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Lipid biomolecules in silica sinters: indicators of microbial biodiversity

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Summary

To explore further the diversity of the microorganisms and their relationship with geothermal sinters, we examined the lipids preserved in six sinters associated with four different hot spring (58–82°C) areas of the Taupo Volcanic Zone (TVZ), New Zealand. These sinters contain microbial remains, but the process of mineralization has rendered them largely unidentifiable. Dominant lipids include free fatty acids, 1,2-diacylglycerophospholipids, 1,2-di-*O*-alkylglycerols, glycerol dialkylglycerol tetraethers and 1-*O*-alkylglycerols. These confirm the presence and, in some cases, high abundances of bacteria in all six sinters and archaea in four of the six sinter samples; in addition, the presence of novel macrocyclic diethers and unusual distributions of monoethers and diethers suggest the presence of previously uncharacterized bacteria. The lipid distributions are also markedly dissimilar among the four sites; for example, novel macrocyclic diethers are restricted to the Rotokawa samples while particularly abundant monoethers occur only in the Orakei Korako sample. Thus, biomarkers can provide crucial insight into the complex community structure of thermophilic microorganisms, including both archaea and bacteria, involved with biogenic silica sinter formation.

Introduction

The study of microbial biomineralization in geothermal systems and their particular role in the formation of siliceous sinters is of broad scientific interest. From their 16S rRNA, many geothermal organisms appear to be quite primitive such that insight into their diversity and ecology is a critical component of origin of life studies and, potentially, astrobiology (Stetter, 1996). In addition, siliceous sinters are of economic interest as they commonly host epithermal gold and silver deposits (Jones *et al*., 2001a).

Finely laminated siliceous sinters are built up from amorphous silica masses that precipitate during cooling of hydrothermal fluids that are supersaturated with silica (e.g. Konhauser *et al*., 2001; Mountain *et al*., 2003). In the past 20 years, a variety of thermophiles and hyperthermophiles has been found in such settings, occurring as mats, in hydrothermal fluids and on the surfaces of and entrained in mineral deposits. These microorganisms can be highly abundant and could play an important role in sinter formation. Association of microorganisms with siliceous sinters has been reported from a range of hot springs in Yellowstone National Park, USA, the Kenyan Rift Valley and the Taupo Volcanic Zone in New Zealand (Jones *et al*., 1996; 1998; 2001b; Renaut *et al*., 1996; Mountain *et al*., 2003).

The Taupo Volcanic Zone (TVZ; Fig. 1) is situated centrally on the North Island of New Zealand. The area is 300 km long and up to 60 km wide, as defined by vent positions and caldera structures, from Mount Ruapehu to White Island, both active volcanoes. The area is the most frequently active and productive silicic volcanic system on Earth, and available data suggest that this has been the case for at least the past 0.34 million years (Wilson *et al*., 1995). Associated with this volcanism are several high-temperature (>250°C) geothermal systems through which a natural heat output of ≈ 4200 MW is channelled (Bibby *et al*., 1995). Steam and gas escaping from these deep hot-water systems appears at the surface as fumarolic activity or may be absorbed by superficial groundwaters to form distinctive hot spring systems (Henley and Ellis, 1983; Fig. 2).

Although their presence has now been confirmed, it is difficult to elucidate the role and nature of microorganisms related to siliceous sinter precipitation because progressive silicification can destroy cytoplasmic details and wall structure making it difficult to identify biosilicified organisms on morphological grounds (Jones *et al*., 1996; 1997). Thus, although the presence of biomineralized microorganisms has been confirmed in a large variety of modern

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Fig. 1. Map of the North Island of New Zealand, showing the Taupo Volcanic Zone and location of hydrothermal springs (white and black circles); those shown in white are the sites investigated in this study.

sinters, it can be difficult to determine which organisms were present during sinter formation. Particularly problematic has been the identification of Archaea in sinters and any assessment of their role in silicification (Renaut and Jones, 2000). Biomarkers (Fig. 3) can be used in the identification of microorganisms. Such efforts have been applied to cold seep authigenic carbonates (Thiel *et al*., 2001; Aloisi *et al*., 2002), while in hydrothermal settings, studies have mainly concerned the mat-building organisms (Dobson *et al*., 1988; Robinson and Eglinton, 1990; Zeng *et al*., 1992a,b; van der Meer *et al*., 2000; Jahnke *et al*., 2004). Here, we report biomarker distributions and abundances for six amorphous silica sinters from four different areas of the TVZ (Fig. 2). These are used to characterize microbial diversity and assess the relative abundances of different microorganisms (e.g. bacteria versus archaea) in siliceous sinter deposits.

Results

Biomarker distributions are heterogeneous among the six samples examined. The most abundant compounds in these samples are free fatty acids, 1,2 diacylglycerophospholipids [analysed as fatty acid methyl esters (FAMEs) after base hydrolysis; Fig. 4] and a variety of neutral ether lipids (Fig. 5), including 1,2-di-*O*-alkylglycerols (diethers), glycerol dialkylglycerol tetraethers (GDGTs) and 1-*O*-alkylglycerols (monoethers).

Phospholipid fatty acids

The abundances of the fatty acids released from saponification of the polar fraction, and thus inferred to derive from hydrolysis of 1,2 diacylglycerophospholipids (**III**, Fig. 3; phospholipid fatty acids, PLFAs), are highly variable with total abundances varying by nearly an order of magnitude among the studied samples (Table 1; representative chromatograms shown in Fig. 4). Inferred PLFA distributions are similarly variable as reflected by large differences in the average chain length and the ratio of unsaturated to saturated components. The Rotokawa samples, characterized by high temperature and low pH, contain very simple fatty acid distributions in the post-hydrolysis polar fractions. Specifically, fatty acids are characterized predominantly by saturated and straight-chain C_{16} and C_{18} components, an absence of branched components and relatively low ratios of unsaturated to saturated PLFAs. This suggests that the fatty acids derive from sources other than bacteria, possibly higher plant debris, and are not present as PLFAs, which appear not to have been well preserved. This is unsurprising considering the high temperatures of these springs, 80–82°C. The free acid fraction does contain much higher abundances of fatty acids (data not shown), consistent with this explanation; however, the free fatty acids have a distribution similar to that expected for higher plant material, and clear evidence for an abundance of bacterial 1,2 diacylglycerophospholipids (relative to other compounds, described below) is weak.

The samples from the other sites contain more abundant inferred PLFAs with more complex distributions. The Orakei Korako sample contains high abundances of predominantly C_{16} and C_{18} PLFAs, but the unsaturated components are of equal or greater abundance than their saturated equivalents. C_{16} and C_{18} components are similarly abundant in the Waiotapu silicate, but also present are high quantities of C_{20} unsaturated and saturated PLFAs, distinguishing it from all other samples. Both Wairakei sinters contain very high abundances of C_{16} and C_{18} saturated PLFAs while unsaturated C_{18} components, branched (*iso* and *anteiso*) and cyclopropyl-bearing C_{16} components and branched (*iso* and *anteiso*) C_{17} components are also present, all occurring in similar distributions in both samples.

Non-isoprenoidal ether lipids

The abundances of ether lipids composed of non-isoprenoidal alkyl moieties (i.e. either straight chain or bearing a single methyl substituent) are quite variable (representative chromatograms shown in Fig. 5; Table 2). 1-*O*-alkylglycerols (monoethers) are highly heterogeneous in their occurrence, being present in only trace

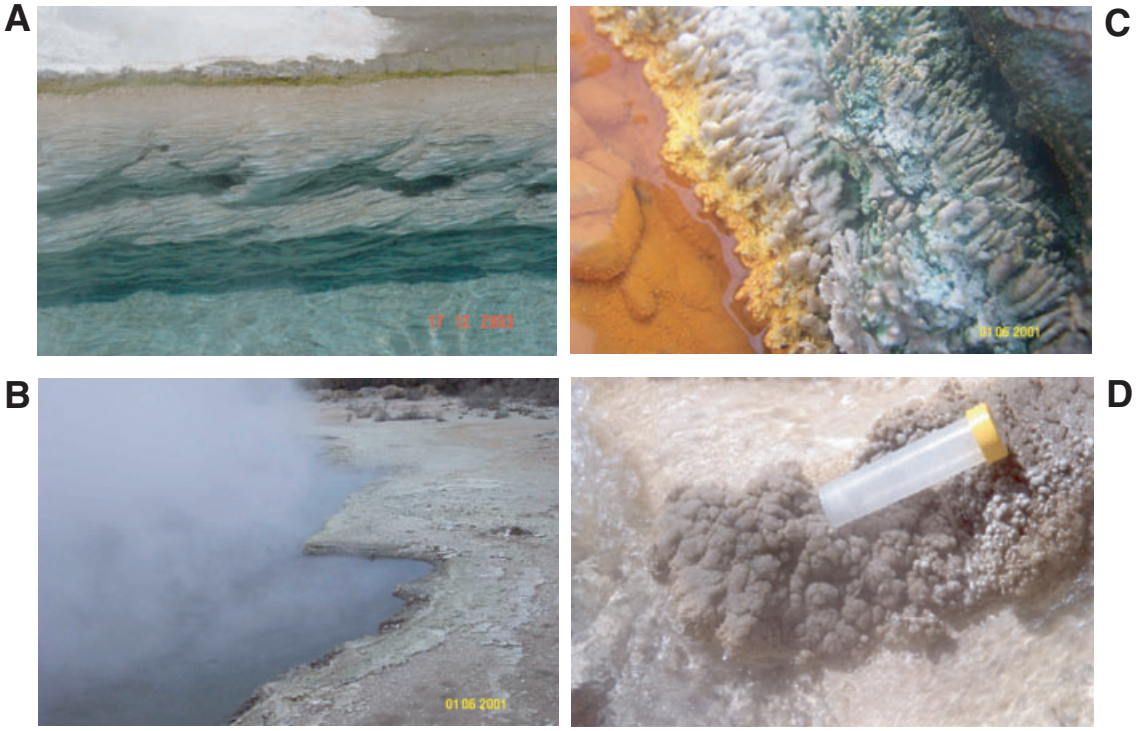

Fig. 2. Photos of sample sites described in this paper.
A. Wastewater drain at the Wairakei Power Station showing subaqueous, fan-like growths of silica sinter (sample site WR-1, T = 65°C, pH 8.5). The top of the photo is bounded by the subaerial top of the concrete divider. Water flows from right to left at ≈0.3 m s^{-1}. Field of view is 1 m in width.
B. South-east margin of the main upflow at Rotokawa (sample site RK-1F, T = 82°C, pH 3.8). Margin of pool is surrounded by spicular microstromatolites composed of amorphous silica.
C. North-east margin of Champagne Pool, Waiotapu (sample site WT-1, T = 75°C, pH 5.5). Subaqueous domal stromatolites are on the left. A rim of native sulfur lies at the air–water interface. This is followed by spicular microstromatolites composed of amorphous silica. The darker material on the right is silica sinter covered by cyanobacteria and algae. Field of view is 40 cm.
D. Nodular subaerial sinter from the Diamond Geyser at Orakei Korako (sample site OK-1D, T = 79°C, pH 8.6). Scale shown by 50 mm plastic tube.

abundances or absent in most samples except in the Orakei Korako sample where they are among the most abundant lipids. In this sample, several homologues – primarily C_{18} and C_{20} – are present. Conversely, 1,2-di-*O*-alkylglycerols (**V**, diethers) are generally more widespread and abundant, with only trace abundances occurring in the Wairakei silicates and more than 10 μg of total diether per gram of rock occurring in Rotokawa 6A. Distributions are also quite variable. The sinter sample from Waiotapu (WT1) contains a range of high-molecular-weight diethers, including a predominance of C_{16}/C_{17}, C_{17}/C_{17} and C_{18}/C_{17}, whereas both samples from the Rotokawa area have a lower molecular weight diether range. The Orakei Korako sinter is unusual in that only two diethers are present, the C_{15}/C_{15} and a C_{19}/C_{18} diether, with no intermediate diethers present.

In addition, in the two samples from the Rotokawa sinter flat, we recovered a novel pseudohomologous series of inferred macrocyclic diethers; these compounds are similar to macrocyclic archaeol (**IX**), which has been found only in the methanogenic archaeon *Methanococcus jannaschi*, but contain *n*-alkyl or simple branched alkyl rather than isoprenoidal components (R. D. Pancost, unpublished data). On structural grounds, the lack of an isoprenoidal hydrocarbon skeleton suggests that an archaeon did not synthesize these compounds; as both Rotokawa sites were deposited under low pH (<4) and high temperature (*c.* 80°C) conditions, it is possible that they derive instead from thermoacidophilic bacteria.

Archaeal ether lipids

Archaeal biomarkers are abundant in all but the Wairakei samples, where they are present but at relatively low concentrations (Table 2). Perhaps because of the high temperatures of the settings (75–82°C, except Wairakei), they are very abundant, commonly occurring at concentrations comparable to those of bacterial lipids. Archaeol, 1,2-di-*O*-phytanyl glycerol (**VIII**, X, X′ = H), is present in four of the six samples (0.13–2.0 μg g^{-1} rock). The greatest abundance of archaeol occurs in the high-temperature sample from Rotokawa (RK-1F, 82°C), in which it is one of the dominant components. Hydroxyarchaeol (**VIII**, X or X′ = OH), found in several species of methanogens, espe-

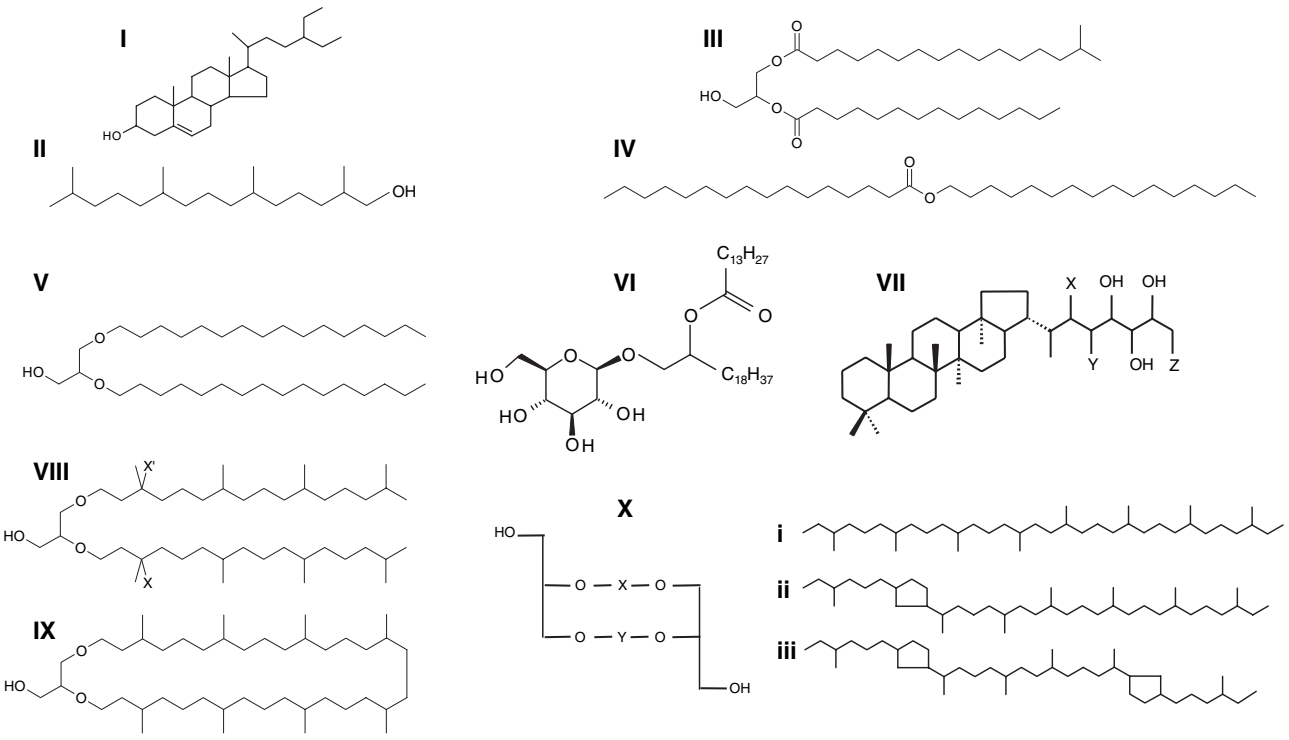

Fig. 3. Structures of compounds discussed in this paper, including those derived from both plant and bacterial sources (**I**, 24-ethylcholesterol; **II**, phytol), plant but predominantly bacterial sources [**III**, 1,2-diacylglycerophospholipid; **IV**, hexadecyl hexadecanoate (wax ester)], solely bacterial sources [**V**, 1,2-di-O-hexadecyl glycerol; **VI**, an alkyl glycoside; **VII**, bacteriohopanepolyol (X, Y can be either H or OH; Z can be a range of different functional groups)] and solely archaeal sources [**VIII**, archaeol (X,X′ = H) or hydroxyarchaeol (either X or X′ = OH); **IX**, macrocyclic archaeol; **X**, glycerol dialkyl glycerol tetraethers (**i**, **ii** and **iii** represent different isoprenoidal skeletons present as X or Y in tetraethers)].

cially *Methanosarcinales* species (Sprott *et al*., 1993; Nishihara and Koga, 1995), and the irregular isoprenoids, crocetene and pentamethylicosene, also associated with methanogenic and methanotrophic archaea (Schouten *et al*., 1997; Elvert *et al*., 1999; Bian *et al*., 2001), are absent. However, the four samples that contain archaeol also contain abundant glycerol dialkylglycerol tetraether lipids (**X**, GDGTs) consisting of two biphytanyl units containing zero to four cyclopentanyl moieties (i.e. Fig. 3i–iii). Although less abundant than bacterial biomarkers in most cases (Table 2), GDGTs are more abundant than archaeol and, in one sample (Rotokawa 1F), they are more abundant than all other compounds in the neutral lipid fraction. Thus, consistent with previous work, it appears that archaeal adaptations to high temperature include high relative abundances of tetraethers in which the biphytanyl units contain multiple cyclopentanyl moieties (Gliozzi *et al*., 1983; Uda *et al*., 2001).

Other lipids

The hopanoids (Fig. 3) are pentacyclic triterpenoids and are membrane components of many bacteria, including cyanobacteria, methanotrophs and aerobic heterotrophic bacteria (Ourisson *et al*., 1987). The most commonly observed hopanoids are diplopterol and bacteriohopanpolyol (**VII**) derivatives. Diplopterol is composed of a C_{30} hopanoid skeleton to which a hydroxyl group is attached at the C_{22} position. Bacteriohopanoids are composed of a C_{35} skeleton in which an *n*-pentyl group is attached to the hopanoid carbon skeleton at the C_{30} position. In all TVZ samples, hopanes are absent and hopanols are present in very low abundances; however, hopanoic acids ranging in carbon number from C_{29} to C_{35}, and especially the C_{31} and C_{32} components, are abundant in the Rotokawa and Orakei Korako sinters. The particularly high abundances of the C_{31} and C_{32} homologues probably result from oxidative cleavage of vicinal diols in tetra- and penta-functionalized bacteriohopanoids respectively (Farrimond *et al*., 2000).

Other lipids present in the total lipid extracts of TVZ siliceous sinter samples include *n*-alkanols and high-molecular-weight *n*-alkanoic acids of either microbial or higher plant origin, alkan-1,2-diols, wax esters (**IV**), tentatively identified alkyl glycosides (**VI**) and polyaromatic hydrocarbons. Of these, the wax esters, alkandiols and glycosides could have particular relevance for the interpretation of microbial communities. Wax esters, compounds comprising fatty acids esterified to long-chain alkanols, are absent in all analysed samples except for

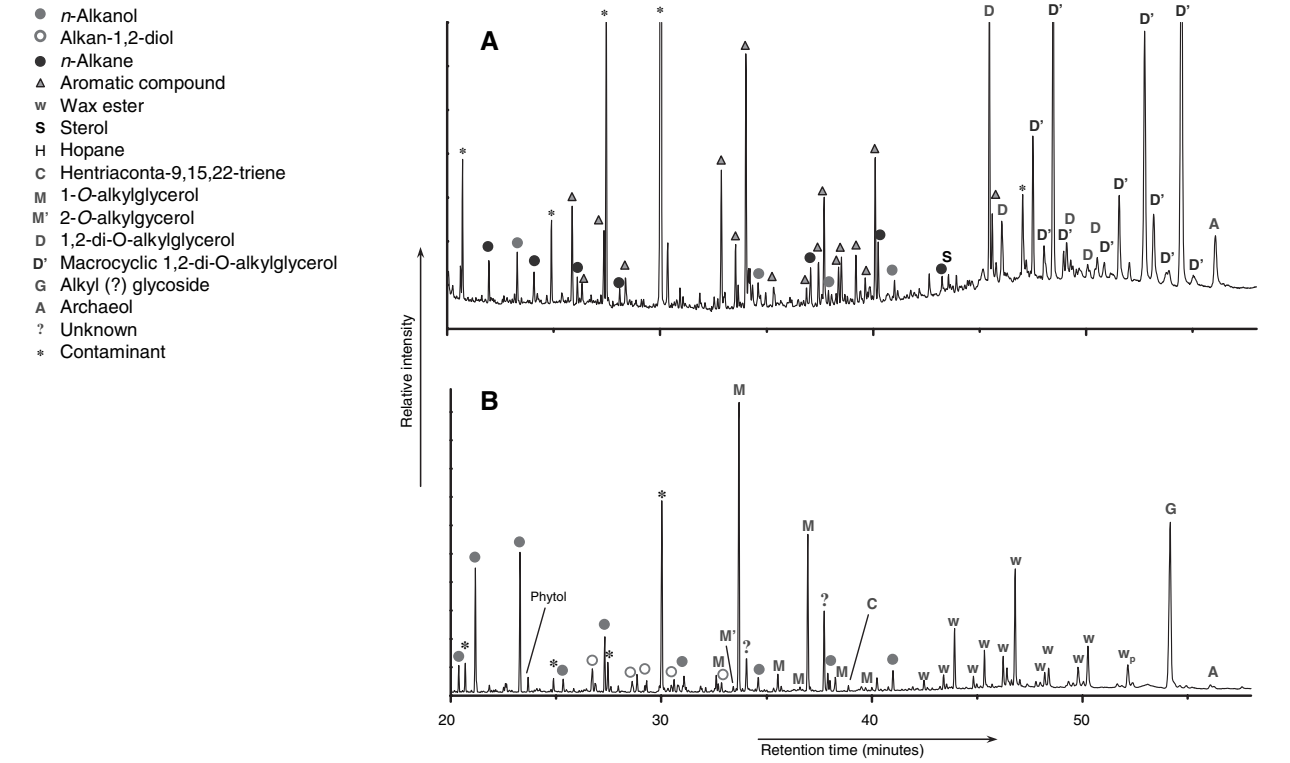

Fig. 4. Partial gas chromatograms of the saponified and methylated phospholipid fractions of the (A) Rotokawa RK-6F and (B) Orakei Korako OK-1D sinters, showing the distribution of predominant PLFAs.

the Orakei Korako sample, in which they were among the most abundant compounds in the neutral lipid fraction and range in carbon number from C_{31} to C_{36}. Similarly, glycosides inferred to bear an *n*-alkyl chain (**VI**) are among the most abundant neutral lipids in the Orakei Korako sinter sample but are present in only low abundances (Warakei 7) or absent in all other samples. Owing to low concentrations of the molecular ion in the mass spectra, it has not been possible to identify the structure of these glycosides. The likely degradation products of alkyl glycosides, *n*-alkyl 1,2-diols, are also abundant in the Orakei Korako sample (0.6 μg g^{-1} dry sediment), where they range in carbon number from C_{15} to C_{18}, but are present in only low or trace abundances in the other samples.

Discussion

Sources of microbial lipids

This is the first report that biomarkers are preserved in sinters and, thus, can be used to evaluate microbial assemblages associated with silica precipitation. However, such an approach must be used with caution: biomarker distributions will reflect a mixture of contributions from the wide range of organisms present, and detailed data on the lipids of different thermophilic organisms are lacking. Nonetheless, certain diagnostic lipids can be used to identify the presence of certain organisms (e.g. Archaea) and obtain semi-quantitative estimates of biomass that can be compared among different sites. Previous organic geochemical analyses of hot spring sediments have focused on the inputs associated with *Cyanobacteria* and *Chloroflexus* species. Biomarkers for these organisms include monomethyl alkanes (Shiea *et al*., 1991) and an all-*cis* hentriaconta-9,15,22-triene (van der Meer *et al*., 1999), respectively, as well as phospholipid fatty acids that are common in all bacteria. However, in all the samples described in this study, methylalkanes are absent, and hentriaconta-9,15,22-triene is present in only low abundances in our samples, suggesting that *Cyanobacteria* and *Chloroflexus* species are not significant components of the silicifying communities – probably reflecting the very high temperatures of these settings.

A possible exception is the Orakei Korako site, characterized by wax esters, alkan-1,2-diols and possible glycosides. Although such compounds could derive from a range of organisms (or groups of organisms), alkan-1,2-diols and wax esters have been reported previously for cyanobacterial/chloroflexus mats in Mushroom and Octopus Springs, Yellowstone, USA (Zeng *et al*., 1992a,b; Ruff-Roberts *et al*., 1994), and such a distribution of neutral lipids is similar to that produced by the

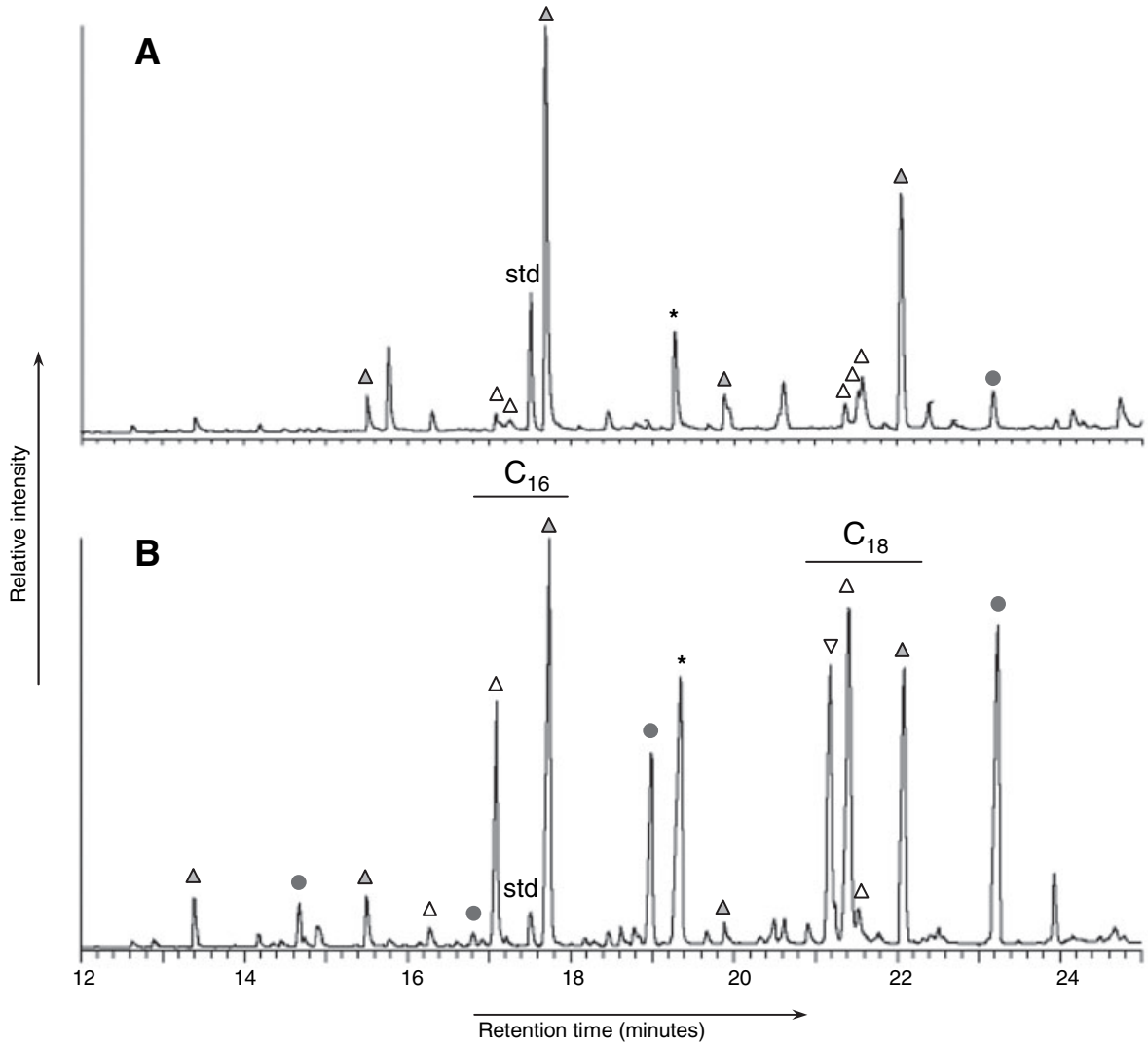

Fig. 5. Partial gas chromatograms of the neutral lipid fractions of the (A) Rotokawa RK-6A and (B) Orakei Korako 1D silicates, showing the distribution of predominant microbial lipids analysed as trimethyl silyl ethers. Solid triangles denote straight-chain, saturated fatty acids whereas open triangles denote either monounsaturated (pointing upwards) or di-unsaturated (pointing downwards) fatty acids.

Table 1. Environmental conditions associated with analysed sinters and the abundances of PLFAs.

						Fatty acid abundances (μg g^{-1} rock) and distributions					
			Concentration (mg l^{-1})							Unsaturated to saturated ratio	
Site	Temp (°C)	pH	SO_4^{2-}	TRS[a]	$SiO_{2(aq)}$	Saturated	Branched	Unsaturated	ACL[b]	C_{16}	C_{18}
Wairakei WR-1	52	8.5	61	0.55	584	2.68	0.38	0.60	17.22	0.01	0.26
Wairakei WR-7	62	8.47	45	2.0	559	0.38	0.10	0.15	17.04	0.04	0.68
Waiotapu WT-1	75	5.61	165	5.6	430	1.03	0.72	0.08	18.28	0.11	0.62
Orakei Korako OK-1D	78	9.01	119	0.34	325	1.20	1.32	0.06	17.13	0.50	2.55
Rotokawa RK-1F	80	2.46	972	12.3	268	1.12	0.39	0.00	17.71	0.02	0.55
Rotokawa RK-6A	82	3.67	420	3.7	336	0.30	0.05	0.00	16.93	0.11	0.33

a. Total reduced sulphur as H_2S.
b. Average chain length of $C_{14–20}$ PLFAs.

Table 2. Abundances (μg g rock^{-1}) of biomarkers (other than PLFAs) in analysed sinters.

	Monoethers		Diethers		Wax esters					
Site	Range	Abundance	Range	Abundance	Range	Abundance	Archaeol	Archaeal GDGTs	Alkyl (?) glycosides	Hopanoic acids
Wairakei WR-1	–	0	–	0	–	0	0	Tr[a]	Tr	–
Wairakei WR-7	–	0	15/15	0.03	–	0	0	Tr	0.18	–
Waiotapu WT-1	C_{20}	0.01	15/17–18/17	5.18	–	0	0.75	3.6	Tr	0.10
Orakei Korako OK-1D	C_{18-21}	5.41	15/15 and 19/18	0.22	C_{30-37}	5.6	0.13	2.6	5.74	0.40
Rotokawa RK-1F	?	0.04	15/15–15/18	0.66	–	0	1.96	31.7	Tr	0.48
Rotokawa RK-6A	–	0	15/15–15/19	11.23	–	0	0.49	5.7	Tr	4.36

a. Tetraethers are present but at abundances too low for quantification (<0.1 μg g^{-1} rock).

thermophilic bacterium *Roseiflexus castenholzii*, a *Chloroflexus* relative. The lipids of *R. castenholzii* are dominated by alkan-1-ol-2-alkanoate moieties glucosidically bound to a hexose sugar (glycoside) (Pond *et al*., 1986; van der Meer *et al*., 2002). Also present in *R. castenholzii* is a series of alkan-1,2-diols ranging in carbon number from C_{19} to C_{21} and wax esters ranging in carbon number from C_{37} to C_{40} with the C_{38} and C_{40} components predominating. However, the optimum growth temperature of *R. castenholzii* is 50°C, considerably lower than the temperatures measured at Orakei Korako, and *R. castenholzii* synthesizes predominantly C_{14}–C_{16} PLFAs. Alternative sources for the alkyl glycosides in the Orakei Korako sample are *Thermomicrobium roseum*, which contains C_{18} to C_{28} diols, and *Thermus* sp., which produce mainly *iso*-C_{18} alkan-1,2-diols also inferred to derive from the alkan-1-ol-2-alkanoate moiety of glycosides (van der Meer *et al*., 2002). Thus, the lipids in the Orakei Korako sinter could derive from relatives of these organisms or a high-temperature relative of *R. castenholzii*; the latter explanation remains an intriguing possibility given the presence, albeit in low abundances, of hentriacontatriene and verrucosan-3β-ol, biomarkers diagnostic for *Chloroflexus* species, in the Orakei Korako sample.

Hopanoid acids occur in four of the studied sinters and are particularly abundant in the Rotokawa 6A sample. Hopanoids have not been found in cultures of anaerobic bacteria and appear to be largely diagnostic for aerobic organisms (Ourisson *et al*., 1987); however, the recent discovery of ^{13}C-depleted hopanoids in Black Sea cold seeps indicates that at least some bacteria can synthesize hopanoids anaerobically (Thiel *et al*., 2003). The occurrence of hopanoids in these samples, including those deposited in anaerobic waters with high HS^- concentrations, is perhaps further evidence of an anaerobic source for these compounds; however, the samples characterized here were all deposited near or just below the air–water interface such that oxygen could have been available to bacteria even at the Waiotapu and Rotokawa sites where waters are anaerobic. Regardless of source, the variability of hopanoic acid distributions and abundances provides evidence for microbial diversity among the studied sinter samples.

A striking characteristic of several examined siliceous sinter samples is the abundance of glycerol dialkyl diethers. There are few known sources of non-isoprenoidal ether lipids, but most are thermophiles or hyperthermophiles. *Aquifex pyrophilus*, a thermophilic hydrogen oxidizer, comprises 66% of the main core lipid as alkyl glycerol diether (Huber *et al*., 1992). Workers have reported different alkyl chain distributions for different *Aquificales* cultures, with Huber and colleagues (1992) reporting C_{16}/C_{16}, C_{17}/C_{17} and C_{17}/C_{18} as the main components and Jahnke and colleagues (2001) reporting C_{18}/C_{18}, C_{18}/C_{20} and $C_{18}/C_{20:1}$ as the main components. The acid methanolysis products of *Ammonifex degensii*, a thermophilic anaerobic bacterium isolated from a neutral volcanic hot spring in Kawah Candradimuka Crater, Indonesia (Huber *et al*., 1996), comprised 85% glycerol diethers, with nine diethers identified. The diethers consist predominantly of C_{16}/C_{16} (34%), C_{16}/C_{17} (18%) and C_{17}/C_{17} (20%) compounds. The hydrophobic residues of *Thermodesulfobacterium commune*, a thermophilic sulfate-reducing anaerobic bacterium, consist of 1,2-dialkylglycerol diethers, with five principal diethers identified: the C_{16}/C_{16}, C_{16}/C_{17}, C_{17}/C_{17}, C_{17}/C_{18} and C_{18}/C_{18} homologues (Langworthy *et al*., 1983). Thus, the distributions of all three organisms are broadly similar to that observed in the Waiotapu sample; in contrast, the Rotokawa sinters contain diethers characterized by shorter chains and, typically, C_{15} alkyl components. The C_{15}/C_{15} diether occurs in most samples and was also detected by Zeng and colleagues (1992a) in cyanobacterial mats from Yellowstone National Park, USA, but the source of this compound remains unknown.

1-*O*-alkylglycerols (monoethers) have been identified in a limited number of settings and organisms, all hyperthermophiles or thermophiles. Monoethers comprise 9% and 11% of the core lipid fractions of *Ammonifex degensii* (Huber *et al*., 1996) and *Thermodesulfobacterium commune* (Langworthy *et al*., 1983), respectively, with alkyl chains ranging from C_{16} to C_{19}. *Clostridium thermosulphurogenes* is also known to produce significant quanti-

ties of monoethers (Langworthy and Pond, 1986). Huber and colleagues (1992) reported that 24% of *Aquifex pyrophilus* core lipid fraction is composed of a single (unidentified) monoether. Recently, however, Jahnke and colleagues (2001) showed that monoethers, specifically those with C_{18} and $C_{20:1}$ alkyl moieties, are the predominant lipids in a variety of *Aquificales* cultures. Similarly, C_{18} and C_{20} 1-*O*-alkylglycerols were major components of the Octopus Spring pink streamer community (Jahnke *et al*., 2001). Thus, the distribution of 1-*O*-alkylglycerols in the Orakei Korako sinter sample is consistent with the presence of *Aquificales* species.

Archaeal biomarkers, including both diethers (archaeol) and tetraethers, occur in all sinter samples from Orakei Korako, Waiotapu and Rotokawa but are lacking in the Wairakei samples. The specific sources of the archaeal lipids are unclear. Ward and colleagues (1985) showed that the abundances of diethers and tetraethers in Yellowstone National Park hot springs tracked methanogen abundances (determined by most-probable-number determinations). However, they observed predominantly archaeol and, to a lesser degree, GDGTs based on two acyclic biphytanyl moieties, whereas the TVZ samples contain predominantly GDGTs with multiple cyclopentane moieties. Other methanogen biomarkers, such as hydroxyarchaeol, crocetene and pentamethylicosene, are absent from all examined samples, suggesting that methanogens are not significant components of the archaeal community. Nonetheless, the occurrence of archaeal diethers and tetraethers reflects the significant contribution by archaea to microbial communities, and the particularly high abundances of archaeal lipids in the Rotokawa 1F sinter sample indicate their importance in particularly extreme (low pH and high temperature) settings.

Microbial communities associated with TVZ sinters

The variations in bacterial lipid distributions, particularly those of ether lipids, between TVZ sinters and cultured organisms suggest that: (i) the organisms present in some TVZ hot springs are distinct from previously cultured organisms; and/or (ii) the capacity for homeoviscous adaptation also extends to these compound classes. The latter explanation is plausible as variation in the carbon number has commonly been reported for the acyl moieties of 1,2 diacylglycerophospholipids. However, higher temperatures are typically associated with longer chain lengths, whereas in our samples, the siliceous sinters deposited at the highest temperatures (Rotokawa, 82°C) contain the lowest molecular weight diethers. It is likely then that, in addition to homeoviscous adaptation, the presence of diverse and uncharacterized microorganisms in these settings accounts for the unusual lipid distributions. The high abundances of unusual macrocyclic diethers offers further evidence that a significant portion of the microbial community in these settings is uncharacterized.

The variability in lipid distributions among organisms due to either physiological or environmental factors, combined with the lack of lipid analyses for the abundant uncultured organisms to be found in any natural setting, makes it difficult to define specific biological assemblages for the TVZ sinter samples. Nonetheless, some general observations can be made and tentative microbial community structures assigned (Table 3). In the relatively lower temperature Wairakei samples, archaeal lipids are present in trace quantities, indicating a lack of such organisms and a predominance of bacteria. At the Orakei Korako site, where sinters were deposited at high temperatures and under slightly alkaline conditions, biomarkers suggesting a high-temperature relative of *Chloroflexus*, 1-*O*-alkylglycerols with a distribution consistent with an *Aquificales* source and archaeal lipids were all identified. The Waiotapu sample was also formed under high temperatures but slightly acidic and anaerobic conditions; like the above sinters, it also contains predominantly bacterial and lesser abundances of archaeal lipids, but 1,2-di-*O*-alkylglycerols predominate and potentially derive from one of several hyperthermophilic – potentially anaerobic – organisms. The Rotokawa sinter samples were formed at relatively high temperatures and low pH, making them the most 'extreme' setting studied; both contain novel macrocyclic diethers, suggesting the presence of an uncharacterized bacterium, but sample RK1F contains predominantly archaeal lipids, while bacterial derived hopanoic acids are particularly abundant in 6A. Owing to

Table 3. Lipids and inferred dominant microorganisms present in TVZ silicates.

Site	Dominant lipids	Inferred dominant microorganisms
Wairakei WR-1	1,2-diacylglycerols	Bacteria; minimal Archaea
Wairakei WR-7	1,2-diacylglycerols	Bacteria; minimal Archaea
Waiotapu WT-1	1,2-diacylglycerols, 1,2-di-O-alkylglycerols	Hyperthermophiles; Archaea
Orakei Korako OK-1D	1,2-diacylglycerols; alkyl glycosides; wax esters; 1-O-alkylglycerols	High-temperature relative of *Chloroflexus*; *Aquificales* species; some Archaea
Rotokawa RK-1F	GDGTs; Archaeol; Macrocyclic diethers	Archaea, lesser abundances of bacteria; novel organism
Rotokawa RK-6A of Archaea	Macrocyclic diethers; GDGTs; hopanoic acids	Novel organism; Bacteria, lesser abundances

the limited sample set, it is difficult to ascribe variations in microbial community structure to specific environmental conditions. However, as expected, Archaea are particularly abundant in the most extreme (hottest, most acidic) settings. In any case, these results clearly illustrate the diversity of organisms associated with biosilicification.

Implications for silicification

Bacterial lipids are present in all and archaeal lipids are present in most of the examined samples, clearly indicating the association of microorganisms with siliceous sinters in these hot springs. Many previous scientists have proposed a causal link between the presence of microorganisms and silicate formation (e.g. Renaut and Jones, 2000 and references therein); we have no direct evidence for that, but our work does provide further evidence for the occurrence of microorganisms in the sinter-forming environment. It has been proposed that these microorganisms facilitate sinter formation by providing reactive sites for silica polymerization (Mountain *et al*., 2003). This role could be played by the various hydroxyl and carboxyl groups on the microbial cell wall or EPS sheath (Renaut *et al*., 1998, Benning *et al*., 2004a), and this may lead to a reduction in activation energy barriers to nucleation, permitting surface chemical interactions that induce further polymerization and aggregation of silica from solution (Konhauser *et al*., 2001; Benning *et al*., 2004b). This passive mediation does not depend on the specific metabolism or ecology of the organisms, but rather upon the presence of a substrate for nucleation (Renaut *et al*., 1998). Indeed, this is consistent with the diversity of microbial biomarkers in the examined sinters, which indicates that diverse communities of bacteria and archaea can be present during silicification.

Conclusions

This work is the first to show that archaeal and bacterial lipids can be recovered from mineral precipitates in hydrothermal systems. As such, it expands on previous analyses of organic biomarkers in hydrothermal settings (e.g. Zeng *et al*., 1992a,b; van der Meer *et al*., 2000), further illustrating that unique and potentially diagnostic lipids are associated with organisms living at high temperatures and, in fact, are likely to reflect an adaptation to those conditions. Moreover, this work has revealed a new compound class, non-isoprenoidal macrocyclic diethers, as well as previously unidentified monoether and diether homologues, and provided the first evidence for the presence of Archaea in sinters and the sinter-forming environment. Lipid distributions are quite variable indicating that: (i) multiple organisms are abundant at individual sites; and (ii) the distribution of microorganisms varies among different sites. This suggests that biogenic silicification is not restricted to a single species or group of organisms. A dynamic interplay between the polymerization of silica in high-temperature geothermal solutions and the presence of a varied microbial community composed of archaea and bacteria could be important for the siliceous sinters to form in such settings.

Experimental procedures

Sample sites

All the samples used in the project were collected from active geothermal pools or streams in the Taupo Volcanic Zone, situated on the North Island, New Zealand. At each site, before sampling, temperatures and pH measurement were carried out, and a fluid sample (for anions, cations and reduced sulphur) was collected. The full chemical composition of the fluids presented in Table 1 as well as further description of the various sampling sites, the mineralogy, geochemistry and, in part, the microbial populations (for Wairakei sample WR-1) are to be found in the paper by Mountain and colleagues (2003).

Waiotapu (WT). The Waiotapu geothermal field is located 23 km SE of Rotorua. This is a region of ash flows and volcaniclastic and lacustrine sediments that have been deposited over the last 300 000 years (Jones *et al*., 2001). The area features mud pools, geysers, fumaroles, hot pools, eruption craters and warm and boiling springs. Sample WT-1 was collected from the Champagne Pool, which occupies a hydrothermal explosion crater formed 600–900 years ago. The pool is ≈60 m in diameter, 150 m in depth and has a surface area of 3000 m^2. Water shallower than 62 m maintains a constant temperature of 75°C as a result of rapid convection and heat loss over the large surface area of the spring (Mountain *et al*., 2003). The water is anaerobic and contains a wide array of trace elements, including Au, Ag, Sb, W and As (Jones *et al*., 2001). Methylated species of Hg, Ge, As, Sb and Te are also observed in these waters (Mountain *et al*., 2003). The pool is rimmed by a subaqueous shelf composed of domal stromatolies containing silicified filamentous, bacilliform and coccoidal microbes (Mountain *et al*., 2003). Above the air–water interface, spicular silica surround the pool. These are composed of predominantly amorphous silica occurring as porous and non-porous laminae. The porous laminae are composed of filamentous and non-filamentous microbes that have undergone variable degrees of silicification (Mountain *et al*., 2003).

Rotokawa (RK). The Rotokawa geothermal field is 10 km north-east of Taupo and has had a history of hydrothermal activity for 20 000 years (Krupp and Seward, 1990). The Sinter Flat area of Rotokawa is a well-defined group of hot springs on the northern margin of Lake Rotokawa that have built up a flat terrace, mostly covered in hot pools (Krupp and Seward, 1990). The waters are turbid as a result of a high concentration of suspended material composed principally of native sulfur, clays and amorphous silica (Mountain

et al., 2003). Sample RK-1F (80°C) is from an ebullient hot spring along the north margin of the sinter flat, and sample RK-6 A (82°C) is from the south shore of the main upflow zone.

Orakei Korako (OK). The Orakei Korako geothermal area is situated on the eastern margin of the Moroa Volcanic Centre, 26 km NE of Taupo. The 2 km^2 area features hot springs, geysers, hydrothermal eruption areas and sinter terraces, with temperatures exceeding 100°C. The waters are near neutral chloride, with a total mineral content lower than at most other sites in the TVZ. The sample studied (OK-1D) originates from the Diamond Geyser with a collection temperature of 78°C. Bacteria have been found in Orakei Korako sinters. The predominant cyanobacterial mat builder is *Chlorogloeopsis* sp. (Shiea *et al*., 1991).

Wairakei (WR). The Wairakei geothermal area is located 9 km north of Taupo and hosts the largest geothermal power plant in New Zealand (Carroll *et al*., 1998). The artificial concrete drains at Wairakei dispose of the wastewater from steam production for the power plant. Wastewater exits flashplants at 100°C and then cools rapidly as it flows through the drains to the Wairakei stream. The sharp drop in temperature causes silica polymerization and silica precipitation. Many filamentous bacteria are present in the subaqueous sinters precipitating from the cooling wastewater. Sample WR-1 is from the main drain. The water at the sampling point has a pH of 8.5, 1900 p.p.m. Cl^- and 570 p.p.m. SiO_2. Scanning electron microscopic studies have shown that the deposits formed in this drain are composed of fine fibres of amorphous silica. These fibres are thought to represent silica sheaths on, or replacements of, filamentous bacteria (Mountain *et al*., 2003). Sample WR-7 is from another drain where silicification occurs mostly on branches and grasses.

Lipid extraction and fractionation

Samples were collected under sterile conditions, immediately sealed in sterile bags and frozen at –20°C and transported to the laboratory. Before analysis, the samples were thawed, rinsed with methanol (MeOH; all solvents are from Rathburn and of either HPLC or glass distilled grade), dried and ground to fine powder in a teemer mill. The powders, in cleaned cellulose thimbles, were placed in a Soxhlet apparatus and extracted for 24 h using a 2:1 azeotrope of dichloromethane (DCM)–methanol (MeOH). An aliquot (50%) of the total lipid extract was separated into neutral, acid and polar (phospholipid) fractions using aminopropyl bond-elut columns (Phenomenex; NH_2, 500 mg, 3 ml). The bond-elut columns were conditioned before separation of fractions by eluting with hexane (9 ml). Samples were loaded on to the column using DCM–MeOH (2:1 v/v) and eluted sequentially with 12 ml DCM–isopropanol (2:1 v/v; neutral fraction), 6 ml of 5% acetic acid in ether followed by 6 ml of 10% acetic acid in ether (combined into acid fraction) and methanol (24 ml; polar fraction). Subsequently, hexadecan-2-ol in DCM was added as an internal standard to the neutral fraction of each sample (42 μg).

Saponification

C_{19} *n*-alkane (200 ng) in hexane was added as an internal standard to the acid and polar (phospholipid) fractions. An aliquot (50%) of each sample was saponified using methanolic sodium hydroxide (1 ml, 0.5 M, 70°C, 1 h). The sample was extracted with hexane, and the extracts were combined and evaporated under nitrogen into a Pyrex culture tube. The sample was methylated with BF3–methanol (70°C, 1 h), and the methyl esters were extracted with chloroform. The sample was concentrated to dryness, redissolved in chloroform and eluted through a sodium sulfate column using DCM to remove water. It is generally assumed that hydrolysis of the polar fraction releases predominantly fatty acids present as phospholipids; however, this fraction is analytically defined, and it is possible that hydrolysable components other than phospholipids are present.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS)

Before analysis, all fractions were silylated with pyridine and BSTFA (25 μl pyridine and 25 μl BSTFA, 70°C, 1 h) to convert alcohols into trimethyl silyl derivatives. Samples were analysed using a Carlo Erba Instruments HRGC 5300 Megaseries gas chromatograph equipped with a Chrompack CP SIL-5CB capillary column (50 m × 0.32 mm internal diameter; 0.12 μm film, dimethylpolysiloxane equivalent) and a flame ionization detector. Hydrogen, at a head pressure of 10 p.s.i., was used as the carrier gas, and samples were injected at 70°C with a temperature programme of 20°C min^{-1} to 130°C and 4°C min^{-1} to 300°C and held for 20 min. GC-MS was performed using a Thermo Finnigan Trace GC interfaced to a Trace MS. The GC column and temperature programme were the same as those described previously, although He was used as the carrier gas. Acquisition of data started 5 min after sample injection by autosampler. Electron ionization (70 eV) was used, and full scan spectra were obtained by scanning the range *m/z* 50–800 at 1 scan s^{-1}.

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