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ORIGINAL ARTICLE

Microbial community dynamics in Inferno Crater Lake, a thermally fluctuating geothermal spring

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Understanding how microbial communities respond and adjust to ecosystem perturbation is often difficult to interpret due to multiple and often simultaneous variations in observed conditions. In this research, we investigated the microbial community dynamics of Inferno Crater Lake, an acidic geothermal spring in New Zealand with a unique thermal cycle that varies between 30 and 80 °C over a period of 40–60 days. Using a combination of next-generation sequencing, geochemical analysis and quantitative PCR we found that the microbial community composition was predominantly chemolithotrophic and strongly associated with the thermal cycle. At temperatures >65 °C, the microbial community was dominated almost exclusively by sulphur-oxidising archaea (*Sulfolobus*-like spp.). By contrast, at mesophilic temperatures the community structure was more mixed, comprising both archaea and bacteria but dominated primarily by chemolithotrophic sulphur and hydrogen oxidisers. Multivariate analysis of physicochemical data confirmed that temperature was the only significant variable associated with community turnover. This research contributes to our understanding of microbial community dynamics in variable environments, using a naturally alternating system as a model and extends our limited knowledge of acidophile ecology in geothermal habitats.
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Introduction

Inferno Crater Lake is an active acidic geothermal spring in the Waimangu-Rotomahana-Tarawera Geothermal System, Rotorua, New Zealand (Figure 1) and is situated in one of the eruption craters formed by the 1886 volcanic eruption of nearby Mt Tarawera (Supplementary Figure 1). The long established hydrothermal system at depth drives the alkali-chloride water to reach the surface, creating a broad array of surface hot springs and fumaroles (Simmons *et al.*, 1993, 1994; Stucker *et al.*, 2016). Due to its location at a topographic high point, Inferno Crater Lake is the only major acidic feature of the valley, and exhibits a stable low pH driven largely by the oxidation of sulphurous compounds in the water column (pH 2.0–2.5; Glover *et al.*, 1994; Simmons *et al.*, 1994). It also has a unique, cyclical thermal profile in which the temperature of the water column fluctuates between 30 and 80 °C during a recurring period of ~40 days, concomitant with a rise and fall in spring water level (Scott, 1994).

The tight coupling between water level and temperature has persisted at Inferno Crater Lake for more than a century. This phenomenon is due to periodic influx and retreat of geothermal fluid and steam via hydrothermal vents on the lake floor, in a manner proposed as synonymous with a subterranean geyser eruption to the regular eruption (Vandemeulebrouck *et al.*, 2008). Each thermal cycle comprises four distinct stages, associated with geothermal fluid fluxes into and out of the crater and seismicity. Each stage has some variability associated with the temperature and duration, however in general the following stages are observed: the first stage ('rise') begins when water level and temperature reach their lowest point (30–40 °C) and ends with a continuous steady rise in temperature (up to 60 °C), generally lasting about five days, but potentially as long as 30 days. During this phase the lake volume increases to about 24 000 m^3. This is followed by a two week 'oscillation' cycle (Stage 2) in which temperature fluctuates between 50 and 60 °C, with accompanying variations in water level and volume increase of an additional 19 400 m^3. Following oscillation, temperature rises to a maximum of ~75–80 °C as water level reaches its peak (65 200 m^3,

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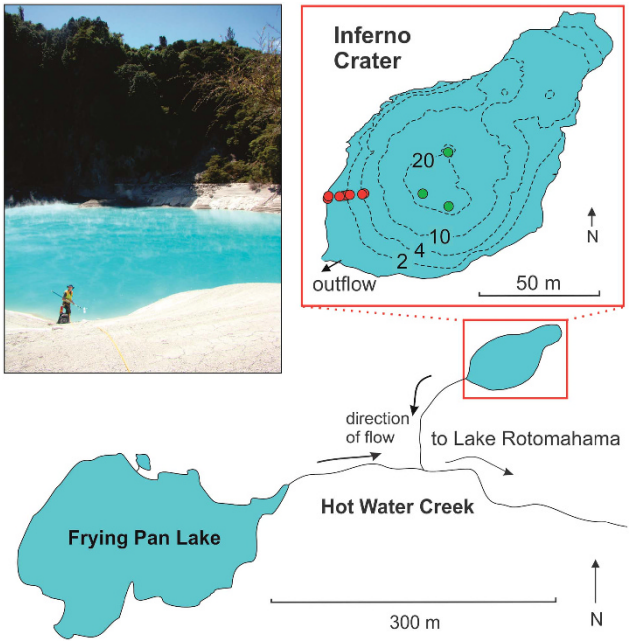

Figure 1 Schematic of the two primary geothermal features at Waimangu Geothermal Valley (adapted from Scott (1994)). During overflow (Stage 3), 75–80 °C water flows from Inferno Crater Lake and joins Hot Water Creek, the main outflow from Frying Pan Lake. Image shows sample collection during Stage 1 ('rise') at Inferno Crater Lake. Inset – Inferno Crater Lake detail: dashed lines; bathymetric depth below overflow, green dots; known vents (Keam, 1981), red dots; sample locations throughout study.

Stage 3; 'overflow'). During this stage, the lake will overflow (average of 79 l s^{-1}) via an outflow channel connected to a circumneutral pH stream (~pH 6.6; 45 °C) that extends down the valley to Lake Rotomahana (Figure 1; Supplementary Figure 1). The overflow lasts ~2 days and is followed by the fourth and final stage ('retreat'); this stage involves a massive subterranean retreat of the water (volume decrease of 45 800 m^3; ~5% of the water volume is lost to evaporation) and temperature down to the minimum, lasting ~20 days (Scott, 1994).

The thermal fluctuations of up to Δ50 °C over the relatively short four stage cycle within the Inferno Crater Lake water body impose unique challenges for microbial life. The spring chemistry and pH remain stable throughout the thermal cycling (Sheppard, 1986; Keywood and Nicholson, 1990), which suggests that the environment should be amenable to a wide range of bacterial and archaeal acidophiles from the phyla *Nitrospirae*, *Proteobacteria*, *Verrucomicrobia*, *Aquificae*, *Firmicutes*, *Crenarchaeota* and *Euryarchaeota* (Shima *et al*., 1994; Siering *et al*., 2006; Op den Camp *et al*., 2009; Kondrat'eva *et al*., 2012; Sharma *et al*., 2012). These acidophiles exhibit temperature growth ranges that span from mesophilic through to thermophilic (35–85 °C). However, there appears to be a clear dichotomy in temperature growth profiles at domain level, with optimal growth temperatures of characterised bacterial representatives being 10–15 °C lower than their archaeal (particularly crenarchaeotal) counterparts (Supplementary Figure 2). The maximum optimum growth temperature for a bacterial acidophile appears to be 60–65 °C (Supplementary Figure 2), and is consistent with observations in molecular surveys of heated acidic environments (Siering *et al*., 2006; Stout *et al*., 2009; Inskeep *et al*., 2010; Kato *et al*., 2011; Mardanov *et al*., 2011; Bohorquez *et al*., 2012; Hedlund *et al*., 2012; Sahm *et al*., 2013). The thermal cycle at Inferno Crater Lake offers a unique opportunity to investigate the response of microbial community structure to environmental condition flux.

In this study, we tested two hypotheses regarding the response of the microbial community to the temperature cycles of the lake. Firstly, we hypothesised, based on published pure-culture studies indicating greater thermotolerance among archaea than bacteria (Supplementary Figure 2), that archaea will dominate the high-temperature stages of Inferno Crater Lake's thermal cycle. If we consider the paucity of subaerial inputs into the spring, then the reciprocal observation of this hypothesis is that only bacterial taxa capable of surviving elevated temperatures via sporulation or similar mechanisms will dominate the microbial community at the lower temperature stages. Secondly, we predicted that changes in the structure of Inferno Crater Lake's microbial community will be niche driven, and strongly correlate with changes in temperature. This second hypothesis is based on previous studies of acidophilic hot spring microbial communities, which have found that bacteria and archaea in these extreme environments are predominantly chemolithotrophs and therefore their persistence in a given environment is largely a product of local physicochemical conditions (Siering *et al*., 2006; Inskeep *et al*., 2010; Kato *et al*., 2011; Hedlund *et al*., 2012). We addressed the first hypothesis using a 16S rRNA gene-directed quantitative PCR assay to measure the relative abundances of archaea and bacteria during each of the four thermal stages across two consecutive cycles. The second hypothesis was tested using 16S rRNA gene amplicon pyrosequencing to identify the microbial community present at the same four sampling points. Microbial community similarity was compared between sampling points and correlated with physicochemical parameters measured alongside each biological sample, using distance-based linear modelling. This is the first microbiological investigation of Inferno Crater Lake, a globally unique geothermal feature which provides a model habitat to investigate niche-driven fluctuations in microbial community assemblages.

Materials and methods

Sampling and water analysis

Water column samples were collected between February and May 2013, from the same location on the western bank of Inferno Crater Lake (Figure 1; 38°16′54.98″S, 176°23′58.69″E – Spring reference #:

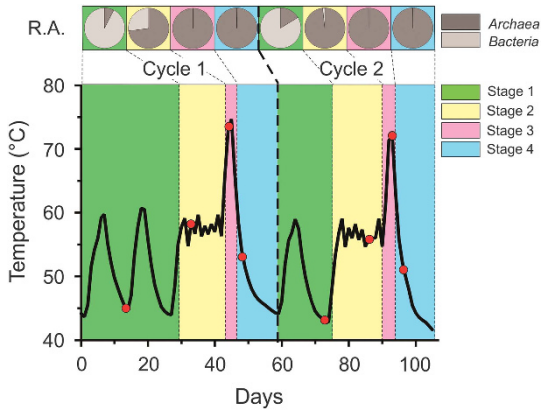

Figure 2 The thermal cycle of Inferno Crater Lake between 11 Feb and 31 May 2013. Samples were collected from all four stages (red dots) over two consecutive cycles; Stage 1 – 'rise' (green), Stage 2 – 'oscillation' (yellow), Stage 3 – 'overflow' (pink) and Stage 4 – 'retreat' (blue). Pie charts represent the relative abundance of bacterial (light grey) and archaeal (dark grey) 16S rRNA gene sequence copy at each time point.

WMF 3026). Sampling times corresponded to representative points within the four distinct stages across two thermal cycles (Figure 2) and are referred to by their cycle number (1 or 2) and their stage number (1–4). The New Zealand Geonet Project (Geonet, 2001) monitors the hydrothermal activity of Inferno Crater Lake; we utilised the real-time telemetry data (temperature and spring height data) to identify appropriate times for sampling (Figure 2). The telemetry thermocouple at Inferno Crater Lake sits at a depth of ca. 10 m (Figure 1). At each time point, water was collected in eight 250 ml sterile tubes and comprised a representative sample that included a mix of surface water and water to a depth of ~30–40 cm. All samples were collected at ca. 80–100 cm from the shoreline. As the Inferno Crater Lake water height varies within the heating cycle, the shoreline varies up to 20 m horizontally and 8 m vertically from maximal edge of the spring; samples were taken in a straight line transect according to period in the cycle (Figure 1). A handheld thermocouple was used to measure temperature at the sampling point and reflected the telemetry data. The samples were immediately returned to the laboratory and processed within 2 h of collection.

A sample for geochemical analysis was also collected at each sampling time point. This consisted of 500 ml of untreated water, 200 ml of syringe-filtered (0.20 μM) water, 200 ml of syringe-filtered and acidified water (0.20 μM filter+2 ml conc. HNO_3), 200 ml of water collected using an evacuated roto-flow flask pre-treated with 50 ml 8 N NaOH for gas analysis (Giggenbach, 1995), and a 500 ml rubber-necked glass bottle filled with water. Sample pH was measured using a benchtop pH metre calibrated for acidic samples.

The aqueous components of the water column including cations, SiO_2 and metals were measured by inductively coupled plasma-optical emission spectroscopy. Ion chromatography (IC) was used to measure SO_4^{2-}, NO_3^- and PO_4^{3-} concentrations. The dissolved gas content (H_2, N_2, CH_4, He, O_2 – Rotoflow flask samples) was determined using gas chromatography. Ammonia (as N-NH_4^+) concentration was determined using ion selective electrode measurement, and sulphide (as H_2S), NO_2^-, Cl^- and CO_2 (as HCO_3^-) concentrations by titration.

DNA extraction, real-time quantitative PCR and 16S rRNA gene amplicon pyrosequencing

Each of the eight 250 ml water samples collected at each time point for molecular analyses was filtered through a sterile 0.20 μM, 25 mM-diameter, cellulose-nitrate filter (Sartorius Stedim Biotech, Goettingen, Germany). Filters were then aseptically transferred to NucleoSpin bead-beating tubes (Macherey-Nagel, Duren, Germany) for DNA extraction. Sterile skim milk solution (200 μl, 50 mg l^{-1} stock solution) was added to the extraction buffer and filtrate (including an extraction negative control) to block binding of DNA to the suspended clays, which are characteristic of Inferno Crater Lake water column samples. Separate extraction controls were used for each sampling time point, and all extraction controls also included skim milk solution. DNA was extracted using a NucleoSpin Soil kit (Macherey-Nagel).

Three DNA extracts from each time point, representing triplicate biological samples, were analysed alongside their respective extraction controls in two separate 16S rRNA gene-targeting quantitative PCR (qPCR) assays to quantify the relative abundance of each prokaryotic domain across two geothermal cycles. The first employed *Bacteria*-specific primers (926f/1062r; (Bacchetti De Gregoris *et al.*, 2011)) while the other used *Archaea*-specific primers (349 f/806r; (Takai and Horikoshi, 2000)). Two replicate 96-well plates were run for each assay in order to obtain a total of four technical replicates per biological repeat (two technical replicates per plate). Accompanying standards, comprising triplicate dilution series of linearised plasmid DNA which included the 16S rRNA genes from laboratory strains of *Thermus filiformis* and *Thermoplasma volcanium* (for *Bacteria* and *Archaea*, respectively), were used for quantification. Additionally, each plate contained triplicate no-template control reactions. All qPCR reactions utilised SYBR Green (Thermo Fisher Scientific, Waltham, MA, USA) chemistry and were performed on a Stratagene Mx3000P qPCR thermocycler, the specific conditions of which are described in detail in the Supplementary Information.

In order to identify changes in microbial community structure across the Inferno Crater Lake thermal cycle, the same DNA extracts analysed using qPCR were also used as templates for amplicon pyrosequencing. The 515f and 806r universal primer pair, which targets both *Bacteria* and *Archaea* (Caporaso *et al.*, 2011), was used to amplify the 16S rRNA gene, with an extra degeneracy introduced to the forward

primer in order to increase coverage of *Archaea* (Supplementary Table 1). The sequencing methodology and conditions under which PCR amplicons were obtained are described in the Supplementary Materials. The purified PCR products obtained from three biological replicates for each sample were diluted to the same volume as determined by the product of lowest concentration (8.35 ng μl^{-1}) and pooled. Sequencing (1/8 plate) was conducted at Macrogen (Seoul, South Korea) on a Roche GS-FLX Titanium Pyrosequencer. Sequence data were deposited in the Sequence Read Archive at NCBI under the accession number SRP066866.

A water column sample was also collected and sterilised (121 °C, 20 min) to check the stability of DNA in the Inferno Crater Lake ecosystem. A laboratory stock (~350 ng) of plasmid DNA (inset: *T. filiformis* 16S rRNA gene), as well as a laboratory strain of *Geobacillus* sp., were separately spiked into sterile water samples (1 ml) and incubated at room temperature for 30 min. DNA was then extracted from the spiked samples, and the resultant extracts amplified by PCR using 9f and 1492r primers (Supplementary Materials).

Bioinformatic and statistical analyses

Raw sequence data returned 130 000 reads with an average read length of 290 bp. A detailed description of data processing is available in the Supplementary Materials. Sequences were processed via the standard mothur pipeline using the default settings (Schloss *et al.*, 2011) and were denoised using the AmpliconNoise algorithm (Quince *et al.*, 2011) to a minimum length of 250 bp. Trimmed FASTA sequences were aligned against the SILVA bacterial 16S rRNA gene sequence database (version LTP 111). Chimeric sequences were detected and removed using UCHIME in association with the SILVA database (Edgar *et al.*, 2011). All remaining sequences were taxonomically classified against the Greengenes database 13_8 using the Bayesian classifier implemented in mothur. Operational taxonomic units (OTUs) were built in mothur, using a sequence similarity distance matrix. Pairwise distances greater than 0.15 were discarded (Schloss *et al.*, 2011) and similar 16S rRNA gene sequences were clustered into OTUs based on a 97% similarity threshold. The number of reads per biological repeat was then rarefied to 3926. Singleton OTUs were removed before subsequent use in analyses of community structure and diversity.

Unless otherwise stated, all statistical analyses were conducted using the PRIMER v6 software package (Clark and Gorley, 2006). To explore beta diversity across the two thermal cycles, rarefied OTU abundances for each sample were analysed together with corresponding physicochemical data. Rarefied OTU sequence counts were pooled for all three subsamples per time point, resulting in a total of 11 778 counts per sample. Raw environmental metadata were screened for missing values and outliers, then visualised on a draftsman scatter plot. Calcium, methane, hydrogen and pH measurements required transformations to account for skew and non-linearity (square root, presence/absence, presence/absence and square root, respectively). Linear correlation coefficients were calculated for pairwise comparison of all environmental variables. Variable pairs with a coefficient greater than absolute (0.7) were considered collinear within the context of the Inferno Crater Lake geothermal system, as the chemistry of the spring water is strongly influenced by deep chloride steam influx during the oscillation and overflow stages (Glover *et al.*, 1994; Simmons *et al.*, 1994). Collinear variables were reduced to one primary representative variable per group in further analyses. This included daily average temperature (which was collinear with chloride), pH (inversely collinear with dissolved oxygen), ammonia (collinear with chloride and carbon dioxide) and boron (collinear with aqueous sulphate). A Bray-Curtis similarity coefficient matrix was constructed for all pairwise OTU count comparisons of sample time points. Hierarchical dendrograms were constructed to visualise trends in sample similarity. Distance linear modelling (DISTLM routine with permutation) was used to model environmental variables against the Bray-Curtis similarity matrix in order to determine which variables best explained similarities between sample communities. DISTLM was conducted using the BEST procedure and adjusted Akaike Information Criterion (AICc) as the selection criteria. Given the small number of samples ($n = 8$), a maximum of two variables was stipulated for mixed models. The following variables were excluded from analysis as they were either deemed biologically irrelevant due to low variation over the two cycles, at values close to the limits of detection, or were confounded with other collinear environmental variables: pH, boron, carbon dioxide, chloride, nitrate, nitrite, phosphate, silica and sodium. All remaining variables were tested without forced inclusion. Marginal tests for each variable were also conducted in the DISTLM routine.

Results and discussion

Inferno Crater Lake is a steam-influenced acidic hot spring that has a thermal cycle which routinely varies in temperature from as low as 30 °C to as high as 80 °C. This semi-predictable thermal cycle offered us a unique opportunity to observe microbial community succession in a natural ecosystem and test a number of hypotheses; namely, that *Archaea* would be the dominant microbial taxon at the high-temperature stages and spore-forming *Bacteria* dominant at the low-temperature stages of Inferno's cycles, and that microbial community diversity would be strongly dependent on physicochemical changes during the cycles. We tested these

Table 1 Physical and chemical parameters of Inferno Crater Lake at each sampling point

Stage	*Cycle 1*				*Cycle 2*			
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Collection date (2013)	15 Feb	8 Mar	18 Mar	22 Mar	13 Apr	29 Apr	5 May	8 May
Average temp. (°C)[a]	45.4	56.7	74.7	54.2	44.6	55.9	71.3	55.9
pH	2.48	2.54	2.59	2.58	2.44	2.53	2.58	2.05
Analyte (mg l^{-1})								
Ammonia	2.1	1.7	1.9	2.1	2.2	1.8	1.8	2
Boron	7	7.7	8.4	8.3	10.2	8	8.4	8.1
Calcium	12.1	11.3	11.6	12.7	12	11.9	2.2	12.1
Chloride	902	879	868	901	897	863	851	875
Lithium	6	6.2	ND	1	6	6	6	5.8
Nitrate	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.
Nitrite	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.
Phosphate	B.d.l.	Bb.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.
Potassium	111	100	89	111	112	106	124	108
Silica (total as SiO_2)	763	740	ND	820	703	707	670	747
Sodium	605	550	510	601	575	576	ND	567
Sulphate	369	329	324	321	245	366	335	348
Sulphide (total as H_2S)	0.004	0.02	0.009	0.01	0.02	0.02	0.03	0.01
Soluble gas concentration (p.p.m.v.)								
Carbon dioxide	163.8	273.8	134.5	83.1	117.4	161.4	151.6	127.1
Hydrogen	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	B.d.l.	0.002	0.002
Hydrogen sulphide	4	5.9	6.1	32.2	3.4	2.3	2.8	8.7
Methane	B.d.l.	B.d.l.	B.d.l.	0.892	0.08	0.143	B.d.l.	0.054
Oxygen	12	1	1	9	9	6	5	16

Abbreviations: ND, not determined; B.d.l., below detection limit.
[a]Temperatures presented are a four point average temperature measured over ± 2 h by telemetry at the time of sampling.

hypotheses by sampling Inferno Crater Lake's water column at each of the four characteristic stages through two complete heating and cooling cycles. For each sampling point we measured the hot spring's water and gas chemistry, and microbial community composition via qPCR and 16S rRNA gene amplicon pyrosequencing.

Temperature is the most variable physicochemical factor across Inferno Crater Lake's cycles

Consistent with previous reports (Keywood and Nicholson, 1990; Simmons *et al*., 1994; Stucker *et al*., 2016), the Inferno Crater Lake chemistry and pH remain relatively stable across the thermal cycle. Geochemical data collected for each sample point across two cycles (Table 1) show little change in any measured analyte over the course of the sampling regime. Of the metadata measured, only temperature varied substantially, along with some minor collinear variations in dissolved gases. The sampling temperatures measured using the handheld thermocouple matched the temperature reported by the telemetry thermocouple at the time of sampling (data not shown) and confirmed the homogeneity of the Inferno Crater Lake ecosystem. During both cycles, the start of Stage 1 was punctuated by what appeared to be false-starts of the 'rise'. These failed cycles have been noted before (Scott, 1994) and often follow a high stand or variation of the cycle. Hence, the water level heated and cooled before the typical rise started (Figure 2). Such 'false-starts' were common in 2013.

Archaeal and bacterial community abundances vary predictably across the thermal cycle

The proportional abundance of *Bacteria* and *Archaea* (estimated by 16S rRNA gene-targeted qPCR) across both cycles shows a clear and repetitive turnover (Figure 2). When proportion data were plotted against water column temperature, it was evident that *Bacteria* dominated only during the low-temperature first stage of the thermal cycle (Figure 3), at temperatures below 45 °C. During Stage 2 (oscillation), *Archaea* began to outnumber *Bacteria*, and by Stage 3 (overflow), *Archaea* were almost exclusively present. *Bacteria* appeared to take some time to re-establish after high-temperature overflow, as *Archaea* remained higher in proportional abundance during stage four of the cycle even as temperatures dropped below 60 °C. This supports culture-based observations that acidophilic *Bacteria* have lower optimal temperature tolerances compared with those of *Archaea*. It remains unknown as to whether bacteria were completely unable to survive at temperatures above 65 °C in this environment. There is evidence, for example, that *Hydrogenobaculum acidiphilum*, a sulphur-requiring hydrogen-oxidising member of the *Aquificales*, has a T_{max} of 80 °C (Shima *et al*., 1994; Stohr *et al*., 2001), and thus the *Hydrogenobaculum* taxa detected in the

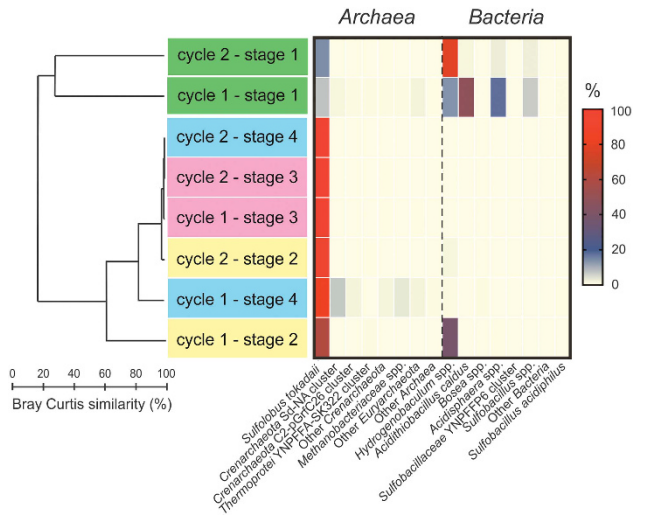

Figure 3 Microbial community composition of Inferno Crater Lake over two thermal cycles. The heatmap shows proportional OTU read abundances, with colour intensity has been scaled nonlinearly to emphasise low- abundance taxa. OTUs with identical taxonomic identities were grouped together along with OTUs with read abundances of $<0.5\%$. The community composition similarity at each stage across both cycles is shown by using a Bray-Curtis similarity tree.

Inferno Crater Lake datasets could conceivably also be able to survive Stage 3. This observation may explain the very low bacterial qPCR abundance signal observed at Stage 3, although we suspect that this was more likely a result of low-level contamination.

Temporal trends in biodiversity

Normalised sequence reads were compared across three biological repeats for each sampling point. The observed biological diversity within Inferno Crater Lake was very low, with only 89 OTUs identified among 112 280 total sequence reads (before normalisation) across all sampling points. A comparison of OTU read abundance across sampling points shows a highly uneven community structure at all high-temperature points across both cycles (Figure 3). Indeed, at all stages except for low-temperature Stage 1, samples were dominated by a single archaeal OTU related to *Sulfolobus tokodaii* (94% 16S rRNA gene sequence similarity to NR_074348 – Supplementary Table 2), an obligately acidophilic, aerobic sulphur oxidiser commonly found in acidic hot springs (Suzuki *et al.*, 2002). Where bacteria were present at higher temperatures during Stage 2, they were singularly dominated by reads of a *Hydrogenobaculum* sp. OTU (100% 16S rRNA gene sequence similarity to NR_025844 – Supplementary Table 2). No bacterial OTUs were detected by pyrosequencing during Stage 3 or 4 of either cycle, suggesting that bacteria do not survive in the water column during this stage or were at numbers below our detection limits. Community structure was moderately less uneven in the low-temperature Stage 1 samples where four bacterial OTUs were prevalent in the first thermal cycle: *Hydrogenobaculum* sp., *Acidithiobacillus caldus*, *Acidisphaera* sp. and *Sulfobacillus acidophilus* (Supplementary Table 2). A comparison of the Stage 1 community composition between cycles shows that the evenness of OTU distribution differs across cycles (Figure 3). While the same OTUs were present across both cycles, in the second cycle, the relative abundance of *Hydrogenobaculum* sp. reads was far greater than in the first. Potentially this is due to stochastic repopulation events or historical contingencies on the structure of bacterial communities in the low-temperature stages, or this may simply be an effect of sample timing, as Cycle 1 Stage 1 samples were taken prior to a 'false start' event. Similarly, Stage 2 samples in Cycle 2 were collected at a longer time interval from the start of the stage compared with their equivalent samples in Cycle 1, which may explain the higher proportional abundance of bacterial reads in Cycle 1 (Figures 2 and 3). Archaeal OTU diversity also appeared to be higher at low-temperature Stage 1, although many of these OTUs could not be taxonomically identified to below order level. This could be the result of the relatively short sequence reads, a lack of representative archaeal sequences in the database used for alignment and classification, or a combination of both. Despite the lack of taxonomic resolution, we did detect typical acidophilic bacterial taxa that have been found elsewhere in geothermal environments, such as *Acidithiobacillus* and *Acidisphaera* spp. (Supplementary Table 3). Surprisingly, several OTUs within the archaeal class *Methanobacteria* were repeatedly detected in both Stage 1 thermal cycles (Figure 3; Supplementary Table 2). Of note was OTU7 which phylogenetically placed in the genus *Methanothermobacter* (Supplementary Figure 3) and was most closely related to *Methanothermobacter crinale* (100% - HQ238273), a thermophilic and neutrophilic methanogen isolated from production water from an oil reservoir (Cheng *et al.*, 2011). Although thermotolerant methanogenic archaea have previously been isolated from geothermal environments, most methanogens have an optimal growth pH near neutral (Barber, 2001; Liu and Whitman, 2008). A few species have been isolated from environments as low as pH 3.6 (Sizova *et al.*, 2003; Cadillo-Quiroz *et al.*, 2006), but this is still substantially higher in pH than was measured in Inferno Crater Lake during this and previous studies (Scott, 1994; Simmons *et al.*, 1994; Vandemeulebrouck *et al.*, 2008). The presence of methanogenic archaeal OTUs in Stage 1 is somewhat perplexing. It is tempting to speculate that these OTUs could be environmental DNA contaminants or possibly of aeolian or chthonic origin. However, we believe that allochthonous DNA is probably not the source of these OTU signals: In preliminary experiments, we spiked DNA and/or neutrophilic laboratory strains directly into sterilised Inferno Crater Lake water

column samples, but were unable to detect corresponding amplification signals via PCR (results not shown). This suggested that either the resultant DNA was adsorbed to the ultra-fine suspended clay in the water column and/or was hydrolysed rapidly due to the sample acidity. While follow-up studies are required, we tentatively consider that the methanogenic OTUs were not artefacts, and are likely a yet-to-be described mesophilic/moderately thermophilic and acid-tolerant strain of *Methanothermobacter*.

Although 16S rRNA gene-based taxonomies do not provide a robust measure of actual metabolic functions within a microbial community, they can be used to explore putative functions (Tringe and Hugenholtz, 2008). The dominance of *Sulfolobus tokodaii* and *Hydrogenobaculum* sp. implies a strong reliance on chemical sources of energy, particularly elemental and reduced inorganic sulphur, as well as hydrogen gas (Hallberg and Johnson, 2001). Dendrogram clustering of samples according to their Bray-Curtis similarities (Figure 3; Supplementary Figure 4) shows two distinct clusters, one containing all of the low-temperature (Stage 1) samples and the other containing the rest of the samples from mid to high temperatures (Stages 2–4). Furthermore, within the mid/high-temperature cluster, all samples group closely (>60% similarity), while the two low-temperature communities split at 20% similarity. This implies that microbial community structure during, and immediately after, overflow was highly similar across both cycles while low-temperature communities varied between thermal cycles, yet still remained distinct from all other cycle stages. DISTLM marginal tests revealed that average temperature (covariant with water level and chloride, both proxies for timing within the cycle and the influx of geothermal water into the lake), ammonia (covariant with chloride and dissolved carbon dioxide), and sulphate (covariant with boron) respectively contributed 42%, 22% and 23% to Bray-Curtis similarity patterns between samples (Table 2). However, average temperature was the only variable that was significant under permutational significance testing (permutational P-value <0.05). When all variables were considered together in a BEST routine DISTLM test, daily average temperature was resolved alone as the contributing explanatory variable that best minimised adjusted Akaike Information Criterion for selection (AICc = 60.891, $R^2 = 0.4193$, Residual Sum of Squares = 7264.9). This lends weight to the hypothesis that communities are structured by physicochemical parameters in this system, particularly temperature which explains up to 42% of the variation seen in OTU relative abundances.

Speculative Inferno Crater Lake ecology

Temporal studies of microbial ecosystems influenced by environmental variation are relatively well documented (Wertz *et al*., 2007; Koenig *et al*., 2011; Gilbert *et al*., 2012; Shade *et al*., 2012; Shade *et al*., 2013). Meta-analysis of these ecosystems show community structure variation is relatively consistent and rapid within a single ecosystem and when comparing similar ecosystems (Shade *et al*., 2013). However, the majority of these studies are non-cyclic and are not influenced by such significant natural temperature variations nor challenging acidic environmental conditions such as observed at Inferno Crater Lake. Inferno Crater Lake is unique in this respect and provided an ideal opportunity to study microbial succession and selection in a naturally-cycling and semi-predictable thermal setting. Communities at all stages of both consecutive cycles were highly uneven and dominated by reads from few OTUs, particularly at high-temperature phases. All proportionally dominant OTUs could be assigned to chemolithotrophic phylotypes that reflected the spring acidity and geochemical landscape over a thermal cycle. In particular, sulphur-based species, such as hydrogen sulphide, elemental sulphur and

Table 2 Marginal tests for environmental variables included in DISTLM

Variable	*Sum of squares (trace)*	*Pseudo-F*	P *(permutation significance test)*	*Proportion of variation explained*
Average temperature	5244.5	4.3316	**0.0463**	**0.41926**
Ammonia (mg l^{-1})	2779.7	1.7142	0.205	0.22221
Calcium (mg l^{-1})[a]	741.01	0.37781	0.5396	0.05592
Potassium (mg l^{-1})	242.65	0.11869	0.8568	0.0194
Sulphate (mg l^{-1})	2851.2	1.7713	0.264	0.22793
Aqueous sulphide (mg l^{-1})	1418.4	0.76736	0.4699	0.11339
Carbon dioxide (p.p.m.v.)	231.87	0.11332	0.8305	0.0185
Hydrogen (p.p.m.v.)[b]	1760.3	0.98257	0.3234	0.14072
Hydrogen sulphide (p.p.m.v.)	335.6	0.16541	0.8815	0.0268
Methane (p.p.m.v.)	739.54	0.37701	0.5922	0.0591
Oxygen (p.p.m.v.)	870.22	0.44861	0.6757	0.0696

Abbreviations: DISTLM, distance linear modelling; p.p.m.v., parts per million by volume.
[a]square root.
[b]presence/absence transformed.
The permutation significance testing values and the proportional contribution of each variable to the Bray-Curtis similarity pattern is presented in third and final columns. Environmental values in bold were identified as being significant ($P<0.05$). P-values were obtained by permutation ($n = 999$).

thiosulfates, and hydrogen gas can support taxa such as *Sulfobacillus*, *Hydrogenobaculum*, *Acidithiobacillus* and *Sulfolobus* spp., and taxa from the family *Methanobacteriaceae* (Boone, 2001; She *et al.*, 2001; Valdes *et al.*, 2008; Romano *et al.*, 2013; Justice *et al.*, 2014) detected in this study (Supplementary Table 3). Interestingly, with the exception of the putative *Methanobacteriaceae*, all detected taxa are obligate or facultative aerobes, reflecting the well mixed nature of Inferno Crater Lake and the general paucity of described acidophilic anaerobes (Prokofeva *et al.*, 2005). While the observed geochemistry and pH explain the observed taxa across the study, they do not resolve the cyclic bacterial and archaeal succession across the cycle. DISTLM analysis resolved temperature as the only significant variable associated with community turnover (Table 2), and no substantial variations in other physicochemical conditions were noted. This is consistent with community succession associated with physicochemical conditions and implies that community structure is strongly niche driven. Why no bacterial signals were detected in the qPCR or amplicon datasets during Stages 3 and 4 ('overflow' and 'recession') is not immediately clear, particularly as *Hydrogenobaculum*, the most abundant bacterial taxon detected throughout the study, can reportedly survive in culture at up to 80 °C (Shima *et al.*, 1994; Stohr *et al.*, 2001; Dopson and Johnson, 2012), the nominal temperature reached at overflow stages. We postulate that, rather than Inferno Crater Lake's temperature exceeding the bacterial survival limit, bacteria are instead simply outcompeted by archaea, particularly *Sulfolobus* spp., which have T_{opt}'s of >65 °C (Huber and Stetter, 2001) and are better adapted to this combination of acidity and high-temperature (Valentine, 2007). This scenario reflects observations from geothermal ecosystem surveys that report archaeal phylotypes generally dominate acidic high-temperature ecosystems in Yellowstone National Park (Meyer-Dombard *et al.*, 2005; Inskeep *et al.*, 2013).

Although this study supported our initial hypotheses, questions arise as to how bacteria might survive the hottest phases of the cycle, considering they were essentially non-detectable at the overflow and early retreat stages. We expected the system to select for spore-forming bacterial taxa, yet only a small proportion of the bacteria detected at Stage 1 have reported spore-forming capability (*Sulfobacillus* spp; Supplementary Table 3). This suggests that bacteria were lost from the system during late oscillation phase and then were re-populated once the temperature dropped during recession. Conversely, archaeal sequences remained proportionally dominant during the recession stages of both cycles, indicating a lag in the re-establishment of *Bacteria* as the dominant domain. This lag indicates that bacteria must repopulate relatively rapidly over a few days during the lowest temperature stage in the cycle. Given the rapid rate at which bacteria become the dominant domain, the massive spring volume (both subaerial and subterranean) which the bacteria must repopulate, and the distinct prominence of a few bacterial phylotypes during the low-temperature points of both cycles, we postulate that Inferno Crater Lake is re-populated from an established refugia population of geothermal-associated moderately thermophilic bacteria rather than stochastic repopulation via aerosol transport from other nearby springs. We can, however, not rule out the possibility of aeolian reintroduction of mesophilic and moderately thermophilic bacteria during Stage 1 of the cycles. The published growth rates of *Hydrogenobaculum* and *Acidithiobacillus caldus* (Hallberg and Lindstrom, 1994; Donahoe-Christiansen *et al.*, 2004) under ideal axenic laboratory conditions could in theory facilitate growth to the observed qPCR cell counts. However, we consider this a remote possibility when considering that the Inferno Crater Lake ecosystem has far from ideal growth conditions. We postulate instead that the primary route for the repopulation of the bacterial community is a subterranean reservoir beneath Inferno Crater Lake (that is, where the Lake water 'retreats' to during Stage 4). This is supported by a lack of obvious or reported subaerial inflows into Inferno Crater Lake (Keam, 1981). In addition, the homogeneous water column temperature and chemistry, and silica sinter lining (Keywood and Nicholson, 1990) diminish the possibility of a substantial mesophilic refugia within the water body or sediments itself. To this end, we propose that Inferno Crater Lake microbial community dynamics and ecology are tightly bound to the thermal hydrology of the system. During Stage 1 ('rise'), a subterranean source of mesophilic acidophiles rapidly repopulates the spring as it gradually increases in volume. *Sulfolobus* spp., from the previous overflow and recession stages (Stages 3 and 4) are also present, but are at the lower end of their temperature growth profiles and are essentially in subsistence/survival mode until the next heating cycle. During the second stage of the thermal cycle (oscillation), the spring increases in temperature to between 55–60 °C over an extended period of time, gradually selecting against most of the Stage 1 bacterial community and favoring remnant crenarchaeotal/*Sulfolobus* populations. Only the hydrogen and sulphur-oxidising bacterium, *Hydrogenobaculum*, remains viable and able to compete with the archaeal species. As the number of temperature oscillations increases, the proportion of bacteria decreases. At overflow (Stage 3) the temperature of the spring sharply increases to an excess of 75 °C and overflows, with only the *Sulfolobus* spp. capable of growth. Any remaining bacteria are washed out during the overflow, and remain absent throughout the 'recession' (Stage 4) until mesophile repopulation in Stage 1 of the next thermal cycle.

In this study, we investigated the microbial community responses to a recurring and unique thermal cycle at Inferno Crater Lake in the

Waimangu-Rotomahana-Tarawera Geothermal System. The microbial community was primarily chemotrophic and acidophilic in nature, reflecting physicochemical conditions over observed cycles. However, the spring temperature was the strongest determinant of microbial community structure, which alternated between a bacterial-dominated system at low temperatures and an archaeal system at elevated temperatures. This study marks the first confirmed observation of a significant wholly time-driven community turnover in an acidic crater lake. The lake's turnover is strongly associated with physicochemical variation, most notably temperature, and represents a clear example of niche-driven community change.

Conflict of Interest

The authors declare no conflict of interest.

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