

## Geomicrobial dynamics of Trans-Himalayan sulfur-borax spring system reveals mesophilic bacteria's resilience to high heat

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**Abbreviations used in the paper:** HTP, highest temperature point; MTP, moderately-high temperature point; LTP, lowest temperature point; RVW, river water; ICPMS, inductively coupled plasma mass spectrometry; VCDT, Vienna Canyon Diablo Troilite; IAEA, International Atomic Energy Agency; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; OTUs, operational taxonomic units; RDP, Ribosomal Database Project; R2A, Reasoner's 2A; MMST, modified basal mineral salt medium supplemented with thiosulfate; LB, Luria broth.

### Abstract

Geomicrobiology of sulfur-boron-dominated, neutral-pH hydrothermal systems was revealed in a Trans-Himalayan spring named *Lotus Pond*, located at 4,436 m, in Puga Valley, Ladakh (India), where water boils at 85°C. Water sampled along *Lotus Pond's* outflow (vent to an adjacent river called *Rulang*), representing an 85-14°C gradient, had high microbial diversity and boron/chloride/sodium/sulfate/thiosulfate concentration; potassium/silicon/sulfide/sulfite was moderately abundant, whereas cesium/lithium small but definite. Majority of the bacterial genera identified in the 85-72°C samples have no laboratory-growth reported at >45°C, and some of those mesophiles were culturable. Sulfur-species concentration and isotope-ratio along the hydrothermal gradient, together with the distribution of genera having sulfur-oxidizing members, indicated chemolithotrophic activities in the 85-72°C sites. While biodiversity increased in the vent-to-river trajectory all-day, maximum rise was invariably between the vent (85-81°C) and the 78-72°C site; below 72°C, diversity increased gradually. Biodiversity of the vent-water exhibited diurnal fluxes relatable to the sub-surface-processes-driven temporal fluxes in physicochemical properties of the discharge. Snow-melts infiltrating (via tectonic faults) the ~160°C geothermal reservoir located within the breccia, at ~450 m depth, apparently transport mesophilic microbes into the thermal waters. As these microorganisms emanate with the vent-water, some remain alive, illustrating that natural bacterial populations are more heat-resilient than their laboratory counterparts.

**Keywords:** Sulfur-boron hot spring; hot spring geomicrobiology; geochemistry of hot spring waters; microbial community dynamics along hydrothermal gradients; mesophilic bacteria in high-temperature habitats; *in situ* resilience to heat

## Introduction

Geochemistry of active hydrothermal systems resemble that of the early Earth (Baross 1998; Patel *et al.* 2015; Ranjan *et al.* 2018) in having copious reducing substances such as hydrogen, hydrogen sulfide, methane and sulfur in their chemical milieu, together with variable quantities of ammonia, carbon dioxide, carbon monoxide, nitrogen, sulfate and thiosulfate, which can all act as substrates for energy-yielding biochemical reactions (Amend and Shock 2001; Amenabar and Boyd 2019). These geochemical features, together with the fact that the comprehensive evolutionary tree of Life has thermophilic/hyperthermophilic chemolithotrophs at its root (Forterre 2015; Hug *et al.* 2016), corroborate the hypothesis that ancient hydrothermal habitats were the potential nurseries of early Life based on inorganic sources of energy (Wächtershäuser 2006; Martin *et al.* 2008; Ghosh and Dam 2009).

Studies of terrestrial hydrothermal vent waters, muds and biofilms, sediments and sub-aqueous sinters and geysers (Blank *et al.* 2002; Lacap *et al.* 2007; Chan *et al.* 2015; Ghosh *et al.* 2015; Roy *et al.* 2016) have elucidated wide-ranging aspects of *in situ* microbial diversity (Cole *et al.* 2013; Huang *et al.* 2013; Arce-Rodríguez *et al.* 2019), ecology (Meyer-Dombard *et al.* 2011; Everroad *et al.* 2012; Wang *et al.* 2013; Payne *et al.* 2019), biogeochemical cycles (Xu *et al.* 1998; Ball *et al.* 2001; Nordstrom *et al.* 2009; Wang *et al.* 2018) and ore-deposition/stromatolite-formation (Walter *et al.* 1972; Jones *et al.* 2000; Berelson *et al.* 2011). Albeit these investigations were spread across geographically/geologically distinct provinces of the world, most of them were based on hot spring systems which discharge low pH sulfurous fluids, generally at low flow rates (Jones *et al.* 2000; Johnson *et al.* 2001, 2003; Owen *et al.* 2008). In contrast, neutral to slightly-alkaline hot springs have remained less explored, presumably since they are geographically infrequent and mostly restricted to areas having high groundwater level and/or huge volumes of melting snow or rain (Skirnisdottir *et al.* 2000; Huang *et al.* 2011). Of the latter type, again, some information of geomicrobiology is available for the systems that are typically rich in sulfide, silicon, chloride, carbonate and bicarbonate, and have overall high levels of salinity and flow rate (Mathrani *et al.* 1993; Atkinson *et al.* 2000; Skirnisdottir *et al.* 2000; Hetzer *et al.* 2007; Roeselers *et al.* 2007; Huang *et al.* 2011); but little geomicrobial information is there on the neutral pH spring systems such as those encountered in the Trans-Himalayas and which discharge hot water typically poor in silicon and total dissolved solids, but rich in boron and sulfur (Garrett 1998; Rai 2001). This said, Trans-Himalayan geothermal systems and their adjoining territories are well explored from the perspectives of energy geophysics, orogeny, magmatism and seismicgeology (Gansser 1964; Viridi *et al.* 1977; Shanker 1988; Singh 1991; Brown 1996; Gokarn *et al.* 2002; Li *et al.* 2003; Harinarayana *et al.*

2006; Azeez and Harinarayana 2007; Craig *et al.* 2013). Spring-waters, sediments and sinters of a few Trans-Himalayan hydrothermal systems have also been studied for their geochemistry (Chowdhury *et al.* 1974; Chowdhury *et al.* 1984; Saxena and D'Amore 1984; Navada and Rao 1991; Tiwari *et al.* 2016; Steller *et al.* 2019), microbial diversity (Roy *et al.* 2016, 2019) and astrobiological implications (Ghosh *et al.* 2012). But such hot springs have seldom been explored *for in situ* microbial community dynamics in relation to the geochemical and geophysical drivers of the system, except for a recent study aimed at defining the geochemical and biophysical parameters which constrain and/or promote microbial mat communities along the hydrothermal gradients of a microbialite spring system called *Shivlinga* located in the Puga geothermal area of eastern Ladakh (Roy *et al.* 2020), within the Indian Trans-Himalayas, and the greater Ladakh-Tibet sulfur-borax spring zone (Garrett 1998; Harinarayana *et al.* 2006).

In the present study, also based on the Puga geothermal area of Ladakh (Figure 1A and B), we explore the aquatic microbiome architecture and geochemistry of a sulfur-borax hot spring (geyser) named *Lotus Pond*, which is situated close to the *Shivlinga* microbialite spring (Figure 2A and B), at an altitude of 4,436 m, where water boils at 85°C. We record and analyze the spatiotemporal fluxes in the physicochemical properties and biodiversity of the spring-water, and then consider the results in the context of the physiography, geology and geophysics of the area to reveal the comprehensive geomicrobial dynamics of the system. Our findings elucidate the potential geodynamic mechanism of microbial transport through Trans-Himalayan hydrothermal reservoirs, and in doing so highlight the significance of the mineralogically peculiar, sulfur-boron hot spring systems in understanding natural bacterial populations' adaptation and resilience to heat.

## **2. Materials and Methods**

### **2.1 Study area: geography and geology**

The *Lotus Pond* hot spring is located (at 33° 13' 46.3" N and 78° 21' 19.7" E) in the eastern flank of the Puga valley (Figure 1A and B), which lies just south of the tectonically-active Indus Suture Zone (ISZ) or the collision junction between the Indian and Asian continental crusts involved in Himalayan orogeny (Gansser 1964). Puga Valley, the most high energy geothermal field in India (Shanker 1988; Harinarayana *et al.* 2006), is connected with Leh, the capital city of the union territory of Ladakh, by an approximately 180 km long metalled road, which runs along the river Indus for the first ~150 km from Leh before veering south across the Indus, near Mahe, to reach the valley of Puga situated at an average altitude of ~4500 m from the sea level.

The Puga geothermal area represents a major crustal subduction zone (Viridi *et al.* 1977) delimited by three distinct tectonic belts within the upper Indus valley (Harinarayana *et al.* 2006). The northern belt is made up of the Indus Group of sedimentary rocks overlying the Ladakh Granites. The central belt (this is same as the ISZ), bordered by the Mahe and Zildat faults in the north and south respectively, features basic, ultrabasic and sedimentary rocks belonging to the Sumdo Group. The southern belt encompasses highly metamorphic, granite-intruded, sedimentary and metasedimentary rocks (Harinarayana *et al.* 2006). While the Zildat fault marks the eastern border of the zone of thermal manifestation within Puga Valley, a regional fault, known as the Kaigar Tso fault, apparently delimits this zone on the western side (Shanker 1991). While most of the surface expressions of geothermal activity, including the *Lotus Pond* spring, are encountered in the eastern part of the 15 km-long and 1 km-wide, east-west orientated Puga Valley, a potential hidden fault, running along the base of the northern hills in the central region of the valley, is thought to be representative of an ancient line of fumarolic activity (Shanker *et al.* 1976).

For a few tens of meters down the surface, the valley of Puga is filled with loose materials (Figure 1C and D) such as recent and sub-recent fluvioglacial and eolian deposits of alluvium or sand containing grains of quartz and mica, and hot spring sinters (Harinarayana *et al.* 2006). This sedimentary stratum is underlain by two geothermal reservoirs at sequential depths (Figure 1C and D). A shallow, ~160°C, heat-reservoir is situated at a depth of ~450 m, within the breccia and fractured upper portions of the basement rocks saturated with a mixture of thermal water and fresh meteoric water, and deep thermal water, respectively (Gupta *et al.* 1974; Singh *et al.* 1983; Shanker 1991; Absar *et al.* 1996; Mishra *et al.* 1996). The basement rocks (gneiss, paragneiss, schists and quartzite), collectively known as Puga Formation, are plausibly of Paleozoic origin; they are intruded by Polokongka La Granite in the west, while in the east Sumdo Formations are exposed; on-surface thermal manifestations are all apparently aligned along the faulted crest of an anticline in the Puga formation (see Harinarayana *et al.* 2006 and references therein). The deeper, main geothermal reservoir featuring ~260°C hot, and progressively cooling, magma chambers (these have aqueous and ionic fluids associated with them) commences at a depth of ~2 km in the upper Himalayan crust, and is underlain by partial rock-melt generated from the Indian continental crust by the high temperature and pressure of its subduction. These rock-melts occur till a depth of ~8 km (and plausibly further below), within the mid-lower Himalayan crust, and the fluid phase associated with them are potentially injected to the upper crust under high pore pressure (Shanker *et al.* 1976; Gupta *et al.* 1983; Shanker 1988; Gokarn *et al.* 2002; Harinarayana *et al.* 2006; Azeez and Harinarayana 2007). Production of the rock-melts being a continuous process in this tectonically active region, geothermal

activity, here, is expected to perpetuate for a long term (Azeez and Harinarayana 2007). In the above geodynamic context it is noteworthy that the shallow and deep geothermal reservoirs of Puga are spatially separated by the metamorphic, granite-intruded basement rocks, which are themselves geophysically non-conductive but can potentially connect the two geothermal reservoirs through the tectonic fault running through them (Shanker 1991; Azeez and Harinarayana 2007). Steep geothermal gradients at shallow depths, high concentrations of cesium in the hot spring discharges and the occurrence of cinnabar encrustations and siliceous sinter deposits around the springs corroborate the association of the Puga hydrothermal discharges with a magmatic source which is in its advanced stage of activity (Chowdhury *et al.* 1974; Chowdhury *et al.* 1984; Saxena and D'Amore 1984; Shanker 1991; Shanker *et al.* 1999; Azeez and Harinarayana 2007). Besides the tectonic fault running through the basement rocks (Shanker 1991), other potential mechanisms of connectivity also exist between the two geothermal reservoirs. For instance, the upward-mobilized, highly-intrusive magmatic body and hot fluid, in the process of getting emplaced into the porous regions of the upper crust, also creates fractures in the basement; the expelled fluids fill up those fractures and continue to move upward into the breccia, thereby linking the shallow geothermal reservoir with the deeper resource-base (Azeez and Harinarayana 2007).

## 2.2 Sample identity

Situated on the bank of a mountain brook called *Rulang*, the *Lotus Pond* spring discharges profuse fumarolic vapor and hot water (Figure 2) with diurnal fluctuations in temperature, flow rate and pH (Figure 3A; Table 1; Table S1). The spring water flows into *Rulang* over centimeters-high terraces of hydrothermal sinters. Thick accretions of geothermal sinters harboring fossilized remains of ancient microbial mat communities also flank the northern side of the *Lotus Pond* spring (Ghosh *et al.* 2012). The trajectory of *Lotus Pond*'s spring-water transit in the vent-to-river direction represents an 85-14°C thermal gradient, within which four distinct sample-sites (Figure 2C) were explored on 20 October 2014 for the spring-/river-water's chemistry and microbial diversity. These sample-sites - designated as HTP (the 85-78°C, highest temperature point), MTP (the 78-70°C, moderately-high temperature point), LTP (the lowest temperature point, situated at the interface between the spring-water's outflow and the *Rulang* river's water current; here the water temperature fluctuates now and then between 25-45°C) and RVW (the 14-24°C, river-water site, situated within *Rulang*'s own water current) - were located at *Lotus Pond*'s vent-center, and 1, 1.5 and 1.8 m away from the vent-center, respectively (Table 1). For each sample-site, *in situ* microbial diversity was revealed at 6:30, 8:30, 14:30 and 20:30 h, while water-chemistry was explored at 10:30 and 17:30 h in addition to the above

four sampling-hours (for chemistry, three sample-replicates were analyzed and averaged on each occasion).

### **2.3 Sample collection for water chemistry**

Water-samples were collected, post filtrations through 0.22  $\mu\text{m}$  cellulose acetate membranes, from individual sample-sites, at different sampling-hours, using separate 25 mL sterile glass-pipettes. Every 100 mL batch of water-sample meant for the quantification of metallic elements was acidified (to  $\text{pH} \leq 2$ ) by adding 69% (w/v)  $\text{HNO}_3$  (400  $\mu\text{L}$ ). 40 mL of each water-sample meant for determining sulfate concentration by gravimetric method was acidified to  $\text{pH} 2.0$  by adding few drops of 1.2 N  $\text{HCl}$ , and then precipitated for  $\text{BaSO}_4$  by adding 1 mL preheated 1 M  $\text{BaCl}_2$  followed by vigorous mixing. Dissolved sulfides were precipitated *in situ* from 25 mL batches of water-samples in the form of  $\text{CdS}$ , by adding 1 mL 2 M  $\text{Cd}(\text{NO}_3)_2$ , inside butyl septum bottles. The bottles were crimp-sealed immediately leaving no head space, and stored at  $4^\circ\text{C}$  until further analysis in the laboratory.  $\text{CdS}$  precipitates meant for sulfur isotope analysis were converted to  $\text{Ag}_2\text{S}$  by adding  $\text{AgNO}_3$  solution. Rate of water discharge at the vent was determined using Soluble-tracer Dilution technique (Rantz 1982).

### **2.4 Analytical techniques**

Concentrations of boron, calcium, lithium, magnesium, and potassium were determined by inductively coupled plasma mass spectrometry (ICPMS) on a iCAP QICPMS (Thermo Fisher Scientific, USA), following manufacturer's protocol; calibration curves were prepared using ICPMS standards supplied by Sigma Aldrich (USA) and VHG Labs Inc. (USA). Based on replicate analyses of the standards, deviations from actual concentrations were  $<2\%$ ,  $<1\%$ ,  $<2.1\%$ ,  $<1\%$  and  $<2.5\%$  for boron, calcium, lithium, magnesium and potassium, respectively. Concentration of sodium was determined using a 240 AA atomic absorption spectrometer (Agilent Technologies, USA), following protocol provided by the manufacturer; standard curves were prepared from Sigma Aldrich AAS standards. Based on multiple analyses of the standard, deviations from actual sodium concentrations were  $<3\%$ . Silicon concentration was determined using a UV-visible spectrophotometer (CARY 100, Varian Deutschland GmbH, Germany), as described previously (Grasshoff *et al.* 1999). Chloride was quantified by precipitation titration with silver nitrate (0.1 N) (Mazumdar *et al.* 2009) using a Titrino 799GPT auto titrator (Metrohm AG, Switzerland).

Thiosulfate and sulfate concentrations were determined by iodometric titration, and gravimetric precipitation with barium chloride, respectively (Kelly and Wood 1994; Alam *et al.* 2013). Sulfite was analyzed spectrophotometrically with pararosaniline hydrochloride (Sigma-

Aldrich) as the indicator (West and Gaeke 1956). Dissolved sulfides that were precipitated as CdS from the water-samples *in situ* were subjected to spectrophotometric measurement as described previously (Cline 1969). Sulfate, sulfite and thiosulfate were additionally quantified in the sulfide-free (CdS-precipitated) water-samples using an ion chromatograph (Basic IC plus 883) equipped with a suppressed conductivity detector and a Metrosep A Supp 5 anion exchange column (all from Metrohm AG, Switzerland).

To determine sulfur isotope ratios, dried and homogenized BaSO<sub>4</sub> or Ag<sub>2</sub>S precipitates were mixed with V<sub>2</sub>O<sub>5</sub>, flash combusted at 1,150°C in an EA1112 elemental analyzer (Thermo Fisher Scientific), and then analyzed using a Thermo Delta V plus continuous flow isotope ratio mass spectrometer (Thermo Fisher Scientific). All results were reported in standard delta notation ( $\delta^{34}\text{S}$ ) as per mil (‰) deviations from the VCDT (Vienna Canyon Diablo Troilite), with reproducibility of  $\pm 0.3$  ‰. IAEA standards S-1, S-2, SO-5 and SO-6 were used for instrument calibration.

## **2.5 Sample collection for analyzing aquatic microbial communities**

At 6:30, 8:30, 14:30 and 20:30 h, water was collected from each of the four sample-sites in multiple 1000 mL batches for culture-independent microbial diversity analysis. Additionally, at all these time-points, plus at 15:00 h, multiple 1000 mL batches of water were collected from the all the four sample-sites for culture-based study and microscopic examination. Each 1000 mL batch of water-sample was passed through sterile 0.22  $\mu\text{m}$  cellulose acetate filter, 4.7 cm in radius (Sartorius Stedim Biotech, Germany), and the filter was inserted into a cryovial containing 5 mL of 50 mM Tris:EDTA (TE, pH 7.8), or 15% glycerol plus 0.9% NaCl solution, according as the sample was meant for community-DNA-based, or culture-based and microscopic, studies. After filter-insertion, each cryovial was sealed with Parafilm (Bemis Company Inc., USA), packed in polyethylene bags, and immediately put into dry ice. Once in the laboratory, the samples meant for culture-independent (community-DNA-based) analysis were stored at -20°C, while those for culture-based and microscopic studies were kept at 4°C. Total community DNA was extracted from the designated samples within 48 h of reaching the laboratory; microscopic examination of the samples was also conducted within the same time frame. For microscopy, the organisms potentially present in the samples were vortexed (for 30 min) out of the filters into the glycerol-NaCl solution, mounted on glass slides, stained using a Gram Stain Kit (HiMedia Laboratories Pvt. Ltd., India), and finally examined under a BX-50 phase contrast microscope (Olympus Corporation, Japan).

## **2.6 Preparation of total community DNA and assessment of bacterial diversity**

Total community DNA was extracted, as described previously (Roy *et al.* 2016), from the microbial cells arrested in the 0.22 µm cellulose acetate filters (Supplementary Methods). V3 regions of all bacterial 16S rRNA genes present in a DNA extract were PCR amplified (using domain-specific primers) and sequenced (on Ion Torrent Personal Genome Machine, Thermo Fisher Scientific) following fusion primer protocol (Ghosh *et al.* 2015; Roy *et al.* 2016; Fernandes *et al.* 2018) (Supplementary Methods). The individual sequence files were deposited to Sequence Read Archive of the National Center for Biotechnology Information, USA, under BioProject PRJNA296849, with distinct run accession numbers (Table S2). All V3 sequence reads obtained for the individual samples were filtered for quality value 20 and length threshold 100 bp; they were then clustered into operational taxonomic units (OTUs), or putative species-level entities unified at the 97% 16S rRNA gene sequence similarity level, using various modules of UPARSE (Edgar 2013); singletons were excluded from final community analyses (Table S2). A Perl programming-script, available within UPARSE, was used to determine Abundance-based Coverage Estimator, and Shannon and Simpson Indices. Taxonomic affiliation of the consensus sequence of each OTU was determined using RDP Classifier (<http://rdp.cme.msu.edu/classifier/classifier.jsp>). Rarefaction analysis for all the sequence datasets [done using Vegan Package in R (R Core Team 2014)] confirmed that their read-contents were sufficient to reveal most of the diversities present in the samples (Figure S1).

## **2.7 Culturing mesophilic bacteria from HTP water-samples**

Sterile 0.22 µm cellulose acetate filters through which batches of 1000 mL HTP water-samples had been passed during sampling, and those which were rehydrated in 5 mL 15% glycerol plus 0.9% NaCl solution, were cut into pieces with sterile scissors, within the same 8 mL cryovials in which the filters were brought from the field. The cryovials were vortexed for 30 min, following which the filter shreds were discarded, and the residual (~5 mL) glycerol-NaCl solutions were added to 25 mL Reasoner's 2A (R2A) or modified basal mineral salt medium supplemented with thiosulfate as the sole source of energy and electrons (MMST). 1 L R2A (pH 7.0) contained 0.5 g proteose peptone, 0.5 g casamino acids, 0.5 g yeast extract, 0.5 g dextrose, 0.5 g soluble starch, 0.3 g K<sub>2</sub>HPO<sub>4</sub>, 0.05 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.3 g sodium pyruvate, while 1 L MMST (pH 7.0) contained 1 g, NH<sub>4</sub>Cl; 2 g, K<sub>2</sub>HPO<sub>4</sub>; 0.75 g, KH<sub>2</sub>PO<sub>4</sub>; 0.5 g, MgSO<sub>4</sub>·7H<sub>2</sub>O; 2.5 g (10 mM), Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O; 50 mg, yeast extract (as growth factor supplement); and 2 mL, trace metals solution (Ghosh and Roy 2006). The mixtures were incubated at 37°C on a rotary shaker (180 rpm) until OD<sub>600</sub> of the R2A cultures reached 0.8, or the pH of the spent MMST medium became <6 due to conversion of thiosulfate to sulfuric acid.



Serial dilutions from the mixed cultures were then plated on R2A- or MMST-agar, and incubated at 37°C. Single colonies of pure strains, apparently unique in terms of colony morphology and/or rate and extent of acid production in chemolithoautotrophic MMST medium, were isolated as pure cultures and maintained in Luria broth (LB) or MMST medium at 37°C.

### 3. Results

#### 3.1 High bacterial diversity, and its spatiotemporal fluxes, along the spring-water transit

All the water-samples, collected round the clock, from the four study-sites of the *Lotus Pond-Rulang* system had considerable density of morphologically diverse microbial cells (phase contrast micrographs for only the 8:30 h samples from HTP, MTP, LTP and RVW have been shown in Figure 4). High levels of bacterial taxonomic diversity were also revealed along *Lotus pond's* hydrothermal gradient when the HTP, MTP, LTP and RVW samples collected at 6:30, 8:30, 14:30 and 20:30 h were explored via PCR-amplification, sequencing and analysis of 16S rRNA gene fragments from the total community DNAs extracted from these samples (OTU-counts determined in this way for the 16 individual water-samples are given in Table 1). OTUs identified from the four samples-sites at two time points each in the forenoon (6:30 and 8:30 h) and afternoon (14:30 and 20:30 h) were first analyzed at the phylum level (Figure 5A-D; Table S3). Results showed the individual communities, especially the one at HTP, to remain largely consistent between 6:30 and 8:30 h (compare Figure 5A and B), and then again between 14:30 and 20:30 h (compare Figure 5C and D). OTU-count at individual sample-sites, however, increased sharply between 8:30 and 14:30 h (compare Figure 5B and C), in view of which OTUs identified in the 8:30 and 14:30 h datasets were further analyzed at the genus level. Consequently, detailed geochemical data from all the four sample-sites were compared for these two time-points only.

For all the individual sample-sites, total number of genera over which the OTUs were distributed increased significantly at 14:30 h, as compared to 8:30 h; but proportions of OTU-affiliation across different phyla remained largely unchanged across the two sampling-hours. For instance, 60-75%, of the OTUs present at any sampling-hour, in any sampling site, were affiliated to *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes* and *Unclassified Bacteria* (Table S3). On the spatial scale, OTU-count increased steadily along the hydrothermal gradient in the vent-to-river trajectory, at all the four sampling-hours, except for a small decrease from LTP to RVW at 20:30 h (Figure 5D). Site to site increases in diversity were maximal between HTP and MTP, where 6.1, 5.8, 4.3 and 4.9 fold increases in OTU-count were recorded at 6:30, 8:30, 14:30 and 20:30 h respectively. OTU-count for almost every individual

phylum detected in the system also increased sharply from HTP to MTP, at all the four sampling-hours, except for *Aquificae* at 14:30 and 20:30 h, and *Deinococcus-Thermus* and *Thermotogae* at 14:30 h (Table S3). Below 72°C, however, OTU-count for individual sample-sites, or the number of phyla over which OTUs were distributed, increased gradually or even decreased in some cases.

### **3.2 Spatiotemporal fluxes in physicochemical parameters account for the biodiversity fluxes**

From the early morning (6:30 h) till the evening (17:30 h), temperature of the vent-water (HTP) decreased gradually from 85°C to 78°C; correspondingly, the rate of vent-water discharge increased from 2 to 3.9 L s<sup>-1</sup>. With the onset of darkness, however, there was a quick rise in the temperature to 82°C (as recorded at 20:30 h), accompanied by a drop in the discharge rate to 3.4 L s<sup>-1</sup> (Table 1). It is known from previous studies that the hydrothermal discharges of Puga come from a reservoir of formation waters trapped in geological sediments (waters occurring naturally within the pores of rocks are called formation/interstitial waters; they are not injected from extraneous sources, and are sometimes present since the formation of the rock). This reservoir, which is distinct from the fresh-water aquifer of the region, is said to be recharged by melting snow (fresh meteoric water) in the higher mountain reaches above an altitude of ~5,600 m (Navada and Rao 1991; for a schematic illustration of this phenomenon see Figure 1). Furthermore, stable isotope ratios determined for hydrogen and oxygen in the hot water discharges of Puga have indicated their brief underground-retention time and moderate interaction with the underlying rocks (Navada and Rao 1991). When these facts are considered alongside the negative correlation observed between the diurnal variations in the temperature and discharge rate of *Lotus Pond* vent-water (Pearson correlation coefficient  $R = -0.9$ , with corresponding probability value  $P = 0.007$ ) it is apparent that with progressive rise in air temperature over the day, snow melts in the higher mountain reaches, and increasing volumes of cold oxygenated water is pumped into the hypothermal reservoir, leading to higher discharge rates and lower temperatures of the vent-water.

The aforesaid geophysical dynamics can be a potent cause of diurnal biodiversity flux in *Lotus Pond*'s vent-water, and consequently in the downstream of it. For instance, in sync with the progressive increase in the rate of vent-water discharge, from the early morning (6:30 h) till the evening (17:30 h), OTU-count increased at all the four sample-sites of the *Lotus Pond-Rulang* system. These hikes, remarkably, were maximal across the forenoon-afternoon divide, i.e. between 8:30 and 14:30 h - maximum increase (2.6 times), at this temporal juncture, was recorded at HTP, whereas minimum increase (1.5 times) was recorded at RVW (compare Figure 5B and C). We, therefore, hypothesized that as more snow-melted water enters the tectonic faults crisscrossing the region (Shanker 1991; Harinarayana *et al.* 2006) and leads to the underlying shallow hydrothermal

reservoir (see schematic illustration in Figure 1), denudation or wearing away of the sediments and breccia introduces more soil/sub-surface microorganisms into the reservoir. These microbes, together with those present in the snow-melts, are potentially transported through the hot-water conduit and eventually ejected through *Lotus Pond* and other vents spread across the valley. That the recharge of the Puga hydrothermal reservoir by melting snow over the day-time indeed causes influx of microorganisms in the vent system was further corroborated by the simultaneous reversing of the trends of biodiversity flux as well as vent-water discharge rate, with the onset of darkness. For instance, at 20:30 h, in sync with the decrease in vent-water discharge there were subtle decreases in the OTU-counts at HTP, MTP and LTP, and a conspicuous (~40%) decrease in the OTU-count at RVW (Table 1). In fact, the decrease in RVW OTU-count at 20:30 h was so sharp that for the first occasion in the entire sampling-day it became even lower than the OTU-count of LTP (notably, at 20:30 h, OTU-count of RVW was almost same as that of MTP). This last observation, together with the elements of consistency detected in the microbial diversity of the individual sample-sites across the day, reflected that the microbial communities along *Lotus Pond*'s spring-water transit were not necessarily being washed away, all the while, into the river, and the higher OTU-count of RVW (as compared to HTP, MTP and LTP) observed throughout the day-time was not due to dumping of microorganisms by *Lotus Pond*'s outflow. Instead, the latter phenomenon seemed attributable to the river's own denudation/transportation dynamics rooted in its visibly higher water flow between 6:30 and 17:30 h, as compared to 20:30 h. Furthermore, in this context, revealed distributions of unique and shared genera across the sample-sites at 8:30 h (Figure S2A; Table S4) and 14:30 h (Figure S2B; Table S5) comprehensively illuminated all potential mechanisms of bacterial diversity flux in the *Lotus Pond-Rulang* system; such data also reflected the consistency of the microbiome in time and space and the contiguous nature of the aquatic communities along *Lotus Pond*'s spring-water transit (see Supplementary Results).

### **3.3 Lotus Pond's spring-water chemistry along the hydrothermal gradient**

All water-samples analyzed along the HTP-RVW hydrothermal gradient contained high concentrations of boron, chloride, sodium, sulfate and thiosulfate (>100 ppm). While potassium, silicon, sulfide and sulfite concentrations were moderately high (up to ~90 ppm), calcium, cesium, lithium and magnesium were present in small but definite amounts (up to ~9 ppm). Trace amounts of rubidium and strontium were also detected in all the samples (0.2-1.4 ppm). The HTP and MTP samples were found to have highest concentrations of boron, lithium, sodium, sulfide, thiosulfate and sulfate (Figure 3A-B; Table S1). From HTP to MTP, at both 8:30 and 14:30 h, there were small but definite increases in the concentrations of boron, thiosulfate and sulfate. Lithium also increased from

HTP to MTP at 8:30 h but remained almost unchanged at 14:30; sodium concentration remained almost unchanged between HTP and MTP at both the sampling-hours.

### **3.4 Sulfur-speciation and chemolithotroph-diversity along Lotus Pond's spring-water transit**

Progressive increase in sulfate concentration and concomitant decrease of dissolved sulfide, thiosulfate and sulfite was observed in the vent-water between 6:30 and 17:30 h. Conversely, after sun-down (between 17:30 and 20:30 h), discharge rate and sulfate concentration decreased, whereas temperature of the vent-water and concentrations of the reduced sulfur species increased (Table 1; Figure 3C; Table S1). Mechanistically, this phenomenon seems to be driven by the incremental recharge of the hydrothermal reservoir with cold oxygenated water during the day-time.

Throughout the day, concentration of thiosulfate in the vent-water was 4-5 times higher than that of sulfide. This indicated that thiosulfate originates in the deeper parts of the hydrothermal system, even as oxidation of dissolved sulfide to thiosulfate may also happen when the upward-moving hot water mixes with the aerated snow-melts in the shallower parts of the system. Abiotic hydrolysis and/or microbial disproportionation of elemental sulfur (Xu *et al.* 1998), which is abundant in the Puga geothermal system (Shanker *et al.* 1976; Harinarayana *et al.* 2006), may also contribute to the formation of thiosulfate, sulfite and/or sulfate in the ascending hydrothermal fluid. Notably, between 6:30 and 17:30 h, increase in sulfate and concomitant decrease in reduced sulfur species did not lower the pH of the vent-water; instead pH increased from 7.2 to 8.0. Diurnal build-up of acidity is plausibly neutralized via potential alkaline buffering by the bicarbonate and borate salts abundant in the Puga hot spring waters (Garrett 1998; Roy *et al.* 2020).

Fluxes in pH and sulfur speciation along *Lotus Pond's* spring-water transit are influenced primarily by aerial oxidation of the reduced sulfur compounds discharged from the vent; so, these fluctuations are different from the sub-surface-processes-driven fluxes recorded for the same parameters in the vent-water over the day (Table 1; Figure 3C; Table S1). In the vent-to-river trajectory, at both 8:30 and 14:30 h, there were significant increases in the concentrations of thiosulfate and sulfate up to LTP (both the sulfur-species, however, declined sharply in RVW); this was accompanied by steady decreases in dissolved sulfide, sulfite, and pH (sulfide and sulfite were no more there in LTP and RVW, at 8:30 h as well as 14:30 h). These trends are apparently attributable to the rapid oxidation of dissolved sulfide and sulfite to thiosulfate/sulfate and sulfate respectively, during transit from HTP to LTP. Notably, the dissolved sulfates precipitated from the HTP water-samples at 8:30 and 14:30 h had similar sulfur isotope ratios ( $\delta^{34}\text{S}$  values 16.3‰ and 16.5‰ VCDT respectively; Table S6). This, on one hand, reflected the steadiness of the

biogeochemical processes related to sulfur cycling in the sub-surface, while on the other, suggested that sulfate supply is largely in excess of what is plausibly reduced microbially and/or precipitated epithermally from the upward-moving geothermal water. However, despite increases in sulfate concentrations from HTP to LTP, at both 8:30 and 14:30 h,  $\delta^{34}\text{S}$  values remained between +16.1‰ and +16.6‰ VCDT for the sulfates precipitated from MTP and LTP at either sampling-hour (Table S6). Albeit we do not know the relative contributions of sulfide and sulfite in the increased sulfate concentrations along *Lotus Pond's* spring-water transit, it seems plausible from the above data that the cumulative sulfur isotope fractionations rendered by the two oxidative processes do not significantly alter the native sulfur isotope ratio of the vent-water sulfate. As observed for dissolved sulfate, sulfur isotope ratio of dissolved sulfide in the HTP water also remained consistent between 8:30 and 14:30 h ( $\delta^{34}\text{S}$  values 4.6‰ and 4.3‰ VCDT respectively), thereby reiterating the steadiness of the biogeochemical sulfur cycle in the vent's sub-surface. In contrast, however, drop in the sulfide concentration of the spring-water in transit from HTP to MTP (followed by complete extinction in LTP), at both the sampling-hours, and concomitant enrichment of  $^{34}\text{S}$  in the dissolved sulfide ( $\Delta\text{S}^{2-}_{\text{MTP-HTP}}$  was 2.4‰ and 1‰ at 8:30 and 14:30 h respectively; Table S6), were consistent with the potential oxidation of sulfide during this passage. Furthermore, in this context, it is noteworthy that the sulfates precipitated from RVW had lower  $\delta^{34}\text{S}$  values (12.4‰ and 13.1‰ VCDT at 8:30 and 14:30 h respectively; Table S6) than their HTP, MTP and LTP counterparts. This indicated that the sulfate-source and/or sulfur biogeochemistry of the river are different from those of the hot spring system.

Variations in the concentrations of sulfur species along *Lotus Pond's* spring-water transit indicated that opportunities for chemolithotrophic lifestyle were aligned anti-parallel to the hydrothermal gradient offering resistance to microbial colonization. Concurrently, 15-33% of the genera detected at the individual sample-sites, at any sampling-hour, have known sulfur-chemolithotrophic members. Number of genera detected in each water-sample is given in Table S1, while the numbers of sulfur-compounds-oxidizing chemolithotrophic genera detected in each sample is given in Table S6; Tables S4 and S5 list all the sulfur-metabolizing attributes that have been reported thus far for the genera identified across *Lotus Pond's* sample-sites at 8:30 and 14:30 h respectively. The number of sulfur-oxidizing genera present at a sample-site, when considered as a percentage of the total number genera identified in that site, was highest for HTP, at both 8:30 h (33% of total genus-count) and 14:30 h (20% of total genus-count). This parameter was remarkably comparable for MTP, LTP and RVW across the two sampling-hours (15-18% of the genera detected individually in these samples have known sulfur-oxidizing members).

In contrast to the rich diversity of sulfur-oxidizing bacteria present along *Lotus Pond's* hydrothermal gradient, very few genera detected at individual sample-sites, at either sampling-hour of the day, encompass species/strains capable of reducing thiosulfate, sulfite and/or sulfate, according to published literature (Figure 3C; Table S6).

### **3.5 Predominance of mesophiles in Lotus Pond's high-temperature sites**

Compositions of the aquatic microbial communities revealed from all the four sample-sites, at both 8:30 and 14:30 h, were remarkably dominated by such bacterial taxa, members of which are incapable of laboratory growth at  $>45^{\circ}\text{C}$ , according to published pure-culture studies. Out of the 57 genera that were detected exclusively at HTP and/or MTP at 8:30 and/or 14:30 h (see Tables S4 and S5 respectively), 41 have no report of laboratory growth at  $>45^{\circ}\text{C}$  (these are listed in Table S7). The overall count of such mesophilic genera in the  $85\text{-}72^{\circ}\text{C}$  temperature-zone of *Lotus Pond* becomes much higher if we consider the genera that were present at LTP and/or RVW in addition to being present at HTP and/or MTP. For instance, out of the total 47 validly published genera detected in HTP at 8:30 h (all these were present in MTP, LTP and/or RVW as well) (Figure S2A; Table S4), only 3 have reports of laboratory growth at  $>80^{\circ}\text{C}$ , 13 are known to grow in the laboratory at  $>45^{\circ}\text{C}$  but not at  $>80^{\circ}\text{C}$ , 31 have no report of laboratory growth at  $>45^{\circ}\text{C}$  (Table S8). Again, out of the total 100 genera that were there in HTP at 14:30 h [71 of these were present in MTP, LTP and/or RVW as well (Figure S2B; Table S5)], only 3 have reports of laboratory growth at  $>80^{\circ}\text{C}$ , 22 are known to grow at  $>45^{\circ}\text{C}$  but not at  $>80^{\circ}\text{C}$ , 63 have no report of laboratory growth at  $>45^{\circ}\text{C}$ ; and there is no information in the literature regarding the upper temperature-limit of laboratory growth for the remaining 12 (Table S9).

The above data were consistent with the findings of a 2013 exploration of the HTP site where 84 out of the 107 genera identified in metataxonomic analysis were found to have no report of laboratory growth above  $45^{\circ}\text{C}$  (Roy *et al.* 2016). Consistent prevalence of mesophiles in *Lotus Pond's* HTP sites is also corroborated by the findings of an environmental 16S rRNA gene cloning and sequencing-based analysis of the *in situ* bacterial diversity undertaken in August 2009. The 50 environmental 16S rRNA gene clones constructed and sequenced in that study using the Bacteria-specific universal primers 27f and 1492r, had yielded 17 species-level clusters unified at the level 97% 16S rRNA gene sequence similarity. Of the 19 type strains of established bacterial species with which the representative clones of the 17 species-level clusters had maximum sequence similarities, only one have laboratory growth reported at  $50^{\circ}\text{C}$ ; all the others have their upper limit of temperature for *in vitro* growth at  $\leq 45^{\circ}\text{C}$  (refer to Supplementary Table S10 for the summary details of the 16S rRNA gene cloning and sequencing-based analysis conducted in 2009).

### 3.6 Some Lotus Pond mesophiles are alive and viable

When two individual sets of residues, obtained from the filtration of two 1000-mL batches of HTP-water (*in situ* temperature 80°C) at 15:00 h, were enriched separately in MMST and R2A broth media at 37°C,  $10^6$  and  $10^9$  CFUs mL<sup>-1</sup> cultures were obtained after 14 h of incubations respectively. Lag phase of both the mixed-cultures was recorded to be approximately 2 h. These cellular yields for the chemolithoautotrophic MMST- and chemoorganoheterotrophic R2A-based enrichment cultures corresponded to an oxidation of 10 mM thiosulfate to an equivalent amount of sulfate (both equal to 20 mM sulfur) and OD<sub>600</sub> of 0.8, respectively. The 65 and 60 min generation times of the two mixed-cell populations, determined from the above data for the MMST- and R2A-broth-enrichments respectively, were used to calculate the potential numbers of live chemolithotrophic and chemoorganotrophic mesophiles in 1000-mL HTP-water as  $1.5 \times 10^5$  and  $7.5 \times 10^7$  cells respectively. These two numbers, in conjunction with the small time-span within which high CFU numbers were reached mL<sup>-1</sup> of the enrichment cultures, indicated that the mesophile populations of the HTP water-sample not only had high cell-counts, but were also metabolically so healthy as to readily divide upon withdrawal of thermal stress. Mesophilic bacterial strains isolated at 37°C, from the two mixed-culture consortia, via dilution plating and iterative steps of dilution streaking on MMST- and R2A-agar plates have been characterized and reported in another study.

### 4. Discussion

The present study revealed the geomicrobiology of one special category of geographically infrequent and biogeochemically less-explored neutral pH hot spring systems that are mineralogically peculiar in being and silica-poor but boron- and sulfur-rich. Bacterial diversity of the explored spring called *Lotus pond* is remarkably higher than most other spring-waters of the world having equivalent temperatures (Figure 6; Table S11). Among the compared hydrothermal sites having temperatures >70°C, none other than *Lotus Pond* MTP and Egedi spring, Eritrea harbors >700 OTUs. Notably, all the >70°C sites compared harbor <100 OTUs (*Lotus Pond* MTP, Egedi, and Hot Springs 2 and 3 in Lake Magadi, Kenya being the exceptions), whereas all the <70°C sites (except Eiland spring, South Africa) harbor >100 OTUs. Furthermore, spring-waters having near-neutral to moderately-alkaline pH, irrespective of their temperatures, are apparently associated with higher microbial diversity. For instance, in the current comparison, none of the hot spring sites having pH <7, except the Icelandic springs P1-P3, were found to have >100 OTUs (Figure 6).

The bacterial diversities of *Lotus Pond*'s high-temperature sites are not only high but also inclusive of several such species-level entities that are affiliated to genera having no member

reported for laboratory growth at  $>45^{\circ}\text{C}$ . Such mesophilic taxa, though unexpected in high-temperature environments, are not totally alien to hot spring habitats; several studies have revealed their presence in geothermal communities across the world (Baker *et al.* 2001; Jiménez *et al.* 2012; Wemheuer *et al.* 2013; Chan *et al.* 2015; Ghosh *et al.* 2015; Menzel *et al.* 2015; Roy *et al.* 2016, 2020). On the other hand, *Lotus Pond*'s spring-water chemistry revealed in this study is consistent with previous chemical analyses of Puga hot spring discharges, which reported the presence of boron, fluorine, silicon, sodium and sulfate in the vent-waters alongside significant amounts of cesium, lithium and rubidium (Chowdhury *et al.* 1974; Chowdhury *et al.* 1984; Saxena and D'Amore 1984; Shanker *et al.* 1999; Rai 2001). In the geomicrobiological context of *Lotus Pond*'s high habitability it is noteworthy that many of the inorganic solutes present in the spring-water are known for their biomacromolecule-stabilizing effects through efficient hydrogen-bonding and enhancement of weak-interactions such as van der Waals and electrostatic forces (Brown 1990; Koynova *et al.* 1997; Mancinelli *et al.* 2007; Roark *et al.* 2012; Okur *et al.* 2017). It, therefore, seems likely that these geochemicals may *in situ* alleviate the biophysical disruption of cell systems by heat, and in doing so allow phylogenetic relatives of mesophilic bacteria survive high temperatures in the Trans-Himalayan sulfur-boron spring system (Roy *et al.* 2020). Whatever may be the geochemical determinants of microbial survival amidst high heat, our preliminary culture-dependent experiments did corroborate that a portion of *Lotus Pond*'s mesophilic diversity was alive and viable. At the same time, the revealed trends of spatiotemporal fluxes in *Lotus Pond*'s biodiversity, together with those in the spring's physicochemical parameters, indicated that the snow-melts entering the numerous tectonic faults of this region (Navada and Rao 1991; Shanker 1991) transport copious soil-/sediment-/snow-dwelling-bacteria into the hydrothermal reservoir, and these are subsequently discharged along with the vented hot waters (Figure 1). Elsewhere in the world also, hot spring waters have been found to get "contaminated" with microbes from various surface/sub-surface sources (Power *et al.* 2018; Colman *et al.* 2019). In the context of Puga, even if such "contamination" is limited to the shallow hydrothermal reservoir, the apparent geomicrobial dynamics of the system implies that a substantial fraction of that transported diversity survives a several-hours-long journey through an approximately  $160^{\circ}\text{C}$  subsurface environment before emanating through the vents. This in turn indicates that for mesophilic bacterial populations in nature, ecosystem-enabled resilience to heat could be considerably higher than the limits observed for the species under laboratory conditions. In the light of the fact that hydrothermal environments are considered to be the habitats of early Life on Earth (Wächtershäuser 2006; Martin *et al.* 2008), the present finding highlights the significance of the Trans-Himalayan sulfur-boron spring systems in our modular understanding of how Life coped with



biophysically extreme conditions, whether in an ancient or modern evolutionary context, on Earth or in potential extra-terrestrial locations.

### **Acknowledgements**

This research was financed by Bose Institute as well as Science and Engineering Research Board (SERB), Government of India (GoI) (SERB grant numbers were SR/FT/LS-204/2009 and EMR/2016/002703). Sri Pankaj Kumar Ghosh (Chinsurah, West Bengal, India) provided additional travel grants for field expeditions. CR and NM received fellowships from University Grants Commission, GoI and SERB, GoI respectively. On field assistance provided by Baishali Ghosh, Asgar Ali, Rimjhim Bhattacharjee, Srabana Bhattacharjee, Bikash Jana, Lotus Sonam and Amrit Pal Singh, over the decade-long exploration of the Puga geothermal area, is gratefully acknowledged.

### **Supplementary material**

The online version of this article contains supplementary materials.

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## Figure legends

**Figure 1.** Geographical location of the Puga valley; and the hypothetical position of the geothermal reservoirs of the area shown in the context of the subsurface sediment and rock layers, together with the geodynamics associated with the reservoirs. (A) Map of northern India showing the location of the Puga geothermal area (indicated by a red shape) in eastern Ladakh and its distance from Leh (indicated by an open circle), the capital of the union territory of Ladakh. (B) Satellite view of the eastern flank of Puga Valley showing the *Lotus Pond* site (indicated by a red shape), *Rulang* river and Sumdo village. (C) Overview of the stratigraphy, and potential physiography of the tectonic faults which crisscross the area adjacent to Puga Valley. These faults act as the conduits for melting snow-waters (indicated by white droplets) to infiltrate the underlying hydrothermal reservoir (red arrows indicate potential strike-slip faults, while yellow and green arrows indicate potential reverse faults). (D) Expanded view of the stratigraphy showing the following layers from the top: (i) loose valley-fill materials (this layer, indicated by gray background color, extends up to a depth of ~65 m from the surface); (ii) fractured breccia hosting the shallow hydrothermal reservoir: upper portions contain fluvio-glacial moraines and sediments of potential Quaternary origin, reconsolidated by the action of hot geothermal fluids (this layer, indicated by cyan background color, is the potential site of snow-melt:hot-water mixing); in the lower portions, saturated with deep thermal water, fragments of gneiss, schists, phyllite and quartzite (components of the basement) predominate in an argillaceous, siliceous matrix (this layer, indicated by blue background color, occurs at a depth of ~450 m); (iii) fractured basement rocks (indicated by orange background color), through which hot fluids and magmatic bodies from the kilometers-deep rock-melts move upwards via tectonic faults and/or narrow feeder channels (indicated by thick blue lines); (iv) magma chambers and partial rock-melts in the upper and mid-lower Himalayan crust respectively (this layer, indicated by red background color, commences at a depth of 2-2.5 km and extends at least up to ~8 km, from the surface); (v) crustal rocks of subducted Indian continental plate (this layer, indicated by dark red color, occurs below 8 km from the surface). Stratigraphic layers indicated in (C) and (D) are not proportional to their actual thickness; color code used to indicate the sediment and rock layers is same for both (C) and (D). Both (A) and (B) were sourced from the publicly available databases <http://maps.google.com/>, while (C) and (D) were reconstructed based on geophysical information available in the published literature (Navada and Rao 1991; Shanker 1991; Harinarayan 2006; Azeez and Harinarayana 2007).

**Figure 2.** The *Lotus Pond* hot spring, the *Rulang* river, and the nearby microbialite hot spring *Shivlinga*, as seen on the sampling-day, 20 October 2014 [*Shivlinga*'s microbial ecology has been explored elsewhere by Roy *et al.* (2016, 2019, 2020)]. (A) Topography of the eastern flank of Puga Valley, where both *Lotus Pond* and *Shivlinga* are situated. (B) *Lotus Pond* at 8:30 h, showing copious fumarolic activity. (C) *Lotus Pond* at 14:30 h, showing almost no fumarolic activity; positions of the four sample-sites HTP (marked as H), MTP (marked as M), LTP (marked as L) and RVW (marked as R) are indicated by red filled circles. In (A) and (B), the cyan and yellow arrows demarcate the locations of *Lotus Pond* and *Shivlinga* respectively.

**Figure 3.** Glyph star plots mapping the data for the major parameters of bacterial diversity and chemistry determined for the water-samples HTP, MTP, LTP and RVW at 8:30 and 14:30 h of 20 October 2014 (procedure involved in the construction of the glyph plots is given in Supplementary Methods). (A) Glyph plots mapping the normalized values for temperature, pH, number of OTUs detected, number of genera detected, number of phyla/classes detected, concentrations of boron, silicon, lithium, sodium, chloride, calcium, potassium, magnesium, rubidium, strontium, cesium, sulfide, thiosulfate, sulfite, and sulfate (indicated by parameter numbers 1 through 20 respectively), across the sample-sites and sampling-hours; each value under a given parameter was normalized by taking its ratio with the highest value available under that parameter (refer to Table S1 for the actual values of all the 20 parameters across the eight samples). (B) Glyph plots mapping the matrix-



standardized values of the same concentrations for boron, silicon, lithium, sodium, chloride, calcium, potassium, magnesium, rubidium, strontium, cesium, sulfide, thiosulfate, sulfite, and sulfate, that are given in Table S1 [parameter numbers are same as those used in (A)]. (C) Glyph plots mapping the normalized values for (a) pH; (b) concentration of sulfide; (c)  $\delta^{34}\text{S}$  of sulfide; concentrations of (d) thiosulfate, (e) sulfite, (f) sulfate, (g)  $\delta^{34}\text{S}$  of sulfate, (h) number of sulfide-, elemental-sulfur-, thiosulfate- and/or sulfite-oxidizing genera present; (i) number of sulfide-oxidizing genera present; (j) number of elemental-sulfur-oxidizing genera present; (k) number of thiosulfate-oxidizing genera present; (l) number of sulfite-oxidizing genera present; (m) number of thiosulfate-, sulfite- and/or sulfate-reducing genera present; (n) number of thiosulfate-reducing genera present; (o) number of sulfite-reducing genera present; (p) number of sulfate-reducing genera present, across the sample-sites and sampling-hours (refer to Table S6 for the actual values of all the 16 parameters across the eight samples).

**Figure 4.** Phase contrast micrographs showing the microbial forms present in the Gram stained, 8:30 h water-samples of HTP, MTP, LTP and RVW. Two representative microscopic fields each (indicated as “a” and “b”) for all the four samples have been shown. For all the eight micrographs, scale bar equals to 10  $\mu\text{m}$ .

**Figure 5.** Microbial community architecture revealed along *Lotus Pond*'s hydrothermal gradient: phylum-/class-level distribution of the OTUs identified in HTP, MTP, LTP and RVW at (A) 6:30 h, (B) 8:30 h, (C) 14:30 h and (D) 20:30 h; color-code for the identification of taxa is common for all the four panels.

**Figure 6.** Bubble plot comparing the *in situ* temperature, pH, and OTU-count of the various *Lotus Pond* samples with the similar parameters reported for other geographically-discrete terrestrial hot springs (procedure involved in the construction of the bubble plot is given in Supplementary Methods). Temperature is plotted along the X-axis, pH along the Y-axis, while individual springs are represented by circles numbered 1 through 37. Size of the circles is proportional to the OTU-count of the springs. Circles representing the *Lotus Pond* sample-sites are in red. Number code for springs: (1) Diretiyanqu, China; (2) Jiemeiquan - Sisters Spring, China; (3) Zhenzhuquan - Pearl Spring, China; (4) Huitaijing - Pregnancy Spring, China; (5) Shuirebaozha - Hydrothermal Explosion, China; (6) Dagunguo - Great Boiling Pot, China; (7) Gumingquan - Drum Beating Spring, China; (8) Gongxiaoshe - Co-op Hotel (side), China; (9) Jinze - Golden Pond Motel, China; (10) Jermuk, Southeast Armenia; (11) Arzakan, Southeast Armenia; (12) Lake Magadi, Hot spring 1, Kenya; (13) Lake Magadi, Hot spring 2, Kenya; (14) Lake Magadi, Hot spring 3, Kenya; (15) Mqhephu hot spring, Limpopo Province of South Africa; (16) Tshipise hot spring, Limpopo Province of South Africa; (17) Sagole hot spring, Limpopo Province of South Africa; (18) Siloam hot spring, Limpopo Province of South Africa; (19) Soutinig hot spring, Limpopo Province of South Africa; (20) Eiland hot spring, Limpopo Province of South Africa; (21) Great Boiling Spring 1002W, Great Basin, USA; (22) Akwar, Eritrea; (23) Egededi, Eritrea; (24) Garbanabra, Eritrea; (25) Gelti, Eritrea; (26) Maiwooi, Eritrea; (27) Icelandic hot springs P0, Iceland; (28) Icelandic hot springs P1, Iceland; (29) Icelandic hot springs P2, Iceland; (30) Icelandic hot springs P3, Iceland; (31) Leirgerdur, Iceland; (32) Hrifla spring, Iceland; (33) *Lotus Pond* HTP, as explored at 17:30 h of 23.07.2013; (34) *Lotus Pond* HTP, as explored at 8:30 h of 20.10.2014; (35) *Lotus Pond* HTP, as explored at 14:30 h of 20.10.2014; (36) *Lotus Pond* MTP, as explored at 8:30 h of 20.10.2014; (37) *Lotus Pond* MTP, as explored at 14:30 h of 20.10.2014. Refer to Table S11 for the references from where all this information were sourced.

**Table 1.** Physicochemical properties<sup>†</sup> of the sample-sites explored along *Lotus Pond*'s spring-water transit representing a hydrothermal gradient in the vent-to-river trajectory (sampling date: 20.10.2014<sup>§</sup>).

	<b>Sample-sites and their distance from the vent-center</b>			
	<b>HTP</b>	<b>MTP</b>	<b>LTP</b>	<b>RVW</b>
	0 m from the vent-center	1 m from the vent-center	1.5 m from the vent-center	1.8 m from the vent-center
<b>Hour of sampling</b>	<b>Temperature (in °C), flow rate (in L s<sup>-1</sup>), pH, OTU-count</b>			
<b>06:30<sup>#</sup></b>	85, 2.0, 7.2, 96	78, ND, 7.2, 584	30-45, ND, 7.0, 735	15, ND, 6.8, 933
<b>08:30<sup>#</sup></b>	84, 2.5, 7.5, 109	74, ND, 7.4, 635	30-45, ND, 7.2, 1013	15, ND, 6.6, 1372
<b>10:30</b>	83, 3.0, 7.6, ND	72, ND, 7.5, ND	30-40, ND, 7.0, ND	17, ND, 6.2, ND
<b>14:30<sup>#</sup></b>	81, 3.5, 7.8, 279	72, ND, 7.5, 1205	32-42, ND, 6.8, 1691	24, ND, 6.2, 2044
<b>17:30</b>	78, 3.8, 8.0, <u>331</u> *	70, ND, 7.7, ND	25-32, ND, 6.5, ND	16, ND, 6.0, ND
<b>20:30<sup>#</sup></b>	82, 3.3, 7.7, 237	72, ND, 7.6, 1158	30-40, ND, 7.2, 1407	14, ND, 6.5, 1166
	<b>Concentrations of sulfide (in μM), thiosulfate (in mM), sulfite (in μM), sulfate (in μM)</b>			
<b>06:30<sup>#</sup></b>	312, 1.6, 344, 693	– ND –	– ND –	– ND –
<b>08:30<sup>#</sup></b>	262, 1.3, 273, 712	120, 1.4, 140, 920	0, 1.5, 0, 1152	0, 0.8, 0, 688
<b>10:30</b>	233, 1.1, 195, 851	– ND –	– ND –	– ND –
<b>14:30<sup>#</sup></b>	178, 0.8, 110, 963	93, 0.9, 15, 1115	0, 1.0, 0, 1281	0, 0.5, 0, 722
<b>17:30</b>	115, 0.5, 24, 1140	– ND –	– ND –	– ND –
<b>20:30<sup>#</sup></b>	150, 0.7, 96, 883	– ND –	– ND –	– ND –

**Table 1.** Physicochemical properties<sup>†</sup> of the sample-sites explored along *Lotus Pond*'s spring-water transit representing a hydrothermal gradient in the vent-to-river trajectory (sampling date: 20.10.2014<sup>§</sup>).

<sup>†</sup> Values given for all the individual parameters are averages of the data obtained from three sampling replicates carried out on 20.10.2014; all the individual data had standard deviations <5% of their corresponding means.

<sup>§</sup> Values of the physicochemical parameters recorded at the sample-sites - HTP, highest temperature point; MTP, moderately-high temperature point; LTP, lowest temperature point; and RVW, river water - on 20.10.2014, were closely similar to those recorded at equivalent locations during the annual explorations of *Lotus Pond* between 2008 and 2013, and then again between 2015 and 2017 (for all the individual parameters, deviations over the years were <5% of the mean values given in this Table).

<sup>#</sup>At 6:30, 8:30, 14:30 and 20:30 h of 20.10.2014, the four sample-sites were studied for both water-chemistry and microbiology; at the other sampling-hours of the day, the sites were studied only for their water-chemistry.

\* 331 OTUs were identified in a separate study of the HTP site (Roy *et al.* 2016) conducted at 17:30 h of a previous sampling day (23.07.2013) when temperature, flow rate and pH at HTP were comparable to those recorded at the same hour of 20.10.2014.

phyla/classes present in the system; others were referred to as minor phyla.

**Fig. 1**

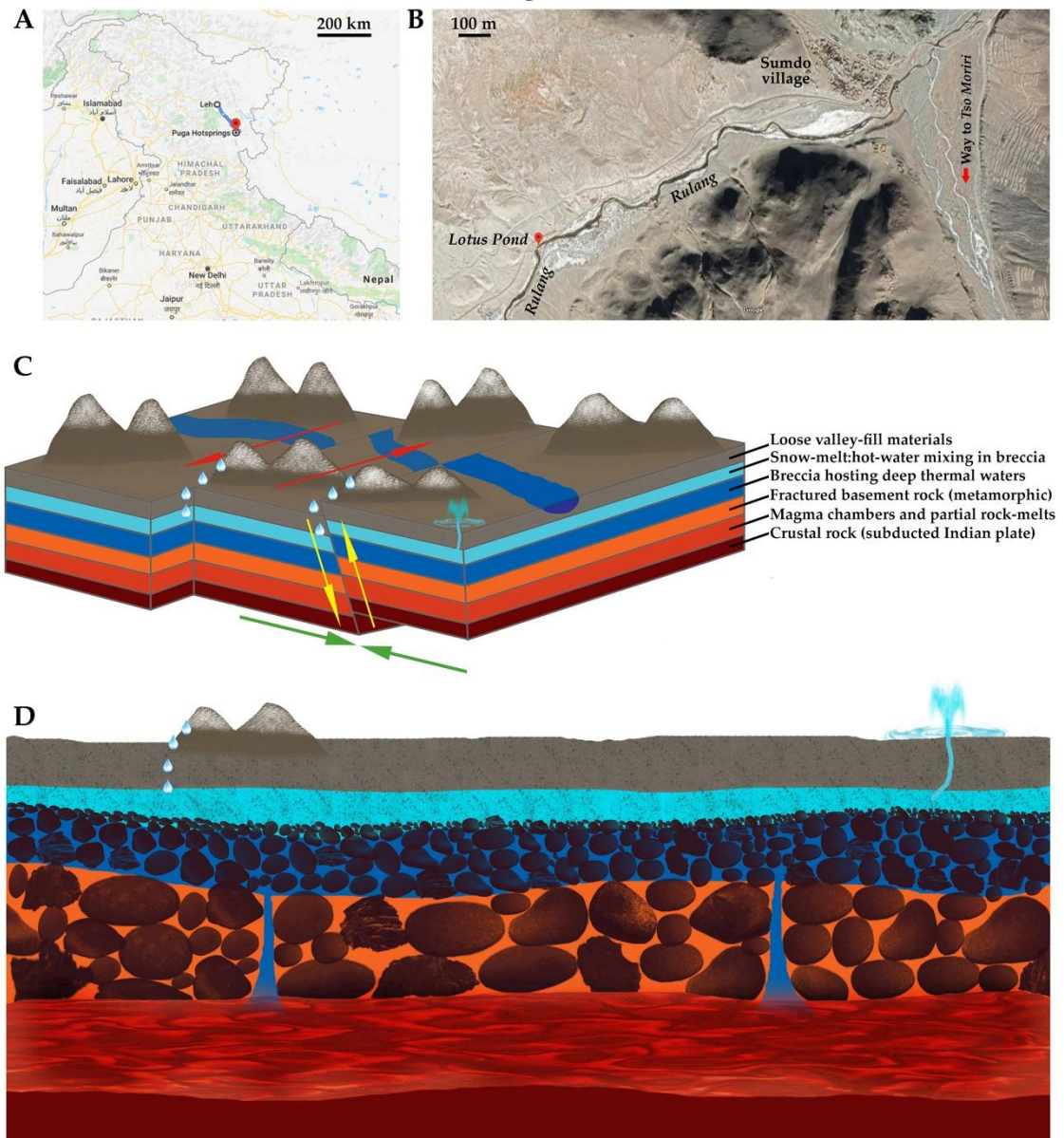
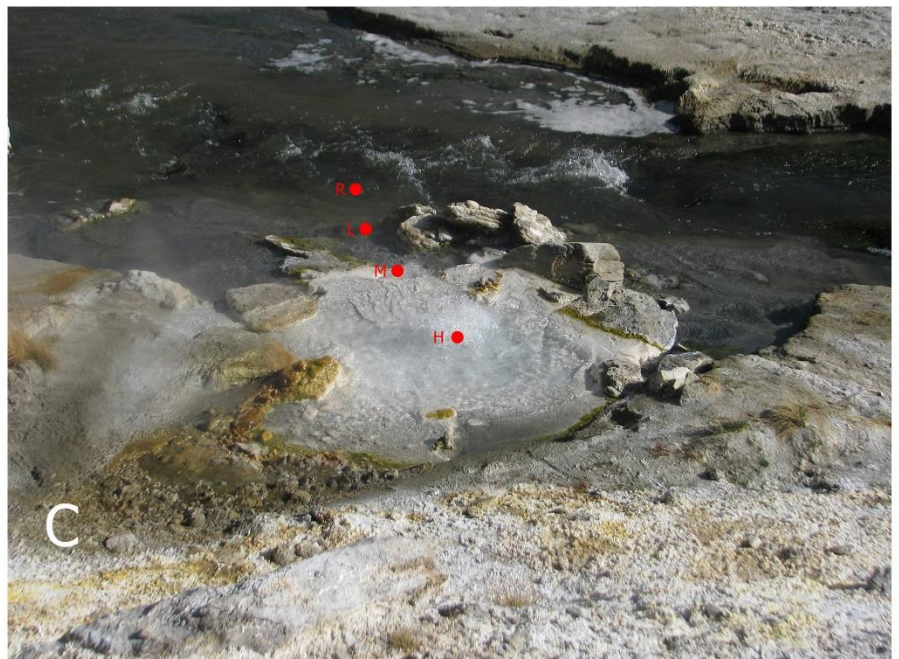
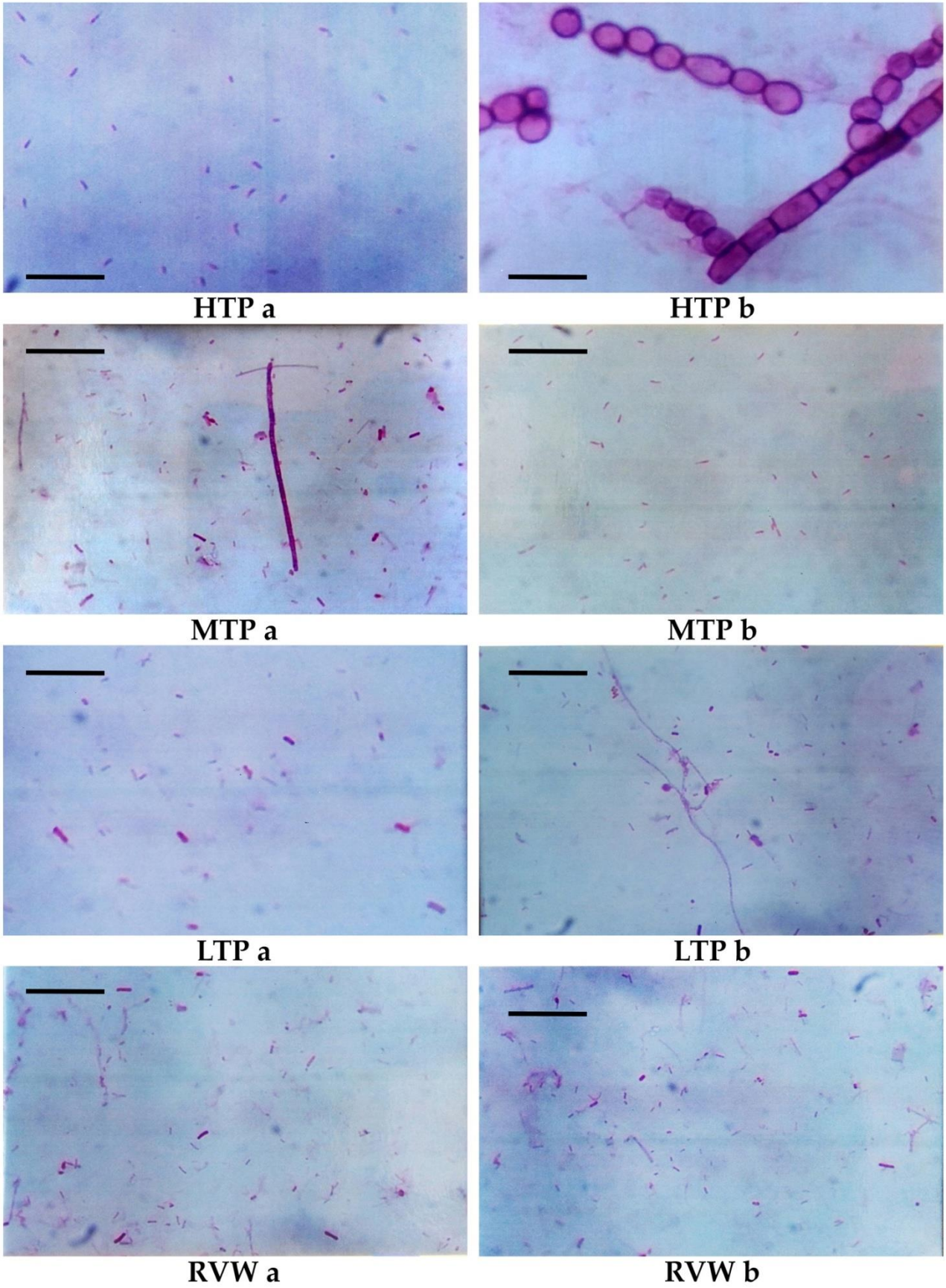


Fig. 2







**Fig 4**

Fig 5

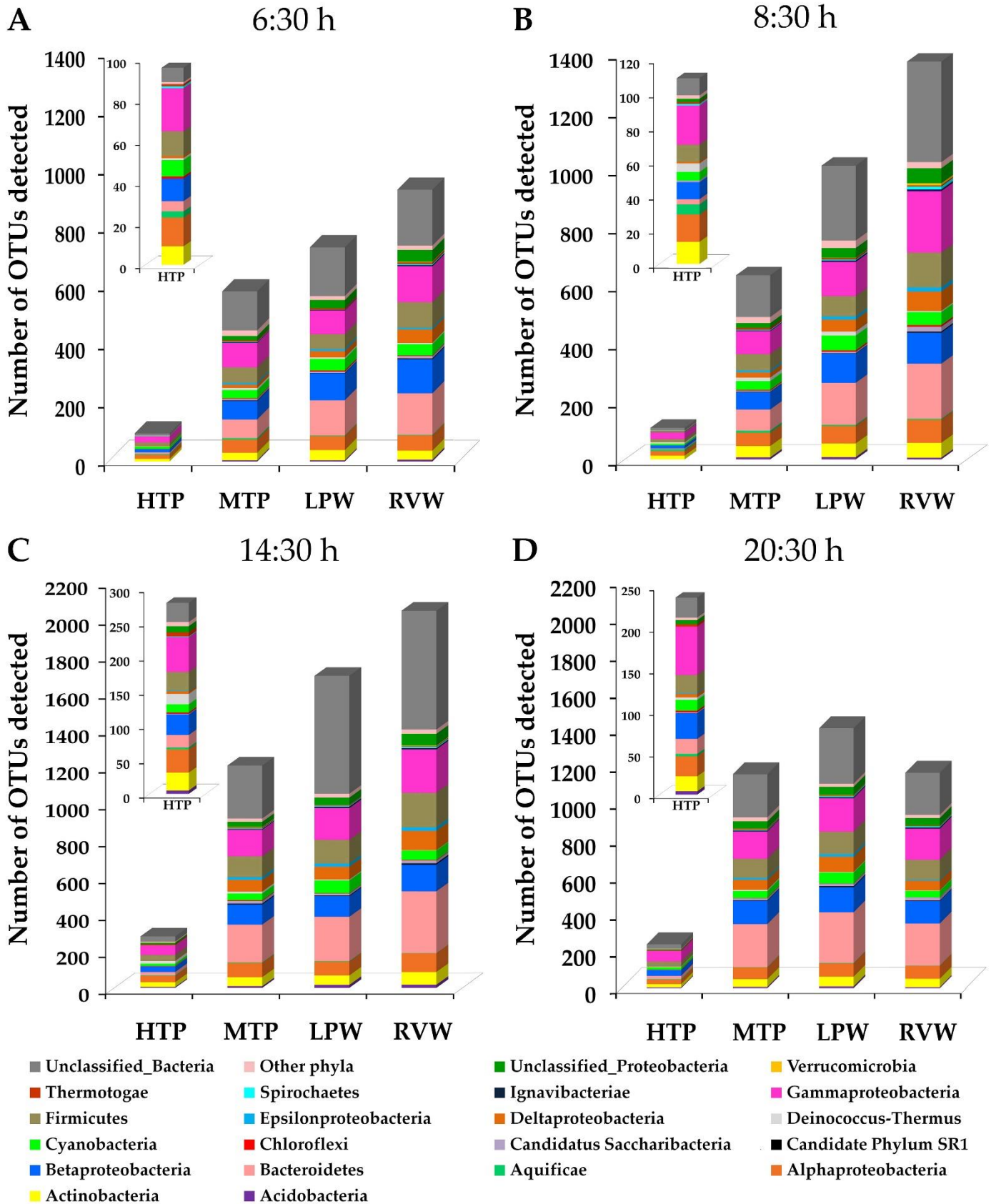
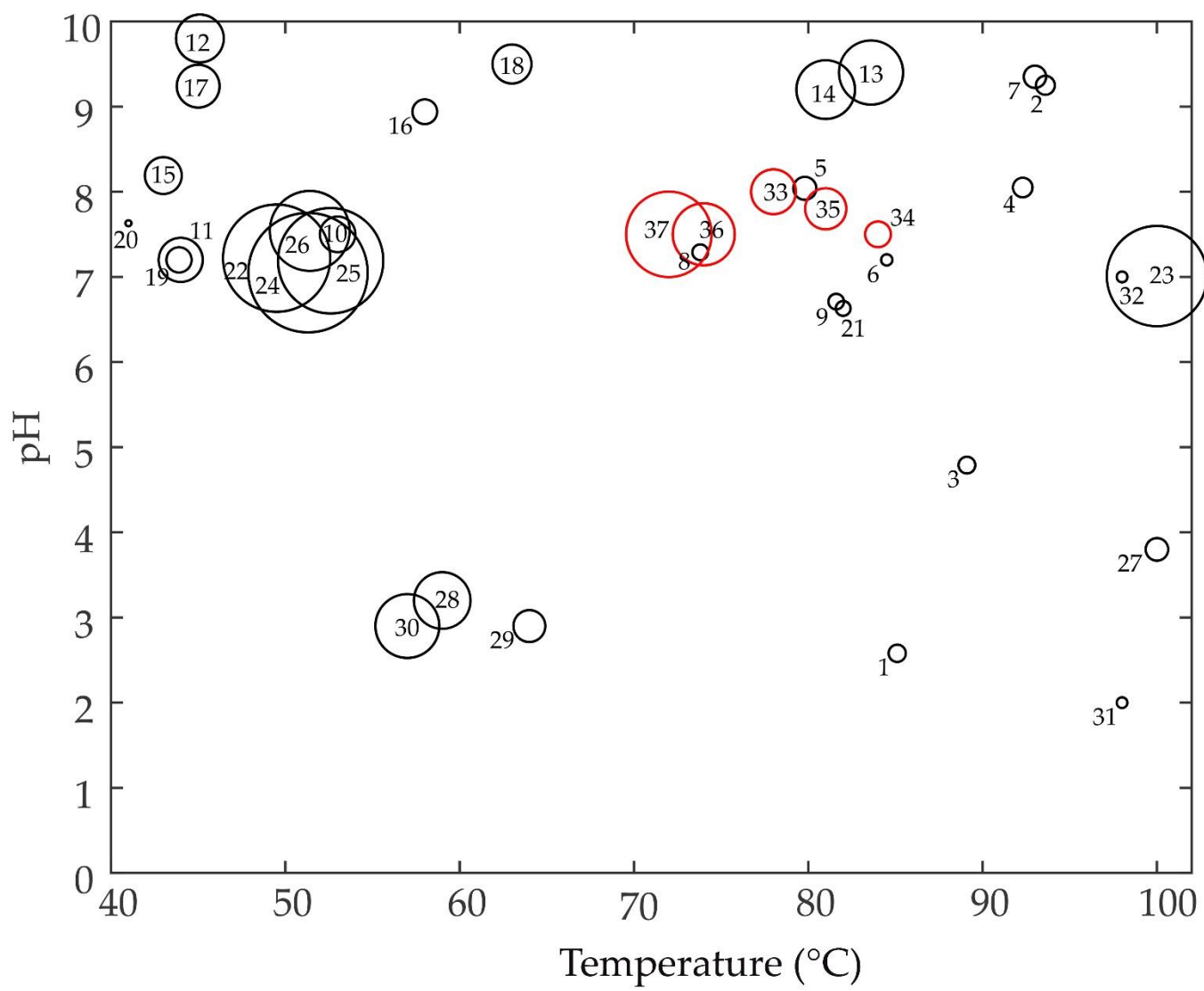


Fig 6





## Supplementary material

### Geomicrobial dynamics of Trans-Himalayan sulfur-borax spring system reveals mesophilic bacteria's resilience to high heat

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## Contents

### Supplementary Methods

Preparation of total environmental DNA from water-samples.

Assessment of microbial diversity within the *Lotus Pond-Rulang* ecosystem.

Bubble and Glyph plots.

References used in Supplementary Methods.

### Supplementary Result

Distribution of unique and shared genera across the sample-sites at 8:30 and 14:30 h.

### Supplementary Note

Additional features of spatiotemporal distribution of genera across the *Lotus Pond-Rulang* ecosystem which suggest that influx of new bacteria from the vent as well as the river, into the hydrothermal territory, increase with the progress of the day.

### Supplementary Tables

**Table S1.** Major parameters of bacterial diversity and water chemistry measured for the HTP, MTP, LTP and RVW water-samples at 8:30 and 14:30 h of 20 October 2014.

**Table S2.** Summary statistics of the OTU analysis of the aquatic communities of HTP, MTP, LTP and RVW conducted at 8:30 and 14:30 h of 20 October 2014.

**Table S3.** Phylum-/class-level assortment of the total number of bacterial OTUs identified at the different sample-sites of the *Lotus Pond-Rulang* ecosystem at 8:30 and 14:30 h of 20 October 2014. Since this Table is more than one page long it has been provided as an MS Excel file named Supplementary\_Dataset.

**Table S4 and S5.** Complete list of the genera identified in the *Lotus Pond-Rulang* ecosystem at 8:30 and 14:30 h of 20 October 2014, together with the information about their presence or absence in the four sample-sites, and their capabilities, if any, to oxidize or reduce sulfur species.

**Table S6.** Concentrations of various redox species of sulfur, and number of sulfur-oxidizing and sulfur-reducing genera present, at the different sample-sites of the *Lotus Pond-Rulang* ecosystem at 8:30 and 14:30 h of 20 October 2014.

**Table S7.** The *Lotus Pond* genera that were detected exclusively at HTP and/or MTP, at 8:30 and/or 14:30 h of 20 October 2014; information about the upper temperature-limit for their laboratory growth is also given.

**Tables S8 and S9.** Highest temperature reported for the laboratory growth of the bacterial genera detected in HTP at 8:30 and 14:30 h of 20 October 2014, respectively.

**Table S10.** Summary of the PCR-amplified 16S rRNA gene cloning and sequencing-based analysis of the aquatic community present at the HTP site in August 2009.

**Table S11.** Number of species-level microbial entities (OTUs) reported from geographically-discrete terrestrial hot springs.

### Supplementary Figures

**Figure S1.** Rarefaction curves showing the proportionality between OTU-level diversity revealed and the number of amplified 16S rRNA gene sequence (V3 region) reads analyzed for the individual water-samples collected from HTP, MTP, LTP and RVW separately at 8:30 and 14:30 h of 20 October 2014.

**Figure S2.** The numbers of genera that are unique to the different sites or common between the sites, across HTP, MTP, LTP and RVW, at 8:30 and 14:30 h.

### Supplementary Methods

#### Preparation of total environmental DNA from water-samples

Total environmental DNA was extracted from the microbial cells arrested in the 0.22  $\mu\text{m}$  cellulose acetate filters, thereby ensuring that no cell-free DNA from the spring/river water-samples infiltrated the preparation (Roy et al., 2016). Each filter was cut into small pieces with sterile scissors, within the TE-containing 8 mL cryovial in which it was brought from Puga. The cryovial was vortexed for 30 min, following which the filter shreds were discarded, and the residual TE was distributed equally into five 1.5 mL microfuge tubes. Each 1.5 mL microfuge tube was centrifuged (10,800 g for 30 min, at 4°C) and then its upper 900  $\mu\text{L}$  supernatant was discarded; the residual 100  $\mu\text{L}$  TE was vortexed for 15 min. Subsequently, the contents of all the five 1.5 mL microfuge tube were pooled up in a fresh 1.5 mL microfuge tube. The pooled TE (500  $\mu\text{L}$ ) was again centrifuged (10,800 g for 30 min, at 4°C), following which the upper 400  $\mu\text{L}$  supernatant was discarded. The remaining 100  $\mu\text{L}$  contained all of the microbial cells that were present in the original 1000 mL water-sample. Total environmental DNA was isolated from this 100  $\mu\text{L}$  cell suspension by the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's protocol. Total environmental DNA yield from all the individual water-samples was  $<30 \text{ ng } 1000 \text{ mL}^{-1}$ , which was quantitatively insufficient for direct shotgun sequencing; so, their microbial diversities were revealed by PCR-amplification and sequencing of the V3 regions of all 16S rRNA genes present in the environmental DNA preparations.

#### Assessment of microbial diversity within the *Lotus Pond-Rulang* ecosystem

V3 regions of all bacterial 16S rRNA genes present in an environmental DNA preparation were PCR amplified using domain-specific universal oligonucleotides, following the fusion primer protocol described previously (Ghosh et al., 2015; Roy et al., 2016; Fernandes et al., 2018). Amplification was carried out using a 16S forward primer prefixed with an Ion Torrent adapter and a unique sample-specific barcode or multiplex identifier in the following 5' to 3' organization: (a) a 26-mer A-linker followed by a 4-mer A-linker key common for all sample-specific primers (see bases in bold fonts in the sequences given below), (b) a 10-mer barcode unique to each sample-specific primer,

which is followed by a common 3-mer barcode adaptor (all denoted as stars in the sequences below), and then (c) the relevant domain-specific universal forward primer in its 5' to 3' direction (see underlined bases in the sequences given below). A reverse primer, in contrast, had (a) a common trP1 adaptor (see bases represented in italics), followed by (ii) the relevant domain-specific universal reverse primer in its 5' to 3' direction (see underlined bases in the sequences given below). In this way, V3 portions of all bacterial 16S rRNA genes present in a sample DNA were amplified using the forward primer 5' - **CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG \*\*\* \*\*** and the reverse primer 5' - *CCT CTC TAT GGG CAG TCG GTG ATA TTA CCG CGG CTG CTG G* - 3' (where the underlined segments represent the universal primers 341f and 515r respectively).

PCR products from all the samples were pooled up at equal concentrations for sequencing on Ion PGM (Thermo Fisher Scientific). Prior to sequencing, size distribution and DNA concentration within the amplicon pool was checked using a Bioanalyzer 2100 (Agilent Technologies) and adjusted to 26 pM. Amplicons were then attached to the surface of Ion Sphere Particles (ISPs) using an Ion Onetouch 200 Template kit (Thermo Fisher Scientific). Manually enriched, templated-ISPs were then sequenced by PGM on an Ion 316 Chip for 500 flows. Before retrieval from the sequencing machine, all reads were filtered by the inbuilt PGM software to remove low-quality and polyclonal-sequences; sequences matching the PGM 3' adaptor were also trimmed. Quality-filtered readsets were exported as fastq files for downstream analyses. The individual sequence files were deposited to Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA), under the BioProject accession number PRJNA296849, with the distinct run accession numbers listed in Supplementary Tables S3.

All V3 sequence reads were filtered a second time for high quality value (QV 20) and length threshold of 100 bp; selected reads were then converted to fasta from fastq using Fastx\_toolkit 0.0.13.2 ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)). Reads obtained for the individual water-samples were clustered into OTUs unified at the 97% 16S rRNA gene sequence similarity level using the various modules of UPARSE (Edgar, 2013), and singletons were discarded. A Perl programming-script, available within UPARSE, was used to determine the Abundance-based Coverage Estimator, and Shannon and Simpson Indices. Rarefaction analysis was carried out using the Vegan Package in R (R Core Team, 2014). The consensus sequence of every OTU was taxonomically classified by the RDP Classifier tool located at <http://rdp.cme.msu.edu/classifier/classifier.jsp>.

### Bubble and Glyph plots

For visual comprehension of the complex information contained in our multidimensional data Glyph and Bubble charts/plots were constructed (Few, 2009; Kelleher and Wagener, 2011; Ware, 2012; Kosara and Mackinlay, 2013). Bubble chart is one category of scatter plot, where one can depict three-dimensional data in a two-dimensional space by using the X and Y axes for two independent variable and size of bubbles for the dependent variable. Multi series bubble chart can also be plotted with different bubble colors/shades to include more than one dependent variable (Few, 2009). The single series bubble plot shown in Figure 6 was constructed using the *Scatter* tool in 'MATLAB 2017b' (Martinez et al., 2017); the syntax used was:

```
scatter(datafile.Temperature(1:32), datafile.pH(1:32), datafile.OTUs(1:32),'k');
```

hold on

```
scatter(datafile.Temperature(33:end), datafile.pH(33:end), datafile.OTUs(33:end),'r');
```

Glyph plots were constructed for visual comparison of the multidimensional geomicrobiological data. A glyph conveys every aspect of a multidimensional data mapped in terms of features such as position, length/size, shape, color, etc. (Ribarsky et al., 1994). To construct the

various Glyph stars of Figure 3, the *Glyphplot* package in ‘MATLAB 2017b’ (Martinez et al., 2017) was used with the syntax:

```
glyphplot(data, 'glyph', 'star', 'Color', 'black', 'standardize', 'column', 'centers', [<co-ordinates of the glyph centres>], 'radius', <radius of the glyphs>).
```

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### Supplementary Result

#### Distribution of unique and shared genera across the sample-sites at 8:30 and 14:30 h

At both the sampling-hours, remarkably high number of genera was found to be present simultaneously in MTP, LTP and RVW: at 8:30 h, 72 out of the total 267 genera detected in the entire *Lotus Pond-Rulang* system; and at 14:30 h, 111 out of the total 316 genera detected in the system, were present at all these three sites. On the other hand, at 8:30 and 14:30 h, 36 and 41 genera were found to be simultaneously present in all four sample-sites respectively. These distributions, together with the presence of unique genera at both ends of the gradient (i.e. at HTP and RVW), and genera that were common and restricted to HTP and MTP, or LTP and RVW, insinuated that movement of bacteria, in this ecosystem, plausibly happens along the vent-to-river as well as river-to-vent trajectories throughout the day. While microbial transport in the vent-to-river trajectory is apparently driven by the hot-water flow, the reverse traffic potentially involves inching of microbes along the wobbly sub-aquatic sediments/sinters, if not swimming against the current of spring-water transit. Another mechanism of microbial flux in the river-to-vent trajectory could be washing of microbes into to the LTP from cooler zones near the banks of the outflow channel, which are connected to the river-water and not always agitated by the hot-water outflow. Supplementary Note discusses additional features of spatiotemporal distribution of genera that suggest that influx of new bacteria from the vent as well as the river (into the hydrothermal territory) increase with the progress

of the day. Furthermore, consistent presence of 28 bacterial genera at both HTP and MTP (irrespective of whether they were also present in LTP and/or RVW) across the forenoon-afternoon divide reflected the consistency of the microbiome in time and space. Likewise, another 103 genera were found to be present in at least one of the two  $\geq 72^{\circ}\text{C}$  sites (i.e. HTP or MTP) at 8:30 as well as 14:30 h, irrespective of whether they were also present in LTP and/or RVW (Tables S4 and S5 show the sample-site-wise distributions of the total 267 and 316 genera identified in the *Lotus Pond-Rulang system* at 8:30 and 14:30 h respectively).

### Supplementary Note

Additional features of spatiotemporal distribution of genera across the *Lotus Pond-Rulang* ecosystem (refer to Figure S2, and Table S4 and S5) which suggest that influx of new bacteria from the vent as well as the river, into the hydrothermal territory, increase with the progress of the day.

- At 8:30 h, only 5 genera were found to be unique to the HTP, while 38 were unique to the RVW. At 14:30 h, 29 and 27 genera were unique to the HTP and RVW respectively.
  - Of the 5 genera unique to the HTP at 8:30 h, 2 were still present as unique to the HTP at 14:30 h while the other 3 were not found anywhere between HTP and RVW, and seemed to have been carried away by the hot-water flow.
  - On the other hand, out of the 29 genera found as unique to the HTP at 14:30 h, 19 were not present in any of the sample-sites at 8:30 h; so they were likely to have been transported along with the sub-surface geothermal waters over the day (the other 10 however, were already there in one or more sample-sites at 8:30 h).

**These distribution patterns indicated that the vent-water's contribution to the influx of new bacteria into the *Lotus Pond* ecosystem increases with the day.**

- Of the 38 genera unique to the RVW at 8:30 h, 27 were found in MTP and/or LTP at 14:30 h, irrespective of whether they were also found in RVW; 1 was found as unique to the HTP while 10 were not detected in any of the sample-sites, not even in RVW.

**This suggested that in the course of the day, some of the river bacteria potentially migrate into the hydrothermal territory even as some get carried away by the river current.**

- Out of the 27 genera unique to RVW at 14:30 h, 19 were nowhere there between the vent and the river at 8:30 h; 6, however, were present at HTP and/or MTP, but not at LTP or RVW; the remaining 2 were present at MTP, LTP and RVW.

**This suggested that very few river bacteria possibly migrate into the hydrothermal territory over the night.**

- Out of 31 genera found as common and restricted to LTP and RVW at 8:30 h, 23 were detected at MTP through RVW at 14:30 h, while 3 were found at HTP through RVW, 2 at MTP and RVW, and 1 each at MTP and LTP, and LTP alone (only 1 was not detected at any of the sample-sites).

**This distribution pattern buttressed the possibility of migration of copious river bacteria into the high temperature zones of *Lotus Pond*'s hydrothermal gradient over the day-time.**

- Out of 12 genera found as common and restricted to LTP and RVW at 14:30 h, 5 were present at HTP or MTP, irrespective of whether they were also found in LTP or RVW; 2 each were found as unique to LTP and RVW while 1 was not detected in any of the sample-sites.
- At 8:30 h, no genus was found as common and restricted to HTP, MTP and LTP, even though all other combinations plausible for the distribution of unique and shared genera across the sample-sites were detected. At 14:30 h, however, all plausible combinations for the distribution of unique and shared genera across the sample-sites were detected, including 8 genera common and restricted to HTP, MTP and LTP.

**This pattern was indicative of microbial transport in the vent-to-river trajectory during the day-time.**

### Supplementary Tables

**Table S1.** Major parameters of bacterial diversity and water chemistry measured for the HTP, MTP, LTP and RVW water-samples at 8:30 and 14:30 h of 20 October 2014.

Serial no.	Physicochemical / microbiological parameters	8:30 h				14:30 h			
		HTP	MTP	LTP	RVW	HTP	MTP	LTP	RVW
1	Temperature (in °C)	84	74	30-45	15	81	72	32-42	24
2	pH	7.5	7.4	7.2	6.6	7.8	7.5	6.8	6.2
3	No. of OTUs detected	109	635	1013	1372	279	1205	1691	2044
4	No. of genera detected	52	160	177	195	100	211	216	219
5	No. of phyla/classes detected	15	34	33	32	20	30	31	34
6	B conc. in water (in ppm)	123.9	145.5	104.4	62.6	126.7	128.8	110.3	66.6
7	Si conc. in water (in ppm)	65.2	67	53.9	36.7	66.7	67	59.3	36
8	Li conc. in water (in ppm)	4.7	5	3.9	2	4.7	4.6	3.6	2.1
9	Na conc. in water (in ppm)	576	521.6	451.3	258	553.5	546.8	531.3	266.8
10	Cl conc. in water (in ppm)	358.8	363.5	287.7	176	370.6	373	320	161
11	Ca conc. in water (in ppm)	2.6	2.6	2.4	2.4	2.1	2.5	2.2	2.2
12	K conc. in water (in ppm)	76.4	89.7	62.8	39.8	75	77.9	69.5	40
13	Mg conc. in water (in ppm)	2.5	2.9	2.9	2.9	2.5	2.5	2.7	2.3
14	Rb conc. in water (in ppm)	1.2	1.4	1	0.6	1.2	1.2	1.1	0.6
15	Sr conc. in water (in ppm)	0.4	0.4	0.3	0.2	0.4	0.4	0.3	0.2
16	Cs conc. in water (in ppm)	9.3	9.2	5.9	3.3	7.5	7.7	6.5	3.5
17	S <sup>2-</sup> conc. in water (in ppm)	8.4	3.8	0	0	5.7	3	0	0
18	S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> conc. in water (in ppm)	145.8	157	168.2	89.7	89.7	100.9	112.1	56.1
19	SO <sub>3</sub> <sup>2-</sup> conc. in water (in ppm)	21.9	11.2	0	0	8.8	1.2	0	0
20	SO <sub>4</sub> <sup>2-</sup> conc. in water (in ppm)	68.4	88.4	110.7	66.1	92.5	107.1	123.1	69.4

**Table S2.** Summary statistics of the OTU analysis of the aquatic communities of HTP, MTP, LTP and RVW conducted at 6:30, 8:30, 14:30 and 20:30 h of 20 October 2014.

Sample Identity	SRA BioSample Accession number	SRA Run Accession number	Total reads	Reads after processing	Reads after de-replication	Total OTUs after removing singletons	Number of Singletons	ACE estimation	Shannon index	Simpson index
<b>Samples collected at 6:30 h</b>										
<b>HTP</b>	SAMN06833865	SRR5482141	37489	18756	11323	96	2131	96	2.8610	0.1170
<b>MTP</b>	SAMN06833866	SRR5482154	100017	53241	28734	584	10929	584	3.7551	0.0855
<b>LTP</b>	SAMN06833867	SRR5482155	125456	62154	35433	735	9563	735	4.2091	0.0637
<b>RVW</b>	SAMN06833868	SRR5482156	119111	55950	29371	933	11323	933	4.6294	0.0450
<b>Samples collected at 8:30 h</b>										
<b>HTP</b>	SAMN06833873	SRR5482162	32864	16009	9363	109	910	109	2.6510	0.1665
<b>MTP</b>	SAMN06833874	SRR5482163	128000	90881	43950	635	4287	635	3.3404	0.1274
<b>LTP</b>	SAMN06833875	SRR5482164	217681	144153	68249	1013	6986	1013	3.7149	0.1113
<b>RVW</b>	SAMN06833876	SRR5482165	228024	157463	84109	1372	7396	1372	4.4025	0.0705
<b>Samples collected at 14:30 h</b>										
<b>HTP</b>	SAMN06833877	SRR5482166	156408	72176	42278	279	4619	279	3.6146	0.0742
<b>MTP</b>	SAMN06833878	SRR5482167	128062	92155	53011	1205	4651	1205	4.9466	0.0389
<b>LTP</b>	SAMN06833879	SRR5482168	215123	123390	60193	1691	5434	1691	4.2222	0.1007
<b>RVW</b>	SAMN06833880	SRR5482169	228477	168605	87944	2044	8413	2044	4.9473	0.0486
<b>Samples collected at 20:30 h</b>										
<b>HTP</b>	SAMN06833869	SRR5482158	93130	46578	25454	237	4497	237	3.7464	0.0642
<b>MTP</b>	SAMN06833870	SRR5482159	115632	55190	30872	1158	11760	1158	5.3514	0.0202
<b>LTP</b>	SAMN06833871	SRR5482160	187074	98723	51289	1407	15445	1407	4.7819	0.0512
<b>RVW</b>	SAMN06833872	SRR5482161	105051	50626	40938	1166	12283	1166	5.1711	0.0302



**TableS3- Copy from last page-**

**Table S4.** Complete list of the 267 genera identified in the *Lotus Pond-Rulang* ecosystem at 8:30 h of 20 October 2014, together with the information about their presence (green shade) or absence (no shade) in the four sample-sites, and their capabilities, if any, to oxidize or reduce sulfur species.

Genus	HTP	MTP	LTP	RVW	Sulfur species oxidized for chemolithotrophic nutrition (Reference)	Sulfur species reduced for anaerobic respiration (Reference)
<i>Aquabacterium</i>						
<i>Leifsonia</i>						
<i>Ralstonia</i>						
<i>Allochromatium</i>					S <sup>0</sup> , HS <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Dahl <i>et al.</i> , 2005)	
<i>Anaerolinea</i>						
<i>Armatimonadetes_gp7</i>						
<i>Caldilinea</i>						
<i>Conexibacter</i>						
<i>Desulfobulbus</i>						SO <sub>4</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Sorokin <i>et al.</i> , 2012)
<i>Silanimonas</i>						
<i>Thermosyntropha</i>						
<i>Alistipes</i>						
<i>Carnobacterium</i>						
<i>Chrysiogenes</i>						
<i>Clostridium XI</i>						
<i>Clostridium XIVa</i>						
<i>Deefgea</i>						
<i>Dethiosulfatibacter</i>						S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Takii <i>et al.</i> , 2007)
<i>Dickeya</i>						
<i>Giesbergeria</i>						
<i>Gillisia</i>						
<i>Gp7</i>						
<i>Hyphomicrobium</i>						
<i>Megamonas</i>						
<i>Methylocystis</i>						
<i>Methylomonas</i>						
<i>Nitriliruptor</i>						
<i>Peredibacter</i>						
<i>Planomicrobium</i>						
<i>Polaromonas</i>						
<i>Polynucleobacter</i>						
<i>Prevotella</i>						

<i>Rheinheimera</i>						
<i>Rhizobacter</i>						
<i>Rhodoferax</i>						
<i>Rickettsia</i>						
<i>Rubrivivax</i>						
<i>Saccharofermentans</i>						
<i>Shewanella</i>						
<i>Sulfurospirillum</i>						S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> (Kodama <i>et al.</i> , 2007)
<i>Sunxiuqinia</i>						
<i>Thiocystis</i>					S <sup>0</sup> , HS <sup>-</sup> (Peduzzi <i>et al.</i> , 2011)	
<i>Acetobacterium</i>						
Acetothermia_genera_incertae_sedis						
<i>Acidovorax</i>						
<i>Aeromonas</i>						
<i>Algoriphagus</i>						
<i>Alkalibacterium</i>						
Aminicenantes_genera_incertae_sedis						
<i>Aquicella</i>						
<i>Arcobacter</i>					HS <sup>-</sup> (Sievert <i>et al.</i> , 2007)	
Atribacteria_genera_incertae_sedis						
<i>Azonexus</i>						
<i>Bacteriovorax</i>						
<i>Brachymonas</i>						
<i>Bradyrhizobium</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Masuda <i>et al.</i> , 2010)	
<i>Calditerrivibrio</i>						
<i>Caloramator</i>						
<i>Cellvibrio</i>						
<i>Chlorobaculum</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , HS <sup>-</sup> (Gregersen <i>et al.</i> , 2011)	
<i>Chloroflexus</i>						
<i>Chlorophyta</i>						
<i>Cloacibacterium</i>						
Clostridium III						
<i>Cryobacterium</i>						
Cryptomonadaceae						
<i>Dechloromonas</i>						
<i>Demequina</i>						
<i>Desulfatirhabdium</i>						S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>4</sub> <sup>2-</sup> (Balk <i>et al.</i> , 2008)
<i>Desulfomicrobium</i>						SO <sub>4</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Roanova <i>et al.</i> , 1988)

<i>Desulfovibrio</i>						SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Mogensen <i>et al.</i> , 2005)
<i>Enticicia</i>						
<i>Ferribacterium</i>						
<i>Fibrobacter</i>						
<i>Flavobacterium</i>						
<i>Geobacter</i>						
<i>Geothrix</i>						
Gp16						
Gp3						
Gp6						
GpI						
GpIIa						
<i>Hydrogenophaga</i>						
<i>Hydrogenophilus</i>						
<i>Ignavibacterium</i>						
<i>Legionella</i>						
<i>Limnohabitans</i>						
<i>Meiothermus</i>						
<i>Methylobacter</i>						
<i>Niabella</i>						
<i>Nocardioides</i>						
<i>Ohtaekwangia</i>						
<i>Paludibacter</i>						
<i>Proteiniclasticum</i>						
<i>Psychroflexus</i>						
<i>Rahnella</i>						
<i>Rhodobacter</i>					HS <sup>-</sup> (Hansen and Imhoff, 1985)	
<i>Roseomonas</i>						
<i>Rubrimonas</i>						
<i>Runella</i>						
Saccharibacteria_genera_incertae_sedis						
<i>Smithella</i>						
Sporolactobacillaceae_incertae_sedis						
SR1_genera_incertae_sedis						
<i>Sulfuricurvum</i>					HS <sup>-</sup> , S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Kodama and Watanabe, 2004)	
<i>Sulfurimonas</i>					S <sup>0</sup> , HS <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Inagaki <i>et al.</i> , 2003)	
<i>Syntrophorhabdus</i>						

<i>Thermanaerovibrio</i>						
<i>Thermodesulfobacterium</i>						SO <sub>4</sub> <sup>2-</sup> (Jeanthon <i>et al.</i> , 2002)
<i>Thermodesulfovibrio</i>						SO <sub>4</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> (Henry <i>et al.</i> , 1994)
<i>Thermoflexus</i>						
<i>Thermotoga</i>						
<i>Thiovirga</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> , HS <sup>-</sup> (Ito <i>et al.</i> , 2005)	
<i>Treponema</i>						
<i>Acinetobacter</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Aguilar <i>et al.</i> , 2008)	
<i>Advenella</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Ghosh <i>et al.</i> , 2005)	
<i>Anoxybacillus</i>					S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Mandal <i>et al</i> Unpublished)	
<i>Azospirillum</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , HS <sup>-</sup> (Lavrinenko <i>et al.</i> , 2010)	
<i>Bacillariophyta</i>						
<i>Bacillus</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Pérez-Ibarra <i>et al.</i> , 2007)	
<i>Beijerinckia</i>						
<i>Brevibacillus</i>						
<i>Brevundimonas</i>						
<i>Burkholderia</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> , S <sup>0</sup> , HS <sup>-</sup> (Wittke <i>et al.</i> , 1997)	
<i>Caldicellulosiruptor</i>						
<i>Clostridium sensu stricto</i>						
<i>Dictyoglomus</i>						
<i>Enhydrobacter</i>						
<i>Escherichia/Shigella</i>						
<i>Fervidobacterium</i>						
<i>Geobacillus</i>						
GpXIII						
<i>Halomonas</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Sorokin, 2003)	
<i>Hydrogenobacter</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Shima and Suzuki, 1993)	
<i>Marinobacter</i>						
<i>Methylobacterium</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Anandham <i>et al.</i> , 2007)	
<i>Propionibacterium</i>						
<i>Providencia</i>						

<i>Pseudomonas</i>					$S_2O_3^{2-}$ (Sorokin <i>et al.</i> , 1999)	
<i>Psychrobacter</i>						
<i>Sediminibacterium</i>						
<i>Staphylococcus</i>						
<i>Streptophyta</i>						
<i>Sulfurihydrogenibium</i>					$S^0$ , $S_2O_3^{2-}$ (Nakagawa <i>et al.</i> , 2005)	
<i>Tepidimonas</i>						
<i>Thermomonas</i>						
<i>Thermus</i>					$S^0$ , $S_2O_3^{2-}$ (Skirnisdottir <i>et al.</i> , 2001)	
<i>Thiobacillus</i>					$S_2O_3^{2-}$ , $S_4O_6^{2-}$ , $S^0$ (Wood and Kelly, 1991)	
<i>Thiofaba</i>					$S_2O_3^{2-}$ , $S^0$ , $HS^-$ , $S_4O_6^{2-}$ (Mori and Suzuki, 2008)	
<i>Thiothrix</i>					$S_2O_3^{2-}$ , $HS^-$ (Chernousova <i>et al.</i> , 2009)	
GpIV						
<i>Acholeplasma</i>						
<i>Bacteroides</i>						
<i>Curvibacter</i>						
<i>Desulfosporosinus</i>						$SO_4^{2-}$ , $S_2O_3^{2-}$ , $S^0$ (Sánchez-Andrea <i>et al.</i> , 2015)
<i>Exiguobacterium</i>						
<i>Fervidicella</i>						
<i>Pleomorphomonas</i>						
<i>Serratia</i>						
<i>Sphingobium</i>						
<i>Streptococcus</i>						
<i>Sulfuricella</i>					$S^0$ , $S_2O_3^{2-}$ (Kojima and Fukui, 2010)	
<i>Brachybacterium</i>						
<i>Dehalogenimonas</i>						
<i>Spirochaeta</i>					$S_2O_3^{2-}$ , $S^0$ , $HS^-$ (Dubinina <i>et al.</i> , 2011)	
<i>Actinomycetospora</i>						
<i>Buttiauxella</i>						
<i>Peptoniphilus</i>						
<i>Planobacterium</i>						
<i>Sphingomonas</i>						
<i>Actinomyces</i>						

<i>Alcanivorax</i>					
<i>Alkaliphilus</i>					
<i>Arenimonas</i>					
<i>Arthrobacter</i>					
<i>Blastocatella</i>					
<i>Comamonas</i>					
<i>Cytophaga</i>					
<i>Deinococcus</i>					
<i>Desulforegula</i>					SO <sub>4</sub> <sup>2-</sup> (Rees and Patel, 2001)
<i>Duganella</i>					
<i>Fluviicola</i>					
<i>Friedmanniella</i>					
<i>Gallicola</i>					
<i>Hermiimonas</i>					
<i>Microvirga</i>					
<i>Ornithinimicrobium</i>					
<i>Panacagrimonas</i>					
<i>Parcubacteria_genera_incertae_sedis</i>					
<i>Pedomicrobium</i>					
<i>Photobacterium</i>					
<i>Piscinibacter</i>					
<i>Porphyromonas</i>					
<i>Sneathiella</i>					
<i>Sphaerotilus</i>					
<i>Thiomonas</i>				S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Vésteinsdóttir <i>et al.</i> , 2011)	
<i>Tindallia</i>					
<i>Actinoalloteichus</i>					
<i>Aminiphilus</i>					
<i>Balneola</i>					
<i>Bosea</i>				S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Das <i>et al.</i> , 1996)	
<i>Cecemia</i>					
<i>Chryseobacterium</i>					
<i>Desulfocapsa</i>					
<i>Elioraea</i>					
<i>Flexibacter</i>					
<i>Formivibrio</i>					
<i>Gemmatimonas</i>					
<i>Ilumatobacter</i>					
<i>Kinneretia</i>					

<i>Kribbella</i>						
<i>Marmoricola</i>						
<i>Methylosoma</i>						
<i>Mycobacterium</i>						
<i>Nitrospira</i>						
<i>Planococcus</i>						
<i>Plesiomonas</i>						
<i>Psychrosinus</i>						
<i>Roseiflexus</i>						
<i>Segetibacter</i>						
<i>Succinispira</i>						
<i>Thermomicrobium</i>						
<i>Thioalkalimicrobium</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , HS <sup>-</sup> , S <sup>0</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Sorokin <i>et al.</i> , 2001)	
<i>Thioalkalivibrio</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Banciu <i>et al.</i> , 2004)	
<i>Tistrella</i>						
<i>Acidaminobacter</i>						
<i>Aerococcus</i>						
<i>Aliidiomarina</i>						
<i>Anaerococcus</i>						
<i>Anaeromyxobacter</i>						
<i>Aquaspirillum</i>						
<i>Arcicella</i>						
<i>Bdellovibrio</i>						
<i>Brevibacterium</i>						
<i>Caldicoprobacter</i>						
<i>Castellaniella</i>						
<i>Desulfomonile</i>						SO <sub>4</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (DeWeerd <i>et al.</i> , 1990)
<i>Desulfonatronum</i>						SO <sub>4</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Zhilina <i>et al.</i> , 2005)
<i>Dyadobacter</i>						
<i>Erysipelothrix</i>						
<i>Faecalibacterium</i>						
<i>Ferruginibacter</i>						
<i>Flectobacillus</i>						
<i>Fusobacterium</i>						
<i>Gaiella</i>						
<i>Gemmobacter</i>						
<i>Halochromatium</i>					HS <sup>-</sup> , S <sup>0</sup> (Imhoff <i>et al.</i> , 1998)	
<i>Heliothrix</i>						

<i>Hyphomonas</i>						
<i>Luteolibacter</i>						
<i>Lysobacter</i>						
<i>Mongoliitalea</i>						
<i>Neochlamydia</i>						
<i>Nesterenkonia</i>						
<i>Olsenella</i>						
<i>Paenisporosarcina</i>						
<i>Prostheco bacter</i>						
<i>Sphaerochaeta</i>						
<i>Stenotrophomonas</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Mandal <i>et al</i> Unpublished)	
<i>Sulfuritalea</i>					S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Kojima and Fukui, 2011)	
<i>Thiocapsa</i>					HS <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Puchkova <i>et al.</i> , 2000)	
<i>Vampirovibrio</i>						
<i>Verrucomicrobium</i>						
<i>Dietzia</i>						
<i>Corynebacterium</i>						
<i>Methylophilus</i>						
<i>Paracoccus</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> , HS <sup>-</sup> , S <sup>0</sup> (Ghosh <i>et al.</i> , 2006)	



**Table S5.** Complete list of the 316 genera identified in the *Lotus Pond-Rulang* ecosystem at 14:30 h of 20 October 2014, together with the information about their presence (green shade) or absence (no shade) in the four sample-sites, and their capabilities, if any, to oxidize or reduce sulfur species.

Genus	HTP	MTP	LTP	RVW	Sulfur species oxidized for chemolithotrophic nutrition (Reference)	Sulfur species reduced for anaerobic respiration (Reference)
<i>Armatimonadetes_gp7</i>						
Gp3						
<i>Pedomicrobium</i>						
<i>Alkanindiges</i>						
<i>Bosea</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Das <i>et al.</i> , 1996)	
<i>Butyricicoccus</i>						
<i>Chlorophyta</i>						
<i>Desulfobulbus</i>						SO <sub>4</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Sorokin <i>et al.</i> , 2012)
<i>Meiothermus</i>						
<i>Nesterenkonia</i>						
<i>Nitrospira</i>						
<i>Peredibacter</i>						
<i>Thermodesulfobacterium</i>						SO <sub>4</sub> <sup>2-</sup> (Jeanthon <i>et al.</i> , 2002)
<i>Thioalkalimicrobium</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , HS <sup>-</sup> , S <sup>0</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Sorokin <i>et al.</i> , 2001)	
<i>Zoogloea</i>						
<i>Alkaliflexus</i>						
<i>Desulfosporosinus</i>						SO <sub>4</sub> <sup>2-</sup> , S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Sánchez-Andrea <i>et al.</i> , 2015)
<i>Fluviicola</i>						
<i>Fusobacterium</i>						
<i>Gemmobacter</i>						
<i>Halanaerobium</i>						
<i>Methylosoma</i>						
<i>Ohtaekwangia</i>						
<i>Pleomorphomonas</i>						
<i>Spirochaeta</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> , HS <sup>-</sup> (Dubinina <i>et al.</i> , 2011)	
<i>Thioalkalivibrio</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Banciu <i>et al.</i> , 2004)	
<i>Vibrio</i>						
<i>Bacillariophyta</i>						
<i>Caldicellulosiruptor</i>						
<i>Enhydrobacter</i>						
GpXIII						

<i>Hydrogenobacter</i>					S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Shima and Suzuki, 1993)	
<i>Streptophyta</i>						
<i>Tepidimonas</i>						
<i>Thermodesulfovibrio</i>						SO <sub>4</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> (Henry <i>et al.</i> , 1994)
<i>Acetobacterium</i>						
Acetothermia_genera_incertae_sedis						
<i>Acholeplasma</i>						
<i>Algoriphagus</i>						
<i>Aliidiomarina</i>						
<i>Alkalibacter</i>						
<i>Alkalibacterium</i>						
<i>Allochromatium</i>					S <sup>0</sup> , HS <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Dahl <i>et al.</i> , 2005)	
Aminicenantes_genera_incertae_sedis						
<i>Aquicella</i>						
<i>Arcicella</i>						
<i>Arcobacter</i>					HS <sup>-</sup> (Sievert <i>et al.</i> , 2007)	
Atribacteria_genera_incertae_sedis						
<i>Azospirillum</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , HS <sup>-</sup> (Lavrinenko <i>et al.</i> , 2010)	
<i>Bacteriovorax</i>						
<i>Bacteroides</i>						
<i>Bdellovibrio</i>						
<i>Calditerrivibrio</i>						
<i>Caloramator</i>						
<i>Candidatus Cloacamonas</i>						
<i>Carnobacterium</i>						
<i>Cellvibrio</i>						
<i>Chlorobaculum</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , HS <sup>-</sup> (Gregersen <i>et al.</i> , 2011)	
<i>Chrysiogenes</i>						
Clostridium III						
<i>Clostridium sensu stricto</i>						
<i>Cryobacterium</i>						
Cryptomonadaceae						
<i>Cytophaga</i>						
<i>Dechloromonas</i>						
<i>Deefgea</i>						
<i>Demequina</i>						

<i>Desulfatirhabdium</i>						S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>4</sub> <sup>2-</sup> (Balk <i>et al.</i> , 2008)
<i>Desulfomicrobium</i>						SO <sub>4</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Rozanova <i>et al.</i> , 1988)
<i>Desulfonatronovibrio</i>						SO <sub>4</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Sorokin <i>et al.</i> , 2012)
<i>Desulfonatronum</i>						SO <sub>4</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Zhilina <i>et al.</i> , 2005)
<i>Dethiosulfatibacter</i>						S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Takii <i>et al.</i> , 2007)
<i>Dickeya</i>						
<i>Dyadobacter</i>						
<i>Ectothiorhodospira</i>						HS <sup>-</sup> , S <sup>0</sup> (Imhoff and Trüper, 1981)
<i>Emticicia</i>						
<i>Erysipelothrix</i>						
<i>Ferribacterium</i>						
<i>Ferruginibacter</i>						
<i>Fervidicella</i>						
<i>Fibrobacter</i>						
<i>Flavobacterium</i>						
<i>Geobacter</i>						
<i>Giesbergeria</i>						
<i>Gillisia</i>						
Gp16						
Gp6						
Gp7						
GpIIa						
<i>Gracilimonas</i>						
<i>Haliea</i>						
<i>Halochromatium</i>						HS <sup>-</sup> , S <sup>0</sup> (Imhoff <i>et al.</i> , 1998)
<i>Hydrogenophaga</i>						
<i>Hydrogenophilus</i>						
<i>Hyphomonas</i>						
<i>Ignavibacterium</i>						
<i>Legionella</i>						
<i>Limnohabitans</i>						
<i>Lysobacter</i>						
<i>Marinomonas</i>						
<i>Megamonas</i>						
<i>Methylobacter</i>						
<i>Methylocystis</i>						
<i>Methylomicrobium</i>						

<i>Methylomonas</i>						
<i>Mongoliitalea</i>						
<i>Niabella</i>						
<i>Nitriliruptor</i>						
<i>Paenisporosarcina</i>						
<i>Paludibacter</i>						
<i>Planomicrobium</i>						
<i>Polaromonas</i>						
<i>Polynucleobacter</i>						
<i>Prevotella</i>						
<i>Proteiniclasticum</i>						
<i>Providencia</i>						
<i>Psychrobacter</i>						
<i>Psychroflexus</i>						
<i>Rahnella</i>						
<i>Rhizobacter</i>						
<i>Rhodobaca</i>						
<i>Rhodobacter</i>					HS <sup>-</sup> (Hansen and Imhoff, 1985)	
<i>Rhodoferax</i>						
<i>Roseomonas</i>						
<i>Rubrimonas</i>						
<i>Runella</i>						
<i>Saccharofermentans</i>						
<i>Sediminibacterium</i>						
<i>Shewanella</i>						
<i>Smithella</i>						
SR1_genera_incertae_sedis						
<i>Sulfuricella</i>					S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Kojima and Fukui, 2010)	
<i>Sulfuricurvum</i>					HS <sup>-</sup> , S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Kodama and Watanabe, 2004)	
<i>Sulfurospirillum</i>						S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> (Kodama <i>et al.</i> , 2007)
<i>Sunxiuqinia</i>						
<i>Syntrophorhabdus</i>						
<i>Thermotoga</i>						
<i>Thioalkalibacter</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> , HS <sup>-</sup> (Banciu <i>et al.</i> , 2008)	
<i>Thiobacillus</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> , S <sup>0</sup> (Wood and Kelly, 1991)	

<i>Thiocapsa</i>					HS <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Puchkova <i>et al.</i> , 2000)	
<i>Thiocystis</i>					HS <sup>-</sup> , S <sup>0</sup> (Peduzzi <i>et al.</i> , 2011)	
<i>Thiomicrospira</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> , S <sup>0</sup> (Sorokin <i>et al.</i> , 2006)	
<i>Thiovirga</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> , HS <sup>-</sup> (Ito <i>et al.</i> , 2005)	
<i>Tissierella</i>						
<i>Trichococcus</i>						
<i>Vampirovibrio</i>						
<i>Acidovorax</i>						
<i>Acinetobacter</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Aguilar <i>et al.</i> , 2008)	
<i>Advenella</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Ghosh <i>et al.</i> , 2005)	
<i>Aeromonas</i>						
<i>Anoxybacillus</i>					S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> (Mandal <i>et al</i> Unpublished)	
<i>Bacillus</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Pérez-Ibarra <i>et al.</i> , 2007)	
<i>Beijerinckia</i>						
<i>Bradyrhizobium</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Masuda <i>et al.</i> , 2010)	
<i>Brevibacillus</i>						
<i>Brevundimonas</i>						
<i>Burkholderia</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> , S <sup>0</sup> , HS <sup>-</sup> (Wittke <i>et al.</i> , 1997)	
<i>Chloroflexus</i>						
<i>Cloacibacterium</i>						
Clostridium XIVa						
<i>Desulfovibrio</i>						SO <sub>3</sub> <sup>2-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , S <sup>0</sup> (Mogensen <i>et al.</i> , 2005)
<i>Dictyoglomus</i>						
<i>Dietzia</i>						
<i>Escherichia/Shigella</i>						
<i>Fervidobacterium</i>						
<i>Geobacillus</i>						
<i>Geothrix</i>						
GpI						
GpIV						
<i>Halomonas</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Sorokin, 2003)	
<i>Hyphomicrobium</i>						

<i>Methylobacterium</i>					$S_2O_3^{2-}$ (Anandham <i>et al.</i> , 2007)	
<i>Nocardioides</i>						
<i>Paracoccus</i>					$S_2O_3^{2-}$ , $S_4O_6^{2-}$ , $HS^-$ , $S^0$ (Ghosh <i>et al.</i> , 2006)	
<i>Pedobacter</i>						
<i>Planococcus</i>						
<i>Propionibacterium</i>						
<i>Pseudomonas</i>					$S_2O_3^{2-}$ (Sorokin <i>et al.</i> , 1999)	
<i>Rheinheimera</i>						
Saccharibacteria_genera_incertae_sedis						
Sporolactobacillaceae_incertae_sedis						
<i>Sulfurihydrogenibium</i>					$S^0$ , $S_2O_3^{2-}$ (Nakagawa <i>et al.</i> , 2005)	
<i>Sulfurimonas</i>					$S^0$ , $HS^-$ , $S_2O_3^{2-}$ (Inagaki <i>et al.</i> , 2003)	
<i>Thermus</i>					$S_2O_3^{2-}$ , $S^0$ (Skirnisdottir <i>et al.</i> , 2001)	
<i>Thiofaba</i>					$S_2O_3^{2-}$ , $S^0$ , $HS^-$ , $S_4O_6^{2-}$ (Mori and Suzuki, 2008)	
<i>Thiothrix</i>					$S_2O_3^{2-}$ , $HS^-$ (Chernousova <i>et al.</i> , 2009)	
<i>Treponema</i>						
<i>Brevibacterium</i>						
<i>Chryseobacterium</i>						
<i>Comamonas</i>						
<i>Corynebacterium</i>						
<i>Deinococcus</i>						
<i>Methyloversatilis</i>						
<i>Pelomonas</i>						
<i>Serratia</i>						
<i>Acidaminobacter</i>						
<i>Anaeromyxobacter</i>						
<i>Campylobacter</i>					$S_2O_3^{2-}$ , $S_4O_6^{2-}$ , $HS^-$ (Voordouw <i>et al.</i> , 1996)	
Clostridium XI						
<i>Curvibacter</i>						
<i>Flectobacillus</i>						
<i>Formivibrio</i>						
<i>Gemmatimonas</i>						
<i>Luteolibacter</i>						

<i>Methylophilus</i>						
<i>Parabacteroides</i>						
<i>Pseudoxanthomonas</i>						
<i>Rickettsia</i>						
<i>Sphaerochaeta</i>						
<i>Steroidobacter</i>						
<i>Sulfuritalea</i>					S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Kojima and Fukui, 2011)	
<i>Thiobaca</i>					HS <sup>-</sup> (Rees <i>et al.</i> , 2002)	
<i>Delftia</i>						
<i>Litoreibacter</i>						
<i>Paenibacillus</i>						
<i>Rhodoplanes</i>						
<i>Staphylococcus</i>						
<i>Achromobacter</i>						
<i>Actinomyces</i>						
<i>Alcanivorax</i>						
<i>Armatimonadetes_gp5</i>						
<i>Brachybacterium</i>						
<i>Cruoricaptor</i>						
<i>Finegoldia</i>						
<i>Gemella</i>						
<i>Gp2</i>						
<i>Klebsiella</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Mason and Kelly, 1988)	
<i>Lysinibacillus</i>						
<i>Marinococcus</i>						
<i>Marmoricola</i>						
<i>Microbacterium</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Anandham <i>et al.</i> , 2008)	
<i>Nevskia</i>						
<i>Patulibacter</i>						
<i>Peptoniphilus</i>						
<i>Photobacterium</i>						
<i>Ralstonia</i>						
<i>Rubrobacter</i>						
<i>Sphingobium</i>						
<i>Sphingomonas</i>						
<i>Sporolituus</i>						
<i>Stenotrophomonas</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (Mandal <i>et al</i> Unpublished)	

<i>Sulfitobacter</i>					$S_2O_3^{2-}$ , $SO_3^{2-}$ (Pukall <i>et al.</i> , 1999)	
<i>Thermoactinomyces</i>						
<i>Truepera</i>						
<i>Turicella</i>						
<i>Variovorax</i>						
<i>Aerococcus</i>						
<i>Alkalilimnicola</i>					$HS^-$ , $S_2O_3^{2-}$ (Hoeft <i>et al.</i> , 2007)	
<i>Aminiphilus</i>						
<i>Aquimonas</i>						
<i>Azospira</i>						
<i>Caldilinea</i>						
Clostridium XVIII						
<i>Desulforegula</i>						$SO_4^{2-}$ (Rees and Patel, 2001)
<i>Euzebya</i>						
Gp4						
<i>Holophaga</i>						
<i>Lactobacillus</i>						
<i>Leptospira</i>						
<i>Marinospirillum</i>						
<i>Mesorhizobium</i>					$S_2O_3^{2-}$ , $S^0$ (Ghosh and Roy, 2006)	
<i>Methylovulum</i>						
<i>Rhodocyclus</i>					$SO_3^{2-}$ (Pfennig, 1978)	
<i>Sphingopyxis</i>						
<i>Actinoalloteichus</i>						
<i>Aquaspirillum</i>						
<i>Arenimonas</i>						
<i>Asticcacaulis</i>						
<i>Azonexus</i>						
<i>Desulfococcus</i>						$SO_4^{2-}$ , $SO_3^{2-}$ (Platen <i>et al.</i> , 1990)
Erysipelotrichaceae_incertae_sedis						
<i>Faecalibacterium</i>						
GpVIII						
<i>Lactococcus</i>						
<i>Oceanibaculum</i>						
<i>Opitutus</i>						
<i>Plesiomonas</i>						
<i>Rhodospirillum</i>					$S_2O_3^{2-}$ , $HS^-$ (Kumar <i>et al.</i> , 2008)	
<i>Roseococcus</i>					$S_2O_3^{2-}$ (Yurkov <i>et al.</i> , 1994)	



<i>Rubrivivax</i>						
<i>Salinicoccus</i>						
Subdivision3_genera_incertae_sedis						
<i>Thermoleophilum</i>						
<i>Alishewanella</i>						
<i>Alkalibaculum</i>						
<i>Alkaliphilus</i>						
<i>Anaerobiospirillum</i>						
<i>Aquabacterium</i>						
<i>Blastocatella</i>						
<i>Brachymonas</i>						
BRC1_genera_incertae_sedis						
<i>Candidatus Endomicrobium</i>						
<i>Dethiobacter</i>						
<i>Friedmanniella</i>						
<i>Haliscomenobacter</i>						
<i>Ilumatobacter</i>						
<i>Jeotgalicoccus</i>						
Latescibacteria_genera_incertae_sedis						
<i>Loktanella</i>						
<i>Magnetococcus</i>					S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> (Bazylnski <i>et al.</i> , 2013)	
<i>Marinimicrobium</i>						
<i>Ornithinimicrobium</i>						
<i>Piscibacillus</i>						
<i>Pseudoflavonifractor</i>						
<i>Pseudorhodobacter</i>						
<i>Rhizobium</i>						
<i>Sphingobacterium</i>						
<i>Terrimonas</i>						
<i>Thermanaerovibrio</i>						
ZB3_genera_incertae_sedis						
<i>Dehalogenimonas</i>						
<i>Marinobacter</i>						
<i>Mycobacterium</i>						
<i>Streptococcus</i>						
<i>Thermomonas</i>						
<i>Exiguobacterium</i>						

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**Table S6.** Concentrations of various redox species of sulfur, and number of sulfur-oxidizing and sulfur-reducing genera present, at the different sample-sites of the *Lotus Pond-Rulang* ecosystem at 8:30 and 14:30 h of 20 October 2014.

Serial no.		8:30 h				14:30 h			
		HTP	MTP	LTP	RVW	HTP	MTP	LTP	RVW
1	pH	7.5	7.4	7.2	6.6	7.8	7.5	6.8	6.2
2	S <sup>2-</sup> concentration* in the water (in mM)	262	120	0	0	178	93	0	0
3	δ <sup>34</sup> S of the dissolved sulfide (in ‰ VCDT)	4.6	7.0	NA	NA	4.3	5.2	NA	NA
4	S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> concentration in the water (in mM)	1.3	1.4	1.5	0.8	0.8	0.9	1	0.5
5	SO <sub>3</sub> <sup>2-</sup> concentration in the water (in μM)	273	140	0	0	110	15	0	0
6	SO <sub>4</sub> <sup>2-</sup> concentration in the water (in μM)	712	920	1152	688	963	1115	1281	722
7	δ <sup>34</sup> S of the dissolved sulfate (in ‰ VCDT)	16.3	16.1	16.2	12.4	16.5	16.5	16.6	13.1
8	Number of S <sup>2-</sup> , S <sup>0</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> and/or SO <sub>3</sub> <sup>2-</sup> oxidizing genera present	17	26	27	30	20	39	37	36
9	Number of S <sup>2-</sup> oxidizing genera present	6	12	13	15	5	21	20	20
10	Number of S <sup>0</sup> oxidizing genera present	9	13	13	17	8	22	21	20
11	Number of S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> oxidizing genera present	16	23	23	25	19	31	31	29
12	Number of SO <sub>3</sub> <sup>2-</sup> oxidizing genera present	0	1	1	2	1	3	2	3
13	Number of S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , SO <sub>3</sub> <sup>2-</sup> and/or SO <sub>4</sub> <sup>2-</sup> reducing genera present	0	8	8	10	2	11	12	8
14	Number of S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> reducing genera present	0	6	7	9	2	9	10	8
15	Number of SO <sub>3</sub> <sup>2-</sup> reducing genera present	0	3	4	6	2	6	7	5
16	Number of SO <sub>4</sub> <sup>2-</sup> reducing genera present	0	7	5	7	1	8	9	5

\* Dissolved sulfide measured collectively using the methylene blue colorimetric method included H<sub>2</sub>S, HS<sup>-</sup> and S<sub>x</sub><sup>2-</sup>.  
NA = Not applicable.

**Table S7.** The 57 *Lotus Pond* genera that were detected exclusively at HTP and/or MTP, at 8:30 and/or 14:30 h of 20 October 2014; information<sup>1</sup> about the upper temperature-limit for their laboratory growth is also given.

Phyla	Genera	Upper limit of temperature (in °C) for growth <i>in vitro</i>	Reference
<b>Genera having no report of laboratory growth above 45°C</b>			
<i>Acidobacteria</i>	<i>Holophaga</i>	35	(Liesack <i>et al.</i> , 1994)
<i>Actinobacteria</i>	<i>Actinomycetospora</i>	30	(Yamamura <i>et al.</i> , 2011)
	<i>Arthrobacter</i>	45	(Dastager <i>et al.</i> , 2014)
	<i>Euzebya</i>	35	(Kurahashi <i>et al.</i> , 2010)
	<i>Leifsonia</i>	28	(Evtushenko <i>et al.</i> , 2000)
	<i>Microbacterium</i>	42	(Krishnamurthi <i>et al.</i> , 2012)
	<i>Patulibacter</i>	39	(Almeida <i>et al.</i> , 2013)
	<i>Turicella</i>	37	(Funke <i>et al.</i> , 1994)
<i>Bacteroidetes</i>	<i>Cruoricaptor</i>	37	(Yassin <i>et al.</i> , 2012)
	<i>Porphyromonas</i>	37	(Willems and Collins, 1995)
	<i>Planobacterium</i>	37	(Peng <i>et al.</i> , 2009)
<i>Firmicutes</i>	<i>Finegoldia</i>	37	(Murdoch and Shah, 1999)
	<i>Gallicola</i>	37	(Ezaki, 2015)
	<i>Gemella</i>	37	(Kilpper-Bälz and Schleifer, 1988)
	<i>Lactobacillus</i>	45	(Kudo <i>et al.</i> , 2012)
	<i>Lysinibacillus</i>	45	(Ahmed <i>et al.</i> , 2007)
	<i>Peptoniphilus</i>	43	(Patel <i>et al.</i> , 2016)
<i>Proteobacteria</i>	<i>Achromobacter</i>	40	(Gomila <i>et al.</i> , 2011)
	<i>Alcanivorax</i>	40	(Rahul <i>et al.</i> , 2014)
	<i>Aquimonas</i>	42	(Saha <i>et al.</i> , 2005)
	<i>Azospira</i>	37	(Lin <i>et al.</i> , 2013)
	<i>Desulforegula</i>	32	(Rees and Patel, 2001)
	<i>Duganella</i>	30	(Li <i>et al.</i> , 2004)
	<i>Hermiimonas</i>	37	(Kämpfer <i>et al.</i> , 2013)
	<i>Klebsiella</i>	37	(Bergey <i>et al.</i> , 1923)
	<i>Mesorhizobium</i>	37	(Martínez-Hidalgo <i>et al.</i> , 2015)
	<i>Methylovulum</i>	34	(Iguchi <i>et al.</i> , 2011)
	<i>Microvirga</i>	45	(Kanso and Patel, 2003)
	<i>Nevskia</i>	25	(Leandro <i>et al.</i> , 2012)
	<i>Panacagrionas</i>	42	(Im <i>et al.</i> , 2010)
	<i>Pedomicrobium</i>	40	(Gebers, 1981)
	<i>Piscinibacter</i>	42	(Stackebrandt <i>et al.</i> , 2009)
	<i>Ralstonia</i>	41	(Yabuuchi <i>et al.</i> , 1995)
	<i>Rhodocyclus</i>	30	(Pfennig, 1978)
	<i>Sneathiella</i>	37	(Jordan <i>et al.</i> , 2007)
	<i>Sphaerotilus</i>	40	(Gridneva <i>et al.</i> , 2011)
	<i>Sphingomonas</i>	40	(Yabuuchi <i>et al.</i> , 1990)
<i>Sphingopyxis</i>	35	(Baik <i>et al.</i> , 2013)	
<i>Sulfobacter</i>	35	(Hong <i>et al.</i> , 2015)	
<i>Variovorax</i>	30	(Willems <i>et al.</i> , 1991)	
<i>Spirochaetes</i>	<i>Leptospira</i>	30	(Faine and Stallmn, 1982)
<b>Genera having report of laboratory growth above 45°C</b>			
<i>Actinobacteria</i>	<i>Actinomyces</i>	55	(An <i>et al.</i> , 2006)
	<i>Rubrobacter</i>	60	(Carreto <i>et al.</i> , 1996)
<i>Deinococcus-Thermus</i>	<i>Truepera</i>	50	(Albuquerque <i>et al.</i> , 2005)
<i>Firmicutes</i>	<i>Marinococcus</i>	50	(Balderrama-Subieta <i>et al.</i> , 2013)
	<i>Sporolituus</i>	60	(Ogg and patel, 2009)
	<i>Thermoactinomyces</i>	60	(Yao <i>et al.</i> , 2014)
	<i>Tindallia</i>	48	(Pikuta <i>et al.</i> , 2003)
<i>Proteobacteria</i>	<i>Alkalilimnicola</i>	55	(Yakimov <i>et al.</i> , 2001)
	<i>Marinospirillum</i>	55	(Namsaraev <i>et al.</i> , 2009)
	<i>Photobacterium</i>	50	(Wang <i>et al.</i> , 2017)
	<i>Thiomonas</i>	50	(Moreira <i>et al.</i> , 1997)

<sup>1</sup> Armatimonadetes\_gp5, Acidobacteria Gp2, Acidobacteria Gp4, Parcubacteria\_genera\_incertae\_sedis and Clostridium XVIII do not have any comparable information.

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**Table S8.** Highest temperature reported for the laboratory growth of the 47 validly published<sup>1</sup>bacterial genera detected in HTP at 8:30 h of 20 October 2014.

Phyla	Genera	Upper limit of temperature (in °C) at which laboratory growth can occur	Reference
Actinobacteria	<i>Actinomycetospora</i>	30	(Yamamura <i>et al.</i> , 2011)
	<i>Brachybacterium</i>	30	(Collins <i>et al.</i> , 1988)
	<i>Corynebacterium</i>	42	(Bernard <i>et al.</i> , 2016)
	<i>Dietzia</i>	30	(Kim <i>et al.</i> , 2011)
	<i>Leifsonia</i>	28	(Evtushenko <i>et al.</i> , 2000)
	<i>Propionibacterium</i>	45	(Koussémon <i>et al.</i> , 2001)
<i>Aquificae</i>	<i>Hydrogenobacter</i>	85	(Takai <i>et al.</i> , 2001)
Bacteroidetes	<i>Planobacterium</i>	37	(Peng <i>et al.</i> , 2009)
	<i>Sediminibacterium</i>	42	(Kang <i>et al.</i> , 2014)
	<i>Sulfurihydrogenibium</i>	80	(O'Neill <i>et al.</i> , 2008)
<i>Chlorobi</i>	<i>Dehalogenimonas</i>	42	(Bowman <i>et al.</i> , 2013)
<i>Deinococcus-Thermus</i>	<i>Thermus</i>	79	(Brock and Freeze, 1969)
Firmicutes	<i>Anoxybacillus</i>	70	(Dulger <i>et al.</i> , 2004)
	<i>Bacillus</i>	70	(Yang <i>et al.</i> , 2013)
	<i>Brevibacillus</i>	65	(Inan <i>et al.</i> , 2012)
	<i>Caldicellulosiruptor</i>	90	(Yang <i>et al.</i> , 2010)
	<i>Dictyoglomus</i>	80	(Saiki <i>et al.</i> , 1985)
	<i>Geobacillus</i>	80	(Coorevits <i>et al.</i> , 2012)
	<i>Peptoniphilus</i>	43	(Patel <i>et al.</i> , 2016)
	<i>Staphylococcus</i>	40	(Fuente <i>et al.</i> , 1985)
Proteobacteria	<i>Acinetobacter</i>	44	(Rooney <i>et al.</i> , 2016)
	<i>Advenella</i>	40	(Shmareva <i>et al.</i> , 2016)
	<i>Aquabacterium</i>	40	(Chen <i>et al.</i> , 2012)
	<i>Azospirillum</i>	37	(Lin <i>et al.</i> , 2013)
	<i>Beijerinckia</i>	30	(Oggerin <i>et al.</i> , 2009)
	<i>Brevundimonas</i>	42	(Choi <i>et al.</i> , 2010)
	<i>Burkholderia</i>	37	(Aizawa <i>et al.</i> , 2011)
	<i>Buttiauxella</i>	42	(Muller <i>et al.</i> , 1996)
	<i>Enhydrobacter</i>	41	(Staley <i>et al.</i> , 1987)
	<i>Escherichia/Shigella</i>	37	(Liu <i>et al.</i> , 2015)
	<i>Halomonas</i>	50	(Guan <i>et al.</i> , 2010)
	<i>Marinobacter</i>	50	(Wang <i>et al.</i> , 2009)
	<i>Methylobacterium</i>	37	(Wood <i>et al.</i> , 1998)
	<i>Methylophilus</i>	37	(Jenkins <i>et al.</i> , 1987)
	<i>Paracoccus</i>	45	(Sun <i>et al.</i> , 2015)
	<i>Providencia</i>	45	(Khunthongpan <i>et al.</i> , 2013)
	<i>Pseudomonas</i>	41	(Wang and Sun, 2016)
	<i>Psychrobacter</i>	38	(Maruyama <i>et al.</i> , 2000)
	<i>Ralstonia</i>	41	(Yabuuchi <i>et al.</i> , 1995)
	<i>Sphingomonas</i>	40	(Yabuuchi <i>et al.</i> , 1990)
	<i>Tepidimonas</i>	65	(Moreira <i>et al.</i> , 2000)
	<i>Thermomonas</i>	50	(Busse <i>et al.</i> , 2002)
	<i>Thiobacillus</i>	42	(Brinkhoff <i>et al.</i> , 1999)
<i>Thiofaba</i>	51	(Mori and Suzuki, 2008)	
<i>Thiothrix</i>	37	(Howarth <i>et al.</i> , 1999)	
<i>Spirochaetes</i>	<i>Spirochaeta</i>	73	(Aksenova <i>et al.</i> , 1992)
<i>Thermotogae</i>	<i>Fervidobacterium</i>	90	(Cai <i>et al.</i> , 2007)

<sup>1</sup> According to <http://www.bacterio.net/>

**Table S9.** Highest temperature reported for laboratory growth of the 88 bacterial genera that were detected in HTP at 14:30 h of 20 October 2014, and for which information is available regarding upper temperature-limit of laboratory growth.

Phyla	Genera	Upper limit of temperature (in °C) at which laboratory growth can occur	Reference
<i>Acidobacteria</i>	<i>Geothrix</i>	35	(Coates <i>et al.</i> , 1999)
<i>Actinobacteria</i>	<i>Actinomyces</i>	55	(An <i>et al.</i> , 2006)
	<i>Brachybacterium</i>	30	(Collins <i>et al.</i> , 1988)
	<i>Brevibacterium</i>	42	(Kim <i>et al.</i> , 2013)
	<i>Corynebacterium</i>	42	(Bernard <i>et al.</i> , 2016)
	<i>Dietzia</i>	30	(Kim <i>et al.</i> , 2011)
	<i>Marmoricola</i>	40	(Lee <i>et al.</i> , 2016)
	<i>Microbacterium</i>	42	(Krishnamurthi <i>et al.</i> , 2012)
	<i>Mycobacterium</i>	43	(Kusunoki and Ezaki, 1992)
	<i>Nocardioides</i>	37	(Prauser, 1976)
	<i>Patulibacter</i>	39	(Almeida <i>et al.</i> , 2013)
	<i>Pedomicrobium</i>	40	(Gebers, 1981)
	<i>Propionibacterium</i>	45	(Koussémon <i>et al.</i> , 2001)
	<i>Rubrobacter</i>	60	(Carreto <i>et al.</i> , 1996)
	<i>Turicella</i>	37	(Funke <i>et al.</i> , 1994)
<i>Aquificae</i>	<i>Hydrogenobacter</i>	85	(Takai <i>et al.</i> , 2001)
<i>Bacteroidetes</i>	<i>Chryseobacterium</i>	37	(Yang <i>et al.</i> , 2015)
	<i>Cloacibacterium</i>	40	(Chun <i>et al.</i> , 2017)
	<i>Cruoricaptor</i>	37	(Yassin <i>et al.</i> , 2012)
	<i>Pedobacter</i>	35	(Zhang <i>et al.</i> , 2015)
	<i>Sulfurihydrogenibium</i>	80	(O'Neill <i>et al.</i> , 2008)
<i>Chlorobi</i>	<i>Dehalogenimonas</i>	42	(Bowman <i>et al.</i> , 2013)
<i>Chloroflexi</i>	<i>Chloroflexus</i>	59	(Gaisin <i>et al.</i> , 2017)
<i>Deinococcus-Thermus</i>	<i>Deinococcus</i>	45	(Hussain <i>et al.</i> , 2016)
	<i>Thermus</i>	79	(Brock and Freeze, 1969)
	<i>Truepera</i>	50	(Albuquerque <i>et al.</i> , 2005)
<i>Firmicutes</i>	<i>Anoxybacillus</i>	70	(Dulger <i>et al.</i> , 2004)
	<i>Bacillus</i>	70	(Yang <i>et al.</i> , 2013)
	<i>Brevibacillus</i>	65	(Inan <i>et al.</i> , 2012)
	<i>Caldicellulosiruptor</i>	90	(Yang <i>et al.</i> , 2010)
	<i>Dictyoglomus</i>	80	(Saiki <i>et al.</i> , 1985)
	<i>Exiguobacterium</i>	49	(Crapart <i>et al.</i> , 2007)
	<i>Finegoldia</i>	37	(Murdoch and Shah, 1999)
	<i>Gemella</i>	37	(Kilpper-Bälz and Schleifer, 1988)
	<i>Geobacillus</i>	80	(Coorevits <i>et al.</i> , 2012)
	<i>Lysinibacillus</i>	45	(Ahmed <i>et al.</i> , 2007)
	<i>Marinococcus</i>	50	(Balderrama-Subieta <i>et al.</i> , 2013)
	<i>Paenibacillus</i>	37	(Shida <i>et al.</i> , 1997)
	<i>Peptoniphilus</i>	43	(Patel <i>et al.</i> , 2016)
	<i>Planococcus</i>	45	(Gan <i>et al.</i> , 2018)
	<i>Sporolituus</i>	60	(Ogg and Patel, 2009)
	<i>Staphylococcus</i>	40	(Fuente <i>et al.</i> , 1985)
<i>Streptococcus</i>	45	(Sherman and Stark, 1931)	
<i>Thermoactinomyces</i>	60	(Yao <i>et al.</i> , 2014)	
<i>Nitrospirae</i>	<i>Thermodesulfovibrio</i>	70	(Sekiguchi <i>et al.</i> , 2008)
<i>Proteobacteria</i>	<i>Achromobacter</i>	40	(Gomila <i>et al.</i> , 2011)
	<i>Acidovorax</i>	37	(Heylen <i>et al.</i> , 2008)
	<i>Acinetobacter</i>	44	(Rooney <i>et al.</i> , 2016)
	<i>Advenella</i>	40	(Shmareva <i>et al.</i> , 2016)
	<i>Aeromonas</i>	37	(Galbis <i>et al.</i> , 2007)
	<i>Alcanivorax</i>	40	(Rahul <i>et al.</i> , 2014)

	<i>Beijerinckia</i>	30	(Oggerin <i>et al.</i> , 2009)
	<i>Bradyrhizobium</i>	37	(Araújo <i>et al.</i> , 2017)
	<i>Brevundimonas</i>	42	(Choi <i>et al.</i> , 2010)
	<i>Burkholderia</i>	37	(Aizawa <i>et al.</i> , 2011)
	<i>Comamonas</i>	44	(Chang <i>et al.</i> , 2002)
	<i>Delftia</i>	45	(Li <i>et al.</i> , 2015)
	<i>Desulfovibrio</i>	37	(Maarel <i>et al.</i> , 1996)
	<i>Enhydrobacter</i>	41	(Staley <i>et al.</i> , 1987)
	<i>Escherichia/Shigella</i>	37	(Liu <i>et al.</i> , 2015)
	<i>Halomonas</i>	50	(Guan <i>et al.</i> , 2010)
	<i>Hyphomicrobium</i>	30	(McDonald <i>et al.</i> , 2001)
	<i>Klebsiella</i>	37	(Bergey <i>et al.</i> , 1923)
	<i>Litoreaibacter</i>	37	(Romanenko <i>et al.</i> , 2011)
	<i>Marinobacter</i>	50	(Wang <i>et al.</i> , 2009)
	<i>Methylobacterium</i>	37	(Wood <i>et al.</i> , 1998)
	<i>Methyloversatilis</i>	37	(Kalyuzhnaya <i>et al.</i> , 2006)
	<i>Nevskia</i>	25	(Leandro <i>et al.</i> , 2012)
	<i>Paracoccus</i>	45	(Sun <i>et al.</i> , 2015)
	<i>Pelomonas</i>	40	(Xie and Yokota, 2005)
	<i>Photobacterium</i>	50	(Wang <i>et al.</i> , 2017)
	<i>Pseudomonas</i>	41	(Wang and Sun, 2016)
	<i>Ralstonia</i>	41	(Yabuuchi <i>et al.</i> , 1995)
	<i>Rheinheimera</i>	35	(Ryu <i>et al.</i> , 2008)
	<i>Rhodoplanes</i>	43	(Okamura <i>et al.</i> , 2009)
	<i>Serratia</i>	35	(Geiger <i>et al.</i> , 2010)
	<i>Sphingobium</i>	37	(Kumari <i>et al.</i> , 2009)
	<i>Sphingomonas</i>	40	(Yabuuchi <i>et al.</i> , 1990)
	<i>Stenotrophomonas</i>	42	(Lee <i>et al.</i> , 2011)
	<i>Sulfitobacter</i>	35	(Hong <i>et al.</i> , 2015)
	<i>Sulfurimonas</i>	40	(Inagaki <i>et al.</i> , 2003)
	<i>Tepidimonas</i>	60	(Chen <i>et al.</i> , 2013)
	<i>Thermomonas</i>	50	(Busse <i>et al.</i> , 2002)
	<i>Thiofaba</i>	51	(Mori and Suzuki, 2008)
	<i>Thiothrix</i>	37	(Howarth <i>et al.</i> , 1999)
	<i>Variovorax</i>	30	(Willems <i>et al.</i> , 1991)
<i>Spirochaetes</i>	<i>Treponema</i>	30	(Evans <i>et al.</i> , 2009)
<i>Thermotogae</i>	<i>Fervidobacterium</i>	90	(Cai <i>et al.</i> , 2007)

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**Table S10.** Summary of the PCR-amplified 16S rRNA gene cloning and sequencing-based analysis of the aquatic community present at the HTP site in August 2009.

Phylum affiliation of the clones	Serial no. Name of the representative 16S rRNA gene clones constructed from total environmental DNA (GenBank/EMBL accession number of the sequence) <u># Number of sibling clones present in the library for that representative</u>	Type strain(s) with which maximum sequence similarity was recorded	Identity (Query coverage)	Upper limit of temperature for growth <i>in vitro</i>	Reference
<i>Bacteroidetes</i>	1. 2010V2W clone B (FR848401) # 3	<i>Lentimicrobium saccharophilum</i> TBC1 <sup>T</sup>	89% (99%)	40	(Sun <i>et al.</i> , 2016)
	2. 2010V2W clone C (FR848402) # 4		89% (97%)		
<i>Actinobacteria</i>	3. clone 2b21 (FN594534) # 2	* <i>Microbacterium schleiferi</i> DSM 20489 <sup>T</sup>	93% (98%)	42	(Krishnamurthi <i>et al.</i> , 2012)
<i>Alphaproteobacteria</i>	4. 2010V2W clone A (FR848400) # 4	<i>Sandaracinobacter sibiricus</i> RB16-17 <sup>T</sup>	92% (98%)	30	(Yurkov <i>et al.</i> , 1997)
		* <i>Pedomicrobium manganicum</i> ATCC 33121 <sup>T</sup>	93% (96%)	40	(Gebbers, 1981)
	5. clone TWC 222 (HE774688) # 1	<i>Rhodobacter capsulatus</i> ATCC 11166 <sup>T</sup> <i>Rhodobacter sediminis</i> N1 <sup>T</sup>	99% (100%)	45	(Subhash <i>et al.</i> , 2016)
<i>Betaproteobacteria</i>	6. clone Puga_TWC_XXX383 (HE798186) # 3	<i>Limnobacter thiooxidans</i> CS-K2 <sup>T</sup>	99% (100%)	50	(Nguyen <i>et al.</i> , 2017)
	7. clone TWC 8 (HE774686) # 1	<i>Hydrogenophaga laconesensis</i> HWB-10 <sup>T</sup>	96% (39%)	37	(Yoon <i>et al.</i> , 2008)
	8. clone TWC 14 (HE774687) # 1	<i>Hydrogenophaga laconesensis</i> HWB-10 <sup>T</sup>	96% (40%)		
	9. Puga_TWC_XXX281 (HE798187) # 2	** <i>Herbaspirillum canariense</i> SUEMI03 <sup>T</sup>	97% (92%)	35	(Baldaniet <i>et al.</i> , 1986)
<i>Oxalicibacterium solurbis</i> NBRC 102665 <sup>T</sup>		37		(Sahin <i>et al.</i> , 2010)	
<i>Gammaproteobacteria</i>	10. clone 2b27 (FN594535) # 4	** <i>Lysobacter enzymogenes</i> DSM 2043 <sup>T</sup>	97% (97%)	40	(Christensen <i>et al.</i> , 1978)
	11. clone 2b28 (FN594536) # 6	** <i>Lysobacter enzymogenes</i> DSM 2043 <sup>T</sup>	99% (98%)		
	12. clone 1b3 (FN594537) # 5	** <i>Lysobacter enzymogenes</i> DSM 2043 <sup>T</sup>	99% (99%)		
	13. clone 1a1 (FN594538) # 4	** <i>Lysobacter enzymogenes</i> DSM 2043 <sup>T</sup>	99% (97%)		
	14. clone 1b16 (FN594539) # 3	** <i>Lysobacter enzymogenes</i> DSM 2043 <sup>T</sup>	96% (98%)		
	15. Puga_TWC_XXX711 (HE798188) # 5	* <i>Acinetobacter junii</i> ATCC 17908 <sup>T</sup>	100% (100%)	44	(Rooney <i>et al.</i> , 2016)
	16. clone TWC 7 (HE774684) # 1	* <i>Pseudomonas putida</i> NBRC 14164 <sup>T</sup>	100% (33%)	41	(Wang and Sun, 2016)
17. clone TWC 15 (HE774685) # 1	<i>Samsonia erythrinae</i> CFBP 5236 <sup>T</sup>	100% (26%)	39	(Sutra <i>et al.</i> , 2001)	

\* These genera were also detected in 2014.

\*\* These genera were also detected in 2013.

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**Table S11.** Number of species-level microbial entities (OTUs)<sup>1</sup> reported from geographically-discrete terrestrial hot springs.

Sl. No.	Name and Geographical Location of the vent	Temperature of the sample-site (in °C)	pH of the sample-site	No. of OTUs <sup>1</sup> identified	Reference
1	Diretuyanqu, China <sup>2</sup>	85.1	2.58	49	(Hou <i>et al.</i> , 2013)
2	Jiemeiquan - Sisters Spring, China <sup>2</sup>	93.6	9.25	59	(Hou <i>et al.</i> , 2013)
3	Zhenzhuquan - Pearl Spring, China <sup>2</sup>	89.1	4.79	46	(Hou <i>et al.</i> , 2013)
4	Huitaijing - Pregnancy Spring, China <sup>2</sup>	92.3	8.05	63	(Hou <i>et al.</i> , 2013)
5	Shuirebaozha - Hydrothermal Explosion, China <sup>2</sup>	79.8	8.04	87	(Hou <i>et al.</i> , 2013)
6	Dagunguo - Great Boiling Pot, China <sup>2</sup>	84.5	7.20	21	(Hou <i>et al.</i> , 2013)
7	Gumingquan - Drum Beating Spring, China <sup>2</sup>	93.0	9.35	82	(Hou <i>et al.</i> , 2013)
8	Gongxiaoshe - Co-op Hotel (side), China <sup>2</sup>	73.8	7.29	41	(Hou <i>et al.</i> , 2013)
9	Jinze - Golden Pond Motel, China <sup>2</sup>	81.6	6.71	40	(Hou <i>et al.</i> , 2013)
10	Jermuk, Southeast Armenia <sup>2,4</sup>	53.0	7.50	209	(Hedlund <i>et al.</i> , 2013)
11	Arzakan, Southeast Armenia <sup>2,4</sup>	44.0	7.20	321	(Hedlund <i>et al.</i> , 2013)
12	Lake Magadi, Hot spring 1, Kenya <sup>3,4</sup>	45.1	9.80	377	(Kambura <i>et al.</i> , 2016)
13	Lake Magadi, Hot spring 2, Kenya <sup>3,4</sup>	83.6	9.40	680	(Kambura <i>et al.</i> , 2016)
14	Lake Magadi, Hot spring 3, Kenya <sup>3,4</sup>	81.0	9.20	568	(Kambura <i>et al.</i> , 2016)
15	Mqhephu hot spring, Limpopo Province of South Africa <sup>3</sup>	43.0	8.19	224	(Tekere <i>et al.</i> , 2015)
16	Tshipise hot spring, Limpopo Province of South Africa <sup>3</sup>	58.0	8.94	101	(Tekere <i>et al.</i> , 2015)
17	Sagole hot spring, Limpopo Province of South Africa <sup>3</sup>	45.0	9.24	303	(Tekere <i>et al.</i> , 2015)
18	Siloam hot spring, Limpopo Province of South Africa <sup>3</sup>	63.0	9.50	251	(Tekere <i>et al.</i> , 2015)
19	Soutinig hot spring, Limpopo Province of South Africa <sup>3</sup>	43.9	7.20	105	(Tekere <i>et al.</i> , 2015)
20	Eiland hot spring, Limpopo Province of South Africa <sup>3</sup>	41.0	7.63	6	(Tekere <i>et al.</i> , 2015)
21	Great Boiling Spring 1002W, Great Basin, USA <sup>3</sup>	82.0	6.63	36	(Cole <i>et al.</i> , 2012)
22	Akwar, Eritrea <sup>3,4</sup>	49.5	7.22	1898	(Ghilamicael <i>et al.</i> , 2017)
23	Elegedi, Eritrea <sup>3,4</sup>	100.0	7.01	1662	(Ghilamicael <i>et al.</i> , 2017)
24	Garbanabra, Eritrea <sup>3,4</sup>	51.3	7.05	2354	(Ghilamicael <i>et al.</i> , 2017)
25	Gelti, Eritrea <sup>3,4</sup>	52.6	7.19	1834	(Ghilamicael <i>et al.</i> , 2017)
26	Maiwooi, Eritrea <sup>3,4</sup>	51.4	7.54	1054	(Ghilamicael <i>et al.</i> , 2017)
27	Icelandic hot springs P0, Iceland <sup>3</sup>	100.0	3.80	84	(Krebs <i>et al.</i> , 2014)
28	Icelandic hot springs P1, Iceland <sup>3</sup>	59.0	3.20	529	(Krebs <i>et al.</i> , 2014)
29	Icelandic hot springs P2, Iceland <sup>3</sup>	64.0	2.90	168	(Krebs <i>et al.</i> , 2014)
30	Icelandic hot springs P3, Iceland <sup>3</sup>	57.0	2.90	674	(Krebs <i>et al.</i> , 2014)
31	Leirgerdur, Iceland <sup>3</sup>	98.0	2.00	19	(Krebs <i>et al.</i> , 2014)
32	Hrifla spring, Iceland <sup>3</sup>	98.0	7.00	19	(Krebs <i>et al.</i> , 2014)
33	Lotus Pond HTP <sup>3</sup> , as explored at 17:30 h of 23.07.2013	78.0	8	331	(Roy <i>et al.</i> , 2016)
34	Lotus Pond HTP <sup>3</sup> , as explored at 8:30 h of 20.10.2014	84.0	7.5	109	(This study)
35	Lotus Pond HTP <sup>3</sup> , as explored at 14:30 h of 20.10.2014	81.0	7.8	279	(This study)
36	Lotus Pond MTP <sup>3</sup> , as explored at 8:30 h of 20.10.2014	74.0	7.5	635	(This study)
37	Lotus Pond MTP <sup>3</sup> , as explored at 14:30 h of 20.10.2014	72.0	7.5	1205	(This study)

<sup>1</sup> In all the reports considered, microbial diversity of aquatic or semi-aquatic communities was estimated in terms of the total number of species-level entities or operational taxonomic units (OTUs) present. OTUs were determined based on high throughput 16S rRNA genes sequence data.

<sup>2</sup> OTU counts for these hydrothermal communities involved both bacteria and archaea.

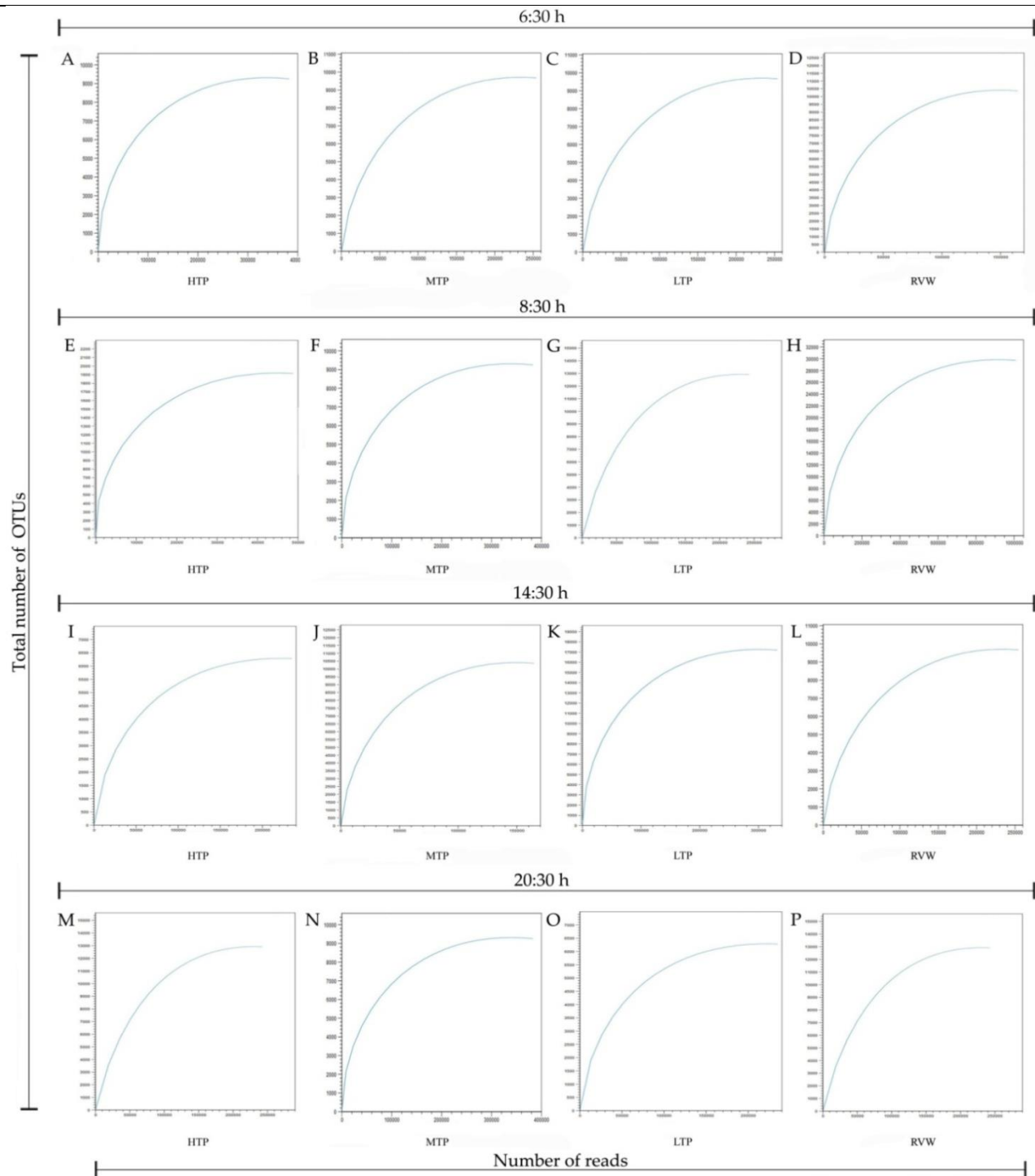
<sup>3</sup> OTU counts for these hydrothermal communities involved only bacteria.

<sup>4</sup> These studies involved semi-aquatic communities; all the other studies involved aquatic communities.

## References used in Table S11

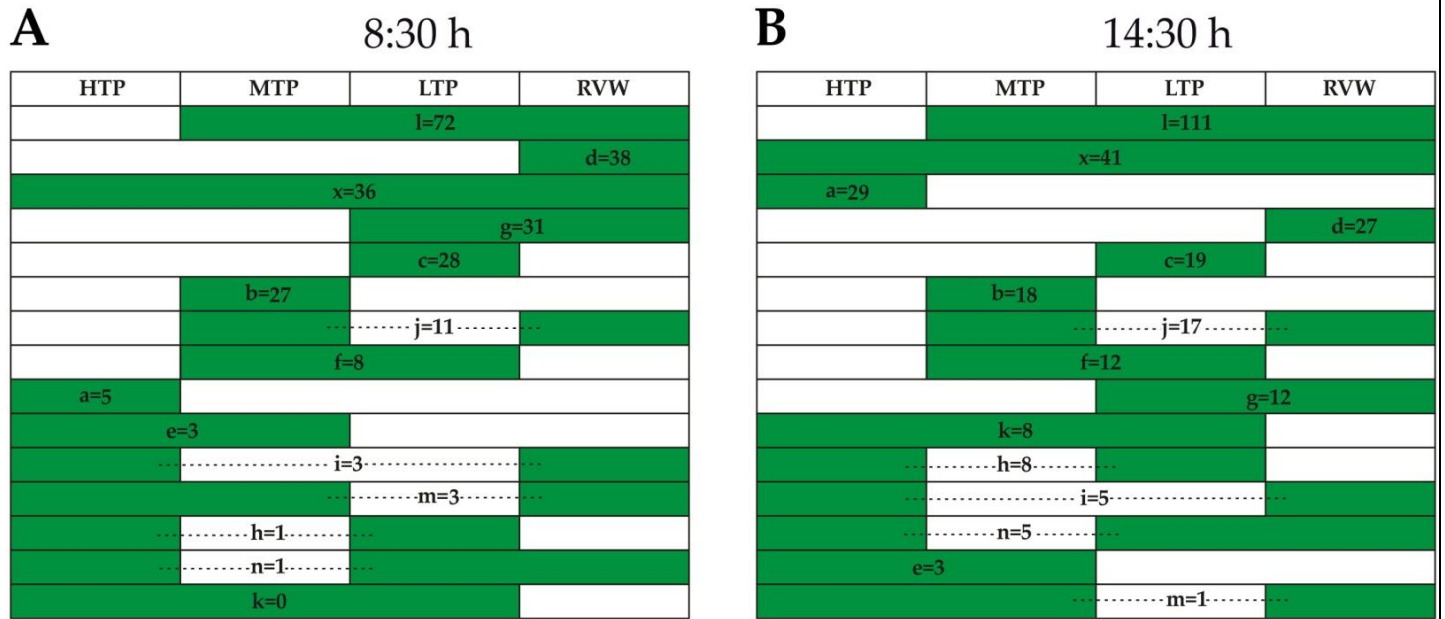
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## Supplementary Figures



**Figure S1.** Rarefaction curves showing the proportionality between OTU-level diversity revealed and the number of amplified 16S rRNA gene sequence (V3 region) reads analyzed for the individual water-samples collected from HTP, MTP, LTP and RVW separately at (A through D) 6:30 h, (E through H) 8:30 h, (I through L) 14:30 h and (M through P) 20:30 h of 20 October 2014. For each of the 16 samples, number of reads used in OTU-building are plotted along the X-axis, while number of OTUs (including singletons) created at the 97% 16S rRNA gene sequence identity level are plotted along the Y-axis.





**Figure S2.** The numbers of genera that are unique to the different sites or common between the sites, across HTP, MTP, LTP and RVW, at (A) 8:30 h and (B) 14:30 h. For both the sampling-hours, a = number of genera that were present only in HTP; b = number of genera that were present only in MTP; c = number of genera that were present only in LTP; d = number of genera that were present only in RVW; e = number of genera that were present in both HTP and MTP, but not elsewhere; f = number of genera that were present in both MTP and LTP but not elsewhere; g = number of genera that were present in both LTP and RVW, but not elsewhere; h = number of genera that were present in both HTP and LTP, but not elsewhere; i = number of genera that were present in both HTP and RVW, but not elsewhere; j = number of genera that were present in both MTP and RVW, but not elsewhere; k = number of genera that were present in HTP, MTP as well as LTP, but not in RVW; l = number of genera that were present in MTP, LTP as well as RVW, but not in HTP; m = number of genera that were present in HTP, MTP as well as RVW, but not in LTP; n = number of genera that were present in HTP, LTP as well as RVW, but not in MTP; x = number of genera that were present in all the four sample-sites.