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# Composition and implications of diverse lipids in New Zealand Geothermal sinters

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## ABSTRACT

Microbial adaptations associated with extreme growth environments, including high temperatures and low pH, are of interest to astrobiologists and origin of life researchers. As part of a survey of microbial lipids present in terrestrial geothermal settings, we examined four silica sinters associated with three different hot spring areas of the Taupo Volcanic Zone (TVZ), New Zealand. Dominant bacterial lipids include free fatty acids, 1,2-diacylglycerophospholipids, 1,2-di-*O*-alkylglycerols, 1-*O*-alkylglycerols, wax esters, alkanols, alkan-1,2-diols and various hopanoids, whereas dominant archaeal lipids include both archaeol and glycerol dialkyl glycerol tetraethers. Although many of these compounds occur in other settings, in the TVZ sinters their distributions (with high abundances of β-OH fatty acids and high-molecular-weight (> $C_{18}$) fatty acyl components) and carbon isotopic compositions (ranging from –40 to +4‰, with up to 25‰ variability in a single sample) are unusual. In addition, we have identified a range of unusual compounds, including novel macrocyclic diethers and hopanoids. The distributions of these compounds differ among the study sites, suggesting that, where preserved in ancient sinters, they could serve as an important tool in studying past hydrothermal environments.

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## INTRODUCTION

The study of hydrothermal microbial ecology and physiology is of broad scientific interest, offering insight into the processes by which mineral deposits form and revealing the ecology of extremeophiles, a critical component of origin of life studies and astrobiology (Stetter, 1996). A variety of thermophiles and hyperthermophiles have been found in such settings, occurring as mats, in hydrothermal fluids and on the surfaces of and entrained in mineral deposits. Of particular interest are silica sinters as they form rapidly in many hydrothermal settings, preserving a chemical signal of the organisms living in such settings (Pancost *et al.*, 2005). Association of micro-organisms 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 (TVZ) in New Zealand (Jones *et al.*, 1996, 1998, 2001a,b; Renaut *et al.*, 1996; Mountain *et al.*, 2003).

The TVZ is situated centrally on the North Island of New Zealand. The area is 300 km long and up to 60 km wide, extending 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 approximately 4200 MW is channelled (Bibby *et al.*, 1995).

Although their presence has now been confirmed, it is difficult to elucidate the role and nature of micro-organisms 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). This is particularly true for old sinters, making it difficult to compare settings of past sinter formation with the modern environment. However, we have recently shown that a wide range of relatively diagnostic lipid biomarkers are preserved in TVZ sinters and it is likely that such compounds, once encapsulated in amorphous silica, could persist for extended periods of time (Pancost *et al.*, 2005). Here we present and discuss biomarker distributions and their carbon isotopic compositions in detail; in combination with previous efforts that have focused on the mat-building organisms in geothermal systems (Dobson *et al.*, 1988; Robinson & Eglinton, 1990; Zeng *et al.*, 1992a,b; van der Meer *et al.*, 2000; Jahnke *et al.*, 2004), it represents an expanded understanding of the diversity and utility of organic biomarkers in investigating geothermal systems. As such, we have three primary goals: (1) assess the structural and carbon isotopic diversity of microbial lipids and compare these data to biomarker distributions in cultured organisms; (2) evaluate the preservation state of lipid biomarkers and the controls on their alteration during sinter formation; and (3) identify unusual biomarker signatures that could have particular chemotaxonomic potential or reveal new insights into the mechanisms by which membrane robustness is maintained 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. At each site, temperature and pH were measured and a fluid sample (for anions, cations and reduced sulfur) was collected. The chemical composition of the fluids is presented in Table 1 and details of individual sites are provided below. Further description of the various sampling sites, the mineralogy and geochemistry can be found in Mountain *et al.* (2003), and a map of the sample area and photos of sample sites are shown in Pancost *et al.* (2005).

*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.*, 2001b). The area features mud pools, geysers, fumaroles, hot pools, eruption craters and warm and boiling springs. Sample WT1 was collected from the Champagne Pool that occupies a hydrothermal explosion crater formed 600–900 years ago. The pool is approximately 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 due to rapid convection and heat loss over the large surface area of the spring (Mountain *et al.*, 2003). The water is anaerobic, with relatively high $HS^-$ concentrations, and contains a wide array of trace elements, including Au, Ag, Sb, W and As (Jones *et al.*, 2001a). 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 stromatolites containing silicified filamentous, bacilliform and coccoidal microbes (Mountain *et al.*, 2003). The sample analysed here is such a stromatolite, forming in anoxic conditions below the air–water interface but with spicular silica deposited above the air-water interface. As with most stromatolites, it is composed of predominantly amorphous silica occurring as porous and nonporous laminae. The porous laminae are composed of filamentous and non-filamentous microbes that have undergone variable degrees of silicification (Mountain *et al.*, 2003).

**Table 1** Environmental conditions associated with analysed sinters

| | | | Concentration (mg $L^{-1}$) | | |
|---|---|---|---|---|---|
| Site | Temp (°C) | pH | $SO_4^{2-}$ | TRS* | $SiO_{2(aq)}$ |
| Waiotapu WT-1 | 75 | 5.61 | 165 | 5.6 | 430 |
| Orakei Korako OK-1D | 78 | 9.01 | 119 | 0.34 | 325 |
| Rotokawa RK-1F | 80 | 2.46 | 972 | 12.3 | 268 |
| Rotokawa RK-6A | 82 | 3.67 | 420 | 3.7 | 336 |

*Total reduced sulfur as $H_2S$.

*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 & 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 & Seward, 1990). The waters are turbid due to a high concentration of suspended material composed principally of native sulfur, clays and amorphous silica (Mountain *et al.*, 2003). Sample RK1F (80 °C) was collected from an ebullient hot spring along the north margin of the sinter flat, whereas sample RK6A (82 °C) was collected from the south shore of the main upflow zone. Both samples consist of microstromatolites that are composed of a multitude of laminations that are made up of either: (a) light coloured silica-rich layers, or darker layers containing clay minerals and sulfur; or (b) orange to yellow interlayers indicating the presence of high concentrations of As and Sb (see Mountain *et al.*, 2003). Microscopic studies of these microstromatolites have shown that they contain very little microbial remnants.

*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 most other sites in the Taupo Volcanic Zone.

The sample studied (OK1D) originates from the Diamond Geyser with a collection temperature of 78 °C. The sample consists of a sinter piece that formed in the main outflow zone of the geyser. The sample was submerged during surge events and exposed during quiescent times and consists fully of amorphous silica that contains a large abundance of microbial remnants in the interlayers. The predominant cyanobacterial mat builder on the surrounding banks is *Chlorogloeopsis* sp. (Shiea *et al.*, 1991).

### Lipid analyses

We have examined the distributions of various lipids, including hopanoids, diacyl and di-*O*-alkyl glycerol lipids (where alkyl chains are linked to glycerol backbones via ester or ether linkages, respectively) and various *n*-alkyl compounds. Lipid analyses were conducted as described elsewhere. Specifically, sample collection, pretreatment, Soxhlet extraction and column chromatographic separation to generate neutral lipid, free fatty acid and phospholipid (PL) fractions were performed as described in Pancost *et al.* (2005). Particular caution is required when interpreting the phospholipid data. Because of the low abundances of lipids in these samples, we extracted large quantities of sinter using a Soxhlet apparatus. Such an approach can result in degradation of phospholipids, via loss of the polar head group; PLs could also degrade during warming of samples during grinding (Macnaughton *et al.*, 1997). However, diacyl glycerols (or diglycerides; formed from dephosphorylation of phospholipids) were not observed in our neutral fractions, replicate extractions yielded similar phospholipid fatty acids (PLFA) profiles and our 'phospholipid fractions' contained expected compounds – sometimes in high abundances – and we presume that there has been minimal loss of phospholipids during analytical work-up. However, this cannot be excluded, and abundances of inferred PLFAs should be interpreted with caution (particularly as absolute abundances); moreover, it is possible that organic matter other than phospholipids contributes to the lipids observed in our saponified 'PLFA' fractions.

Gas chromatography and gas chromatography–mass spectrometry were also performed as described in Pancost *et al.* (2005); briefly, a Chrompack CP SIL-5CB capillary column (50 m × 0.32 mm i.d.; 0.12 μm film, dimethylpolysiloxane equivalent) was used, and samples were injected at 70 °C with a temperature program of 20 °C $min^{-1}$ to 130 °C and at 4 °C $min^{-1}$ to 300 °C and held for 20 min. GC-isotope ratio monitoring mass spectrometry (GC-IRMS) analysis was performed using a ThermoFinnigan DeltaPlus–XP mass spectrometer interfaced to a gas chromatograph via a ConFlo combustion interface. The same column and temperature program were used as in the case of gas chromatography. $\delta^{13}C$ values are reported in standard per mil notation (vs. V-PDB standard) and were obtained by at least two analyses (with the average values reported here). Bacteriohopanoid analyses, including high temperature gas chromatography, liquid chromatography–mass spectrometry (LC–MS) and treatment with periodic acid and sodium borohydride to convert bacteriohopanepolyol (BHP) to more readily analysable terminal alcohols, were all performed as described in Talbot *et al.* (2005).

Glycerol dialkyl glycerol diether (GDGT) analyses were performed on total lipid extracts using both high temperature gas chromatography (HTGC) and LC–MS. The latter analyses were performed using an HP 1100 series (Palo-Alto, CA, USA) LC–MS equipped with an auto-injector and Chemstation chromatography manager software. Separation was achieved on an Prevail Cyano column (2.1 × 150 mm, 3 μm; Alltech, Deerfield, IL, USA), maintained at 30 °C. Injection volumes varied from 1 to 5 μL. Tetraethers were eluted isocratically with 99% A and 1% B for 5 min, followed by a linear gradient to 1.8% B in 45 min, where A = hexane and B = propanol. Flow rate was 0.2 mL $min^{-1}$. After each analysis the column was cleaned by back-flushing hexane/propanol (90:10, v/v) at 0.2 mL $min^{-1}$ for 10 min. Detection was achieved using atmospheric pressure positive ion chemical ionization mass spectrometry (APCI-MS) of the eluent. Conditions for APCI-MS were as follows: nebulizer pressure 60 psi, vaporizer temperature 400 °C, drying gas ($N_2$) flow 6 L $min^{-1}$ and temperature 200 °C, capillary voltage –3 kV, corona 5 μA (~3.2 kV). Positive ion spectra were generated by scanning m/z 950–1450 in 1.9 s. LC–MS was used to determine relative GDGT abundances, whereas HTGC was used for quantification of total absolute abundances. HTGC was performed on a Hewlett Packard 5890 Series II GC, equipped with a flame ionization detector, and using hydrogen as the carrier gas and a head pressure of 1.5 psi. Samples were run over an SGE HT5 (5% phenyl equivalent, polycarborane siloxane), 6 m by 0.53 mm aluminium clad column with the following temperature program: 50 °C (1 min) to 140 °C at 20 °C $min^{-1}$ followed by 140 °C to 420 °C (10 min) at 7 °C $min^{-1}$. GDGT peaks were identified based on comparisons with standards (2,3,2′,3′-tetra-*O*-dibiphytanyl-di-sn-glycerol-1′-β-glucosyl-1-phosphoryl-3″-sn-glycerol sodium salt standard, subsequently converted into a GDGT; Universal Biologicals Ltd, Cambridge, UK), and GDGTs identified in cold seep samples using LC–MS.

## RESULTS

### Free fatty acids

The saponified acid fractions contain a variety of alkanoic (**Ia, Ib, Ic, Id** Appendix) and hydroxy alkanoic acids (Table 2; Fig. 1), the summed abundances of which vary by nearly an order of magnitude. Because this fraction was saponified, it contains both free fatty acids but also perhaps fatty acyl moieties of glycolipids that can also elute in this fraction. However, analysis of a select sample without saponification suggests that most compounds in this fraction derive from free fatty acids, and for simplicity this term is used throughout.

**Table 2** Abundances of free fatty acids and phospholipid fatty acids (ng $g^{-1}$ rock)

| | RK6A neut | | RK1F neut | | OK1D neut | | WT1 neut | |
|---|---|---|---|---|---|---|---|---|
| | PLFA | Free FA | PLFA | Free FA | PLFA | Free FA | PLFA | Free FA |
| *n*-C14:0 | 0.003 | 0.064 | – | 0.060 | 0.028 | 0.11 | 0.003 | 0.19 |
| *i*-15:0 | 0.001 | 0.011 | – | 0.013 | 0.027 | 0.36 | – | 0.018 |
| *ai*-15:0 | 0.001 | 0.007 | – | 0.009 | 0.022 | 0.15 | – | 0.014 |
| *n*-C15:0 | 0.005 | 0.049 | – | 0.044 | 0.032 | 0.14 | 0.004 | 0.10 |
| *n*-C16:2 | – | – | – | – | 0.012 | | – | – |
| br-C16:0 | – | 0.008 | – | 0.036 | 0.005 | 0.012 | 0.005 | 0.024 |
| br-C16:0 | 0.003 | – | – | 0.015 | 0.19 | 0.30 | 0.014 | 0.013 |
| *n*-C16:1 | 0.004 | – | – | 0.004 | – | 0.05 | – | 0.013 |
| *n*-16:0 | 0.076 | 1.1 | 0.18 | 1.3 | 0.38 | 1.3 | 0.15 | 1.7 |
| β-OH-C14:0 | – | 0.006 | 0.046 | 0.030 | – | 0.019 | 0.027 | 0.30 |
| *i*-C17:0 | – | 0.022 | – | 0.049 | – | 0.80 | 0.006 | 0.031 |
| *ai*-C17:0 | – | 0.044 | 0.006 | 0.12 | – | 0.42 | 0.018 | 0.084 |
| β-OH-brC15:0 | – | – | 0.059 | – | – | | – | – |
| *n*-C17:0 | 0.005 | 0.070 | 0.011 | 0.070 | 0.018 | 0.47 | 0.006 | 0.17 |
| br-C18:0 | – | 9 | – | 0.087 | 0.007 | – | – | 0.019 |
| br-C18:0 | – | – | – | – | 0.015 | – | – | – |
| *n*-C18:2 | – | – | 0.083 | – | 0.25 | – | 0.047 | – |
| *n*-C18:1 | 0.006 | – | 0.098 | – | 0.38 | – | 0.067 | 0.045 |
| *n*-C18:1 | 0.007 | 0.14 | 0.035 | 0.18 | – | – | 0.019 | 0.095 |
| β-OH-brC16:0 | – | – | – | – | – | – | 0.01 | 0.12 |
| *n*-C18:0 | 0.044 | 0.98 | 0.38 | 0.89 | 0.25 | 2.6 | 0.24 | 2.6 |
| β-OH-C16:0 | – | 0.008 | 0.087 | 0.074 | – | 0.041 | 0.12 | 0.29 |
| br-C19:0 | – | – | – | – | – | 0.15 | – | – |
| br-C19:0 | – | 0.026 | – | – | – | 0.13 | – | 0.14 |
| *n*-C19:0 | 0.004 | 0.029 | 0.008 | 0.14 | 0.006 | 0.14 | 0.029 | 0.65 |
| β-OH-C17:0 | – | – | – | 0.011 | – | – | 0.004 | 0.085 |
| *n*-C20:2 | – | – | – | – | – | – | 0.14 | – |
| *n*-C20:1 | – | – | – | – | – | – | 0.079 | 0.18 |
| *n*-C20:0 | 0.007 | 0.18 | 0.042 | 0.20 | 0.025 | 1.2 | 0.12 | 2.2 |
| β-OH-C18:0 | 0.005 | – | 0.055 | 0.051 | – | 0.061 | 0.12 | 0.46 |
| C21:cy | – | – | – | – | – | 0.76 | – | – |
| *n*-21:0 | 0.009 | 0.12 | – | – | 0.004 | 0.1 | – | 0.28 |
| β-OH-C19:0 | – | – | – | – | – | 0.013 | 0.023 | 0.11 |
| *n*-22:0 | – | 0.50 | – | 0.29 | – | 0.30 | – | 0.28 |
| β-OH-C20:0 | – | – | – | – | – | – | 0.046 | 0.79 |
| *n*-C23:0 | – | 0.27 | – | – | – | 0.13 | – | – |
| *n*-C24:0 | – | 1.5 | – | 0.51 | – | 0.19 | – | 0.24 |
| *n*-C25:0 | – | 0.31 | – | 0.095 | – | 0.068 | – | – |
| *n*-C26:0 | – | 1.7 | – | 0.41 | – | 0.14 | – | 0.15 |
| *n*-C27:0 | – | 0.25 | – | 0.033 | – | 0.03 | – | – |
| *n*-C28:0 | – | 1.0 | – | 0.16 | – | 0.140 | – | 0.098 |
| *ai*-C29:0 | – | 0.14 | – | – | – | – | – | – |
| *n*-C29:0 | – | 0.32 | – | 0.029 | – | 0.028 | – | – |
| *ai*-C30:0 | – | 0.14 | – | – | – | – | – | – |
| *n*-C30:0 | – | 0.86 | – | 0.11 | – | 0.078 | – | 0.072 |
| *ai*-C31:0 | – | 0.20 | – | – | – | – | – | – |
| *n*-C31:0 | – | 0.17 | – | – | – | 0.011 | – | – |
| *n*-C32:0 | – | 0.51 | – | 0.051 | – | 0.061 | – | 0.026 |
| *n*-C33:0 | – | 0.085 | – | – | – | – | – | – |
| *n*-C34:0 | – | 0.40 | – | 0.038 | – | 0.011 | – | 0.016 |

The Rotokawa samples are dominated by saturated and straight-chain components ranging in carbon number from $C_{12}$ to $C_{35}$ (Fig. 1C,D). Also present are relatively low abundances of $C_{15–18}$ branched fatty acids and, in the crust (RK6A), high-molecular-weight (HMW) *anteiso* (e.g. **Ib**, albeit with chain lengths of $C_{29}$–$C_{31}$; *anteiso* structure tentatively identified on the basis of mass spectral characteristics, Matsumoto *et al.*, 1992) branched alkanoic acids ($C_{27}$ to $C_{32}$) characterized by a slight odd-over-even predominance. Unsaturated fatty acids are present in very low abundances in RK1F and absent in RK6A. Both Rotokawa acid fractions also contain hydroxy alkanoic acids. ω-OH fatty acids with $C_{22}$ and $C_{24}$ chain

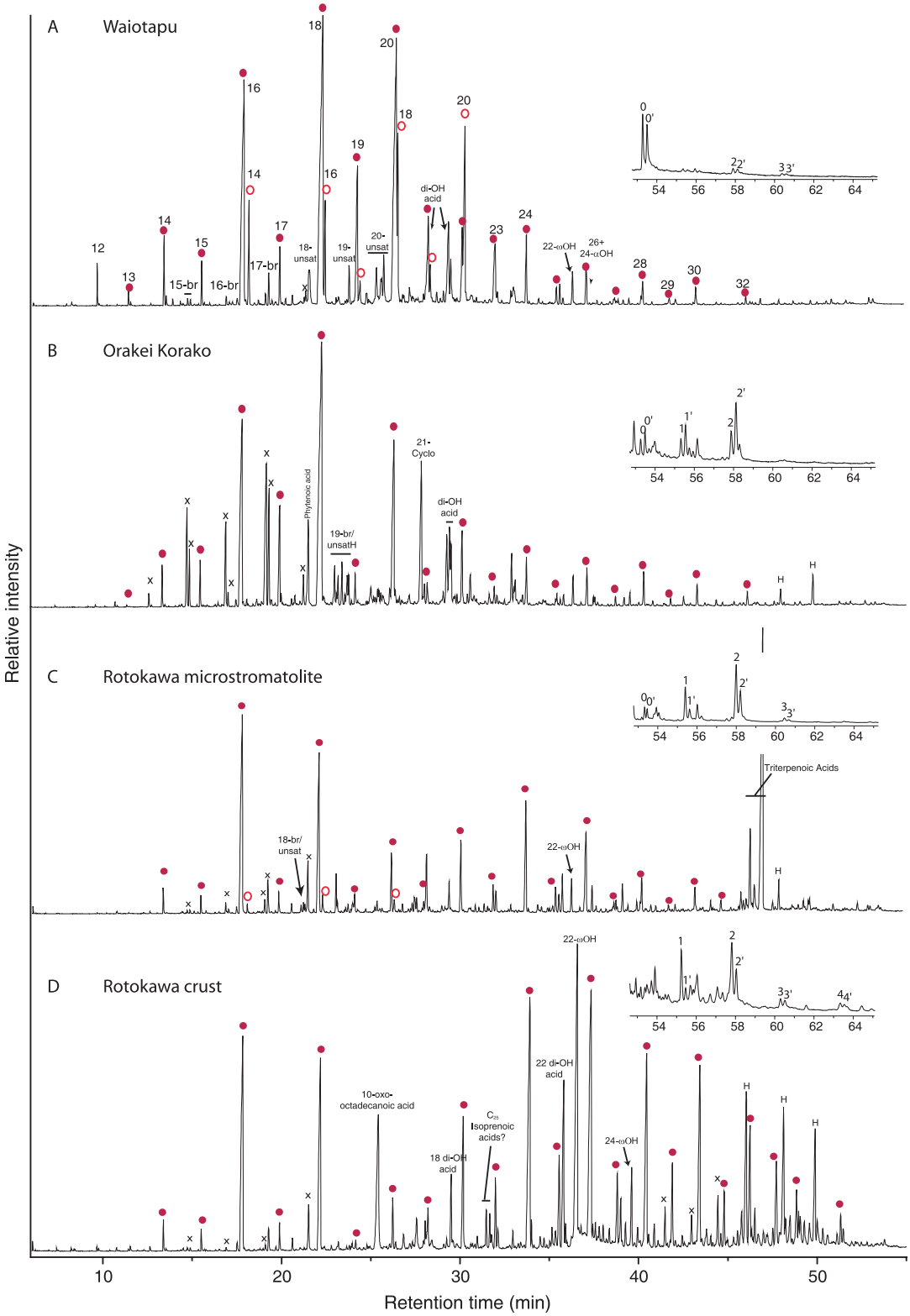


**Fig. 1** Partial gas chromatograms showing the saponified acid fractions, including free fatty acids and hopanoic acids, for the (A) Waiotapu sinter (WT1), (B) Orakei Korako sinter (OK1D), (C) Rotokawa microstromatolite (RK1F) and (D) Rotokawa crust (RK6A). Numbers denote the number of carbon atoms in the fatty acid, with closed circles identifying *n*-alkanoic acids, x identifying branched alkanoic acids, H identifying hopanoic acids and open circles identifying β-OH alkanoic acids. The inserts show the area of the chromatogram where biphytane diacids and ω-OH acids elute, denoted by 0 and 0′, respectively (the numbers denote the number of cyclopentyl moieties).

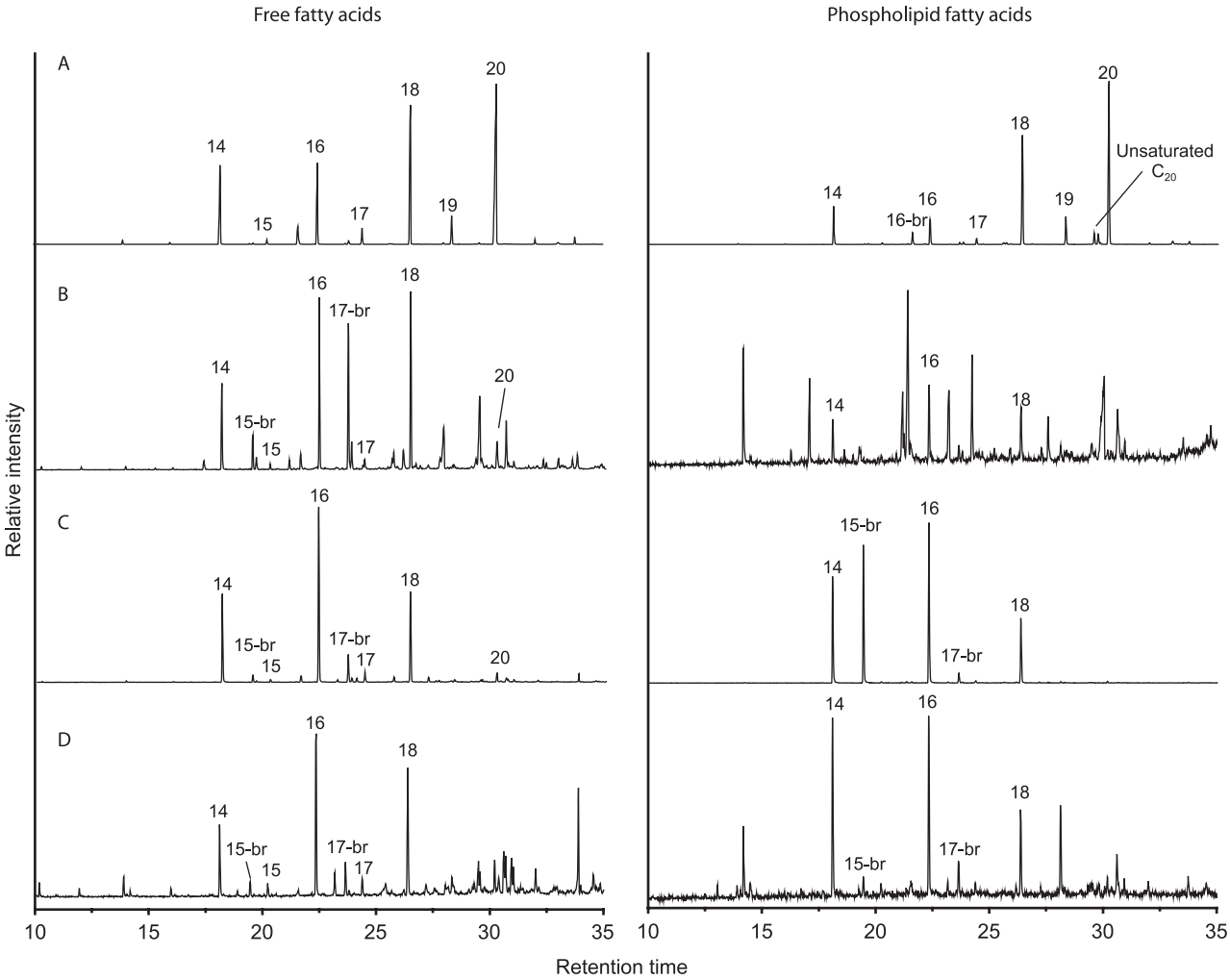


**Fig. 2** Partial *m/z* 175 mass chromatograms showing the distribution of β-OH fatty acids released by saponification of the acid and phospholipid fractions of the (A) Waiotapu sinter, (B) Orakei Korako sinter, (C) Rotokawa microstromatolite and (D) Rotokawa crust. Numbers denote the number of carbon atoms in the fatty acid.

lengths are present in both samples but are particularly abundant in RK6A. RK1F, the microstromatolite, contains relatively high abundances of β-OH alkanoic acids (**II**, Fig. 2C,D). Their distributions differ from those of the non-hydroxylated alkanoic acids; $C_{14}$, $C_{16}$ and $C_{18}$ straight-chain and $C_{15}$ and $C_{17}$ branched (*iso* (**Ia**) and *anteiso* (**Ib**)) components are all particularly abundant. The carbon isotopic compositions of Rotokawa fatty acids are highly variable, with values ranging from –26‰ to –40‰ and even-carbon-number fatty acids being enriched in $^{13}C$ relative to odd-carbon-number homologues (Fig. 3; Table 4).

The Orakei Korako sample contains fatty acids ranging in carbon number from 13 to 32, dominated by the low-molecular-weight (LMW) components and with particularly high abundances of the $C_{16}$, $C_{18}$ and $C_{20}$ straight-chain components (Fig. 1B and Table 2) and the $C_{15}$ to $C_{19}$ branched (mainly *iso* but with subordinate quantities of the *anteiso*) components. Unsaturated fatty acids are absent except for a few low abundance $C_{19}$ components, although a $C_{21}$ fatty acid bearing a cyclopropyl ring is abundant (**Id**). β-OH alkanoic acids are present but only in relatively low abundances and with a distribution similar to that of the nonhydroxylated fatty acids (Fig. 2B). The carbon isotopic compositions of OK1D fatty acids suggest that they derive from multiple sources. The HMW components ($C_{22}$–$C_{32}$) have $\delta^{13}C$ values consistent with a higher plant origin (–28.1 to –33.3‰). The LMW components' $\delta^{13}C$ values vary from +3.9‰ ($C_{21cy}$ FA), to the best of our knowledge the most enriched natural abundance value ever reported for a fatty acid, to –22.0‰ ($C_{16}$ FA; Table 4).

The Waiotapu sinter is dominated by even-carbon-number alkanoic acids and β-OH alkanoic acids (Figs 1A and 2A). The nonhydroxylated fatty acids range in carbon number from $C_{12}$ to $C_{32}$ with an even-over-odd homologue predominance throughout and particularly high abundances of the $C_{16}$ to $C_{20}$ components. Branched and unsaturated LMW alkanoic acids are present but in relatively low abundances: branched

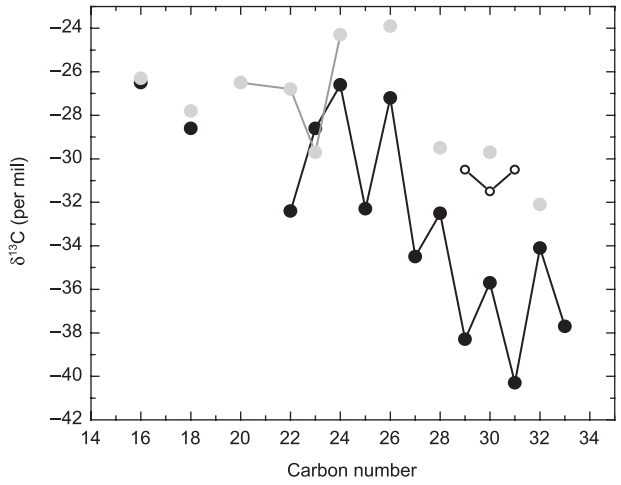


**Fig. 3** Carbon isotopic compositions of straight-chain and branched fatty acids in the Rotokawa, TVZ sinters. Black circles denote fatty acids from RK6A, whereas grey circles denote RK1F; open circles denote *anteiso*-branched fatty acids in the RK6A sinter.

fatty acids are mainly represented by $C_{17}$ *iso* and lesser amounts of *anteiso* components, whereas unsaturated fatty acids are represented by $C_{18}$ and $C_{19}$ components. The carbon isotopic compositions of WT1 fatty acids exhibit similar variability as observed in the OK1D fatty acids and also appear to derive from multiple sources. $C_{24}$–$C_{32}$ homologues have $\delta^{13}C$ values ranging from −29.2 to −31.6‰. LMW components vary from −11.5‰ ($C_{19}$ FA) to −24.6‰ ($C_{16}$ FA). β-OH alkanoic acids are abundant and have distributions similar to those of their nonhydroxylated counterparts.

### Phospholipid fatty acids (PLFAs)

The fatty acids released by saponification of the phospholipid fraction are inferred to derive from hydrolysis of 1,2-diacylglycerophospholipids (I). Their abundances vary by nearly an order of magnitude among the studied samples (Fig. 4 and Table 2). PLFA distributions are also variable as reflected by large differences in the average chain length and the ratio of unsaturated to saturated components.

The Rotokawa samples contain very simple PLFA distributions characterized predominantly by saturated and straight-chain $C_{16}$ and $C_{18}$ components, very low abundances of branched components and relatively low ratios of unsaturated to saturated PLFAs (Fig. 4C,D). Previously, we proposed that such simple distributions suggest the presence in the 'phospholipid fraction' of fatty acyl components ultimately derived from allochthonous sources and perhaps plant debris (Pancost *et al.*, 2005). Alternatively, the abundance of free fatty acids in the acid fraction suggests that PLFAs could have been hydrolysed to their constituent fatty acids and, thus, not preserved intact at this site. Rotokawa microstromatolite (RK1F) PLFA fractions also contain significant quantities of hydroxy alkanoic acids (Fig. 2C).

The Orakei Korako sample contains high abundances of predominantly $C_{16}$ and $C_{18}$ PLFAs, with lesser abundances of $C_{14}$ and $C_{20}$ components (Fig. 4B). Odd-chain PLFAs ($C_{15}$ and $C_{17}$) and branched PLFAs, represented primarily by *iso*- and *anteiso*-$C_{15}$ and $C_{17}$ components, are present in much lower abundances. Unsaturated components, in contrast to the free acid fraction, are abundant with a $C_{16:1}$, a $C_{18:1}$ and a $C_{18:2}$ component dominating. The latter is unusual as di-unsaturated PLFAs are typically attributed to photosynthetic organisms and their presence in such high temperature deposits is unusual; however, it is also present in the Rotokawa microstromatolite and Waiotapu sinter. As with the free acid fraction, β-OH alkanoic acids ($C_{14}$, $C_{16}$ and $C_{18}$) are present but only in relatively low abundances.

In contrast to the other sinters, the Waiotapu sinter is dominated by $C_{20}$ as well as $C_{16}$ and $C_{18}$ PLFAs (Fig. 4A). Odd-carbon-number fatty acids and branched fatty acids are present in relatively low abundances, but unsaturated even-numbered PLFAs are almost as abundant as their saturated counterparts. As with the free acid fraction, β-OH alkanoic acids are abundant and have distributions similar to those of their nonhydroxylated counterparts; also present are a group of $C_{20}$ unsaturated β-OH alkanoic acids, also reflecting characteristics of the nonhydroxylated fatty acid distribution (Fig. 2A).

### *n*-alcohols and phytol

*n*-alkanols are generally nonspecific biomarkers and occur in a range of environmental settings. The Orakei Korako sinter contains relatively abundant LMW alkanols (***IV***; Fig. 5), ranging in carbon number from $C_{12}$ to $C_{28}$, but dominated by the $C_{16}$, $C_{18}$ and $C_{20}$ components, similar to the free alkanoic acid and PLFA distributions, and the $C_{17}$ component, unlike the acid distibutions. As with the comparable fatty acids, the carbon isotopic compositions of these alcohols are highly variable, with $C_{20}$ having a $\delta^{13}C$ value of 0.7‰, the highest reported value for an *n*-alkanol, and the $C_{16}$ alcohol $\delta^{13}C$ value being −14.5‰. Phytol (**III**), a $C_{20}$ isoprenoidal alcohol often derived from the ester-linked phytyl moiety in chlorophyll and, hence, associated with photosynthetic activity, is also present. This distribution of alcohols is similar to that previously reported for an Orakei Korako photosynthetic bacterial mat (Shiea *et al.*, 1991). A homologous series of *n*-alkanols ranging in carbon number from $C_{18}$ to $C_{28}$ and dominated by the even-carbon-number homologues is present in the neutral fractions of both RK6A and WT1 but at much lower abundances than the Orakei Korako alkanols.

### Alkyl monoethers and diethers

The abundances of ether lipids comprised of nonisoprenoidal alkyl moieties (i.e. either straight-chain or bearing a single methyl

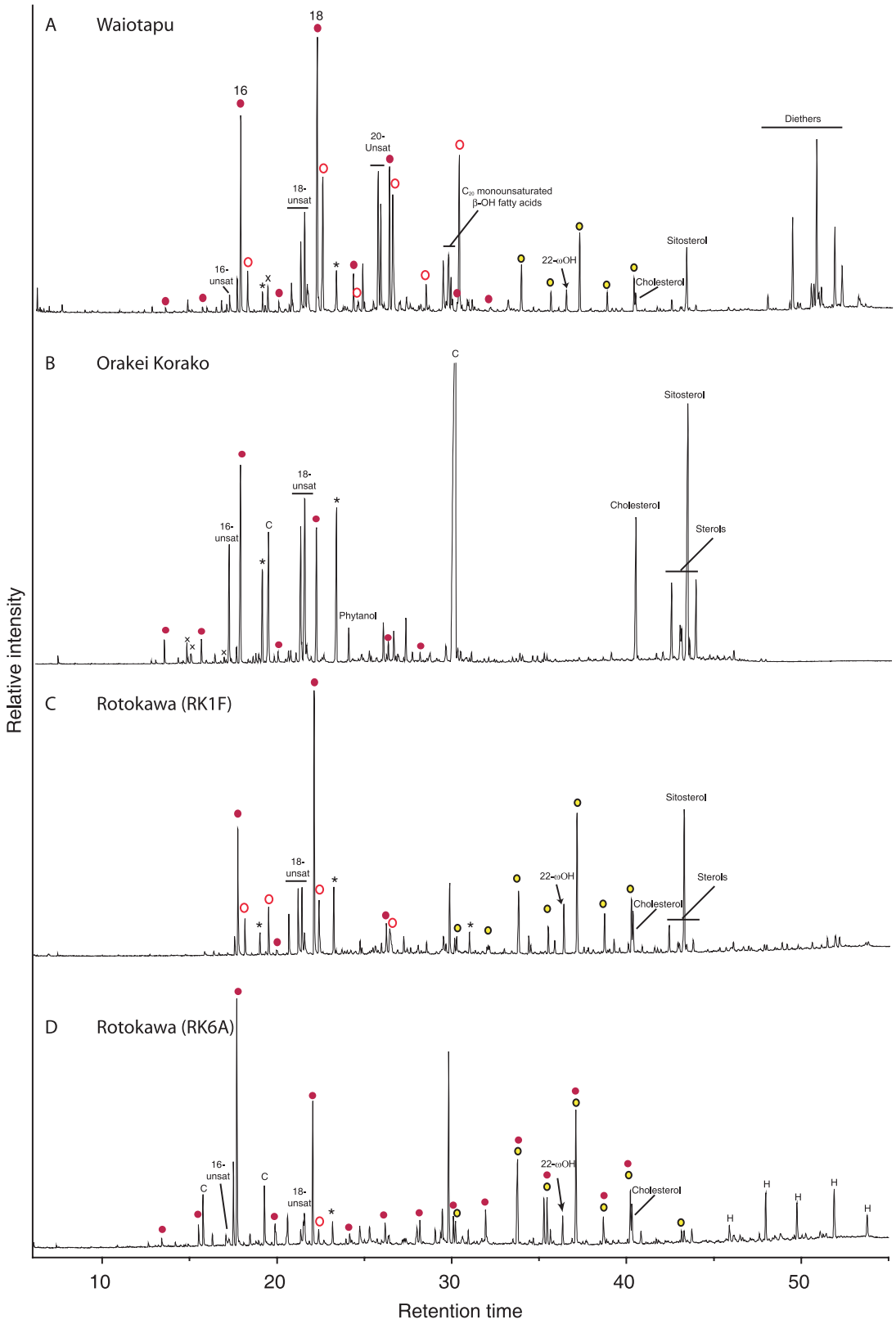


**Fig. 4** Partial gas chromatograms showing the saponified phospholipid fractions of the (A) Waiotapu sinter, (B) Orakei Korako sinter, (C) Rotokawa microstromatolite and (D) Rotokawa crust. Numbers denote the number of carbon atoms in the fatty acid, with closed circles identifying *n*-alkanoic acids, x identifying branched alkanoic acids, open circles identifying β-OH alkanoic acids, grey-filled circles identifying α-OH alkanoic acids and H identifying hopanoic acids.

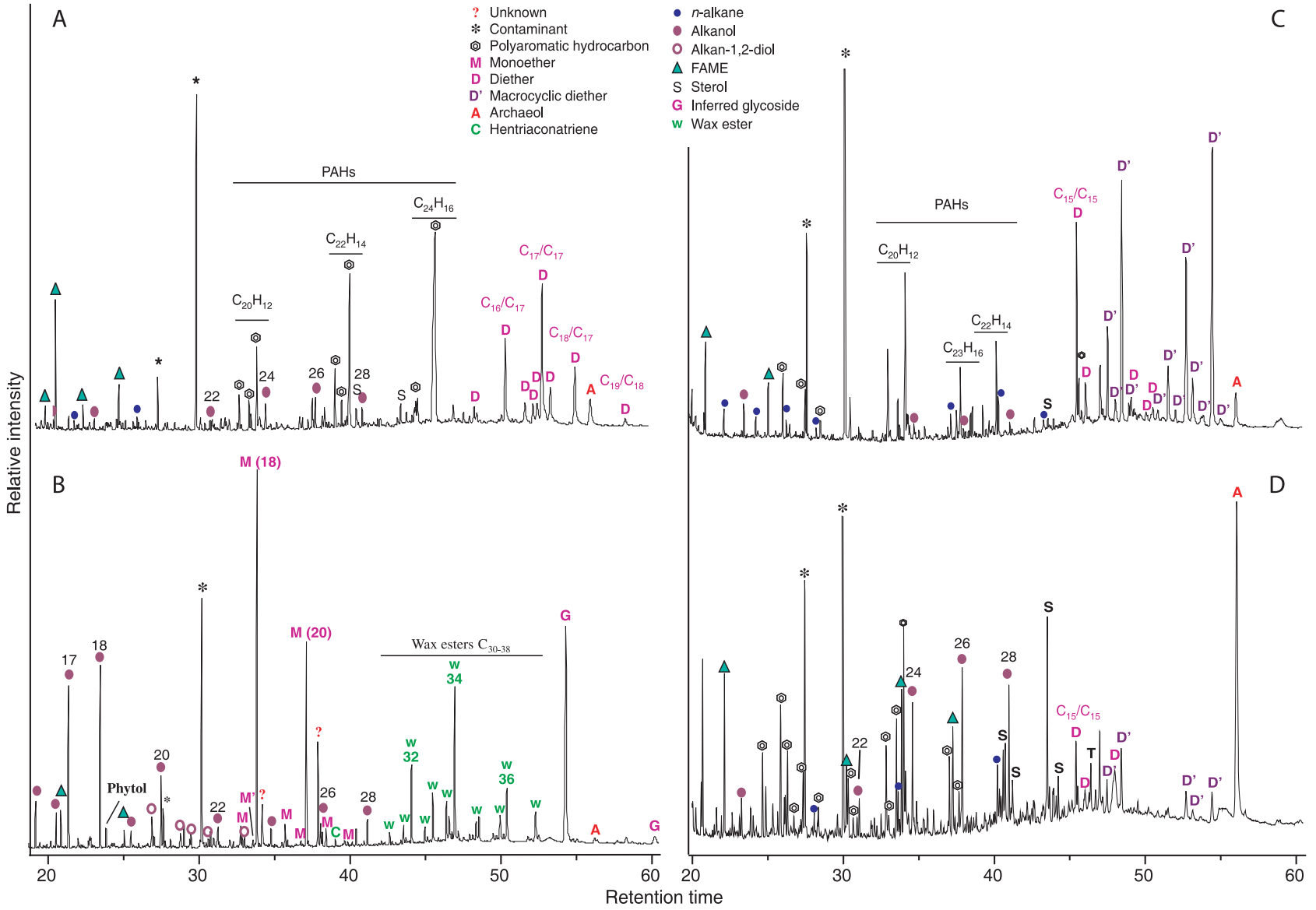


**Fig. 5** Partial gas chromatograms of the neutral fractions of the (A) Waiotapu sinter, (B) Orakei Korako sinter, (C) Rotokawa crust (RK6A) and (D) Rotokawa microstromatolite (RK1F). Numbers denote the number of carbon atoms in the compounds (except for monoethers [number of carbon atoms in alkyl chain] and diethers [number of carbon atoms in two alkyl chains]). Note that (B) and (C) were previously published in Pancost *et al*. (2005) and are included here for comparison.

**Table 3** Abundances (μg $g^{-1}$ rock) of lipid biomarkers in the neutral fractions

| | Sample† | RK6A neut | RK1F neut | OK1D neut | WT1 neut |
|---|---|---|---|---|---|
| Diethers | C15/C15 | 1.4 | 0.19 | 0.03 | – |
| | C15/C16* | 0.38 | 0.06 | – | – |
| | C15/C17* | 0.89 | – | – | – |
| | C15/C17* | 0.08 | – | – | – |
| | C17/unsat* | – | 0.39 | – | – |
| | C15/C17 | – | – | – | 0.20 |
| | C15/C18* | 0.20 | 0.02 | – | – |
| | C15/C18* | 0.04 | – | – | – |
| | C15/C19* | 0.06 | – | – | – |
| | C16/C17 | – | – | – | 1.3 |
| | C15/C19* | 0.09 | – | – | – |
| | C17/C17 | – | – | – | 2.3 |
| | C18/C17 | – | – | – | 1.3 |
| | C19/C18* | – | – | – | 0.15 |
| | C19/C18* | – | – | 0.19 | – |
| Monoethers | br-C18 | – | – | 0.18 | – |
| | C18 | – | 0.04 | 3.1 | – |
| | C19 | – | – | 0.03 | – |
| | C20 | – | – | 1.8 | 0.01 |
| | C21:1 | – | – | 0.12 | – |
| | C25 | – | – | 0.03 | – |
| Macrocyclic diethers | C30 | 0.85 | 0.10 | – | – |
| | C31′ | 0.19 | – | – | – |
| | C31 | 1.9 | 0.18 | – | – |
| | C32 | 0.22 | – | – | – |
| | C33′ | 0.74 | 0.05 | – | – |
| | C33 | 0.16 | – | – | – |
| | C34 | 1.9 | 0.12 | – | – |
| | C34′ | 0.69 | 0.03 | – | – |
| | C35 | 3.9 | 0.15 | – | – |
| Glycosides | 1 | – | – | 5.2 | – |
| | 2 | – | – | 0.41 | – |
| Alkane-1,2-diols | *n*-C14 | – | – | 0.015 | – |
| | br-C15 | – | – | 0.24 | – |
| | *n*-C15 | – | – | 0.03 | – |
| | br-C16 | – | – | 0.11 | – |
| | *n*-C16 | – | – | 0.12 | – |
| | *n*-C17 | – | – | 0.06 | – |
| | *n*-C18 | – | – | 0.09 | – |
| | br-C20* | – | – | – | 0.01 |
| | *n*-C20* | – | – | – | 0.04 |
| Alkan-1-ols | *n*-C14 | – | – | 0.02 | – |
| | br-C15 | – | – | 0.03 | – |
| | *n*-C15 | – | – | 0.10 | – |
| | *n*-C16 | – | – | 0.37 | – |
| | br-C17 | – | – | 0.27 | – |
| | br-C17 | – | – | 0.02 | – |
| | *n*-C17 | – | – | 1.3 | – |
| | *n*-C18 | 0.18 | – | 1.4 | 0.08 |
| | *n*-C19 | – | – | 0.12 | – |
| | *n*-C20 | – | – | 0.52 | 0.02 |
| | *n*-C21 | – | – | | – |
| | *n*-C22 | 0.04 | – | 0.12 | – |
| | *n*-C24 | 0.06 | – | 0.14 | 0.16 |
| | *n*-C26 | 0.05 | – | 0.17 | 0.20 |
| | *n*-C28 | 0.06 | – | 0.15 | 0.12 |
| Archaeal lipids | Archaeol | 0.49 | 1.96 | 0.13 | 0.75 |
| | ΣGDGTs | 5.7 | 31.7 | 2.6 | 5.3 |

†Identification of some components was incomplete; an * indicates that the alkyl chain length has only been tentatively identified, while′ after a number indicates that it is the subordinate, earlier-eluting isomer (inferred to have additional branching).

**Table 4** Stable carbon isotopic compositions of lipid biomarkers in the neutral and acid fractions

| | Sample | RK6A neut | RK1F neut | OK1D neut | WT1 neut |
|---|---|---|---|---|---|
| Diethers | C15/C15 | –16.9 | –17.4 | – | |
| | C16/C17 | – | – | – | –25.8 |
| | C17/C17 | – | – | – | –25.3 |
| | C18/C17 | – | – | – | –25.6 |
| | C19/C18 | – | – | – | –24.4 |
| Monoethers | C18 | – | – | –1.0 | – |
| | C20 | – | – | –3.7 | – |
| Macrocyclic diethers | C31 | –14.8 | –23.7 | – | – |
| | C34 | –14.8 | – | – | – |
| | C35 | –14.0 | – | – | – |
| Alkan-1-ols | *n*-C16 | – | – | –14.5 | – |
| | br-C17 | – | – | –13.0 | – |
| | *n*-C17 | – | – | –12.5 | – |
| | *n*-C18 | – | – | –2.0 | – |
| | *n*-C19 | – | – | –4.2 | – |
| | *n*-C20 | – | – | 0.7 | – |
| Wax esters | C31 | – | – | –15.5 | – |
| | br-C32 | – | – | –17.3 | – |
| | C32 | – | – | –15.4 | – |
| | br-C33 | – | – | –16.0 | – |
| | C33 | – | – | –15.0 | – |
| | C34 | – | – | –14.7 | – |
| | C35 (plus phyt)* | – | – | –15.4 | – |
| | C36 | – | – | –15.0 | – |
| | C39:1 (phyt)* | – | – | –9.0 | – |
| Free fatty acids | C16 | –26.5 | –26.3 | –22.0 | –24.6 |
| | C17 | – | – | –11.5 | –14.6 |
| | C18 | –28.6 | –27.8 | –8.4 | –15.6 |
| | C19 | – | – | –10.5 | –11.5 |
| | C20 | – | –26.5 | 0.0 | –12.6 |
| | C21:cy | – | – | 3.9 | – |
| | C21 | – | – | –27.0 | –18.9 |
| | β-OH-C20:0 | – | – | – | –10.3 |
| | C22 | –32.4 | –26.8 | –24.8 | –21.1 |
| | C23 | –28.6 | –29.7 | –30.9 | –28.3 |
| | C24 | –26.6 | –24.3 | –28.1 | –29.2 |
| | C25 | –32.3 | – | –30.9 | –31.3 |
| | C26 | –27.2 | –23.9 | –29.1 | –30.2 |
| | C27 | –34.5 | – | –31.9 | – |
| | C28 | –32.5 | –29.5 | –29.9 | –31.5 |
| | *ai*-C29 | –30.5 | – | – | – |
| | C29 | –38.3 | – | –33.3 | – |
| | *ai*-C30 | –31.5 | – | – | – |
| | C30 | –35.7 | –29.7 | –31.9 | –29.5 |
| | *ai*-C31 | –30.5 | – | – | – |
| | C31 | –40.3 | – | – | – |
| | C32 | –34.1 | –32.1 | –29.3 | –31.6 |
| | C33 | –37.7 | – | – | – |
| | ω-OH-C22 | – | –31.5 | – | – |
| Hopanoic acids | C31 αβ | –33.1 | – | – | – |
| | C31 ββ | –34.3 | – | – | – |
| | C32 αβ | –32.3 | – | – | – |
| Archaeal lipids | Archaeol | –12.9 | –8.2 | –2.6 | –23.0 |

*phyt refers to wax ester with a phytenyl moiety.

substituent) are variable (Fig. 5; Table 3). 1-*O*-alkylglycerols (**VII**; monoethers) are present in trace abundances or absent in Rotokawa and Waiotapu sinters but among the most abundant lipids in the Orakei Korako sinter (Fig. 5B). The most abundant components are the $C_{18}$ and $C_{20}$ homologues,

but also present is a $C_{21}$ component bearing a double bond (or cyclopropyl group) and a branched $C_{18}$ monoether. Their stable carbon isotopic compositions are generally high (Table 4).

Unlike the monoethers, the 1,2-di-*O*-alkylglycerols (**V**; diethers) are more widespread, with abundances ranging from *c.* 0.2 μg $g^{-1}$ rock in the Orakei Korako sinter to 5.2 μg $g^{-1}$ rock in the Waiotapu sinter (Table 3 and Fig. 5A). Distributions are also variable. The Waiotapu sinter contains a range of HMW diethers, including a predominance of $C_{16}/C_{17}$, $C_{17}/C_{17}$ and $C_{18}/C_{17}$ components (subscripts denote the carbon chain length of the two alkyl components). Their $\delta^{13}C$ values are similar to those of fatty acids, ranging from –24.4 to –25.8‰. Both samples from the Rotokawa area have a lower-molecular-weight range of diethers, dominated by the $C_{15}/C_{15}$ component. Although identification of all diethers in the Rotokawa samples is difficult due to co-elution, they all bear an *m/z* 299 fragment, suggesting that each contains a $C_{15}$ alkyl chain at the *sn*-2 position; this suite of diethers is similar to one of the series of diethers previously observed at cold seeps (Pancost *et al.*, 2001). The Orakei Korako sinter is unusual in that only two diethers are present, the $C_{15}/C_{15}$ and a $C_{19}/C_{18}$ diethers, with no other diethers detected.

The Waiotapu 'phospholipid fraction' also contains abundant diethers and in distributions identical to those in the neutral fraction (Fig. 4A). Repeated solid phase extraction (SPE) columns with lower sample loading indicates that the diethers were indeed preserved in the sinter as intact phospholipids. The polar head group must have been removed during our saponification step, but that is not typically used for quantitative preparation of phospholipids and abundances should be interpreted with caution.

In the two samples from the Rotokawa sinter flat, we recovered a novel series of compounds (Fig. 5C,D), characterized by an *m/z* 145 base peak in their mass spectra. That and other mass spectral features are characteristic for macrocyclic archaeol (**XIII**), which has been found only in the methanogenic archaeon *Methanococcus jannaschi* (Comita *et al.*, 1984), and macrocyclic archaeols bearing cyclopentyl moieties, which have only been reported for a few methane seeps (Stadnitskaia *et al.*, 2005). Thus, it appears likely that the novel Rotokawa compounds are related compounds, where the alkyl component ranges in carbon number from $C_{30}$ to $C_{35}$ (e.g. **VI**). Although the precise structures could not be elucidated, their retention indices suggest that the alkyl moieties of the diethers contain one or more methyl branches but are not isoprenoidal. The macrocyclic diether $\delta^{13}C$ values are *c.* 2–4‰ enriched (values range from –14 to –14.8‰) relative to cooccurring diethers in RK6A, and it is unclear whether they derive from a different source.

### Bacteriohopanoids

The hopanoids are pentacyclic triterpenoids and are membrane components of many bacteria, including cyanobacteria, methanotrophs, and aerobic heterotrophic bacteria (Ourisson *et al.*, 1987; Rohmer *et al.*, 1992). The most commonly observed hopanoids are bacteriohopanepolyol (BHP) derivatives (**VIII**), comprising a $C_{35}$ skeleton in which an *n*-pentyl group is attached to the hopanoid carbon skeleton at the C-30 position. $C_{31}$ and $C_{32}$ hopanols, commonly found in natural settings due to oxidative cleavage of vicinal diols in penta- and tetra-functionalized bacteriohopanoids, respectively (Rohmer *et al.*, 1984; Farrimond *et al.*, 2000), are present in only trace concentrations in our samples. In contrast, intact bacteriohopanetetrol and bacteriohopanepentol are present in concentrations ranging from 0.021 to 0.29 μg $g^{-1}$ rock and from 0 to 0.32 μg $g^{-1}$ rock, respectively (Table 5; Talbot *et al.*, 2005), with lowest concentrations in the Waiotapu sinter. Hopanoic acids (**IX**) also occur in all four samples and are particularly abundant in the Rotokawa 6A sample but present at low abundances in the Waiotapu sinter (Figs 1 and 6; Table 5). Both the biological 17β,21β(H) and thermally stable 17α,21β(H) (and to a lesser degree, the 17β,21α(H)) configurations of hopanoic acids are present. In addition, we also observed a group of 32,35-anhydrobacteriohopanoids (Talbot *et al.*, 2005): a dihydroxylated component (32,35-

**Table 5** Distributions and abundances of hopanoids (μg $g^{-1}$ rock)

| | Intact bacteriohopanoids | | | | | Hopanoids released by periodic acid treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | BHT | Pentol | A1 | A2 | A3 | P1† | P2 | P3 | 32ββ-ol | 31ββ-ol |
| RK6A | 0.13 | 0.03 | 2.1 | 2.40 | 2.80 | 0.91 | 0.46 | 0.46 | 0.16 | 0.19 |
| RK1F | 0.18 | 0.14 | 0.12 | 0.13 | 0.16 | 0.03 | 0.02 | 0.02 | 0.08 | 0.09 |
| OK1 | 0.29 | 0.32 | – | – | – | – | – | – | 0.06 | 0.09 |
| WT1 | 0.02 | – | 0.01 | – | – | – | – | – | 0.04 | 0.03 |
| | | | | | Hopanoic acids | | | | | |
| | 30ββ | 31αβ | 31βα | 31ββ | 32αβ | 32βα | 32ββ | 33αβ | 33βα | 33ββ |
| RK6A | 0.10 | 1.2 | 0.18 | 1.0 | 0.61 | 0.18 | 0.86 | – | – | 0.19 |
| RK1F | – | 0.13 | – | 0.18 | 0.06 | 0.02 | 0.08 | – | – | – |
| OK1 | – | 0.03 | – | 0.10 | 0.03 | 0.02 | 0.20 | – | – | 0.02 |
| WT1 | – | 0.02 | – | 0.03 | 0.01 | – | 0.03 | – | – | 0.01 |

†P1, P2 and P3 are thought to be products of acid treatment of A1, A2 and A3, respectively (Talbot *et al.*, 2005)

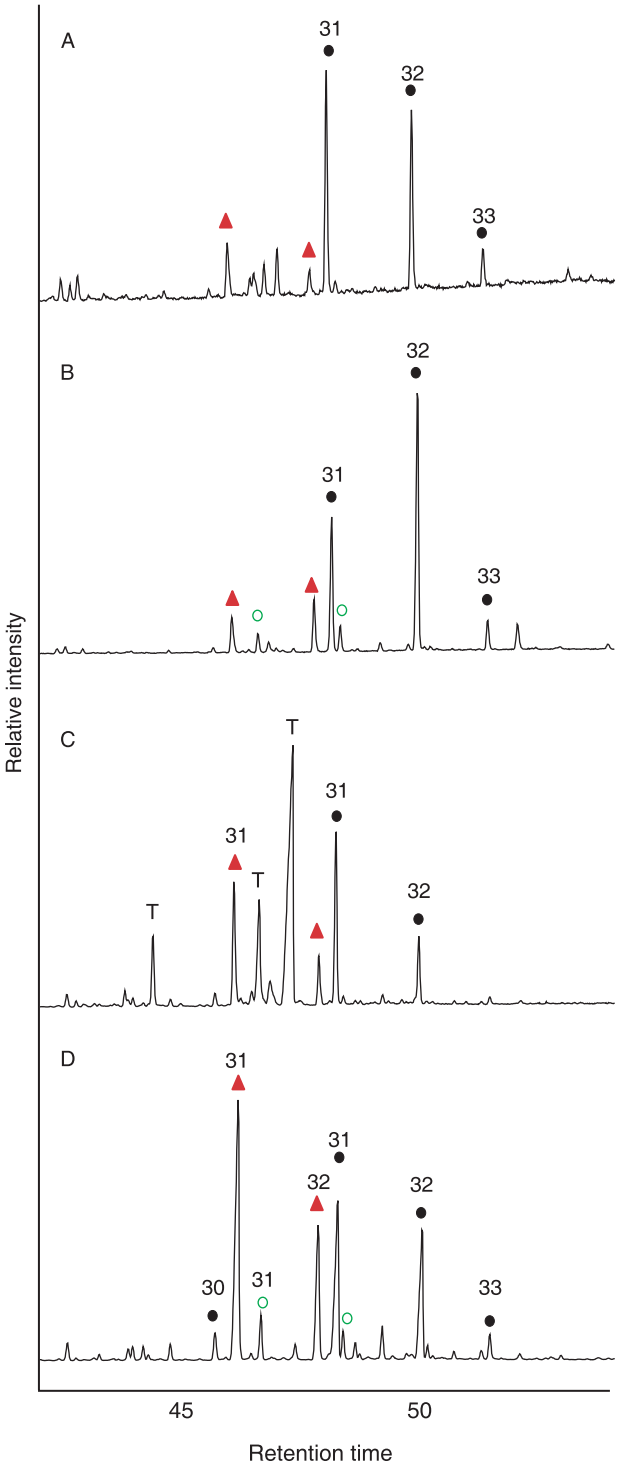


**Fig. 6** Partial *m/z* 191mass chromatograms showing the distribution of hopanoic acids present in the free acid fraction of the (A) Waiotapu sinter, (B) Orakei Korako sinter, (C) Rotokawa microstromatolite and (D) Rotokawa crust. Triangles denote 17α,21β(H) isomers, open circles denote 17β,21α(H) isomers and closed circles denote 17β,21β(H) isomers, while numbers denote carbon numbers and T denotes triterpenoic acids of inferred higher plant origin.

anhydrobacteriohopanetetrol; A1) that has been previously identified in a marine sponge (Costantino *et al.*, 2001) and deep marine sediments (Bednarczyk *et al.* in press) and two trihydroxylated components (A2 and A3).

### Biomarkers for cyanobacteria and green non-sulfur bacteria

Previous organic geochemical analyses of hot spring sediments have focused on the inputs associated with Cyanobacteria and *Chloroflexus* relatives. Biomarkers for geothermal cyanobacteria include monomethyl alkanes (Shiea *et al.*, 1991), and biomarkers for green nonsulfur bacteria include alkyl glycosides (***XI***) and alkan-1,2-diols (Pond *et al.*, 1986; van der Meer *et al.*, 2002), verrucosan-2β-ol (Hefter *et al.*, 1993), wax esters (***X***; Knudsen *et al.*, 1982; van der Meer *et al.*, 2000) and an all-*cis* hentriaconta-9,15,22-triene (van der Meer *et al.*, 1999). In all the sinters described in this study methylalkanes are absent; biomarkers for green nonsulfur bacteria are discussed below.

Wax esters, compounds comprising fatty acids esterified to long-chain alkanols, were detected only in the Orakei Korako sinter, in which they are among the most abundant compounds in the neutral lipid fraction and range in carbon number from $C_{30}$ to $C_{41}$. The distribution of the wax esters is complex, comprising several homologous series (Fig. 7). The dominant series comprises straight-chain wax esters, ranging in carbon number from $C_{30}$ to $C_{38}$ and dominated by even-carbon-number homologues ($C_{32}$, $C_{34}$ and $C_{36}$); each peak represents a range of compounds with different distributions of alkyl and acyl components (e.g. the $C_{32}$ wax esters include compounds with $C_{14}$ to $C_{17}$ fatty acyl components, of which the $C_{15}$ fatty acyl-bearing wax ester is predominant). A second series comprises (methyl?) branched isomers, ranging in carbon number from $C_{30}$ to $C_{36}$ with the $C_{32}$ and $C_{34}$ homologues being most abundant. A third series of wax esters comprises a group of compounds in which a phytene moiety is ester-bound to an unsaturated ($C_{16:1}$ to $C_{21:1}$) fatty acyl moiety (Fig. 7A). Yet another homologous series comprises wax esters bearing a straight-chain unsaturated component esterified to a $C_{18:1}$ fatty acyl moiety. A common origin seems plausible for many of the wax esters observed in the Orakei Korako sample. Indeed, both straight-chain and branched wax esters have been observed in cultured *Roseiflexus yellowstonii* (van der Meer, Schouten, Ward and Sinninghe Damsté, unpublished data). However, the differences in their carbon isotopic compositions suggest that the phytene-bearing wax esters (–9‰) have a different origin than the straight-chain wax esters (–15‰).

Like wax esters, glycosides inferred to bear an *n*-alkyl chain are among the most abundant neutral lipids in the Orakei Korako sinter sample but are present in only low abundances or absent in all other samples. Due to low molecular ion intensities in the mass spectra it was not possible to identify their structure. The likely degradation products of alkyl glycosides, *n*-alkan-1,2-diols, are also abundant in the Orakei Korako sample (0.6 μg g dry sediment$^{-1}$), where they range in carbon

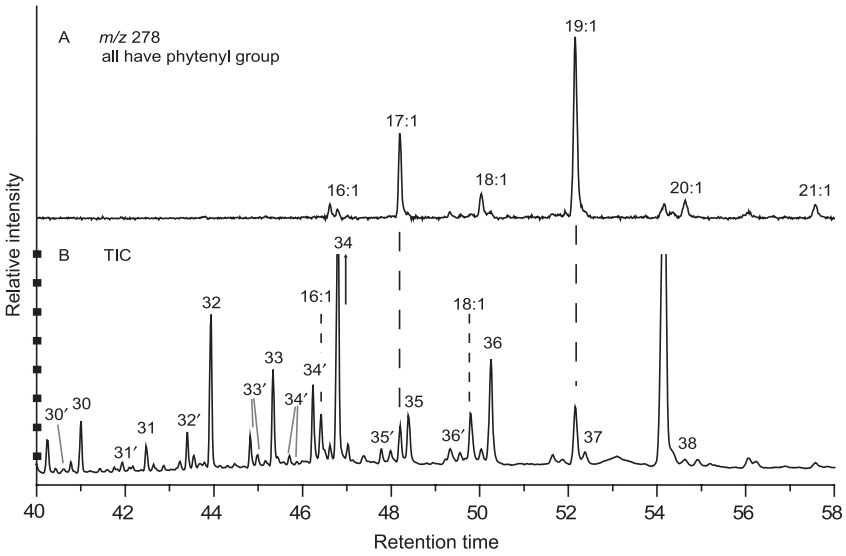


**Fig. 7** Partial *m/z* 278 mass chromatograms (A; derived from the $[M\text{-}RCOOH]^+$ ion of wax esters bearing a phytenyl component, respectively) and total ion current gas chromatogram (B) of Orakei Korako neutral lipid fraction. In a, numbers denote carbon number of acyl component, and in b, numbers denote total number of carbon atoms (16:1 and 18:1 denote wax esters that contain a $C_{18:1}$ fatty alkyl component and a C16:1 or C18:1 fatty acyl component, respectively).

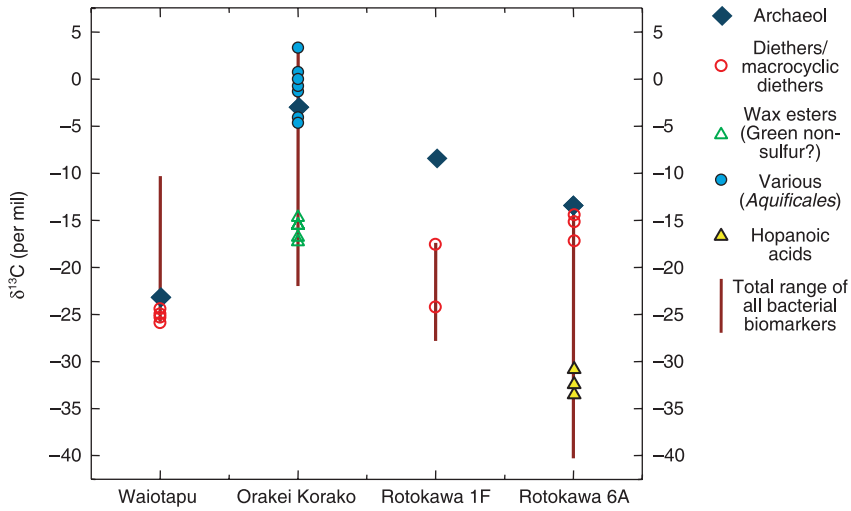


**Fig. 8** Comparison of archaeal and bacterial biomarker $\delta^{13}C$ values in the four analysed sinters. Vertical lines represent the range of $\delta^{13}C$ values for inferred bacterial biomarkers.

number from $C_{15}$ to $C_{18}$, but are present in only low or trace abundances in the other samples.

Shiea *et al.* (1991) reported that *Chloroflexus aurantiacus* contains $C_{29}$ to $C_{32}$ mono-, di- and triunsaturated alkenes as major components of the hydrocarbon fraction, with the all-*cis* hentriaconta-9,15,22-triene ($C_{31:3}$) as the dominant component (van der Meer *et al.*, 1999). Similarly, the rare diterpene verrucosan-2β-ol, occurs in *C. aurantiacus* in concentrations comparable to hopanoids in bacteria (Hefter *et al.*, 1993). Both of these biomarkers occur in the Orakei Korako sample, albeit only in trace abundances, and are absent in the other studied sinters.

## Archaeal biomarkers

Archaeal biomarkers are abundant in all four samples, commonly occurring at concentrations comparable to those of bacterial lipids (Table 2). Archaeol (**XII**; 1,2-di-*O*-phytanyl glycerol) concentrations range from 0.13 to 2.0 μg $g^{-1}$ rock and are greatest in the low pH Rotokawa sinter (RK1F, 82 °C) where it is one of the dominant components (Fig. 5). Its carbon isotopic composition, like those of bacterial biomarkers, is highly variable, ranging from –23.0 to –2.6‰; archaeol is typically enriched in $^{13}C$ relative to bacterial lipids, but the magnitude of that enrichment is variable (Fig. 8). Hydroxyarchaeol (**XII**, where either X or X′ = OH), found in several species of methanogens (Sprott *et al.*, 1993; Nishihara & Koga, 1995) and anaerobic methanotrophs (e.g. Hinrichs *et al.*, 1999; Pancost *et al.*, 2000), 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.

All four of the TVZ silicates contain GDGTs (**XIV**), as revealed by both HTGC and LC–APCI–MS (Fig. 9). GDGTs are among the most abundant compounds in all four sinters; their abundances correlate with those of archaeol, but the

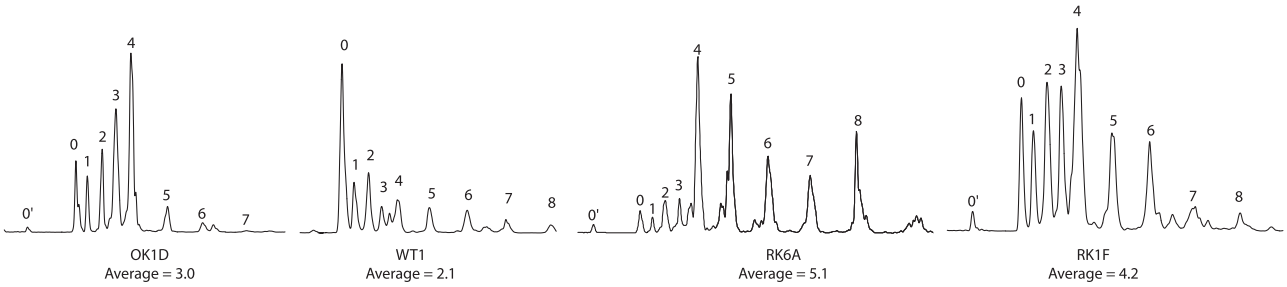


**Fig. 9** Partial HPLC–APCI–MS total ion current chromatograms showing the distribution or archaeal glycerol dialkyl glycerol diethers. Numbers denote the number of cyclopentyl moieties in the GDGTs; 0′ denotes a GDGT where one of the alkyl chains is a biphytanyl unit and the other is open, comprised of two separate phytanyl units.

GDGTs are five to 15 times more abundant. As expected, a wide variety of tetraether lipids are present, with the total number of cyclopentane moieties ranging from none to eight; thus, all nine of the GDGT configurations comprising two biphytanyl moieties with zero to four cyclopentyl groups and previously observed in thermophilic archaea are present (De Rosa & Gambacorta, 1988). For the most part, the GDGT distributions are dominated by those components with multiple cyclopentyl moieties; the exception to this is the Waiotapu sinter which is dominated by the GDGT lacking cyclopentyl groups. In addition, there appears to be a number of other GDGT isomers, reflected by the several smaller peaks eluting between or co-eluting with the identified GDGTs.

We also tentatively identified a number of biphytane α,ω-diacids (**XV**) and ω-OH acids (**XVI**) in the free acid fractions (Fig. 1). In general, the distributions of these compounds with respect to the number of cyclopentyl moieties is the same as the distributions of the GDGTs; for example the Waiotapu acids and GDGTs are dominated by components lacking cyclopentyl moieties, whereas the RK1F acids and GDGTs are dominated by components with 1 or 2 cylcopentyl moieties. This suggests that diacids and hydroxy acids derive from postdepositional oxidation of the GDGTs.

## DISCUSSION

### Preservation of lipid biomarkers

All samples were cleaned via extraction with methanol prior to being ground, such that all detected compounds derive from micro-organisms actually encased in the silica matrix. These compounds include a wide range of functionalized lipids, such as diethers and alcohols, but also their inferred biological precursors, such as intact phospholipids and bacteriohopanoids. Preservation of phospholipids is particularly surprising as they are thought to be rapidly enzymatically degraded upon death of the organism; thus, it is possible that the silicification process has enhanced their preservation. Similarly, intact bacteriohopanoid polyols were detected in all four sinters and occur in abundances comparable to those of hopanoic acids, their degradation products.

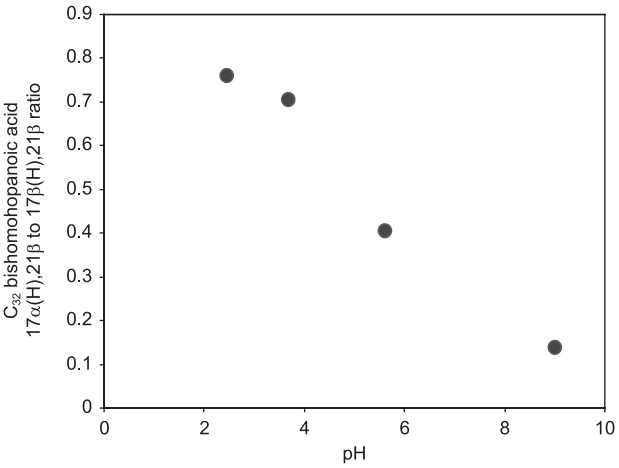


**Fig. 10** Cross-plot of pH against the ratio of the 17α,21β(H) to the 17β,21β(H) bishomohopanoic acid isomers.

However, most compounds have been diagenetically or thermally altered. Despite the presence of some intact phospholipids, many of the free acids probably derive from hydrolysis of the biological precursor membrane lipids. Also, the presence of abundant hopanoic acids suggests that oxidative cleavage of vicinal diols in the bacteriohopanpolyol chain has occurred. The presence of biphytane α,ω diacids and ω-OH acids suggests that oxidative degradation of archaeal GDGTs might also occur. However, the source of oxygen in such reactions is unclear – especially in the anaerobic settings.

Other controls on biomarker preservation/alteration include the high temperatures and variable pH values of the waters. In particular, the transformation from biological hopanoids with the 17β,21β(H) configuration to those with the thermally stable 17α,21β(H) configuration is directly related to thermal stress (e.g. Peters & Moldowan, 1991). However, the presence of 'thermally mature' hopanoids in acidic peat bog settings (Quirk *et al.*, 1984; Pancost *et al.*, 2003) indicates that they can also be formed by acid catalyzed reactions. There have been reports of *de novo* synthesis of 17α,21β(H) hopanoids (Rosa-Putra *et al.*, 2001), but in this setting, the extreme conditions and the high abundances of 17α,21β(H) hopanoids suggest that they are alteration products of

biological precursors. Indeed, in our analysed sinters (all formed at similar temperatures), the extent of the transformation from 17β,21β(H) to 17α,21β(H) hopanoids correlates with pH (Fig. 10); curiously, this is observed only for the $C_{31}$ and $C_{32}$ hopanoic acids, with only the 17β,21β(H) isomer of trishomohopanoic acid being present.

### Sources of bacterial lipids

Previous work (Mountain *et al.*, 2003) on TVZ sinters indicates that those at Waiotapu and Orakei Korako form at a rate of *c.* 0.02 mm $d^{-1}$, whereas those at Rotokawa form about an order of magnitude more slowly. At such rates, the sinters analysed here formed over *c.* 5–50 years and record a microbial signal integrated over those timescales.

#### *Orakei Korako*

The Orakei Korako sinter contains abundant and diverse archaeal and bacterial lipids. The bacterial lipids appear to derive largely from *Aquificales* species and green nonsulfur bacteria, although compounds such as hopanoids that probably derive from neither are also abundant.

Biomarkers for the *Aquificales* include 1-*O*-alkylglycerols and certain fatty acids. 1-*O*-alkylglycerols (monoethers) have been identified in a limited number of organisms, all of which are hyperthermophiles or thermophiles (including *Ammonifex degensii* (Huber *et al.*, 1996*)*; *Thermodesulfobacterium commune* (Langworthy *et al.*, 1983*)*; *Clostridium thermosulfurogenes* (Langworthy & Pond, 1986); and *Aquifex pyrophilus* (Huber *et al.*, 1992*)*). Recently, however, Jahnke *et al.* (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, and that in *Thermocrinis ruber*, diethers are absent. Similarly, $C_{18}$ and $C_{20}$ 1-*O*-alkylglycerols are major components of the Octopus Spring pink streamer community (Jahnke *et al.*, 2001). Thus, the dominance of $C_{18}$, $C_{20}$ and $C_{20:1}$ 1-*O*-alkylglycerols in the Orakei Korako sinter sample and the relative dearth of diethers is consistent with the presence of *Aquificales* species and particularly *T. ruber*. Other compounds probably derived from *Aquificales* species are the $C_{18}$, $C_{20}$ and $C_{21cy}$ fatty acids (Jahnke *et al.*, 2001). Consistent with a common origin, the $\delta^{13}C$ values of the above fatty acids and monoethers range from +3.9 to –8.4‰, a relatively narrow range given the total range of lipid $\delta^{13}C$ values observed in this sinter (+3.9 to –33.3‰) and the fact that the –8.4‰ value is for the $C_{18}$ fatty acid, likely derived from multiple sources. These isotopic distributions offer further evidence that these lipids derive from *Aquificales* species – a similar depletion for $C_{21cy}$ relative to $C_{20}$ has been observed for *T. ruber* (Jahnke *et al.*, 2001) – and perhaps provides insight into the metabolism of the *Aquificales* species. The observed values are among the highest measured, considerably higher than even those of the co-occurring green nonsulfur bacterial biomarkers that are usually $^{13}C$-enriched due to utilization of the 3-hydroxypropionate pathway (see below). Jahnke *et al.* (2001) have shown that carbon isotope fractionation by *Aquificales* species is dependent on the carbon substrate, with formate assimilation resulting in a rather large isotope effect (19.7‰) but $CO_2$ assimilation resulting in little isotope discrimination (3.3‰). Unfortunately, we do not know the DIC $\delta^{13}C$ values required to constrain this explanation, but obviously $CO_2$ assimilation is most consistent with our data; future work will focus on resolving the origin of such unusual biomarker carbon isotopic compositions.

Biomarkers for green nonsulfur bacteria include verrucosan-2β-ol, wax esters, all-*cis* hentriaconta-9,15,22-triene, the inferred glycosides and the 1,2-diols. Alkan-1,2-diols and wax esters have been previously reported 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 thermophilic bacterium *Roseiflexus castenholzii*, a close phylogenetic relative of *Chloroflexus* species. However, the specific distributions observed here are not directly comparable to those that have been previously reported. In chloroflexus mats, all-*cis* hentriaconta-9,15,22-triene can be present in abundances comparable to other bacterial compounds such as fatty acids and wax esters (van der Meer *et al.*, 2000); however, in the Orakei Korako sinter, hentriaconta-9,15,22-triene and verrucosan-2β-ol are present in only trace abundances. Also, cultures of *C. aurantiacus* (Shiea *et al.*, 1991) and *R. castenholzii* (van der Meer *et al.*, 2002) contain wax esters with a strong even-over-odd carbon number predominance with the former also containing abundant mono-unsaturated components; however, neither of these distributions is consistent with previous investigations of hot spring photoautotrophic bacterial mats (Dobson *et al.*, 1988; Shiea *et al.*, 1991; Zeng *et al.*, 1992a; van der Meer *et al.*, 2000), which did not observe unsaturated wax esters and instead reported the presence of abundant *iso* and/or *anteiso* branched wax esters. Our wax ester distribution shares characteristics with both field and culture studies, containing both unsaturated and branched components, as well as compounds with phytenyl components that on the basis of their $\delta^{13}C$ values likely derive from an alternative source.

Although the Orakei Korako lipid distributions differ in their detail to those of *Chloroflexus* relatives, the presence of such a wide range of compounds known to occur in *C. aurantiacus* and *R. castenholzii* suggests that *Chloroflexus* relatives were important components of the microbial population at this site. However, the optimum growth temperatures of *C. aurantiacus* and *R. castenholzii* are considerably lower than the temperatures measured at Orakei Korako and it is unlikely that these specific organisms lived in this setting. Instead, we suggest that this material derives from allochthonous organic matter, most likely photosynthetic mats surrounding the hot spring (Diamond Geyser) from which this sinter was collected. An external input could explain the large differences in the

$\delta^{13}C$ values of wax ester and *Aquificales* biomarkers. Alkanols and phytol could also derive from such allochthonous inputs; Shiea *et al.* (1991) found *n*-$C_{17}$ and $C_{18}$ alkanols predominating over phytol and other branched or unsaturated alcohols in photosynthetic bacterial mats, including a mat from the Orakei Korako site. However, not all alkanols appear to derive from allochthonous sources; although the $\delta^{13}C$ values for the $C_{16}$, $C_{17}$ and branched $C_{17}$ alkanols are all similar to those of the wax esters (*c.* –12.5 to –14.5‰), $\delta^{13}C$ values of the $C_{18}$ to $C_{20}$ alkanols are much higher (+0.7 to –4.2‰) and similar to those of the *Aquificales* biomarkers.

The OK1D sinter also contains abundant bacteriohopanetetrol and –pentol as well as their oxidative cleavage products, bishomohopanoic and homohopanoic acid, respectively (Table 5). Neither *Aquificales* nor *Chloroflexus* species are known to synthesize hopanoids, and these compounds likely represent yet a third group of bacteria present in this setting. Hopanoids are not typically 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 (Thiel *et al.*, 2003), the presence of hopanoids in anaerobic enrichments comprised largely of Annamox bacteria (Sinninghe Damsté *et al.*, 2004), and the discovery of hopanoids in anaerobic *Geobacter* species (Härtner *et al.*, 2005) indicates that at least some anaerobic bacteria can synthesize hopanoids.

Similarly, neither branched fatty acids nor unsaturated PLFAs have been reported in high abundance in *Aquificales* or *Chloroflexus* species and the source(s) of these compounds remains unclear. The branched fatty acids range in carbon number from $C_{14}$ to $C_{19}$ with the $C_{15}$ to $C_{17}$ components being particularly abundant and, curiously, only a minimal odd-over-even carbon number predominance. These characteristics of the fatty acid distribution were not described in a previous study of an Orakei Korako photosynthetic bacterial mat (Shiea *et al.*, 1991), where instead a distribution dominated by $C_{16}$ and (to a lesser degree) $C_{18}$ fatty acids was observed and branched acids were present in subordinate quantities.

The archaeal lipids are dominated by GDGTs, consistent with the high growth temperature. Although archaeol is present, the GDGTs are over an order of magnitude more abundant, with summed abundances comparable to those of the bacterial lipids (Table 3). Moreover, the GDGTs are dominated by components bearing one to four cyclopentyl moieties; this suggests a predominant source from hyperthermophilic crenarchaeota, because methanogens, including hyperthermophilic ones, are typically characterized by GDGTs comprised of acyclic biphytanes (Koga *et al.*, 1993, 1998 but see also Gattinger *et al.*, 2002 and Pancost *et al.*, 2003). The carbon isotopic composition of archaeol is –2.6‰, higher than most previously reported values but similar to the $\delta^{13}C$ values of *Aquificales* biomarkers (Table 4) and $\delta^{13}C$ of archaeal lipids inferred to derive from $CO_2$-utilizing methanogens in the Lost City hydrothermal field (Kelley *et al.*, 2005). Thus, the high $\delta^{13}C$ values could reflect utilization of similar highly $^{13}C$-enriched substrates, potentially DIC (Craig, 1963; Kelley *et al.*, 2005), by these different groups of organisms.

### *Waiotapu*

The Waiotapu sinter, like that from Orakei Korako, contains a variety of bacterial and archaeal lipids, but the distributions are considerably different. The bacterial lipids are dominated by the di-*O*-alkyl glycerol diethers and various free and pospholipid fatty acids. The archaeal lipids comprise both archaeol and GDGTs.

There are few known sources of nonisoprenoidal ether lipids, but, like the sources of the 1-*O*-alkylglycerols, all are thermophiles or hyperthermophiles. Workers have reported different alkyl chain distributions for different *Aquificales* cultures, with Huber *et al.* (1992) reporting $C_{16}/C_{16}$, $C_{17}/C_{17}$ and $C_{17}/C_{18}$ as the main components and Jahnke *et al.* (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 *A. 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 but the $C_{16}/C_{16}$ (34%), $C_{16}/C_{17}$ (18%) and $C_{17}/C_{17}$ (20%) compounds being predominant. The hydrophobic residues of *T. commune*, a thermophilic sulphate-reducing anaerobic bacterium, consist of 1,2-di-*O*-alkylglycerol 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). The lack of other biomarkers for *Aquificales* species (e.g. 1-*O*-alkylglycerols) suggests that these organisms were not the source of the Waiotapu diethers. However, both the diether distributions and the anaerobic conditions prevailing at the Waiotapu pond are consistent with a source from either *A. degensii* or *T. commune*. The carbon isotopic compositions of the Waiotapu diethers range from –24.4 to –25.8‰; the significance of these values is difficult to interpret in the absence of DIC $\delta^{13}C$ values, but they are depleted in $^{13}C$ by up to 10‰ relative to cooccurring fatty acids, suggesting a different source.

The free and phospholipid fatty acids likely derive from multiple sources. The HMW fatty acids (> $C_{22}$) have distributions and $\delta^{13}C$ values consistent with a higher plant origin (Tables 2 and 4), although a bacterial origin cannot be excluded. The LMW fatty acids ($C_{16}$ to $C_{22}$) have $\delta^{13}C$ values ranging from –11.5‰ ($C_{19}$) to –24.6‰ ($C_{16}$) and likely derive from mixing of multiple sources as discussed above for the Orakei Korako fatty acids (Table 4). The β-OH fatty acid distribution is similar to that of the fatty acids and the $C_{20}$ fatty acid and β-OH fatty acid have similar $\delta^{13}C$ values, suggesting a common source.

Hopanoids are present in the Waiotapu sinter, albeit in very low abundances (Table 5). This is consistent with recent observations that hopanoids do occur in anaerobic bacteria (see above) but are more common in aerobic bacteria (Rohmer

*et al.*, 1984). Whether the Waiotapu hopanoids are derived from a specific anaerobic organism is unclear: the sinters precipitate near the water surface and it is possible that aerobic organisms might live near the air–water interface.

The archaeal lipids comprise both archaeol and GDGTs; the latter are particularly abundant, with summed abundances being greater than that of any bacterial-derived lipids. A unique feature of the Waiotapu archaeal lipid distribution is the predominance of a GDGT lacking cyclopentyl moieties; as it was formed at temperatures similar to those of the sinters, this probably reflects a different archaeal assemblage rather than environmental conditions. In particular, this could reflect a dominance of euryarchaeota and particularly methanogens (e.g. Koga *et al.*, 1993, 1998), consistent with the anaerobic setting. The $\delta^{13}C$ value of archaeol is –23‰, depleted in $^{13}C$ relative to fatty acids but similar to the $\delta^{13}C$ values of bacterial diethers (Table 4; Fig. 8).

### *Rotokawa*

The Rotokawa sinters contain the most unusual distribution of microbial lipids, including HMW branched and straight-chain fatty acids, diethers and novel macrocyclic diethers and novel bacteriohopanoids, all presumably derived from bacteria. Also present are a range of archaeal lipids, including archaeol and GDGTs with 0–8 cyclopentyl moieties.

The 1,2-di-*O*-alkyl glycerols are dominated by the 1,2-di-*O*-pentadecyl glycerol (Table 3), a distribution unlike those previously reported for cultured organisms. Its $\delta^{13}C$ value is –16.9‰, slightly enriched in $^{13}C$ compared to the fatty acids and hopanoids, suggesting it derives from a different source, but similar to those of the macrocyclic diethers (Table 4). The macrocyclic diethers are not present in the sinters examined from the Waitotapu and Orakei Korako areas (as well as other areas analysed but not discussed here), and given the low pH of the Rotokawa setting, it is possible that they derive from thermoacidophiles. They are present in both RK6A and RK1F, although concentrations in the former are over an order of magnitude higher.

In both sinters, the free and phospholipid fatty acids are dominated by the $C_{16}$ and $C_{18}$ straight-chain homologues with only small amounts of branched and unsaturated components (Table 2). The free $C_{16}$ and $C_{18}$ fatty acids have $\delta^{13}C$ values of *c.* –26‰ and –28‰, respectively, with little difference between the two samples. It is likely that the low-molecular-weight components derive from bacteria, but the origin of the HMW fatty acids is less clear; such compounds are not typically found in bacteria but could possibly derive from terrestrial (higher plant leaf wax) contamination (Eglinton *et al.*, 1962). Given the fact that TVZ sinters are known to precipitate on higher plant material (e.g. Jones *et al.*, 1997), the latter explanation cannot be excluded; however, the Rotokawa crust (RK6A) contains the most abundant HMW acids but lacks steroid or triterpenoid higher plant biomarkers that do occur in other sinters (e.g. RK1F). The carbon isotopic distributions of the HMW fatty acids suggest an origin from multiple sources. In the crust (RK6A), $C_{24}$ to $C_{34}$ fatty acid $\delta^{13}C$ values vary by nearly 15‰, with the odd-carbon-number homologues being strongly depleted in $^{13}C$ relative to the even-carbon-number acids and all fatty acids becoming more depleted with increasing molecular weight (Fig. 3; Table 4). This suggests mixing of at least two sources of fatty acids; consistent with this explanation is the similar but less dramatic variation in the microstromatolite HMW fatty acid $\delta^{13}C$ values, also suggesting mixing albeit in different ratios. Another group of alkyl acids are the β-OH fatty acids. Although present in both sinters, they are particularly abundant in RK1F and in distributions dissimilar to those of the nonhydroxylated alkanoic acids.

Also present, and likely derived from a bacterial source, are HMW *anteiso* branched alkanoic acids ($C_{27}$ to $C_{32}$), characterized by a slight odd-over-even predominance. These compounds have previously been reported in cryptoendolithic microorganisms that had colonized Antarctic rocks (Matsumoto *et al.*, 1992) and in acidic freshwater lakes (Fukushima *et al.*, 2005) and are not expected to derive from higher plants that synthesize straight-chain waxes and membrane lipids. These compounds have $\delta^{13}C$ values of –30.5% to –31.5‰, but their carbon number range corresponds to the straight-chain fatty acids with the most depleted $\delta^{13}C$ values (–35.7% to –40.3‰), suggesting a separate source.

Hopanoids, including both bacteriohopanetetrol and -pentol, a group of three 32,35-anhydrobacteriohopanoids and a range of hopanoic acids presumably derived from more functionalized compounds, are present in both Rotokawa sinters (Table 5; Figure). Their $\delta^{13}C$ values range from –32.3 to –34.3‰, relatively low but consistent with the HMW fatty acid $\delta^{13}C$ values. The 32,35-anhydrobacteriohopanoids are unusual, and although they occur in all of our hot spring samples and have been observed elsewhere (Bednarczyk *et al.* in press), they are unusually abundant in the Rotokawa sinters. It is unclear whether they are biosynthesized or represent a diagenetic rearrangement of bacteriohopanepolyols under the extreme temperature and pH conditions found here.

Archaeal lipids include archaeol and GDGTs with 0–8 cyclopentyl moieties of inferred crenarchaeal origin. The GDGTs are over an order of magnitude more abundant than archaeol and their abundances are comparable to those of bacterial lipids in the crust (RK6A) and far greater than the bacterial lipids in the microstromatolite (RK1F). As the latter was deposited under the lowest pH conditions examined, it is possible that the predominance of archaea is an ecological response to these very extreme conditions. Similarly, the high proportions of GDGTs and the high number of cyclopentyl moieties in those GDGTs (average ring number >4; Fig. 9) could be physiological responses to low pH (e.g. Macalady *et al.*, 2004). For both samples, archaeol $\delta^{13}C$ values are higher than those of all bacterial biomarkers; in the case of RK1F, archaeol is enriched by *c.* 8‰. Thus, unlike the other sites,

archaeol $\delta^{13}C$ values suggest a decoupling of archaeal and bacterial ecology.

### Novel or unusual lipids as guides to uncharacterized organisms

The analyses of TVZ sinters revealed a wide variety of lipids typically found in nonextreme and hot spring settings. Also present, however, are both novel compounds and compounds that are rarely observed and could derive from previously uncharacterized organisms. These include:

**1)** Macrocyclic diethers (Rotokawa sinters), occurring in abundances and distributions consistent with a thermoacidophile bacterial source.

**2)** A predominance of the $C_{15}/C_{15}$ diether – previously observed in Yellowstone hot springs (Zeng *et al.*, 1992a) but not previously documented for cultured bacteria.

**3)** Wax esters comprising a phytenyl moiety esterified to an unsaturated acyl moiety (Orakei Korako sinter).

**4)** HMW alkanoic acids in Rotokawa sinters; due to the possibility of higher plant contributions, it is difficult to precisely characterize their distribution and, indeed, there could be several isotopically distinct populations.

**5)** HMW branched alkanoic acids (Rotokawa crust); these occurred in distributions dissimilar to the HMW straight-chain acids and were absent in the Rotokawa microstromatolite, suggesting they derive from a different source than the other HMW acids.

**6)** 32,35-anhydrohopanoids; although present in other environments, the very high abundances of these compounds in the Rotokawa samples suggest a specific source from high temperature, acidic settings.

In addition to the above, we observe much higher concentrations of β-OH alkanoic acids relative to nonhydroxylated alkanoic acids in the Waiotapu and Rotokawa microstromatolite samples than are typically reported for environmental samples. Such compounds are typically associated with lipopolysaccharides of gram-negative bacteria, but can also be present as the fatty acyl components of PLFAs. Here, it is unclear what their source is and thus, whether the high β-OH alkanoic acid concentrations of these two sinters represents high abundances of gram-negative bacteria, enhanced lipopolysaccharide production or a particularly high abundance of these compounds in bacterial membranes.

### Proxies for past environmental conditions in hydrothermal settings

One of the advantages of biomarkers over other microbial tracers (e.g. DNA or RNA) is that they are relatively well preserved on long geological timescales. Thus, their preservation in silica sinters indicates that they could be used to examine past changes in environmental conditions. Indeed, the presence of highly functionalized compounds, including intact phospholipids, in these sinters suggests that silicification facilitates biochemical preservation.

Future work is certainly required but this initial survey has highlighted a variety of possible indicators. The most obvious approach is to use biomarkers to profile microbial communities from which environmental conditions can be inferred. Biomarkers for green nonsulfur bacteria, *Aquificales* species and Archaea have all been recovered from our samples and could be used to infer past environmental conditions. For example, the relatively high abundances of archaeal lipids in all of our samples are consistent with the high temperature and of these settings. However, pH is also important, consistent with the fact that archaeal lipids are dominant in RK1F, the sinter associated with the lowest pH.

Other adaptations to extreme conditions are the abundances and relative distributions of diethers. Molecular modelling indicates that the difference in membrane ordering between diesters and diethers is minimal (Paltauf, 1983); however, the latter are more resistant to hydrolysis and this could be an important adaptation to the extreme temperature and pH conditions at these sites (e.g. Russell & Fukunaga, 1990). Indeed, ether lipids are important constituents of the biomarker distributions in all analysed sinters. The abundance in archaea of GDGTs relative to diethers also increases with growth temperature (Gliozzi *et al.*, 1983; Uda *et al.*, 2001), and this is reflected in all of our samples by the high GDGT to archaeol ratios (Table 3). GDGTs might also be advantageous in low pH settings, where they help maintain high pH gradients across the cell membrane (e.g. Macalady *et al.*, 2004). Similarly, the presence of macrocyclic diethers could be used as tracers for low pH and high temperature conditions; previous workers have shown that with increasing growth temperature, the proportion of macrocyclic diether in *M. jannaschii* increases (Sprott *et al.*, 1991). Studies of liposomes based on macrocyclic diethers indicate that their presence results in decreased membrane fluidity and proton permeability (Arakawa *et al.*, 2001). It is likely that the novel macrocyclic diethers described here serve a similar role in bacterial membranes; because they are especially abundant in the settings with low pH, we suggest that it is the decreased proton permeability afforded by such structures that governs their occurrence. Thus, ratios of macrocyclic diether vs. total diethers, GDGTs vs. archaeol or diethers vs. diesters could be used as indicators of pH or temperature, with future work on modern settings being used to calibrate relationships.

The distributions of the acyl component of 1,2-diacyl glycerols also reflect environmental conditions. In particular, the degree of unsaturation of fatty acyl components is thought to decrease (e.g. Russell, 1984) while the average chain length (Russell, 1984) and degree of branching (e.g. Kaneda, 1977; Fulco & Fujii, 1980; Russell, 1984) is thought to increase with increasing growth temperature. In our samples, we sometimes observe abundant branched fatty acids, including HMW branched fatty acids (Rotokawa crust), but not in all samples.

Similarly, some contain abundant unsaturated fatty acids, which is unexpected, while other sinters contain very low abundances of unsaturated components. Perhaps, the most consistent response to high temperatures is the increase in fatty acyl chain length with the $C_{20}$ and $C_{22}$ components being important in the Orakei Korako and Waiotapu sinters. Such components are not significant in the Rotokawa sinters, but those samples contain HMW ($C_{24}$–$C_{32}$) fatty acids that appear to be of bacterial origin and could potentially by adaptations to the extreme acidity and temperature.

The abundance of hopanoids could provide insight into redox conditions in past environments. As described above, it is now known that hopanoids occur in both anaerobic and aerobic bacterial membranes; however, they appear to be far more common in the latter. Consistent with that, the anoxic Waiotapu site contained hopanoids in abundances about an order of magnitude lower than in the other sinters. Also, the extent of hopanoid stereochemcial transformation could provide a proxy for past pH; however, interpretation of such ratios in older sinters will be more problematic as both temperature and pH, as well as extent of exposure to each of these, will have to be considered.

## CONCLUSIONS

Our previous paper (Pancost *et al.*, 2005) revealed that biomarker lipids are present in silica sinters formed in hot springs. This more detailed discussion highlights the exceptional variety in those structures and their carbon isotopic compositions. A range of functionalities, including ether lipids (of both bacterial and archaeal origin), ester lipids, alkyl glycosides, and wax esters are present as is a range of hydrocarbon skeletons, including hopanoids and branched- and straight-chain fatty acids ranging in carbon number from $C_{12}$ to $C_{34}$. The carbon isotopic compositions of these compounds range from –40.5‰ to +3.9‰ (with nearly as great a range sometimes occurring in a single sample); such a range of values has little precedent and likely reflects a range of carbon assimilation pathways being utilized by the organisms thriving in a given setting. Clearly, future work is necessary, but this work reveals the potential use of biomarkers in the characterization of past microbial communities and as guides for the identification of new organisms.

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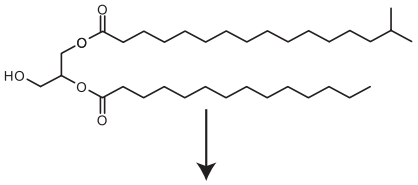
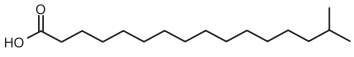
**Appendix** Structures of Biomarkers

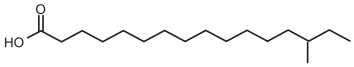
**I; Diglyceride** (Phospho- and glycolipids have a variety of polar headgroups attached to glycerol)



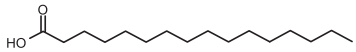
**Ia**; Various fatty acids; can be free or derive from **I**; chain length can vary



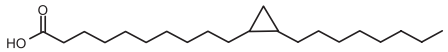
**Ib**



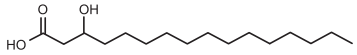
**Ic**



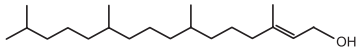
**Id**



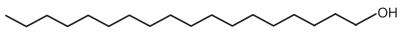
**II; β-OH acids**



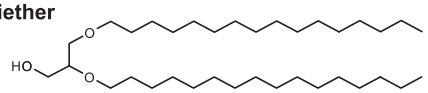
**III; Phytol**



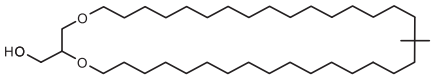
**IV; *n*-alkanols**



**V; Diether**

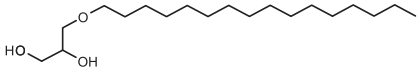


**VI; Macrocyclic diether**

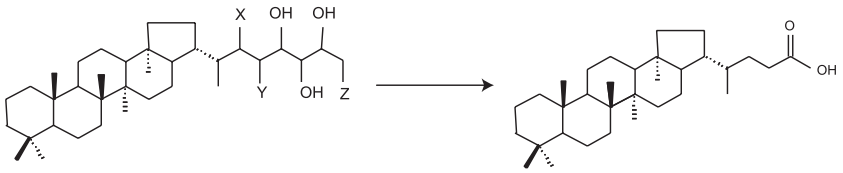


Location of methyl group(s) unknown

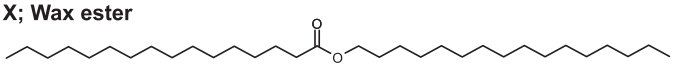
**VII; Monoether**



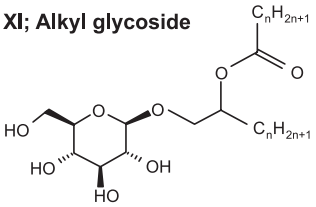
**VIII; Bacteriohopanpolyol** → **IX; Bishomohopanoic acid**



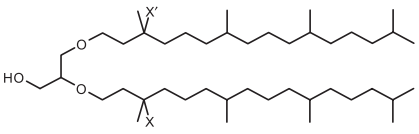
**X; Wax ester**



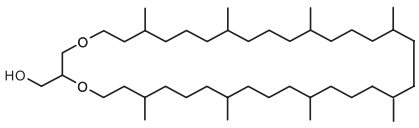
**XI; Alkyl glycoside**



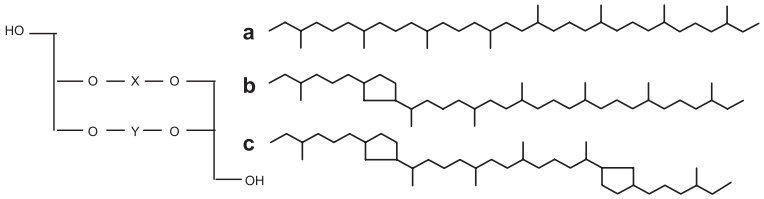
**XII; Archaeal diethers (archaeol: X, X′ = H)**



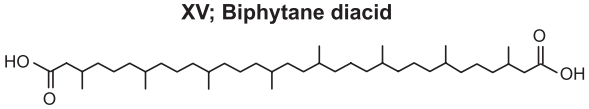
**XIII; Macrocyclic archaeol**



**XIV; Glycerol dialkyl glycerol tetraethers (GDGTs)**



**XV; Biphytane diacid**



**XVI; Biphytane ω-OH acid**