

Distribution of ether lipids and composition of the archaeal community in terrestrial geothermal springs: impact of environmental variables

Wei Xie,^{1,2} Chuanlun L. Zhang,^{1,2*} Jinxiang Wang,² Yufei Chen,¹ Yuanqing Zhu,^{1,3} José R. de la Torre,⁴ Hailiang Dong,^{5,6} Hilairy E. Hartnett,^{7,8} Brian P. Hedlund⁹ and Martin G. Klotz^{10**}

¹State Key Laboratory of Marine Geology, Tongji University, Shanghai 200092, China.

²Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA.

³Seismological Bureau of Shanghai, Shanghai 201203, China.

⁴Department of Biology, San Francisco State University, San Francisco, CA 94132, USA.

⁵State Key Laboratory of Biogeology and Environmental Geology & Institute of Earth Sciences, China University of Geosciences, Beijing 100083, China.

⁶Department of Geology and Environmental Earth Science, Miami University, Oxford, OH 45056, USA.

⁷School of Earth and Space Exploration and

⁸Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA.

⁹School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154, USA.

¹⁰Department of Biological Sciences, University of North Carolina, Charlotte, NC 28223, USA.

Summary

Archaea can respond to changes in the environment by altering the composition of their membrane lipids, for example, by modification of the abundance and composition of glycerol dialkyl glycerol tetraethers (GDGTs). Here, we investigated the abundance and proportions of polar GDGTs (P-GDGTs) and core GDGTs (C-GDGTs) sampled in different seasons from Tengchong hot springs (Yunnan, China), which encompassed a pH range of 2.5–10.1 and a temperature range of 43.7–93.6°C. The phylogenetic composition of the archaeal community (reanalysed

from published work) divided the Archaea in spring sediment samples into three major groups that corresponded with spring pH: acidic, circumneutral and alkaline. Cluster analysis showed correlation between spring pH and the composition of P- and C-GDGTs and archaeal 16S rRNA genes, indicating an intimate link between resident Archaea and the distribution of P- and C-GDGTs in Tengchong hot springs. The distribution of GDGTs in Tengchong springs was also significantly affected by temperature; however, the relationship was weaker than with pH. Analysis of published datasets including samples from Tibet, Yellowstone and the US Great Basin hot springs revealed a similar relationship between pH and GDGT content. Specifically, low pH springs had higher concentrations of GDGTs with high numbers of cyclopentyl rings than neutral and alkaline springs, which is consistent with the predominance of high cyclopentyl ring-characterized *Sulfolobales* and *Thermoplasmatales* present in some of the low pH springs. Our study suggests that the resident Archaea in these hot springs are acclimated if not adapted to low pH by their genetic capacity to effect the packing density of their membranes by increasing cyclopentyl rings in GDGTs at the rank of community.

Introduction

Archaea constitute one of the three domains of life on Earth (Woese *et al.*, 1990). A large number of archaeal species produce specialized cytoplasmic membranes composed of isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) (see Fig. S1 for structures of major GDGTs), which resist delamination and protect against proton permeability (van de Vossenberg *et al.*, 1998; Macalady *et al.*, 2004; Valentine, 2007). The presence of polar GDGTs (P-GDGTs) is assumed to reflect living organisms (e.g. Petsch *et al.*, 2001; Biddle *et al.*, 2006; Lipp *et al.*, 2008) because, upon cell death, the P-GDGTs may rapidly degrade into more recalcitrant core GDGTs (C-GDGTs) by cleavage or degradation of the polar head groups similar to phospholipid fatty acids (White *et al.*, 1979; Harvey *et al.*, 1986). However, a later report

Received 7 January, 2014; accepted 8 August, 2014. For correspondence. *E-mail archaea.zhang@gmail.com; Tel. 86-21-65982012; Fax 86-21-65988808. **E-mail martin.g.klotz@gmail.com; Tel. 0017046875465; Fax 0017046871488.

indicated that P-GDGs can be persistent under certain conditions (Logemann *et al.*, 2011). In contrast, C-GDGs can be preserved in the geological record for more than 120 million years (e.g. Kuypers *et al.*, 2001). Another investigation revealed a similarly extended persistence of long-chain glycolipids from heterocystous N₂-fixing cyanobacteria (Bauersachs *et al.*, 2010).

The composition of GDGTs is affected by several physico-chemical factors, which have been investigated under laboratory conditions and in natural environments. Because the packing density of the archaeal membrane is affected by and, potentially, reflective of their growth temperature or pH, the average number of cyclopentane moieties per GDGT lipid (the 'ring index', RI) has been used as a proxy to reflect temperature or pH (Pearson *et al.*, 2004; Schouten *et al.*, 2007; Boyd *et al.*, 2011). Studies with *Sulfolobus solfataricus* (De Rosa *et al.*, 1980), *Thermoplasma acidophilum* (Uda *et al.*, 2001) and *Acidilobus sulfurireducens* (Boyd *et al.*, 2011) revealed that the ring indices of their core GDGTs increased with incubation temperature. In natural environments, however, chemical variables other than temperature or pH appear to affect archaeal community structure and thus the ring indices as well (Pearson *et al.*, 2008; Li *et al.*, 2013; Paraiso *et al.*, 2013); for example, the RI of C-GDGs was found to correlate with the concentration of bicarbonate in some hot springs of the US Great Basin (Pearson *et al.*, 2004).

Tengchong, in central-western Yunnan Province, Southwestern China, is known for its geothermal features (Liao and Guo, 1986; Tong and Zhang, 1989; Wang *et al.*, 2000). The Rehai and Ruidian geothermal fields in Tengchong are two localities of intense hydrothermal activity with numerous springs and pools. A wide physico-chemical diversity of springs (temperature to > 97°C; pH ranges from < 1.8 to ≥ 9.3; granite hosted versus carbonate hosted) provide numerous niches for extremophiles, including thermophilic and thermoacidophilic Archaea. These archaeal communities have been studied by traditional culturing approaches (reviewed in Hedlund *et al.*, 2012) as well as via culture-independent methods that provide a deep census (Huang *et al.*, 2010; Jiang *et al.*, 2010; Song *et al.*, 2010; 2013; Pagaling *et al.*, 2012; Hou *et al.*, 2013). The Rehai and Ruidian geothermal fields provide an excellent opportunity to investigate the relationship between GDGTs, archaeal community composition and physico-chemical conditions. Recently, Wu and colleagues (2013) reported on the distribution of C-GDGs from hot springs of Tengchong and other locations in Yunnan Province as a function of temperature and pH; however, the study lacked data regarding the presence and distribution of P-GDGs as well as phylogenetic information on the resident archaeal community. This limited set of data prevented the examination of whether

the resident archaeal community structure correlates with the abundance and distribution of GDGTs. Furthermore, the global patterning of GDGTs and the underpinning environmental and biological variables are not well understood in hot springs.

In this study, we show that pH affects the distribution of GDGTs in terrestrial hot springs at the global scale whereas temperature affects ether lipid distribution only in samples with similar phylogenetic composition and within individual geographical regions. Dissolved oxygen (limited to Tengchong dataset) also correlated with differences in lipid distribution, which may be attributed to differential degradation of GDGTs under different redox conditions. The lipid composition and the archaeal community structure correlated only at the local level. The dominance of pH over temperature and other locally relevant factors including oxygen concentration and archaeal community structure is a significant improvement upon our previous finding (Pearson *et al.*, 2004; 2008; Zhang *et al.*, 2006) and could provide additional insight on the acclimation and, perhaps, adaption of Archaea to the extreme environments of the geothermal systems.

Results

Physical and chemical characteristics of sampling sites

The springs were divided into five major groups based on visual appearance: (i) large pools with standing water, which included Dagunguo (Dgg), Gongxiaoshe [including GxsS (carbonate-rich sediment at bottom) and GxsB (sinter sample chipped from the sides of the spring)] and Jinze (Jz); (ii) high-discharge, fast-flowing springs with small source pools, which included Gumingquan [including GmqS (source), GmqC (channel) and GmqP (streamer pool)] and Jiemeiquan [including JmqR (right pool) and JmqL (left pool)]; (iii) shallow acidic pools, which included the Diretiyan (Drty) pools and Zhenzhuquan (Zzq); (iv) shallow springs with multiple sources, which included Shuirebaozha (Srbz), Direchi (Drc), left and right Wumingquan (WmqL, WmqR); and (v) small outflow channels containing microbial mats at different locations, which included the upper and lower parts of Shiziwei (SzwU and SzwD respectively) and the upper, middle and lower parts of Shiziyao (SzyS, SzyM and SzyD respectively). Springs Gxs and Jz were located in the town of Ruidian, while all others were located in the Rehai geothermal field, which is about 40 km south of Ruidian (refer to maps in Hou *et al.*, 2013). The physico-chemical parameters of all springs are listed in Table S1. The temperature (43.7°C) of the Srbz spring was significantly lower in July 2012 than it was in January (79.8°C) and June (72.1°C) of 2011, which may be attributed to an

unusually shallow water table. Several hot springs, including WmqL, WmqR and Shiziyao, were only sampled in June 2011 because they were dry in January 2011 and July 2012. In all, the temperatures and pH values were relatively stable in other springs and ranged from 55.1°C to 93.6°C and from 2.5 to 9.4 respectively. Soils near the hot springs were collected in June 2011 to evaluate the extent to which GDGTs in the hot springs were impacted by run-off from surrounding soils.

Abundance of archaeal lipids in Tengchong hot springs and surrounding soils

All hot spring samples contained measurable concentrations of P- and/or C-GDGTs plus P-archaeol (40 out of 46 samples) and C-archaeol (44/46) (Table S1; see Fig. S1 for the structure of archaeol); the latter are mainly present in the membranes of *Euryarchaeota* such as methanogens. Absolute abundance of P-GDGTs ranged from 0.4 to 15 912 ng lipid g⁻¹ dry mass. Concentrations of C-GDGTs ranged from less than 0.7 to 10 292 ng lipid g⁻¹ dry mass. The levels of total P- and C-GDGTs in hot spring samples from June 2011 were correlated, as shown by a linear regression analysis of log-transformed absolute abundances of the P- versus C-GDGTs ($R^2 = 0.73$; Fig. S2). To assess whether P- and/or C-GDGTs in the hot springs may have been introduced from the surrounding soil, pertinent soil samples were collected in June 2011. While the (log-transformed) concentrations of P and C-GDGTs in these soil samples were positively correlated ($R^2 = 0.80$; Fig. S2), the slope and y-intercept were different from that of the hot springs (Fig. S2). The average concentrations of P- and C-GDGTs were about 22-fold and 25-fold higher, respectively, in hot spring samples than in the pertinent surrounding soil samples. In addition, the average concentrations of P- and C-archaeol were about 16-fold and 13-fold higher, respectively, in hot spring samples than in the soil samples (Fig. S3).

Cluster analysis

Cluster analysis was performed to evaluate the distribution of P- and C-GDGTs from all three sampling periods. For P-GDGTs, the samples could be clustered into three major groups that corresponded to the pH rather than temperature at the sample site (Fig. 1): group 1 (pH = 2.8 ± 0.3 , T = $69.3 \pm 12.2^\circ\text{C}$; $n = 11$) was characterized by relatively high abundance of GDGT-4, GDGT-5 and GDGT-6; group 2 (pH = 6.0 ± 1.7 , T = $73.1 \pm 20.6^\circ\text{C}$; $n = 9$) was similar to group 1, but contained less GDGT-6 and GDGT-7 and more GDGT-0 and GDGT-1; and group 3 (pH = 8.8 ± 0.8 , T = $78.1 \pm 11.8^\circ\text{C}$; $n = 21$) was characterized by high abundance of GDGT-0, GDGT-1, GDGT-2

and GDGT-3 (Fig. 1). In addition, Gxs in January and June 2011 were clustered together and were characterized by high abundance of crenarchaeol. For the C-GDGTs, the samples also clustered into three major groups that corresponded with pH and were similar to clusters formed by analysis of P-GDGTs (Fig. S4): group 1 (pH = 3.0 ± 0.6 , T = $70.7 \pm 15.2^\circ\text{C}$; $n = 14$), group 2 (pH = 7.5 ± 0.8 , T = $68.1 \pm 15.2^\circ\text{C}$; $n = 8$) and group 3 (pH = 8.5 ± 1.0 , T = $79 \pm 11.5^\circ\text{C}$; $n = 24$). Most sites sampled over multiple seasons clustered together, indicating that the distribution of P- and C-GDGTs in those springs was relatively stable across different seasons.

While relative abundances of most individual P-GDGT and C-GDGT values were highly correlated with each other (Figs 2 and S5), one exception was the proportion of P-GDGT-0 and C-GDGT-0, which was not significantly correlated ($R^2 = 0.21$; Fig. 2). In contrast to samples with higher dissolved oxygen content ($950 \pm 461 \mu\text{g l}^{-1}$, $n = 13$), those with lower dissolved oxygen content ($222 \pm 118 \mu\text{g l}^{-1}$, $n = 6$) had higher relative abundance of P-GDGT-0 compared with C-GDGT-0, suggesting that degradation rates of P-GDGT-0 and/or C-GDGT-0 might be different under very low oxygen conditions (Fig. S5), although we note that dissolved oxygen concentrations in these springs are virtually always substantially under saturated.

Physical or chemical factors controlling the distribution of P- and C-GDGTs

Redundancy analysis (RDA) of the data was performed to examine whether the observed GDGT patterns correlated significantly with environmental variables. RDA1 and RDA2 explained 90% and 10%, respectively, of the variance in the archaeal P-GDGTs. The results of this analysis (Fig. 3) showed that the pH vector was highly significant and nearly parallel to RDA1, indicating that the RDA1 axis correlated strongly with pH. RDA1 clearly distinguished the P-GDGT distribution in low pH samples from those in neutral or high pH samples, and pH was identified as the most significant environmental factor ($P = 0.001$). Low pH samples were close to the vectors for P-GDGT-5 and -6, while neutral or high pH samples corresponded with the vectors for P-GDGT-0, -1, -2, -3, -4 and crenarchaeol-isomer.

Temperature was identified as another significant environmental factor ($P = 0.004$) contributing to GDGT distribution in hot springs (Fig. 3). However, the vector representing temperature pointed close to RDA2, which only contributed 10% to the distribution of P-GDGTs. The results of RDA based on C-GDGT composition were similar to that of P-GDGTs, indicating that pH is a dominant factor controlling the distribution of P- and C-GDGTs

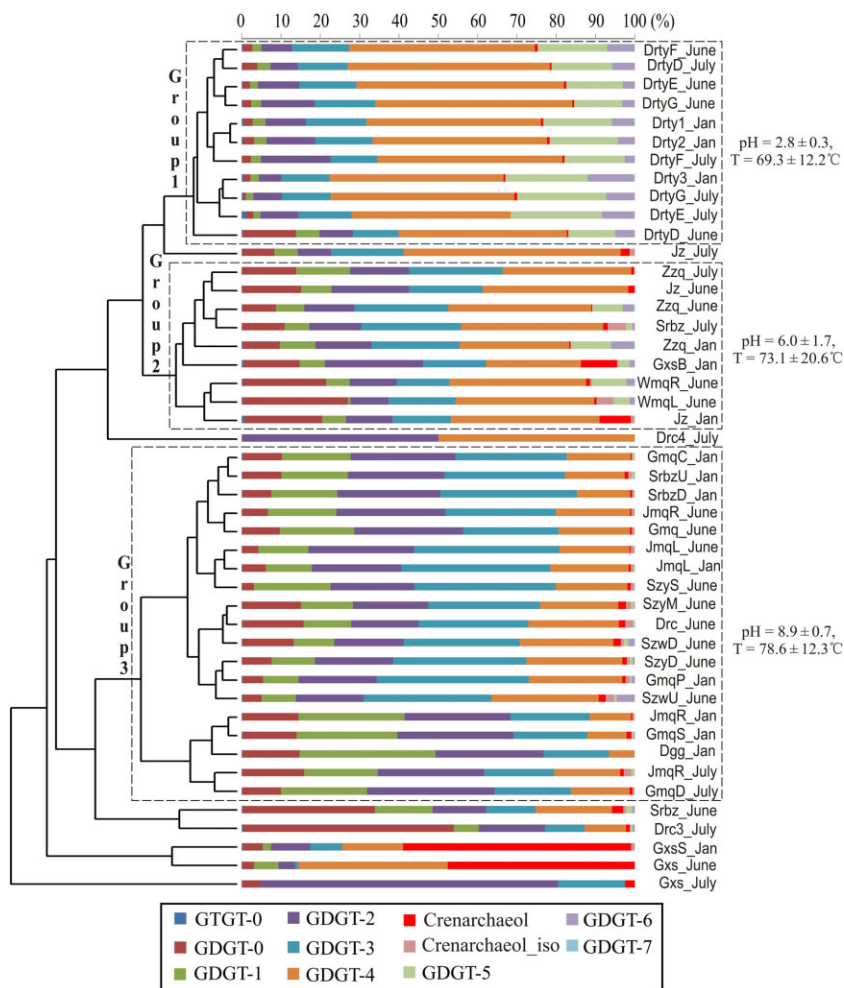


Fig. 1. Cluster analysis of P-GDGTs from Tengchong hot spring sediments collected in January 2011, June 2011 and July 2012. Sample names are shown on the right of the figure. GDGTs are colour coded and shown at the bottom of the figure. Those samples are clustered into three groups that correspond to differences in pH: group 1 (pH = 2.8 ± 0.3 , $T = 69.3 \pm 12.2^\circ\text{C}$; $n = 11$), group 2 (pH = 6.0 ± 1.7 , $T = 73.1 \pm 20.6^\circ\text{C}$; $n = 9$) and group 3 (pH = 8.9 ± 0.7 , $T = 78.6 \pm 12.3^\circ\text{C}$; $n = 19$). The samples in corresponding groups are boxed with dash lines.

in Tengchong hot springs (Fig. S6). Other factors that were investigated, including dissolved oxygen, sulfide, ammonia, nitrate plus nitrite, sulfate and ferrous iron, were not significantly correlated ($P > 0.05$) with the distributions of P- or C-GDGTs.

Regression analyses of RI and environmental variables

Multiple regression analysis was performed to quantitatively investigate the relationship between P-RI and C-RI values and environmental variables. Two equations, including eight environmental variables, best described the relationship between geochemical data and P-RI or C-RI respectively. The root mean square errors of Eqs 1 and 2 are 0.19 and 0.29 respectively.

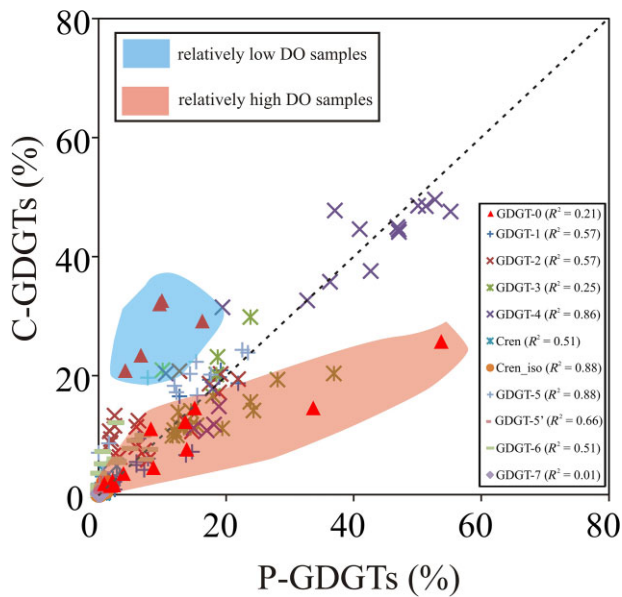
$$P\text{-RI} = 4.1 - 0.37 \times pH + 0.02 \times T - 0.24 \times [DO] + 0.05 \times [S^{2-}] - 0.04 \times [NH_4^+] + 0.003 \times [NO_2^- + NO_3^-] - 0.0001 \times [SO_4^{2-}] - 0.01 \times [FE^{2+}] \quad (1)$$

($n = 16$, $R^2 = 0.94$, $RMSE = 0.19$, $P < 0.01$)

$$C\text{-RI} = 4.69 - 0.3 \times pH - 0.001 \times T + 0.32 \times [DO] - 0.01 \times [S^{2-}] - 0.01 \times [NH_4^+] - 0.07 \times [NO_2^- + NO_3^-] + 0.0003 \times [SO_4^{2-}] - 0.01 \times [FE^{2+}] \quad (2)$$

($n = 16$, $R^2 = 0.91$, $RMSE = 0.29$, $P < 0.01$)

The coefficients of regression between RI and pH (0.37 for P-RI and 0.3 for C-RI) as well as RI and dissolved oxygen (0.24 for P-RI and 0.32 for C-RI) were significantly higher than those for correlations of RI with other environmental factors (< 0.1 for both P- and C-RI) in the equations. The correlation between P- and C-RI and pH was significant ($R^2 = 0.87$ and 0.89 respectively; Fig. 4A), consistent with the cluster analyses and RDA. The results also indicate that dissolved oxygen correlates significantly with P- and C-RI, even when two points with unusually high dissolved oxygen values, Drc and Zzq in July 2012, were considered outliers and thus excluded ($R^2 = 0.58$ and 0.73 respectively; Fig. 4B). The P- or C-RI showed a positive correlation with dissolved oxygen values within the range of 140–



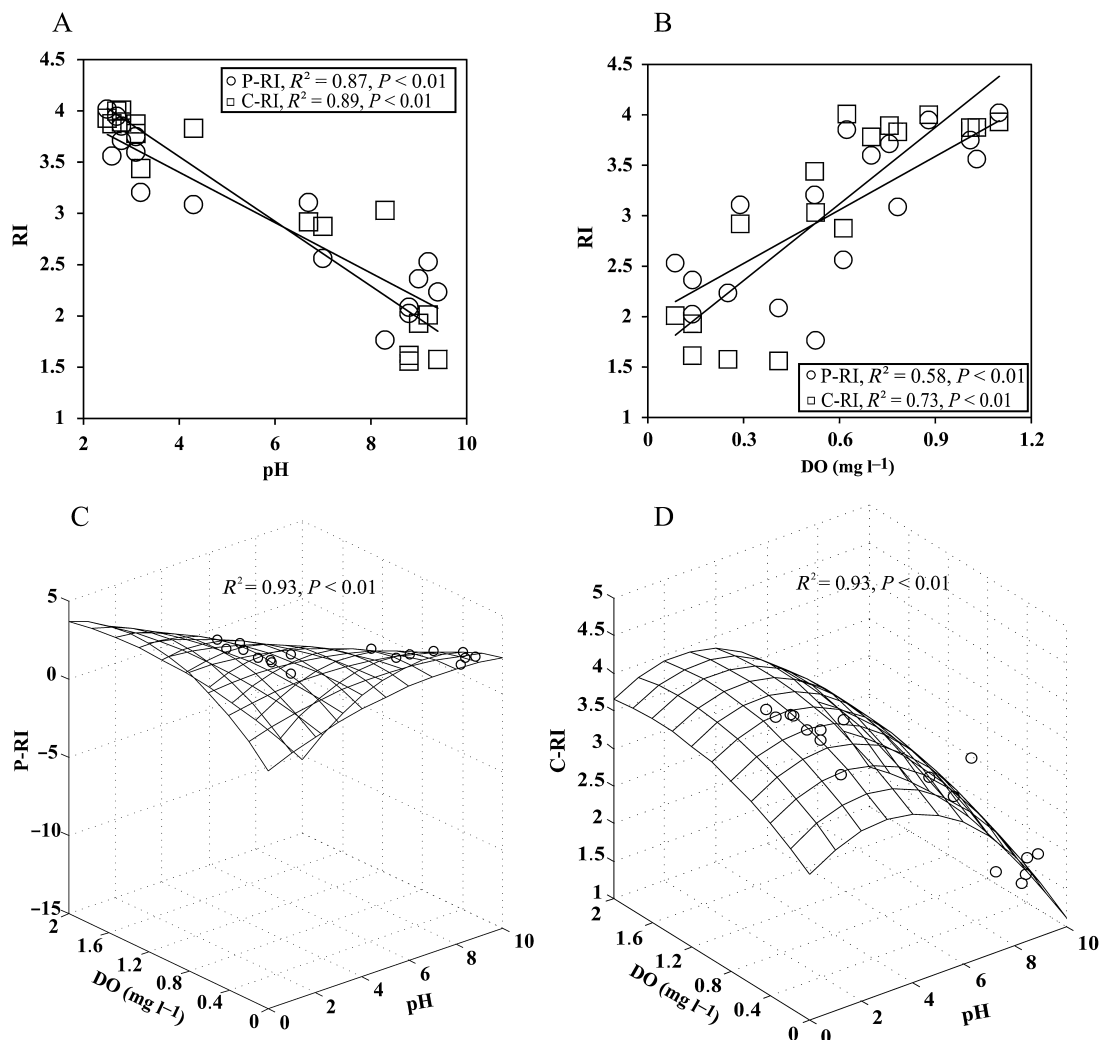


Fig. 4. Simple linear regression plots for relationship (A) between P- or C-RI and pH ($P\text{-RI} = 4.39 - 0.25 \times \text{pH}$, $R^2 = 0.87$; $C\text{-RI} = 4.81 - 0.32 \times \text{pH}$, $R^2 = 0.89$), (B) between P- or C-RI and dissolved oxygen [$P\text{-RI} = 2 + 1.77 \times (\text{DO})$, $R^2 = 0.58$; $C\text{-RI} = 1.59 + 2.53 \times (\text{DO})$, $R^2 = 0.73$]; curved surfaces show relationships (C) between P-RI and pH and dissolved oxygen [$P\text{-RI} = 0.27 + 0.65 \times \text{pH} + 6.8 \times [\text{DO}] - 0.04 \times [\text{pH}]^2 - 2.6 \times [(\text{DO})]^2 - 0.8 \times \text{pH} \times [\text{DO}]$] and (D) between C-RI and pH and dissolved oxygen [$C\text{-RI} = 2.9 + 0.25 \times \text{pH} + 1 \times [\text{DO}] - 0.04 \times [\text{pH}]^2 - 0.3 \times [(\text{DO})]^2 - 0.02 \times \text{pH} \times [\text{DO}]$]. Samples for analysis were collected in June 2011 and July 2012. January 2011 data are not included because there is no dissolved oxygen data. The determination coefficient and P value are shown on the top of the each diagram.

temperature ($R^2 = 0.86$ and 0.85 for P-RI and C-RI values respectively; Fig. S8). Samples from group 3 were characterized by high abundance of *Thaumarchaeota* or *Aigarchaeota* and were located in Ruidian (Fig. S7). Ruidian hot springs have similar pHs to some of the Rehai hot springs but are carbonate hosted and have a distinct chemistry. The relative abundance of *Thaumarchaeota* in the sediment from Gxs (GxsS) was significantly higher than in the sinter from Gxs (GxsB) (Fig. S7). Likewise, the P-crenarchaeol in GxsS was also more abundant than in GxsB (Fig. 1), suggesting that the *Thaumarchaeota* in the Gxs spring resided mainly in the sediment rather than the sinter.

Comparison with other geothermal systems

Previously published data exist for the abundance and distribution of GDGTs and corresponding 16S rRNA pyrotag sequence data of samples from hot springs in Tibet (Li *et al.*, 2013; Wang *et al.*, 2013), Yellowstone National Park (YNP) (Boyd *et al.*, 2013) and the US Great Basin (Table S1; Cole *et al.*, 2013), which were re-analysed to compare the pertinent relationships between archaeal community structure and distribution of P-GDGTs in hot springs from different regions (Figs 5 and 6; Table S2). The phylogenetic pattern consisted of six major groups: (i) group 1 (*Thaumarchaeota*/

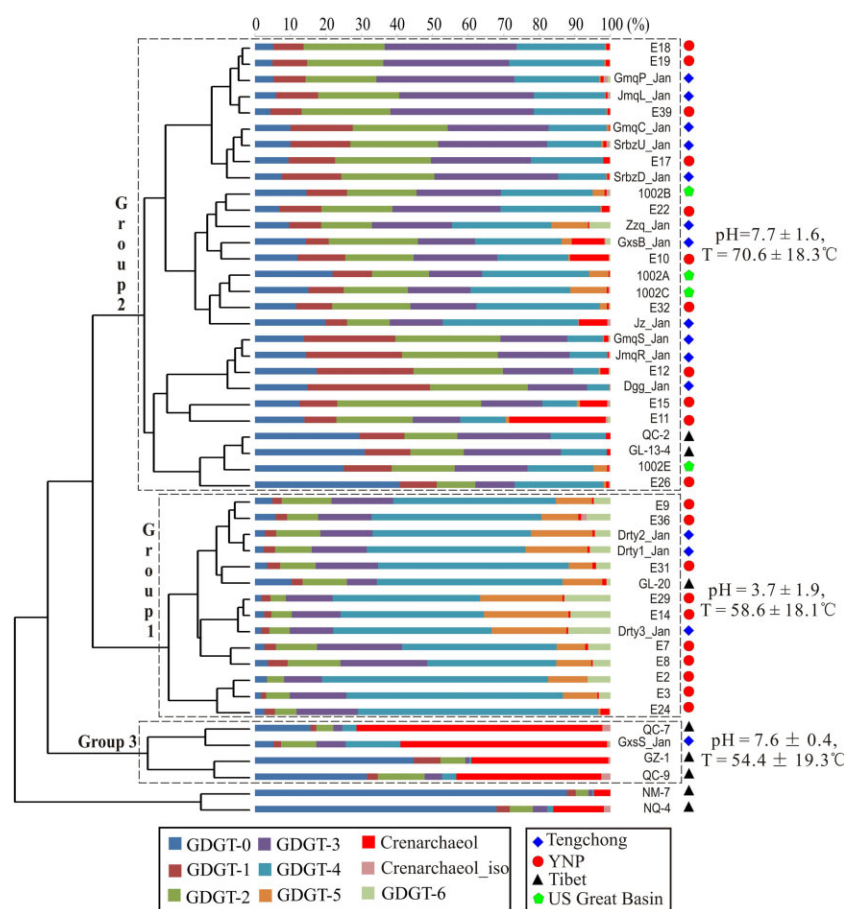


Fig. 5. Cluster analysis based on phylogenetic composition of Archaea from Tengchong, Tibet, YNP and the US Great Basin hot springs at the taxonomic rank of order. Sample names are shown on the right of the figure. The orders are colour coded and shown at the bottom of the figure. Those samples are majorly clustered into six groups: group 1 ($\text{pH} = 7.5 \pm 1.0$, $T = 60.1 \pm 17.5^\circ\text{C}$; $n = 16$), group 2 ($\text{pH} = 7.9 \pm 1.9$, $T = 78.8 \pm 10.3^\circ\text{C}$; $n = 13$), group 3 ($\text{pH} = 5.2 \pm 2.9$, $T = 52.3 \pm 19.4^\circ\text{C}$; $n = 11$), group 4 ($\text{pH} = 2.8 \pm 0.3$, $T = 30.9 \pm 11.8^\circ\text{C}$; $n = 2$), group 5 ($\text{pH} = 7.2 \pm 0.3$, $T = 44.5 \pm 2.1^\circ\text{C}$; $n = 2$) and group 6 ($\text{pH} = 3.1 \pm 1.0$, $T = 73.9 \pm 16.8^\circ\text{C}$; $n = 4$). The samples in corresponding groups are boxed with dash lines. Those data were re-analysed from Hou and colleagues (2013) (Tengchong), Wang and colleagues (2013) (Tibet), Boyd and colleagues (2013) (YNP) and Cole and colleagues (2013) (US Great Basin). MBGA: marine benthic group A; MCG: miscellaneous crenarchaeotic group; pUWA2: an environmental sequence from acidic hot spring water (Takai and Sako, 1999); AcrA07: an environmental sequence of halophilic crenarchaeota from a hypersaline endoevaporitic microbial mat (Sorensen *et al.*, 2005); YLA114: an environmental sequence of Parvarchaea from Yellowstone Lake (Kan *et al.*, 2011).

Aigarchaeota-abundant group; $n = 16$), (ii) group 2 (*Desulfurococcales/Thermoproteales*-abundant group; $n = 13$), (iii) group 3 ('high-diversity' group; $n = 11$), (iv) group 4 (*Thermoplasmatales*-abundant group; $n = 2$), (v) group 5 (YLA114-abundant group; $n = 2$) and (vi) group 6 (*Sulfolobales*-abundant group; $n = 4$) (Fig. 5). In contrast to Tengchong hot spring samples, which were mainly clustered into a *Desulfurococcales/Thermoproteales*-abundant group and a *Sulfolobales*-abundant group, samples from Tibet, YNP and the US Great Basin were mainly represented by the 'high-diversity' group and the *Thaumarchaeota/Aigarchaeota*-abundant group. On the other hand, clustering based on archaeal lipid composition demonstrated that samples from different regions clustered into three major groups that corresponded with sample pH (Fig. 6): group 1 ($\text{pH} = 3.7 \pm 1.9$; $n = 14$), group 2 ($\text{pH} = 7.7 \pm 1.6$; $n = 28$) and group 3 ($\text{pH} = 7.6 \pm 0.4$, $T = 54.4 \pm 19.3^\circ\text{C}$; $n = 4$). The low pH samples (group 1) had high abundance of GDGT-5 and GDGT-6, while most neutral-high pH samples (group 2) had relatively high abundance of GDGT-0, -1, -2, -3 and -4. Group 3 consisted of samples with abundant crenarchaeol.

RDA of the expanded datasets showed that both pH and temperature are significantly correlated with the archaeal phylogenetic composition and corresponding lipid distribution (Fig. 7A and B respectively). The RDA1 in Fig. 7A and B explained 64% and 96% of the total variance respectively. Figure 7A shows that both pH and temperature correlate significantly with the phylogenetic composition of the archaeal community at the order level, whereas Fig. 7B shows a dominant relationship between pH and archaeal lipid content, with a weaker but still significant correlation between temperature and archaeal lipid content.

Spearman's rho analyses were further used to investigate the relationships among pH, temperature, archaeal composition and individual P-GDGs. The results (Table S3) indicated that both pH and temperature were statistically significantly correlated with four of 18 archaeal genera, which is consistent with the RDA results, demonstrating that pH and temperature equally contributed to the structure of the archaeal community in those hot springs. On the other hand, pH was significantly correlated with the abundance of all nine individual P-GDGs (positively correlated with GDGT-0, -1, -2, -3,

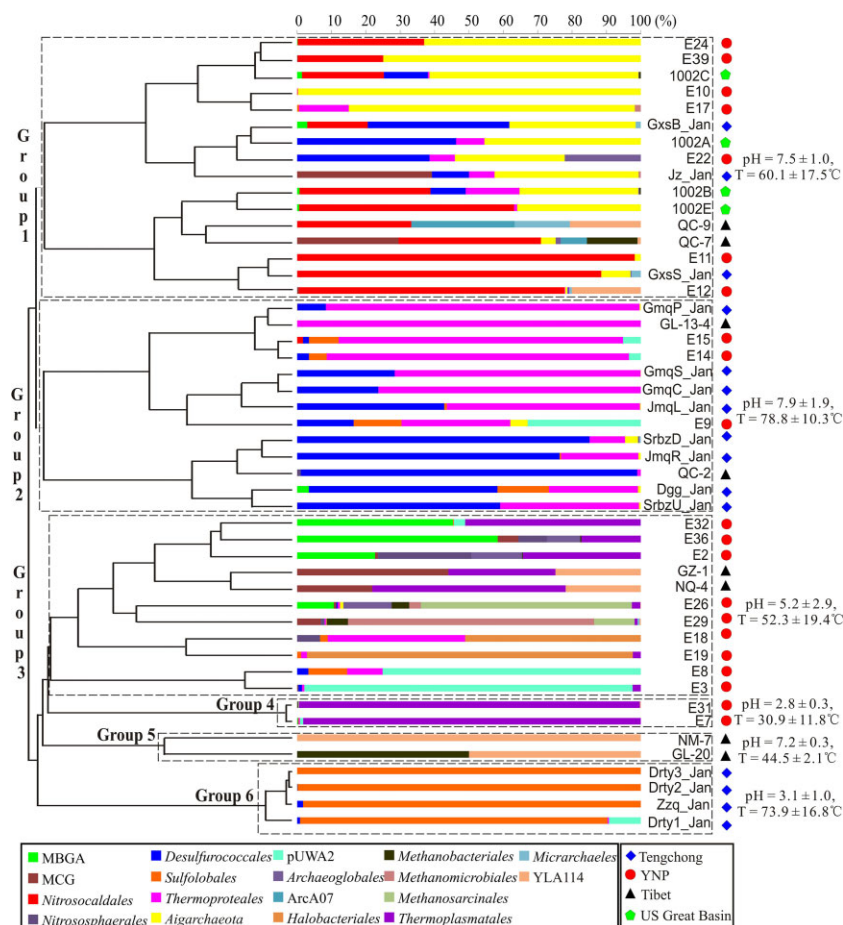


Fig. 6. Cluster analyses of P-GDGTs from Tengchong hot spring samples in January 2011, from Tibet, YNP and the US Great Basin. Sample names are shown on the right of the figure. GDGTs are colour coded and shown at the bottom of the figure. Those samples are majorly clustered into three groups: group 1 (pH = 3.7 ± 1.9 , T = $58.6 \pm 18.1^\circ\text{C}$; n = 14), group 2 (pH = 7.7 ± 1.6 , T = $70.6 \pm 18.3^\circ\text{C}$; n = 28) and group 3 (pH = 7.6 ± 0.4 , T = $54.4 \pm 19.3^\circ\text{C}$; n = 4). The samples in corresponding groups are boxed with dash lines.

crenarchaeol and crenarchaeol-isomer and negatively correlated with GDGT-4, -5 and -6), while temperature just correlated with two of them (positively correlated with GDGT-1, -2). The results of Spearman's rho analyses were consistent with the results of the RDA, demonstrating that pH and temperature equally contributed to the structure of the archaeal community, but pH was the dominant correlate with the distribution of P-GDGTs. The Spearman's rho analyses identified several statistically significant relationships between the abundance of certain archaeal genera and P-RI. P-RI was positively correlated with both presence and abundance of *Sulfolobales*, *Nitrososphaerales* and pUWA2, and negatively correlated with ArcA07 and YLA114.

Discussion

Origin of archaeal lipids in the hot springs

In situ production of archaeal GDGTs in hot springs is supported by two lines of evidence. Firstly, although significant correlations between P- and C-GDGTs were found for both the hot spring and surrounding soil sites, the slope and y-intercept were different. Secondly, the

mean concentration of both GDGTs and archaeol were higher in hot spring sediments than surrounding soils, suggesting that GDGT and archaeol structures identified in the hot springs were predominantly autochthonous rather than the result of exogenous input. The interpretation of these results is consistent with previous observations from hot springs in the US Great Basin (Pearson *et al.*, 2004; Zhang *et al.*, 2006; Paraiso *et al.*, 2013), YNP (Boyd *et al.*, 2013) and Tibet (He *et al.*, 2012; Li *et al.*, 2013).

Relative importance of pH, temperature, dissolved oxygen and community structure in shaping the distribution of P- and C-GDGTs

The number of cyclopentyl rings in GDGTs, expressed in the form of the RI, is known to correlate with temperature (Uda *et al.*, 2001; Boyd *et al.*, 2011; Paraiso *et al.*, 2013) and pH (Boyd *et al.*, 2011; 2013). In contrast, Pearson and colleagues (2004) observed that bicarbonate, but not temperature, correlated with the RI in alkaline hot springs in the US Great Basin while Li and colleagues (2013) reported that multiple environmental factors could significantly impact the RI in Tibet. The analysis of GDGTs

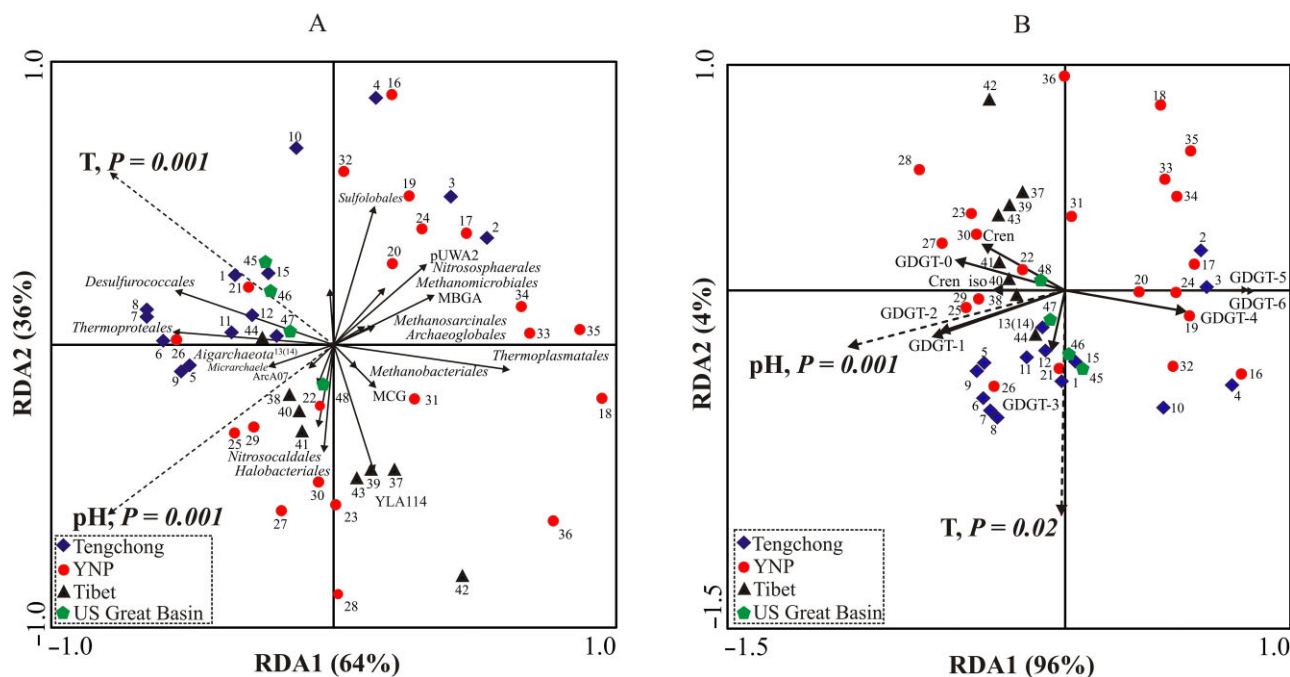


Fig. 7. RDA ordination diagrams of (A) major archaeal community structure and (B) archaeal lipids with environmental variables from hot springs. Correlations between environmental variables and RDA axes are represented by the length and angle of dashed arrows (environmental factor vectors). Solid arrows represent the relative abundance of (A) archaeal orders or (B) P-GDGs. The numbers on the samples correspond with the sample numbers in Table S2.

presented here revealed that the composition of P- and C-GDGs was correlated primarily with pH on a global scale and secondarily correlated with temperature, dissolved oxygen and archaeal community structure (see details below).

pH. The negative correlation between pH and RI was revealed by RDA and multiple regression analysis for Tengchong hot spring samples and is consistent with previous reports on pure cultures (Macalady *et al.*, 2004; Boyd *et al.*, 2011) and samples from natural environments (Pearson *et al.*, 2008; Boyd *et al.*, 2013). On the other hand, our results are in contrast with published work on the euryarchaeon *T. acidophilum* (Shimada *et al.*, 2008) and with results from hot springs in Tibet (Li *et al.*, 2013). One reason for the discrepancy between these studies may be that different Archaea respond to environmental cues with different changes in the composition of their lipids. This interpretation is supported by different relationships between GDGT compositions and environmental factors in Tengchong harbouring different Archaea. For example, the P-RI and C-RI values in the *Desulfurococcates*- and *Thermoproteales*-dominated group were correlated with temperature (Fig. S8), whereas the P-RI and C-RI values in the *Sulfolobales*-dominated group were not correlated with temperature. An important observation based on pooling of hot spring P-GDGT data

from Tengchong, Tibet, YNP and the US Great Basin is the dominance of the effect of pH on the distribution of P-GDGs over any biogeographic patterns or temperature, as described below.

Temperature. Although it has been demonstrated that the number of cyclopentyl rings increases with temperature in some pure cultures (De Rosa *et al.*, 1980; Uda *et al.*, 2001; Shimada *et al.*, 2008; Boyd *et al.*, 2011) and natural environments (Schouten *et al.*, 2002; 2003; Li *et al.*, 2013; Paraiso *et al.*, 2013), temperature showed less impact than pH on the distribution of P- or C-GDGs in both Tengchong hot springs and the expanded dataset. The impact of temperature on the distribution of P- or C-GDGs could be detected in Tengchong hot springs only in the *Desulfurococcates*- and *Thermoproteales*-dominated group having a narrow pH range (9.0 ± 0.9 , $n = 6$). In the expanded dataset, temperature did correlate with archaeal lipid content but was much less significant than pH. Similarly, Li and colleagues (2013) only found a positive correlation between temperature and P-RI in a subgroup of samples having a narrow pH range (7.7 ± 0.5 , $n = 12$) in Tibetan hot springs. Paraiso and colleagues (2013) found that temperature and pH both correlated strongly with the relative abundance of lipids sampled in US Great Basin hot springs that had a pH range of 6.8–10.1. Lastly, Boyd and colleagues (2013) did

not find any correlation between temperature and RI in sediment samples from YNP hot springs having a pH range of 2.06–9.57. These studies demonstrate that the influence of temperature on GDGT content decreases with expanding pH range in terrestrial geothermal environments.

Dissolved oxygen. Dissolved oxygen significantly correlated with P-RI and C-RI values, indicating that the oxygen concentration, or redox status, may affect the distribution of GDGTs in Tengchong hot springs. Paraiso and colleagues (2013) reported that oxygen correlated with the distribution of GDGTs in US Great Basin hot springs, albeit with moderate significance, and suggested that the relationship may result from a co-variation between oxygen and temperature. Considering that there was co-variation between oxygen and pH for the Tengchong samples, oxygen or redox status may or may not directly affect the distribution of P- and C-GDGTs in Tengchong springs. The observed difference between polar and core GDGT substructures specifically for C-GDGT-0 and P-GDGT-0 was similar to observations reported for YNP hot springs (Boyd *et al.*, 2013). In addition, samples revealing high differences between P- and C-GDGT-0 lipid species were retrieved from sites characterized by relatively low oxygen concentrations (Figs 2 and S5). The observed differences between P- and C-GDGT-0 lipid species might be due to different oxygen-dependent degradation rates of this particular GDGT structure and, in particular, of the polar moieties. Further studies are needed to investigate the impact of dissolved oxygen on the distribution of GDGTs under experimental conditions.

Relationship with community structure. Archaeal 16S rRNA gene and lipid profiles from Tengchong hot springs collected in samples of January 2011 were investigated to address the effect of community structure on the archaeal lipid profile. The hot spring samples from Tengchong were classified into three major groups based on 16S rRNA pyrosequencing analyses: a *Desulfurococcales*- and *Thermoproteales*-abundant group, a *Sulfolobales*-abundant group and a *Thaumarchaeota*-*Aigarchaeota*-abundant group. It is known that *Desulfurococcales* produce a high proportion of GDGT-0 (Lanzotti *et al.*, 1989; Blöchl *et al.*, 1997; Huber *et al.*, 1998; Jahn *et al.*, 2004; Schouten *et al.*, 2007) while *Thermoproteales* produce a mixture of GDGT-0, -1, -2, -3 and -4 (Langworthy and Pond, 1986; Thurl and Schäfer, 1988; Trincone *et al.*, 1992; Völkl *et al.*, 1993; Itoh *et al.*, 1999, 2003; Schouten *et al.*, 2007). Consequently, RI values from the *Desulfurococcales*- and *Thermoproteales*-abundant group of springs were relatively low. In contrast, GDGT-5, -6, -7 and -8 are frequently found in

Sulfolobales (De Rosa and Gambacorta, 1988; Takayanagi *et al.*, 1996; Sturt *et al.*, 2004; Schouten *et al.*, 2007), resulting in high RI values in the *Sulfolobales*-dominated group of springs. *Thaumarchaeota* synthesize crenarchaeol and GDGTs decorated with 0–3 cyclopentyl moieties (Schouten *et al.*, 2008; Pitcher *et al.*, 2010, 2011), which result in low RI values for the *Thaumarchaeota*-*Aigarchaeota*-abundant group of springs. The significant correlation between the taxonomic structure of the archaeal community and the GDGT lipid composition suggests that the composition of archaeal lipids in Tengchong hot springs reflects the resident community structure. Although Briggs and colleagues (2013) reported significant temporal change in archaeal taxonomic compositions because of high rainfall influx during the monsoon season, lipid samples collected from most sites over multiple seasons clustered together, indicating that the distribution of P- and C-GDGTs in those springs was relatively stable and not affected by archaeal community dynamics. Whereas only pH had a significant influence on the structure of the archaeal community and, consequently, the distribution of GDGTs in Tengchong hot springs, pH and temperature appeared to control the structure of the archaeal community equally when the Tengchong data were extended to include data from Tibet, YNP and the US Great Basin hot springs. Only five in 18 genera were correlated with P-RI, indicating a more complex relationship between archaeal community structure and the distribution of P-GDGTs. Nevertheless, the distribution of P-GDGTs still exhibited a dominant correlation with pH, suggesting that the resident Archaea in these hot springs are acclimated if not adapted to low pH by their genetic capacity to effect the packing density of their membranes by increasing cyclopentyl rings in GDGTs at the rank of community.

Conclusions

Comparative analysis of the abundance of P- and C-GDGTs in hot springs and their surrounding soils revealed that archaeal lipids in both fractions were predominantly produced *in situ*. Cluster analyses of P- and C-GDGTs were similar to phylogenetic clusters, suggesting that environmental factors control both the phylogenetic and GDGT compositions that are intimately linked in Tengchong hot springs. pH appears to be the dominant factor affecting the distribution of P- or C-GDGTs in terrestrial hot springs at the global scale whereas temperature appears to affect lipid distribution when the pH and/or phylogenetic composition are similar. Furthermore, dissolved oxygen or redox status may affect GDGT profiles because of differential degradation of individual GDGT species under different redox conditions, which is also secondary control on GDGT composition.

Experimental procedures

Field measurements and sample collection

Field measurements and sample collection in Tengchong and the US Great Basin were conducted as follows. Water temperature and pH were measured with portable meters (LaMotte, MD, USA). Hot spring water was sampled without disrupting the sediment as to not affect the bulk water chemistry. Total ammonia, nitrate plus nitrite, sulfate, total sulfide, ferrous iron, dissolved oxygen were measured colourimetrically using Hach test kits (Hach Chemical, IA, USA) in the field following procedures described previously (Zhang *et al.*, 2008; Dodsworth *et al.*, 2012). Briefly, water samples for measurements of total ammonia, sulfate, nitrate plus nitrite and ferrous iron were filtered (0.20 µm polyethersulfone membrane filters, Pall Corp., NY) and allowed to cool to ambient temperature prior to analysis. Ammonia was determined using salicylate oxidation method. Nitrate plus nitrite was determined by cadmium reduction of nitrate and subsequent diazotization of nitrite. Sulfate was determined by barium reaction. Ferrous iron was measured with 1,10-phenanthroline indicator. Dissolved oxygen and sulfide were measured immediately after sampling. Dissolved oxygen measurements were made using the HRDO Accuvac ampule method (Hach, high range) or the Indigo Carmine method (Hach, low range), avoiding contacting with the atmosphere. Sulfide was measured with the Pomeroy methylene blue method to prevent heat inactivation of reagents and to allow rapid analysis prior to oxidation.

A total of 46 samples from 15 geothermal springs were collected with sterile spatulas and spoons; each sample was homogenized in a pre-sterilized aluminum pan before allocation in different containers. Another 10 samples were collected from surrounding soils in June 2011. Four sediment samples from Great Boiling Spring in the US Great Basin were collected in February 2010. All the samples for lipid and DNA analyses were immediately frozen on dry ice and kept at -80°C in the laboratory.

Lipid extraction and fractionation

All the samples were freeze-dried and ground to fine powder. For samples from January 2011, about 5 g of the powder were extracted at room temperature using a sonication method (Schouten *et al.*, 2007; Wei *et al.*, 2011) with methanol (MeOH) (twice), MeOH-dichloromethane (DCM) (1:1, v/v; twice) and DCM (twice). The total lipid extract (TLE) was fractionated over a pre-activated silica gel chromatography into apolar and polar (C-GDGTs and P-GDGTs) fractions using hexane-DCM (9:1, v/v) and DCM-MeOH (1:1, v/v) as eluents respectively. The polar fraction was amended with a known amount of a C₄₆ internal GDGT standard, divided into two equal aliquots and evaporated under N₂. One aliquot was re-dissolved in hexane-isopropanol (99:1, v/v) and filtered with a 0.45 µm membrane to obtain the C-GDGTs and then for high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS) analysis. The other aliquot was acid hydrolysed by refluxing it in 2 ml of 5% HCl in MeOH for 3 h at 70°C. The solution was cooled to room temperature, adjusted to pH 5

with KOH-MeOH (1:1, v/v) and partitioned by addition of bidistilled water and DCM to reach a ratio of 1:1 H₂O-MeOH. The mixture was washed two more times with DCM. All the DCM phases were dried under N₂ and processed as described above for the C-GDGTs. The abundance of P-GDGTs was calculated as the difference between hydrolysed and non-hydrolysed fractions.

For samples from Tengchong in June 2011 and July 2012 and samples from the US Great Basin in February 2010, we used a modified Bligh and Dyer method (Sturt *et al.*, 2004) using a mixture of MeOH, DCM and phosphate buffer at pH 7.4 (2:1:0.8 v/v) for four times. After sonication for 5–10 min, additional DCM and buffer were added to the mixture to achieve a final MeOH/DCM/buffer ratio of 1:1:0.8. The phases were separated and the extraction repeated three more times. The extracts were separated into an organic phase and an aqueous phase by centrifugation at 2500 r.p.m. for 10 min; the bottom DCM phase was collected using a glass pipette, which was repeated three times. The TLEs were dried under a stream of nitrogen gas at about 37°C. A known amount of C₄₆ compound was added as internal standard to the TLEs. The TLEs were equally divided into two parts. One half, named F₁, was dried under a stream of nitrogen gas at about 37°C and dissolved in hexane/isopropanol (99:1, v/v) and filtered using a 0.45 µm polytetrafluoroethylene (PTFE) filter for further analysis. The other half, named F₂, was acid hydrolysed as described above. The hydrolysed fractions were dissolved in hexane/isopropanol (99:1, v/v) and filtered using a 0.45 µm PTFE filter for further analysis. Core lipid in the sediment samples were directly quantified from F₁. Polar lipid was indirectly quantified by the difference between F₂ and F₁.

Analysis and quantification of GDGTs

P- and C-GDGTs samples were analysed using published methods (Hopmans *et al.*, 2000). Analyses were performed using an Agilent 1200 HPLC device connected to an Agilent 6460 triple-quadrupole mass spectrometer. Separation was achieved on an Alltech Prevail Cyano column (150 × 2.1 mm, 3 µm) maintained at 30°C. Injection volume was 10 µl. For the first 5 min, GDGTs were eluted isocratically with 99% A (hexane) and 1% B (isopropanol), followed by a linear gradient to 1.8% B in 45 min with a constant flow rate of 0.2 ml min⁻¹. After each analysis, the column was cleaned by back-flushing hexane-propanol (9:1, v/v) at 0.2 ml min⁻¹ for 10 min. Quantification of archaeol (m/z⁻¹ 653), GDGTs (m/z⁻¹ 1302, 1300, 1298, 1296, 1294, 1292, 1290 and 1288) and a C₄₆ GDGT internal standard (m/z⁻¹ 744) was achieved by integration of the extracted peak areas. GDGTs were quantified by integration of peak area from the extracted ion chromatogram. The GDGT-4 peak area from extracted ion chromatogram was corrected by subtracting 46% of crenarchaeol (Hopmans *et al.*, 2000).

Calculation of RI

RI was calculated according to an equation described by Uda and colleagues (2004) as follows:

$$RI = \frac{GDGT-1 + 2 \times GDGT-2 + 3 \times GDGT-3 + 4 \times GDGT-4 + 5 \times GDGT-5 + 6 \times GDGT-6 + 7 \times GDGT-7}{GDGT-0 + GDGT-1 + GDGT-2 + GDGT-3 + GDGT-4 + GDGT-5 + GDGT-6 + GDGT-7} \quad (3)$$

The structures of GDGT-0 to -7 are referred to Fig. S1.

Pyrosequencing data

To examine the phylogenetic composition of microbial communities associated with the lipid analyses, previously published, pyrosequenced amplified 16S rRNA gene datasets were downloaded from the Short Read Archive database at NCBI: Tengchong (SRA056421; Hou *et al.*, 2013), Tibet (SRA061437; Wang *et al.*, 2013), YNP (SRR648329; Boyd *et al.*, 2013) and US Great Basin (SRX201239, SRX201242, SRX201245 and SRX201246; Cole *et al.*, 2013). To ensure that all analyses were comparable, we re-analysed all the datasets using a single pipeline. Pyrosequencing data were converted to sequence reads using MOTHUR software (Schloss *et al.*, 2009) and were analysed using the QIIME standard pipeline (Caporaso *et al.*, 2010). Sequence reads were first filtered by removing low-quality or ambiguous reads. Sequences that passed the initial quality control were checked with CHIMERASLAYER (Haas *et al.*, 2011). The chimeric sequences were excluded for further analysis. The remaining high quality 16S rRNA sequences were clustered into OTUs using UCLUST (Edgar, 2010) with 97% sequence identity threshold. Taxonomy was assigned using the Ribosomal Database Project classifier 2.2 (minimum confidence of 80%) (Cole *et al.*, 2009). Then all the archaeal taxonomies at the rank of order were chosen to recalculate the proportion.

Statistical methods

Clustering of samples by lipid profiles and archaeal community composition was performed using the base program in R 2.12.1 [freeware available at <http://cran.r-project.org/> (Maindonald, 2007)]. The relative abundances of P-GDGTs and C-GDGTs and identified archaeal taxons from hot spring sediments were imported into R 2.12.1. A hierarchical clustering tree was generated by Heatmap command in R package (Maindonald, 2007). Relationship between distribution of P- and C-GDGTs and environmental factors were explored with RDA using the software CANOCO [version 4.5; Microcomputer Power (Ter Braak and Smilauer, 2002)]. Variable significance was examined by Monte-Carlo significance test with permutation number of 999. Spearman's rho values were calculated in SPSS Statistics 16.1 (SPSS, Chicago, IL, USA) to identify correlative relationships among community structure, distribution of GDGTs and environmental variables. Equations to predict relationship between environmental parameters and P- or C-RI based on the distribution of GDGTs was derived using multiple regression analysis (Peterse *et al.*, 2012) by MATLAB software (freeware available at <http://www.mathworks.com/>, Version 7.11.0, The Mathworks, Natick, MA, USA). The units of the regression equations are as Table S1 shown; one exception is the dissolved oxygen, which is transferred to milligram per litre). Samples having all the eight measured variables were included into the multiple regress analyses (four exceptions

were the Gxs in June 2011 and July 2012 having high abundance of crenarchaeol, Drc and Zzq in July 2012 having outliers DO values).

Acknowledgements

We thank the Tengchong Partnership for International Research and Education team and the staff from the Yunnan Tengchong Volcano and Spa Tourist Attraction Development Corporation for their assistance. We also wish to thank two anonymous reviewers and the handling editor for their constructive and helpful comments. This study was funded by the National Natural Science Foundation of China Grants #41373072 and #40972211, the US National Science Foundation Grants #ETBC-1024614 and #OISE-0968421, the '1000 Talents Program' and the State Key Laboratory of Marine Geology at Tongji University. M. G. K. was supported by Visiting Scholar Funds of Tongji University and Research Incentive Funds from the University of North Carolina at Charlotte. B. P. H. was supported by a generous donation from Greg Fullmer through the UNLV Foundation.

References

- Bauersachs, T., Speelman, E.N., Hopmans, E.C., Reichart, G.-J., Schouten, S., and Damsté, J.S.S. (2010) Fossilized glycolipids reveal past oceanic N₂ fixation by heterocystous cyanobacteria. *Proc Natl Acad Sci USA* **107**: 19190–19194.
- Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sorensen, K.B., Anderson, R., *et al.* (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc Natl Acad Sci USA* **103**: 3846–3851.
- Blöchl, E., Rachel, R., Burggraf, S., Hafenbradl, D., Jannasch, H.W., and Stetter, K.O. (1997) *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113°C. *Extremophiles* **1**: 14–21.
- Boyd, E.S., Pearson, A., Pi, Y., Li, W.J., Zhang, Y.G., He, L., *et al.* (2011) Temperature and pH controls on glycerol dibiphytanyl glycerol tetraether lipid composition in the hyperthermophilic crenarchaeon *Acidilobus sulfurireducens*. *Extremophiles* **15**: 59–65.
- Boyd, E.S., Hamilton, T.L., Wang, J.X., He, L., and Zhang, C.L. (2013) The role of tetraether lipids in the adaptation of thermophilic archaea to acidity. *Front Microbiol* **4**: 62. doi:10.3389/fmicb.2013.00062.
- Briggs, B.R., Brodie, E.L., Tom, L.M., Dong, H.L., Jiang, H.C., Huang, Q.Y., *et al.* (2013) Seasonal patterns in microbial communities inhabiting the hot springs of Tengchong, Yunnan Province, China. *Environ Microbiol* **16**: 1579–1591.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.
- Cole, J.K., Peacock, J.P., Dodsworth, J.A., Williams, A.J., Thompson, D.B., Dong, H.L., *et al.* (2013) Sediment microbial communities in Great Boiling Spring are controlled by temperature and distinct from water communities. *ISME J* **7**: 718–729.

- Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., *et al.* (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37**: D141–D145.
- De Rosa, M., and Gambacorta, A. (1988) The lipids of archaebacteria. *Prog Lipid Res* **27**: 153–175.
- De Rosa, M., Esposito, E., Gambacorta, A., Nicolaus, B., and Bu'Lock, J.D. (1980) Effects of temperature on ether lipid composition of *Caldariella acidophila*. *Phytochemistry* **19**: 827–831.
- Dodsworth, J.A., McDonald, A.I., and Hedlund, B.P. (2012) Calculation of total free energy yield as an alternative approach for predicting the importance of potential chemolithotrophic reactions in geothermal springs. *FEMS Microbiol Ecol* **81**: 446–454.
- Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.
- Haas, B.J., Gevers, D., Earl, A.M., Feldgarden, M., Ward, D.V., Giannoukos, G., *et al.* (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res* **21**: 494–504.
- Harvey, H.R., Fallon, R.D., and Patton, J.S. (1986) The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. *Geochim Cosmochim Acta* **50**: 795–804.
- He, L., Zhang, C.L., Dong, H.L., Fang, B., and Wang, G.H. (2012) Distribution of glycerol dialkyl glycerol tetraethers in Tibetan hot springs. *Geosci Front* **3**: 289–300.
- Hedlund, B.P., Cole, J.K., Williams, A.J., Hou, W., Zhou, E., Li, W., *et al.* (2012) A review of the microbiology of the Rehai geothermal field in Tengchong, Yunnan Province, China. *Geosci Front* **3**: 273–288.
- Hopmans, E.C., Schouten, S., Pancost, R.D., van der Meer, M.T., and Sinninghe Damsté, J.S. (2000) Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun Mass Spectrom* **14**: 585–589.
- Hou, W.G., Wang, S., Dong, H.L., Jiang, H.C., Briggs, B.R., Peacock, J.P., *et al.* (2013) A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China using 16S rRNA gene pyrosequencing. *PLoS ONE* **8**: e53350.
- Huang, Q.Y., Jiang, H.C., Zhang, C.L., Li, W.J., Deng, S.C., Yu, B.S., *et al.* (2010) Abundance of ammonia-oxidizing microorganisms in response to environmental variables of hot springs in Yunnan Province, China. *Wei Sheng Wu Xue Bao* **50**: 132–136.
- Huber, R., Dyba, D., Huber, H., Burggraf, S., and Rachel, R. (1998) Sulfur-inhibited *Thermosphaera aggregans* sp. nov., a new genus of hyperthermophilic archaea isolated after its prediction from environmentally derived 16S rRNA sequences. *Int J Syst Bacteriol* **48**: 31–38.
- Itoh, T., Suzuki, K., Sanchez, P.C., and Nakase, T. (1999) *Caldivirga maquilingensis* gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *Int J Syst Bacteriol* **49**: 1157–1163.
- Itoh, T., Suzuki, K., Sanchez, P., and Nakase, T. (2003) *Caldisphaera lagunensis* gen. nov., sp. nov., a novel thermoacidophilic crenarchaeote isolated from a hot spring at Mt Maquiling, Philippines. *Int J Syst Evol Microbiol* **53**: 1149–1154.
- Jahn, U., Summons, R., Sturt, H., Grosjean, E., and Huber, H. (2004) Composition of the lipids of *Nanoarchaeum equitans* and their origin from its host *Ignicoccus* sp. strain KIN4/I. *Arch Microbiol* **182**: 404–413.
- Jiang, H.C., Huang, Q.Y., Dong, H.L., Wang, P., Wang, F.P., Li, W.J., *et al.* (2010) RNA-based investigation of ammonia-oxidizing archaea in hot springs of Yunnan Province, China. *Appl Environ Microbiol* **76**: 4538–4541.
- Kan, J., Clingenpeel, S., Macur, R.E., Inskeep, W.P., Lovallo, D., Varley, J., *et al.* (2011) Archaea in Yellowstone Lake. *ISME J* **5**: 1784–1795.
- Kuypers, M.M., Blokker, P., Erbacher, J., Kinkel, H., Pancost, R.D., Schouten, S., *et al.* (2001) Massive expansion of marine archaea during a mid-Cretaceous oceanic anoxic event. *Science* **293**: 92–95.
- Langworthy, T.A., and Pond, J.L. (1986) Archaeobacterial ether lipids and chemotaxonomy. *Syst Appl Microbiol* **7**: 253–257.
- Lanzotti, V., Trincone, A., Nicolaus, B., Zillig, W., Rosa, M.D., and Gambacorta, A. (1989) Complex lipids of *Pyrococcus* and AN1, thermophilic members of archaebacteria belonging to *Thermococcales*. *Biochim Biophys Acta* **1004**: 44–48.
- Li, F.Y., Zhang, C.L., Dong, H.L., Li, W.J., and Williams, A. (2013) Environmental controls on the distribution of archaeal lipids in Tibetan hot springs: insight into the application of organic proxies for biogeochemical processes. *Environ Microbiol Rep* **5**: 868–882.
- Liao, Z., and Guo, G. (1986) Geology of the Tengchong geothermal field and surrounding area, west Yunnan, China. *Geothermics* **15**: 339–345.
- Lipp, J.S., Morono, Y., Inagaki, F., and Hinrichs, K.U. (2008) Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature* **454**: 991–994.
- Logemann, J., Graue, J., Köster, J., Engelen, B., Rullkötter, J., and Cypionka, H. (2011) A laboratory experiment of intact polar lipid degradation in sandy sediments. *Biogeosciences* **8**: 2547–2560.
- Macalady, J.L., Vestling, M.M., Baumler, D., Boekelheide, N., Kaspar, C.W., and Banfield, J.F. (2004) Tetraether-linked membrane monolayers in *Ferroplasma* spp: a key to survival in acid. *Extremophiles* **8**: 411–419.
- Maindonald, J.H. (2007) *Data Analysis and Graphics Using R: An Example-Based Approach*. New York, USA: Cambridge University Press.
- Pagaling, E., Grant, W.D., Cowan, D.A., Jones, B.E., Ma, Y., Ventosa, A., *et al.* (2012) Bacterial and archaeal diversity in two hot spring microbial mats from the geothermal region of Tengchong, China. *Extremophiles* **16**: 607–618.
- Paraiso, J.J., Williams, A.J., Huang, Q., Wei, Y., Dijkstra, P., Hungate, B.A., *et al.* (2013) The distribution and abundance of archaeal tetraether lipids in US Great Basin hot springs. *Front Microbiol* **4**: 247. doi:10.3389/fmicb.2013.00247.
- Pearson, A., Huang, Z.Y., Ingalls, A.E., Romanek, C.S., Wiegel, J., Freeman, K.H., *et al.* (2004) Nonmarine crenarchaeol in Nevada hot springs. *Appl Environ Microbiol* **70**: 5229–5237.

- Pearson, A., Pi, Y.D., Zhao, W.D., Li, W.J., Li, Y.L., Inskeep, W., *et al.* (2008) Factors controlling the distribution of archaeal tetraethers in terrestrial hot springs. *Appl Environ Microbiol* **74**: 3523–3532.
- Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., *et al.* (2012) Revised calibration of the MBT–CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochim Cosmochim Acta* **96**: 215–229.
- Petsch, S.T., Eglington, T.I., and Edwards, K.J. (2001) 14C-dead living biomass: evidence for microbial assimilation of ancient organic carbon during shale weathering. *Science* **292**: 1127–1131.
- Pitcher, A., Rychlik, N., Hopmans, E.C., Spieck, E., Rijpstra, W.I., Ossebaer, J., *et al.* (2010) Crenarchaeol dominates the membrane lipids of *Candidatus Nitrososphaera gargensis*, a thermophilic group I.1b Archaeon. *ISME J* **4**: 542–552.
- Pitcher, A., Wuchter, C., Siedenberg, K., Schouten, S., and Sinninghe Damsté, J.S. (2011) Crenarchaeol tracks winter blooms of ammonia-oxidizing Thaumarchaeota in the coastal North Sea. *Limnol Oceanogr* **56**: 2308–2318.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., *et al.* (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.
- Schouten, S., Hopmans, E.C., Schefuß, E., and Sinninghe Damsté, J.S. (2002) Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? *Earth Planet Sci Lett* **204**: 265–274.
- Schouten, S., Wakeham, S.G., Hopmans, E.C., and Sinninghe Damsté, J.S. (2003) Biogeochemical evidence that thermophilic archaea mediate the anaerobic oxidation of methane. *Appl Environ Microbiol* **69**: 1680–1686.
- Schouten, S., van der Meer, M.T., Hopmans, E.C., Rijpstra, W.I., Reysenbach, A.L., Ward, D.M., *et al.* (2007) Archaeal and bacterial glycerol dialkyl glycerol tetraether lipids in hot springs of Yellowstone National Park. *Appl Environ Microbiol* **73**: 6181–6191.
- Schouten, S., Hopmans, E.C., Baas, M., Boumann, H., Standfest, S., Konneke, M., *et al.* (2008) Intact membrane lipids of ‘*Candidatus Nitrosopumilus maritimus*,’ a cultivated representative of the cosmopolitan mesophilic group I Crenarchaeota. *Appl Environ Microbiol* **74**: 2433–2440.
- Shimada, H., Nemoto, N., Shida, Y., Oshima, T., and Yamagishi, A. (2008) Effects of pH and temperature on the composition of polar lipids in *Thermoplasma acidophilum* HO-62. *J Bacteriol* **190**: 5404–5411.
- Song, Z.Q., Chen, J.Q., Jiang, H.C., Zhou, E.M., Tang, S.K., Zhi, X.Y., *et al.* (2010) Diversity of Crenarchaeota in terrestrial hot springs in Tengchong, China. *Extremophiles* **14**: 287–296.
- Song, Z.Q., Wang, F.P., Zhi, X.Y., Chen, J.Q., Zhou, E.M., Liang, F., *et al.* (2013) Bacterial and archaeal diversities in Yunnan and Tibetan hot springs, China. *Environ Microbiol* **15**: 1160–1175.
- Sorensen, K.B., Canfield, D.E., Teske, A.P., and Oren, A. (2005) Community composition of a hypersaline endoevaporitic microbial mat. *Appl Environ Microbiol* **71**: 7352–7365.
- Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., and Hinrichs, K.U. (2004) Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry–new biomarkers for biogeochemistry and microbial ecology. *Rapid Commun Mass Spectrom* **18**: 617–628.
- Takai, K., and Sako, Y. (1999) A molecular view of archaeal diversity in marine and terrestrial hot water environments. *FEMS Microbiol Ecol* **28**: 177–188.
- Takayanagi, S., Kawasaki, H., Sugimori, K., Yamada, T., Sugai, A., Ito, T., *et al.* (1996) *Sulfolobus hakonensis* sp. nov., a novel species of acidothermophilic archaeon. *Int J Syst Bacteriol* **46**: 377–382.
- Ter Braak, C., and Smilauer, P. (2002) *Canoco for Windows Version 4.5*. Wageningen, the Netherlands: Biometris-Plant Research International.
- Thurl, S., and Schäfer, W. (1988) Lipids from the sulphur-dependent archaebacterium *Thermoproteus tenax*. *Biochim Biophys Acta* **961**: 253–261.
- Tong, W., and Zhang, M. (1989) *Tengchong Geothermics*. Beijing, China: Science Press.
- Trincone, A., Nicolaus, B., Palmieri, G., De Rosa, M., Huber, R., Huber, G., *et al.* (1992) Distribution of complex and core lipids within new hyperthermophilic members of the archaea domain. *Syst Appl Microbiol* **15**: 11–17.
- Uda, I., Sugai, A., Itoh, Y.H., and Itoh, T. (2001) Variation in molecular species of polar lipids from *Thermoplasma acidophilum* depends on growth temperature. *Lipids* **36**: 103–105.
- Uda, I., Sugai, A., Itoh, Y.H., and Itoh, T. (2004) Variation in molecular species of core lipids from the order *Thermoplasmatales* strains depends on the growth temperature. *J Oleo Sci* **53**: 399–404.
- Valentine, D.L. (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nat Rev Microbiol* **5**: 316–323.
- van de Vossenberg, J.L., Driessen, A.J., and Konings, W.N. (1998) The essence of being extremophilic: the role of the unique archaeal membrane lipids. *Extremophiles* **2**: 163–170.
- Völkl, P., Huber, R., Drobner, E., Rachel, R., Burggraf, S., Trincone, A., *et al.* (1993) *Pyrobaculum aerophilum* sp. nov., a novel nitrate-reducing hyperthermophilic archaeum. *Appl Environ Microbiol* **59**: 2918–2926.
- Wang, F., Peng, Z.C., Chen, W.J., Wang, Z.R., Yang, J.W., Zhang, Z.L., *et al.* (2000) High-precision thermal ionization mass spectrometry dating of young volcanic rocks by using U-series method. *Chin Sci Bull* **45**: 83–87.
- Wang, S., Hou, W.G., Dong, H.L., Jiang, H.C., Huang, L.Q., Wu, G., *et al.* (2013) Control of temperature on microbial community structure in hot springs of the Tibetan Plateau. *PLoS ONE* **8**: e62901.
- Wei, Y.L., Wang, J.X., Liu, J., Dong, L., Li, L., Wang, H., *et al.* (2011) Spatial variations in archaeal lipids of surface water and core-top sediments in the South China Sea: implications for paleoclimate studies. *Appl Environ Microbiol* **77**: 7479–7489.

- White, D., Davis, W., Nickels, J., King, J., and Bobbie, R. (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**: 51–62.
- Woese, C.R., Kandler, O., and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* **87**: 4576–4579.
- Wu, W.Y., Zhang, C.L., Wang, H.Y., He, L., Li, W.J., and Dong, H.L. (2013) Impacts of temperature and pH on the distribution of archaeal lipids in Yunnan hot springs, China. *Front Microbiol* **4**: 312. doi:10.3389/fmicb.2013.00312.
- Zhang, C.L., Pearson, A., Li, Y.L., Mills, G., and Wiegel, J. (2006) Thermophilic temperature optimum for crenarchaeol synthesis and its implication for archaeal evolution. *Appl Environ Microbiol* **72**: 4419–4422.
- Zhang, C.L., Ye, Q., Huang, Z.Y., Li, W.J., Chen, J.Q., Song, Z.Q., *et al.* (2008) Global occurrence of archaeal *amoA* genes in terrestrial hot springs. *Appl Environ Microbiol* **74**: 6417–6426.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Structures of GDGTs presented in the terrestrial geothermal springs and the surrounding soil. GTGT-0 is glycerol trialkyl glycerol tetraether without any cyclopentyl ring.

Fig. S2. Correlations of abundances of P-GDGTs and C-GDGTs (in log values) from Tengchong hot spring sediments and surrounding soils in June 2011.

Fig. S3. Comparison of concentrations of archaeol and isoprenoid lipids from hot springs and surrounding soils in June 2011.

Fig. S4. Cluster analysis of C-GDGTs from Tengchong hot spring sediments in January 2011, June 2011 and July 2012. Sample names are shown on the right of the figure; GDGTs are colour coded and shown at the bottom of the figure. Samples are clustered into three groups: group 1 (pH = 3.0 ± 0.7 , $T = 70.7 \pm 15.2^\circ\text{C}$; $n = 14$), group 2 (pH = 7.5 ± 0.8 , $T = 68.1 \pm 15.2^\circ\text{C}$; $n = 8$) and group 3

(pH = 8.5 ± 1.0 , $T = 79.0 \pm 11.5^\circ\text{C}$; $n = 24$). Samples in corresponding groups are boxed with dash lines.

Fig. S5. Difference (in %) between P-GDGTs and corresponding C-GDGTs from Tengchong hot spring samples having measurable dissolved oxygen (DO) concentrations. Sample names are shown on the right, followed by corresponding dissolved oxygen ($\mu\text{g l}^{-1}$).

Fig. S6. RDA ordination plots for the first two principal dimensions of the relationship between the distribution of C-GDGTs and relevant environmental parameters from Tengchong in January 2011, June 2011 and July 2012. Correlations between environmental variables and RDA axes are represented by the length and angle of dash arrows (environmental factor vectors). Solid arrows stand for the proportion of P-GDGTs.

Fig. S7. Cluster analysis based on archaeal phylogenetic composition of Tengchong hot springs in January 2011 at the rank of order. MCG: miscellaneous crenarchaeotal group. Sample names are shown on the right of the figure. Samples are clustered into three groups that correspond to pH: group 1 (pH = 3.1 ± 1.0 , $T = 73.9 \pm 16.8^\circ\text{C}$; $n = 4$), group 2 (pH = 8.8 ± 0.8 , $T = 85.6 \pm 5.8^\circ\text{C}$; $n = 8$) and group 3 (pH = 7.0 ± 0.4 , $T = 77.1 \pm 5.7^\circ\text{C}$; $n = 2$). Groups are delineated by dashed lines.

Fig. S8. Regression plots for temperature and P- or C-RI in group 2 from Tengchong hot springs in January 2011 [see Fig. S8; *Desulfurococcales*- and *Thermoproteales*-dominated group; P- or C-RI of *SrbzU* and *SrbzD* did not fit the equation, which might be because *Srbz* pool was sourced by surface run-off (Hou *et al.*, 2013), which may deliver more soil contamination than other hot springs].

Table S1. Lipid and chemistry data for isoprenoidal GDGTs from Tengchong hot springs in January 2011, June 2011, July 2012 and US Great Basin hot spring in October 2010.

Table S2. Composition and relative abundance (%) of Archaea and distribution of P-GDGTs from Tengchong hot springs in January 2011, YNP hot springs in June through August of 2008, Tibet hot springs in June 2010 and US Great Basin hot springs in October 2010.

Table S3. The non-parametric correlation results of archaeal composition, P-GDGTs (relative abundance) and environmental variables from Tengchong hot springs in January 2011, YNP hot springs in June through August of 2008, Tibet hot springs in June 2010 and US Great Basin hot springs in October 2010.