO7P+       | M+H     | 734.6077        | 734.6058         | 2.5            | 3.E+09             |
| C34:1 AEG-PDME  | 89.4        | C41H83NO7P+       | M+H     | 732.5913        | 732.5902         | 1.6            | 2.E+10             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds       | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:2 AEG-PDME  | 90.4        | C41H81NO7P+       | M+H     | 730.5758        | 730.5745         | 1.8            | 2.E+11             |
| <b>PC-AEG</b>   |             |                   |         |                 |                  |                |                    |
| C30:0 AEG-PC    | 114.0       | C38H79NO7P+       | M+H     | 692.559         | 692.5589         | 0.1            | 6.E+10             |
| C30:1 AEG-PC    | 116.4       | C38H77NO7P+       | M+H     | 690.5432        | 690.5432         | 1.5            | 9.E+10             |
| C31:1 AEG-PC    | 113.5       | C38H79NO7P+       | M+H     | 704.5602        | 704.5589         | 1.9            | 4.E+10             |
| C32:0 AEG-PC    | 110.0       | C40H83NO7P+       | M+H     | 720.5915        | 720.5902         | 2.3            | 9.E+10             |
| C32:1 AEG-PC    | 112.1       | C40H81NO7P+       | M+H     | 718.5758        | 718.5745         | 1.8            | 9.E+11             |
| C32:2 AEG-PC    | 115.4       | C40H79NO7P+       | M+H     | 716.5602        | 716.5589         | 1.8            | 3.E+11             |
| C33:1 AEG-PC    | 110.6       | C41H83NO7P+       | M+H     | 732.5915        | 732.5902         | 1.8            | 2.E+11             |
| C33:2 AEG-PC    | 112.8       | C41H81NO7P+       | M+H     | 730.5763        | 730.5745         | 2.4            | 3.E+10             |
| C36:1-AEG-PC    | 104.8       | C44H89NO7P+       | M+H     | 774.6385        | 774.6395         | 1.8            | 1.E+11             |
| C36:2-AEG-PC    | 108.2       | C44H87NO7P+       | M+H     | 772.623         | 772.6215         | 2.0            | 5.E+11             |
| C36:3-AEG-PC    | 110.2       | C44H85NO7P+       | M+H     | 770.6071        | 770.6058         | 1.7            | 4.E+11             |
| C36:4-AEG-PC    | 113.4       | C44H83NO7P+       | M+H     | 768.5913        | 768.5902         | 1.5            | 3.E+11             |
| <b>PE-DEG</b>   |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PE    | 85.2        | C35H75NO6P+       | M+H     | 636.5336        | 636.5327         | 1.4            | 2.E+10             |
| C30:1-DEG-PE    | 85.5        | C35H73NO6P+       | M+H     | 634.5181        | 634.517          | 1.7            | 4.E+10             |
| C30:2-DEG-PE    | 86.3        | C35H71NO6P+       | M+H     | 632.5021        | 632.5014         | 1.2            | 1.E+10             |
| C31:0-DEG-PE    | 84.6        | C36H77NO6P+       | M+H     | 650.5497        | 650.5483         | 2.2            | 7.E+09             |
| C31:1-DEG-PE    | 85.2        | C36H75NO6P+       | M+H     | 648.5339        | 648.5327         | 2.0            | 5.E+10             |
| C31:2-DEG-PE    | 85.8        | C36H73NO6P+       | M+H     | 646.5182        | 646.517          | 1.8            | 4.E+09             |
| C32:0-DEG-PE    | 84.1        | C37H79NO6P+       | M+H     | 664.5652        | 664.564          | 1.9            | 7.E+09             |
| C32:1-DEG-PE    | 84.7        | C37H77NO6P+       | M+H     | 662.5496        | 662.5483         | 2.0            | 7.E+10             |
| C32:2-DEG-PE    | 85.4        | C37H75NO6P+       | M+H     | 660.534         | 660.5327         | 2.0            | 3.E+11             |
| C33:1-DEG-PE    | 84.4        | C38H79NO6P+       | M+H     | 676.5649        | 676.564          | 1.4            | 2.E+11             |
| C33:2-DEG-PE    | 85.1        | C38H77NO6P+       | M+H     | 674.5490        | 674.5483         | 1.0            | 1.E+11             |
| C34:0-DEG-PE    | 83.5        | C39H83NO6P+       | M+H     | 692.5958        | 692.5953         | 0.8            | 6.E+08             |
| C34:1-DEG-PE    | 84.0        | C39H81NO6P+       | M+H     | 690.5801        | 690.5796         | 0.8            | 4.E+09             |
| C34:2-DEG-PE    | 84.7        | C39H79NO6P+       | M+H     | 688.5650        | 688.5640         | 1.5            | 5.E+11             |
| C35:2-DEG-PE    | 84.3        | C40H81NO6P+       | M+H     | 702.5807        | 702.5769         | 1.5            | 5.E+10             |
| <b>PME-DEG</b>  |             |                   |         |                 |                  |                |                    |
| C29:0-DEG-PME   | 87.4        | C35H75NO6P+       | M+H     | 636.5338        | 636.5327         | 1.8            | 3.E+10             |
| C29:1-DEG-PME   | 88.4        | C35H73NO6P+       | M+H     | 634.5175        | 634.5170         | 0.7            | 3.E+09             |
| C30:0-DEG-PME   | 87.2        | C36H77NO6P+       | M+H     | 650.5496        | 650.5483         | 2.0            | 4.E+11             |
| C30:1-DEG-PME   | 87.7        | C36H75NO6P+       | M+H     | 648.5338        | 648.5327         | 1.8            | 6.E+10             |
| C30:2-DEG-PME   | 88.5        | C36H73NO6P+       | M+H     | 646.5184        | 646.5170         | 2.1            | 5.E+09             |
| C31:0-DEG-PME   | 86.5        | C37H79NO6P+       | M+H     | 664.5652        | 664.5640         | 1.9            | 7.E+10             |
| C31:1-DEG-PME   | 87.2        | C37H77NO6P+       | M+H     | 662.5495        | 662.5483         | 1.8            | 3.E+11             |
| C32:0-DEG-PME   | 86.2        | C38H81NO6P+       | M+H     | 678.5807        | 678.5796         | 1.7            | 3.E+10             |
| C32:1-DEG-PME   | 86.8        | C38H79NO6P+       | M+H     | 676.5649        | 676.5640         | 1.4            | 1.E+11             |
| C32:2-DEG-PME   | 87.4        | C38H77NO6P+       | M+H     | 674.5493        | 674.5483         | 1.5            | 2.E+11             |
| C33:0-DEG-PME   | 85.7        | C39H83NO6P+       | M+H     | 692.5958        | 692.5953         | 0.8            | 3.E+09             |
| C33:1-DEG-PME   | 86.2        | C39H81NO6P+       | M+H     | 690.5803        | 690.5796         | 1.0            | 8.E+10             |
| C33:2-DEG-PME   | 87.0        | C39H79NO6P+       | M+H     | 688.5648        | 688.5640         | 1.3            | 1.E+11             |
| C34:2-DEG-PME   | 86.7        | C40H81NO6P+       | M+H     | 702.5803        | 702.5769         | 1.0            | 2.E+11             |
| <b>PDME-DEG</b> |             |                   |         |                 |                  |                |                    |
| C28:0-DEG-PDME  | 89.8        | C35H75NO6P+       | M+H     | 636.5338        | 636.5327         | 1.8            | 3.E+09             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C29:0-DEG-PDME | 89.3        | C36H77NO6P+       | M+H     | 650.5502        | 650.5483         | 2.9            | 8.E+09             |
| C30:0-DEG-PDME | 88.9        | C37H79NO6P+       | M+H     | 664.5653        | 664.5640         | 2.0            | 4.E+10             |
| C31:0-DEG-PDME | 88.4        | C38H81NO6P+       | M+H     | 678.5797        | 678.5796         | 0.1            | 1.E+10             |
| C31:1-DEG-PDME | 88.9        | C38H79NO6P+       | M+H     | 676.5651        | 676.5640         | 1.6            | 5.E+10             |
| C31:2-DEG-PDME | 89.7        | C38H77NO6P+       | M+H     | 674.5490        | 674.5483         | 1.0            | 2.E+10             |
| C32:0-DEG-PDME | 87.8        | C39H83NO6P+       | M+H     | 692.5964        | 692.5953         | 1.6            | 2.E+10             |
| C32:1-DEG-PDME | 88.3        | C39H81NO6P+       | M+H     | 690.5807        | 690.5796         | 1.6            | 5.E+10             |
| C32:2-DEG-PDME | 89.0        | C39H79NO6P+       | M+H     | 688.5651        | 688.5640         | 1.7            | 9.E+10             |
| C33:1-DEG-PDME | 87.9        | C40H83NO6P+       | M+H     | 704.5964        | 704.5953         | 1.7            | 1.E+12             |
| C33:2-DEG-PDME | 88.7        | C40H81NO6P+       | M+H     | 702.5808        | 702.5769         | 1.7            | 3.E+11             |
| C34:0-DEG-PDME | 86.8        | C41H87NO6P+       | M+H     | 720.6274        | 720.6266         | 1.2            | 5.E+10             |
| C34:2-DEG-PDME | 88.3        | C41H83NO6P+       | M+H     | 716.5964        | 716.5953         | 1.6            | 2.E+12             |
| <b>1G-Sph</b>  |             |                   |         |                 |                  |                |                    |
| C33:0-Sph-1G   | 74.7        | C39H78NO8+        | M+H     | 688.5732        | 688.5722         | 1.4            | 3.E+10             |
| C33:1-Sph-1G   | 75.0        | C39H76NO8+        | M+H     | 686.5576        | 686.5565         | 1.5            | 1.E+11             |
| C33:2-Sph-1G   | 75.4        | C39H74NO8+        | M+H     | 684.5421        | 684.5409         | 1.8            | 9.E+09             |
| C34:0-Sph-1G   | 74.4        | C40H80NO8+        | M+H     | 702.5878        | 702.5878         | 1.7            | 4.E+10             |
| C34:1-Sph-1G   | 74.8        | C40H78NO8+        | M+H     | 700.5732        | 700.5722         | 1.5            | 1.E+11             |
| C34:2-Sph-1G   | 75.2        | C40H76NO8+        | M+H     | 698.5576        | 698.5565         | 1.4            | 5.E+10             |
| C35:0-Sph-1G   | 74.2        | C41H82NO8+        | M+H     | 716.6035        | 716.6035         | 1.4            | 1.E+10             |
| C35:1-Sph-1G   | 74.5        | C41H80NO8+        | M+H     | 714.5887        | 714.5878         | 1.3            | 3.E+11             |
| C35:2-Sph-1G   | 74.9        | C41H78NO8+        | M+H     | 712.5732        | 712.5722         | 1.5            | 1.E+11             |
| C36:0-Sph-1G   | 74.0        | C42H84NO8+        | M+H     | 730.6198        | 730.6191         | 0.8            | 2.E+10             |
| C36:1-Sph-1G   | 74.3        | C42H82NO8+        | M+H     | 728.6042        | 728.6035         | 1.0            | 1.E+11             |
| C36:2-Sph-1G   | 75.4        | C42H80NO8+        | M+H     | 726.5889        | 726.5878         | 1.4            | 5.E+11             |
| C36:3-Sph-1G   | 75.8        | C42H78NO8+        | M+H     | 724.5731        | 724.5722         | 1.3            | 4.E+10             |
| C37:1-Sph-1G   | 74.1        | C43H84NO8+        | M+H     | 742.6199        | 742.6191         | 1.0            | 4.E+10             |
| C37:2-Sph-1G   | 74.4        | C43H82NO8+        | M+H     | 740.6042        | 740.6035         | 1.0            | 2.E+11             |
| C37:3-Sph-1G   | 75.5        | C43H80NO8+        | M+H     | 738.5889        | 738.5878         | 1.4            | 4.E+10             |
| C38:1-Sph-1G   | 73.8        | C44H86NO8+        | M+H     | 756.6353        | 756.6348         | 0.7            | 5.E+10             |
| C38:2-Sph-1G   | 74.9        | C44H84NO8+        | M+H     | 754.6201        | 754.6191         | 1.2            | 9.E+11             |
| C38:3-Sph-1G   | 75.2        | C44H82NO8+        | M+H     | 752.6042        | 752.6035         | 0.9            | 8.E+10             |
| C40:0-Sph-1G   | 74.5        | C46H92NO8+        | M+H     | 786.5731        | 786.6817         | -2.7           | 3.E+09             |
| C40:1-Sph-1G   | 74.8        | C46H90NO8+        | M+H     | 784.6668        | 784.6661         | 0.9            | 7.E+09             |
| C40:2-Sph-1G   | 74.5        | C46H88NO8+        | M+H     | 782.6511        | 782.6504         | 0.8            | 6.E+10             |
| C40:3-Sph-1G   | 74.8        | C46H86NO8+        | M+H     | 780.6355        | 780.6348         | 0.9            | 6.E+10             |

**(3) Natural chimney sample: NC-HSC**

**PG-DAG**

|              |      |              |       |          |          |     |        |
|--------------|------|--------------|-------|----------|----------|-----|--------|
| C32:0 DAG-PG | 81.8 | C38H79NO10P+ | M+NH4 | 740.5449 | 740.5436 | 1.7 | 5.E+10 |
| C32:1 DAG-PG | 82.3 | C38H77NO10P+ | M+NH4 | 738.5291 | 738.5280 | 1.6 | 6.E+11 |
| C32:2 DAG-PG | 82.8 | C38H75NO10P+ | M+NH4 | 736.5132 | 736.5123 | 1.3 | 2.E+11 |
| C33:1 DAG-PG | 82.0 | C39H79NO10P+ | M+NH4 | 752.5439 | 752.5436 | 0.4 | 1.E+11 |
| C34:0 DAG-PG | 81.2 | C40H83NO10P+ | M+NH4 | 768.5757 | 768.5749 | 1.1 | 2.E+10 |
| C34:1 DAG-PG | 81.6 | C40H81NO10P+ | M+NH4 | 766.5602 | 766.5593 | 1.3 | 6.E+12 |
| C34:2 DAG-PG | 82.2 | C40H79NO10P+ | M+NH4 | 764.5446 | 764.5436 | 1.2 | 4.E+12 |
| C35:1 DAG-PG | 81.4 | C41H83NO10P+ | M+NH4 | 780.5753 | 780.5749 | 0.5 | 1.E+11 |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C35:2 DAG-PG   | 82.0        | C41H81NO10P+      | M+NH4   | 778.5602        | 778.5593         | 1.2            | 8.E+10             |
| C36:0 DAG-PG   | 80.6        | C42H87NO10P+      | M+NH4   | 796.6072        | 796.6062         | 1.3            | 4.E+12             |
| C36:1 DAG-PG   | 81.0        | C42H85NO10P+      | M+NH4   | 794.5916        | 794.5906         | 1.3            | 4.E+12             |
| C36:2 DAG-PG   | 81.5        | C42H83NO10P+      | M+NH4   | 792.5760        | 792.5749         | 1.4            | 1.E+13             |
| C37:1 DAG-PG   | 80.8        | C43H87NO10P+      | M+NH4   | 808.6071        | 808.6062         | 1.1            | 2.E+11             |
| C38:1 DAG-PG   | 80.5        | C44H89NO10P+      | M+NH4   | 822.6222        | 822.6219         | 0.4            | 4.E+10             |
| C38:2 DAG-PG   | 81.0        | C44H87NO10P+      | M+NH4   |                 | 820.6062         |                | 3.E+11             |
| C39:1-DAG-PG   | 81.1        | C45H91NO10P+      | M+NH4   | 836.6376        | 836.6375         | 0.1            | 1.E+11             |
| C39:2-DAG-PG   | 81.5        | C45H89NO10P+      | M+NH4   | 834.6232        | 834.6219         | 1.6            | 3.E+11             |
| C39:3-DAG-PG   | 81.6        | C45H87NO10P+      | M+NH4   | 832.6069        | 832.6062         | 0.9            | 1.E+11             |
| <b>PC-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C32:0 DAG-PC   | 120.0       | C40H81NO8P+       | M+H     | 734.5706        | 734.5694         | 1.6            | 3.E+10             |
| C34:1 DAG-PC   | 119.8       | C42H83NO8P+       | M+H     | 760.5862        | 760.5851         | 1.4            | 1.E+12             |
| C34:2 DAG-PC   | 119.9       | C42H81NO8P+       | M+H     | 758.5703        | 758.5694         | 1.1            | 9.E+10             |
| C35:1 DAG-PC   | 119.4       | C43H85NO8P+       | M+H     | 774.6022        | 774.6007         | 1.9            | 4.E+11             |
| C36:2 DAG-PC   | 119.9       | C44H85NO8P+       | M+H     | 786.6023        | 786.6007         | 2.0            | 3.E+13             |
| C37:2 DAG-PC   | 118.0       | C45H87NO8P+       | M+H     | 800.6174        | 800.6164         | 1.2            | 3.E+11             |
| <b>PE-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PE   | 89.1        | C35H71NO8P+       | M+H     | 664.4927        | 664.4912         | 2.2            | 4.E+12             |
| C30:1 DAG-PE   | 89.7        | C35H69NO8P+       | M+H     | 662.4771        | 662.4755         | 2.4            | 6.E+12             |
| C30:2 DAG-PE   | 90.4        | C35H67NO8P+       | M+H     | 660.4615        | 660.4599         | 2.4            | 7.E+11             |
| C31:0 DAG-PE   | 88.7        | C36H73NO8P+       | M+H     | 678.5087        | 678.5068         | 2.7            | 1.E+11             |
| C31:1 DAG-PE   | 89.3        | C36H71NO8P+       | M+H     | 676.4925        | 676.4912         | 1.9            | 4.E+11             |
| C32:0 DAG-PE   | 88.2        | C37H75NO8P+       | M+H     | 692.5237        | 692.5225         | 1.8            | 2.E+12             |
| C32:1 DAG-PE   | 88.8        | C37H73NO8P+       | M+H     | 690.5084        | 690.5068         | 2.3            | 4.E+13             |
| C32:2 DAG-PE   | 89.5        | C37H71NO8P+       | M+H     | 688.4926        | 688.4912         | 2.0            | 1.E+13             |
| C33:0 DAG-PE   | 88.0        | C38H77NO8P+       | M+H     | 706.5383        | 706.5381         | 0.3            | 7.E+10             |
| C33:1 DAG-PE   | 88.5        | C38H75NO8P+       | M+H     | 704.5239        | 704.5225         | 2.0            | 7.E+11             |
| C33:2 DAG-PE   | 89.0        | C38H73NO8P+       | M+H     | 702.5083        | 702.5068         | 2.1            | 2.E+11             |
| C34:0 DAG-PE   | 87.4        | C39H79NO8P+       | M+H     | 720.5547        | 720.5538         | 1.3            | 2.E+11             |
| C34:1 DAG-PE   | 88.0        | C39H77NO8P+       | M+H     | 718.5395        | 718.5381         | 1.9            | 1.E+13             |
| C34:2 DAG-PE   | 88.6        | C39H75NO8P+       | M+H     | 716.5237        | 716.5225         | 1.7            | 1.E+13             |
| C35:0 DAG-PE   | 87.1        | C40H81NO8P+       | M+H     | 734.5709        | 734.5694         | 1.9            | 2.E+10             |
| C35:1 DAG-PE   | 87.6        | C40H79NO8P+       | M+H     | 732.5552        | 732.5538         | 2.0            | 4.E+11             |
| C35:2 DAG-PE   | 88.3        | C40H77NO8P+       | M+H     | 730.5392        | 730.5381         | 1.5            | 3.E+11             |
| C36:0 DAG-PE   | 86.7        | C41H83NO8P+       | M+H     | 748.5861        | 748.5851         | 1.3            | 1.E+11             |
| C36:1 DAG-PE   | 87.1        | C41H81NO8P+       | M+H     | 746.5708        | 746.5694         | 1.8            | 3.E+12             |
| C36:2 DAG-PE   | 87.8        | C41H79NO8P+       | M+H     | 744.5551        | 744.5538         | 1.8            | 9.E+12             |
| C36:3 DAG-PE   | 88.3        | C41H77NO8P+       | M+H     | 742.5387        | 742.5381         | 0.8            | 2.E+11             |
| C36:4 DAG-PE   | 89.0        | C41H75NO8P+       | M+H     | 740.5234        | 740.5225         | 1.3            | 2.E+11             |
| C37:1 DAG-PE   | 86.8        | C42H83NO8P+       | M+H     | 760.5862        | 760.5851         | 1.4            | 1.E+11             |
| C38:1 DAG-PE   | 86.6        | C43H85NO8P+       | M+H     | 774.6016        | 774.6007         | 1.2            | 2.E+10             |
| C38:2 DAG-PE   | 87.1        | C43H83NO8P+       | M+H     | 772.5858        | 772.5851         | 1.0            | 1.E+11             |
| <b>PME-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PME  | 92.3        | C36H73NO8P+       | M+H     | 678.5091        | 678.5068         | 3.3            | 3.E+10             |
| C32:0 DAG-PME  | 91.3        | C38H77NO8P+       | M+H     | 706.5380        | 706.5381         | -0.2           | 2.E+10             |
| C32:1 DAG-PME  | 91.9        | C38H75NO8P+       | M+H     | 704.5236        | 704.5225         | 1.6            | 5.E+11             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C32:2 DAG-PME  | 92.6        | C38H73NO8P+       | M+H     | 702.5079        | 702.5068         | 1.5            | 2.E+11             |
| C34:2 DAG-PME  | 91.7        | C40H77NO8P+       | M+H     | 730.5393        | 730.5381         | 1.6            | 4.E+11             |
| <b>APT-DAG</b> |             |                   |         |                 |                  |                |                    |
| C32:0-DAG-APT  | 83.3        | C40H81NO11P+      | M+H     | 782.5552        | 782.5542         | 1.3            | 5.E+10             |
| C32:1-DAG-APT  | 84.7        | C40H79NO11P+      | M+H     | 780.5396        | 780.5385         | 1.3            | 2.E+11             |
| C32:2-DAG-APT  | 85.3        | C40H77NO11P+      | M+H     | 778.5239        | 778.5229         | 1.3            | 7.E+10             |
| C34:0-DAG-APT  | 83.8        | C42H85NO11P+      | M+H     | 810.5865        | 810.5855         | 1.2            | 6.E+10             |
| C34:1-DAG-APT  | 84.0        | C42H83NO11P+      | M+H     | 808.5706        | 808.5698         | 0.9            | 2.E+11             |
| C34:2-DAG-APT  | 84.5        | C42H81NO11P+      | M+H     | 806.5551        | 806.5542         | 1.2            | 2.E+11             |
| <b>1G-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-1G   | 73.0        | C39H78NO10+       | M+NH4   | 720.5632        | 720.5620         | 1.7            | 4.E+10             |
| C30:1 DAG-1G   | 73.3        | C39H76NO10+       | M+NH4   | 718.5475        | 718.5464         | 1.6            | 3.E+11             |
| C30:2 DAG-1G   | 73.8        | C39H74NO10+       | M+NH4   | 716.5312        | 716.5307         | 0.6            | 8.E+10             |
| C32:1 DAG-1G   | 72.9        | C41H80NO10+       | M+NH4   | 746.5790        | 746.5777         | 1.8            | 6.E+10             |
| <b>PE-AEG</b>  |             |                   |         |                 |                  |                |                    |
| C31:1-AEG-PE   | 86.5        | C36H73NO7P+       | M+H     | 662.5130        | 662.5119         | 1.6            | 1.E+11             |
| C32:0-AEG-PE   | 85.6        | C37H77NO7P+       | M+H     | 678.5448        | 678.5432         | 2.3            | 3.E+10             |
| C32:1-AEG-PE   | 86.0        | C37H75NO7P+       | M+H     | 676.5284        | 676.5276         | 1.3            | 3.E+11             |
| C32:2-AEG-PE   | 87.3        | C37H73NO7P+       | M+H     | 674.5128        | 674.5119         | 1.2            | 7.E+10             |
| C33:1-AEG-PE   | 85.6        | C38H77NO7P+       | M+H     | 690.5444        | 690.5432         | 1.7            | 5.E+11             |
| C33:2-AEG-PE   | 86.4        | C38H75NO7P+       | M+H     | 688.5288        | 688.5276         | 1.8            | 4.E+10             |
| C34:1-AEG-PE   | 85.8        | C39H79NO7P+       | M+H     | 704.5598        | 704.5589         | 1.3            | 2.E+11             |
| C34:2-AEG-PE   | 86.6        | C39H77NO7P+       | M+H     | 702.5443        | 702.5432         | 1.5            | 5.E+11             |
| <b>PME-AEG</b> |             |                   |         |                 |                  |                |                    |
| C31:0-AEG-PME  | 88.1        | C37H77NO7P+       | M+H     | 678.5445        | 678.5432         | 1.9            | 4.E+10             |
| C31:1-AEG-PME  | 88.8        | C37H75NO7P+       | M+H     | 676.5291        | 676.5276         | 2.3            | 8.E+10             |
| <b>APT-AEG</b> |             |                   |         |                 |                  |                |                    |
| C36:0 AEG-APT  | 86.4        | C44H91NO10P+      | M+H     | 824.6379        | 824.6375         | 0.4            | 4.E+10             |
| C38:1 AEG-APT  | 85.9        | C46H93NO10P+      | M+H     | 850.6541        | 850.6532         | 1.1            | 2.E+10             |
| <b>PE-DEG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PE   | 84.8        | C35H75NO6P+       | M+H     | 636.5334        | 636.5327         | 1.2            | 2.E+11             |
| C30:1-DEG-PE   | 85.3        | C35H73NO6P+       | M+H     | 634.5184        | 634.5170         | 2.2            | 2.E+11             |
| C31:1-DEG-PE   | 85.0        | C36H75NO6P+       | M+H     | 648.5333        | 648.5327         | 1.0            | 1.E+11             |
| C32:0-DEG-PE   | 84.1        | C37H79NO6P+       | M+H     | 664.5651        | 664.5640         | 1.8            | 2.E+10             |
| C32:1-DEG-PE   | 84.6        | C37H77NO6P+       | M+H     | 662.5493        | 662.5483         | 1.5            | 2.E+11             |
| C32:2-DEG-PE   | 85.1        | C37H75NO6P+       | M+H     | 660.5339        | 660.5327         | 1.9            | 1.E+11             |
| C33:0-DEG-PE   | 83.7        | C38H81NO6P+       | M+H     | 678.5802        | 678.5796         | 0.9            | 9.E+10             |
| C33:1-DEG-PE   | 84.2        | C38H79NO6P+       | M+H     | 676.5651        | 676.5640         | 1.6            | 3.E+11             |
| C33:2-DEG-PE   | 84.7        | C38H77NO6P+       | M+H     | 674.5483        | 674.5483         | 2.0            | 8.E+10             |
| C34:0-DEG-PE   | 83.4        | C39H83NO6P+       | M+H     | 692.5961        | 692.5953         | 1.3            | 6.E+11             |
| C34:1-DEG-PE   | 83.9        | C39H81NO6P+       | M+H     | 690.5807        | 690.5796         | 1.6            | 2.E+11             |
| C34:2-DEG-PE   | 84.5        | C39H79NO6P+       | M+H     | 688.5649        | 688.5640         | 1.4            | 1.E+12             |
| C35:0-DEG-PE   | 83.0        | C40H85NO6P+       | M+H     | 706.6119        | 706.6109         | 1.5            | 3.E+11             |
| C35:1-DEG-PE   | 83.5        | C40H83NO6P+       | M+H     | 704.5963        | 704.5953         | 1.4            | 3.E+11             |
| C35:2-DEG-PE   | 84.2        | C40H81NO6P+       | M+H     | 702.5804        | 702.5796         | 1.1            | 1.E+11             |
| C36:0-DEG-PE   | 82.7        | C41H87NO6P+       | M+H     | 720.6276        | 720.6266         | 1.4            | 1.E+11             |
| C36:1-DEG-PE   | 83.1        | C41H85NO6P+       | M+H     | 718.6116        | 718.6109         | 0.9            | 1.E+11             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C36:2-DEG-PE   | 83.6        | C41H83NO6P+       | M+H     | 716.5962        | 716.5953         | 1.3            | 7.E+10             |
| <b>PME-DEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PME  | 86.9        | C36H77NO6P+       | M+H     | 650.5496        | 650.5483         | 1.9            | 2.E+11             |
| C31:0-DEG-PME  | 86.3        | C37H79NO6P+       | M+H     | 664.5655        | 664.5640         | 2.3            | 1.E+11             |
| C31:1-DEG-PME  | 86.9        | C37H77NO6P+       | M+H     | 662.5500        | 662.5483         | 2.6            | 1.E+11             |
| <b>APT-DEG</b> |             |                   |         |                 |                  |                |                    |
| C33:0 DEG-APT  | 85.5        | C41H87NO9P+       | M+H     | 768.6115        | 768.6113         | 0.3            | 4.E+11             |
| C34:0 DEG-APT  | 85.2        | C42H89NO9P+       | M+H     | 782.6279        | 782.6269         | 1.2            | 3.E+12             |
| C34:1 DEG-APT  | 85.8        | C42H87NO9P+       | M+H     | 780.6119        | 780.6113         | 0.7            | 4.E+11             |
| C35:0 DEG-APT  | 84.9        | C43H91NO9P+       | M+H     | 796.6431        | 796.6426         | 0.7            | 2.E+12             |
| C35:1 DEG-APT  | 85.4        | C43H89NO9P+       | M+H     | 794.6282        | 794.6269         | 1.6            | 1.E+12             |
| C36:0 DEG-APT  | 84.6        | C44H93NO9P+       | M+H     | 810.6594        | 810.6582         | 1.4            | 7.E+11             |
| C36:1 DEG-APT  | 85.1        | C44H91NO9P+       | M+H     | 808.6436        | 808.6426         | 1.3            | 7.E+11             |
| C36:2 DEG-APT  | 85.4        | C44H89NO9P+       | M+H     | 806.6280        | 806.6269         | 1.3            | 6.E+10             |

**(4) Natural chimney sample: NC-HRV**

**PG-DAG**

|              |      |              |       |          |          |     |        |
|--------------|------|--------------|-------|----------|----------|-----|--------|
| C30:0 DAG-PG | 82.7 | C36H75NO10P+ | M+NH4 | 712.5136 | 712.5123 | 1.8 | 2.E+11 |
| C30:1 DAG-PG | 83.1 | C36H73NO10P+ | M+NH4 | 710.4979 | 710.4967 | 1.7 | 2.E+11 |
| C30:2 DAG-PG | 83.7 | C36H71NO10P+ | M+NH4 | 708.5546 | 708.4810 | 1.5 | 1.E+10 |
| C31:0 DAG-PG | 82.3 | C37H77NO10P+ | M+NH4 | 726.5295 | 726.5280 | 2.2 | 5.E+10 |
| C31:1 DAG-PG | 82.8 | C37H75NO10P+ | M+NH4 | 724.5134 | 724.5123 | 1.5 | 3.E+11 |
| C31:2 DAG-PG | 83.3 | C37H73NO10P+ | M+NH4 | 722.5703 | 722.5694 | 1.2 | 1.E+10 |
| C32:0 DAG-PG | 82.0 | C38H79NO10P+ | M+NH4 | 740.5444 | 740.5436 | 1.1 | 4.E+10 |
| C32:1 DAG-PG | 82.3 | C38H77NO10P+ | M+NH4 | 738.5295 | 738.5280 | 2.1 | 1.E+12 |
| C32:2 DAG-PG | 82.9 | C38H75NO10P+ | M+NH4 | 736.5135 | 736.5123 | 1.6 | 8.E+11 |
| C32:3 DAG-PG | 83.4 | C38H73NO10P+ | M+NH4 | 734.4974 | 734.4967 | 1.1 | 2.E+10 |
| C33:0 DAG-PG | 81.6 | C39H81NO10P+ | M+NH4 | 754.5597 | 754.5593 | 0.6 | 2.E+10 |
| C33:1 DAG-PG | 82.1 | C39H79NO10P+ | M+NH4 | 752.5444 | 752.5436 | 1.0 | 2.E+11 |
| C33:2 DAG-PG | 82.5 | C39H77NO10P+ | M+NH4 | 750.5287 | 750.5280 | 1.0 | 2.E+11 |
| C34:0 DAG-PG | 81.4 | C40H83NO10P+ | M+NH4 | 768.5753 | 768.5749 | 0.4 | 4.E+09 |
| C34:1 DAG-PG | 81.7 | C40H81NO10P+ | M+NH4 | 766.5601 | 766.5593 | 1.0 | 2.E+11 |
| C34:2 DAG-PG | 82.2 | C40H79NO10P+ | M+NH4 | 764.5447 | 764.5436 | 1.4 | 2.E+12 |
| C34:3 DAG-PG | 82.8 | C40H77NO10P+ | M+NH4 | 762.5288 | 762.5280 | 1.1 | 1.E+11 |
| C34:4 DAG-PG | 83.4 | C40H75NO10P+ | M+NH4 | 760.5130 | 760.5123 | 0.9 | 1.E+10 |
| C35:1 DAG-PG | 81.5 | C41H83NO10P+ | M+NH4 | 780.5759 | 780.5749 | 1.2 | 7.E+10 |
| C35:2 DAG-PG | 81.9 | C41H81NO10P+ | M+NH4 | 778.5601 | 778.5593 | 1.1 | 1.E+11 |
| C36:1 DAG-PG | 81.1 | C42H85NO10P+ | M+NH4 | 794.5912 | 794.5906 | 0.9 | 1.E+10 |
| C36:2 DAG-PG | 81.6 | C42H83NO10P+ | M+NH4 | 792.5757 | 792.5749 | 1.1 | 3.E+11 |
| C36:3 DAG-PG | 82.0 | C42H81NO10P+ | M+NH4 | 790.5599 | 790.5593 | 0.9 | 1.E+11 |
| C36:4 DAG-PG | 82.5 | C42H79NO10P+ | M+NH4 | 788.5442 | 788.5436 | 0.7 | 3.E+10 |

**PC-DAG**

|              |       |             |     |          |          |     |        |
|--------------|-------|-------------|-----|----------|----------|-----|--------|
| C33:0 DAG-PC | 119.4 | C41H83NO8P+ | M+H | 748.5864 | 748.5851 | 1.7 | 2.E+11 |
| C33:1 DAG-PC | 120.0 | C41H81NO8P+ | M+H | 746.5715 | 746.5694 | 2.1 | 2.E+12 |
| C34:0 DAG-PC | 117.6 | C42H85NO8P+ | M+H | 762.6013 | 762.6007 | 0.1 | 6.E+10 |
| C34:1 DAG-PC | 119.8 | C42H83NO8P+ | M+H | 760.5851 | 760.5851 | 1.8 | 2.E+13 |
| C34:2 DAG-PC | 119.8 | C42H81NO8P+ | M+H | 758.5715 | 758.5694 | 2.7 | 9.E+11 |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C35:1 DAG-PC  | 118.5       | C43H85NO8P+       | M+H     | 774.6022        | 774.6007         | 1.9            | 1.E+12             |
| C35:2 DAG-PC  | 119.8       | C43H83NO8P+       | M+H     | 772.5862        | 772.5851         | 1.4            | 3.E+11             |
| C36:1 DAG-PC  | 115.6       | C44H87NO8P+       | M+H     | 788.6172        | 788.6164         | 1.0            | 3.E+12             |
| C36:2 DAG-PC  | 119.1       | C44H85NO8P+       | M+H     | 786.6015        | 786.6007         | 1.0            | 1.E+13             |
| C36:3 DAG-PC  | 120.0       | C44H83NO8P+       | M+H     | 784.5864        | 784.5851         | 1.6            | 2.E+12             |
| C37:2 DAG-PC  | 117.1       | C45H87NO8P+       | M+H     | 800.6175        | 800.6164         | 1.4            | 4.E+11             |
| C37:3 DAG-PC  | 119.1       | C45H85NO8P+       | M+H     | 798.6016        | 798.6007         | 1.1            | 1.E+11             |
| C38:1 DAG-PC  | 111.6       | C46H91NO8P+       | M+H     | 816.6491        | 816.6477         | 1.7            | 6.E+10             |
| C38:2 DAG-PC  | 114.7       | C46H89NO8P+       | M+H     | 814.6332        | 814.6320         | 1.4            | 2.E+12             |
| C38:3 DAG-PC  | 116.7       | C46H87NO8P+       | M+H     | 812.6176        | 812.6164         | 1.4            | 4.E+12             |
| C38:4 DAG-PC  | 119.9       | C46H85NO8P+       | M+H     | 810.6017        | 810.6007         | 1.3            | 1.E+12             |
| C38:5 DAG-PC  | 119.8       | C46H83NO8P+       | M+H     | 808.5853        | 808.5851         | 0.3            | 2.E+11             |
| C40:2 DAG-PC  | 110.2       | C48H93NO8P+       | M+H     | 842.6647        | 842.6633         | 1.7            | 9.E+10             |
| C40:3 DAG-PC  | 113.1       | C48H91NO8P+       | M+H     | 840.6488        | 840.6477         | 1.3            | 3.E+11             |
| C40:4 DAG-PC  | 115.3       | C48H89NO8P+       | M+H     | 838.6331        | 838.6320         | 1.2            | 3.E+11             |
| <b>PE-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PE  | 89.3        | C35H71NO8P+       | M+H     | 664.4932        | 664.4912         | 3.1            | 8.E+11             |
| C30:1 DAG-PE  | 89.9        | C35H69NO8P+       | M+H     | 662.4775        | 662.4755         | 3.0            | 3.E+12             |
| C30:2 DAG-PE  | 90.6        | C35H67NO8P+       | M+H     | 660.4618        | 660.4599         | 2.9            | 5.E+11             |
| C31:0 DAG-PE  | 88.9        | C36H73NO8P+       | M+H     | 678.5085        | 678.5068         | 2.5            | 3.E+11             |
| C31:1 DAG-PE  | 89.5        | C36H71NO8P+       | M+H     | 676.4930        | 676.4912         | 2.7            | 2.E+12             |
| C31:2 DAG-PE  | 90.0        | C36H69NO8P+       | M+H     | 674.4771        | 674.4755         | 2.3            | 2.E+11             |
| C32:0 DAG-PE  | 88.5        | C37H75NO8P+       | M+H     | 692.5237        | 692.5225         | 1.8            | 2.E+11             |
| C32:1 DAG-PE  | 88.9        | C37H73NO8P+       | M+H     | 690.5085        | 690.5068         | 2.5            | 7.E+12             |
| C32:2 DAG-PE  | 89.7        | C37H71NO8P+       | M+H     | 688.4930        | 688.4912         | 2.6            | 7.E+12             |
| C32:3 DAG-PE  | 90.5        | C37H69NO8P+       | M+H     | 686.4770        | 686.4755         | 2.1            | 2.E+11             |
| C32:4 DAG-PE  | 91.2        | C37H67NO8P+       | M+H     | 684.4611        | 684.4599         | 1.7            | 2.E+10             |
| C33:0 DAG-PE  | 88.0        | C38H77NO8P+       | M+H     | 706.5396        | 706.5381         | 2.1            | 2.E+11             |
| C33:1 DAG-PE  | 88.5        | C38H75NO8P+       | M+H     | 704.5240        | 704.5225         | 2.2            | 1.E+12             |
| C33:2 DAG-PE  | 89.2        | C38H73NO8P+       | M+H     | 702.5082        | 702.5068         | 1.9            | 1.E+12             |
| C34:0 DAG-PE  | 87.7        | C39H79NO8P+       | M+H     | 720.5549        | 720.5538         | 1.6            | 3.E+10             |
| C34:1 DAG-PE  | 88.1        | C39H77NO8P+       | M+H     | 718.5392        | 718.5381         | 1.6            | 5.E+11             |
| C34:2 DAG-PE  | 88.7        | C39H75NO8P+       | M+H     | 716.5240        | 716.5225         | 2.1            | 6.E+12             |
| C34:3 DAG-PE  | 89.3        | C39H73NO8P+       | M+H     | 714.5081        | 714.5068         | 1.7            | 2.E+11             |
| C35:1 DAG-PE  | 87.8        | C40H79NO8P+       | M+H     | 732.5550        | 732.5538         | 1.7            | 1.E+11             |
| C35:2 DAG-PE  | 88.5        | C40H77NO8P+       | M+H     | 730.5393        | 730.5381         | 1.6            | 5.E+11             |
| C36:1 DAG-PE  | 87.5        | C41H81NO8P+       | M+H     | 746.5706        | 746.5694         | 1.6            | 2.E+11             |
| C36:2 DAG-PE  | 88.0        | C41H79NO8P+       | M+H     | 744.5553        | 744.5538         | 2.0            | 9.E+11             |
| C36:3 DAG-PE  | 88.3        | C41H77NO8P+       | M+H     | 742.5395        | 742.5381         | 1.8            | 5.E+11             |
| C36:4 DAG-PE  | 88.9        | C41H75NO8P+       | M+H     | 740.5235        | 740.5225         | 1.4            | 1.E+11             |
| C38:2 DAG-PE  | 86.9        | C43H83NO8P+       | M+H     | 772.5861        | 772.5851         | 1.3            | 1.E+11             |
| C38:3 DAG-PE  | 87.4        | C43H81NO8P+       | M+H     | 770.5701        | 770.5694         | 0.9            | 1.E+11             |
| C38:5 DAG-PE  | 88.7        | C43H77NO8P+       | M+H     | 766.5392        | 766.5381         | 1.5            | 2.E+11             |
| C38:6 DAG-PE  | 89.4        | C43H75NO8P+       | M+H     | 764.5231        | 764.5225         | 0.9            | 1.E+11             |
| C40:2 DAG-PE  | 86.2        | C45H87NO8P+       | M+H     | 800.6177        | 800.6164         | 1.7            | 4.E+10             |
| C40:3 DAG-PE  | 86.7        | C45H85NO8P+       | M+H     | 798.6015        | 798.6007         | 1.0            | 6.E+10             |
| C40:4 DAG-PE  | 87.3        | C45H83NO8P+       | M+H     | 796.5856        | 796.5851         | 0.6            | 3.E+10             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds       | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C40:5 DAG-PE    | 87.9        | C45H81NO8P+       | M+H     | 794.5703        | 794.5694         | 1.1            | 3.E+10             |
| C40:6 DAG-PE    | 88.7        | C45H79NO8P+       | M+H     | 792.5552        | 792.5538         | 1.8            | 1.E+11             |
| <b>PME-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C29:0 DAG-PME   | 93.1        | C35H71NO8P+       | M+H     | 664.4932        | 664.4912         | 3.0            | 7.E+10             |
| C30:0 DAG-PME   | 92.8        | C36H73NO8P+       | M+H     | 678.5085        | 678.5068         | 2.5            | 4.E+11             |
| C30:1 DAG-PME   | 93.0        | C36H71NO8P+       | M+H     | 676.4925        | 676.4912         | 1.9            | 3.E+11             |
| C31:0 DAG-PME   | 92.0        | C37H75NO8P+       | M+H     | 692.5244        | 692.5225         | 2.8            | 2.E+11             |
| C31:1 DAG-PME   | 92.8        | C37H73NO8P+       | M+H     | 690.5087        | 690.5068         | 2.7            | 7.E+11             |
| C32:0 DAG-PME   | 91.6        | C38H77NO8P+       | M+H     | 706.5400        | 706.5381         | 2.6            | 1.E+11             |
| C32:1 DAG-PME   | 92.1        | C38H75NO8P+       | M+H     | 704.5244        | 704.5225         | 2.7            | 3.E+12             |
| C32:2 DAG-PME   | 92.8        | C38H73NO8P+       | M+H     | 702.5086        | 702.5068         | 2.5            | 2.E+12             |
| C33:0 DAG-PME   | 91.1        | C39H79NO8P+       | M+H     | 720.5538        | 720.5538         | 2.2            | 4.E+10             |
| C33:1 DAG-PME   | 91.6        | C39H77NO8P+       | M+H     | 718.5400        | 718.5381         | 2.7            | 3.E+11             |
| C34:1 DAG-PME   | 91.1        | C40H79NO8P+       | M+H     | 732.5553        | 732.5538         | 2.1            | 3.E+11             |
| C34:2 DAG-PME   | 91.7        | C40H77NO8P+       | M+H     | 730.5400        | 730.5381         | 2.5            | 5.E+12             |
| C34:3 DAG-PME   | 92.6        | C40H75NO8P+       | M+H     | 728.5240        | 728.5225         | 2.1            | 1.E+11             |
| C35:1 DAG-PME   | 90.5        | C41H81NO8P+       | M+H     | 746.5710        | 746.5694         | 2.2            | 6.E+10             |
| C35:2 DAG-PME   | 91.4        | C41H79NO8P+       | M+H     | 744.5557        | 744.5538         | 2.5            | 2.E+11             |
| C36:2 DAG-PME   | 90.8        | C42H81NO8P+       | M+H     | 758.5707        | 758.5694         | 1.7            | 1.E+12             |
| C36:3 DAG-PME   | 91.2        | C42H79NO8P+       | M+H     | 756.5553        | 756.5538         | 2.0            | 4.E+11             |
| C37:2 DAG-PME   | 90.5        | C43H83NO8P+       | M+H     | 772.5861        | 772.5851         | 1.3            | 4.E+10             |
| C37:3 DAG-PME   | 90.8        | C43H81NO8P+       | M+H     | 770.5705        | 770.5694         | 1.4            | 4.E+10             |
| <b>PDME-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PDME  | 95.5        | C37H75NO8P+       | M+H     | 692.5247        | 692.5225         | 3.2            | 8.E+10             |
| C30:1 DAG-PDME  | 95.8        | C37H73NO8P+       | M+H     | 690.5092        | 690.5068         | 3.5            | 6.E+11             |
| C31:0 DAG-PDME  | 94.7        | C38H77NO8P+       | M+H     | 706.5404        | 706.5381         | 3.2            | 3.E+10             |
| C31:1 DAG-PDME  | 95.1        | C38H75NO8P+       | M+H     | 704.5245        | 704.5225         | 2.8            | 2.E+11             |
| C32:0 DAG-PDME  | 94.0        | C39H79NO8P+       | M+H     | 720.5557        | 720.5538         | 2.7            | 6.E+10             |
| C32:1 DAG-PDME  | 94.7        | C39H77NO8P+       | M+H     | 718.5402        | 718.5381         | 2.9            | 6.E+12             |
| C32:2 DAG-PDME  | 95.5        | C39H75NO8P+       | M+H     | 716.5240        | 716.5225         | 2.6            | 4.E+12             |
| C33:1 DAG-PDME  | 94.3        | C40H79NO8P+       | M+H     | 732.5557        | 732.5538         | 2.6            | 1.E+11             |
| C34:1 DAG-PDME  | 93.4        | C41H81NO8P+       | M+H     | 746.5710        | 746.5694         | 2.1            | 3.E+11             |
| C34:2 DAG-PDME  | 94.3        | C41H79NO8P+       | M+H     | 744.5559        | 744.5538         | 2.9            | 6.E+12             |
| C34:3 DAG-PDME  | 94.7        | C41H77NO8P+       | M+H     | 742.5397        | 742.5381         | 2.2            | 4.E+10             |
| C36:2 DAG-PDME  | 93.2        | C43H83NO8P+       | M+H     | 772.5862        | 772.5851         | 1.5            | 2.E+11             |
| C36:3 DAG-PDME  | 93.4        | C43H81NO8P+       | M+H     | 770.5709        | 770.5694         | 1.9            | 2.E+11             |
| C36:4 DAG-PDME  | 94.3        | C43H79NO8P+       | M+H     | 768.5552        | 768.5538         | 1.9            | 2.E+10             |
| C36:5 DAG-PDME  | 94.5        | C43H77NO8P+       | M+H     | 766.5374        | 766.5381         | -0.9           | 1.E+11             |
| <b>APT-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C30:1-DAG-APT   | 85.7        | C38H75NO11P+      | M+H     | 752.5087        | 752.5072         | 1.9            | 5.E+09             |
| C30:2-DAG-APT   | 86.3        | C38H73NO11P+      | M+H     | 750.4924        | 750.4916         | 1.1            | 2.E+10             |
| C32:1-DAG-APT   | 84.8        | C40H79NO11P+      | M+H     | 780.5392        | 780.5385         | 0.9            | 6.E+09             |
| C32:2-DAG-APT   | 85.4        | C40H77NO11P+      | M+H     | 778.5240        | 778.5229         | 1.5            | 5.E+09             |
| <b>DGTS</b>     |             |                   |         |                 |                  |                |                    |
| C32:0-DGTS      | 84.0        | C42H82NO7+        | M+H     | 712.6097        | 712.6086         | 1.5            | 3.E+10             |
| C32:1-DGTS      | 84.5        | C42H80NO7+        | M+H     | 710.5929        | 710.5929         | 1.0            | 3.E+11             |
| C32:2-DGTS      | 85.1        | C42H78NO7+        | M+H     | 708.5778        | 708.5773         | 0.8            | 3.E+10             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:0-DGTS    | 83.1        | C44H86NO7+        | M+H     | 740.6411        | 740.6399         | 1.7            | 5.E+09             |
| C34:1-DGTS    | 83.6        | C44H84NO7+        | M+H     | 738.6254        | 738.6242         | 1.5            | 2.E+11             |
| C34:2-DGTS    | 84.2        | C44H82NO7+        | M+H     | 736.6086        | 736.6086         | 1.6            | 2.E+11             |
| C36:1-DGTS    | 82.8        | C46H88NO7+        | M+H     | 766.6556        | 766.6555         | 0.1            | 2.E+10             |
| C36:2-DGTS    | 83.4        | C46H86NO7+        | M+H     | 764.6405        | 764.6399         | 0.8            | 6.E+11             |
| C36:3-DGTS    | 83.8        | C46H84NO7+        | M+H     | 762.6249        | 762.6242         | 0.9            | 2.E+11             |
| <b>1G-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-1G  | 73.0        | C39H78NO10+       | M+NH4   | 720.5636        | 720.5620         | 2.2            | 1.E+11             |
| C30:1 DAG-1G  | 72.5        | C39H76NO10+       | M+NH4   | 718.5474        | 718.5464         | 1.5            | 8.E+10             |
| C32:1 DAG-1G  | 72.1        | C41H80NO10+       | M+NH4   | 746.5785        | 746.5777         | 1.1            | 7.E+10             |
| C32:2 DAG-1G  | 72.4        | C41H78NO10+       | M+NH4   | 744.5631        | 744.5620         | 1.5            | 1.E+11             |
| C34:0 DAG-1G  | 72.8        | C43H86NO10+       | M+NH4   | 776.6253        | 776.6246         | 0.9            | 3.E+10             |
| C36:2 DAG-1G  | 72.5        | C45H86NO10+       | M+NH4   | 800.6257        | 800.6246         | 1.3            | 9.E+10             |
| C38:2 DAG-1G  | 72.1        | C47H90NO10+       | M+NH4   | 828.6568        | 828.6559         | 1.1            | 4.E+10             |
| <b>PG-AEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-AEG-PG  | 80.5        | C36H74O9P+        | M+H     | 681.5075        | 681.5065         | 1.5            | 4.E+10             |
| C30:1-AEG-PG  | 80.8        | C36H72O9P+        | M+H     | 679.4916        | 679.4908         | 1.1            | 4.E+10             |
| C31:0-AEG-PG  | 80.0        | C37H76O9P+        | M+H     | 695.5231        | 695.5221         | 1.4            | 1.E+11             |
| C31:1-AEG-PG  | 80.5        | C37H74O9P+        | M+H     | 693.5074        | 693.5065         | 1.4            | 9.E+10             |
| C31:2-AEG-PG  | 81.1        | C37H72O9P+        | M+H     | 691.4924        | 691.4908         | 2.2            | 3.E+09             |
| C32:0-AEG-PG  | 79.8        | C39H78O9P+        | M+H     | 709.5386        | 709.5378         | 1.1            | 1.E+10             |
| C32:1-AEG-PG  | 80.1        | C39H76O9P+        | M+H     | 707.5229        | 707.5221         | 1.1            | 8.E+10             |
| C32:2-AEG-PG  | 80.7        | C39H74O9P+        | M+H     | 705.5073        | 705.5065         | 1.1            | 2.E+10             |
| C33:0-AEG-PG  | 79.5        | C40H80O9P+        | M+H     | 723.5543        | 723.5534         | 1.1            | 5.E+09             |
| C33:1-AEG-PG  | 79.7        | C40H78O9P+        | M+H     | 721.5386        | 721.5378         | 1.1            | 1.E+11             |
| C33:2-AEG-PG  | 80.4        | C40H76O9P+        | M+H     | 719.5227        | 719.5221         | 0.8            | 2.E+10             |
| C34:1-AEG-PG  | 79.5        | C40H80O9P+        | M+H     | 735.5548        | 735.5534         | 1.9            | 1.E+10             |
| C34:2-AEG-PG  | 80.2        | C40H78O9P+        | M+H     | 733.5387        | 733.5378         | 1.2            | 5.E+10             |
| <b>PC-AEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-AEG-PC  | 113.0       | C38H79NO7P+       | M+H     | 692.5604        | 692.5589         | 2.2            | 4.E+11             |
| C31:0-AEG-PC  | 110.8       | C39H81NO7P+       | M+H     | 706.5762        | 706.5745         | 2.3            | 6.E+10             |
| C32:0-AEG-PC  | 109.9       | C40H83NO7P+       | M+H     | 720.5921        | 720.5902         | 2.7            | 9.E+11             |
| C32:1-AEG-PC  | 112.2       | C40H81NO7P+       | M+H     | 718.5764        | 718.5745         | 2.6            | 1.E+12             |
| C32:2-AEG-PC  | 114.9       | C40H79NO7P+       | M+H     | 716.5602        | 716.5589         | 1.9            | 9.E+11             |
| C33:1-AEG-PC  | 110.8       | C41H83NO7P+       | M+H     | 732.5919        | 732.5902         | 2.4            | 1.E+12             |
| C34:1-AEG-PC  | 108.4       | C42H85NO7P+       | M+H     | 746.6077        | 746.6058         | 2.6            | 2.E+12             |
| C34:2-AEG-PC  | 111.4       | C42H83NO7P+       | M+H     | 744.5923        | 744.5902         | 2.8            | 2.E+12             |
| C34:3-AEG-PC  | 113.5       | C42H81NO7P+       | M+H     | 742.5762        | 742.5745         | 2.3            | 3.E+11             |
| C34:4-AEG-PC  | 117.1       | C42H79NO7P+       | M+H     | 740.5609        | 740.5589         | 2.8            | 6.E+10             |
| C35:1-AEG-PC  | 106.8       | C43H87NO7P+       | M+H     | 760.6226        | 760.6215         | 1.4            | 1.E+11             |
| C36:1-AEG-PC  | 104.8       | C44H89NO7P+       | M+H     | 774.6393        | 774.6371         | 2.8            | 3.E+11             |
| C36:2-AEG-PC  | 108.2       | C44H87NO7P+       | M+H     | 772.6234        | 772.6215         | 2.5            | 1.E+12             |
| C36:3-AEG-PC  | 110.0       | C44H85NO7P+       | M+H     | 770.6079        | 770.6058         | 2.7            | 4.E+11             |
| C36:4-AEG-PC  | 111.9       | C44H83NO7P+       | M+H     | 768.5921        | 768.5902         | 2.5            | 4.E+11             |
| C38:1-AEG-PC  | 103.7       | C46H93NO7P+       | M+H     | 802.6714        | 802.6684         | 3.8            | 3.E+10             |
| C38:2-AEG-PC  | 104.6       | C46H91NO7P+       | M+H     | 800.6544        | 800.6528         | 2.1            | 3.E+11             |
| C38:3-AEG-PC  | 106.3       | C46H89NO7P+       | M+H     | 798.6387        | 798.6371         | 1.9            | 1.E+11             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds       | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C38:4-AEG-PC    | 108.6       | C46H87NO7P+       | M+H     | 796.6227        | 796.6215         | 1.6            | 2.E+11             |
| C40:2-AEG-PC    | 103.3       | C48H95NO7P+       | M+H     | 828.6857        | 828.6841         | 2.0            | 2.E+11             |
| C40:3-AEG-PC    | 104.4       | C48H93NO7P+       | M+H     | 826.6703        | 826.6684         | 2.3            | 3.E+11             |
| C40:4-AEG-PC    | 105.0       | C48H91NO7P+       | M+H     | 824.6548        | 824.6528         | 2.5            | 9.E+10             |
| <b>PE-AEG</b>   |             |                   |         |                 |                  |                |                    |
| C30:1-AEG-PE    | 86.9        | C35H71NO7P+       | M+H     | 648.4981        | 648.4963         | 2.8            | 3.E+11             |
| C30:2-AEG-PE    | 88.5        | C35H69NO7P+       | M+H     | 646.4823        | 646.4806         | 2.6            | 7.E+10             |
| C31:1-AEG-PE    | 86.6        | C36H73NO7P+       | M+H     | 662.5139        | 662.5119         | 2.9            | 2.E+12             |
| C31:2-AEG-PE    | 87.3        | C36H71NO7P+       | M+H     | 660.4980        | 660.4963         | 2.7            | 8.E+10             |
| C32:0-AEG-PE    | 88.5        | C37H77NO7P+       | M+H     | 678.5432        | 678.5432         | 2.2            | 3.E+11             |
| C32:1-AEG-PE    | 86.2        | C37H75NO7P+       | M+H     | 676.5276        | 676.5276         | 1.9            | 1.E+12             |
| C32:2-AEG-PE    | 86.8        | C37H73NO7P+       | M+H     | 674.5132        | 674.5119         | 2.0            | 7.E+11             |
| C32:3-AEG-PE    | 87.4        | C37H71NO7P+       | M+H     | 672.4979        | 672.4963         | 2.4            | 2.E+10             |
| C33:0-AEG-PE    | 85.3        | C38H79NO7P+       | M+H     | 692.5599        | 692.5589         | 1.5            | 2.E+10             |
| C33:1-AEG-PE    | 85.8        | C38H77NO7P+       | M+H     | 690.5449        | 690.5432         | 2.4            | 4.E+12             |
| C33:2-AEG-PE    | 86.5        | C38H75NO7P+       | M+H     | 688.5291        | 688.5279         | 2.2            | 7.E+11             |
| C34:1-AEG-PE    | 85.5        | C39H79NO7P+       | M+H     | 704.5600        | 704.5589         | 1.6            | 1.E+11             |
| C34:2-AEG-PE    | 86.1        | C39H77NO7P+       | M+H     | 702.5447        | 702.5432         | 2.1            | 2.E+12             |
| C35:1-AEG-PE    | 85.1        | C40H81NO7P+       | M+H     | 718.5758        | 718.5745         | 1.8            | 1.E+11             |
| C35:2-AEG-PE    | 85.7        | C40H79NO7P+       | M+H     | 716.5601        | 716.5589         | 1.7            | 4.E+11             |
| C36:1-AEG-PE    | 84.6        | C41H83NO7P+       | M+H     | 732.5912        | 732.5902         | 1.4            | 1.E+10             |
| C36:2-AEG-PE    | 85.3        | C41H81NO7P+       | M+H     | 730.5757        | 730.5745         | 1.7            | 7.E+11             |
| C36:3-AEG-PE    | 85.8        | C41H79NO7P+       | M+H     | 728.5607        | 728.5589         | 2.5            | 2.E+10             |
| C36:4-AEG-PE    | 86.3        | C41H77NO7P+       | M+H     | 726.5446        | 726.5432         | 1.8            | 7.E+09             |
| C36:5-AEG-PE    | 86.2        | C41H75NO7P+       | M+H     | 724.5258        | 724.5276         | -2.4           | 5.E+10             |
| <b>PME-AEG</b>  |             |                   |         |                 |                  |                |                    |
| C33:0-AEG-PME   | 87.6        | C39H81NO7P+       | M+H     | 706.5756        | 706.5745         | 1.6            | 2.E+10             |
| C33:1-AEG-PME   | 88.0        | C39H79NO7P+       | M+H     | 704.5609        | 704.5589         | 2.9            | 4.E+11             |
| C33:3-AEG-PME   | 88.3        | C39H75NO7P+       | M+H     | 700.5264        | 700.5276         | -1.6           | 1.E+10             |
| C33:4-AEG-PME   | 89.0        | C39H73NO7P+       | M+H     | 698.5110        | 698.5119         | -1.3           | 3.E+10             |
| C34:1-AEG-PME   | 87.6        | C40H81NO7P+       | M+H     | 718.5756        | 718.5745         | 1.5            | 3.E+10             |
| C34:2-AEG-PME   | 88.5        | C40H79NO7P+       | M+H     | 716.5590        | 716.5589         | 0.2            | 2.E+11             |
| C35:1-AEG-PME   | 87.1        | C41H83NO7P+       | M+H     | 732.5917        | 732.5902         | 2.1            | 5.E+10             |
| <b>PDME-AEG</b> |             |                   |         |                 |                  |                |                    |
| C32:0-AEG-PDME  | 90.0        | C39H81NO7P+       | M+H     | 706.5751        | 706.5745         | 0.8            | 3.E+10             |
| C33:1-AEG-PDME  | 90.1        | C40H81NO7P+       | M+H     | 718.5761        | 718.5745         | 2.2            | 4.E+11             |
| C34:2-AEG-PDME  | 90.5        | C41H81NO7P+       | M+H     | 730.5762        | 730.5745         | 2.3            | 2.E+11             |
| <b>PG-DEG</b>   |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PG    | 78.9        | C36H76O8P+        | M+H     | 667.5286        | 667.5272         | 2.1            | 3.E+11             |
| C30:1-DEG-PG    | 79.2        | C36H74O8P+        | M+H     | 665.5129        | 665.5116         | 2.0            | 1.E+11             |
| C30:2-DEG-PG    | 79.7        | C36H72O8P+        | M+H     | 663.4971        | 663.4959         | 1.7            | 4.E+09             |
| C31:0-DEG-PG    | 78.5        | C37H78O8P+        | M+H     | 681.5439        | 681.5429         | 1.6            | 8.E+10             |
| C31:1-DEG-PG    | 78.9        | C37H76O8P+        | M+H     | 679.5280        | 679.5272         | 1.2            | 2.E+11             |
| C31:2-DEG-PG    | 79.4        | C37H74O8P+        | M+H     | 677.5120        | 677.5116         | 0.6            | 9.E+09             |
| C32:0-DEG-PG    | 78.3        | C38H80O8P+        | M+H     | 695.5594        | 695.5585         | 1.2            | 1.E+11             |
| C32:1-DEG-PG    | 78.6        | C38H78O8P+        | M+H     | 693.5439        | 693.5429         | 1.4            | 3.E+11             |
| C32:2-DEG-PG    | 79.0        | C38H76O8P+        | M+H     | 691.5277        | 691.5272         | 0.7            | 1.E+11             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C33:0-DEG-PG   | 78.1        | C39H82O8P+        | M+H     | 709.5750        | 709.5742         | 1.2            | 1.E+10             |
| C33:1-DEG-PG   | 78.3        | C39H80O8P+        | M+H     | 707.5598        | 707.5585         | 1.8            | 3.E+11             |
| C33:2-DEG-PG   | 78.8        | C39H78O8P+        | M+H     | 705.5438        | 705.5429         | 1.3            | 2.E+11             |
| C34:0-DEG-PG   | 77.8        | C40H84O8P+        | M+H     | 723.5906        | 723.5898         | 1.0            | 7.E+10             |
| C34:1-DEG-PG   | 78.1        | C40H82O8P+        | M+H     | 721.5748        | 721.5742         | 0.9            | 4.E+10             |
| C34:2-DEG-PG   | 78.6        | C40H80O8P+        | M+H     | 719.5593        | 719.5585         | 1.1            | 7.E+11             |
| C35:0-DEG-PG   | 77.6        | C41H86O8P+        | M+H     | 737.6060        | 737.6055         | 0.7            | 9.E+09             |
| C35:1-DEG-PG   | 77.9        | C41H84O8P+        | M+H     | 735.5906        | 735.5898         | 1.1            | 3.E+10             |
| C35:2-DEG-PG   | 78.3        | C41H82O8P+        | M+H     | 733.5753        | 733.5742         | 1.5            | 1.E+11             |
| <b>PE-DEG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PE   | 85.0        | C35H75NO6P+       | M+H     | 636.5342        | 636.5327         | 2.4            | 1.E+12             |
| C30:1-DEG-PE   | 85.4        | C35H73NO6P+       | M+H     | 634.5186        | 634.5170         | 2.4            | 2.E+12             |
| C30:2-DEG-PE   | 86.0        | C35H71NO6P+       | M+H     | 632.5029        | 632.5014         | 2.4            | 3.E+11             |
| C30:3-DEG-PE   | 86.9        | C35H69NO6P+       | M+H     | 630.4871        | 630.4857         | 2.3            | 6.E+09             |
| C31:0-DEG-PE   | 84.5        | C36H77NO6P+       | M+H     | 650.5499        | 650.5483         | 2.4            | 2.E+11             |
| C31:1-DEG-PE   | 85.0        | C36H75NO6P+       | M+H     | 648.5342        | 648.5327         | 2.4            | 2.E+12             |
| C31:2-DEG-PE   | 85.6        | C36H73NO6P+       | M+H     | 646.5183        | 646.5170         | 2.0            | 1.E+11             |
| C32:0-DEG-PE   | 84.0        | C37H79NO6P+       | M+H     | 664.5656        | 664.5640         | 2.5            | 2.E+11             |
| C32:1-DEG-PE   | 84.7        | C37H77NO6P+       | M+H     | 662.5501        | 662.5483         | 2.7            | 1.E+12             |
| C32:2-DEG-PE   | 85.3        | C37H75NO6P+       | M+H     | 660.5342        | 660.5327         | 2.3            | 2.E+12             |
| C32:3-DEG-PE   | 85.9        | C37H73NO6P+       | M+H     | 658.5186        | 658.5170         | 2.5            | 3.E+10             |
| C32:4-DEG-PE   | 86.7        | C37H71NO6P+       | M+H     | 656.5032        | 656.5014         | 2.9            | 1.E+10             |
| C33:0-DEG-PE   | 83.8        | C38H81NO6P+       | M+H     | 678.5803        | 678.5796         | 1.1            | 1.E+10             |
| C33:1-DEG-PE   | 84.3        | C38H79NO6P+       | M+H     | 676.5653        | 676.5640         | 2.0            | 3.E+12             |
| C33:2-DEG-PE   | 84.9        | C38H77NO6P+       | M+H     | 674.5497        | 674.5483         | 2.0            | 1.E+12             |
| C34:0-DEG-PE   | 83.4        | C39H83NO6P+       | M+H     | 692.5963        | 692.5953         | 1.4            | 3.E+10             |
| C34:1-DEG-PE   | 83.8        | C39H81NO6P+       | M+H     | 690.5807        | 690.5796         | 1.6            | 2.E+11             |
| C34:2-DEG-PE   | 84.6        | C39H79NO6P+       | M+H     | 688.5654        | 688.5640         | 2.1            | 7.E+12             |
| C34:3-DEG-PE   | 85.2        | C39H77NO6P+       | M+H     | 686.5496        | 686.5483         | 1.9            | 2.E+10             |
| C35:1-DEG-PE   | 83.4        | C40H83NO6P+       | M+H     | 704.5964        | 704.5953         | 1.7            | 5.E+11             |
| C35:2-DEG-PE   | 84.2        | C40H81NO6P+       | M+H     | 702.5807        | 702.5769         | 1.6            | 1.E+12             |
| C36:2-DEG-PE   | 83.8        | C41H83NO6P+       | M+H     | 716.5962        | 716.5953         | 1.3            | 3.E+11             |
| <b>PME-DEG</b> |             |                   |         |                 |                  |                |                    |
| C29:0-DEG-PME  | 87.3        | C35H75NO6P+       | M+H     | 636.5344        | 636.5327         | 2.8            | 4.E+11             |
| C30:0-DEG-PME  | 87.0        | C36H77NO6P+       | M+H     | 650.5498        | 650.5483         | 2.3            | 3.E+12             |
| C30:1-DEG-PME  | 87.5        | C36H75NO6P+       | M+H     | 648.5342        | 648.5327         | 2.4            | 5.E+11             |
| C30:2-DEG-PME  | 88.2        | C36H73NO6P+       | M+H     | 646.5184        | 646.5170         | 2.2            | 4.E+10             |
| C31:0-DEG-PME  | 86.5        | C37H79NO6P+       | M+H     | 664.5652        | 664.5640         | 1.9            | 8.E+11             |
| C31:1-DEG-PME  | 87.0        | C37H77NO6P+       | M+H     | 662.5494        | 662.5483         | 1.7            | 2.E+12             |
| C31:2-DEG-PME  | 87.8        | C37H75NO6P+       | M+H     | 660.5341        | 660.5327         | 2.2            | 6.E+10             |
| C32:0-DEG-PME  | 86.0        | C38H81NO6P+       | M+H     | 678.5811        | 678.5796         | -1.3           | 2.E+11             |
| C32:1-DEG-PME  | 86.6        | C38H79NO6P+       | M+H     | 676.5647        | 676.5640         | 1.1            | 8.E+11             |
| C32:2-DEG-PME  | 87.3        | C38H77NO6P+       | M+H     | 674.5493        | 674.5483         | 1.5            | 8.E+11             |
| C33:0-DEG-PME  | 85.5        | C39H83NO6P+       | M+H     | 692.5963        | 692.5953         | 1.4            | 4.E+10             |
| C33:1-DEG-PME  | 86.1        | C39H81NO6P+       | M+H     | 690.5801        | 690.5796         | 0.8            | 3.E+11             |
| C33:2-DEG-PME  | 86.9        | C39H79NO6P+       | M+H     | 688.5650        | 688.5640         | 1.5            | 4.E+11             |
| C34:0-DEG-PME  | 85.1        | C40H85NO6P+       | M+H     | 706.6118        | 706.6109         | 1.3            | 1.E+10             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:1-DEG-PME  | 85.7        | C40H83NO6P+       | M+H     | 704.5960        | 704.5953         | 1.1            | 3.E+10             |
| C34:2-DEG-PME  | 86.5        | C40H81NO6P+       | M+H     | 702.5804        | 702.5769         | 1.2            | 9.E+11             |
| C35:0-DEG-PME  | 84.6        | C41H87NO6P+       | M+H     | 720.6278        | 720.6266         | 1.7            | 2.E+10             |
| C35:1-DEG-PME  | 85.2        | C41H85NO6P+       | M+H     | 718.6119        | 718.6109         | 1.4            | 1.E+11             |
| C35:2-DEG-PME  | 86.1        | C41H83NO6P+       | M+H     | 716.5962        | 716.5953         | 1.3            | 1.E+11             |
| PDME-DEG       |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PDME | 88.8        | C37H79NO6P+       | M+H     | 664.5657        | 664.5640         | 2.6            | 7.E+10             |
| C31:0-DEG-PDME | 88.3        | C38H81NO6P+       | M+H     | 678.5807        | 678.5796         | 1.6            | 3.E+10             |
| C31:4-DEG-PDME | 89.1        | C38H73NO6P+       | M+H     | 670.5192        | 670.5170         | 3.3            | 6.E+10             |
| C32:0-DEG-PDME | 87.8        | C39H83NO6P+       | M+H     | 692.5960        | 692.5953         | 1.1            | 2.E+10             |
| C32:1-DEG-PDME | 88.4        | C39H81NO6P+       | M+H     | 690.5796        | 690.5796         | 0.0            | 5.E+10             |
| C33:1-DEG-PDME | 87.9        | C40H83NO6P+       | M+H     | 704.5965        | 704.5953         | -1.6           | 7.E+11             |
| C33:2-DEG-PDME | 88.8        | C40H81NO6P+       | M+H     | 702.5798        | 702.5769         | 0.3            | 9.E+10             |
| C34:0-DEG-PDME | 86.7        | C41H87NO6P+       | M+H     | 720.6279        | 720.6266         | 1.9            | 2.E+11             |
| C34:2-DEG-PDME | 88.3        | C41H83NO6P+       | M+H     | 716.5959        | 716.5953         | 1.0            | 7.E+11             |
| DPG            |             |                   |         |                 |                  |                |                    |
| C60:1-DPG      | 84.6        | C69H136NO17P2+    | M+NH4   | 1312.9240       | 1312.9278        | -2.9           | 1.E+10             |
| C60:2-DPG      | 84.7        | C69H134NO17P2+    | M+NH4   | 1310.9120       | 1310.9122        | -0.1           | 3.E+10             |
| C61:1-DPG      | 84.2        | C70H138NO17P2+    | M+NH4   | 1326.9897       | 1326.9888        | 0.7            | 2.E+10             |
| C61:2-DPG      | 84.3        | C70H136NO17P2+    | M+NH4   | 1324.9269       | 1324.9278        | -0.7           | 2.E+10             |
| C62:0-DPG      | 83.4        | C71H142NO17P2+    | M+NH4   | 1342.9738       | 1342.9748        | -0.7           | 6.E+09             |
| C62:1-DPG      | 84.2        | C71H140NO17P2+    | M+NH4   | 1340.9570       | 1340.9591        | -1.5           | 2.E+10             |
| C62:2-DPG      | 84.3        | C71H138NO17P2+    | M+NH4   | 1338.9418       | 1338.9435        | -1.3           | 4.E+10             |
| C62:3-DPG      | 84.6        | C71H136NO17P2+    | M+NH4   | 1336.9266       | 1336.9278        | -0.9           | 3.E+10             |
| C63:1-DPG      | 84.0        | C72H142NO17P2+    | M+NH4   | 1354.9739       | 1354.9748        | -0.6           | 8.E+09             |
| C63:2-DPG      | 83.9        | C72H141NO17P2+    | M+NH4   | 1352.9585       | 1352.9591        | -0.4           | 3.E+10             |
| C63:3-DPG      | 84.3        | C72H139NO17P2+    | M+NH4   | 1350.9420       | 1350.9435        | -1.1           | 2.E+10             |
| C64:2-DPG      | 83.6        | C73H142NO17P2+    | M+NH4   | 1366.9722       | 1366.9748        | -1.9           | 6.E+10             |
| C64:3-DPG      | 84.1        | C73H140NO17P2+    | M+NH4   | 1364.9574       | 1364.9591        | -1.2           | 9.E+10             |
| C64:4-DPG      | 84.3        | C73H138NO17P2+    | M+NH4   | 1362.9437       | 1362.9435        | 0.2            | 7.E+10             |
| C65:2-DPG      | 83.4        | C74H144NO17P2+    | M+NH4   | 1380.9883       | 1380.9904        | -1.5           | 2.E+10             |
| C65:3-DPG      | 83.8        | C74H142NO17P2+    | M+NH4   | 1378.9729       | 1378.9748        | -1.3           | 3.E+10             |
| C65:4-DPG      | 84.2        | C74H140NO17P2+    | M+NH4   | 1376.9580       | 1376.9591        | -0.8           | 2.E+10             |
| C66:2-DPG      | 83.6        | C75H146NO17P2+    | M+NH4   | 1395.0002       | 1395.0061        | -4.2           | 2.E+10             |
| C66:3-DPG      | 83.6        | C75H144NO17P2+    | M+NH4   | 1392.9900       | 1392.9904        | -0.3           | 6.E+10             |
| C66:4-DPG      | 83.8        | C75H142NO17P2+    | M+NH4   | 1390.9718       | 1390.9748        | -2.1           | 7.E+10             |
| C66:5-DPG      | 84.2        | C75H140NO17P2+    | M+NH4   | 1388.9554       | 1388.9591        | -2.6           | 1.E+10             |
| C67:2-DPG      | 83.5        | C76H148NO17P2+    | M+NH4   | 1409.0160       | 1409.0217        | -4.1           | 9.E+09             |
| C67:3-DPG      | 83.3        | C76H146NO17P2+    | M+NH4   | 1407.0046       | 1407.0061        | -1.0           | 3.E+10             |
| C67:4-DPG      | 83.8        | C76H144NO17P2+    | M+NH4   | 1404.9880       | 1404.9904        | -1.7           | 2.E+10             |
| C68:2-DPG      | 82.9        | C77H150NO17P2+    | M+NH4   | 1423.0371       | 1423.0374        | -0.2           | 4.E+09             |
| C68:3-DPG      | 83.1        | C77H148NO17P2+    | M+NH4   | 1421.0200       | 1421.0217        | -1.2           | 2.E+10             |
| C68:4-DPG      | 83.2        | C77H146NO17P2+    | M+NH4   | 1419.0050       | 1419.0061        | -0.7           | 6.E+10             |
| C68:5-DPG      | 83.7        | C77H144NO17P2+    | M+NH4   | 1416.9877       | 1416.9904        | -1.9           | 1.E+10             |
| C68:6-DPG      | 84.0        | C77H142NO17P2+    | M+NH4   | 1414.9702       | 1414.9748        | -3.2           | 6.E+09             |
| C69:3-DPG      | 83.0        | C78H150NO17P2+    | M+NH4   | 1435.0360       | 1435.0374        | -0.9           | 9.E+09             |
| C69:4-DPG      | 83.2        | C78H148NO17P2+    | M+NH4   | 1433.0188       | 1433.0217        | -2.0           | 1.E+10             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C70:4-DPG     | 82.7        | C79H150NO17P2+    | M+NH4   | 1447.0336       | 1447.0375        | -2.6           | 2.E+10             |
| C70:5-DPG     | 83.0        | C79H148NO17P2+    | M+NH4   | 1445.0215       | 1445.0217        | -0.2           | 8.E+09             |
| C72:4-DPG     | 82.4        | C81H154NO17P2+    | M+NH4   | 1475.0669       | 1475.0687        | -1.2           | 2.E+10             |
| C72:5-DPG     | 82.6        | C81H152NO17P2+    | M+NH4   | 1473.0504       | 1473.0530        | -1.8           | 1.E+10             |
| C72:6-DPG     | 82.8        | C81H150NO17P2+    | M+NH4   | 1471.0343       | 1471.0374        | -2.1           | 1.E+10             |
| <b>1G-Sph</b> |             |                   |         |                 |                  |                |                    |
| C32:0-Sph-1G  | 74.9        | C38H76NO8+        | M+H     | 674.5577        | 674.5565         | 1.7            | 6.E+10             |
| C32:1-Sph-1G  | 75.2        | C38H74NO8+        | M+H     | 672.5423        | 672.5409         | 2.1            | 2.E+10             |
| C33:0-Sph-1G  | 74.6        | C39H78NO8+        | M+H     | 688.5729        | 688.5722         | 1.1            | 8.E+10             |
| C33:1-Sph-1G  | 74.9        | C39H76NO8+        | M+H     | 686.5576        | 686.5565         | 1.6            | 1.E+11             |
| C34:0-Sph-1G  | 74.4        | C40H80NO8+        | M+H     | 702.5885        | 702.5878         | 0.9            | 1.E+11             |
| C34:1-Sph-1G  | 74.7        | C40H78NO8+        | M+H     | 700.5732        | 700.5722         | 1.4            | 5.E+10             |
| C34:2-Sph-1G  | 75.0        | C40H76NO8+        | M+H     | 698.5577        | 698.5565         | 1.6            | 5.E+10             |
| C35:0-Sph-1G  | 74.1        | C41H82NO8+        | M+H     | 716.6042        | 716.6035         | 1.1            | 5.E+10             |
| C35:1-Sph-1G  | 74.4        | C41H80NO8+        | M+H     | 714.5887        | 714.5878         | 1.2            | 3.E+11             |
| C35:2-Sph-1G  | 74.8        | C41H78NO8+        | M+H     | 712.5735        | 712.5722         | 1.8            | 1.E+11             |
| C36:0-Sph-1G  | 73.9        | C42H84NO8+        | M+H     | 730.6198        | 730.6191         | 0.9            | 7.E+10             |
| C36:1-Sph-1G  | 74.2        | C42H82NO8+        | M+H     | 728.6042        | 728.6035         | 1.0            | 4.E+11             |
| C36:2-Sph-1G  | 74.6        | C42H80NO8+        | M+H     | 726.5890        | 726.5878         | 1.6            | 4.E+11             |
| C36:3-Sph-1G  | 75.7        | C42H78NO8+        | M+H     | 724.5724        | 724.5722         | 0.4            | 2.E+10             |
| C37:1-Sph-1G  | 74.0        | C43H84NO8+        | M+H     | 742.6199        | 742.6191         | 1.0            | 1.E+11             |
| C37:2-Sph-1G  | 74.3        | C43H82NO8+        | M+H     | 740.6045        | 740.6035         | 1.3            | 2.E+11             |
| C38:1-Sph-1G  | 73.7        | C44H86NO8+        | M+H     | 756.6353        | 756.6348         | 0.7            | 2.E+11             |
| C38:2-Sph-1G  | 74.8        | C44H84NO8+        | M+H     | 754.6201        | 754.6191         | 1.3            | 1.E+12             |
| C38:3-Sph-1G  | 75.1        | C44H82NO8+        | M+H     | 752.6040        | 752.6035         | 0.7            | 5.E+10             |
| C40:0-Sph-1G  | 74.4        | C46H92NO8+        | M+H     | 786.6822        | 786.6817         | 0.6            | 1.E+11             |
| C40:1-Sph-1G  | 74.7        | C46H90NO8+        | M+H     | 784.6666        | 784.6661         | 0.6            | 2.E+10             |
| C40:2-Sph-1G  | 75.0        | C46H88NO8+        | M+H     | 782.6512        | 782.6504         | 1.0            | 7.E+10             |

**(5) Reference sample:RF-SO**

**PG-DAG**

|              |      |              |       |          |          |      |        |
|--------------|------|--------------|-------|----------|----------|------|--------|
| C30:0 DAG-PG | 82.0 | C36H75NO10P+ | M+NH4 | 712.5115 | 712.5123 | -1.2 | 2.E+10 |
| C30:1 DAG-PG | 82.5 | C36H73NO10P+ | M+NH4 | 710.4955 | 710.4967 | -1.6 | 9.E+09 |
| C30:2 DAG-PG | 82.9 | C36H71NO10P+ | M+NH4 | 708.4797 | 708.4810 | -1.9 | 6.E+08 |
| C31:0 DAG-PG | 81.8 | C37H77NO10P+ | M+NH4 | 726.5276 | 726.5280 | -0.5 | 2.E+10 |
| C31:1 DAG-PG | 82.1 | C37H75NO10P+ | M+NH4 | 724.5111 | 724.5123 | -1.7 | 1.E+10 |
| C32:0 DAG-PG | 81.4 | C38H79NO10P+ | M+NH4 | 740.5426 | 740.5436 | -1.4 | 4.E+10 |
| C32:1 DAG-PG | 81.8 | C38H77NO10P+ | M+NH4 | 738.5270 | 738.5280 | -1.2 | 2.E+11 |
| C32:2 DAG-PG | 82.3 | C38H75NO10P+ | M+NH4 | 736.5113 | 736.5123 | -1.4 | 9.E+10 |
| C32:3 DAG-PG | 82.9 | C38H73NO10P+ | M+NH4 | 734.4951 | 734.4967 | -2.1 | 5.E+09 |
| C32:4 DAG-PG | 83.5 | C38H71NO10P+ | M+NH4 | 732.4807 | 732.4810 | -0.5 | 8.E+08 |
| C33:0 DAG-PG | 81.1 | C39H81NO10P+ | M+NH4 | 754.5585 | 754.5593 | -1.0 | 3.E+10 |
| C33:1 DAG-PG | 81.6 | C39H79NO10P+ | M+NH4 | 752.5428 | 752.5436 | -1.0 | 1.E+10 |
| C33:2 DAG-PG | 81.9 | C39H77NO10P+ | M+NH4 | 750.5264 | 750.5280 | -2.1 | 1.E+10 |
| C34:0 DAG-PG | 80.9 | C40H83NO10P+ | M+NH4 | 768.5734 | 768.5749 | -2.0 | 9.E+10 |
| C34:1 DAG-PG | 81.2 | C40H81NO10P+ | M+NH4 | 766.5583 | 766.5593 | -1.2 | 3.E+11 |
| C34:2 DAG-PG | 81.6 | C40H79NO10P+ | M+NH4 | 764.5423 | 764.5436 | -1.7 | 3.E+11 |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:3 DAG-PG  | 82.1        | C40H77NO10P+      | M+NH4   | 762.5270        | 762.5280         | -1.2           | 5.E+09             |
| C35:1 DAG-PG  | 80.9        | C41H83NO10P+      | M+NH4   | 780.5743        | 780.5749         | -0.8           | 4.E+10             |
| C35:2 DAG-PG  | 81.4        | C41H81NO10P+      | M+NH4   | 778.5579        | 778.5593         | -1.8           | 3.E+10             |
| C36:0 DAG-PG  | 80.0        | C42H87NO10P+      | M+NH4   | 796.6052        | 796.6062         | -1.3           | 7.E+10             |
| C36:1 DAG-PG  | 80.5        | C42H85NO10P+      | M+NH4   | 794.5895        | 794.5906         | -1.3           | 1.E+10             |
| C36:2 DAG-PG  | 81.1        | C42H83NO10P+      | M+NH4   | 792.5738        | 792.5749         | -1.4           | 2.E+11             |
| <b>PC-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PC  | 103.5       | C38H77NO8P+       | M+H     | 706.5377        | 706.5381         | -0.6           | 2.E+11             |
| C30:1 DAG-PC  | 104.2       | C38H75NO8P+       | M+H     | 704.5225        | 704.5225         | 0.1            | 6.E+10             |
| C31:0 DAG-PC  | 103.0       | C39H79NO8P+       | M+H     | 720.5539        | 720.5538         | 0.2            | 1.E+11             |
| C31:1 DAG-PC  | 103.4       | C39H77NO8P+       | M+H     | 718.5383        | 718.5381         | 0.2            | 4.E+10             |
| C32:0 DAG-PC  | 102.5       | C40H81NO8P+       | M+H     | 734.5695        | 734.5694         | 0.1            | 4.E+12             |
| C32:1 DAG-PC  | 103.0       | C40H79NO8P+       | M+H     | 732.5547        | 732.5538         | 1.3            | 3.E+12             |
| C32:2 DAG-PC  | 103.6       | C40H77NO8P+       | M+H     | 730.5382        | 730.5381         | 0.1            | 4.E+11             |
| C33:0 DAG-PC  | 102.5       | C41H83NO8P+       | M+H     | 748.5848        | 748.5851         | -0.3           | 2.E+11             |
| C33:2 DAG-PC  | 103.2       | C41H79NO8P+       | M+H     | 744.5540        | 744.5538         | 0.2            | 9.E+10             |
| C34:0 DAG-PC  | 102.1       | C42H85NO8P+       | M+H     | 762.5998        | 762.6007         | -1.2           | 2.E+11             |
| C34:1 DAG-PC  | 102.5       | C42H83NO8P+       | M+H     | 760.5845        | 760.5851         | -0.7           | 5.E+12             |
| C34:2 DAG-PC  | 102.7       | C42H81NO8P+       | M+H     | 758.5692        | 758.5694         | -0.4           | 2.E+12             |
| C34:3 DAG-PC  | 103.5       | C42H79NO8P+       | M+H     | 756.5538        | 756.5538         | 0.0            | 3.E+11             |
| C35:0 DAG-PC  | 102.0       | C43H87NO8P+       | M+H     | 776.6161        | 776.6164         | -0.3           | 2.E+10             |
| C35:1 DAG-PC  | 102.2       | C43H85NO8P+       | M+H     | 774.5999        | 774.6007         | -1.1           | 3.E+11             |
| C35:2 DAG-PC  | 102.5       | C43H83NO8P+       | M+H     | 772.5839        | 772.5851         | -1.6           | 2.E+11             |
| C35:3 DAG-PC  | 103.2       | C43H81NO8P+       | M+H     | 770.5692        | 770.5694         | -0.3           | 3.E+10             |
| C35:4 DAG-PC  | 103.4       | C43H79NO8P+       | M+H     | 768.5542        | 768.5538         | 0.5            | 4.E+09             |
| C36:0 DAG-PC  | 102.0       | C44H89NO8P+       | M+H     | 790.6308        | 790.6320         | -1.6           | 5.E+09             |
| C36:1 DAG-PC  | 102.0       | C44H87NO8P+       | M+H     | 788.6142        | 788.6164         | -2.8           | 4.E+11             |
| C36:2 DAG-PC  | 102.2       | C44H85NO8P+       | M+H     | 786.5999        | 786.6007         | -1.0           | 2.E+12             |
| C36:3 DAG-PC  | 102.5       | C44H83NO8P+       | M+H     | 784.5840        | 784.5851         | -1.4           | 5.E+11             |
| C37:0 DAG-PC  | 100.8       | C45H91NO8P+       | M+H     | 804.6470        | 804.6477         | -0.9           | 1.E+09             |
| C37:1 DAG-PC  | 102.0       | C45H89NO8P+       | M+H     | 802.6317        | 802.6320         | -0.4           | 4.E+10             |
| C37:2 DAG-PC  | 102.2       | C45H87NO8P+       | M+H     | 800.6153        | 800.6164         | -1.4           | 7.E+10             |
| C37:3 DAG-PC  | 102.2       | C45H85NO8P+       | M+H     | 798.5997        | 798.6007         | -1.3           | 2.E+10             |
| C37:4 DAG-PC  | 102.5       | C45H83NO8P+       | M+H     | 796.5825        | 796.5851         | -3.3           | 9.E+09             |
| C38:1 DAG-PC  | 101.1       | C46H91NO8P+       | M+H     | 816.6480        | 816.6477         | 0.4            | 5.E+10             |
| C38:2 DAG-PC  | 102.0       | C46H89NO8P+       | M+H     | 814.6314        | 814.6320         | -0.8           | 2.E+11             |
| C38:3 DAG-PC  | 102.1       | C46H87NO8P+       | M+H     | 812.6155        | 812.6164         | -1.0           | 1.E+11             |
| C38:4 DAG-PC  | 102.5       | C46H85NO8P+       | M+H     | 810.5993        | 810.6007         | -1.8           | 2.E+11             |
| C38:5 DAG-PC  | 102.7       | C46H83NO8P+       | M+H     | 808.5842        | 808.5851         | -1.1           | 1.E+11             |
| C38:6 DAG-PC  | 103.4       | C46H81NO8P+       | M+H     | 806.5688        | 806.5694         | -0.8           | 8.E+10             |
| C39:1 DAG-PC  | 101.0       | C47H93NO8P+       | M+H     | 830.6620        | 830.6633         | -1.5           | 3.E+09             |
| C39:2 DAG-PC  | 101.0       | C47H91NO8P+       | M+H     | 828.6477        | 828.6477         | 0.0            | 8.E+09             |
| C39:3 DAG-PC  | 101.9       | C47H89NO8P+       | M+H     | 826.6314        | 826.6320         | -0.8           | 8.E+09             |
| C39:4 DAG-PC  | 102.2       | C47H87NO8P+       | M+H     | 824.6151        | 824.6164         | -1.6           | 4.E+09             |
| C40:1 DAG-PC  | 99.9        | C48H95NO8P+       | M+H     | 844.6793        | 844.6790         | 0.4            | 1.E+09             |
| C40:2 DAG-PC  | 100.7       | C48H93NO8P+       | M+H     | 842.6635        | 842.6633         | 0.1            | 2.E+10             |
| C40:3 DAG-PC  | 101.5       | C48H91NO8P+       | M+H     | 840.6473        | 840.6477         | -0.5           | 2.E+10             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C40:4 DAG-PC   | 102.0       | C48H89NO8P+       | M+H     | 838.6317        | 838.6320         | -0.4           | 2.E+10             |
| <b>PE-DAG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PE   | 87.6        | C35H71NO8P+       | M+H     | 664.4902        | 664.4912         | -1.4           | 1.E+11             |
| C30:1 DAG-PE   | 88.0        | C35H69NO8P+       | M+H     | 662.4743        | 662.4755         | -1.9           | 1.E+11             |
| C30:2 DAG-PE   | 88.6        | C35H67NO8P+       | M+H     | 660.4590        | 660.4599         | -1.4           | 1.E+10             |
| C31:0 DAG-PE   | 87.0        | C36H73NO8P+       | M+H     | 678.5062        | 678.5068         | -1.0           | 3.E+10             |
| C31:1 DAG-PE   | 87.7        | C36H71NO8P+       | M+H     | 676.4903        | 676.4912         | -1.3           | 4.E+10             |
| C31:2 DAG-PE   | 88.2        | C36H69NO8P+       | M+H     | 674.4747        | 674.4755         | -1.2           | 2.E+09             |
| C32:0 DAG-PE   | 86.6        | C37H75NO8P+       | M+H     | 692.5211        | 692.5225         | -2.1           | 4.E+10             |
| C32:1 DAG-PE   | 87.1        | C37H73NO8P+       | M+H     | 690.5062        | 690.5068         | -1.0           | 5.E+11             |
| C32:2 DAG-PE   | 87.7        | C37H71NO8P+       | M+H     | 688.4906        | 688.4912         | -0.8           | 3.E+11             |
| C32:3 DAG-PE   | 88.6        | C37H69NO8P+       | M+H     | 686.4744        | 686.4755         | -1.6           | 5.E+09             |
| C33:0 DAG-PE   | 86.3        | C38H77NO8P+       | M+H     | 706.5372        | 706.5381         | -1.3           | 3.E+10             |
| C33:1 DAG-PE   | 86.9        | C38H75NO8P+       | M+H     | 704.5217        | 704.5225         | -1.1           | 8.E+10             |
| C34:0 DAG-PE   | 86.0        | C39H79NO8P+       | M+H     | 720.5532        | 720.5538         | -0.9           | 1.E+10             |
| C34:1 DAG-PE   | 86.4        | C39H77NO8P+       | M+H     | 718.5366        | 718.5381         | -2.1           | 2.E+11             |
| C34:2 DAG-PE   | 86.9        | C39H75NO8P+       | M+H     | 716.5216        | 716.5225         | -1.2           | 3.E+11             |
| C34:3 DAG-PE   | 87.7        | C39H73NO8P+       | M+H     | 714.5054        | 714.5068         | -2.0           | 3.E+09             |
| C35:0 DAG-PE   | 85.6        | C40H81NO8P+       | M+H     | 734.5677        | 734.5694         | -2.4           | 3.E+09             |
| C35:1 DAG-PE   | 86.0        | C40H79NO8P+       | M+H     | 732.5530        | 732.5538         | -1.1           | 4.E+10             |
| C35:2 DAG-PE   | 86.6        | C40H77NO8P+       | M+H     | 730.5363        | 730.5381         | -2.6           | 3.E+10             |
| C36:0 DAG-PE   | 85.1        | C41H83NO8P+       | M+H     | 748.5836        | 748.5851         | -2.0           | 2.E+09             |
| C36:1 DAG-PE   | 85.6        | C41H81NO8P+       | M+H     | 746.5677        | 746.5694         | -2.3           | 2.E+10             |
| C36:2 DAG-PE   | 86.0        | C41H79NO8P+       | M+H     | 744.5531        | 744.5538         | -0.9           | 9.E+10             |
| C36:3 DAG-PE   | 86.6        | C41H77NO8P+       | M+H     | 742.5364        | 742.5381         | -2.4           | 9.E+09             |
| C36:4 DAG-PE   | 86.9        | C41H75NO8P+       | M+H     | 740.5217        | 740.5225         | -1.0           | 7.E+09             |
| C38:0 DAG-PE   | 84.7        | C43H87NO8P+       | M+H     | 776.6155        | 776.6164         | -1.1           | 6.E+08             |
| C38:1 DAG-PE   | 84.7        | C43H85NO8P+       | M+H     | 774.6001        | 774.6007         | -0.8           | 5.E+09             |
| C38:2 DAG-PE   | 85.2        | C43H83NO8P+       | M+H     | 772.5837        | 772.5851         | -1.8           | 2.E+10             |
| C38:3 DAG-PE   | 85.8        | C43H81NO8P+       | M+H     | 770.5679        | 770.5694         | -1.9           | 2.E+10             |
| C38:4 DAG-PE   | 86.2        | C43H79NO8P+       | M+H     | 768.5526        | 768.5538         | -1.6           | 3.E+10             |
| C40:1 DAG-PE   | 84.0        | C45H89NO8P+       | M+H     | 802.6317        | 802.6320         | -0.5           | 1.E+09             |
| C40:2 DAG-PE   | 84.5        | C45H87NO8P+       | M+H     | 800.6147        | 800.6164         | -2.0           | 7.E+09             |
| C40:3 DAG-PE   | 85.0        | C45H85MO8P+       | M+H     | 798.6001        | 798.6007         | -0.8           | 7.E+09             |
| C40:4 DAG-PE   | 85.8        | C45H83NO8P+       | M+H     | 796.5836        | 796.5851         | -1.8           | 4.E+09             |
| C42:2 DAG-PE   | 83.8        | C47H91NO8P+       | M+H     | 828.6464        | 828.6477         | -1.6           | 1.E+09             |
| C42:3 DAG-PE   | 84.3        | C47H89NO8P+       | M+H     | 826.6299        | 826.6320         | -2.5           | 3.E+09             |
| C42:4 DAG-PE   | 85.0        | C47H87NO8P+       | M+H     | 824.6149        | 824.6164         | 1.4            | 9.E+08             |
| <b>PME-DAG</b> |             |                   |         |                 |                  |                |                    |
| C32:0 DAG-PME  | 88.5        | C38H77NO8P+       | M+H     | 706.5375        | 706.5381         | -0.9           | 2.E+10             |
| C32:1 DAG-PME  | 88.9        | C38H75NO8P+       | M+H     | 704.5217        | 704.5225         | -1.1           | 1.E+11             |
| C32:2 DAG-PME  | 89.6        | C38H73NO8P+       | M+H     | 702.5065        | 702.5068         | -0.4           | 2.E+10             |
| C33:0 DAG-PME  | 88.0        | C39H79NO8P+       | M+H     | 720.5524        | 720.5538         | -2.0           | 2.E+10             |
| C33:1 DAG-PME  | 88.5        | C39H77NO8P+       | M+H     | 718.5372        | 718.5381         | -1.3           | 1.E+10             |
| C34:0 DAG-PME  | 87.7        | C40H81NO8P+       | M+H     | 734.5679        | 734.5694         | -2.0           | 4.E+09             |
| C34:1 DAG-PME  | 88.2        | C40H79NO8P+       | M+H     | 732.5530        | 732.5538         | -1.0           | 2.E+10             |
| C34:2 DAG-PME  | 88.8        | C40H77NO8P+       | M+H     | 730.5367        | 730.5381         | -1.9           | 2.E+11             |

(Continued to next page)

**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds       | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:3 DAG-PME   | 89.4        | C40H75NO8P+       | M+H     | 728.5217        | 728.5225         | -1.1           | 1.E+09             |
| C35:0 DAG-PME   | 87.1        | C41H83NO8P+       | M+H     | 748.5835        | 748.5851         | -2.1           | 3.E+09             |
| C35:1 DAG-PME   | 87.7        | C41H81NO8P+       | M+H     | 746.5679        | 746.5694         | -2.0           | 4.E+09             |
| <b>PDME-DAG</b> |             |                   |         |                 |                  |                |                    |
| C32:0 DAG-PDME  | 90.2        | C39H79NO8P+       | M+H     | 720.5531        | 720.5538         | -0.9           | 4.E+09             |
| C32:1 DAG-PDME  | 90.8        | C39H77NO8P+       | M+H     | 718.5375        | 718.5381         | -0.9           | 3.E+10             |
| C33:0 DAG-PDME  | 90.1        | C40H81NO8P+       | M+H     | 734.5684        | 734.5694         | -1.5           | 2.E+09             |
| C34:0 DAG-PDME  | 89.4        | C41H83NO8P+       | M+H     | 748.5840        | 748.5851         | -1.5           | 1.E+09             |
| C34:1 DAG-PDME  | 90.1        | C41H81NO8P+       | M+H     | 746.5683        | 746.5694         | -1.5           | 7.E+09             |
| C34:2 DAG-PDME  | 90.8        | C41H79NO8P+       | M+H     | 744.5530        | 744.5538         | -1.1           | 5.E+10             |
| <b>PG-AEG</b>   |             |                   |         |                 |                  |                |                    |
| C30:0-AEG-PG    | 79.9        | C36H74O9P+        | M+H     | 681.5055        | 681.5065         | -1.5           | 4.E+09             |
| C31:0-AEG-PG    | 79.5        | C37H76O9P+        | M+H     | 695.5204        | 695.5221         | -2.5           | 8.E+09             |
| C31:1-AEG-PG    | 80.0        | C37H74O9P+        | M+H     | 693.5051        | 693.5065         | -2.0           | 6.E+08             |
| C32:0-AEG-PG    | 79.2        | C39H78O9P+        | M+H     | 709.5364        | 709.5378         | -1.9           | 4.E+09             |
| C32:1-AEG-PG    | 79.8        | C39H76O9P+        | M+H     | 707.5204        | 707.5221         | -2.5           | 3.E+09             |
| C33:0-AEG-PG    | 78.8        | C40H80O9P+        | M+H     | 723.5518        | 723.5534         | -2.3           | 1.E+09             |
| C33:1-AEG-PG    | 79.3        | C40H78O9P+        | M+H     | 721.5365        | 721.5378         | -1.8           | 3.E+09             |
| C34:0-AEG-PG    | 78.5        | C40H82O9P+        | M+H     | 737.5677        | 737.5691         | -1.9           | 1.E+09             |
| C34:1-AEG-PG    | 78.9        | C40H80O9P+        | M+H     | 735.5518        | 735.5534         | -2.3           | 4.E+09             |
| C34:2-AEG-PG    | 79.9        | C40H78O9P+        | M+H     | 733.5372        | 733.5378         | -0.9           | 2.E+09             |
| C35:0-AEG-PG    | 78.3        | C41H84O9P+        | M+H     | 751.5825        | 751.5847         | -3.0           | 1.E+09             |
| C35:1-AEG-PG    | 78.7        | C41H82O9P+        | M+H     | 749.5686        | 749.5691         | -0.7           | 8.E+08             |
| C36:0-AEG-PG    | 78.1        | C42H86O9P+        | M+H     | 765.5997        | 765.6004         | -0.9           | 5.E+08             |
| C36:2-AEG-PG    | 78.8        | C42H84O9P+        | M+H     | 761.5662        | 761.5691         | -3.8           | 7.E+08             |
| <b>PC-AEG</b>   |             |                   |         |                 |                  |                |                    |
| C32:0-AEG-PC    | 100.7       | C40H83NO7P+       | M+H     | 720.5904        | 720.5902         | 0.3            | 2.E+11             |
| C32:1-AEG-PC    | 101.1       | C40H81NO7P+       | M+H     | 718.5751        | 718.5745         | 0.8            | 1.E+11             |
| C32:2-AEG-PC    | 102.8       | C40H79NO7P+       | M+H     | 716.5599        | 716.5589         | 1.4            | 1.E+10             |
| C33:0-AEG-PC    | 100.3       | C41H85NO7P+       | M+H     | 734.6053        | 734.6058         | -0.6           | 8.E+09             |
| C33:1-AEG-PC    | 100.8       | C41H83NO7P+       | M+H     | 732.5894        | 732.5902         | -1.0           | 2.E+10             |
| C34:1-AEG-PC    | 100.1       | C42H85NO7P+       | M+H     | 746.6059        | 746.6058         | 0.1            | 1.E+11             |
| C34:2-AEG-PC    | 100.9       | C42H83NO7P+       | M+H     | 744.5900        | 744.5902         | -0.2           | 1.E+11             |
| C34:3-AEG-PC    | 102.4       | C42H81NO7P+       | M+H     | 742.5736        | 742.5745         | -1.2           | 2.E+10             |
| C35:1-AEG-PC    | 99.9        | C43H87NO7P+       | M+H     | 760.6229        | 760.6215         | 1.8            | 7.E+09             |
| C36:1-AEG-PC    | 99.1        | C44H89NO7P+       | M+H     | 774.6369        | 774.6371         | -0.3           | 2.E+10             |
| C36:2-AEG-PC    | 100.1       | C44H87NO7P+       | M+H     | 772.6215        | 772.6215         | 0.1            | 2.E+10             |
| C36:3-AEG-PC    | 100.3       | C44H85NO7P+       | M+H     | 770.6059        | 770.6058         | 0.1            | 4.E+10             |
| C36:4-AEG-PC    | 102.2       | C44H83NO7P+       | M+H     | 768.5892        | 768.5902         | -1.2           | 2.E+10             |
| <b>PE-AEG</b>   |             |                   |         |                 |                  |                |                    |
| C32:0-AEG-PE    | 84.1        | C37H77NO7P+       | M+H     | 678.5416        | 678.5432         | -2.3           | 1.E+10             |
| C32:1-AEG-PE    | 85.0        | C37H75NO7P+       | M+H     | 676.5266        | 676.5276         | -1.5           | 4.E+10             |
| C32:2-AEG-PE    | 85.8        | C37H73NO7P+       | M+H     | 674.5107        | 674.5119         | -1.8           | 9.E+09             |
| C33:0-AEG-PE    | 83.7        | C38H79NO7P+       | M+H     | 692.5579        | 692.5589         | -1.5           | 5.E+09             |
| C33:1-AEG-PE    | 84.4        | C38H77NO7P+       | M+H     | 690.5423        | 690.5432         | -1.3           | 2.E+10             |
| C33:2-AEG-PE    | 85.0        | C38H75NO7P+       | M+H     | 688.5265        | 688.5279         | -1.6           | 3.E+09             |
| C34:0-AEG-PE    | 83.3        | C39H81NO7P+       | M+H     | 706.5729        | 706.5745         | -2.3           | 4.E+09             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:1-AEG-PE  | 84.0        | C39H79NO7P+       | M+H     | 704.5583        | 704.5589         | -0.7           | 3.E+10             |
| C34:2-AEG-PE  | 85.0        | C39H77NO7P+       | M+H     | 702.5424        | 702.5432         | -1.2           | 7.E+10             |
| C34:3-AEG-PE  | 85.4        | C39H75NO7P+       | M+H     | 700.5265        | 700.5276         | -1.5           | 2.E+09             |
| C35:2-AEG-PE  | 84.4        | C40H79NO7P+       | M+H     | 716.5578        | 716.5589         | -1.5           | 2.E+10             |
| C36:1-AEG-PE  | 82.9        | C41H83NO7P+       | M+H     | 732.5889        | 732.5902         | -1.7           | 9.E+09             |
| C36:2-AEG-PE  | 84.0        | C41H81NO7P+       | M+H     | 730.5740        | 730.5745         | -0.7           | 1.E+11             |
| C36:3-AEG-PE  | 84.7        | C41H79NO7P+       | M+H     | 728.5577        | 728.5589         | -1.5           | 2.E+10             |
| C36:4-AEG-PE  | 85.1        | C41H77NO7P+       | M+H     | 726.5419        | 726.5432         | -1.8           | 3.E+09             |
| C36:5-AEG-PE  | 85.8        | C41H75NO7P+       | M+H     | 724.5262        | 724.5276         | -1.8           | 3.E+09             |
| C36:6-AEG-PE  | 86.2        | C41H73NO7P+       | M+H     | 722.5103        | 722.5119         | -2.2           | 3.E+09             |
| C37:2-AEG-PE  | 83.7        | C42H83NO7P+       | M+H     | 744.5899        | 744.5902         | -0.4           | 5.E+10             |
| C37:3-AEG-PE  | 84.2        | C42H81NO7P+       | M+H     | 742.5730        | 742.5745         | -2.0           | 9.E+09             |
| C37:4-AEG-PE  | 84.7        | C42H79NO7P+       | M+H     | 740.5572        | 740.5589         | -2.3           | 1.E+09             |
| C38:1-AEG-PE  | 82.1        | C43H83NO7P+       | M+H     | 760.6194        | 760.6215         | -2.7           | 2.E+09             |
| C38:2-AEG-PE  | 83.3        | C43H81NO7P+       | M+H     | 758.6046        | 758.6058         | -1.6           | 6.E+10             |
| C38:3-AEG-PE  | 83.7        | C43H79NO7P+       | M+H     | 756.5899        | 756.5902         | -0.3           | 1.E+11             |
| C38:4-AEG-PE  | 84.4        | C43H77NO7P+       | M+H     | 754.5739        | 754.5745         | -0.9           | 3.E+10             |
| C39:2-AEG-PE  | 82.9        | C44H75NO7P+       | M+H     | 772.6203        | 772.6215         | -1.5           | 1.E+10             |
| C39:3-AEG-PE  | 83.5        | C44H73NO7P+       | M+H     | 770.6044        | 770.6058         | -1.8           | 3.E+10             |
| C39:4-AEG-PE  | 84.0        | C44H71NO7P+       | M+H     | 768.5892        | 768.5902         | -1.2           | 7.E+09             |
| C40:2-AEG-PE  | 82.5        | C45H77NO7P+       | M+H     | 786.6353        | 786.6371         | -2.3           | 3.E+09             |
| C40:3-AEG-PE  | 83.0        | C45H75NO7P+       | M+H     | 784.6202        | 784.6215         | -1.6           | 2.E+10             |
| C40:4-AEG-PE  | 83.7        | C45H73NO7P+       | M+H     | 782.6053        | 782.6058         | -0.7           | 2.E+10             |
| <b>PG-DEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PG  | 78.4        | C36H76O8P+        | M+H     | 667.5262        | 667.5272         | -1.6           | 3.E+10             |
| C30:1-DEG-PG  | 78.8        | C36H74O8P+        | M+H     | 665.2291        | 665.5116         | -1.2           | 7.E+08             |
| C31:0-DEG-PG  | 78.1        | C37H78O8P+        | M+H     | 681.5451        | 681.5429         | -1.2           | 1.E+10             |
| C31:1-DEG-PG  | 78.5        | C37H76O8P+        | M+H     | 679.5251        | 679.5272         | -3.1           | 2.E+09             |
| C32:0-DEG-PG  | 77.8        | C38H80O8P+        | M+H     | 695.5569        | 695.5585         | -2.3           | 1.E+10             |
| C32:1-DEG-PG  | 77.4        | C38H78O8P+        | M+H     | 693.5416        | 693.5429         | -1.8           | 1.E+10             |
| C33:0-DEG-PG  | 77.6        | C39H82O8P+        | M+H     | 709.5722        | 709.5742         | -2.8           | 4.E+09             |
| C33:1-DEG-PG  | 78.0        | C39H80O8P+        | M+H     | 707.5567        | 707.5585         | -2.6           | 4.E+09             |
| C33:2-DEG-PG  | 78.2        | C39H78O8P+        | M+H     | 705.5428        | 705.5429         | -0.1           | 6.E+08             |
| C34:0-DEG-PG  | 77.4        | C40H84O8P+        | M+H     | 723.5878        | 723.5898         | -2.8           | 4.E+09             |
| C34:1-DEG-PG  | 77.6        | C40H82O8P+        | M+H     | 721.5742        | 721.5742         | -3.1           | 2.E+09             |
| C34:2-DEG-PG  | 78.3        | C40H80O8P+        | M+H     | 719.5568        | 719.5585         | -2.5           | 7.E+09             |
| C35:0-DEG-PG  | 77.1        | C41H86O8P+        | M+H     | 737.6036        | 737.6055         | -2.5           | 1.E+09             |
| C35:1-DEG-PG  | 77.5        | C41H84O8P+        | M+H     | 735.5880        | 735.5898         | -2.5           | 7.E+08             |
| C35:2-DEG-PG  | 78.1        | C41H82O8P+        | M+H     | 733.5731        | 733.5742         | -1.4           | 1.E+09             |
| <b>PE-DEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PE  | 83.5        | C35H75NO6P+       | M+H     | 636.5314        | 636.5327         | -2.0           | 8.E+10             |
| C30:1-DEG-PE  | 83.9        | C35H73NO6P+       | M+H     | 634.5159        | 634.5170         | -1.7           | 6.E+09             |
| C31:0-DEG-PE  | 82.9        | C36H77NO6P+       | M+H     | 650.5472        | 650.5483         | -1.7           | 1.E+10             |
| C31:1-DEG-PE  | 83.5        | C36H75NO6P+       | M+H     | 648.5311        | 648.5327         | -2.4           | 6.E+09             |
| C32:0-DEG-PE  | 82.6        | C37H79NO6P+       | M+H     | 664.5628        | 664.5640         | -1.7           | 5.E+09             |
| C32:1-DEG-PE  | 83.5        | C37H77NO6P+       | M+H     | 662.5470        | 662.5483         | -2.0           | 3.E+10             |
| C32:2-DEG-PE  | 83.7        | C37H75NO6P+       | M+H     | 660.5319        | 660.5327         | -1.1           | 7.E+09             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C33:0-DEG-PE   | 82.3        | C38H81NO6P+       | M+H     | 678.5780        | 678.5796         | -2.4           | 3.E+09             |
| C33:1-DEG-PE   | 82.9        | C38H79NO6P+       | M+H     | 676.5624        | 676.5640         | -2.2           | 1.E+10             |
| C33:2-DEG-PE   | 83.3        | C38H77NO6P+       | M+H     | 674.5467        | 674.5483         | -2.4           | 3.E+09             |
| C34:0-DEG-PE   | 81.9        | C39H83NO6P+       | M+H     | 692.5939        | 692.5953         | -2.0           | 6.E+10             |
| C34:1-DEG-PE   | 82.6        | C39H81NO6P+       | M+H     | 690.5786        | 690.5796         | -1.5           | 6.E+09             |
| C34:2-DEG-PE   | 83.5        | C39H79NO6P+       | M+H     | 688.5627        | 688.5640         | -1.8           | 8.E+10             |
| C36:0-DEG-PE   | 81.3        | C41H87NO6P+       | M+H     | 720.6251        | 720.6266         | -2.1           | 2.E+09             |
| C36:2-DEG-PE   | 82.3        | C41H83NO6P+       | M+H     | 716.5942        | 716.5953         | -1.4           | 2.E+09             |
| <b>PME-DEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PME  | 83.5        | C36H77NO6P+       | M+H     | 650.5475        | 650.5483         | -1.2           | 1.E+10             |
| C31:0-DEG-PME  | 83.9        | C37H79NO6P+       | M+H     | 664.5624        | 664.5640         | -2.3           | 7.E+09             |
| C32:0-DEG-PME  | 83.5        | C38H81NO6P+       | M+H     | 678.5782        | 678.5796         | -2.1           | 4.E+09             |
| <b>DPG</b>     |             |                   |         |                 |                  |                |                    |
| C60:0-DPG      | 83.3        | C69H138NO17P2+    | M+NH4   | 1314.9441       | 1314.9435        | 0.5            | 2.E+09             |
| C60:1-DPG      | 83.3        | C69H136NO17P2+    | M+NH4   | 1312.9281       | 1312.9278        | 0.2            | 5.E+08             |
| C60:2-DPG      | 83.5        | C69H134NO17P2+    | M+NH4   | 1310.9099       | 1310.9122        | -1.7           | 2.E+08             |
| C61:0-DPG      | 82.8        | C70H140NO17P2+    | M+NH4   | 1328.9584       | 1328.9591        | -0.5           | 9.E+08             |
| C61:1-DPG      | 83.0        | C70H138NO17P2+    | M+NH4   | 1326.9403       | 1326.9435        | -2.4           | 8.E+08             |
| C62:0-DPG      | 82.8        | C71H142NO17P2+    | M+NH4   | 1342.9718       | 1342.9748        | -2.2           | 1.E+09             |
| C62:1-DPG      | 82.8        | C71H140NO17P2+    | M+NH4   | 1340.9603       | 1340.9591        | 0.9            | 2.E+09             |
| C62:2-DPG      | 83.0        | C71H138NO17P2+    | M+NH4   | 1338.9403       | 1338.9435        | -2.3           | 1.E+09             |
| C62:3-DPG      | 83.3        | C71H136NO17P2+    | M+NH4   | 1336.9253       | 1336.9278        | -1.9           | 6.E+08             |
| C63:0-DPG      | 82.3        | C72H144NO17P2+    | M+NH4   | 1356.9857       | 1356.9904        | -3.5           | 1.E+09             |
| C63:1-DPG      | 82.5        | C72H142NO17P2+    | M+NH4   | 1354.9703       | 1354.9748        | -3.3           | 2.E+09             |
| C63:2-DPG      | 82.8        | C72H141NO17P2+    | M+NH4   | 1352.9572       | 1352.9591        | -1.4           | 1.E+09             |
| C63:3-DPG      | 82.9        | C72H139NO17P2+    | M+NH4   | 1350.9452       | 1350.9435        | 1.3            | 5.E+08             |
| C64:0-DPG      | 82.0        | C73H146NO17P2+    | M+NH4   | 1371.0040       | 1371.0061        | -1.5           | 1.E+09             |
| C64:1-DPG      | 82.1        | C73H144NO17P2+    | M+NH4   | 1368.9897       | 1368.9904        | -0.5           | 3.E+09             |
| C64:2-DPG      | 82.5        | C73H142NO17P2+    | M+NH4   | 1366.9734       | 1366.9748        | -1.0           | 8.E+09             |
| C64:3-DPG      | 82.9        | C73H140NO17P2+    | M+NH4   | 1364.9561       | 1364.9591        | -2.2           | 4.E+09             |
| C64:4-DPG      | 83.0        | C73H138NO17P2+    | M+NH4   | 1362.9425       | 1362.9435        | -0.7           | 1.E+10             |
| C64:5-DPG      | 83.5        | C73H136NO17P2+    | M+NH4   | 1360.9221       | 1360.9278        | -4.2           | 5.E+08             |
| C65:0-DPG      | 81.7        | C74H148NO17P2+    | M+NH4   | 1385.0219       | 1385.0217        | 0.1            | 9.E+08             |
| C65:1-DPG      | 82.0        | C74H146NO17P2+    | M+NH4   | 1383.0046       | 1383.0061        | -1.0           | 2.E+09             |
| C65:2-DPG      | 82.3        | C74H144NO17P2+    | M+NH4   | 1380.9888       | 1380.9904        | -1.2           | 3.E+09             |
| C65:3-DPG      | 82.8        | C74H142NO17P2+    | M+NH4   | 1378.9694       | 1378.9748        | -3.9           | 1.E+09             |
| C65:4-DPG      | 82.8        | C74H140NO17P2+    | M+NH4   | 1376.9596       | 1376.9591        | 0.4            | 1.E+09             |
| C65:5-DPG      | 83.3        | C74H138NO17P2+    | M+NH4   | 1374.9436       | 1374.9435        | 0.1            | 4.E+08             |
| C66:0-DPG      | 81.6        | C75H150NO17P2+    | M+NH4   | 1399.0369       | 1399.0374        | -0.3           | 1.E+09             |
| C66:1-DPG      | 81.9        | C75H148NO17P2+    | M+NH4   | 1397.0165       | 1397.0217        | -3.7           | 2.E+09             |
| C66:2-DPG      | 82.0        | C75H146NO17P2+    | M+NH4   | 1395.0049       | 1395.0061        | -0.8           | 9.E+09             |
| C66:3-DPG      | 82.3        | C75H144NO17P2+    | M+NH4   | 1392.9890       | 1392.9904        | -1.0           | 1.E+10             |
| C66:4-DPG      | 82.6        | C75H142NO17P2+    | M+NH4   | 1390.9740       | 1390.9748        | -0.5           | 5.E+09             |
| C66:5-DPG      | 82.8        | C75H140NO17P2+    | M+NH4   | 1388.9591       | 1388.9591        | 0.0            | 4.E+09             |
| C67:0-DPG      | 81.3        | C76H152NO17P2+    | M+NH4   | 1413.0516       | 1413.0530        | -1.0           | 6.E+08             |
| C67:1-DPG      | 81.5        | C76H150NO17P2+    | M+NH4   | 1411.0369       | 1411.0374        | -0.3           | 1.E+09             |
| C67:2-DPG      | 81.8        | C76H148NO17P2+    | M+NH4   | 1409.0195       | 1409.0217        | -1.5           | 3.E+09             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(5) Reference sample:RF-SO**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C67:3-DPG     | 82.1        | C76H146NO17P2+    | M+NH4   | 1407.0045       | 1407.0061        | -1.1           | 2.E+09             |
| C67:4-DPG     | 82.1        | C76H144NO17P2+    | M+NH4   | 1404.9882       | 1404.9904        | -1.6           | 9.E+08             |
| C67:5-DPG     | 82.6        | C76H142NO17P2+    | M+NH4   | 1402.9750       | 1402.9748        | 0.2            | 8.E+08             |
| C68:0-DPG     | 81.3        | C77H154NO17P2+    | M+NH4   | 1427.0659       | 1427.0687        | -1.9           | 5.E+08             |
| C68:1-DPG     | 81.6        | C77H152NO17P2+    | M+NH4   | 1425.0461       | 1425.0530        | -4.8           | 1.E+09             |
| C68:2-DPG     | 81.6        | C77H150NO17P2+    | M+NH4   | 1423.0366       | 1423.0374        | -0.5           | 5.E+09             |
| C68:3-DPG     | 81.7        | C77H148NO17P2+    | M+NH4   | 1421.0216       | 1421.0217        | -0.1           | 9.E+09             |
| C68:4-DPG     | 82.1        | C77H146NO17P2+    | M+NH4   | 1419.0057       | 1419.0061        | -0.2           | 7.E+09             |
| C68:5-DPG     | 82.5        | C77H144NO17P2+    | M+NH4   | 1416.9868       | 1416.9904        | -2.5           | 2.E+09             |
| C68:6-DPG     | 82.8        | C77H142NO17P2+    | M+NH4   | 1414.9730       | 1414.9748        | -1.2           | 3.E+09             |
| C69:2-DPG     | 81.3        | C78H152NO17P1+    | M+NH4   | 1437.0516       | 1437.0530        | -1.0           | 1.E+09             |
| C69:3-DPG     | 81.7        | C78H150NO17P2+    | M+NH4   | 1435.0347       | 1435.0374        | -1.9           | 3.E+09             |
| C69:4-DPG     | 82.0        | C78H148NO17P2+    | M+NH4   | 1433.0176       | 1433.0217        | -2.9           | 1.E+09             |
| C70:2-DPG     | 81.4        | C79H154NO17P0+    | M+NH4   | 1451.0632       | 1451.0687        | -3.7           | 1.E+09             |
| C70:3-DPG     | 81.3        | C79H152NO17P1+    | M+NH4   | 1449.0529       | 1449.0530        | -0.1           | 3.E+09             |
| C70:4-DPG     | 81.7        | C79H150NO17P2+    | M+NH4   | 1447.0374       | 1447.0375        | -0.7           | 3.E+09             |
| C70:5-DPG     | 81.8        | C79H148NO17P2+    | M+NH4   | 1445.0187       | 1445.0217        | -2.1           | 8.E+08             |
| C70:6-DPG     | 82.3        | C79H146NO17P2+    | M+NH4   | 1443.0034       | 1443.0061        | -1.8           | 8.E+08             |
| <b>1G-Sph</b> |             |                   |         |                 |                  |                |                    |
| C34:0-Sph-1G  | 74.4        | C40H80NO8+        | M+H     | 702.5860        | 702.5878         | -2.6           | 8.E+09             |
| C34:1-Sph-1G  | 74.8        | C40H78NO8+        | M+H     | 700.5706        | 700.5722         | -2.3           | 2.E+09             |
| C35:0-Sph-1G  | 74.2        | C41H82NO8+        | M+H     | 716.6018        | 716.6035         | -2.4           | 1.E+10             |
| C35:1-Sph-1G  | 75.2        | C41H80NO8+        | M+H     | 714.5859        | 714.5878         | -2.7           | 2.E+10             |
| C36:0-Sph-1G  | 73.9        | C42H84NO8+        | M+H     | 730.6176        | 730.6191         | -2.2           | 2.E+10             |
| C36:1-Sph-1G  | 75.4        | C42H82NO8+        | M+H     | 728.6031        | 728.6035         | -2.0           | 7.E+09             |
| C36:2-Sph-1G  | 75.1        | C42H80NO8+        | M+H     | 726.5865        | 726.5878         | -1.9           | 7.E+09             |
| C37:1-Sph-1G  | 75.2        | C43H84NO8+        | M+H     | 742.6180        | 742.6191         | -1.6           | 7.E+09             |
| C38:1-Sph-1G  | 75.0        | C44H86NO8+        | M+H     | 756.6311        | 756.6348         | -2.3           | 4.E+10             |
| C38:2-Sph-1G  | 74.7        | C44H84NO8+        | M+H     | 754.6168        | 754.6191         | -3.1           | 2.E+10             |

**(6) Reference sample:RF-AS**

**PG-DAG**

|              |      |              |       |          |          |      |        |
|--------------|------|--------------|-------|----------|----------|------|--------|
| C30:0 DAG-PG | 82.1 | C36H75NO10P+ | M+NH4 | 712.5143 | 712.5123 | 2.9  | 4.E+10 |
| C30:1 DAG-PG | 82.5 | C36H73NO10P+ | M+NH4 | 710.6989 | 710.4967 | 3.2  | 1.E+11 |
| C30:2 DAG-PG | 83.0 | C36H71NO10P+ | M+NH4 | 708.4833 | 708.4810 | 3.3  | 1.E+10 |
| C32:0 DAG-PG | 81.3 | C38H79NO10P+ | M+NH4 | 740.5469 | 740.5436 | 4.4  | 3.E+10 |
| C32:1 DAG-PG | 81.8 | C38H77NO10P+ | M+NH4 | 738.5303 | 738.5280 | 3.1  | 5.E+11 |
| C32:2 DAG-PG | 82.3 | C38H75NO10P+ | M+NH4 | 736.5126 | 736.5123 | 0.4  | 8.E+11 |
| C32:3 DAG-PG | 82.8 | C38H73NO10P+ | M+NH4 | 734.4999 | 734.4967 | 4.4  | 2.E+11 |
| C32:4 DAG-PG | 83.5 | C38H71NO10P+ | M+NH4 | 732.4827 | 732.4810 | 2.3  | 2.E+10 |
| C33:0 DAG-PG | 81.1 | C39H81NO10P+ | M+NH4 | 754.5605 | 754.5593 | 1.7  | 2.E+10 |
| C33:1 DAG-PG | 81.6 | C39H79NO10P+ | M+NH4 | 752.5461 | 752.5436 | 3.3  | 8.E+10 |
| C33:2 DAG-PG | 82.0 | C39H77NO10P+ | M+NH4 | 750.5276 | 750.5280 | -0.5 | 4.E+10 |
| C34:0 DAG-PG | 80.8 | C40H83NO10P+ | M+NH4 | 768.5783 | 768.5749 | 4.4  | 1.E+10 |
| C34:1 DAG-PG | 81.1 | C40H81NO10P+ | M+NH4 | 766.5613 | 766.5593 | 2.7  | 4.E+11 |
| C34:2 DAG-PG | 81.7 | C40H79NO10P+ | M+NH4 | 764.5469 | 764.5436 | 4.3  | 1.E+12 |
| C34:3 DAG-PG | 82.0 | C40H77NO10P+ | M+NH4 | 762.5311 | 762.5280 | 4.2  | 9.E+10 |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(6) Reference sample:RF-AS**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:4 DAG-PG  | 82.7        | C40H75NO10P+      | M+NH4   | 760.5109        | 760.5123         | -1.9           | 8.E+09             |
| C36:0 DAG-PG  | 80.1        | C42H87NO10P+      | M+NH4   | 796.6078        | 796.6062         | 2.1            | 1.E+10             |
| C36:1 DAG-PG  | 80.5        | C42H85NO10P+      | M+NH4   | 794.5925        | 794.5906         | 2.4            | 2.E+10             |
| C36:2 DAG-PG  | 81.0        | C42H83NO10P+      | M+NH4   | 792.5771        | 792.5749         | 2.7            | 6.E+11             |
| <b>PC-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PC  | 104.6       | C38H77NO8P+       | M+H     | 706.5381        | 706.5425         | -0.6           | 2.E+12             |
| C30:1 DAG-PC  | 105.6       | C38H75NO8P+       | M+H     | 704.5225        | 704.5225         | 0.0            | 7.E+11             |
| C31:0 DAG-PC  | 104.0       | C39H79NO8P+       | M+H     | 720.5543        | 720.5538         | 0.7            | 4.E+11             |
| C31:1 DAG-PC  | 104.6       | C39H77NO8P+       | M+H     | 718.5380        | 718.5381         | -0.2           | 2.E+11             |
| C32:0 DAG-PC  | 103.6       | C40H81NO8P+       | M+H     | 734.5699        | 734.5694         | 0.6            | 5.E+12             |
| C32:1 DAG-PC  | 104.1       | C40H79NO8P+       | M+H     | 732.5560        | 732.5538         | 3.0            | 1.E+13             |
| C32:2 DAG-PC  | 105.0       | C40H77NO8P+       | M+H     | 730.5377        | 730.5355         | -0.6           | 2.E+12             |
| C33:0 DAG-PC  | 103.3       | C41H83NO8P+       | M+H     | 748.5846        | 748.5851         | -0.7           | 2.E+11             |
| C33:1 DAG-PC  | 103.5       | C41H81NO8P+       | M+H     | 746.5696        | 746.5694         | 0.3            | 2.E+12             |
| C33:2 DAG-PC  | 104.1       | C41H79NO8P+       | M+H     | 744.5555        | 744.5538         | 2.3            | 4.E+11             |
| C34:1 DAG-PC  | 103.2       | C42H83NO8P+       | M+H     | 760.5869        | 760.5851         | 2.4            | 3.E+13             |
| C34:2 DAG-PC  | 103.7       | C42H81NO8P+       | M+H     | 758.5702        | 758.5694         | 1.0            | 1.E+13             |
| C34:3 DAG-PC  | 104.4       | C42H79NO8P+       | M+H     | 756.5560        | 756.5538         | 3.0            | 4.E+12             |
| C35:1 DAG-PC  | 102.9       | C43H85NO8P+       | M+H     | 774.6000        | 774.6007         | -0.9           | 1.E+12             |
| C35:2 DAG-PC  | 103.2       | C43H83NO8P+       | M+H     | 772.5856        | 772.5851         | 0.6            | 9.E+11             |
| C35:3 DAG-PC  | 103.7       | C43H81NO8P+       | M+H     | 770.5693        | 770.5694         | -0.2           | 2.E+11             |
| C36:1 DAG-PC  | 102.9       | C44H87NO8P+       | M+H     | 788.6172        | 788.6164         | 1.0            | 1.E+12             |
| C36:2 DAG-PC  | 103.0       | C44H85NO8P+       | M+H     | 786.6016        | 786.6007         | 1.1            | 9.E+12             |
| C36:3 DAG-PC  | 103.3       | C44H83NO8P+       | M+H     | 784.5850        | 784.5851         | -0.1           | 6.E+12             |
| C36:4 DAG-PC  | 104.1       | C44H81NO8P+       | M+H     | 782.5693        | 782.5668         | -0.2           | 3.E+12             |
| C36:5 DAG-PC  | 105.0       | C44H79NO8P+       | M+H     | 780.5538        | 780.5538         | 0.0            | 3.E+12             |
| C37:2 DAG-PC  | 102.9       | C45H87NO8P+       | M+H     | 800.6154        | 800.6164         | -1.3           | 4.E+11             |
| C37:3 DAG-PC  | 103.2       | C45H85NO8P+       | M+H     | 798.6017        | 798.6007         | 1.3            | 4.E+11             |
| C37:4 DAG-PC  | 103.6       | C45H83NO8P+       | M+H     | 796.5851        | 796.5824         | -1.9           | 8.E+10             |
| C38:2 DAG-PC  | 102.8       | C46H89NO8P+       | M+H     | 814.6319        | 814.6320         | -0.2           | 1.E+12             |
| C38:3 DAG-PC  | 102.9       | C46H87NO8P+       | M+H     | 812.6164        | 812.6164         | 0.8            | 2.E+12             |
| C38:4 DAG-PC  | 103.2       | C46H85NO8P+       | M+H     | 810.6014        | 810.6007         | 0.8            | 1.E+12             |
| C38:5 DAG-PC  | 103.8       | C46H83NO8P+       | M+H     | 808.5858        | 808.5851         | 0.9            | 2.E+12             |
| C38:6 DAG-PC  | 104.6       | C46H81NO8P+       | M+H     | 806.5686        | 806.5694         | -1.0           | 1.E+12             |
| C40:2 DAG-PC  | 102.0       | C48H93NO8P+       | M+H     | 842.6641        | 842.6633         | 0.9            | 5.E+10             |
| C40:3 DAG-PC  | 102.4       | C48H91NO8P+       | M+H     | 840.6478        | 840.6477         | 0.1            | 2.E+11             |
| C40:4 DAG-PC  | 103.0       | C48H89NO8P+       | M+H     | 838.6314        | 838.6320         | -0.7           | 2.E+11             |
| C40:5 DAG-PC  | 103.1       | C48H87NO8P+       | M+H     | 836.6178        | 836.6164         | 1.7            | 3.E+11             |
| <b>PE-DAG</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG-PE  | 87.4        | C35H71NO8P+       | M+H     | 664.4932        | 664.4912         | 3.0            | 2.E+11             |
| C30:1 DAG-PE  | 87.9        | C35H69NO8P+       | M+H     | 662.4780        | 662.4755         | 3.8            | 2.E+12             |
| C30:2 DAG-PE  | 88.6        | C35H67NO8P+       | M+H     | 660.4597        | 660.4599         | -0.3           | 4.E+11             |
| C31:0 DAG-PE  | 87.1        | C36H73NO8P+       | M+H     | 678.5094        | 678.5068         | 3.8            | 7.E+10             |
| C31:1 DAG-PE  | 87.5        | C36H71NO8P+       | M+H     | 676.4930        | 676.4912         | 2.8            | 2.E+11             |
| C31:2 DAG-PE  | 88.1        | C36H69NO8P+       | M+H     | 674.4766        | 674.4755         | 1.5            | 4.E+10             |
| C32:0 DAG-PE  | 86.6        | C37H75NO8P+       | M+H     | 692.5231        | 692.5225         | 0.9            | 7.E+10             |
| C32:1 DAG-PE  | 87.1        | C37H73NO8P+       | M+H     | 690.5094        | 690.5112         | -2.6           | 2.E+12             |

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**Appendix Table A3.3.** Detail information of all non-archaeal IPLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(6) Reference sample:RF-AS**

| Compounds     | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|---------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C32:2 DAG-PE  | 87.8        | C37H71NO8P+       | M+H     | 688.4940        | 688.4955         | -2.3           | 4.E+12             |
| C32:3 DAG-PE  | 88.4        | C37H69NO8P+       | M+H     | 686.4766        | 686.4755         | 1.5            | 3.E+11             |
| C32:4 DAG-PE  | 89.3        | C37H67NO8P+       | M+H     | 684.4611        | 684.4599         | 1.7            | 2.E+10             |
| C33:0 DAG-PE  | 86.3        | C38H77NO8P+       | M+H     | 706.5405        | 706.5425         | -2.8           | 5.E+10             |
| C33:1 DAG-PE  | 86.8        | C38H75NO8P+       | M+H     | 704.5226        | 704.5225         | 0.2            | 2.E+11             |
| C33:2 DAG-PE  | 87.4        | C38H73NO8P+       | M+H     | 702.5060        | 702.5068         | -1.1           | 2.E+11             |
| C34:0 DAG-PE  | 85.9        | C39H79NO8P+       | M+H     | 720.5565        | 720.5538         | 3.7            | 2.E+10             |
| C34:1 DAG-PE  | 86.4        | C39H77NO8P+       | M+H     | 718.5410        | 718.5381         | 3.9            | 2.E+11             |
| C34:2 DAG-PE  | 86.9        | C39H75NO8P+       | M+H     | 716.5252        | 716.5225         | 3.8            | 2.E+12             |
| C34:3 DAG-PE  | 87.5        | C39H73NO8P+       | M+H     | 714.5085        | 714.5068         | 2.4            | 1.E+11             |
| C34:4 DAG-PE  | 88.0        | C39H71NO8P+       | M+H     | 712.4930        | 712.4912         | 2.5            | 3.E+10             |
| C35:1 DAG-PE  | 88.1        | C40H79NO8P+       | M+H     | 732.5565        | 732.5538         | 3.7            | 2.E+11             |
| C35:2 DAG-PE  | 88.8        | C40H77NO8P+       | M+H     | 730.5408        | 730.5381         | 3.6            | 2.E+12             |
| C35:3 DAG-PE  | 89.2        | C40H75NO8P+       | M+H     | 728.5231        | 728.5225         | 0.8            | 9.E+10             |
| C36:2 DAG-PE  | 86.2        | C41H79NO8P+       | M+H     | 744.5564        | 744.5538         | 3.5            | 3.E+11             |
| C36:3 DAG-PE  | 86.6        | C41H77NO8P+       | M+H     | 742.5384        | 742.5355         | 4.0            | 2.E+11             |
| C36:4 DAG-PE  | 87.2        | C41H75NO8P+       | M+H     | 740.5239        | 740.5225         | 1.9            | 5.E+10             |
| C38:2 DAG-PE  | 85.3        | C43H83NO8P+       | M+H     | 772.5859        | 772.5851         | 1.0            | 2.E+10             |
| C38:3 DAG-PE  | 85.7        | C43H81NO8P+       | M+H     | 770.5720        | 770.5694         | 3.3            | 2.E+10             |
| C38:4 DAG-PE  | 86.5        | C43H79NO8P+       | M+H     | 768.5562        | 768.5538         | 3.1            | 3.E+10             |
| <b>PE-DEG</b> |             |                   |         |                 |                  |                |                    |
| C30:0-DEG-PE  | 83.3        | C35H75NO6P+       | M+H     | 636.5333        | 636.5327         | 1.1            | 6.E+10             |
| C30:1-DEG-PE  | 83.9        | C35H73NO6P+       | M+H     | 634.5171        | 634.5170         | 0.1            | 4.E+10             |
| C30:2-DEG-PE  | 84.5        | C35H71NO6P+       | M+H     | 632.5031        | 632.5014         | 2.7            | 1.E+10             |
| C34:1-DEG-PE  | 83.5        | C39H81NO6P+       | M+H     | 688.5654        | 688.5640         | 2.1            | 8.E+10             |
| C36:0-DEG-PE  | 83.6        | C41H87NO6P+       | M+H     | 720.6292        | 720.6266         | 3.7            | 1.E+11             |

**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(1) Artificial chimney sample: AC-C0016A**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| <b>CL-DAGs</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DAG      | 37.5        | C33H65O5          | M+H     | 541.4849        | 541.4827         | 4.2            | 2.E+10             |
| C30:1 DAG      | 38.7        | C33H63O5          | M+H     | 539.4690        | 539.4670         | 3.7            | 4.E+10             |
| C30:2 DAG      | 40.3        | C33H61O5          | M+H     | 537.4534        | 537.4514         | 3.8            | 1.E+10             |
| C31:0 DAG      | 37.1        | C34H67O5          | M+H     | 555.4999        | 555.4983         | 2.9            | 8.E+09             |
| C31:1 DAG      | 38.2        | C34H65O5          | M+H     | 553.4847        | 553.4827         | 3.8            | 1.E+09             |
| C32:0 DAG      | 36.6        | C35H69O5          | M+H     | 569.5157        | 569.5140         | 3.0            | 2.E+10             |
| C32:1 DAG      | 37.6        | C35H67O5          | M+H     | 567.5001        | 567.5140         | 3.2            | 4.E+10             |
| C32:2 DAG      | 39.1        | C35H65O5          | M+H     | 565.4844        | 565.4827         | 3.2            | 1.E+11             |
| C33:0 DAG      | 36.3        | C36H71O5          | M+H     | 583.5319        | 583.5453         | 3.9            | 5.E+09             |
| C33:1 DAG      | 37.4        | C36H69O5          | M+H     | 581.5159        | 581.5296         | 3.3            | 3.E+10             |
| C33:2 DAG      | 38.6        | C36H67O5          | M+H     | 579.5001        | 579.4983         | 3.0            | 7.E+09             |
| C34:0 DAG      | 35.7        | C37H73O5          | M+H     | 597.5474        | 597.5453         | 3.5            | 8.E+10             |
| C34:1 DAG      | 36.8        | C37H71O5          | M+H     | 595.5316        | 595.5296         | 3.3            | 4.E+10             |
| C34:2 DAG      | 38.2        | C37H69O5          | M+H     | 593.5140        | 593.5140         | 3.2            | 6.E+10             |
| C35:0 DAG      | 35.1        | C38H75O5          | M+H     | 611.5624        | 611.5609         | 2.4            | 4.E+09             |
| C35:1 DAG      | 36.5        | C38H73O5          | M+H     | 609.5466        | 609.5453         | 2.3            | 1.E+10             |
| C35:2 DAG      | 37.7        | C38H71O5          | M+H     | 607.5316        | 607.5296         | 3.3            | 8.E+09             |
| C36:0 DAG      | 35.0        | C39H77O5          | M+H     | 625.5781        | 625.5766         | 2.5            | 1.E+10             |
| C36:1 DAG      | 36.1        | C39H75O5          | M+H     | 623.5626        | 623.5609         | 2.8            | 1.E+10             |
| C36:2 DAG      | 37.1        | C39H73O5          | M+H     | 621.5469        | 621.5453         | 2.6            | 4.E+10             |
| C36:3 DAG      | 38.5        | C39H71O5          | M+H     | 619.5312        | 619.5296         | 2.6            | 1.E+10             |
| C36:4 DAG      | 40.3        | C39H69O5          | M+H     | 617.6153        | 617.5140         | 2.2            | 6.E+09             |
| C37:0 DAG      | 34.8        | C40H79O5          | M+H     | 639.5940        | 639.5922         | 2.8            | 7.E+09             |
| C37:1 DAG      | 35.8        | C40H77O5          | M+H     | 637.5778        | 637.5766         | 2.0            | 1.E+10             |
| C37:2 DAG      | 36.9        | C40H75O5          | M+H     | 635.4508        | 635.5609         | -1.5           | 5.E+09             |
| C38:0 DAG      | 34.1        | C41H81O5          | M+H     | 653.6096        | 653.6079         | 2.7            | 4.E+09             |
| C38:1 DAG      | 35.1        | C41H79O5          | M+H     | 651.5936        | 651.5922         | 2.1            | 3.E+09             |
| C38:2 DAG      | 36.6        | C41H77O5          | M+H     | 649.5785        | 649.5766         | 3.0            | 7.E+09             |
| <b>CL-AEGs</b> |             |                   |         |                 |                  |                |                    |
| C31:0 AEG      | 11.4        | C34H69O4          | M+H     | 541.5213        | 541.5190         | 4.2            | 6.E+09             |
| C31:1 AEG      | 12.2        | C34H67O4          | M+H     | 539.5054        | 539.5034         | 3.8            | 1.E+10             |
| C32:0 AEG      | 11.2        | C35H71O4          | M+H     | 555.5364        | 555.5347         | 3.1            | 1.E+10             |
| C32:1 AEG      | 11.9        | C35H69O4          | M+H     | 553.5211        | 553.5190         | 3.6            | 2.E+10             |
| C32:2 AEG      | 12.9        | C35H67O4          | M+H     | 551.5055        | 551.5034         | 3.8            | 2.E+10             |
| C33:0 AEG      | 10.9        | C36H73O4          | M+H     | 569.5833        | 569.5503         | 2.9            | 1.E+10             |
| C33:1 AEG      | 11.6        | C36H71O4          | M+H     | 567.5366        | 567.5347         | 3.4            | 3.E+10             |
| C33:2 AEG      | 12.5        | C36H69O4          | M+H     | 565.5206        | 565.5190         | 2.8            | 4.E+09             |
| C34:0 AEG      | 10.8        | C37H75O4          | M+H     | 583.5678        | 583.5660         | 3.1            | 2.E+10             |
| C34:1 AEG      | 11.5        | C37H73O4          | M+H     | 581.5522        | 581.5503         | 3.3            | 4.E+10             |
| C34:2 AEG      | 12.4        | C37H71O4          | M+H     | 579.5368        | 579.5347         | 3.7            | 2.E+10             |
| C35:0 AEG      | 10.6        | C38H77O4          | M+H     | 597.5839        | 597.5816         | 3.8            | 1.E+10             |
| C35:1 AEG      | 11.2        | C38H75O4          | M+H     | 595.5678        | 595.5660         | 3.1            | 2.E+10             |
| C35:2 AEG      | 11.8        | C38H73O4          | M+H     | 593.5528        | 593.5503         | 4.1            | 4.E+09             |

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**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(1) Artificial chimney sample: AC-C0016A**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| <b>CL-DEGs</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DEG      | 8.9         | C33H69O3          | M+H     | 513.5261        | 513.5241         | 3.9            | 4.E+11             |
| C30:1 DEG      | 9.3         | C33H67O3          | M+H     | 511.5103        | 511.5085         | 3.6            | 9.E+10             |
| C30:2 DEG      | 9.8         | C33H65O3          | M+H     | 509.4948        | 509.4928         | 3.9            | 1.E+10             |
| C31:0 DEG      | 8.7         | C34H71O3          | M+H     | 527.5418        | 527.5398         | 3.9            | 1.E+11             |
| C31:1 DEG      | 9.2         | C34H69O3          | M+H     | 525.5261        | 525.5241         | 3.8            | 1.E+11             |
| C31:2 DEG      | 9.7         | C34H67O3          | M+H     | 523.5106        | 523.5085         | 4.0            | 6.E+09             |
| C32:0 DEG      | 8.6         | C35H73O3          | M+H     | 541.5576        | 541.5554         | 3.9            | 2.E+11             |
| C32:1 DEG      | 9.0         | C35H71O3          | M+H     | 539.5418        | 539.5398         | 3.8            | 1.E+11             |
| C32:2 DEG      | 9.6         | C35H69O3          | M+H     | 537.5262        | 537.5241         | 3.8            | 1.E+11             |
| C32:3 DEG      | 10.2        | C35H67O3          | M+H     | 535.5105        | 535.5085         | 3.8            | 1.E+10             |
| C32:4 DEG      | 10.8        | C35H65O3          | M+H     | 533.4949        | 533.4928         | 3.8            | 5.E+09             |
| C33:0 DEG      | 8.5         | C36H75O3          | M+H     | 555.5731        | 555.5711         | 3.7            | 6.E+11             |
| C33:1 DEG      | 9.0         | C36H73O3          | M+H     | 553.5576        | 553.5554         | 4.0            | 2.E+11             |
| C33:2 DEG      | 9.3         | C36H71O3          | M+H     | 551.5410        | 551.5398         | 2.3            | 1.E+10             |
| C34:0 DEG      | 8.4         | C37H77O3          | M+H     | 569.5887        | 569.5867         | 3.6            | 2.E+12             |
| C34:1 DEG      | 8.7         | C37H75O3          | M+H     | 567.5731        | 567.5867         | 3.5            | 3.E+11             |
| C34:2 DEG      | 9.5         | C37H73O3          | M+H     | 565.5573        | 565.5554         | 3.2            | 4.E+11             |
| C35:0 DEG      | 8.3         | C38H79O3          | M+H     | 583.6044        | 583.6024         | 3.5            | 7.E+11             |
| C35:1 DEG      | 8.6         | C38H77O3          | M+H     | 581.5887        | 581.5867         | 3.4            | 7.E+11             |
| C35:2 DEG      | 9.0         | C38H75O3          | M+H     | 579.5729        | 579.5711         | 3.1            | 5.E+10             |
| C36:0 DEG      | 8.1         | C39H81O3          | M+H     | 597.6201        | 597.6180         | 3.4            | 9.E+11             |
| C36:1 DEG      | 8.5         | C39H79O3          | M+H     | 595.6004        | 595.6024         | 3.5            | 4.E+11             |
| C36:2 DEG      | 8.9         | C39H77O3          | M+H     | 593.5887        | 593.5867         | 3.4            | 1.E+11             |
| C37:1 DEG      | 8.4         | C40H81O3          | M+H     | 609.6197        | 609.6180         | 2.7            | 3.E+12             |
| C37:2 DEG      | 8.7         | C40H79O3          | M+H     | 607.6024        | 607.6024         | 3.1            | 1.E+11             |
| C38:0 DEG      | 7.9         | C41H85O3          | M+H     | 625.6509        | 625.6493         | 2.6            | 8.E+10             |
| C38:1 DEG      | 8.3         | C41H83O3          | M+H     | 623.6351        | 623.6337         | 2.2            | 6.E+10             |
| C38:2 DEG      | 8.6         | C41H81O3          | M+H     | 621.6196        | 621.6180         | 2.5            | 4.E+11             |
| C39:1 DEG      | 8.1         | C42H85O3          | M+H     | 637.6510        | 637.6493         | 2.6            | 3.E+11             |
| C39:2 DEG      | 8.4         | C42H83O3          | M+H     | 635.6354        | 635.6337         | 2.7            | 6.E+10             |

**(2) Artificial chimney sample: AC-C0016B**

|           |      |          |     |          |          |     |        |
|-----------|------|----------|-----|----------|----------|-----|--------|
| C30:0 DAG | 37.6 | C33H65O5 | M+H | 541.4897 | 541.4827 | 3.7 | 4.E+10 |
| C30:1 DAG | 39.0 | C33H63O5 | M+H | 539.4689 | 539.4670 | 3.6 | 1.E+11 |
| C30:2 DAG | 40.4 | C33H61O5 | M+H | 537.4533 | 537.4514 | 3.6 | 1.E+11 |
| C30:3 DAG | 42.4 | C33H65O5 | M+H | 535.4375 | 535.4357 | 3.4 | 8.E+10 |
| C31:0 DAG | 37.2 | C34H67O5 | M+H | 555.5001 | 555.4983 | 3.3 | 2.E+10 |
| C31:1 DAG | 38.5 | C34H65O5 | M+H | 553.4845 | 553.4827 | 3.3 | 4.E+10 |
| C31:2 DAG | 39.9 | C34H63O5 | M+H | 551.4694 | 551.4670 | 4.4 | 1.E+10 |
| C32:0 DAG | 36.5 | C35H69O5 | M+H | 569.5157 | 569.5140 | 3.0 | 5.E+10 |
| C32:1 DAG | 37.9 | C35H67O5 | M+H | 567.5001 | 567.5140 | 3.2 | 4.E+11 |
| C32:2 DAG | 39.6 | C35H65O5 | M+H | 565.4843 | 565.4827 | 2.9 | 7.E+11 |
| C32:3 DAG | 41.2 | C35H63O5 | M+H | 563.4686 | 563.4670 | 2.9 | 4.E+11 |
| C32:4 DAG | 43.3 | C35H61O5 | M+H | 561.4530 | 561.4514 | 2.9 | 1.E+11 |
| C33:0 DAG | 36.0 | C36H71O5 | M+H | 583.5313 | 583.5453 | 2.8 | 2.E+10 |

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**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C33:1 DAG      | 37.4        | C36H69O5          | M+H     | 581.5157        | 581.5296         | 3.0            | 9.E+10             |
| C33:2 DAG      | 38.9        | C36H67O5          | M+H     | 579.4999        | 579.4983         | 2.8            | 4.E+10             |
| C33:3 DAG      | 40.6        | C36H65O5          | M+H     | 577.4846        | 577.4827         | 3.3            | 1.E+10             |
| C34:0 DAG      | 35.4        | C37H73O5          | M+H     | 597.5469        | 597.5453         | 2.8            | 5.E+10             |
| C34:1 DAG      | 37.0        | C37H71O5          | M+H     | 595.5315        | 595.5296         | 3.2            | 8.E+11             |
| C34:2 DAG      | 38.4        | C37H69O5          | M+H     | 593.5157        | 593.5140         | 3.0            | 7.E+11             |
| C34:3 DAG      | 40.2        | C37H67O5          | M+H     | 591.5002        | 591.4983         | 3.2            | 1.E+12             |
| C35:1 DAG      | 36.3        | C38H73O5          | M+H     | 609.5468        | 609.5453         | 2.5            | 5.E+10             |
| C35:2 DAG      | 37.9        | C38H71O5          | M+H     | 607.5316        | 607.5296         | 3.2            | 4.E+10             |
| C35:3 DAG      | 39.8        | C38H69O5          | M+H     | 605.5156        | 605.5140         | 2.8            | 2.E+10             |
| C36:0 DAG      | 34.5        | C38H75O5          | M+H     | 625.5780        | 625.5766         | 2.3            | 1.E+10             |
| C36:1 DAG      | 35.8        | C38H75O5          | M+H     | 623.5624        | 623.5609         | 2.4            | 2.E+11             |
| C36:2 DAG      | 37.4        | C38H73O5          | M+H     | 621.5468        | 621.5453         | 2.5            | 5.E+11             |
| C36:3 DAG      | 39.0        | C38H71O5          | M+H     | 619.5310        | 619.5296         | 2.3            | 3.E+11             |
| C36:4 DAG      | 40.6        | C38H69O5          | M+H     | 617.5155        | 617.5140         | 2.5            | 3.E+11             |
| C38:0 DAG      | 33.7        | C40H79O5          | M+H     | 653.6093        | 653.6079         | 2.2            | 7.E+09             |
| C38:1 DAG      | 35.0        | C40H79O5          | M+H     | 651.5936        | 651.5922         | 2.1            | 3.E+10             |
| C38:2 DAG      | 36.2        | C40H77O5          | M+H     | 649.5781        | 649.5766         | 2.3            | 2.E+11             |
| C38:3 DAG      | 38.3        | C40H75O5          | M+H     | 647.5625        | 647.5609         | 2.5            | 1.E+11             |
| C38:4 DAG      | 39.8        | C40H73O5          | M+H     | 645.5467        | 645.5453         | 2.2            | 1.E+11             |
| <b>CL-AEGs</b> |             |                   |         |                 |                  |                |                    |
| C30:0 AEG      | 12.6        | C33H67O4          | M+H     | 527.5053        | 527.5034         | 3.6            | 7.E+10             |
| C30:1 AEG      | 13.2        | C33H65O4          | M+H     | 525.4895        | 525.4877         | 3.4            | 6.E+10             |
| C31:0 AEG      | 12.3        | C34H69O4          | M+H     | 541.5211        | 541.5190         | 3.8            | 5.E+11             |
| C31:1 AEG      | 13.0        | C34H67O4          | M+H     | 539.5054        | 539.5034         | 3.7            | 3.E+11             |
| C31:2 AEG      | 13.9        | C34H65O4          | M+H     | 537.4895        | 537.4877         | 3.3            | 2.E+10             |
| C32:0 AEG      | 12.1        | C35H71O4          | M+H     | 555.5364        | 555.5347         | 3.2            | 7.E+10             |
| C32:1 AEG      | 12.8        | C35H69O4          | M+H     | 553.5210        | 553.5190         | 3.5            | 3.E+11             |
| C32:2 AEG      | 13.7        | C35H67O4          | M+H     | 551.5053        | 551.5034         | 3.5            | 2.E+11             |
| C33:0 AEG      | 11.9        | C36H73O4          | M+H     | 569.5521        | 569.5503         | 3.1            | 2.E+10             |
| C33:1 AEG      | 12.6        | C36H71O4          | M+H     | 567.5365        | 567.5347         | 3.2            | 1.E+12             |
| C33:2 AEG      | 13.4        | C36H69O4          | M+H     | 565.5207        | 565.5190         | 2.9            | 2.E+11             |
| C33:3 AEG      | 14.4        | C36H67O4          | M+H     | 563.5050        | 563.5034         | 2.9            | 2.E+10             |
| C34:0 AEG      | 11.7        | C37H75O4          | M+H     | 583.5676        | 583.5660         | 2.7            | 2.E+10             |
| C34:1 AEG      | 12.3        | C37H73O4          | M+H     | 581.5521        | 581.5503         | 3.0            | 1.E+11             |
| C34:2 AEG      | 13.1        | C37H71O4          | M+H     | 579.5364        | 579.5347         | 3.0            | 8.E+11             |
| C35:0 AEG      | 11.6        | C38H77O4          | M+H     | 597.5833        | 597.5816         | 2.7            | 2.E+10             |
| C35:1 AEG      | 12.1        | C38H75O4          | M+H     | 595.5678        | 595.5660         | 3.1            | 6.E+10             |
| C35:2 AEG      | 12.9        | C38H73O4          | M+H     | 593.5520        | 593.5503         | 2.8            | 1.E+11             |
| C35:3 AEG      | 13.7        | C38H71O4          | M+H     | 591.5362        | 591.5347         | 2.5            | 2.E+10             |
| C35:4 AEG      | 14.7        | C38H69O4          | M+H     | 589.5209        | 589.5190         | 3.2            | 2.E+10             |
| C36:0 AEG      | 11.4        | C39H79O4          | M+H     | 611.5988        | 611.5973         | 2.4            | 1.E+10             |
| C36:1 AEG      | 12.0        | C39H77O4          | M+H     | 609.5833        | 609.5216         | 2.6            | 3.E+10             |
| C36:2 AEG      | 12.7        | C39H75O4          | M+H     | 607.5678        | 607.5660         | 3.0            | 3.E+11             |
| C36:3 AEG      | 13.6        | C39H73O4          | M+H     | 605.5519        | 605.5503         | 2.6            | 2.E+10             |
| C36:4 AEG      | 14.4        | C39H71O4          | M+H     | 603.5361        | 603.5347         | 2.4            | 3.E+10             |

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**Appendix Table A3.3.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(2) Artificial chimney sample: AC-C0016B**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C38:1 AEG      | 11.5        | C41H81O4          | M+H     | 637.6141        | 637.6129         | 2.4            | 9.E+09             |
| C38:2 AEG      | 12.2        | C41H79O4          | M+H     | 635.5989        | 635.5973         | 2.6            | 2.E+10             |
| C38:3 AEG      | 13.0        | C41H77O4          | M+H     | 633.5829        | 633.5816         | 2.0            | 7.E+09             |
| <b>CL-DEGs</b> |             |                   |         |                 |                  |                |                    |
| C30:0 DEG      | 9.6         | C33H69O3          | M+H     | 513.5261        | 513.5241         | 3.8            | 1.E+12             |
| C30:1 DEG      | 10.0        | C33H67O3          | M+H     | 511.5104        | 511.5085         | 3.8            | 7.E+11             |
| C30:2 DEG      | 10.6        | C33H65O3          | M+H     | 509.4947        | 509.4928         | 3.6            | 2.E+11             |
| C30:3 DEG      | 11.3        | C33H63O3          | M+H     | 507.4791        | 507.4772         | 3.7            | 9.E+10             |
| C30:4 DEG      | 12.1        | C33H61O3          | M+H     | 505.4635        | 505.4615         | 3.9            | 3.E+10             |
| C31:0 DEG      | 9.3         | C34H71O3          | M+H     | 527.5418        | 527.5398         | 3.9            | 4.E+11             |
| C31:1 DEG      | 9.8         | C34H69O3          | M+H     | 525.5260        | 525.5241         | 3.6            | 1.E+12             |
| C31:2 DEG      | 10.5        | C34H67O3          | M+H     | 523.5104        | 523.5085         | 3.6            | 1.E+11             |
| C32:0 DEG      | 9.2         | C35H73O3          | M+H     | 541.5575        | 541.5554         | 3.8            | 4.E+11             |
| C32:1 DEG      | 9.7         | C35H71O3          | M+H     | 539.5419        | 539.5398         | 4.0            | 1.E+12             |
| C32:2 DEG      | 10.2        | C35H69O3          | M+H     | 537.5263        | 537.5241         | 4.1            | 3.E+12             |
| C32:3 DEG      | 10.9        | C35H67O3          | M+H     | 535.5104        | 535.5085         | 3.6            | 9.E+10             |
| C32:4 DEG      | 11.7        | C35H65O3          | M+H     | 533.4948        | 533.4928         | 3.6            | 1.E+11             |
| C33:0 DEG      | 9.1         | C36H75O3          | M+H     | 555.5729        | 555.5711         | 3.4            | 6.E+11             |
| C33:1 DEG      | 9.5         | C36H73O3          | M+H     | 553.5577        | 553.5554         | 4.1            | 3.E+12             |
| C33:2 DEG      | 10.0        | C36H71O3          | M+H     | 551.5419        | 551.5398         | 3.9            | 1.E+12             |
| C34:0 DEG      | 9.0         | C37H77O3          | M+H     | 569.5887        | 569.5867         | 3.6            | 4.E+12             |
| C34:1 DEG      | 9.4         | C37H75O3          | M+H     | 567.5728        | 567.5867         | 3.1            | 9.E+11             |
| C34:2 DEG      | 9.8         | C37H73O3          | M+H     | 565.5574        | 565.5554         | 3.4            | 5.E+12             |
| C35:0 DEG      | 8.8         | C38H79O3          | M+H     | 583.6043        | 583.6024         | 3.3            | 2.E+12             |
| C35:1 DEG      | 9.2         | C38H77O3          | M+H     | 581.5886        | 581.5867         | 3.3            | 2.E+12             |
| C35:2 DEG      | 9.7         | C38H75O3          | M+H     | 579.5728        | 579.5711         | 2.9            | 8.E+11             |
| C36:0 DEG      | 8.8         | C39H81O3          | M+H     | 597.6198        | 597.6180         | 3.0            | 1.E+12             |
| C36:1 DEG      | 9.1         | C39H79O3          | M+H     | 595.6041        | 595.6024         | 2.8            | 1.E+12             |
| C36:2 DEG      | 9.5         | C39H77O3          | M+H     | 593.5886        | 593.5867         | 3.1            | 5.E+11             |
| C37:0 DEG      | 8.6         | C40H83O3          | M+H     | 611.6353        | 611.6337         | 2.6            | 7.E+10             |
| C37:1 DEG      | 9.0         | C40H81O3          | M+H     | 609.6194        | 609.6180         | 2.3            | 4.E+11             |
| C37:2 DEG      | 9.4         | C40H79O3          | M+H     | 607.6041        | 607.6024         | 2.9            | 2.E+11             |
| C37:3 DEG      | 9.8         | C40H77O3          | M+H     | 605.5883        | 605.5867         | 2.5            | 4.E+10             |
| C38:0 DEG      | 8.5         | C41H85O3          | M+H     | 625.6507        | 625.6493         | 2.2            | 2.E+10             |
| C38:1 DEG      | 8.8         | C41H83O3          | M+H     | 623.6350        | 623.6337         | 2.1            | 6.E+10             |
| C38:2 DEG      | 9.2         | C41H81O3          | M+H     | 621.6194        | 621.6180         | 2.2            | 1.E+11             |
| C39:1 DEG      | 8.7         | C42H85O3          | M+H     | 637.6508        | 637.6493         | 2.2            | 2.E+10             |
| C39:2 DEG      | 9.2         | C42H83O3          | M+H     | 635.6354        | 635.6337         | 2.7            | 2.E+10             |

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**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| DAG       |             |                   |         |                 |                  |                |                    |
| C32:0 DAG | 36.2        | C35H69O5          | M+H     | 569.5161        | 569.5140         | 3.7            | 2.E+10             |
| C32:1 DAG | 37.4        | C35H67O5          | M+H     | 567.5002        | 567.4983         | 3.3            | 4.E+10             |
| C32:2 DAG | 38.5        | C35H65O5          | M+H     | 565.4845        | 565.4827         | 3.3            | 2.E+10             |
| C34:0 DAG | 35.3        | C37H73O5          | M+H     | 597.5466        | 597.5453         | 2.2            | 4.E+10             |
| C34:1 DAG | 36.2        | C37H71O5          | M+H     | 595.5314        | 595.5296         | 3.1            | 7.E+10             |
| C34:2 DAG | 37.9        | C37H69O5          | M+H     | 593.5159        | 593.5140         | 3.2            | 4.E+10             |
| C36:1 DAG | 35.1        | C38H75O5          | M+H     | 623.5625        | 623.5609         | 2.6            | 5.E+10             |
| C36:2 DAG | 36.6        | C38H73O5          | M+H     | 621.5471        | 621.5453         | 2.9            | 5.E+10             |
| AEG       |             |                   |         |                 |                  |                |                    |
| C32:1 AEG | 11.8        | C35H69O4          | M+H     | 553.5212        | 553.5190         | 4.0            | 1.E+11             |
| C33:0 AEG | 11.0        | C36H73O4          | M+H     | 569.5520        | 569.5503         | 2.9            | 1.E+11             |
| C33:1 AEG | 11.7        | C36H71O4          | M+H     | 567.5366        | 567.5347         | 3.4            | 2.E+11             |
| C33:2 AEG | 12.1        | C36H69O4          | M+H     | 565.5211        | 565.5190         | 3.6            | 3.E+10             |
| C34:0 AEG | 11.0        | C37H75O3          | M+H     | 583.5678        | 583.5660         | 3.1            | 5.E+11             |
| C34:1 AEG | 11.5        | C37H73O4          | M+H     | 581.5522        | 581.5503         | 3.3            | 2.E+11             |
| C34:2 AEG | 12.3        | C37H71O4          | M+H     | 579.5366        | 579.5347         | 3.3            | 4.E+11             |
| C35:0 AEG | 10.7        | C38H77O4          | M+H     | 597.5836        | 597.5816         | 3.3            | 5.E+11             |
| C35:1 AEG | 11.2        | C38H75O4          | M+H     | 595.5677        | 595.5660         | 3.0            | 2.E+11             |
| C35:2 AEG | 11.9        | C38H73O4          | M+H     | 593.5518        | 593.5503         | 2.5            | 4.E+10             |
| C36:0 AEG | 10.6        | C39H79O4          | M+H     | 611.5990        | 611.5973         | 2.8            | 5.E+11             |
| C36:1 AEG | 11.1        | C39H77O4          | M+H     | 609.5833        | 609.5216         | 2.7            | 2.E+11             |
| C36:2 AEG | 11.6        | C39H75O4          | M+H     | 607.5681        | 607.5660         | 3.5            | 5.E+10             |
| C37:0 AEG | 10.3        | C40H81O4          | M+H     | 625.6141        | 625.6129         | 1.9            | 2.E+10             |
| C37:1 AEG | 10.8        | C40H79O4          | M+H     | 623.5990        | 623.5973         | 2.7            | 6.E+10             |
| C38:0 AEG | 10.1        | C41H83O4          | M+H     | 639.6302        | 639.6286         | 2.5            | 1.E+11             |
| C38:1 AEG | 10.5        | C41H81O4          | M+H     | 637.6146        | 637.6129         | 2.5            | 4.E+11             |
| C38:2 AEG | 11.0        | C41H79O4          | M+H     | 635.5992        | 635.5973         | 3.0            | 4.E+10             |
| DEG       |             |                   |         |                 |                  |                |                    |
| C30:0 DEG | 8.9         | C33H69O3          | M+H     | 513.5262        | 513.5241         | 4.0            | 5.E+11             |
| C31:0 DEG | 8.8         | C34H71O3          | M+H     | 527.5419        | 527.5398         | 4.0            | 1.E+11             |
| C31:1 DEG | 9.3         | C34H69O3          | M+H     | 525.5261        | 525.5241         | 3.8            | 4.E+11             |
| C32:0 DEG | 8.7         | C35H73O3          | M+H     | 541.5578        | 541.5554         | 4.4            | 1.E+12             |
| C32:1 DEG | 9.1         | C35H71O3          | M+H     | 539.5418        | 539.5398         | 3.8            | 4.E+11             |
| C32:2 DEG | 9.7         | C35H69O3          | M+H     | 537.5262        | 537.5241         | 3.8            | 2.E+11             |
| C33:0 DEG | 8.6         | C36H75O3          | M+H     | 555.5732        | 555.5711         | 3.9            | 8.E+12             |
| C33:1 DEG | 9.0         | C36H73O3          | M+H     | 553.5577        | 553.5554         | 4.1            | 5.E+12             |
| C33:2 DEG | 9.3         | C36H71O3          | M+H     | 551.5417        | 551.5398         | 3.6            | 1.E+11             |
| C34:0 DEG | 8.5         | C37H77O3          | M+H     | 569.5867        | 569.5867         | 4.2            | 5.E+13             |
| C34:1 DEG | 8.9         | C37H75O3          | M+H     | 567.5732        | 567.5867         | 3.7            | 1.E+13             |
| C34:2 DEG | 9.5         | C37H73O3          | M+H     | 565.5574        | 565.5554         | 3.4            | 7.E+12             |
| C35:0 DEG | 8.4         | C38H79O3          | M+H     | 583.6046        | 583.6024         | 3.7            | 2.E+13             |
| C35:1 DEG | 8.7         | C38H77O3          | M+H     | 581.5888        | 581.5867         | 3.6            | 2.E+13             |
| C35:2 DEG | 9.1         | C38H75O3          | M+H     | 579.5729        | 579.5711         | 3.2            | 1.E+12             |
| C35:3 DEG | 9.6         | C38H73O3          | M+H     | 577.5577        | 577.5554         | 3.9            | 6.E+10             |

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**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(3) Natural chimney sample: NC-HSC**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C36:0 DEG | 8.2         | C39H81O3          | M+H     | 597.6201        | 597.6180         | 3.5            | 2.E+13             |
| C36:1 DEG | 8.6         | C39H79O3          | M+H     | 595.6043        | 595.6024         | 3.3            | 1.E+13             |
| C36:2 DEG | 8.9         | C39H77O3          | M+H     | 593.5887        | 593.5867         | 3.3            | 2.E+12             |
| C37:0 DEG | 8.1         | C40H83O3          | M+H     | 611.6353        | 611.6337         | 2.6            | 6.E+11             |
| C37:1 DEG | 8.4         | C40H81O3          | M+H     | 609.6197        | 609.6180         | 2.7            | 6.E+12             |
| C37:2 DEG | 8.8         | C40H79O3          | M+H     | 607.6044        | 607.6024         | 3.3            | 1.E+12             |
| C38:0 DEG | 7.9         | C41H85O3          | M+H     | 625.6511        | 625.6493         | 2.8            | 5.E+11             |
| C38:1 DEG | 8.2         | C41H83O3          | M+H     | 623.6354        | 623.6337         | 2.7            | 9.E+11             |
| C38:2 DEG | 8.7         | C41H81O3          | M+H     | 621.6198        | 621.6180         | 2.8            | 2.E+12             |
| C39:1 DEG | 8.1         | C42H85O3          | M+H     | 637.6511        | 637.6493         | 2.7            | 3.E+12             |
| C39:2 DEG | 8.5         | C42H83O3          | M+H     | 635.6356        | 635.6337         | 3.0            | 3.E+11             |
| C41:1 DEG | 7.9         | C44H89O3          | M+H     | 665.6824        | 665.6806         | 2.6            | 8.E+11             |
| C41:2 DEG | 8.2         | C44H87O3          | M+H     | 663.6672        | 663.6650         | 3.3            | 2.E+11             |
| C41:3 DEG | 8.2         | C44H85O3          | M+H     | 677.6821        | 677.6806         | 2.2            | 6.E+11             |

**(4) Natural chimney sample: NC-HRV**

DAG

|           |      |          |     |          |          |     |        |
|-----------|------|----------|-----|----------|----------|-----|--------|
| C30:0 DAG | 37.7 | C33H65O5 | M+H | 541.4847 | 541.4827 | 3.7 | 5.E+10 |
| C30:1 DAG | 39.0 | C33H63O5 | M+H | 539.4689 | 539.4670 | 3.6 | 1.E+11 |
| C30:2 DAG | 40.7 | C33H61O5 | M+H | 537.4532 | 537.4514 | 3.5 | 6.E+10 |
| C30:3 DAG | 42.7 | C33H59O5 | M+H | 535.4374 | 535.4357 | 3.1 | 7.E+09 |
| C31:0 DAG | 37.2 | C34H67O5 | M+H | 555.5001 | 555.4983 | 3.2 | 3.E+10 |
| C31:1 DAG | 38.4 | C34H65O5 | M+H | 553.4847 | 553.4827 | 3.8 | 1.E+11 |
| C31:2 DAG | 40.2 | C34H63O5 | M+H | 551.4688 | 551.4670 | 3.3 | 1.E+10 |
| C32:0 DAG | 36.6 | C35H69O5 | M+H | 569.5153 | 569.5140 | 2.4 | 5.E+10 |
| C32:1 DAG | 38.0 | C35H67O5 | M+H | 567.4999 | 567.5140 | 2.9 | 4.E+11 |
| C32:2 DAG | 39.7 | C35H65O5 | M+H | 565.4843 | 565.4827 | 2.9 | 4.E+11 |
| C32:3 DAG | 41.6 | C35H63O5 | M+H | 563.4687 | 563.4670 | 3.0 | 6.E+10 |
| C33:0 DAG | 36.3 | C36H71O5 | M+H | 583.5308 | 583.5453 | 2.1 | 2.E+10 |
| C33:1 DAG | 37.6 | C36H69O5 | M+H | 581.5156 | 581.5296 | 2.9 | 9.E+10 |
| C33:2 DAG | 39.0 | C36H67O5 | M+H | 579.5000 | 579.4983 | 2.9 | 5.E+10 |
| C34:0 DAG | 35.5 | C37H73O5 | M+H | 597.5472 | 597.5453 | 3.3 | 3.E+10 |
| C34:1 DAG | 37.1 | C37H71O5 | M+H | 595.5314 | 595.5296 | 3.0 | 6.E+11 |
| C34:2 DAG | 38.5 | C37H69O5 | M+H | 593.5158 | 593.5140 | 3.1 | 7.E+11 |
| C34:3 DAG | 40.5 | C37H67O5 | M+H | 591.5001 | 591.4983 | 3.1 | 2.E+11 |
| C34:4 DAG | 42.2 | C37H65O5 | M+H | 589.4844 | 589.4827 | 3.0 | 5.E+10 |
| C35:0 DAG | 35.4 | C38H75O5 | M+H | 611.5623 | 611.5609 | 2.3 | 1.E+10 |
| C35:1 DAG | 36.6 | C38H73O5 | M+H | 609.5468 | 609.5453 | 2.6 | 6.E+10 |
| C35:2 DAG | 38.1 | C38H71O5 | M+H | 607.5314 | 607.5296 | 3.0 | 5.E+10 |
| C36:0 DAG | 35.0 | C39H77O5 | M+H | 625.5778 | 625.5766 | 2.0 | 1.E+10 |
| C36:1 DAG | 36.2 | C39H75O5 | M+H | 623.5624 | 623.5609 | 2.5 | 2.E+11 |
| C36:2 DAG | 37.5 | C39H73O5 | M+H | 621.5453 | 621.5453 | 2.5 | 5.E+11 |
| C36:3 DAG | 39.1 | C39H71O5 | M+H | 619.5311 | 619.5296 | 2.4 | 3.E+11 |
| C36:4 DAG | 40.9 | C39H69O5 | M+H | 617.5154 | 617.5140 | 2.3 | 2.E+11 |
| C37:1 DAG | 35.8 | C40H77O5 | M+H | 637.5776 | 637.5766 | 1.6 | 2.E+10 |
| C37:2 DAG | 37.1 | C40H75O5 | M+H | 635.5618 | 635.5609 | 1.5 | 2.E+10 |
| C38:1 DAG | 35.3 | C41H79O5 | M+H | 651.5937 | 651.5922 | 2.3 | 3.E+10 |

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**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and reference samples from the Iheya North field (continued).

**(4) Natural chimney sample: NC-HRV**

| Compounds      | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|----------------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C38:2 DAG      | 36.5        | C41H77O5          | M+H     | 649.5781        | 649.5766         | 2.3            | 2.E+11             |
| C38:3 DAG      | 38.1        | C41H75O5          | M+H     | 647.5625        | 647.5609         | 2.5            | 1.E+11             |
| C38:4 DAG      | 38.6        | C41H73O5          | M+H     | 645.5469        | 645.5453         | 2.6            | 1.E+11             |
| C40:1 DAG      | 34.6        | C43H83O5          | M+H     | 679.6235        | 679.6235         | 2.3            | 6.E+09             |
| C40:2 DAG      | 35.4        | C43H81O5          | M+H     | 677.6088        | 677.6079         | 1.3            | 5.E+10             |
| C40:3 DAG      | 36.3        | C43H79O5          | M+H     | 675.5936        | 675.5922         | 2.1            | 4.E+10             |
| <b>CL-AEGs</b> |             |                   |         |                 |                  |                |                    |
| C30:0 AEG      | 11.8        | C33H67O4          | M+H     | 527.5053        | 527.5034         | 3.9            | 2.E+11             |
| C30:1 AEG      | 12.5        | C33H65O4          | M+H     | 525.4897        | 525.4877         | 3.9            | 1.E+11             |
| C31:0 AEG      | 11.6        | C34H69O4          | M+H     | 541.5212        | 541.5190         | 4.0            | 1.E+12             |
| C31:1 AEG      | 12.2        | C34H67O4          | M+H     | 539.5055        | 539.5034         | 3.9            | 1.E+12             |
| C31:2 AEG      | 13.1        | C34H65O4          | M+H     | 537.4899        | 537.4877         | 4.0            | 4.E+10             |
| C32:0 AEG      | 11.4        | C35H71O4          | M+H     | 555.5365        | 555.5347         | 3.3            | 1.E+11             |
| C32:1 AEG      | 12.0        | C35H69O4          | M+H     | 553.5211        | 553.5190         | 3.8            | 3.E+11             |
| C32:2 AEG      | 12.8        | C35H67O4          | M+H     | 551.5054        | 551.5034         | 3.7            | 2.E+11             |
| C33:0 AEG      | 11.2        | C36H73O4          | M+H     | 569.5522        | 569.5503         | 3.4            | 4.E+10             |
| C33:1 AEG      | 11.8        | C36H71O4          | M+H     | 567.5365        | 567.5347         | 3.2            | 1.E+12             |
| C33:2 AEG      | 12.5        | C36H69O4          | M+H     | 565.5208        | 565.5190         | 3.1            | 2.E+11             |
| C34:0 AEG      | 11.1        | C37H75O4          | M+H     | 583.5672        | 583.5660         | 2.1            | 1.E+10             |
| C34:1 AEG      | 11.6        | C37H73O4          | M+H     | 581.5522        | 581.5503         | 3.2            | 1.E+11             |
| C34:2 AEG      | 12.3        | C37H71O4          | M+H     | 579.5366        | 579.5347         | 3.3            | 6.E+11             |
| C35:0 AEG      | 10.9        | C38H77O4          | M+H     | 597.5834        | 597.5816         | 3.0            | 1.E+10             |
| C35:1 AEG      | 11.4        | C38H75O4          | M+H     | 595.5679        | 595.5660         | 3.3            | 6.E+10             |
| C35:2 AEG      | 12.1        | C38H73O4          | M+H     | 593.5523        | 593.5503         | 3.3            | 1.E+11             |
| C35:3 AEG      | 12.9        | C38H71O4          | M+H     | 593.5363        | 591.5347         | 2.8            | 1.E+10             |
| C36:1 AEG      | 10.9        | C39H77O4          | M+H     | 609.5831        | 609.5216         | 2.4            | 4.E+10             |
| C36:2 AEG      | 11.9        | C39H75O4          | M+H     | 607.5679        | 607.5660         | 3.1            | 2.E+11             |
| <b>CL-DEG</b>  |             |                   |         |                 |                  |                |                    |
| C30:0 DEG      | 9.1         | C33H69O3          | M+H     | 513.5262        | 513.5241         | 4.0            | 1.E+12             |
| C30:1 DEG      | 9.5         | C33H67O3          | M+H     | 511.5105        | 511.5085         | 4.0            | 7.E+11             |
| C30:2 DEG      | 10.1        | C33H65O3          | M+H     | 509.4947        | 509.4928         | 3.7            | 1.E+11             |
| C30:3 DEG      | 10.7        | C33H63O3          | M+H     | 507.4790        | 507.4772         | 3.5            | 2.E+10             |
| C31:0 DEG      | 8.9         | C34H71O3          | M+H     | 527.5418        | 527.5398         | 3.9            | 3.E+11             |
| C31:1 DEG      | 9.4         | C34H69O3          | M+H     | 525.5262        | 525.5241         | 4.0            | 1.E+12             |
| C31:2 DEG      | 9.9         | C34H67O3          | M+H     | 523.5105        | 523.5085         | 3.9            | 1.E+11             |
| C32:0 DEG      | 8.8         | C35H73O3          | M+H     | 541.5575        | 541.5554         | 3.8            | 2.E+11             |
| C32:1 DEG      | 9.2         | C35H71O3          | M+H     | 539.5419        | 539.5398         | 4.0            | 9.E+11             |
| C32:2 DEG      | 9.7         | C35H69O3          | M+H     | 537.5262        | 537.5241         | 3.9            | 1.E+12             |
| C32:3 DEG      | 10.3        | C35H67O3          | M+H     | 535.5106        | 535.5085         | 3.9            | 4.E+10             |
| C32:4 DEG      | 11.2        | C35H65O3          | M+H     | 533.4949        | 533.4928         | 3.8            | 4.E+10             |
| C33:0 DEG      | 8.6         | C36H75O3          | M+H     | 555.5729        | 555.5711         | 3.2            | 6.E+10             |
| C33:1 DEG      | 9.0         | C36H73O3          | M+H     | 553.5576        | 553.5554         | 4.0            | 2.E+12             |
| C33:2 DEG      | 9.5         | C36H71O3          | M+H     | 551.5420        | 551.5398         | 4.0            | 7.E+11             |
| C34:0 DEG      | 8.5         | C37H77O3          | M+H     | 569.5886        | 569.5867         | 3.2            | 3.E+11             |
| C34:1 DEG      | 8.9         | C37H75O3          | M+H     | 567.5729        | 567.5867         | 3.3            | 2.E+11             |

(Continued to next page)

**Appendix Table A3.4.** Detail information of all non-archaeal CLs observed in artificial chimneys, natural chimneys and**(4) Natural chimney sample: NC-HRV**

| Compounds | RT<br>(min) | Predicted formula | Adducts | Obs. <i>m/z</i> | Theo. <i>m/z</i> | Error<br>(ppm) | Peak area/g<br>TOC |
|-----------|-------------|-------------------|---------|-----------------|------------------|----------------|--------------------|
| C34:2 DEG | 9.4         | C37H73O3          | M+H     | 565.5574        | 565.5554         | 3.6            | 3.E+12             |
| C34:3 DEG | 9.9         | C37H71O3          | M+H     | 563.5417        | 563.5398         | 3.5            | 4.E+10             |
| C35:0 DEG | 8.4         | C38H79O3          | M+H     | 583.6041        | 583.6024         | 3.0            | 6.E+10             |
| C35:1 DEG | 8.8         | C38H77O3          | M+H     | 581.5886        | 581.5867         | 3.2            | 8.E+10             |
| C35:2 DEG | 9.2         | C38H75O3          | M+H     | 579.5729        | 579.5711         | 3.1            | 4.E+11             |
| C36:0 DEG | 8.3         | C39H81O3          | M+H     | 597.6193        | 597.6180         | 2.6            | 3.E+10             |
| C36:1 DEG | 8.6         | C39H79O3          | M+H     | 595.6043        | 595.6024         | 3.3            | 4.E+10             |
| C36:2 DEG | 9.1         | C39H77O3          | M+H     | 593.5886        | 593.5867         | 3.2            | 2.E+11             |
| C37:1 DEG | 8.5         | C40H81O3          | M+H     | 609.6196        | 609.6180         | 2.5            | 3.E+10             |
| C37:2 DEG | 8.9         | C40H79O3          | M+H     | 607.6041        | 607.6024         | 2.8            | 1.E+10             |
| C37:3 DEG | 9.4         | C40H77O3          | M+H     | 605.5886        | 605.5867         | 3.1            | 2.E+10             |

**(5) Reference sample:RF-SO**

Not detected.

**(5) Reference sample:RF-AS**

Not detected.