Differential depth distribution of microbial function and putative symbionts through sediment-hosted aquifers in the deep terrestrial subsurface

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An enormous diversity of previously unknown bacteria and archaea has been discovered recently, yet their functional capacities and distributions in the terrestrial subsurface remain uncertain. Here, we continually sampled a CO2-driven geyser (Colorado Plateau, Utah, USA) over its 5-day eruption cycle to test the hypothesis that stratified, sandstone-hosted aquifers sampled over three phases of the eruption cycle have microbial communities that differ both in membership and function. Genome-resolved metagenomics, single-cell genomics and geochemical analyses confirmed this hypothesis and linked microorganisms to groundwater compositions from different depths. Autotrophic Candidatus “Altiarchaeum sp.” and phylogenetically deep-branching nanoarchaeae dominate the deepest groundwater. A nanoarchaeon with limited metabolic capacity is inferred to be a potential symbiont of the Ca. “Altiarchaeum”. Candidate Phyla Radiation bacteria are also present in the deepest groundwater and they are relatively abundant in water from intermediate depths. During the recovery phase of the geyser, microaerophilic Fe- and S-oxidizers have high in situ genome replication rates. Autotrophic Sulfurimonas sustained by aerobic sulfide oxidation and with the capacity for N2 fixation dominate the shallow aquifer. Overall, 104 different phylum-level lineages are present in water from these subsurface environments, with uncultivated archaea and bacteria partitioned to the deeper subsurface.

Much remains to be learned about how microbial communities in the deep terrestrial subsurface vary with depth due to limited access to samples without contamination from drilling fluids or sampling equipment. Studies to date have analyzed samples acquired by drilling1,2, from deep mines3,4, subsurface research laboratories5–7 and sites of groundwater discharge8–11. These investigations have shown that the terrestrial subsurface is populated by a vast array of previously undescribed archaea and bacteria. At one site, an aquifer in Colorado (Rifle, USA), the diversity spans much of the tree of life12 and includes organisms of the Candidate Phyla Radiation (CPR)13, which may comprise more than 50% of all bacterial diversity14, and many other previously undescribed bacterial lineages. Also present in the terrestrial subsurface are previously unknown or little known archaea, including members of the DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaeas)12,13, Altiarchaeum13, Lokarchaeota14 and Ainarchaeota13.

A major question in subsurface microbiology relates to how organisms, and their capacities for carbon, nitrogen and sulfur cycling, vary along depth transects through the terrestrial subsurface. Some evidence pointing to taxonomic variation between 9 m and 52 m below the surface was obtained via a massive 16S ribosomal RNA gene survey at the Hanford Site15. Similar variation and change of two functional genes were also detected for two shallow aquifers that were accessed via drilling in Germany16. However, major groups of archaea and bacteria may have been overlooked due to sampling17 and primer bias18,19,20,21 and the spatial variation in metabolic functions over depth transects including the deep subsurface (100 m below the ground) remains unexplored.

Crystal Geyser is a cold-water, CO2-driven geyser located geologically within the Paradox Basin, Colorado Plateau, Utah, USA22. Originally an abandoned oil exploration well, the 800-m deep vertical borehole has served as a geyser conduit whose regular and significant flow rate (since 1936) provides uncontaminated access to deep groundwater12. Time-series geochemical data collected over the ca. 5-day eruption cycle suggest that Crystal Geyser is primarily sourced from the Navajo Sandstone, with increased contributions from the shallower Entrada Sandstone during major eruptions, and increased fraction of deeper water during minor eruptions23,24. A survey of ribosomal proteins predicted from metagenome sequences from Crystal Geyser microbial communities revealed the existence of a large phylotypic diversity of previously unknown bacteria and archaea in this system25, and a genomic resolution study documented a high incidence of carbon-fixation pathways26.

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A remaining question relates to the source regions and distributions of these organisms. Here, we tracked the microbiology and the associated geyser discharge geochemistry continuously throughout the full 5-day geyser eruption cycle to test the hypothesis that groundwater from stratified aquifers sampled at different stages of the cycle has microbial communities that differ in both membership and function. Our analyses made use of a comprehensive collection of more than 1,000 newly reconstructed genomes, both from metagenomes and single cells, as well as detailed physical and chemical information that enabled linking of fluids to their groundwater source regions.

**Results**

Continuous in situ (downhole) monitoring of the geyser water pressure throughout the field campaign defined the regular ~5-day period of the eruption cycle (Supplementary Fig. 1). Sampling was conducted over a complete eruption cycle (24–29 May, 2015) during which microbial cells were continuously collected onto 0.1 μm filters. Time series of downhole temperature, electrical conductivity, total dissolved gas pressure and water samples (for major ion, trace metal and dissolved gas analyses) were collected to associate specific microorganisms with water from different geyser eruption intervals and relative aquifer depths (Supplementary Fig. 1).

Time series of water pressure, electrical conductivity and temperature showed three Crystal Geyser eruption phases previously observed\(^1\): the recovery (relatively low water level, no eruptions, light CO\(_2\) bubbling), minor eruptions (short eruptions of ~10 min every hour with elevated CO\(_2\) discharge) and major eruptions (constant eruption and heavy CO\(_2\) discharge\(^2\); Fig. 1). Average chloride concentrations ([Cl\(^-\)]) and baseline water temperature (16.9 °C; also observed in a year-long monitoring period; Supplementary Fig. 1d) indicate that, overall, the geyser water is primarily sourced from ~320 to 480 m depth, which mainly corresponds to the Navajo aquifer; Supplementary Fig. 1a–d). In minor eruptions, elevated electrical conductivity and [Cl\(^-\)] indicate increased contribution from deeper, more saline water (that is, possibly the Wingate aquifer or Paradox brine sourced from even greater depth; Supplementary Fig. 1c). In the major eruption phase, decreased electrical conductivity and [Cl\(^-\)] and elevated Ca, Sr and Fe concentrations, were consistent with an increased contribution from the shallower Entrada aquifer (Supplementary Fig. 1b). During the eruption-free recovery phase, in which the Crystal Geyser borehole slowly refilled after the major eruption phase, electrical conductivity gradually increased with the relative contribution of deeper groundwater up to (and during) the minor eruptions. During this time the water level increased ~3.5 m over 33.5 h, potentially enabling microbes to thrive in microaerophilic borehole-affected conditions. To simplify terminology, we henceforth refer to the source water compositions as relatively ‘deep’ during the minor eruptions, ‘intermediate’ (and borehole-affected) during the recovery phase and ‘shallow’ during the major eruptions. Similar phase variations in the relative depths of water composition were recently observed\(^3\).

Analysis of microbial community composition in the 2015 bulk samples made use of a relatively comprehensive database of genomic information for the Crystal Geyser system (see Supplementary Fig. 2 for sample processing and analysis overview). The genomic dataset included previously reported draft genomes from this system and new genomes reconstructed from size-fractionated samples obtained in April and October of 2014 (Supplementary Table 1). Samples included a post-0.2 μm fraction collected onto a 0.1-μm filter to enrich for community members with ultra-small cell sizes. Binning of assembled metagenomes from 27 different samples from five time points in 2014 used seven different algorithms (see Supplementary Methods) and resulted in 30,574 genomic bins (with multiple bins for the same genome generated by different algorithms). Selection of the best quality bin generated for any organism in each sample resulted in 5,795 bins for bacteria and archaea, of which 2,216 were considered to be at least medium quality (>70% completeness based on single-copy genes with less than three multiple single-copy genes). After curation based on guanine-cytosine content, coverage and taxonomy the database contains 1,215 genome sequences for 503 different archaeal and bacterial species (for details on genome numbers for each step, please see Supplementary Fig. 2; genome completeness is provided in Supplementary Table 2).

To augment the genome-resolved metagenomics, we acquired 206 single amplified genomes (SAGs) from cells collected at one time point in the minor eruption and one time point during the recovery phase. SAGs were chosen for full sequencing and analysis if PCR-screening for 16S rRNA genes was positive, agnostic to the specific sequence. Only SAGs with assembly size of >100 kbp after multi-step contamination screening were considered further. This set comprised 183 SAGs, seven of which were of sufficient quality to be classified as medium-quality draft genomes (>70% complete, less than three multiple single-copy genes). We required alignments ≥98% nucleotide identity over >30% of the SAG to establish a match between SAGs and genomes from metagenomes. This approach was chosen because almost all of the SAGs were less complete than related draft genomes from metagenomes (Supplementary Fig. 3). In general, SAG sequences aligned well to

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**Fig. 1** Crystal Geyser’s 5-day eruption cycle measured during the 2015 sampling period exhibited variations in downhole water pressure and electrical conductivity that define three phases. In each phase, electrical conductivity (EC) and geochemical measurements (6,710 measurements each, no technical replicates; Supplementary Fig. 1) are used to identify relative depths of source water compositions: intermediate for the recovery phase (2,330 measurements), deep for the minor eruptions (2,820 measurements) and shallow for the major eruptions (1,560 measurements). The numbered horizontal grey bars indicate the time periods for each metagenomic sample (25 samples in total) and coloured numbers indicate the grouping of samples from each phase.
the sequences of genomes from the metagenomes (Supplementary Fig. 3). We found that >70% of the SAGs (145, of which five were draft quality) were represented in the set of 503 draft-quality genomes from the metagenomes. Conversely, 63 of the 503 genomes from metagenomes were also detected by single-cell genomics. Two draft-quality SAG genomes were not binned from the metagenomes and thus were added to the database. One SAG is entirely absent in the metagenomes based on sequences of the ribosomal protein S3 and read mapping, and probably derived from a very rare organism. The 505 genomes in the database (Supplementary Table 2), which were derived via dereplication from a total set of 1,208 genomes (984 genomes from metagenomes, 222 genomes from a previous study\(^7\) and two single-cell genomes), represent archaeal and bacterial species that belong to 104 different phylum-level lineages (Fig. 2). Nine lineages were named as they were represented by least two genomes with significant phylogenetic distance to neighbouring phyla and thus may constitute previously unrecognized phylum-level lineages. In addition, six genomes may be from previously unknown phylum-level lineages but the lineages are currently only represented by a single genome. The majority of diversity was attributed to members of the CPR (Fig. 2).

Mapping of metagenome reads to the set of 505 genomes showed that the genomes account for ~50% of the sequence data collected through the 2015 eruption cycle (Supplementary Table 4) and thus is representative of the community found in the Crystal Geyser ecosystem (Supplementary Fig. 4; morphological diversity of organisms is provided in Supplementary Fig. 5). Analysis of the community structure of the 25 different metagenomes using this approach revealed strong shifts in microbial composition over the cycle (Fig. 3b; Supplementary Table 5). The accuracy of relative abundance measures of individual genomes was confirmed for three species using quantitative digital droplet PCR (Supplementary Fig. 6). No physical or geochemical factor besides time, which corresponds to the source regions of the sampled water, could explain the observed changes in community composition (based on multivariate statistics; Supplementary Table 6). The community was dominated by species of the taxa Candidatus “Altiarchaeum”, Sulfurovum, Piscirickettsiaceae, Gallionellaceae and Betaproteobacteria (in order of decreasing abundance, Fig. 3c). The set of CPR and archaea from the DPANN superphylum showed several peaks in relative abundance at several time points during the eruption cycle (Fig. 3d,e). Both groups had the highest cumulative abundance during the minor eruptions, when groundwater from the deepest source was sampled. The most abundant CPR (Moranbacteria\(^1\)) was, however, prominent during the recovery phase (Fig. 3d). Overall, the cumulative abundances of DPANN and other archaea were significantly higher in the deep groundwater compared to shallow or intermediate (Supplementary Fig. 7).

**Fig. 2 | Diversity of recovered genomes based on 16 concatenated ribosomal proteins.** Genomes were reconstructed for organisms from 104 different phylum-level lineages; 503 different lineages are shown (two lineages did not exhibit >50% alignment coverage and are thus not displayed). Phylog in bold were assigned names in this study. The scale corresponds to per cent average amino acid substitution over the alignment. Asterisks mark yet-to-be-cultivated phyla, which thus have a Candidatus status. OD1, Parcubacteria. A full tree with reference sequences can be found in Supplementary File 1.
Fig. 3 | Hydrogeology and community composition of subsurface fluids sourced from Crystal Geyser throughout an entire eruption cycle. a, The Crystal Geyser site lies within one of the several natural CO₂ reservoirs within the Paradox Basin. The CO₂ was probably generated from thermal decomposition of Pennsylvanian-aged carbonate rocks. CO₂ gas and brine formed by groundwater dissolution of Paradox evaporites migrate via faults and fractures.

b, The community profile of 505 organisms strongly followed the succession of the geyser eruptions (blue lines, NMDS). One data point corresponds to one metagenomic sample. The samples show a clear pattern following the succession of the geyser cycle. c, Entire community profile of 505 organisms tracked across the 5-day cycle of the geyser. Each colour corresponds to one genome. d, e, Profiles of the CPR and DPANN community, respectively, show an increase in the overall abundance during the minor eruptions when groundwater has the deepest source composition. f, Downhole electrical conductivity time series during the sampling of the cycle illustrating the individual phases of the geyser (6,710 samples were measured, see Fig. 1 and Supplementary Fig. 1). Number of biological replicates in panels b–e was 24. EC, electrical conductivity; GW, groundwater.
When analysed one at a time, the majority of organisms (289, ~57%) were significantly enriched (false discovery rate-corrected P value <0.05) in one specific phase of the geyser and could thus be sourced to one of the groundwater depths (Fig. 4a–c). The shallowest groundwater was mainly populated by one Sulfurimonas sp. along with a few other bacteria and some archaea. Based on the genome sequence of Sulfurimonas sp., this organism was inferred to be a chemolithoautotroph, capable of nitrogen and carbon fixation as well as sulfide oxidation through oxygen respiration (Supplementary Table 7). The capacity for carbon fixation via the low-cost reductive TCA cycle (two ATP per pyruvate8) coupled to oxygen respiration may provide an ecological advantage for this species and is also indicative of microaerophilic conditions in the relatively shallow aquifer. In contrast to the shallow source, groundwater from intermediate depths had a great diversity of different organisms, the majority of which belonged to the CPR. The most abundant organism was a member of the Gallionellaceae, a family of bacteria well known for microaerophilic iron and sulfur oxidation at Crystal Geyser8,9. This organism also exhibited the highest genome replication rates of all bacteria in the study (average in situ replication rate (iRep) value of 2.5, maximum iRep value of 4.2; Supplementary Table 8), suggesting that it was also proliferating in the geyser conduit over the 33.5h of the recovery phase. Its growth was probably favoured by microaerophilic conditions as well as sulfide and reduced iron in the geyser fluids. Potentially, other microorganisms enriched in this fraction may also have favoured the conditions in the borehole over the 33.5-hour recovery phase, during which the geyser had no water discharge. Consequently, the community sampled from the recovery phase represents the community from intermediate depths with distortions from microbial growth in the borehole. When deeper groundwater was discharged, the abundances of different DPANN archaea and Ca. "Altiaarchaeum" were significantly increased. Diverse members of the CPR were still present in deep groundwater, although at low relative abundance.

The shallowest groundwater had a substantially higher capacity for microbial sulfide oxidation, nitrogen fixation and oxygen respiration, probably due to the presence of atmospheric gases. In contrast, the intermediate source and borehole community had the highest microbial capacity for reduction of various nitrogen compounds as well as thiosulfate disproportionation, metal reduction and oxidation. The deepest groundwater was enriched in several bacteria with the capacity for sulfitreduction, with carbon fixation mediated by the Ca. "Altiaarchaeum". The capacity for oxygen respiration decreased with increasing depth to the sourced groundwater.
Previously, we reported the operation of three carbon fixation pathways in bacteria and archaea from the Crystal Geyser communities, each of which requires substantially different amounts of energy\(^1\). While the Wood–Ljungdahl pathway requires approximately 1 mol of ATP for the generation of 1 mol pyruvate, the Calvin–Benson–Bassham cycle and the reverse TCA cycle require approximately 1 mol of ATP for the generation of 1 mol pyruvate, the Calvin–Benson–Bassham cycle and the reverse TCA cycle require 7 mol and 2 mol, respectively\(^{29}\). Here, we show that the three carbon fixation pathways used by Crystal Geyser microorganisms were most abundant in different eruption phases, which reflect varying depths of source water composition (and borehole; Fig. 4e). The reductive TCA cycle was associated with a *Sulfurimonas* sp. that dominates the shallowest groundwater and also has the capacity for N\(_2\) fixation. The Calvin–Benson–Bassham cycle was enriched in bacteria associated with the intermediate groundwater as well as the borehole, and the Wood–Ljungdahl pathway was encoded in *Ca. Altarchaeum* and Deltaproteobacteria genomes, and thus most highly represented in the deepest groundwater (Fig. 4d). The microbiome is dominated by *Ca. Altarchaeum* (SM1) and their putative DPANN symbionts and populated by many CPR and other bacteria, some of which are probably symbiotic partners for CPR. We envision facile distribution of the very small CPR and DPANN cells through the sandstone pore spaces, providing periodic opportunities for establishment of the symbiont-host interactions that are probably required for CPR and DPANN cell replication. This figure provides a conceptual diagram of generalized microbial habitats in the aquifer based on an approximate pore size of sandstone. However, we note the subsurface is a heterogeneous three-dimensional system and physical properties will vary substantially\(^4\). The Carmel and Kayenta formations are expected to act as aquitards (confining barriers) that separate the high permeability sandstone aquifers (Fig. 3a), with each aquifer largely confined, both hydrologically and microbiologically, from other aquifers by low-permeability units probably contributing to the distinctive microbial communities associated with the three relative groundwater source depths as documented in the study.

**Fig. 5 | Putative symbiotic interaction of *Ca. Altarchaeum* and *Ca. H. crystalense*.** a, Linear correlation analysis of relative abundance of the two archaea across 25 metagenome samples (full cycle of the geyser). b, Scanning electron micrograph of what are inferred to be *Ca. Altarchaeum* ("SM1") taken during the minor eruptions of the geyser. Tiny cell-like structures appear to be attached to the surface ("?N"). This structure was observed in two out of five samples taken for scanning electron microscopy analysis from the geyser fluids. cor., correlation coefficient. More images are available under Supplementary Fig. 9.

**Fig. 6 | Conceptual representation of a relatively stable microbiome in deeper sandstone aquifer sources.** The microbiome is dominated by *Ca. Altarchaeum* (SM1) and their putative DPANN symbionts and populated by many CPR and other bacteria, some of which are probably symbiotic partners for CPR. We envision facile distribution of the very small CPR and DPANN cells through the sandstone pore spaces, providing periodic opportunities for establishment of the symbiont-host interactions that are probably required for CPR and DPANN cell replication. This figure provides a conceptual diagram of generalized microbial habitats in the aquifer based on an approximate pore size of sandstone. However, we note the subsurface is a heterogeneous three-dimensional system and physical properties will vary substantially\(^6\). The Carmel and Kayenta formations are expected to act as aquitards (confining barriers) that separate the high permeability sandstone aquifers (Fig. 3a), with each aquifer largely confined, both hydrologically and microbiologically, from other aquifers by low-permeability shale/mudstone units\(^6\). This physical separation by low-permeability units probably contributes to the distinctive microbial communities associated with the three relative groundwater source depths as documented in the study.
this may be provided by scanning electron microscope images, which showed small rounded structures of approximately 0.15-µm diameter attached to larger cells (Fig. 5b, Supplementary Fig. 9). We infer that the larger cells are Ca. "Altiaarchaeum", based on the distinct hami-like appendages18, and that Ca. "H. crystallense" are epsymbionts. Interestingly, both genomes exhibited very high levels of fragmentation, an indication of high levels of strain heterogeneity within both populations. The diversification of the Ca. "Altiaarchaeum" host in its deep subsurface habitat might drive coevolution of Ca. "H. crystallense". The shared characteristic of strain heterogeneity may also support the inference of their interaction.

Discussion

Our microbiological investigation clearly demonstrated a strong stratification of microbial community composition and microbial function with relative groundwater source depths. Groundwater sampled from all three relative depths was dominated by autotrophs. The main pathway used for carbon fixation in the deeper subsurface is the one with the lowest energy cost, the Wood–Ljungdahl pathway, possibly because the deep biosphere is the most energy limited. Use of this pathway for provision of organic carbon was reported recently for other deep biosphere communities12. This pathway is also central to metabolism of methanogen, archaeal autotrophs found in the deep subsurface34. Reliance on the nitric acid for sample preservation37. The bottles were frozen for transport to the University of Calgary. Alkalinity was measured in the laboratory using an Orion Autochemistry 960 Autotitrator with 0.2 N sulfuric acid within 1 month of collection and expressed as HCO3 concentration. Major element and trace metal were collected hourly at a pumping rate of 0.2 l min⁻¹ over the trace metals were collected approximately every 10 min. Samples were field filtered to remove bacteria and temperature were monitored using a Solinst LTC logger located in Crystal geyser genome database. The genome database was constructed from genomes, from metagenomes and from single-cell genomes (SAGs) collected in 2014. First, all curated, newly binned genomes from metagenomes (985 in total) were covered with 222 previously published genomes and clustered based on 98% nucleotide identity. One representative of each genome cluster was chosen based on the highest completeness (single-copy genes) and lowest amount of contamination (multiple single-copy genes) following the formula: $score = single-copy genes - 2x multiple single-copy genes$⁻¹. In cases of ties, the genome with the highest N50 was chosen. The resulting 503 archaea and bacteria were then compared against draft-quality SAGs (at least 70% complete) using 98% nucleotide identity. Two draft-quality SAGs were not covered by the genomes from metagenomes and were thus added to the Crystal Geyser database that consists of 505 archaeal and bacterial species used for downstream analyses. A schematic overview of the procedure is presented in Supplementary Fig. 2.

Comparison of genomes from metagenomes to SAGs. Whole-genome alignment of genomes from metagenomes was performed at 98% nucleotide identity. If a SAG shared more than 30% of its genomic content with a genome from a metagenome (which were at least 70% complete), the SAG was considered to be represented by the genome from the metagenome (Supplementary Fig. 4).

Phylogeny of bacteria and archaea. Phylogenetic placements of the 505 archaea and bacteria in the Crystal Geyser database were determined from a tree computed from 16 ribosomal proteins and included 3,609 sequences (including reference sets from previous studies)12. Bacterial ribosomal proteins were extracted using Neoarchaeum equitans as described earlier14. Two draft-quality SAGs were not covered by the genomes from metagenomes and were thus added to the Crystal Geyser database that consists of 505 archaeal and bacterial species used for downstream analyses. A schematic overview of the procedure is presented in Supplementary Fig. 2.

Tracking community members across time. In 2015, time resolution of the Crystal Geyser community was achieved by near-continuous filtration of groundwater onto 0.1-µm filters (ZTECG, Graver Technologies, Glasgow, USA) that we recovered at 25 different time points over a time course of nearly 5 days (114h). Filtration was performed for an average of 4.6h per sample and sampling spanned one entire eruption cycle of the geyser (Fig. 1). Metagenomic DNA was extracted from the samples and paired-end sequenced (Illumina HiSeq 2500, Supplementary Table 1). Reads of 150 bp were quality filtered (see Supplementary Methods) and mapped onto the de-replicated genome set of 505 organisms using bowtie2 (default settings), allowing three mismatches per 150-bp read (98% identity)14. Read coverage was normalized by genome size and relative abundances of each genome in each sample were normalized by number of reads per sample using the equation $A = \frac{N_r}{N_s}$, where $A$ is the relative abundance of the genome in a particular sample, $N_r$ is the maximum number of reads of all metagenome samples, $N_s$ is the total number of reads of that particular sample, $r$ is the number of reads of that particular sample that mapped to the genome, $l$ is the average read length and $g$ is the length of the genome.

The reads were calculated with one mismatch per read as described earlier14. Relative abundance measure using metagenomics was confirmed using quantitative digital droplet PCR (ddPCR), for which the method can be found in the Supplementary Methods.
Microbial metabolism from genomics. Microbial metabolism from genomics was predicted as described earlier11,12. In brief, genes for each genome were predicted using prodigal13 with the respective genetic code and key metabolic genes for various sulfur, nitrogen, hydrogen and metal redox processes were predicted using HHMs14,15. In addition, functional predictions against KEGG were performed on the basis of HHMs including hits with e-values E-10. As such, the genetic potential of organisms for carbon fixation and oxygen respiration was based on the presence of all key enzymes and a pathway coverage of at least 60% in the KEGG module.

Microbial community statistics. Ordination analyses of microbial community structure was performed using a Bray–Curtis distance measure and non-metric multidimensional scaling (NMDS) in the R programming environment16. Influence of environmental factors as provided in Supplementary Table 1 were determined by BioSVA (Bray–Curtis dissimilarity, Spearman correlation) and plotted onto the NMDS17. Microbial source tracking of organisms and changes in microbial metabolism between different groundwater source depths based on cumulative abundance of organisms was performed using analysis of variance coupled to a Tukey honest significant difference post hoc test. Sample designations of the different depths correspond to those provided in Fig. 1. All P values that were affected by multiple testing were corrected for false discovery rate using the Benjamini–Hochberg procedure18.

Scanning electron microscopy. Methods can be found in the supplementary documents.

Data availability. SRA accession numbers for metagenomes of each sample are provided in Supplementary Table 1. All genomes from metagenomes included in this study were deposited at NCBI under Bioprojects PRJNA362739, PRJNA349044 and PRJNA297582. Genomes from metagenomes and single-cell genomes are also available under: https://ggbase.berkeley.edu/CG_2014_505_non-redundant_genomes.organsisms, http://ggbase.berkeley.edu/CG_2014_genomes_from_metagenomes/organsisms and http://ggbase.berkeley.edu/CG_2014_SAGs.organsisms.

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Author contributions

A.J.P., B.L., J.B.E., K.A., M.C.R. and JFB sampled the ecosystem. B.L. and M.C.R. conducted hydrogeological and geochemical analyses. A.J.P. performed genome-resolved metagenomics, phylogenetic, metabolic and community analyses. D.E.G.M. performed digital droplet PCR. C.M.K.S. and B.C.T. provided software. J.I. and T.W. performed single-cell genomics. A.J.P., J.I. and R.R.M. performed analyses of single-cell genomes. M.S. and A.K. carried out scanning electron microscopy. A.J.P. and J.F.B. designed the study. A.J.P. and J.F.B. wrote the paper with input from B.L. and M.C.R. All authors revised the manuscript.

Competing interests

The authors declare no competing financial interests.

Additional information

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### Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - Sample size was determined based on hydrogeological properties of subsurface fluids. This is elucidated in Figure 1 and Supplementary Figure 1 in detail.

2. **Data exclusions**
   - Describe any data exclusions.
   - The continuous data collected over the eruption cycle of the geyser was categorized based on hydrogeological measurements. Samples taken during the transition between the categories were excluded if they showed properties of both categories (Figure 1 and Supplementary Figure 1).

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - Biological replicates are represented by the samples of the different categories (determined via hydrogeological measurements). At least four samples per category were used to ensure statistical robustness.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - n/a

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - n/a

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - n/a
   - Confirmed

   - The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

   - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

   - A statement indicating how many times each experiment was replicated

   - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

   - A description of any assumptions or corrections, such as an adjustment for multiple comparisons

   - The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted

   - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

   - Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

| publicly available code written R, shell, python or ruby |
| --- |

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

| n/a |
| --- |

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

9. Antibodies

| n/a |
| --- |

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

10. Eukaryotic cell lines

| n/a |
| --- |

a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

| n/a |
| --- |

Provide details on animals and/or animal-derived materials used in the study.

Policy information about studies involving human research participants

12. Description of human research participants

| n/a |
| --- |

Describe the covariate-relevant population characteristics of the human research participants.