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12. Data Report: Intact Membrane Lipids as Indicators of Subsurface Life in Cretaceous and Paleogene Sediments from Sites 1257 and 1258[1]

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ABSTRACT

We report results from the analysis of intact polar lipids (IPLs) in sediments from Ocean Drilling Program Sites 1257 and 1258. IPLs, constituting the cell membranes of living organisms, were detected in organic-lean sediments but not in underlying organic-rich black shales. Microbial activity in organic-lean sediments is likely due to sulfate-dependent oxidation of methane whereas difficulties detecting IPLs in black shales are interpreted to result from unfavorable signal-to-noise ratios due to low cell concentrations in combination with extremely high analytical noise created by uncharacterized organic matrix. IPLs found are consistent with a low-diversity community of archaea and bacteria. The concentrations of IPLs are more than one order of magnitude lower than those in Neogene deep subsurface sediments at the Peruvian margin, suggestive of significantly lower cell concentrations in Demerara Rise. This finding is consistent with inferred low rates of subsurface microbial activity.

INTRODUCTION

Glycerol-based phospho- and glycolipids are the major structural components of cell membranes and typically compose 2%–6% of the dry weight of a cell (e.g., Langworthy et al., 1983; Ferrante et al., 1990). Because of their susceptibility toward hydrolytic cleavage of the bond

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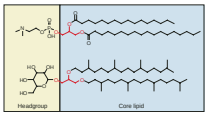
between polar headgroup and glycerol, their presence in environmental samples is viewed as an indicator of living matter (White et al., 1979).

Although structurally diverse, membrane lipids of all organisms share some basic features; usually a glycerol molecule linked to two fatty acids or fatty alcohols through ester- or ether-linkages, respectively, forms the core lipid. The headgroup is bonded to the remaining hydroxyl group on the glycerol molecule and may consist of a sugar moiety (Fig. F1, bottom structure) or a phosphate group, commonly bonded to another functional group such as N,N-dimethylethanolamine (Fig. F1, top structure), glycerol, choline, and so on.

F1. Structures of typical intact polar lipids, p. 7.



Fundamental differences between core lipids of archaea and bacteria/eukarya allow easy distinction; archaea contain relatively simple lipids based on isoprenoid fatty alcohols attached to the glycerol via ether linkages. Bacterial and eukaryotic membrane lipids are more diverse and generally contain fatty acids bonded via ester linkages to glycerol. Ether-linked fatty alcohols are observed in some bacteria (e.g., Sturt et al., 2004).

The diversity of membrane lipids in prokaryotes enables us to develop a picture of the general types of organisms present in a sample, and the distribution of intact polar lipids (IPLs) can be related to the composition of a prokaryotic community (Rütters et al., 2002a, 2002b; Sturt et al., 2004; Biddle et al., 2006).

The study of microbial life and processes in the marine subsurface is a major priority of oceanic deep drilling in the twenty-first century. Our objectives were to detect and characterize prokaryotic subsurface life in Cretaceous and Mesozoic sediment drilled during Ocean Drilling Program (ODP) Leg 207. Previous work on sediments from ODP Legs 190 (Zink et al., 2003) and 201 (Sturt et al., 2004; Biddle et al., 2006) has demonstrated the usefulness of IPL analysis for subsurface biogeochemistry.

The studied samples from ODP Sites 1257 and 1258 come from two distinct sedimentary environments, (i.e., Cretaceous black shales and Cretaceous and Paleogene nannofossil chalks and clays overlying the black shales). The black shale horizons are considered to be biologically active (i.e., methane diffusing out of black shales results from slow microbial degradation of sedimentary organic material) (Meyers et al., 2004; Arndt et al., 2006). In addition, elevated activity is expected in sediments overlying black shales at depths where methane is consumed at the expense of sulfate (e.g., Arndt et al., 2006).

MATERIALS AND METHODS

Analytical Protocol

Full details of the analytical procedures are presented in Sturt et al. (2004) and Biddle et al. (2006). Briefly, sediments were extracted using a modified Bligh and Dyer procedure, and sample cleanup was effected by open-column chromatography using silica adsorbent and 10 increasingly polar mixtures of solvents giving eight fractions containing various classes of compounds (Rütters et al., 2002b). Fractions 7 (glycolipids) and 8 (phospholipids) were analyzed using the high-performance liquid chromatography/electrospray ionization multistage mass spectrometry (HPLC-MS^n) procedures described by Sturt et al. (2004).

The softly ionizing electrospray procedure produces almost exclusively molecular ions, which is of great benefit when complex samples are analyzed. For clarity, results are presented as chromatograms in den-

sity map form, where mass to charge ratio (m/z) is plotted on the y-axis and time on the x-axis. Increasing relative intensity is plotted in darker shades of gray to black, causing eluting compounds to show up as distinct spots against a pale background. Archaeal lipids with their relatively simple system of core lipids (mainly archaeols and glyceroldialkylglyceroltetraethers [GDGTs]) generally show up as single signals, whereas bacterial lipids with a variety of fatty acid chain lengths show up as a series of closely eluting signals but with molecular ions varying by 14 Da (i.e., a CH_2 unit). The density map represents one "layer" of data, the base peak from each full scan (500–2000 m/z) is isolated and fragmented giving MS^2 spectral data, and the base peak from that MS^2 spectrum is further isolated and fragmented giving MS^3 spectral data. Also, data are recorded in both positive and negative ion modes (as two separate chromatographic runs) giving complimentary structural information. Only the positive ion density maps are shown here.

Samples

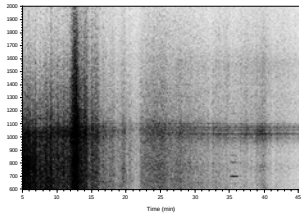
With the exception of one sample from Hole 1257C, all studied samples are from Hole 1258B (Table T1). Samples from both organic-rich black shales and organic-lean nannofossil chalks and clays have been analyzed.

T1. List of samples analyzed for IPLs, p. 10.

RESULTS AND DISCUSSION

From organic-rich black shales of Hole 1258B, typically 30 g of wet sediment was extracted. From the resulting highly concentrated polar fractions, aliquots equivalent to an extract of ~5 g of sediment were analyzed by HPLC-MS^n (Table T1). These sample concentrations proved to be insufficient for the detection of IPLs (Fig. F2). The organic-rich black shales are particularly challenging for the detection of miniscule quantities of IPLs because high quantities of fossil refractory organic carbon are difficult to separate from the polar lipids and contribute to a highly complex analytical background matrix that possibly causes ion suppression and dilution of analyte ions in the ion trap of the mass spectrometer. Figure F2 shows a representative density map plot from Sample 207-1258B-52R-2, 80–90 cm. After the dark shading in the density map is normalized to the signal intensity, the overall dark color indicates the lack of any compounds eluting above the relatively high background. Determination of prokaryotic cell numbers by catalyzed reporter deposition–fluorescence in situ hybridization (CARD-FISH) (**Schippers and Neretin,** this volume) suggests very low concentrations of cells, typically below 10^5 cells/cm^3.

F2. Density map of typical sample in positive ion mode, p. 8.



Following evidence for diagenetically active sediments being not only in black shales but also in horizons overlying the black shales (Arndt et al., 2006), we selected samples from the respective organic-lean intervals (Table T1). As a result of much more favorable signal-to-noise ratios, we were able to analyze samples as highly concentrated solutions, equivalent to injection of an extract from 69 and 120 g of sediment. IPLs were detected in these two samples and are described in detail below.

Table T2 shows that IPLs were found in both of the large sediment samples extracted. The more deeply buried, older Sample 207-1258B-39R-5, 130–140 cm, contained an order of magnitude less lipids than Sample 207-1257C-5R-2, 130–140 cm (Table T2). Absolute concentra-

T2. Relative signal intensity of IPLs, Sites 1257 and 1258, p. 11.

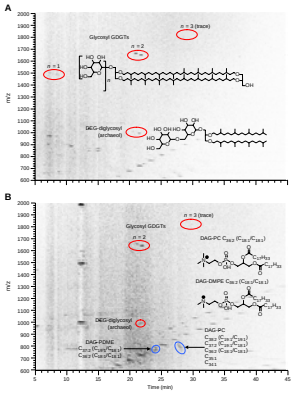
tions of these lipids cannot be computed at this point because of the lack of authentic standards, but we can compare the analytical IPL response to that in other environments that contain similar or identical compounds. For example, in deeply buried sediments off Peru typically containing 2×10^6 to 7×10^6 prokaryotic cells/cm^3 (total cells hybridizing to either an archaeal or bacterial FISH probe) (Biddle et al., 2006), archaeal IPLs are on average 20 times more abundant than in the two samples from Demerara Rise in which IPLs were detected. Hence, the IPL abundance is consistent with low prokaryotic cell numbers 10^5 cells/cm^3, which is reasonably consistent with the findings of **Schippers and Neretin** (this volume).

The archaeal lipids found are exclusively glycolipids, with mainly the GDGT core lipid. Related lipids have been detected in deeply buried sediments recovered during Leg 201 off Peru, where parallel phylogenetic analysis based on extractable 16S ribosomal ribonucleic acid suggests that they derive from sedimentary Crenarchaeota (Biddle et al., 2006). The glycolipids found herein contained no cyclopentyl rings within the GDGT macrocycle, a feature more common to the few Euryarchaeota we have studied that contain the GDGT core lipid, though the Euryarchaeota generally have a mixture of glycolipids and phospholipids in their membranes (H.F. Fredricks and K.-U. Hinrichs, unpubl. data). In Sample 207-1257C-5R-2, 130–140 cm (Table **T2;** Fig. **F3**), two unusual core lipids were observed that are 14 Da larger than the usual di-phytanyl archaeol and the di-biphytanyl GDGT core lipid. These so-called "DEG + 14-diglycosyl" and "GDGT + 14-diglycosyl" lipids are clearly identified as archaeol- and GDGT-like IPLs by their MS^n signals, but how the extra 14 Da is incorporated into the core lipid is unclear; the slightly shorter retention time (i.e., corresponding to a lower polarity) is consistent with an additional (nonpolar) CH_2 unit, though this has not been observed in any archaea to our knowledge. No nonitol-based GDGT core lipids were observed as found in various Leg 201 sediments (Biddle et al., 2006; Sturt et al., 2004) and observed members of the crenarchaeotal order Sulfolobales (Langworthy et al., 1974; Sugai et al., 1995).

The small range of bacterial lipids found is intriguing. Phosphatidylcholine (PC) lipids are relatively rare among bacteria. PC lipid is more common in Eukaryotes, where it is usually the most abundant lipid (e.g., Raetz, 1986). However, it is present in about 10% of bacterial species (Sohlenkamp et al., 2003) and observed in a significant number of proteobacterial isolates from the deep subsurface (Schubotz, 2005). Phosphatidyldimethylethanolamine (PDME) is tentatively identified based on the neutral loss of 169 Da in a fragmentation pattern common to most phospholipids. It is observed in Sample 207-1257C-5R-2, 130–140 cm, and because it contains the same core lipids as the PC species found, we suggest that it is derived from organisms similar to or related to those containing the PC species. PDME is reported as an intermediate in the biosynthesis of PC via methylation of phosphatidylethanolamine (Sohlenkamp et al., 2003) but is relatively rare in bacteria. Fang et al. (2000) reported PDME in barophilic bacteria from the Marianas Trench, along with PC and several other phospholipids.

The pattern and abundance of lipids observed in these Leg 207 Demerara Rise sediments is different from those of the Peru margin (Biddle et al., 2006; Sturt et al., 2004). Although no bacterial lipids were observed in the Peru margin sediments, the seemingly ubiquitous diglycosyl-GDGTs were observed in both locations. The Peru margin showed at

F3. Positive ion density map of glycolipid and phospholipid fractions, p. 9.



least an order of magnitude higher abundance of lipids. This is consistent with higher microbial activity and cell concentration in younger Peru margin sediments containing organic material of higher reactivity.

CONCLUSIONS

1. IPLs from both archaea and bacteria were detected in organic-lean Paleogene sediments recovered during Leg 207. In contrast, we were not able to detect IPLs in organic-rich black shales, presumably due to higher detection limit caused by a polar organic matrix that was inseparable from polar fractions containing IPLs.
2. The IPL abundance is consistent with very low cell concentrations reported by **Schippers and Neretin** (this volume).

ACKNOWLEDGMENTS

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Figure F1. Structures of typical intact polar lipids found in membranes of bacteria (top, phosphatidyl-N,N-dimethylethanolamine) and archaea (bottom, monoglycosyl diglyceride).

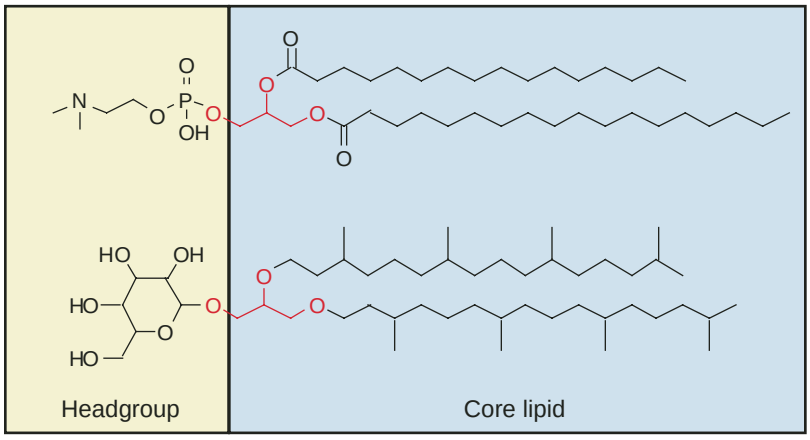

Figure F2. Density map of typical sample (Sample 207-1258B-52R-2, 80–90 cm) in positive ion mode. The vertical lines of darker (e.g., ~13 min) or lighter (~21 min) shading indicate enhancement or suppression of ionization, most likely due to the elution of noncharacterized and chromatographically nonresolved compounds from the HPLC column. The abundance of ions between 1000 and 1100 m/z (mass to charge ratio) resembles column or system bleed.

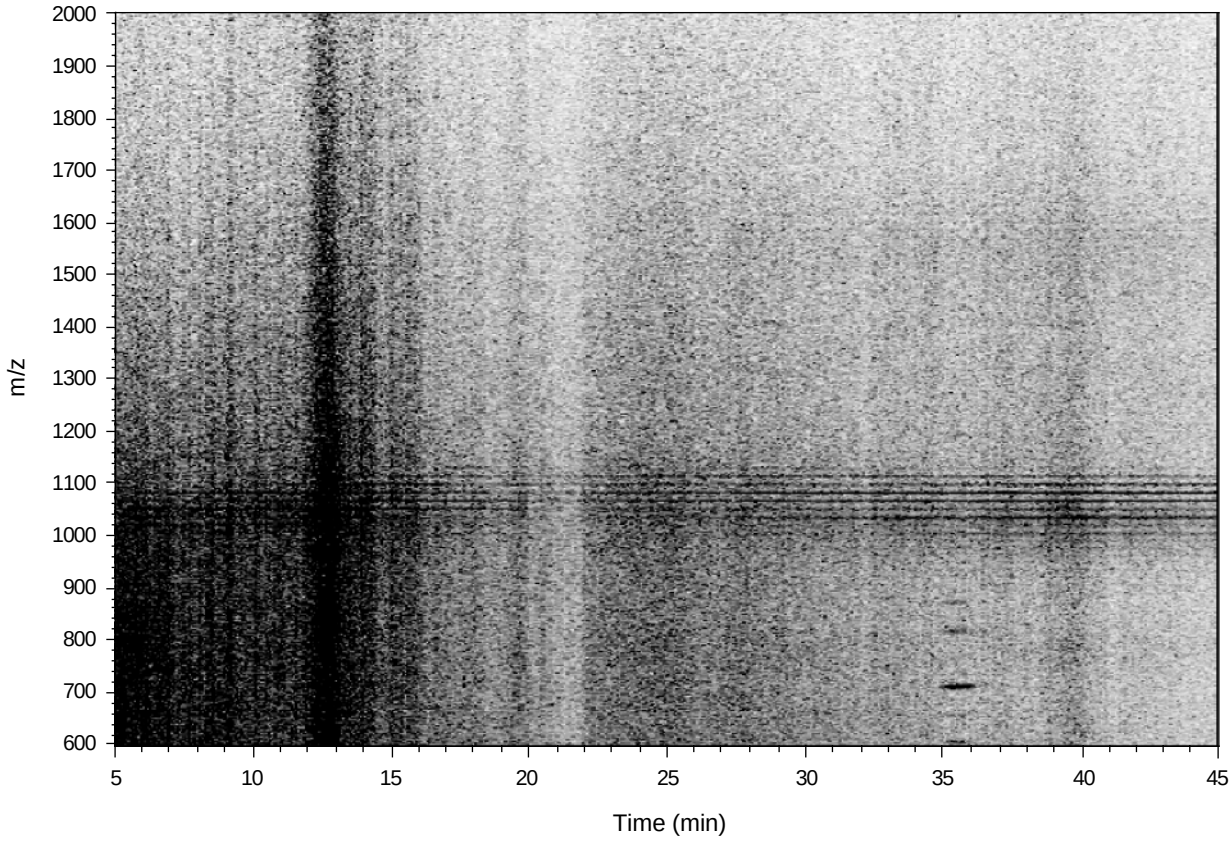

Figure F3. Positive ion density map plots of (**A**) glycolipid and (**B**) phospholipid fractions from Sample 207-1257C-5R-2, 130–140 cm; 123.3 mbsf. The two signals corresponding to the diglycosyl GDGT and archaeol species are the regular core lipid and unusual "+14" Da species, see text for discussion. Unmarked signals observed in the density maps were either identified as contaminants or are unidentified compounds showing no characteristics of IPLs. m/z = mass to charge ratio.

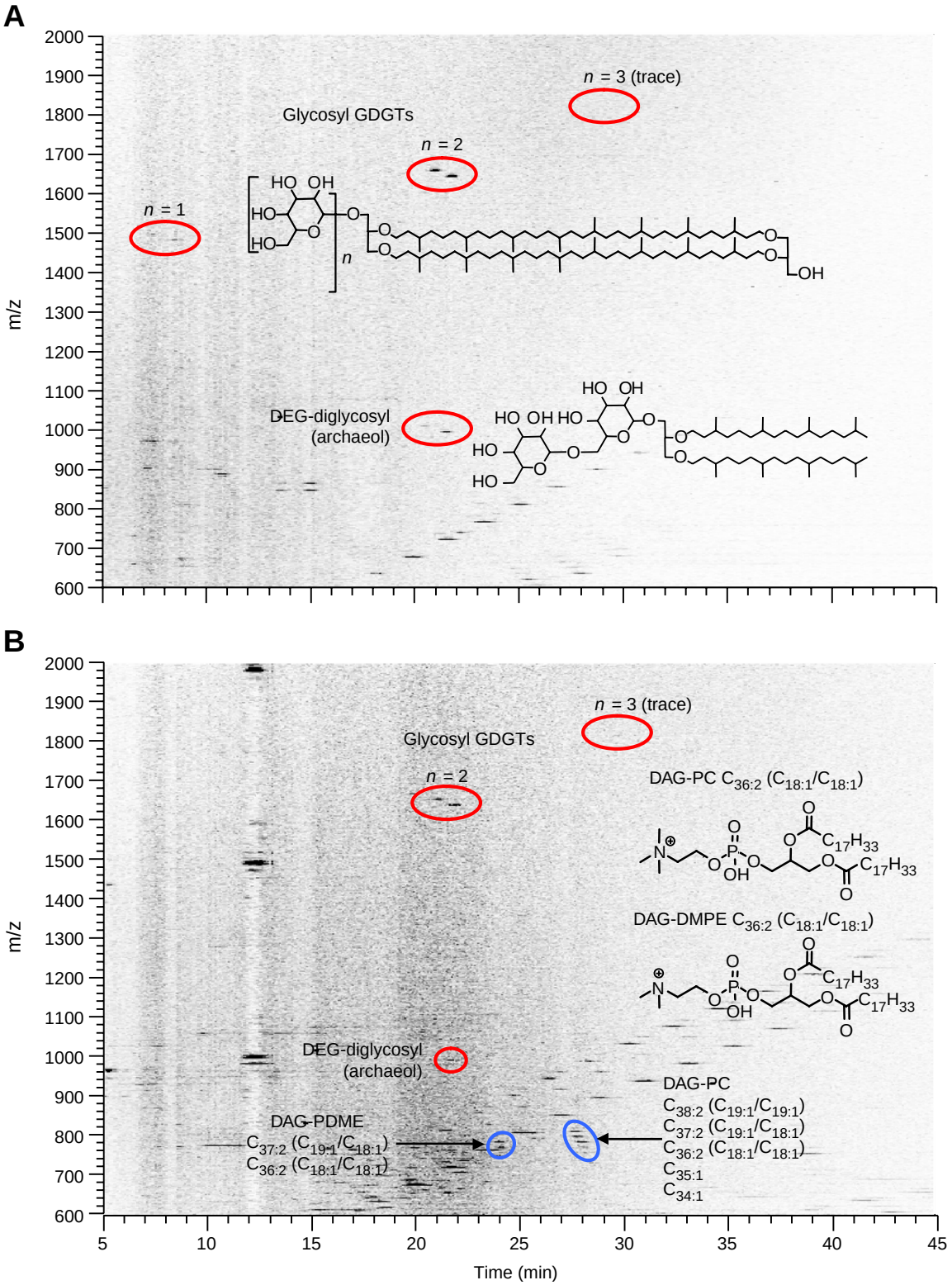

Table T1. List of samples analyzed for intact polar lipids (IPLs).

Core, section, interval (cm)	Depth (mbsf)	Lithology	Injected sample aliquot equivalent to *n* g sediment	IPLs detected?
207-1258B-				
40R-1, 80–90	369.4	Calcareous nannofossil clay	5	No
42R-1, 110–120	384.9		5	No
43R-1, 120–130	390.5		5	No
45R-2, 54–65	399.0	Laminated black shale/limestone w/organic matter	5	No
46R-1, 44–54	403.5		5	No
51R-2, 13–20	427.9		5	No
52R-2, 80–90	433.8		5	No
53R-1, 100–110	436.9		5	No
54R-3, 20–30	444.6		5	No
55R-3, 68–78	448.4		5	No
207-1257C-				
5R-2, 130–140	123.3	Calcareous nannofossil clay or chalk	120	Yes
207-1258B-				
39R-5, 130–140	366.5		69	Yes

Table T2. Relative signal intensity of intact polar lipids (IPLs) in two samples, Sites 1257 and 1258.

	Compound	Relative intensity (%) Sample 207-1257C-5R-2	Sample 207-1258B-39R-5
Archaeal lipids:	GDGT-monoglycosyl	2	5
	DEG-diglycosyl	11	3
	"DEG+14-diglycosyl"	2	
	GDGT-diglycosyl	28	6
	"GDGT+14-diglycosyl"	11	
	GDGT-triglycosyl	Tr	
Bacterial lipids:	DAG-PDME ($C_{37:2}$)	7	
	DAG-PDME ($C_{36:2}$)	4	
	DAG-PC ($C_{38:2}$)	9	3
	DAG-PC ($C_{37:2}$)	15	8
	DAG-PC ($C_{36:2}$)	10	
	DAG-PC ($C_{35:1}$)	1	
	DAG-PC ($C_{34:1}$)	Tr	
	Total:	100	25

Notes: Data are semiquantitative and result from peak areas of individual compound per gram of sediment, where the sum of peak areas of all IPLs/g dry sediment in Sample 207-1257C-5R-2, 130–140 cm, is equivalent to 100%. Note that response factors of individual IPLs are not known; consequently the relative signal intensity is not necessarily equivalent to relative abundance. Tr = the ion and corresponding MS^2 data was observed but the peak was not quantifiable. The carbon numbers refer to the total number of carbon atoms in the two fatty acids on the diacylglycero- (DAG) lipids. Diethylglycero- (DEG) lipids are all $C_{40:0}$ and assumed to be archaeal. N.B. compounds written in quotation marks remain to be fully identified and are named for their characteristics.