INTRODUCTION

Isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) as essential membrane lipids of Archaea widely occur in natural environments (Schoenen et al., 2013 and references therein). They can contain up to eight cyclopentyl moieties (Trincone et al., 1988; Schoenen et al., 2000, 2007). GDGTs with fewer than six cyclopentyl rings (except for GDGT-4) are ubiquitous and often abundant in low temperature environments (see reviews by Pearson and Ingalls, 2013; Schoenen et al., 2013). However, GDGTs with five to eight cyclopentyl moieties are mainly found in geothermal environments (Schoenen et al., 2007; Boyd et al., 2013; Li et al., 2013). In addition to these GDGTs, a structurally unusual GDGT, thaumarchaeol that contains one cyclohexyl ring in addition to four cyclopentyl rings is also widespread (Pearson et al., 2008; He et al., 2013; Pitcher et al., 2009a; He et al., 2012; Boyd et al., 2013; Li et al., 2013).

Proxies based on GDGT distribution have been shown to correlate with physical, chemical, or biological parameters. Studies of thermophilic cultures showed that the number of rings (ring index, RI) increased with increasing temperature (Uda et al., 2001; Shimada et al., 2008; Boyd et al., 2011) and decreased (Shimada et al., 2008) or increased (Boyd et al., 2011) with decreasing pH. In normal marine sediments thaumarchaeol and its isomer are predominantly from deposition of planktonic Thaumarchaeota, whereas in gas hydrate- or cold seep-dominated environments, methanotrophic Archaea performing anaerobic oxidation of methane constitute the majority of the archaeal community, which synthesizes mostly GDGTs-1, -2, and -3 but not thaumarchaeol and its isomer. Based on the relative abundances of GDGTs-1, -2, and -3 and thaumarchaeol and its isomer, a methane index (MI) was developed as an indicator for anaerobic oxidation of methane associated with destabilization of gas hydrates (Zhang et al., 2011). Furthermore, the ratio of thaumarchaeol (Thaum) to GDGT-0 (caldarchaeol) correlated with the relative abundance of Thaumarchaeota to Euryarchaeota in natural settings (Zhang et al., 2006; Turich et al., 2007; Yang et al., 2010), which is consistent with the notion that thaumarchaeol is the biomarker for Thaumarchaeota and GDGT-0 the major component of euryarchaeotal membrane lipids (Schouten et al., 2007).

In geothermal springs of Yellowstone, Great Basin, Kamchatka, and Tibet, multiple factors vary simultaneously, and GDGT distribution shows various correlations with environmental or biological parameters in different regions (Pearson et al., 2008; He et al., 2012; Boyd et al., 2013; Li et al., 2013; Paraiso et al., 2013). The goal of this study was to determine how GDGT distribution might be affected by multiple parameters such as temperature, pH, and nutrients in Yunnan hot springs. Cluster analysis was
performed to show the patterns of GDGT distribution in these hot springs, which resulted in two major groups (Group 1 and Group 2). Regression analysis showed that these two groups had distinct responses to temperature and pH, suggesting that impacts of environmental variables on the distribution of GDGTs need to be evaluated in the context of lipid classification.

MATERIALS AND METHODS

FIELD SAMPLING

Twenty-six samples were collected from hot springs in Yunnan, China. Ten of them were from Rehai geothermal field in Tengchong (TC), nine from Longling (LL), six from Eryuan (EY), and one from Anning (AN; Figure 1). Temperature and pH were determined using a portable thermometer and pH meter, respectively. The concentrations of ammonium (NH$_4^+$), nitrite (NO$_2^-$), nitrate (NO$_3^-$), sulfide (S$_2^-$), sulfate (SO$_4^{2-}$), and ferrous iron (Fe$^{2+}$) were measured in the field by portable Hach kits according to the manufacturer’s instructions (Hach Company, Loveland, CO, USA).

Microbial mats and surface sediments were sampled by a hand scoop, which was pre-sterilized with 75% ethanol and dried before each new sample was taken. Samples were collected into sterile 50 ml Falcon tubes, immediately preserved in liquid nitrogen in the field, and stored at −80°C in the laboratory until further analysis.

ARCHAEAL LIPID EXTRACTION

Samples were freeze-dried and ground to fine powder. Approximately 5 g of each powder sample was used to extract lipids according to a modified Bligh–Dyer technique (Bligh and Dyer, 1959; Zhang et al., 2012). A single phase solvent mixture (2:1:0.8, v/v/v) of MeOH, dichloromethane (DCM) and phosphate buffer (pH 7.4) was added to the sample in a centrifuge tube. The sample was sonicated (15 min) and then centrifuged (5 min, 2500 rpm). The extract was collected into a labeled glass tube. This procedure was repeated twice. DCM and phosphate buffer were added to the combined extract at 1:1:0.9 (v/v/v), achieving phase separation. The bottom DCM phase was collected into a 40 ml glass tube. The residue was rinsed with DCM (twice) and the DCM
analysis was performed according to Wei et al. (2011) using an Agilent 6460 HPLC joined to a MS. Separation was achieved on Alltech Prevail Cyan column (150 × 2.1 mm, 3 μm) maintained at 90 °C. A 5-μl volume of each sample was injected. GDGTs were eluted isocratically with 99% hexane and 1% isopropanol for 5 min, followed by a gradient to 1.8% isopropanol for 45 min at a flow rate of 0.2 ml/min. The column was cleaned after each analysis by back-flushing hexane-propanol (90:10, v/v) for 10 min. The relative abundances of GDGTs were quantified by integration of the peak areas from extracted single-ion chromatograms and comparison with the peak area of C40. IS. The abundance of GDGT-4 was obtained after accounting for the isotope effect by thaumarchaeol on the peak area of GDGT-4 (He et al., 2012).

**STATISTICAL ANALYSIS**

Cluster analysis was performed using the R program according to Li et al. (2013). The relative abundance of GDGTs was used to construct Euclidean distance matrices describing the dissimilarity of the lipid distributions between samples, generating a hierarchical clustering tree (Turich et al., 2007; Pearson et al., 2008; Li et al., 2013). To measure the robustness of the clustering results, principal component analysis was also performed using CANOCO for Windows version 4.5. Regression analysis and bivariate correlation analysis were conducted using the SPSS software to evaluate the relationships of GDGTs with environmental parameters. A significant correlation was defined when $P < 0.05$.

**CALCULATIONS OF RI AND MI**

RI was calculated following the equation modified from Schouten et al. (2007). MI was calculated by the equation defined by Zhang et al. (2011).

$$RI = \frac{GDGT-1 + 2 \times GDGT-2 + 3 \times GDGT-3 + 4 \times GDGT-4 + 5 \times GDGT-5 + 6 \times GDGT-6}{100}$$

$$MI = \frac{GDGT-1 + GDGT-2 + GDGT-3}{GDGT-1 + GDGT-2 + GDGT-3 + \text{Thaum} + \text{Thaum isomer}}$$

**RESULTS**

**WATER CHEMISTRY**

Hot springs in Yunnan, China, exhibited diverse physical and geochemical conditions (Table 1). For example, temperature ranged from 39.0 to 94.0 °C and pH from 2.35 to 9.11. The concentration of sulfate varied from 5.0 to 500.0 mg/L and was below 2000 mg/L in most springs. The concentrations of ammonium, nitrite and nitrate were below 0.34 mg/L, 0.17 mg/L, and 0.69 mg/L, respectively. The concentration of ferrous iron was no more than 1.00 mg/L with the exception of TC-2 (17.00 mg/L). The concentration of sulfuric acid was no more than 4.80 mg/L. Overall, these hot springs were characterized as low inorganic energy sources (Zhang et al., 2008).

**GDGT CONCENTRATIONS AND GDGT-BASED PROXIES**

GDGT-0 to GDGT-6, thaumarchaeol (Thaum) and its regioisomer (Thaum.isomer; Figure 2) were detected in Yunnan hot springs (Table 2). The concentration of the total GDGTs ranged from 1.43 to 993.83 ng/g, the most abundant GDGTs occurred in TC-5 and the least abundant in EY-3 (Table 2). The values of RI ranged from 0.14 to 3.99, those of Thaum/(Thaum + GDGT-0) from 0 to 0.89, and those of MI from 0.14 to 1.0 (Table 3).

**CLUSTER ANALYSIS**

Cluster analysis was conducted to examine the patterns of GDGT distribution. The results from R clustering method using Euclidean distance as the metric showed that all samples fell into two major groups (Group 1 and Group 2; Figure 3). The samples within Group 1 were primarily from Tengchong plus some samples from Longling. It was further divided into two subgroups (Group 1.1 and Group 1.2); Group 1.1 was distinguished from Group 1.2 by relatively more abundant GDGT-3 and GDGT-4. The samples within Group 2 were exclusively from Eryuan plus the remaining samples that were not found in Group 1. Group 2 was also divided into two subgroups (Group 2.1 and Group 2.2); Group 2.1 was defined by the dominance of GDGT-0 and Group 2.2 was characterized by the dominance of thaumarchaeol. The results were consistent with those from principle component analysis method using CANOCO for Windows version 4.5 (Figure A1 in Appendix).

**CORRELATIONS OF INDIVIDUAL GDGTs AND ORGANIC PROXIES WITH ENVIRONMENTAL VARIABLES**

For total samples, correlations of individual GDGTs or organic proxies with environmental variables were examined. No significant correlations existed between individual GDGTs and any environmental variables (data not shown). However, some correlations were found between the organic proxies and temperature (Figure 4). Thaum/(Thaum + GDGT-0) showed a negative correlation with temperature (Figure 4A). MI ($R^2 = 0.40, P = 0.008$; Figure 4B) and RI ($R^2 = 0.34, P = 0.002$; Figure 4C) showed positive correlations...
with temperature. Significant correlations of these proxies with other factors such as pH and nutrients were not observed (data not shown).

When samples were divided into Group 1 and Group 2, temperature was the dominant factor for Group 1, and pH was the significant factor for Group 2 (Figure 5). In Group 1, Thaum/(Thaum + GDGT-0) negatively correlated with temperature ($R^2 = 0.31, P = 0.032$; Figure 5A) and MI positively correlated with temperature ($R^2 = 0.52, P = 0.002$; Figure 5B); the results were similar to the observation of the total samples (Figures 4A,B). In Group 2, Thaum/(Thaum + GDGT-0) negatively correlated with pH ($R^2 = 0.37, P = 0.044$, Figure 5C). In either group, correlations between nutrients and the organic proxies were insignificant (data not shown).

### DISCUSSION

**ORIGIN AND DISTRIBUTION OF ARCHAEAL GDGTs FROM CLUSTER-GENERATED GROUPS**

Recent studies of hot springs in Tibet (He et al., 2012; Li et al., 2013), and Great Basin (Pearson et al., 2008; Paraiso et al., 2013) revealed that GDGTs from these hot springs were primarily produced autotrophically and not the result of soil contamination. Although soils surrounding Yunnan hot springs were not sampled in this study, they were collected in later field trips (Xie et al., unpublished), and showed an obvious difference in GDGT distribution from geothermal springs (Figure A2 in Appendix), suggesting that GDGTs in the hot springs are dominated by those

## Table 1 | Location and water chemistry of Yunnan hot springs.

| Sample | GPS | pH | $T$ ($^\circ$C) | $SO_4^{2-}$ (mg/L) | $NO_3^-$ (mg/L) | $NO_2^-$ (mg/L) | $NH_4^+$ (mg/L) | $Fe^{3+}$ (mg/L) | $S^{2-}$ (mg/L) |
|--------|-----|----|---------------|-------------------|-----------------|----------------|----------------|----------------|----------------|
| TC-1b  | N 24°57’12.7” | E 98°26’17.4” | 784 | 84.0 | 300.0 | 0.05 | 0.51 | 0.02 | BDL | 0.31 |
| TC-2b  | N 24°57’12.7” | E 98°26’17.4” | 2.35 | 870 | 500.0 | 0.03 | 0.06 | 1.18 | 1700 | 0.21 |
| TC-3b  | N 24°57’12.8” | E 98°26’17.5” | 9.00 | 94.0 | 6.5 | 0.04 | 0.56 | 0.01 | BDL | 4.80 |
| TC-4  | N 24°57’12.9” | E 98°26’17.6” | 9.00 | 94.0 | 6.5 | 0.04 | 0.56 | 0.01 | BDL | 4.80 |
| TC-5b  | N 24°57’12.10” | E 98°26’17.7” | 3.50 | 89.5 | 70.0 | 0.03 | 0.19 | 0.34 | 1.0 | 0.04 |
| TC-6  | N 24°57’12.11” | E 98°26’17.8” | 8.80 | 89.0 | 6.0 | 0.04 | 0.69 | BDL | BDL | 3.40 |
| TC-7  | N 24°57’12.15” | E 98°26’17.7” | 6.78 | 78.0 | 45.0 | 0.14 | 0.41 | BDL | 0.06 | 0.10 |
| TC-8  | N 24°57’15.6” | E 98°26’15.7” | 6.69 | 64.8 | 33.0 | 0.16 | 0.47 | BDL | 0.04 | BDL |
| TC-9  | N 24°57’15.8” | E 98°26’15.8” | 762 | 52.2 | BDL | BDL | BDL | BDL | BDL |
| TC-10 | N 24°57’15.9” | E 98°26’15.9” | 4.50 | 72.0 | 12.0 | 0.05 | 0.47 | BDL | 0.02 | BDL |
| LL-1  | N 24°39’37.2” | E 98°43’11.9” | 6.78 | 56.7 | 15.0 | 0.01 | 0.03 | BDL | 0.01 | BDL |
| LL-2  | N 24°39’37.3” | E 98°43’11.10” | 707 | 44.5 | 29.0 | 0.01 | BDL | 0.03 | BDL | 0.02 |
| LL-3  | N 24°39’37.3” | E 98°43’10.4” | 6.30 | 60.0 | 40.0 | 0.00 | BDL | BDL | BDL | BDL |
| LL-4  | N 24°39’37.4” | E 98°40’03.5” | 6.30 | 60.0 | 40.0 | 0.00 | BDL | BDL | BDL | BDL |
| LL-5  | N 24°39’37.5” | E 98°40’03.6” | 8.22 | 86.0 | 46.0 | 0.01 | 0.03 | BDL | BDL | 0.05 |
| LL-6  | N 24°39’37.6” | E 98°40’03.7” | 9.11 | 82.0 | 42.0 | 0.01 | 0.02 | BDL | 0.02 | BDL |
| LL-7  | N 24°39’37.7” | E 98°40’03.8” | 6.67 | 55.0 | 47.0 | 0.01 | BDL | BDL | 0.05 | BDL |
| LL-8  | N 24°39’37.8” | E 98°40’03.9” | 6.79 | 39.0 | 72.0 | 0.01 | BDL | BDL | 0.03 | BDL |
| LL-9  | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-10 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-11 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-12 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-13 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-14 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-15 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-16 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-17 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-18 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-19 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-20 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
| LL-21 | N 24°39’37.9” | E 98°40’03.9” | 8.01 | 50.0 | 100.0 | 0.07 | BDL | BDL | 0.03 | BDL |
produced in situ rather than contamination from surrounding soils.

Studies based on pure cultures of thermophilic Archaea showed that the GDGT composition varies from species to species (Macalady et al., 2004; Schouten et al., 2007; de la Torre et al., 2008; Boyd et al., 2011). GDGTs with 0–4 cyclopentyl rings are likely derived from thermophilic Crenarchaeota and Euryarchaeota (Schouten et al., 2007, 2013). GDGT-5 and GDGT-6 are produced predominantly by the orders Sulfolobales and Thermoplasmatales, which optimally grow at low pH (Schouten et al., 2007, 2013).
Indeed, GDGT-5 and GDGT-6 occurred in acidic hot springs of Tengchong with abundant Sulfolobales and Thermoplasmatales (Hou et al., 2013), such as TC-2 and TC-5 in Group 1 (Table 2; Figure 3). Similar observations were made in Yellowstone (Pearson et al., 2008; Boyd et al., 2013), Kamchatka (Pearson et al., 2008), and Tibet (He et al., 2012; Li et al., 2013). Moreover, all retrieved archaeal species from TC-2 (Hou et al., 2013) and TC-5 (Song et al., 2010) were grouped into the Sulfolobales order. In summary, the distribution of archaeal membrane lipids corroborated 16S rRNA gene sequence data to show that Archaea in Group 1 hot springs were dominated by thermophilic Crenarchaeota and Euryarchaeota.

GDGT-0 has been identified in Euryarchaeota, Crenarchaeota, as well as Thaumarchaeota (Brochier-Armanet et al., 2008; de la Torre et al., 2008; Spang et al., 2010). Archaeal cultivation studies showed that Euryarchaeota with the exception of Thermoplasmatales, DHVE-2 cluster, Thermococcus aggregans, Methanopyrus kandleri, and methanotrophic ANME-1, predominantly produce GDGT-0 (Schouten et al., 2013). GDGTs from samples in Group 2.1 exhibited a dominance of GDGT-0 (Figure 3), implicating that Archaea in this subgroup may be dominated by Euryarchaeota. The presence of GDGTs with cyclopentyl moieties other than GDGT-0 (Figure 3) indicated that other Archaea may be present as a minor component of the archaeal community in these hot springs.

Thaumarchaeol, originally called crenarchaeol, was considered a biomarker for mesophilic Crenarchaeota (Schouten et al., 2002, 2003; Sinninghe Damsté et al., 2002); however, the lineage linked to this group of species remains to be determined. The occurrence of Thaumarchaeol in a few samples with high GDGT-0 contents suggests a minor contribution of this archaeal lineage to these hot springs.

### Table 2 | Relative abundances of GDGTs for core lipids from Yunnan hot springs.

| Samplea | Total lipids (ng/g) | % GDGTs | GDGT0 | GDGT-1 | GDGT-2 | GDGT-3 | GDGT-4 | Thaum. | Thaum. isomer | GDGT-5 | GDGT-6 |
|---------|---------------------|---------|-------|--------|--------|--------|--------|--------|-----------|--------|--------|
| TC-1b   | 331.5               | 36      | 20    | 15     | 16     | 13     | 0      | 0      | 1         | 0      | 0      |
| TC-2b   | 501.7               | 2       | 2     | 6     | 13     | 51     | 0      | 0      | 15        | 12     |        |
| TC-3b   | 62.5                | 5       | 6     | 11    | 23     | 52     | 1      | 0      | 1         | 0      | 1      |
| TC-4    | 15.6                | 14      | 15    | 24    | 16     | 28     | 1      | 0      | 2         | 1      |        |
| TC-5b   | 993.8               | 5       | 5     | 8     | 17     | 37     | 1      | 0      | 19        | 9      |        |
| TC-6    | 706.1               | 34      | 29    | 29    | 2      | 7      | 0      | 0      | 0         | 0      | 0      |
| TC-7    | 2.2                 | 8       | 5     | 11    | 17     | 45     | 2      | 0      | 10        | 1      | 1      |
| TC-8b   | 61.7                | 21      | 9     | 10    | 10     | 26     | 12     | 2      | 7         | 3      |        |
| TC-9    | 119                 | 42      | 4     | 4     | 5      | 17     | 24     | 0      | 3         | 0      |        |
| TC-10   | 276                 | 17      | 25    | 25    | 21     | 10     | 0      | 0      | 1         | 1      |        |
| LL-1    | 18                  | 28      | 7     | 8     | 4      | 2      | 51     | 0      | 0         | 0      |        |
| LL-2a   | 26.4                | 75      | 2     | 1     | 1      | 7      | 14     | 0      | 0         | 0      |        |
| LL-3a   | 193.1               | 33      | 9     | 11    | 15     | 24     | 4      | 1      | 2         | 1      |        |
| LL-4    | 121.6               | 32      | 9     | 10    | 15     | 26     | 3      | 1      | 3         | 1      |        |
| LL-5    | 41.9                | 17      | 9     | 14    | 24     | 40     | 1      | 0      | 1         | 0      |        |
| LL-6    | 192.8               | 7       | 9     | 9     | 14     | 24     | 46     | 1      | 0         | 1      |        |
| LL-7a   | 36.2                | 29      | 4     | 5     | 13     | 31     | 14     | 0      | 3         | 0      |        |
| LL-8    | 28.8                | 14      | 5     | 9     | 12     | 25     | 34     | 0      | 0         | 0      |        |
| LL-9    | 25.7                | 14      | 18    | 20    | 19     | 18     | 10     | 1      | 0         | 0      |        |
| EY-1    | 57.4                | 75      | 2     | 3     | 2      | 0      | 17     | 1      | 0         | 0      |        |
| EY-2    | 82.3                | 69      | 2     | 3     | 1      | 11     | 23     | 0      | 0         | 0      |        |
| EY-3b   | 1.4                 | 71      | 0     | 0     | 0      | 10     | 18     | 0      | 0         | 0      |        |
| EY-4    | 22.5                | 10      | 3     | 6     | 3      | 4      | 65     | 7      | 0         | 1      |        |
| EY-5    | 43.3                | 49      | 3     | 3     | 2      | 3      | 35     | 4      | 0         | 0      |        |
| EY-6    | 68.4                | 73      | 2     | 2     | 1      | 8      | 15     | 0      | 0         | 0      |        |
| AN-1    | 16.1                | 7       | 8     | 0     | 2      | 27     | 56     | 0      | 0         | 0      |        |

* Samples a and b: Dagunguo, TC-2; Diretiyanqu, TC-3; Yanjingquan, TC-4; Yanjingquan, TC-6; Zhenzhuquan, TC-5; Dawumingquan, TC-7; Gumingquan, TC-8; Wumingxiaoxizhongyou, TC-9; Wumingxiaoxizhongyou, TC-10; Hamazui, TC-9; Dahebianzhongyou, LL-1; Dahebianzaotang, LL-2; Banglazhangshangxiao #1, LL-3; Banglazhangshangxiao #2, LL-4; Banglazhangshangxiao #3, LL-5; Banglazhangshangxiao #4, LL-6; Niujieyongpingzaotang, EY-3; Xiashankouchitan, EY-4; Xiaoxiaqiangxia, EY-5; Shibeicunlaizitang #1, EY-6; Shibeicunlaizitang #2, AN-1; Tianxiadiyitang.

b Chemistry that has been reported in Jiang et al. (2010).

cGDGT-0 has been identified in Euryarchaeota, Crenarchaeota, as well as Thaumarchaeota (Brochier-Armanet et al., 2008; de la Torre et al., 2008; Spang et al., 2010). Archaeal cultivation studies showed that Euryarchaeota with the exception of Thermoplasmatales, DHVE-2 cluster, Thermococcus aggregans, Methanopyrus kandleri, and methanotrophic ANME-1, predominantly produce GDGT-0 (Schouten et al., 2013). GDGTs from samples in Group 2.1 exhibited a dominance of GDGT-0 (Figure 3), implicating that Archaea in this subgroup may be dominated by Euryarchaeota. The presence of GDGTs with cyclopentyl moieties other than GDGT-0 (Figure 3) indicated that other Archaea may be present as a minor component of the archaeal community in these hot springs.

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with this lipid has recently been reclassified as a separate phylum of Archaea called Thaumarchaeota (Broich-Armanet et al., 2008; Pester et al., 2011; Simonneau Damitè et al., 2012). Subsequent studies revealed that thaumarchaeol could also be synthesized in geothermal environments by thermophilic archaea (Pearson et al., 2004). Previous studies showed that amoA genes were present in Yunnan hot springs (Zhang et al., 2008; Jiang et al., 2010; Hedlund et al., 2012) where thaumarchaeol occurred. GDGTs from the hot springs falling in Group 2.2 were dominated by thaumarchaeol, which indicates that Thaumarchaeota and the process of ammonia oxidation might occurred in hot springs belonging to this group (Figure 3).

Thaumarchaeol abundance showed no correlation with the concentration of ammonium in Yunnan hot springs, this may be due to the contamination of hot spring sediments with cre- narcal from surrounding soils adjacent to the springs. Also, ammonium concentration was low (below detection limit) in half of the springs studied (Table 1) and the small size and uneven distribution of ammonium concentrations may have obscured the correlation between ammonium and thaumarchaeol. In addition, ammonia-oxidizing Archaia remove ammonia from their environment and the concentration of ammonia in a spring is dependent on the flux of ammonia, including production/delivery and removal, so it is not surprising that no clear correlation exists.

**FACTORS CONTROLLING GDGT-BASED PROXIES: INSIGHTS ON THE APPLICATION OF PROXIES**

In chemically relatively stable environments like the ocean, GDGT-based proxies have been developed to discern temperature or other factors that control archaeal lipid distribution. However, in terrestrial geothermal springs, dynamic geobiological interactions may result in compounded physical, chemical, and biological influences on GDGT-based proxies.

Recent molecular studies have showed that hot springs in Yunnan harbored diverse archaeal populations (Song et al., 2010; Hedlund et al., 2012; Hou et al., 2013) and that the community structures were influenced by the combination of temperature and pH (Hou et al., 2013). In this study, Thaumarchaeota correlated negatively with temperature in Group 1 (Figure 5A) and negatively with pH in Group 2 (Figure 5C, pH 6–8), suggesting that the relative abundance of Thaumarchaeota to Euryarchaeota was predominantly affected by temperature in Group 1 and by pH in Group 2. The observation in Group 1 is consistent with that of the Great Basin (Zhang et al., 2008), which may indicate a similar archaeal community structure between Group 1 of Yunnan hot springs and hot springs of the Great Basin reported in Zhang et al. (2006). In hydrate-impacted marine sediments, the process of anaerobic methane oxidation can be identified by high MI values (MI > 0.85; Zhang et al., 2011). In this study, MI values of most samples in Group 1 (Figure 5B) surpassed those of hydrate-impacted marine sediments (Zhang et al., 2011). It is unknown, however, whether anaerobic methane oxidation occurred in Yunnan hot springs because of the lack of data on genes associated with methane-oxidizing organisms (e.g., Orphan et al., 2002) or on carbon isotopes of archaeal membrane lipids (e.g., Hinrichs et al., 1996; Zhang et al., 2003, 2011). On the other hand, MI increased with increasing temperature (Figure 4B), which indicates that MI

**Table 3 | Proxies based on GDGTs from Yunnan hot springs.**

| Sample | Thaumarchaeota | Methane index | Ring index |
|--------|----------------|---------------|------------|
| TC-1a  | 0.08           | 0.99          | 1.52       |
| TC-2b  | 0.24           | 0.98          | 3.99       |
| TC-3b  | 0.21           | 0.96          | 3.11       |
| TC-4   | 0.05           | 0.99          | 2.35       |
| TC-5b  | 0.09           | 0.98          | 3.67       |
| TC-6   | 0.00           | 1.00          | 1.17       |
| TC-7   | 0.36           | 0.36          | 2.14       |
| TC-8   | 0.37           | 0.67          | 2.14       |
| TC-9   | 0.36           | 0.36          | 1.12       |
| TC-10  | 0.03           | 0.99          | 1.89       |
| LL-1   | 0.65           | 0.27          | 0.44       |
| LL-2a  | 0.15           | 0.25          | 0.36       |
| LL-3a  | 0.11           | 0.68          | 1.87       |
| LL-4   | 0.08           | 0.69          | 1.97       |
| LL-5   | 0.07           | 0.98          | 2.76       |
| LL-6   | 0.08           | 0.99          | 2.94       |
| LL-7   | 0.33           | 0.61          | 1.92       |
| LL-8   | 0.71           | 0.43          | 1.59       |
| LL-9   | 0.40           | 0.84          | 1.88       |
| EY-1   | 0.19           | 0.27          | 0.14       |
| EY-2   | 0.25           | 0.19          | 0.16       |
| EY-3   | 0.20           | 0.00          | 0.41       |
| EY-4   | 0.86           | 0.14          | 0.49       |
| EY-5   | 0.42           | 0.18          | 0.27       |
| EY-6   | 0.17           | 0.22          | 0.39       |
| AN-1   | 0.83           | 0.15          | 1.23       |

*TC-1, Dagunguo; TC-2, Deyi; TC-3, Yangjingshan; TC-4, Yangjing; TC-5, Wumingpor; TC-6, Wumingshuang; TC-7, Wumingxi; TC-8, Wumingxi; TC-9, Wumingxi; TC-10, Haimao; LL-1, Dahebianzhongyou; LL-2, Dahebianzhong; LL-3, Banglazhangshangxiao #1; LL-4, Banglazhangshangxiao #2; LL-5, Banglazhangshangxiao #3; LL-6, Banglazhangshangxiao #4; LL-7, Banglazhangshangxiao #5; LL-8, Banglazhangshangxiao #6; LL-9, Xiaoxiaqiangxia; EY-1, Yunnanxi; EY-2, Yunnanxi; EY-3, Yunnanxi; EY-4, Yunnanxi; EY-5, Yunnanxi; EY-6, Yunnanxi; AN-1, Yunnanxi.*

*Chemoautotrophic ammonia oxidation (de la Torre et al., 2008).*
FIGURE 3 | Cluster analysis of archaeal lipids in hot spring sediments by R clustering method. Sample names are shown on the right of the figure. GDGTs are color coded and shown at the bottom of the figure: TC-1, Dagunguo; TC-2, Gshtayou; TC-3, Yejingquan; TC-4, Yejingquan; TC-5, Zhenzhuquan; TC-6, Gumingquan; TC-7, Dawumingquan; TC-8, Wumingxaszhangqu; TC-9, Wumingxaszhangqu; TC-10, Hama; LL-1, Dalileibchanqou; LL-2, Dalileibchanqou; LL-3, Banglazhangshangxiao #1; LL-4, Banglazhangshangxiao #1; LL-5, Banglazhangshangxiao #1; LL-6, Banglazhangshangxiao #3; LL-7, Banglazhangshangxiao #6; LL-8, Banglazhangshangxiao #8; LL-9, Xiaoxiaqiangxia; EY-1, Xishankoudaotian; EY-2, Xishankoudaotian; EY-3, Niujieyongpingzaotang; EY-4, Niujieyongpingshougongjing; EY-5, Shibeicunlaizitang #1; EY-6, Shibeicunlaizitang #2; AN-1, Tianxiadiyitang.
FIGURE 4 | Correlations between organic proxies and environmental parameters using total samples. (A) Thaum/(Thaum + GDGT-0) and temperature; (B) MI and temperature; (C) RI and temperature. Thaum, Thaumarchaeol.

FIGURE 5 | Correlations between organic proxies and environmental parameters in two clustering groups according to Figure 3. (A) Thaum/(Thaum + GDGT-0) and temperature in Group 1; (B) MI and temperature in Group 1; (C) Thaum/(Thaum + GDGT-0) and pH in Group 2. Thaum, Thaumarchaeol.
mainly reflects temperature control on GDGT distribution rather than the process of anaerobic methane oxidation in the hot spring environment.

Ring index was found to correlate with temperature or nitrite in subgroups of GDGTs in Tibetan hot springs (Li et al., 2013). In this study, RI did not show any correlation with temperature or other chemical variables in subgroups. In general, GDGTs in Group 2 were dominated by GDGT-0 or thaumarcheol (two compounds that are not counted in the RI calculation), which resulted in RI values in Group 2 being lower than in Group 1.

In addition, weak correlations falling below their confidence threshold were observed between GDGT-based proxies and nutrient concentrations (data not shown), which is in contrast to the results from Boyd et al. (2013) and Li et al. (2013). This is possibly due to the small amount of data used in this study compared to those reported by Boyd et al. (2013) and Li et al. (2013).

In summary, samples with different patterns of GDGT distribution were clustered into two major groups (Group 1 and Group 2). RI and Thaum/(Thaum + GDGT-0) were found to correlate with temperature in Group 1, and only Thaum/(Thaum + GDGT-0) correlated with pH in Group 2. The inconsistency of the relationships of organic proxies with environmental parameters using different samples reminds us to be specific when addressing environmental impacts on lipid distributions. This study also demonstrates that the microbial community structure should be taken into consideration in the application of organic proxies.

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APPENDIX

FIGURE A1 | Clustering results from Yunnan hot springs by principal component analysis. Solid circles with brown, yellow, blue, and red color represent samples from Group 1.1, Group 1.2, Group 2.1, and Group 2.2 in Figure 3, respectively. Thaum, Thaumarchaeol. Thaum., Thaumarchaeol.
FIGURE A2 | Cluster analysis of archaeal lipids from Yunnan hot spring sediments in this study and surrounding soils (Xie et al., unpublished data). Samples named with “S” are from soils surrounding hot springs. TC-1, Diegang; TC-2, Diretiyanqu; TC-3, Yajiangqu; TC-4, Yangynqu; TC-5, Zhenzhuquan; TC-6, Gumingquan; TC-7, Dongzhuquan; TC-8, Wumingxiao; TC-9, Wumingxiqiao; TC-10, Hamazui; LL-1, Shuirebaozao; LL-2, Shuirebaozao #2; LL-3, Banglazhang; LL-4, Banglazhang #1; LL-5, Banglazhang #2; LL-6, Banglazhang #3; LL-7, Banglazhang #4; LL-8, Banglazhang #5; EY-1, Xiashankoudaotian; EY-2, Xiashankoudi; EY-3, Niujieyongpingtang; EY-4, Niujieyongpingtang #1; EY-5, Shuirebaozao #1; EY-6, Shuirebaozao #2; EY-7, Shuirebaozao #3; EY-8, Shuirebaozao #4; EY-9, Shuirebaozao #5; EY-10, Shuirebaozao #6; EY-11, Shuirebaozao #7; EY-12, Shuirebaozao #8; EY-13, Shuirebaozao #9; AN-1, Tianxian; Srbz 1, Shuirebaozao #1; Srbz 2, Shuirebaozao #2; Zzqr, Zhenzhuquan right; DrtyC, Diretiyan #C; DrtyD, Diretiyan #D; DrtyF, Diretiyan #F; DrtyG, Diretiyan #G; Jmqr, Jiemeiquan right; Gmqd, Gumingquan down; Jz, Jieming; Gxs, Gongxiaoshe.