Greater temporal changes of sediment microbial community than its waterborne counterpart in Tengchong hot springs, Yunnan Province, China

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Temporal variation in geochemistry can cause changes in microbial community structure and diversity. Here we studied temporal changes of microbial communities in Tengchong hot springs of Yunnan Province, China in response to geochemical variations by using microbial and geochemical data collected in January, June and August of 2011. Greater temporal variations were observed in individual taxa than at the whole community structure level. Water and sediment communities exhibited different temporal variation patterns. Water communities were largely stable across three sampling times and dominated by similar microbial lineages: Hydrogenobaculum in moderate-temperature acidic springs, Sulfolobus in high-temperature acidic springs, and Hydrogenobacter in high-temperature circumneutral to alkaline springs. Sediment communities were more diverse and responsive to changing physicochemical conditions. Most of the sediment communities in January and June were similar to those in waters. However, the August sediment community was more diverse and contained more anaerobic heterotrophs than the January and June: Desulfurella and Acidicaldus in moderate-temperature acidic springs, Ignisphaera and Desulfurococcus in high-temperature acidic springs, the candidate division OP1 and Fervidobacterium in alkaline springs, and Thermus and GAL35 in neutral springs. Temporal variations in physicochemical parameters including temperature, pH, and dissolved organic carbon may have triggered the observed microbial community shifts.

Spatial censuses of microbial community structure have been well-studied in diverse hot springs1–8 and these studies have shown that physicochemical parameters of hot springs such as temperature, pH and nutrient supply are important drivers for shaping microbial diversity and community structure as well as metabolism9–16. In contrast, only a limited number of studies in the last decade have explored temporal changes in microbial communities by using microbial and geochemical data collected in January, June and August of 2011. Greater temporal variations were observed in individual taxa than at the whole community structure level. Water and sediment communities exhibited different temporal variation patterns. Water communities were largely stable across three sampling times and dominated by similar microbial lineages: Hydrogenobaculum in moderate-temperature acidic springs, Sulfolobus in high-temperature acidic springs, and Hydrogenobacter in high-temperature circumneutral to alkaline springs. Sediment communities were more diverse and responsive to changing physicochemical conditions. Most of the sediment communities in January and June were similar to those in waters. However, the August sediment community was more diverse and contained more anaerobic heterotrophs than the January and June: Desulfurella and Acidicaldus in moderate-temperature acidic springs, Ignisphaera and Desulfurococcus in high-temperature acidic springs, the candidate division OP1 and Fervidobacterium in alkaline springs, and Thermus and GAL35 in neutral springs. Temporal variations in physicochemical parameters including temperature, pH, and dissolved organic carbon may have triggered the observed microbial community shifts.
The hot springs in Tengchong County, Yunnan Province of China, are located in a subtropical area with heavy temporal monsoon rainfall (rainy season May-October), and these springs represent a wide range of microbial niches for highly diverse Achaetes, Bacteria, and viruses. Rehai and Diantan (formally Ruidian) are two main geothermal areas within the Tengchong geothermal system. A previous study showed that Rehai springs contained similar sediment and water communities in winter, while Diantan sediment communities harbored very different communities from those in water. Despite these results, little is known about how microbial community structure changes over time in these springs, and if this change is similar between water and sediment. The effects of environmental change on microbial community study are, to some extent, dependent on the community of interest; in other words, a subset of a community and the entire community structure may respond to different environmental parameters. For example, a previous study showed that significant seasonal patterns were observed for some individual species but not for the entire community structure. In the Tengchong hot springs, dominant species were similar between sediment and water in winter season, but no systematic studies have been performed to compare water and sediment communities in response to temporal geochemical changes. Hence, it is imperative to compare and contrast temporal variations in water and sediment microbial diversity and community structure in Tengchong hot springs, at different taxonomic levels (whole community and individual microbial lineages).

Therefore, temporal changes of microbial communities across three sampling time points in the Tengchong hot springs were studied by using high-throughput sequencing of 16S rRNA genes integrated with extensive biochemical analyses. The goals of this study were: (1) determine the temporal variability of microbial lineages in individual hot springs in correlation with geochemical conditions; and (2) compare the response patterns between sediment and water communities to temporal variations in geochemistry.

**Methods**

**Sampling sites description.** Three field trips were made to Tengchong, Yunnan Province, China in 2011: one in the dry season (January) and two in the rainy season (June and August). A total of nine springs across a range of pH and temperature were studied by using high-throughput sequencing of 16S rRNA genes integrated with extensive biochemical analyses. The goals of this study were to: (1) determine the temporal variability of microbial lineages in individual hot springs in correlation with geochemical conditions; and (2) compare the response patterns between sediment and water communities to temporal variations in geochemistry.

**Field measurements and sample collection.** Concentrations of ammonium (NH$_4^+$), total sulfate (SO$_4^{2-}$), ferrous iron (Fe$^{2+}$), nitrate (NO$_3^-$), and nitrite (NO$_2^-$) were measured in the field with spectrophotometric Hach kits (Hach Chemical Co., IA, USA). Water samples for laboratory measurements, e.g., cations, anions, dissolved organic carbon (DOC), and total nitrogen (TN), were collected by filtration of spring water through 0.45 μm Whatman GF/F filter. Water samples for DOC and cations were acidified and stored at 4°C. Depending on spring turbidity, various volumes of water were filtered through the 0.22 μm syringe polyethersulfone (PES) membrane filters (Pall Corp., NY) to collect biomass. Sediment samples were collected with sterile spatulas and spoons, and homogenized in a pre-sterilized aluminum pan and then placed into 50 mL sterilized Teflon tubes. Sediment samples for geochemical analyses, including total organic carbon (TOC) and mineralogy, were stored on ice until analyses.

**DNA extraction and pyrosequencing.** DNA was extracted from biomass-containing filters or 0.5 g sediment samples using the FastDNA SPIN Kit for Soil (MP Biomedical, OH, USA). The extracted DNA was amplified using a universal modified primer set 5′-GTGYCAGCMGCCGCGGTAA-3′ (E2) and 5′-GACGGGCGGTGTGTRCA-3′ as previously described. Unique 8-bp barcodes for each sample were added at the 5′-end of both the forward and reverse primers to demultiplex sequences. Each 25 μl PCR system contained 10 ng template DNA, 1× PCR buffer, 400 nM each primer, 200 μM dNTPs, and 0.3 unit Taq polymerase (Takara, Dalian, China). PCR and purification of the amplified products were performed as previously described. Pyrosequencing was performed with a 454 GS FLX Titanium technology (454 Life Sciences, Branford, CT, USA) at the Chinese National Human Genome Center in Shanghai.

**Data processing and statistic analyses.** The sequence data for the January samples have been used in another publication and data for the June and August samples were analyzed together with new data. All these data were processed by Mothur and QIIME. Sequences that had an average quality score of lower than 27 over a window size of 50 nt were removed from subsequent analysis. OTU clusters at 97% identity sequence were determined using the UCLAST algorithm. The first sequence from each OTU was picked as a representative and taxonomy was assigned using the QIIME representative using the ribosome database project (RDP) classifier algorithm.

**Results**

**Water and sediment geochemistry.** Consistent with previous results, DOC and TN of spring waters and TOC of sediments were higher in samples of the rainy season (June and August) than in those from the dry season (January), reflecting the increased monthly precipitation from January to August (Table 1). Acidic springs and neutral-alkaline springs exhibited different water chemistry. In all three sampling times moderate-temperature acidic springs were dominated by high levels of sulfate and ferrous ion followed by calcium and potassium (Table S1 and Fig. S1a). The concentrations of these ions increased from January to June, and then decreased in August. In neutral-alkaline springs, waters exhibited high levels of chloride and sodium concentrations followed by less abundant potassium and calcium (Table S1 and Fig. S1b). Chloride concentration decreased from January to June, whereas the concentrations of cations (potassium, sodium...
and calcium) generally increased from January to June, but decreased in August samples (Table S1 and Fig. S1b).

There were no uniform temporal trends of changes among ions in high-temperature acidic springs (Table S1 and Fig. S1c). These spring water experienced great temporal variations in physicochemical conditions (Table 1, Table S1 and Fig. S1c). For example, pH in Zhenzhuquan (Zzq) ranged from 4.8 in January to 4.7 in June to 6.1 in August (Table 1). In this spring, Fe\(^{2+}\) pH in Zhenzhuquan (Zzq) ranged from 4.8 in January to 4.7 in June to 6.1 in August (Table 1, Table S1 and Fig. S1c). For example, in August samples (Table S1 and Fig. S1b).

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Three groups of major minerals in hot spring sediments were identified (quartz, K-feldspar, and clay minerals such as kaolinite, smectite and illite) (Table 2); however, abundance of these minerals differed between acidic and circumneutral-alkaline springs: Quartz and kaolinite were more abundant in acidic springs, whereas more K-feldspar was detected in circumneutral-alkaline springs. Clay minerals were comparable in all the investigated springs except Gongxiaosheng spring (GxsB), where aragonite was the predominant mineral (Table 2).

**Microbial diversity.** Both hot spring sediment and water experienced large temporal variations in microbial diversity. All phylotypes collected from all sites were grouped according to sampling time to evaluate temporal effects on microbial diversity (Fig. 1 and Table S2). We found that more phylotypes were observed in the rainy season, especially in August (Fig. 1a). Furthermore, the August community (n=285) contained more unique taxa (the OTUs only found in one sampling time point) than January (n=80) and June (n=87) (Fig. 1a). The temporal effect could also be observed in equitability, with higher equitability values in sediment than its water counterpart in the rainy season (June and August), but in the dry season (January) the equitability values were similar between sediment and water (except in GxsB) (Fig. 1b). Positively linear relationship between richness (Chao1) and phylogenetic diversity, as well as between evenness (Equitability) and phylogenetic diversity were observed (Fig. 1c), which suggested sediment community in the rainy season, especially in August, was composed of a group of highly diverse microbes.

**Limited temporal changes observed for the entire microbial community structure.** Different statistical analyses were employed to evaluate the temporal effects on the entire microbial community.
Table 2 | Quantitative mineralogy in June sediments as determined by X-ray diffraction (unit: %)

| SampleID | Quartz | K-feldspar | Albite | Calcite | Aragonite | Dolomite | Halite |
|----------|--------|------------|--------|---------|-----------|----------|--------|
| Drty-1.6 | 52.7   | 3.8        | 2.9    | 1.4     | 1.4       | 1.4      | 0.8    |
| Drty-2.6 | 44.7   | 9.7        | 4      | 1.7     | 1.7       | 1.7      | 0.6    |
| Drty-3.6 | 44.9   | 5.7        | 5.1    | 0.6     | 0.6       | 0.6      | 0.4    |
| GmqP.6   | 23.8   | 38.7       | 4      | 2.5     | 2.5       | 2.5      | 0.6    |
| JmqR.6   | 14.6   | 39.9       | 2.1    | 3.4     | 3.4       | 3.4      | 1.3    |
| Zzq.6    | 9.4    | 46.2       | 0.7    | 2.9     | 2.9       | 2.9      | 0.9    |
| SrbzD.6  | 27.8   | 26.9       | 4.4    | 1.1     | 1.1       | 1.1      | 0.6    |
| Jz.6     | 20.7   | 19.7       | 3.7    | 12.1    | 12.1      | 12.1     | 0.3    |

| SampleID | Gypsum | Barite | Sulfur | Kaolinite | Smectite | Illite | Biotite |
|----------|--------|--------|--------|-----------|----------|--------|---------|
| Drty-1.6 | 2.4    | 1      | 0.6    | 9.5       | 8.4      | 9.5    | 5.5     |
| Drty-2.6 | 1.6    | 0.8    | 0.9    | 14.9      | 14.5     | 14.9   | 3.6     |
| Drty-3.6 | 1.3    | 1.7    | 1.1    | 30.9      | 8.3      | 30.9   | 4.1     |
| GmqP.6   | 1.8    | 0.6    | 0.6    | 17.9      | 5        | 17.9   | 4.9     |
| JmqR.6   | 2      | 1.1    | 7.5    | 17.4      | 4.7      | 17.4   | 4.9     |
| Zzq.6    | 2.9    | 0.5    | 0.6    | 11.2      | 20.2     | 11.2   | 3.3     |
| SrbzD.6  | 0.7    | 0.3    | 1.4    | 10.7      | 14.3     | 10.7   | 13      |
| GxsB.6   | 5.6    | 0.6    | 1.5    | 7.8       | 26.1     | 7.8    | 2.4     |
| Jz.6     | 3.6    | 0.6    | 1.5    | 7.8       | 26.1     | 7.8    | 2.4     |

Figure 1 | Temporal changes in microbial diversity. (a) Microbial community relatedness between different sampling time points. More taxa were observed in the rainy season June and August, and the August community also harbored the largest number of unique taxa (n = 285, only occurred at one sampling time point). (b) Phylogenetic diversity in paired sediment and water samples for January, June and August. (c) Positively linear relationship between richness (i.e. Chao1) and phylogenetic diversity, as well as between evenness (i.e. Equitability) and phylogenetic diversity.
structure, but the results were dependent on the methods used. Although more phylotypes were observed in the rainy season, their abundances were so low (Figure S3, the “rare lineages” category) that their emergence could not be detected by the statistical methods (MRPP, ANODIS, and ANOSIM). Therefore the statistical results barely showed significant change in the entire community structure across the three sampling times (Table 3, Jan vs. Jun vs. Aug, p > 0.05). The communities were predominantly composed of phyla Crenarchaeota (30%–35% of the total sequences in each group) and Aquificae (43%–48%) across January, June and August (Fig. S3). However, temporal changes in some springs were observed when NMDS ordinations were used to visualize the temporal difference in microbial community structure (Figs. 2a & S4). In this case, pH was an important factor in shaping the microbial community distribution patterns (Fig. 2b). The pH effect was also confirmed by the significance tests (Table 3, acidic vs. neutral vs. alkaline, p < 0.05).

When we further investigated the microbial community structure separately for each sampling time, limited changes were observed from January and June to August (Fig. S4). For example, acidic springs formed two distinct clusters according to temperature in both January and June (Fig. S4a and S4b) but in August no temperature-dependent clusters were observed for acidic springs. Furthermore two high-temperature acidic springs (Zhenzhuquan and Diretiyanq-1) were far apart from each other in August (Fig. S4c). Some other environmental parameters that constrained the microbial distribution patterns were identified, such as total organic carbon in sediments and nitrogen species in water (Fig. S4). Major chemical ions such as sulfate, ferrous iron, sodium, chloride and fluoride also showed significant correlations with microbial community distribution patterns; however, they were also co-variied with pH, making it difficult to distinguish between pH and these chemical ion effects.

Greater temporal changes in sediment community than water community. Although limited temporal changes were observed in the entire community across the three sampling times, greater temporal variations were observed when the whole community was separated into water and sediment communities (Fig. 3, Fig 4 and Table 4). The overall average dissimilarity for the sediment communities across January, June and August was 83.54%, much higher than the overall average dissimilarity of 50.21% for the water communities over the same time period (Table 4). This difference suggested that sediment community displayed more pronounced temporal change than water community. A similar pattern was also revealed on the clustering tree: short branches indicated a high similarity (the lowest Bray-Curtis similarity value was 0.6, except Shuirebaozha spring) between the water communities from the three sampling times (Fig. 3a), whereas a low similarity (the lowest value was about 0.1) was observed between the three sediment communities (Fig. 3b). SIMPER analysis was performed to rank the contribution of individual taxa to the observed temporal differences in microbial communities (Table 4). The results showed that the lower dissimilarity for the water communities across January, June and August could be ascribed to relatively small abundance changes of top 14 taxa (contribution > 1%) across the three sampling times, whereas the higher dissimilarity for the sediment communities was due to larger abundance changes of top 22 taxa (contribution > 1%) across the same time period (Table 4).

When examined for individual springs, the dominant taxa within the water community did not show pronounced temporal changes (Fig. 3a, 3b and 4b) and the Mantel test confirmed the significant similarities (p < 0.01) in water communities across different sampling times (Fig. 4b). The circumneutral–alkaline spring community was mainly composed of Hydrogenobacter (59%–96% of total sequences in each spring) (Fig. 4a), except the Shuirebaozha spring (SrbzD.W). The dominant taxa in the Shuirebaozha spring changed from Persephonella and Fervidobacterium in June to Persephonella and Hydrogenobacter in August (Fig. 4a). In acidic springs, Zhenzhuquan (Zzq.W) was the only spring from which water samples were collected at all three time points. In this spring, communities between January and June were highly similar to each other (Fig. 4b) and both were predominated by Sulfolobus (Fig. 4a), but the August communities were significantly different from January and June (Fig. 4b), with Hydrogenobacter being the most abundant taxon in August followed by Sulfolobus (Fig. 4a).

In sediments, specific microbial lineages exhibited major changes across the three sampling times, especially from June to August (Fig. 3b, 3c and 4d), except the Jinze spring (Jz.S), where the sediment community composition remained similar across the three sampling times. In the two alkaline springs Gumingquan (GmqP.S) and Jiemiejuan (JmqRS) which were characterized by fast-flowing and high discharges, the Mantel test showed high similarity (Mantel r = 0.97 for Gumingquan and 0.79 for Jiemiejuan) between January and June sediment community structures (Fig. 4d). A single lineage Hydrogenobacter was the dominant constituent in both January and June communities but Sulphophobococcus emerged in June (Fig. 4c). Low similarity (Mantel r = 0.25 for Gumingquan and 0.5 for Jiemiejuan) was observed between June and August community structures (Fig. 4d), largely because Persephonella and OP1 became the dominant member in August Gumingquan and Jiemiejuan sediments, respectively (Fig. 4c). Likewise, the alkaline Shuirebaozha spring also displayed a strong temporal variation in its sediment microbial community structure (Fig. 4c and 4d).

Similar to these alkaline springs, high-temperature acidic springs Zhenzhuquan (Zqz.S) and Diretiyan-1 (Drtiy-1.S) also exhibited important temporal changes in sediment community structures. Sulfolobus was the dominant member in these two springs in both January and June communities, but in the August community, Desulfococcus substituted Sulfolobus in Zhenzhuquan and Ignispaera replaced Sulfolobus in Diretiyan-1 (Fig. 4c). This change was confirmed by the Mantel test showing a high similarity between January and June but a low similarity between January and August.

Table 3 | Dissimilarity tests between different microbial groups as defined either temporally or geochemically. P-values indicating significance differences between the groups are highlighted in bold

| Data sets | MRPP δ | P-value | ANODIS R-Square | P-value | ANOSIM R | P-value |
|-----------|---------|---------|----------------|---------|----------|---------|
| Jan vs. Jun vs. Aug | 0.9019 | 0.742 | 0.02173 | 0.369 | -0.02149 | 0.732 |
| Rehai vs. Ruidian | 0.8748 | 0.004 | 0.06081 | 0.003 | 0.1483 | 0.025 |
| Acid vs. neutral vs. Alkaline | 0.8216 | 0.001 | 0.16176 | 0.001 | 0.836 | 0.001 |
| Water vs. Sediment | 0.8709 | 0.001 | 0.06437 | 0.002 | 0.0917 | 0.02 |

*only contained samples that are paired among three sample times (13 samples for each sampling time point).*

*only contained springs that have paired water and sediment samples at a given time (16 samples [8 for water and 8 for sediment] for January, 14 samples [7 for water and 7 for sediment] for June, and 12 samples [6 for water and 6 for sediment] for August).*
community structures (Fig. 4d). However, a slight temporal change in moderate-temperature acidic spring Diretiyanqu-3 (Drty-3.S) community structure was observed. In Diretiyanqu-3, the abundance of *Hydrogenobaculum* decreased over time (47% in January vs. 26% in June vs. 5% in August), but those of *Acidicaldus* (10% vs. 26% vs. 43%) and *Desulfurella* (24% vs. 24% vs. 34%) increased (Fig. 4c). Another moderate-temperature acidic spring, Diretiyanqu-2 (Drty-2.S), was an exception to the above observations, e.g., the June and August community structures were similar to each other, but they were different from that in January (Fig. 4c and 4d).

Similar to a previous microbial census study in January, Diantan sediments contained more diverse microbial lineages than Rehai sediments. Dramatic temporal changes were observed for the Gongxiaoshe spring sediments (GxsB.S), especially in August (Fig. 4c and 4d). Specifically, the neutral Gongxiaoshe spring contained similar microbial lineages in January and June, including Candidatus *Nitrosocaldus*, *Armatimonadetes*, *Thermus*, *Geothermobacterium*, GAL35, O1aA90 and OP1 (Fig. 4c). However, in August, a single dominant lineage *Thermus* emerged, followed by less abundant *Geothermobacterium* and *Chloroflexus*, and this large change in microbial community structure from June to August was confirmed by the extremely low value of 0.11 in the Mantel test (Fig. 4d).

**Discussion**

Previous studies on Tengchong hot springs using pyrosequencing and Phylochip have provided a census of overall microbial community and temporal change (January vs. June). According to those results, non-endemic microbes which were flushed into springs due to enhanced rain influx may have accounted for the temporal changes in microbial community distribution pattern from January to June. With the use of the pyrosequencing method and one more sampling time point (January vs. June vs. August), we were not only able to further document the temporal variability of the overall hot spring communities, but also to reveal the temporal changes of individual genera in individual springs, and compare the temporal variability between sediments and water communities. The exact reasons accounting for the temporal changes in microbial community structure were difficult to establish, but the results from temporal changes of geochemistry in combination with the physiology of the culture representatives could shed light on these observed temporal changes of individual lineages.

Our data indicated that pH was a primary factor in determining the overall community assembly across the studied time points, followed by temperature and DOC. Temporal changes in these geochemical parameters may have induced microbial community shifts. For example, the dominant microbial lineages in the high-temperature acidic Zhenzhuquan spring (Zzq) changed from *Sulfolobus* in both sediments and water in January and June to microaerophilic *Hydrogenobacter* in the water and anaerobic *Desulfococcus* in the sediments in August. This significant change was possibly due to the pH increase from 4.7–4.8 in January and June to 6.1 in August. This high pH in August exceeded the growth pH range for *Sulfolobus* (0.9–5.8), but fit well with the pH range of *Hydrogenobacter* (near neutral pH) and *Desulfococcus* (pH 6.0–6.5).

The reasons for the decrease of *Sulfolobus* in another high-temperature acidic spring, Diretiyanqu-1, may be different. In this spring, pH also increased over time (from 2.6 to 5.0), but did not exceed the upper pH limit for *Sulfolobus*. Instead, high ion accumulations of NH$_4^+$, K$^+$, and Na$^+$ in August could have inhibited the growth of *Sulfolobus* species by affecting its RNA polymerase activity. Indeed, NH$_4^+$ (1.3 mM) and K$^+$ (9.4 mM) concentrations in August were much higher than those in natural environments where *Sulfolobus sulfataricus* have been isolated (1 mM for both ions). In addition, the high concentration of DOC in Diretiyanqu-1 observed in August would have facilitated the rapid growth of chemoorganotrophic microorganisms such as *Ignisphaera*. In these two high-temperature acidic springs (Zzq and Drty-1), due to these temporal changes of microbial lineages, microbial functions, as inferred from the microbial physiology, could have changed correspondingly: dominance of sulfur-oxidation in January and June by *Sulfolobus*-related species to sulfate-reduction by *Desulfococcus* and *Ignisphaera* related species in August. This functional shift was supported by the observed decrease in sulfate concentration from January to June to August.
Consistent with our previous results\(^9\), our data suggest that several genera of *Desulfococcales* tolerated a wider range of pH than their optimal pH conditions. For example, a previous study showed that *Ignisphaera* is a moderate acidophile with a pH range of 5.4 to 7.0 (optimum pH 6.4)\(^4\). However, this genus was present in acidic Tengchong springs with pH <5. Likewise, culture-independent
studies detected 16S rRNA sequences related to *Ignisphaera* in a Great Basin spring with pH 7.242 although its abundance was low. Another lineage *Sulfophobococcus* was previously reported in environments with a pH range of 6.5–8.5 and a low ion strength (<0.2% w/v NaCl)43, however, this lineage was detected in alkaline Teng-chong springs with pH less than 9. Similarly, *Desulfurella*, which is considered as a neutrophile44, was found in acidic springs in the present study, co-existing with *Hydrogenobaculum* and such co-occurrence has been observed in another acidic spring, i.e. Dragon spring in Yellowstone National Park (YNP)45. Collectively, these results suggest that some uncultured members within these lineages can tolerate a broader pH range in complex natural environments than in defined culture media38,43. Furthermore, microbes in natural environments can be present in micro-environments within sediments or surrounded by their own cell aggregates to avoid unfavorable conditions. For example, *Sulfophobococcus*43 and *Ignisphaera*41 were reported to create aggregates, so this mechanism may help them inhabit higher and lower pH environments, respectively, than their optimal pH conditions.

In addition to pH and water chemistry, a large temperature change also could induce microbial change. For example, in Gumingquan, the sediment community shifted from *Hydrogenobacter* in January and June to *Persephonella* in August. This shift corresponded to a temperature decrease (from 93°C in January to 83°C in June, to 69°C in August). The dominance of *Persephonella* at a moderate temperature e.g. 69°C was consistent with the recent results showing that *Persephonella* was isolated from worldwide springs with temperature no more than 70°C46. Members in *Persephonella*47 and *Hydrogenobacter*48 are all obligate H2-oxidizing chemolithotrophs, suggesting that chemolithotrophic growth was a dominant process in Gumingquan for all three sampling times. Overall, these results confirmed that time-course changes of microbial species were associated with the changing environmental conditions49. These results are consistent with previous studies in hot springs that microbial community structure and diversity in hot springs were significantly affected by physicochemical parameters such as pH, temperature and water/sediments chemistry3,4,9,10,19,35,50–54.
It is unexpected that sediment communities exhibited more dramatic temporal changes than their water counterparts. To our knowledge, only one other study in a Great Basin spring of the United States showed greater temporal change in sediment community than in water\(^1\). The major change observed in the sediment communities of Tengchong was the flourish of heterotrophs or fermenters in August sediments, suggesting that the elevated bioavailable nutrients in the August sediments may have favored the growth of heterotrophs. A previous study has shown that nutrient amendment can drive dramatic microbial community shifts in a groundwater ecosystem\(^5\), and our results suggest that similar shifts are possible in hot springs. Indeed, DOC in June and August were much higher than that in January, apparently due to high rainfall in June and August. However, it is interesting to note that despite high DOC in June, the sediment community structures did not shift until August. This lag in their response time suggests that sediment microbial communities need time to acclimate themselves to the new geochemical conditions. This kind of lag time is commonly observed when microorganisms collected from natural environments are cultivated in laboratory defined media. The length of lag time is dependent on how different the new condition is from the original one. A previous cultivation study of thermophilic *Nitrospira* reported that the lag time ranged from 2 to 4 months depending on different NO\(_2\) concentrations used in media\(^5\). In comparison, our results suggested a fairly rapid adaption of sediment microbial community with a time-lag period of only a few months (May to August, May is the start of rainy season, but no samples were collected). However, our insufficient sampling time resolution did not allow any quantitative estim-

### Table 4 | SIMPER analysis identifies the top individual taxa that contributed at least ~1% to the dissimilarity between the microbial communities from three sampling times.

**Water: January vs. June vs. August**

Overall average dissimilarity 50.21%

| Genus Order | Phylum | Contri. % | Mean abund. Jan (%) | Mean abund. Jun (%) | Mean abund. Aug (%) |
|-------------|--------|-----------|---------------------|---------------------|---------------------|
| Hydrogenobacter | Aquificaceae | Aquificae | 35.19 | 70.1 | 53.9 | 61.4 |
| Sulfobolus | Sulfolobales | Crenarchaeota | 22.54 | 18.5 | 16.3 | 4.19 |
| Persephonella | Hydrogenothermaceae | Aquificae | 11.27 | 0.07 | 7.51 | 11.1 |
| Fervidobacterium | Thermotogales | Thermotogae | 4.89 | 0.49 | 6.58 | 0.42 |
| Pyrolobus | Thermoproteales | Crenarchaeota | 4.24 | 1.02 | 3.35 | 3.31 |
| Thermus | Thermales | Deinococcus-Thermus | 4.21 | 1.79 | 3.99 | 2.89 |
| Sulfophobococcus | Desulfurococcales | Crenarchaeota | 2.67 | 2.17 | 2.17 | 0.92 |
| Caldibacterium | Thermodesulfobacteriales | Thermodesulfobacteriia | 1.71 | 2.45 | 0.34 | 0.4 |
| unclassified genus | Thermotogales | Thermotogae | 1.51 | 0.02 | 2.11 | 0.12 |
| Desulfuroccus | Desulfurococcales | Crenarchaeota | 1.46 | 1.07 | 0.22 | 1.26 |
| unclassified genus | GAL35 | | 1.46 | 0.04 | 0.68 | 1.56 |
| unclassified genus | Crenarchaeota | | 0.98 | 0.01 | 0.0 | 1.42 |
| Tepidimonas | Burkholderiales | Beta-proteobacteria | 0.96 | 0 | 0.1 | 1.34 |

**Sediment: January vs. June vs. August**

Overall average dissimilarity 83.54%

| Genus Order | Phylum | Contri. % | Mean abund. Jan (%) | Mean abund. Jun (%) | Mean abund. Aug (%) |
|-------------|--------|-----------|---------------------|---------------------|---------------------|
| Sulfolobus | Sulfolobales | Crenarchaeota | 15.93 | 12.7 | 24.7 | 10.4 |
| Hydrogenobacter | Aquificaceae | Aquificae | 15.25 | 29.8 | 14.1 | 5.4 |
| Hydrogenobaculum | Aquificaceae | Aquificae | 6.08 | 11.6 | 4.2 | 1.67 |
| Acidicola | Rhodospirillales | Alpha-proteobacteria | 5.14 | 6.14 | 3.69 | 4.99 |
| unclassified genus | GAL35 | | 4.95 | 4 | 5.91 | 3.91 |
| unclassified genus | OP1 | | 4.83 | 2.98 | 3.53 | 8.38 |
| Ignisphaera | Desulfurococcales | Crenarchaeota | 4.54 | 0.18 | 0.81 | 10.6 |
| Sulfobacterium | Desulfurococcales | Crenarchaeota | 4.12 | 2.23 | 6.08 | 3.77 |
| Thermus | Thermales | Deinococcus-Thermus | 3.5 | 1.13 | 1.12 | 7.21 |
| Desulfura | Desulfurococcales | Delta-proteobacteria | 3.46 | 2.98 | 2.86 | 3.83 |
| Persephonella | Aquificaceae | Aquificae | 3.33 | 0.66 | 0.51 | 7.3 |
| Fervidobacterium | Thermotogales | Thermotogae | 3.12 | 0.77 | 6.16 | 1.19 |
| Desulfurococci | Desulfurococcales | Crenarchaeota | 2.95 | 0.09 | 0.99 | 6.46 |
| unclassified genus | Crenarchaeota | | 1.72 | 1.38 | 2.18 | 1.22 |
| unclassified genus | Thermoplasmatales | Eurysarchaeota | 1.65 | 1.69 | 2.54 | 0.19 |
| unclassified genus | Aquificae | | 1.37 | 1.62 | 0.98 | 1.49 |
| Caldibacterium | Thermodesulfobacteriales | Thermodesulfobacteriia | 1.23 | 2.81 | 0.47 | 0.27 |
| Geothermobacterium | Thermodesulfobacteriales | Thermodesulfobacteriia | 1.19 | 1.5 | 0.53 | 1.41 |
| Candidatus Nitrospira | Nitrospira | Thaumarchaeota | 1.05 | 0.95 | 1.75 | 0.06 |
| unclassified genus | OIaA90 | | 0.99 | 1.44 | 1.14 | 0.1 |

*Order level for most bacteria and archaea and family level for Aquificales.

*Phylum level for most bacteria and class level for archaea and Proteobacteria.

*Minor group* represents the sum of microbial lineages with abundance ~5% of the total sequences in each sample.
ate of this lag time. More frequent sampling will be conducted to confirm this preliminary finding.

Although heavy surface runoff in the rainy season could have brought soil microbes into hot springs, our results showed that the abundant heterotrophs in the August sediments were all hyperthermophiles or thermophiles, which were not likely to be derived from surrounding soil environments. Our results are consistent with a recent lipid biomarker study where glycerol dialkyl glycerol tetraether from spring sediments were distinctly different from surrounding soils (Wu et al., personal communication). These heterotrophs occurring in August grew either by anaerobic respiration (such as Desulfococcus, Acidicladus and Desulfurella using sulfate, ferric iron and sulfur as an electron acceptor, respectively) or fermentation (such as Ignisphaera, Thermus, Fervidobacterium, Sulfophobococcus and GAL352). These physiological characteristics suggest that they may have initially inhabited heterogeneous and anaerobic micro-environments within spring sediments in January, but when the conditions became favorable (e.g., high TOC and nutrients), these minor microbes may have flourished and even become predominant. If so, this speculation would support a hypothesis that is widely applied to marine environments: “rare biosphere” acts as a seed bank in terms of the whole community or their genes.

Relative to sediment communities, water communities did not show much temporal change. We speculate that this was likely due to fundamental differences between sediment and water environment. In hot spring sediments micro-niches and physical barriers can host diverse and heterogeneous microbial communities which would result in high richness and evenness. High richness, evenness, and phylogenetic diversity in sediment, especially in the rainy season, may suggest a more complex and dynamic sediment community that interacts with the changing environmental conditions. Changes in geochemical conditions may cause previously dominant members to diminish and previously minor members to flourish, because minor members under previously sub-optimal conditions may find new conditions to be optimal for their growth. However, in water, constant mixing and lack of physical barrier would result in less diverse microbial community. Future work is necessary to focus on temporal changes at the functional/activity level with a higher temporal resolution.

Conclusions

Limited temporal changes were observed in the entire community across springs and sampling times, but greater temporal changes were observed for specific microbial lineages in individual springs, likely because of the higher sensitivity of these genera to environmental changes. Water and sediment communities responded differently to temporal physicochemical changes. Whereas water communities were stable from January to June to August, sediment communities were more responsive to temporal geochemical changes. Specifically more abundant anaerobic heterotrophs and/ or fermenters occurred in August sediment relative to January and June. The hyperthermophilic and thermophilic nature of these heterotrophs suggests that they were not transported into hot springs from the surroundings by increased surface runoff in August, but rather their occurrence or even dominance was due to large temporal variations of physicochemical conditions (i.e. pH, temperature and DOC) in these springs.

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**Author contributions**
H.D. conceived the research idea and led the study. S.W. performed this study; W.H. collected samples and performed DNA extraction. H.J. collected samples and help with interpreting the data. Q.H. and B.B. performed total organic carbon and total nitrogen analyses, and analysis of major ions. L.H. performed field Hach kit measurements. The manuscript was written by S.W. and H.D. with contributions from all co-authors.

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