Tectonic and Geological Setting Influence Hot Spring Microbiology

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Running Title: Tectonic influences on hot spring communities

Originality-Significance Statement

Despite decades of investigations of hot spring microbial populations and their associations with volcanic settings, little is known of how differences in tectonic and geologic settings influence hot spring geochemical and microbial compositions globally. Here, coordinated microbial community and geochemical compositional analyses were conducted for 87 hot springs from Iceland, Yellowstone National Park, and Japan to elucidate these geo-microbial associations. Similar geophysical processes controlled hot spring geochemistry among regions, coinciding with similar patterns of microbial taxonomic compositions and diversity. However, region-specific differences in geochemistry were observed that are attributable to differences in geologic setting and tectonic characteristics. These differences corresponded to nearly completely distinct sub-genus level (i.e., 16S rRNA gene amplicon sequence variant; ASV) microbial community compositions among regions, although some cosmopolitan ASVs were present in multiple regions. These results argue against a predominant influence of dispersal limitation shaping the global biogeography of hot spring taxa, but rather suggest that local geologic and tectonic characteristics shape hydrothermal geochemistry that then selects for region-specific microbial community compositions. Consequently, these data advance our understanding of the co-evolution of global hydrothermal systems and their microbial consortia, in addition to providing new insights into the mechanisms underlying the global biogeography of thermophilic microorganisms.

Summary

Hydrothermal systems form at divergent and convergent boundaries of lithospheric plates and within plates due to weakened crust and mantle plumes, playing host to diverse microbial ecosystems. Little is known of how differences in tectonic setting influence the geochemical and microbial compositions of these hydrothermal ecosystems. Here, coordinated geochemical and microbial community analyses were conducted on 87 high-temperature (>65^{o}C) water and sediment samples from hot springs in Yellowstone National Park, Wyoming, USA (n=41; mantle plume setting), Iceland (n=41, divergent boundary), and Japan (n=5; convergent boundary). Region-specific variation in geochemistry and sediment-associated 16S rRNA gene amplicon sequence variant (ASV) composition was observed, with 16S rRNA gene assemblages being nearly completely distinguished by region and pH being the most explanatory parameter within regions. Several low abundance ASVs exhibited cosmopolitan distributions across regions, while most high-abundance ASVs were only identified in specific regions. The presence of some cosmopolitan ASVs across regions argues against dispersal limitation primarily shaping the distribution of taxa among regions. Rather, the results point to local tectonic and geologic characteristics shaping the geochemistry of continental hydrothermal systems that then select for distinct microbial assemblages. These results provide new insights into the co-evolution of hydrothermal systems and their microbial communities.

Introduction

Hydrothermal systems are found on all continents and are likely to have been present since early in Earth's history (Djokic *et al.*, 2017). Hydrothermal systems support diverse thermophilic microbial assemblages (Barns *et al.*, 1994, Hugenholtz *et al.*, 1998, Power *et al.*, 2018, Colman *et al.*, 2019) and have recorded some of the earliest evidence of microbial life (Van Kranendonk *et al.*, 2008, Djokic *et al.*, 2017). Common among hydrothermal systems is a source of heat, a source of water, and rock permeability that permits fluid circulation and convection to the surface (Hutchinson, 1978). Hydrothermal systems are categorized as volcanic and non-volcanic systems (Goff & Janik, 2000), the heat source for the former being magma and the latter, a body of hot rock (Arnórsson *et al.*, 2007).

Many volcanic and continental hydrothermal systems have been studied from geochemical or hydrologic perspectives, including for example at convergent subduction boundaries as in New Zealand (Rowland & Simmons, 2012), Russia (Taran, 2009), South America (Wrage *et al.*, 2017), China (Guo & Wang, 2012), and Japan (Allis & Yusa, 1989); at mantle plume hot spots as in Yellowstone National Park (YNP; Wyoming, USA) (Fournier, 1989); at mantle plumes in spreading zones as in Iceland (Arnórsson, 1995); and at back-arc basin spreading zones as in Italy (Duchi *et al.*, 1995). In addition to reflecting local tectonic, volcanic, and hydrologic characteristics, individual hydrothermal systems are variably influenced by the subsurface process of fluid phase separation, water-rock reactions, volcanic gas input, and mixing with infiltrating oxidized meteoric fluids (Fournier, 1989, Nordstrom *et al.*, 2005, Arnórsson *et al.*, 2007, Nordstrom *et al.*, 2009). Together, these features of hydrothermal systems result in extraordinary geochemical variation, both within and among systems.

Surface hydrothermal features (e.g., hot springs, mudpots, geyser, and fumaroles) have also been investigated to evaluate the composition and geochemical influences on their microbial communities, including in the Taupo Volcanic Zone of New Zealand (Power *et al.*, 2018), the Tengchong Volcanic Field of China (Hou *et al.*, 2013), YNP (Mitchell, 2009, Inskeep *et al.*, 2013, Colman *et al.*, 2019), and Iceland (Castenholz, 1969, Moreras-Marti *et al.*, 2021), among others. Overall, a growing consensus is that pH and, to a lesser extent, temperature primarily explain the distributions of microbial taxa within geothermal systems, regardless of which hydrothermal system is being considered (Mitchell, 2009, Boyd *et al.*, 2010, Inskeep *et al.*, 2013, Power *et al.*, 2018, Colman *et al.*, 2019, Moreras-Marti *et al.*, 2021). Nevertheless, few studies have evaluated the associations of geochemistry with microbial community compositions across hydrothermal systems that exhibit differing tectonic, geological, and hydrologic settings. Among the few such studies, pH, followed by temperature were again the primary factors shaping archaeal community compositions from Tengchong, YNP, and the Great Basin (United States) (Xie *et al.*, 2015). More recently, associations between microbial community compositions and tectonic characteristics have been observed across localized South American subduction zones including for 21 hot springs of the Northern Costa Rican convergent margin (Fullerton *et al.*, 2021, Rogers *et al.*, 2022) and for 14 hot springs of the convergent margin underlying the Peruvian Andes (Upin *et al.*, 2023). These studies have suggested that surface hot spring microbial communities closely align with the geochemical consequences of subsurface processes that are related to tectonic characteristics (e.g., differential volatile or heat delivery). Nevertheless, while these few comparative studies have provided some insight into the predominant geochemical factors that shape continental hot spring microbial communities, the importance of such factors have yet to be examined across the various types of tectonic settings (e.g., convergent vs. divergent vs. mantle plume type volcanism) that host hydrothermal systems.

Here, we conducted a large-scale comparison of high-temperature hot springs from YNP, Iceland, and Japan, sampled at spring sites where temperature and geochemical conditions preclude photosynthesis. The absence of photosynthetic populations results in hot spring microbial communities dependent on chemical energy (i.e., chemotrophy) that should more closely reflect geochemical differences among hot springs within and between regions. The heat and volatiles that drive the hydrothermal system in YNP occur due to hot spot (mantle plume) volcanism that has resulted in largely rhyolitic eruptions (Huang *et al.*, 2015). As such, rhyolite (silica-rich, iron-poor rock) comprises the major bedrock type in YNP (Christiansen, 2001). In contrast, the heat and volatiles that drive the hydrothermal system in Iceland are due to a mantle plume and spreading center volcanism associated with the Mid-Atlantic Ridge, largely resulting in basaltic (silica-poor, iron-rich) bedrock, with some localized areas of rhyolitic or andesitic (moderate silica and iron compositions) bedrock (Arnórsson, 1995). The hydrothermal system of Beppu, Japan, arises from subduction type volcanism associated with the Ryukyu volcanic arc, resulting in primarily andesitic bedrock in the region (Allis & Yusa, 1989). Sediment and water samples from hot springs were subjected to molecular and aqueous geochemical analyses to identify potential geologic and geochemical differences associated with host springs and how such differences may shape the compositions of their microbial communities. We hypothesized that universal drivers (e.g., pH and temperature) would influence microbial community compositions from each region at a first order. Further, we hypothesized that region-specific geochemical factors associated with each tectonic/geological regime would have a higher contribution to differences in community composition than what could be attributed to dispersal-related mechanisms associated with biogeographic patterns.

Experimental Procedures

Sample collection and geochemical analyses

Water and sediment/gravel samples were collected from hot springs during 2012-2019 as detailed in **Supplementary Table S1**. In field measurements of water/substrate (i.e., sediment/gravel) temperature and pH were used to select sampling points from the springs and discharge channels. Samples were collected and preserved on-site as previously described (Podar *et al.*, 2020). Briefly, sediment/gravel samples were collected with sterile instruments (spatulas or a stainless steel can at the end of a telescopic pole, depending on accessibility), poured hot into sterile Pyrex tubes or bottles as slurries, sealed with butyl rubber stoppers without air, cooled to ambient temperature, and transported cold within hours after collection. Sediment slurry aliquots were then taken at the laboratory upon return from the field and stored at -80^{o}C until subsequent molecular analyses. To ensure that sampling strategy and the inability to immediately freeze samples did not noticeably influence community compositional observations, comparisons to hot spring sediment samples preserved by immediate freezing as part of a separate study were conducted (described below). Images of sampling locations in hot springs are provided in **Supplementary File 1**.

Samples of some hot spring waters for geochemical analyses were filtered through 0.2 µm PVDF syringe membranes (Millipore, MA, USA) and stored at 4^{o}C in sterile, metal-free polypropylene containers (VWR, PA, USA) prior to analyses. The waters were submitted to the University of Tennessee Water Quality Core Facility for chemical analyses of select analytes.

Metal concentrations were measured by inductively coupled argon plasma optical emission spectrometry using a Thermo-Scientific iCAP 7400 mass spectrometer. Due to the inability to transport strong acid while sampling in some areas, waters were acidified upon return to the laboratory and prior to analysis. Consequently, metal concentrations may represent modest underestimates relative to actual concentrations. Major ion concentrations were measured by ion chromatography using a Thermo-Scientific Dionex ICS-2100 column (anions) or ICS-1100 column (cations), with background suppression to enable low detection limits. Data from the GeoFluids database for 663 Icelandic hot springs were collected for contextualizing the geochemical data reported here (Stefánsson *et al.*, 2016), in addition to temperature and pH data for 7,706 thermal features in YNP from the YNP research coordination network database (http://rcn.montana.edu), and ion data for 488 YNP springs from a previously published report (Ball *et al.*, 2002).

16S rRNA amplicon gene sequencing and processing.

Total DNA was extracted from microbial community samples using the MO BIO/Qiagen DNeasy PowerSoil (Maryland, USA) or Zymo (California, USA) soil DNA extraction kits (based on availability), following manufacturer protocols. PCR amplicons were generated by targeting the V4 hypervariable regions of 16S rRNA genes, using previously described procedures (Lundberg *et al.*, 2013). Libraries were prepared and sequenced at Oak Ridge National Laboratory using a previously described workflow (Podar *et al.*, 2020). Briefly, universal bacterial/archaeal primers (515F/806R) were used in combination with modified versions that increase coverage of Crenarchaeota and Nanoarchaeota-related taxa that have been previously underrepresented in 16S rRNA gene surveys using the standard 515F/806R primers

(Walters *et al.* 2016). These modifications included 20% of the primer pool comprising 515FCren (5'-GTGKCAGCMGCCGCGGTAA-3'), 5% 515FNano (5'-GTGGCAGYCGCCRCGGKAA-3'), and 5% 805RNano (5'-GGAMTACHGGGGTCTCTAAT-3') (Podar *et al.* 2020). Twelve-nucleotide barcode oligonucleotides were added to the amplicons in a second-stage amplification to enable multiplex sequencing. Final amplicons were pooled, purified, and sequenced on the Illumina MiSeq platform (2 x 250 bp paired-end reads) according to manufacturer protocols. Resultant paired-end sequences were imported into QIIME1 (Caporaso *et al.*, 2010) and demultiplexed, followed by importing into QIIME2 (Bolyen *et al.*, 2019) and subsequent adapter and primer removal using the QIIME2-implemented cutadapt program (Martin, 2011) using paired-end mode and default parameters. Paired reads were then joined using vsearch (Rognes *et al.*, 2016) and the resulting sequences were denoised using the DEBLUR algorithm (Amir *et al.*, 2017) to generate high-quality amplicon sequence variants (ASVs). Forward and reverse reads were trimmed to 200 bp prior to denoising and ASV generation. Sequences were then classified using the SILVA 132 database (Quast *et al.*, 2013) using the sk-learn package, as implemented in QIIME2. Contaminant sequences (unclassified to the domain level, chloroplasts, and mitochondria) were removed and sequence data was rarefied to 6,000 sequences per sample based on the lowest number of available sequences per sample.

Statistical analysis

Statistical analysis of 16S rRNA gene ASVs was conducted using the ASV feature table and Bray-Curtis dissimilarity matrix that were generated in QIIME2. The Shannon Diversity index was calculated to assess community diversity using the diversity function of the vegan software package (v.2.5-7) (Oksanen *et al.*, 2017) for R (v.4.0.4). Shannon Diversity values were assessed for normality using a Shapiro-Wilk normality test in R. Following confirmation of normality ($p > 0.05$), diversity values were subjected to Pearson correlation analysis in R using the cor.test function and with comparison to relevant geochemical parameters (i.e., temperature and pH). The Bray-Curtis matrix was subjected to non-metric multidimensional scaling ordination using the metaMDS function of vegan (stress of 0.156, indicating a good fit). Geochemical parameters were fit to the resulting ordination using the envfit function of vegan and the significance of fits was evaluated using 999 permutations (and by omitting samples without available geochemical data). An analysis of similarities test (ANOSIM) was used to evaluate the association of region of origin to community compositional variation. SIMPER analysis was also conducted, as implemented in vegan, to assess the contributions of individual ASVs to differences between community types that were delineated by region (Iceland or YNP) and for ranges of spring pH that included mid-pH (pH 5-7) and high pH (pH >7) groups. Samples from springs with pH < 5 and those from Japanese hot springs were not considered in these analyses, since both groups contained few samples from which to robustly evaluate differences compared to other groups. Only ASVs contributing up to 70% of the variation between groups are shown in the results for brevity. Lastly, network analysis was conducted based on correlations in the relative abundances of ASVs that represented ≥5.0% relative abundance in at least one of the 87 samples (n=125 ASVs). Pearson correlations were calculated between abundances, and only those correlations exhibiting $p \leq 0.05$ were used to construct the network (n=330 correlations). All but four of these correlations were positive. The correlations were treated as "edges", with ASVs as nodes, and then imported into Cytoscape (v. 3.8.2) (Smoot *et al.*, 2011). The default layout (prefuse, force-directed, based on correlation *R* values) was used for visualization and network layout, without further modification.

To assess potential influences on community composition from the lack of immediate freezing, the relative abundances of taxa for the 41 YNP hot spring communities of this study were compared to those of 37 YNP hot spring metagenomes generated from immediately frozen sediment samples that are a component of an ongoing study (Colman, unpublished data). Differences in the 16S rRNA gene-based and metagenome-based taxonomic compositions (via NMDS ordination of a Bray-Curtis dissimilarity matrix describing variation in taxonomic relative abundances) were not observed and both exhibited similar and overlapping pH-dependent distributions of taxa, as observed here and elsewhere (described in detail in the Results and Discussion). Moreover, 16S rRNA gene profiles for samples from the same spring and from different years/sampling points were nearly identical to each other and clustered with the metagenomic sample collected from a different year from the same spring. Taken together, these results reveal a minimal, if any, influence of sampling storage and transport on the community compositions described here.

Data availability

The 16S rRNA gene sequence data have been submitted to the NCBI database under BioProject accession PRJNA916746. Additional processed datasets are available upon request from the authors.

Results and Discussion

Sample site descriptions. To begin to evaluate how different tectonic and geologic settings influence the geochemistry and microbial biodiversity of hot springs, microbial community samples for DNA-based analyses and water samples for geochemical analyses were collected from select locations in YNP, Iceland, and Japan (**Supplementary Fig. 1; Supplementary Table S1**). Samples were collected from 25, 28, and 5 different hot springs from each location, respectively (**Supplementary Table S1, Supplementary File S1**).

In YNP, samples were collected from 7 geographically distinct hot spring areas including the Gibbon Geyser Basin, Hayden Valley, Heart Lake Geyser Basin, Lower Geyser Basin, Norris Geyser Basin, the Norris-Mammoth Corridor, Potts Geyser Basin, and the Upper Geyser Basin (**Supplementary Fig. 1; Supplementary Table 1**). Samples from Iceland were collected from 8 areas, including the Flúðir, Geysir, Borgarfjörður, Torfajökull, Kerlingarfjöll, Hveragerði, Ölkelduháls, and Reykjanes areas. The geologic settings of the sampled Icelandic areas have been described as primarily containing basalt as the primary bedrock, with the exception of the primarily rhyolitic bedrock of the Torfajökull region and the mixed basalt/rhyolite bedrock of the Geysir and Kerlingarfjöll areas (Macdonald *et al.*, 1990, Flude *et al.*, 2010, Ármannsson, 2016). The Beppu geothermal region on the island of Kyushu was the only hydrothermal area sampled in Japan. Only hot springs with a temperature >65°C and sampling locations that did not exhibit visible evidence for photosynthesis (i.e., visual pigmentation) were sampled from each region. Photosynthetic organisms have an empirical upper limit of ~74^{o}C in neutral to alkaline hot springs of YNP and ~54^{o}C in acidic hot springs (Brock, 1967, Castenholz, 1969, Boyd et al., 2010, Cox *et al.*, 2011, Boyd *et al.*, 2012). Within these temperature realms, sulfide at concentrations > 5 µM also further restricts the presence of photosynthetic organisms (Castenholz, 1976, Cox *et al.*, 2011, Boyd *et al.*, 2012). The upper temperature limit for photosynthesis in Iceland is, however, lower than in YNP (Castenholz, 1969), perhaps due to generally higher sulfide concentrations in Icelandic springs (Castenholz, 1976).

Hot spring geochemistry among regions. The hot spring samples that were collected spanned the range of pH (pH~2-10) commonly observed in hot springs in continental hydrothermal fields (Brock, 1971). The 41 samples from YNP were obtained from springs spanning a pH range of 2.2-9.6 (68-90^{o}C), while the 41 from Iceland were collected from springs spanning pH 2.0-9.0 (72-100^{o}C) (**Fig. 1a**). Representative springs from most pH and temperature ranges were sampled from Iceland and YNP, with the exception of acidic springs ($pH < 5$) that were relatively under-sampled in this study compared to their distributions in both regions (**Fig. 1a**). The five samples from Japan spanned a narrower range of pH (3.7-6.0) and temperature (67-71^{o}C), owing to the smaller sampling effort conducted only within the Beppu geothermal system (**Fig. 1a; Supplementary Table S1**).

The geochemistry of surface hot springs is controlled by the ascent of deeply-sourced hydrothermal fluids containing volcanically-derived gases and solutes from high-temperature water-rock interactions (Fournier, 1989, Nordstrom *et al.*, 2005, Arnórsson *et al.*, 2007). Upon ascension, the waters can undergo decompressional boiling and phase separation into a vapor phase enriched in volatile elements (e.g., H_2S and CO_2) and a liquid phase containing non-volatile elements that do not partition into the vapor phase (e.g., Cl^-; Na^+; and Mg^{2+}) (Fournier, 1989, Nordstrom *et al.*, 2005, Arnórsson *et al.*, 2007, Nordstrom *et al.*, 2009). The condensation of gas- rich vapor and subsequent oxidation of H_2S in the near surface upon interaction with oxygenated near-surface waters leads to the production of sulfate (SO_4^{2-}) as sulfuric acid

(H_2SO_4), in a process potentially mediated in part by thermophilic microorganisms (Schoen, 1969, Mosser *et al.*, 1973, Nordstrom *et al.*, 2009, Colman *et al.*, 2018, Colman *et al.*, 2022). The production of H_2SO_4 leads to water acidification. In contrast, the boiled liquid water depleted in volatile acids (i.e., CO_2 and H_2S) exhibits neutral to alkaline pH (Fournier, 1989, Nordstrom *et al.*, 2005, Arnórsson *et al.*, 2007). These processes consequently lead to the bimodal distribution of pH observed within, and among, global continental hydrothermal systems (Brock, 1971).

The SO_4^{2-} concentrations in hot springs reflect the input of volcanic gas into hydrothermal systems, with SO_4^{2-} concentrations also being influenced by boiling, condensation, oxidation processes, and water availability (Arnórsson *et al.*, 2007, Nordstrom *et al.*, 2009). Acidic hot springs ($pH < 5$) tend to have the highest SO_4^{2-} concentrations, because acidity results from sulfuric acid (Arnórsson, 1995, Nordstrom *et al.*, 2005, Nordstrom *et al.*, 2009). These springs tend to be enriched in potential substrates for microbial chemotrophy like sulfur and metals dissolved from rocks and minerals (Ball *et al.*, 2002, Kaasalainen & Stefansson, 2012). Increased SO_4^{2-} concentrations in thermal waters can also occur due to input of seawater in coastal hydrothermal areas. Chloride (Cl^-) is instead used as a proxy for sourcing of deep thermal waters. Variation in Cl^- concentrations of hydrothermal waters relates to the source water type, i.e., seawater, magmatic fluid (i.e., as HCl), or meteoric water (Arnórsson *et al.*, 2007). For meteoric water sourced hydrothermal fluids, Cl^- concentration variability is largely influenced by the host rock composition. The YNP caldera, where the majority of hydrothermal activity of YNP is located, is largely filled with rhyolite that display elevated Cl concentrations (Christiansen, 2001). Consequently, leaching of Cl^- upon water-rock interaction is the main source of Cl^- in YNP hydrothermal fluids (Fournier 1989). In contrast, basalt displays low concentrations of Cl compared to rhyolites, as reflected in lower Cl^- concentrations of basalt-hosted hydrothermal fluids (Arnórsson, 1995, Arnórsson *et al.*, 2007). These deeper-sourced boiled liquid waters tend to be gas-poor, oxidant-poor, and circumneutral or slightly alkaline in pH (Fournier, 1989). Based on the framework outlined above, SO_4^{2-} and Cl^- concentrations are often used to constrain the sources of fluids to hydrothermal waters, including in YNP (Fournier, 1989, Nordstrom *et al.*, 2009), Iceland (Arnórsson, 1995, Arnórsson *et al.*, 2007), and Japan (Allis & Yusa, 1989).

Although the temperature and pH profiles of springs sampled from each location were similar, the spring waters exhibited different overall geochemical compositions. SO_4^{2-} concentrations were generally similar in the Icelandic and YNP springs (**Fig. 1b**), while concentrations from the four acidic Japanese springs were among the highest (**Fig. 1b**; **Supplementary Table S1**). Some of the thermal fluids in the Beppu geothermal region of Japan are partially sourced by seawater (general SO_4^{2-} concentration of ~2,800 ppm), which may be responsible for the higher SO_4^{2-} concentrations in these springs (Allis & Yusa, 1989) (**Fig. 1c**). The highest SO_4^{2-} concentrations in YNP were detected in acidic springs (**Fig. 1c**), consistent with them being buffered by sulfuric acid derived from H_2S input and subsequent H_2S oxidation in the near-surface (Arnórsson *et al.*, 2007, Nordstrom *et al.*, 2009). SO_4^{2-} concentrations were not measured for the two Icelandic springs sampled here with pH < 5. However, previous geochemical analyses of Icelandic springs have revealed SO_4^{2-} concentrations in acid-sulfate waters (Kaasalainen & Stefansson, 2012) that are of equivalent magnitude to those of YNP reported here (e.g., as in **Fig. 1b**). Moderately acidic springs (pH 5-7) in YNP and Iceland exhibited similar SO_4^{2-} concentrations (**Fig. 1c**), consistent with these springs being influenced by elevated gas (e.g., H_2S) input into meteoric waters, or otherwise from their sourcing via mixtures of meteoric and hydrothermal waters. SO_4^{2-} concentrations in the higher pH (>7) waters of YNP were the lowest among all sample groups (**Fig. 1c**), consistent with these being liquid dominated systems with minimal vapor phase input (Fournier, 1989). Interestingly, the SO_4^{2-} concentrations of higher pH (>7) waters of Iceland were even higher than in the mid-pH springs of either Iceland or YNP (**Fig. 1c**). These data are consistent with other observations across Icelandic springs (Kaasalainen & Stefansson, 2012) and could suggest that these springs received increased input of volcanic gas input relative to YNP springs and/or are less diluted by meteoric water than mid-pH waters in Iceland/YNP.

Cl^- concentrations varied markedly among the three regions. Specifically, Icelandic hot spring waters generally contained the lowest Cl^- concentrations (**Fig. 1b, Supplementary Fig. 3**), while the YNP spring waters contained considerably higher Cl^- concentrations, and four of the Japanese springs contained the highest Cl^- concentrations (**Fig. 1b, Supplementary Fig. 3**). The higher Cl^- concentrations in hot spring waters from the Beppu region of Japan likely reflects partial sourcing by seawater (Allis & Yusa, 1989), (general ~19,000 ppm Cl^- concentration) (Drever, 1997). The observed variation in Cl^- concentrations between the sampled Icelandic and YNP springs is potentially associated with differences in water sources, local rock, and thus water-rock interactions. These differences could be partially due to differences in rhyolitic (YNP) vs. basaltic (Iceland) rock, for reasons outlined above. Moreover, these results are consistent with previous studies showing differences in Cl^- concentrations between Icelandic and YNP hot springs (Planer-Friedrich *et al.*, 2020). Overall, the geochemistry of the waters analyzed here reflected a primary influence from decompressional boiling of ascending hydrothermal waters, subsequent phase separation, and further geochemical alteration of the phase fluids via several processes. In addition, differences were observed in the geochemical profiles among regions that are likely due, at least in part, to different sourcing of waters and different bedrock that the waters interacted with among regions.

Microbial taxonomic composition across regions

16S rRNA gene amplification and sequencing resulted in a total of 469,800 high-quality reads comprising 4,952 ASVs distributed across 87 samples. Overall diversity levels (as reflected by the Shannon diversity index) were generally similar among communities from all three regions, with Shannon diversity indices generally being significantly inversely correlated with spring temperature (Pearson's R: -0.36, $p < 0.001$; **Supplementary Fig. 4A**) and significantly positively correlated with spring pH (Pearson's R: 0.30, $p < 0.01$; **Supplementary Fig. 4B**). Despite the positive correlation with spring pH, diversity notably peaked at slightly acidic pH values, then decreased, consistent with patterns of functional and phylogenetic diversity of YNP hot spring metagenomes that have been suggested to result from increased mixing of hydrothermal and/or meteoric water types, resulting in slightly acidic pH values (Colman *et al.* 2019, Fernandes-Martins *et al.* 2023). These patterns are overall consistent with trends in diversity that have been reported for numerous other globally distributed hot spring systems (Miller *et al.*, 2009, Mitchell, 2009, Sharp *et al.*, 2014, Power *et al.*, 2018, Upin *et al.*, 2023), suggesting similar, perhaps universal, controls on microbial diversity across regions. Dominant taxa were also generally similar across regions, with Crenarchaeota (including the orders Desulfurococcales, Thermoproteales, Caldarchaeales, and others) and Aquificota (previously the Aquificae; including the genera *Thermocrinis*, *Sulfurihydrogenibium*, *Hydrogenobaculum,* and *Hydrogenobacter*) constituting the majority of the communities (**Supplementary Figs. 5 and 6**).

Aquificota are considered the dominant primary producers in non-photosynthetic high-temperature hot springs, with distinct genera distributed among springs with distinct pH realms and geochemistry. In particular, *Hydrogenobaculum* is abundant in low pH springs, *Sulfurihydrogenibium* generally dominate mid-pH sulfidic springs, and *Thermocrinis*/*Hydrogenobacter* dominate circumneutral to alkaline springs (Reysenbach, 2005, Mitchell, 2009, Reysenbach *et al.*, 2009, Hou *et al.*, 2013, Inskeep *et al.*, 2013, Power *et al.*, 2018). Nearly all isolated Aquificota strains from hot springs are capable of fixing inorganic carbon (Reysenbach *et al.*, 2009) that is driven by the oxidation of inorganic volatiles (e.g., H_2, H_2S or its derivatives $S_2O_3^-$ and/or S^0) that is generally coupled to reduction of O_2 or sometimes NO_3^- (Reysenbach *et al.*, 2009, Hedlund *et al.*, 2015, Reysenbach *et al.* 2015). Consequently, the dominance of the aforementioned genera in the hot springs analyzed here (**Supplementary Figs. 5** and **6**) is consistent with their general dominance in hot springs globally. Among the Crenarchaeota, genera from the Desulfurococcales and Thermoproteales primarily include species involved in sulfur metabolism (e.g., S^0 or SO_3^{2-}/SO_4^{2-} reduction, $S_2O_3^-$ oxidation) and are typically facultative or obligate heterotrophs (Huber *et al.*, 2006, Huber & Stetter, 2006). The Crenarchaeota are also abundant members of high-temperature springs globally (Hou *et al.*, 2013, Inskeep *et al.*, 2013), including the genus *Pyrobaculum* (Thermoproteales) and the order Sulfolobales that were abundant members of springs analyzed here (**Supplementary Figs. 5** and **6**) and that have been shown to typically reduce sulfur species (e.g., SO_3^{2-} and S^0) and oxidize sulfur species (e.g., S^0), respectively (Huber *et al.*, 1987, Jay *et al.*, 2015, Lewis *et al.*, 2021). Nevertheless, many dominant Crenarchaeota of hot springs have yet to be cultivated (Inskeep *et al.*, 2013, Jay *et al.*, 2016), leading to some uncertainty in their metabolism. Metagenomic and functional genomic analyses have nevertheless suggested potentially versatile metabolic physiologies for some of the lineages that were abundant in springs from all three regions, including chemoorganoheterotrophy in Caldarchaeales (previously Aigarchaeota) (Hedlund *et al.*, 2015, Buessecker *et al.*, 2022), carbon monoxide metabolism in Geoarchaeales (Kozubal *et al.*, 2013), aerobic ammonia oxidation by some Nitrososphaeria (previously Thaumarchaeota; De la Torre *et al.* 2008), and Fe(III), S^0, or SO_3^{2-}/SO_4^{2-} reduction by other Nitrososphaeria (Daebeler *et al.*, 2018, Payne *et al.*, 2019, Kato *et al.*, 2021).

Variation in hot spring pH largely correlated to variation in the taxonomic composition of communities in all three regions (**Supplementary Figs. 5** and **6**), although this was most apparent for the Icelandic and YNP communities, owing to the wider breadth of sampling in these regions. These results support previous analyses of hot spring taxonomic and functional diversity across YNP (Mitchell, 2009, Inskeep *et al.*, 2013, Colman *et al.*, 2016), New Zealand (Power *et al.*, 2018), Iceland (Moreras-Marti *et al.*, 2021), and China (Xie *et al.*, 2015) that together indicate pH to be the dominant factor influencing community structure, often followed secondarily by hot spring temperature. Since pH (hydrogen ion concentrations) is dictated by and involved in many of the chemical reactions that occur in springs, it can be considered an "umbrella" parameter that captures variation in the availability and speciation of sulfur, iron, arsenic, carbon, and other elements of springs that are relevant for microbial metabolism (Shock *et al.*, 2010, Colman *et al.*, 2019). Since chemosynthetic metabolisms are dependent on chemical energy (i.e., via element cycling), and microorganisms often inherit components of their metabolic capacity vertically, it is perhaps not surprising then that pH is such a strong predictor of the taxonomic composition of high temperature hot spring communities.

Non-metric multidimensional scaling (NMDS) of ASV compositions in the 87 samples revealed nearly complete segregation in the composition of microbial communities when comparing YNP and Icelandic/Japanese springs (**Fig. 2**). In particular, NMDS axis 1 separated YNP communities from Icelandic spring communities, with the five Japanese springs also distinguished from the YNP springs along NMDS axis 1, but only partially distinguished from Icelandic springs (**Fig. 2**). Accordingly, the region of origin was very significantly associated with variation in community composition (ANOSIM R: 0.68, $p < 0.001$). Fluoride (F^-) concentrations and pH were the parameters that correlated best with community compositional variation (R^2=0.7294, $p < 0.001$ and R^2=0.6604, $p < 0.001$, respectively, envfit analysis) and were highly associated with NMDS axes 1 and 2, respectively (**Fig. 3; Supplementary Fig. 7**).

F^- concentrations in hydrothermal waters are largely due to water-rock interactions that should generally differ between YNP and Icelandic geologic settings, since F tends to be substantially more abundant in rhyolites than in basalts (Nordstrom, 2022). Thus, the clear differentiation of YNP and Icelandic/Japanese hot spring communities along NMDS axis 1 reflects differences in their respective geologic settings. Several other significant correlates to the NMDS ordination including silica (SiO_2; $R^2 = 0.45$, $p < 0.001$), magnesium (Mg^{2+}; $R^2 = 0.39$, $p < 0.001$), potassium (K^+; $R^2 = 0.37$, $p < 0.001$), calcium (Ca^{2+}; $R^2 = 0.23$, $p < 0.01$), Cl^- ($R^2 = 0.23$, $p < 0.01$), sodium (Na^+; R^2=0.19, $p < 0.01$), and arsenic (As; $R^2 = 0.18$, $p < 0.05$) concentrations exhibited variation that was largely associated with differences in YNP and Iceland hot spring communities (**Fig. 3; Supplementary Fig. 7**). Again, such variation potentially reflects differences in high-temperature water-rock interactions that occur in the aquifers sourcing these springs that are hosted in rhyolitic (SiO_2, Cl, and As) or basaltic bedrock (Mg and Ca) (Arnórsson, 1995, Planer-Friedrich *et al.*, 2020, McCleskey *et al.*, 2022).

Several other measured parameters were significantly correlated with the NMDS ordination, albeit to a lesser extent than pH. These included SO_4^{2-} (or sulfuric acid; $R^2 = 0.4513$, $p < 0.001$) that buffers acidic hot springs and concentrations of heavy metals such as iron (Fe; $R^2 = 0.24$, $p < 0.001$ and aluminum, Al; $R^2 = 0.24$, $p < 0.01$) that are leached from bedrock by acid and that are consequently more concentrated in acidic waters (Ball *et al.*, 2002, Kaasalainen & Stefansson, 2012). Lastly, temperature was also significantly associated with the variation in the clustering of communities in the NMDS ordination, but to a much lesser extent ($R^2 = 0.34$, $p < 0.001$) than pH or several of the other geochemical factors related to water-rock interactions in different regions. Consequently, these results suggest that geologic setting and its controls on the chemistry of waters is the most important factor associated with community compositional differences of hot springs across regions at the ASV level. Moreover, geologic setting is often related to the type of volcanism of a region that itself is related to tectonic setting. Consequently, these results suggest that tectonic settings ultimately control global hot spring community compositions.

The six Torfajökull Icelandic hot spring samples were taken from regions with rhyolitic bedrock (Macdonald *et al.*, 1990), in addition to 1 and 8 samples from the Kerlingarfjöll and Geysir areas, respectively, that contain both rhyolite and basalt host rock (Flude *et al.*, 2010, Ármannsson, 2016). A comparison of the community differences (as Bray-Curtis distances) between these 15 microbial communities and those from Iceland and YNP did not suggest any closer affiliation with YNP communities compared to the rest of the Icelandic spring communities (**Fig. 2**). However, the number of shared ASVs was generally higher between these Icelandic communities and those from other Icelandic rhyolite or mixed basalt-rhyolite bedrock regions relative to the number of shared ASVs with communities in regions without rhyolite bedrock, when considering a similar pH range (**Supplementary Fig. 8**). Given that Icelandic hot spring communities within rhyolite settings did not share noticeably higher ASVs with YNP hot spring communities (that primarily derive from rhyolite settings), these results likely reflect additional geologic, hydrologic, or geochemical influences on communities that differ between YNP and Iceland hydrothermal systems, aside from host rock setting alone. Such influences could include differing hydrologic dynamics (and thus, water-rock interactions) and different concentrations of gases (e.g., H_2S and/or H_2) that were not measured here. In addition, the numbers of ASVs shared among Icelandic springs, regardless of host rock, were similar for three comparisons, with minimal sharing of ASVs between these communities and YNP communities (Supplementary Fig. 8). It is consequently possible that some effect of dispersal limitation could partially explain these results (discussed in greater detail below).

The observed differences in hot spring microbial composition among different geologic settings is likely due to differences in the availability of substrates to support chemosynthetic microbial metabolisms in each region. While there are clear differences in the chemical composition of springs, as reflected by metal and ion concentrations, it is likely that other non-measured analytes also directly contribute to differentiation in community composition via their direct roles in microbial metabolism, including gases like H_2, H_2S, or CH_4 sourced from volcanic activity or water-rock interactions (Lowenstern *et al.*, 2015, Lindsay *et al.*, 2019), or their chemical derivatives (e.g., $S_2O_3^-$ and S^0) (Colman *et al.*, 2019). Some of these potential substrates are differentially distributed among hot spring types within and among regions (Lowenstern *et al.*, 2015, Amenabar & Boyd, 2019, Lindsay *et al.*, 2019, Planer-Friedrich *et al.*, 2020) and would be expected to be generally different among geologic settings, owing to differing water-rock interactions. For example, a recent analysis observed distinct distributions and abundances of thiolated metals in Icelandic and YNP hot springs, with concentrations being higher in Icelandic hot springs (Planer-Friedrich *et al.*, 2020). Given the observed capacity for dominant hot spring taxa like *Thermocrinis* spp. and *Pyrobaculum* spp. to metabolize or produce thiolated metals (e.g., thioarsenates) (Hartig *et al.*, 2014, Jay *et al.*, 2015), differences in the availability of such compounds may help explain differences in the distributions and abundances of these species among regions. Moreover, other substrates have been inferred to support the metabolism of abundant hot spring taxa, such as formate, CO, H_2, CH_4, polysulfides, and NH_4^+ (Windman *et al.*, 2007, Inskeep *et al.*, 2013, Urschel *et al.*, 2015, Daebeler *et al.*, 2018, Lindsay *et al.*, 2019). Consequently, further robust geochemical analyses, combined with analyses of the functional potential of organisms, could help resolve mechanisms underlying the differentiation of taxa observed here among regions.

Region-specific taxonomic groups

SIMPER analysis was used to identify the ASVs that most contributed to differentiation of communities in YNP and Iceland hot springs (hot spring communities from Japan were excluded from this analysis due to sample size) (**Fig. 4, 5**). Comparisons were conducted individually for communities from springs with pH 5-7 (**Fig. 4**) and those with pH > 7 (**Fig. 5**) to isolate taxonomic differentiation between regions, while accounting for the significant influences of pH on overall taxonomic distributions within regions, as discussed above.

Most of the high abundance ASVs in the YNP and Iceland communities were specifically found in springs of only one region, consistent with the NMDS analysis, while genera were often equally dominant among regions (**Fig. 4, Fig. 5**). Considering mid-pH springs (pH 5-7) *Sulfurihydrogenibium* ASV1 and ASV3 were exclusively dominant in YNP hot spring communities, while *Sulfurihydrogenibium* ASV2, *Thermocrinis* ASV1, two *Thermoproteus tenax* ASVs, and two *Pyrobaculum* ASVs (among others) were exclusively dominant in Iceland hot spring communities (**Fig. 4**). Likewise, *Thermocrinis* ASV1, *Thermus* ASV1, multiple *Pyrobaculum* ASVs, and multiple *Ignisphaera* ASVs were exclusively dominant in pH > 7 communities from Iceland (**Fig. 5**). In contrast, several other *Thermocrinis* ASVs, *Thermus* ASV2, Gearchaeales ASV1, and several *Candidatus* Caldarchaeum ASVs were exclusively dominant in YNP hot spring communities with pH > 7 (**Fig. 5**).

Notably, while most of the above dominant ASVs were exclusively found in one region, several exhibited minor abundances (i.e., < 1%) in one or a few hot springs from the region where they were sub-dominant. These included *Sulfurihydrogenibium* ASV2, the *Thermofilum* ASV, and the uncultured Thaumarchaeota ASV (**Fig. 4**) in the mid pH communities (pH 5-7), along with uncultured Acetothermia and *Fervidibacteria* ASV1 in the higher pH (pH >7) communities (**Fig. 5**). These observations suggest that different tectonic and geologic settings select for sub-genus level (i.e., species or strain level) variants, and that these geographically distinct variants are unlikely to arise primarily due to dispersal limitation between regions, since several archaeal and bacterial ASVs were identified in springs of both regions, although most only exhibited minor abundances in their secondary region. Alternatively, it may be possible that cosmopolitan ASVs inhabit niches that do not vary substantively among regions, whereas the geographically-differentiated variants inhabit niches that vary among regions.

A previous comparison of the microbial communities along two hot spring outflows of Iceland and one in YNP also identified the presence of ASV types unique to each region (Podar *et al.*, 2020), suggesting the presence of putatively endemic species. This interpretation is consistent with earlier analyses of the distribution of thermophilic taxa in globally distributed hot springs (Papke *et al.*, 2003, Whitaker *et al.*, 2003). In these studies, variation in measured chemical analytes failed to adequately explain the variation in Sulfolobales isolate genotypes or cyanobacterial 16S rRNA gene/internal transcribed spacer phylotypes, which was used to suggest the presence of microbial endemism to each region due to geographic isolation. The results reported here support an alternative hypothesis wherein thermophilic taxa can be globally distributed among hot springs and are selected for by distinct geochemical environments that are characteristic of each region. Nevertheless, higher resolution genomic data collected from multiple globally distributed regions is needed to rigorously test each of these hypotheses. It should also be noted that the 16S rRNA gene sequence data counts from each sample were sub-sampled to the same level to allow direct comparisons of diversity levels among samples. This method is commonly employed in 16S rRNA gene-based studies but can decrease sensitivity in detecting low abundance taxa due to their preferential removal during sub-sampling (McMurdie and Holmes 2014). However, decreased sensitivity is minimized when compositional variation and overdispersion among samples is high (Hong *et al.* 2022), consistent with the substantial turnover in spring taxa within and among regions in this study. Thus, lost sensitivity due to rarefying data is likely minimal in this study. Nevertheless, it is possible that additional rare taxa could have been missed due to this approach, causing an underestimation of the level of cosmopolitan taxa observed among regions.

To assess whether ASV cohorts are specific to regions, as opposed to individual ASVs, a network analysis was conducted based on correlations of the relative abundances of abundant ASVs (>5% in any sample; n=125) from hot springs in all three geographic regions (**Fig. 6**). Consistent with analyses of individual ASVs, the network revealed largely YNP- and Iceland-specific ASV cohorts, with a lesser extent of Japanese-specific ASV cohorts due to the limited sampling of Japanese springs. Of the 331 statistically significant correlations among ASV distributions, all but four were positive. The four significant negative correlations were present between ASV4648 (*Thermocrinis* sp. [Aquificales]; indicated with an asterisk in **Fig. 6**) that was only abundant in Icelandic springs, and whose abundances were negatively correlated to three ASVs only identified in YNP, and one (Gearchaeales ASV3598; ‘c’ in **Fig. 6**) that was predominantly found in YNP communities and a single Japanese spring community (**Supplementary Fig. 9**). Among the 125 abundant ASVs, only nine were abundant in hot spring communities in more than one region (**Supplementary Fig. 9**), but several of these ASVs were abundant in multiple hot springs from multiple regions and spanned the range of relative abundances as well as taxonomic groups. Indeed, some of the cosmopolitan bacterial (*Sulfurihydrogenibium*; Aquificales) and archaeal (*Pyrobaculum* and *Ca*. Caldarchaeum) ASVs are from genera that are abundant in hot springs globally (Huber *et al.*, 1987, Inskeep *et al.*, 2013, Power *et al.*, 2018) and that are putative autotrophs (Reysenbach *et al.*, 2009, Nunoura *et* *al.*, 2011). Thus, the identification of cosmopolitan ASVs across geographically separated hydrothermal systems suggest that geographic barriers to dispersal may not by themselves necessarily limit the distributions of thermophilic microbial taxa. Rather, most populations may be capable of dispersing across these distances and once established, evolve to increase their fitness in the prevailing geochemical regimes that accompany different tectonic and geological settings. Nevertheless, the relative influences of dispersal limitation and localized selection likely varies for individual taxa due to characteristics that could promote or inhibit movement including physiological (e.g., spore formation and physiological tolerances), ecological (e.g., population sizes and niche breadth), and environmental (e.g., strength of ecological filter) characteristics, among other factors (Custer et al. 2022).

Conclusions

Coordinated geochemical and microbial community compositional analyses of non-photosynthetic hot springs in three globally distributed, but geologically distinct, regions revealed similar universal influences on hot spring microbial community diversity and broad taxonomic compositions. Concomitantly, nearly completely unique microbial community compositions were observed among regions at finer taxonomic levels represented by amplicon sequence variants (ASVs). The predominant factors that influence hot spring microbial communities were similar among springs sampled from the three regions (i.e., pH and temperature). Thus, the nearly complete separation of ASVs among regions suggests that either 1) dispersal limitation resulted in distinct endemic communities among the regions or that 2) tectonic and geologic characteristics specific to each region generated distinct geochemical environments that then selected for differentially-adapted species among regions. Little evidence was observed to support the former assertion, because i) several taxonomically diverse ASVs were present and even abundant in hot springs of multiple regions, ii) the three regions exhibited similar universal geophysical controls on overall geochemical variation but distinct geochemical compositions due to their specific tectonic and geologic settings, and iii) microbial community variation closely recapitulated geochemical variation across regions. Consequently, these results support that local geochemical conditions in hot springs driven by the localized geologic and tectonic settings influence microbial community compositions to a greater extent than biogeographic patterning due to geographic barriers to dispersal. In other words, the rate of initial adaptive evolution to local conditions in each geographic region must have exceeded the rate of dispersal between geographic regions, allowing for speciation (and an adaptive advantage to local conditions) to occur at the sub-genus level in some lineages. Taken together, these results provide new insights into the co-evolution of microorganisms and hydrothermal systems, the trajectories of which likely differ based on tectonic and geologic global settings. Such observations further contribute to our incipient understanding of the co-evolution of life and Earth. Increased resolution of the genomic differences underlying the populations that are closely related, but differentially distributed among hot springs from different regions will help illuminate the ecological and evolutionary mechanisms underlying their observed distributions.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest to report.

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Figure Legends

Fig. 1. Overview of variation in select geochemical measurements in hot springs sampled from Iceland, Yellowstone National Park (YNP), U.S.A., and Japan. A) Temperature and pH of hot spring water samples collected this study are shown in large circles colored by region, as indicated above the plot. Data from 663 Icelandic hot springs within the GeoFluids database (Stefánsson *et al.* 2016) are shown for context in in light black, while data from 7,706 thermal features in the YNP research coordination network database (http://rcn.montana.edu) are shown in light blue. **B)** Log10 transformed sulfate (SO_4^{2-}) and chloride (Cl^-) concentrations in hot spring waters, with points colored by region as in panel **A**. The light black points are from the same database as in **A** and the light blue points derive from 488 samples reported in Ball *et al.* 2002. **C)** Log10 concentrations of SO_4^{2-} in hot spring waters sampled in this study and delineated by pH < 5, pH 5-7, and pH > 7 within each geographic region. Shaded boxes show the interquartile ranges, the white lines show the distribution medians, and the whiskers show the range for each distribution, with individual data points also plotted on top of the boxplots. Samples from Iceland taken from springs with pH < 5 do not have coordinated anion measurements and are thus not included here.

Fig. 2. Nonmetric multidimensional scaling analysis (NMDS) of 16S rRNA gene amplicon sequence variants (ASVs) from hot spring communities in Iceland, Yellowstone National Park (U.S.A.), and Japan. Communities identified by 16S rRNA gene ASVs are visually distinguished based on region and pH, as indicated in the legend.

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination of community 16S rRNA gene amplicon sequence variants (ASVs) overlaid by measured geochemical parameters.

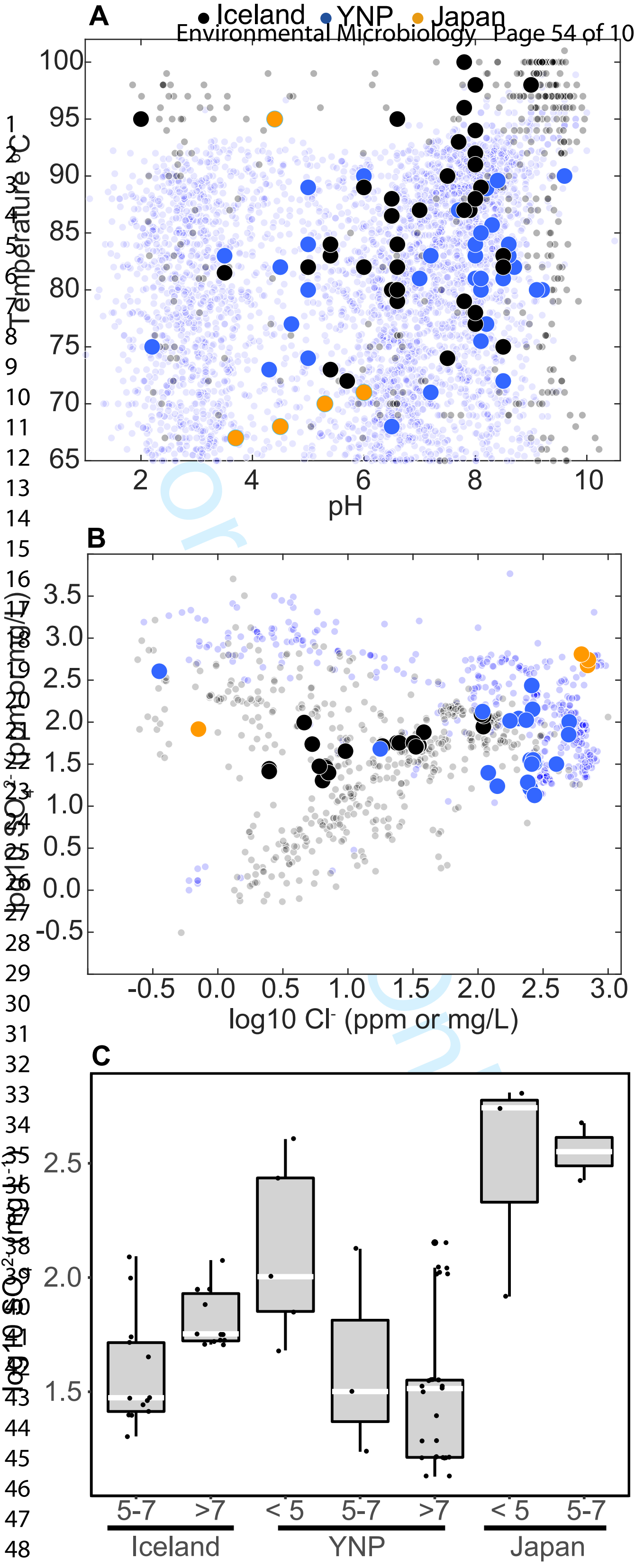
The NMDS ordinations are the same as shown in **Fig. 2**. All tested parameters were significantly ($p \leq 0.05$) associated with the ordination based on factor fitting using the envfit function for vegan (R^2 values are shown in parentheses and significance levels are indicated by asterisks; ***, $p < 0.001$; **, $p < 0.01$; *, $p \leq 0.05$). Samples without geochemical measurements for a given parameter are not included in the individual panels. The NMDS axis values are not shown but are the same as in **Fig. 2**. Visualization of parameter association with community compositional variation was facilitated by log10-transformed parameters, where indicated in each plot.

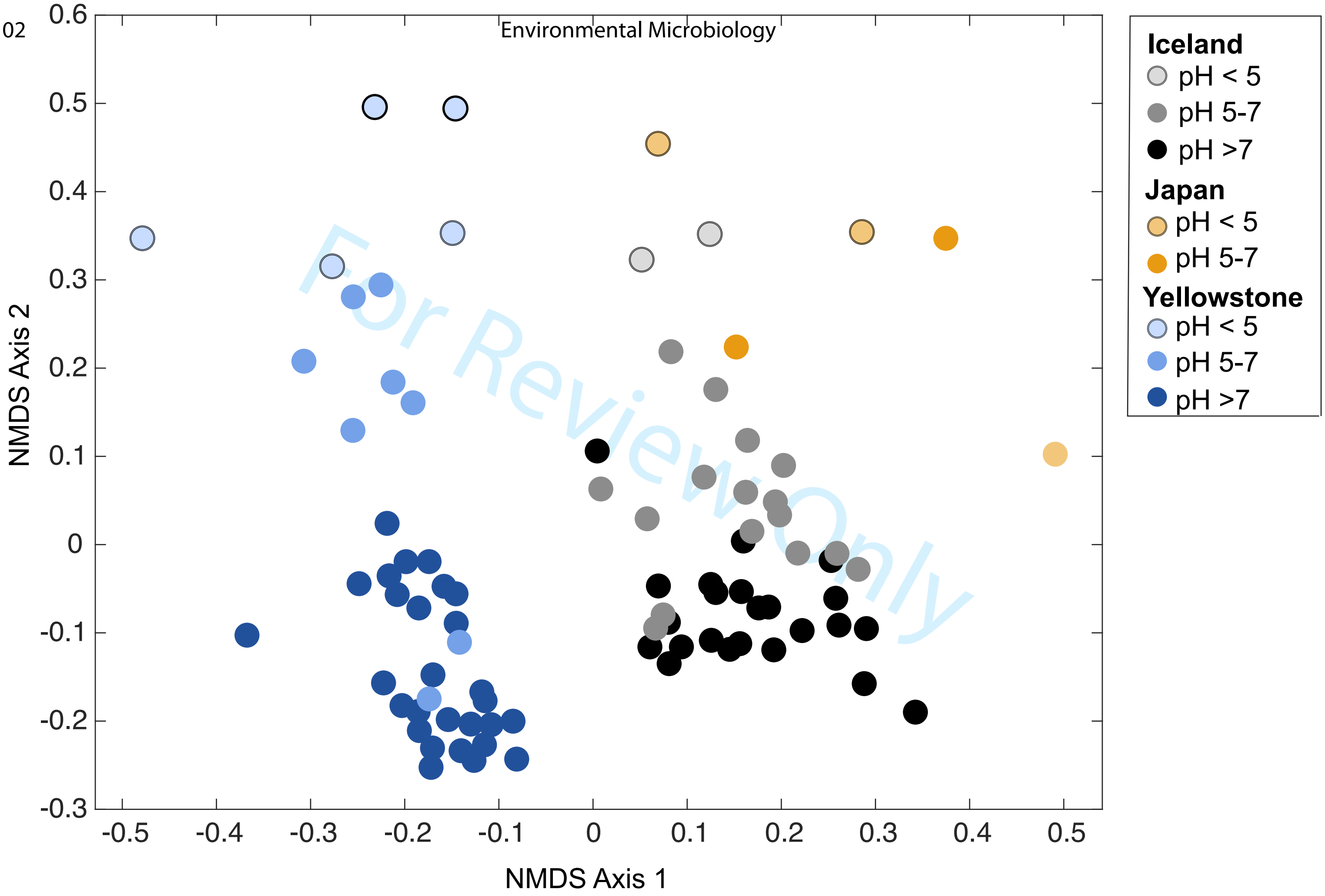
Fig. 4. The relative abundances of 16S rRNA gene amplified sequence variants (ASVs) in communities from hot springs (pH 5 – 7 only) in Iceland and Yellowstone National Park (YNP; U.S.A. that most contributed to region-based differences. The taxonomic classification of each ASV is given on the left (to the highest level of classification) and the ASVs are ordered based on taxonomic classification, with Archaea shown in tan on the top and Bacteria in green at the bottom. The SIMPER contribution scores, as calculated from Bray-Curtis community differences, are shown to the right for each ASV. Only the OTUs that contributed to 70% of the cumulative overall variation between regional communities are shown (n=39). Samples are shown as columns and are arranged in order of ascending pH for communities from Iceland (left) and YNP (right). The relative abundances are log10 transformed to facilitate comparison and visualization, as indicated by the scale at the bottom left.

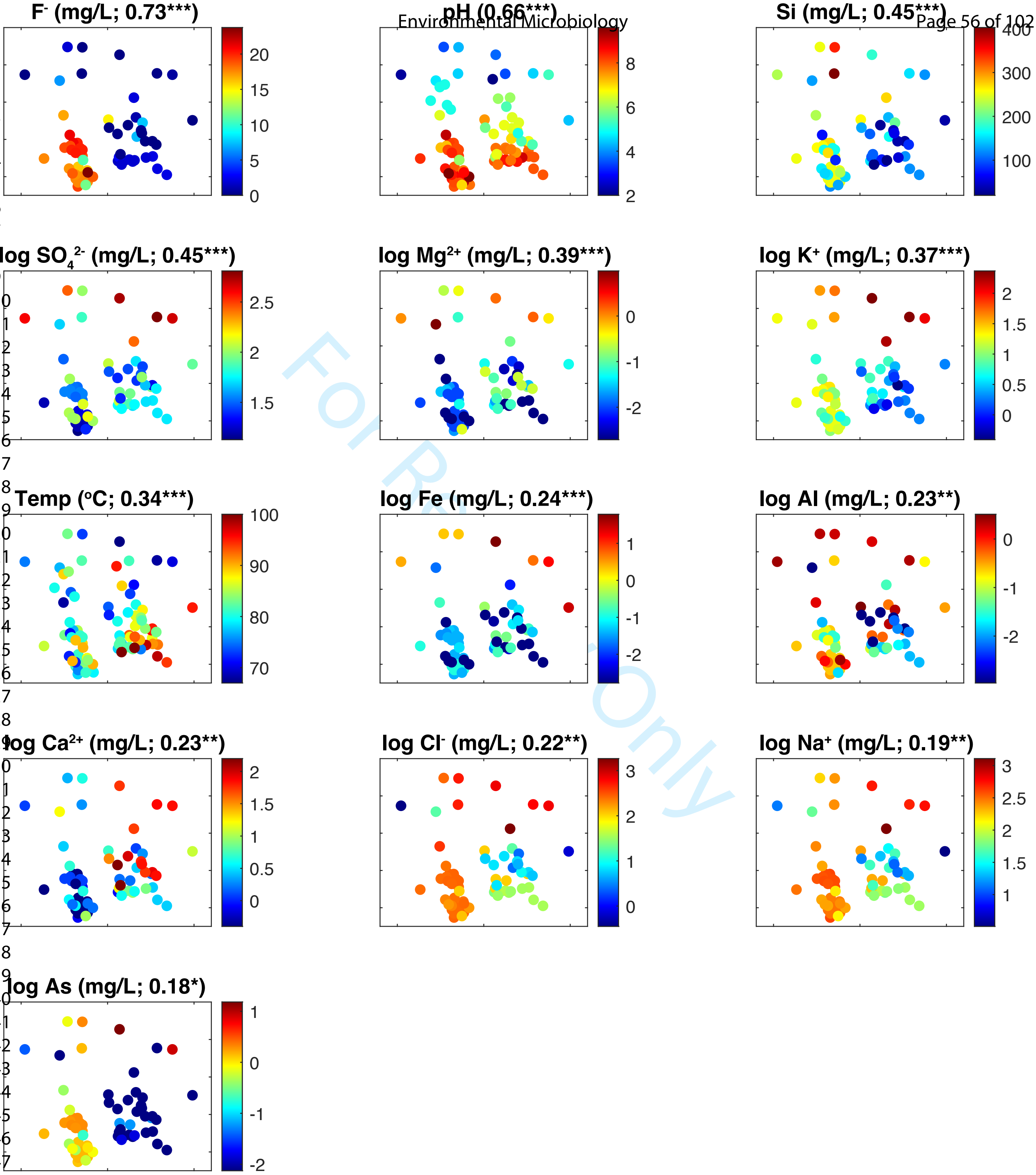
Fig. 5. The relative abundances of 16S rRNA gene amplified sequence variants (ASVs) in communities from hot springs (pH >7 only) in Iceland and Yellowstone National Park (YNP, U.S.A.) that most contributed to region-based differences. The taxonomic

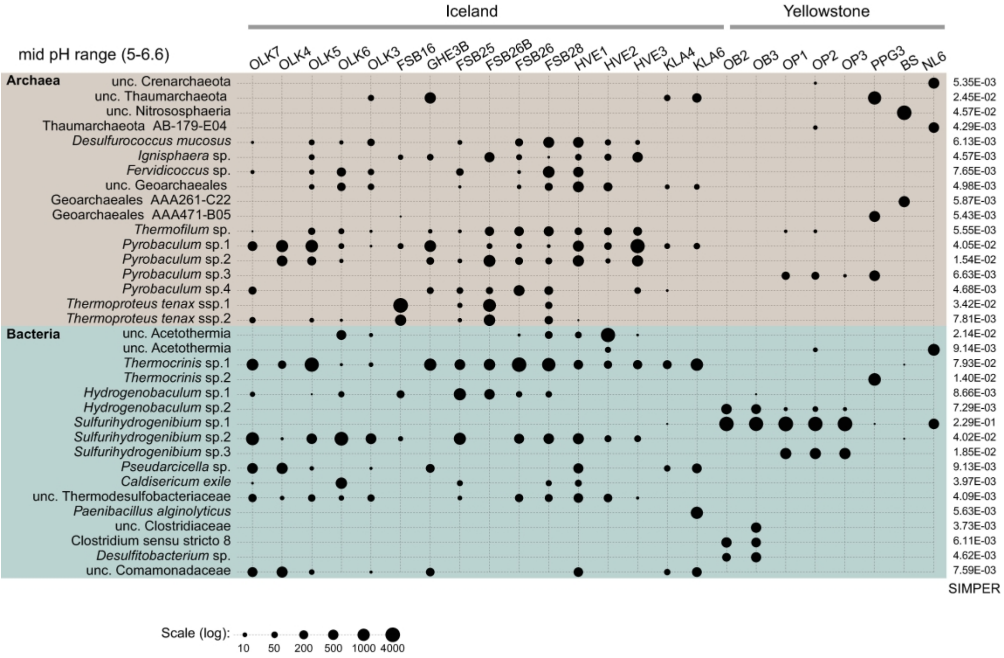
classification of each ASV is given on the left (to the highest level of classification) and the ASVs are ordered based on taxonomic classification, with Archaea shown in tan on the top and Bacteria in green at the bottom. The SIMPER contribution scores, as calculated from Bray-Curtis community differences, are shown to the right for each ASV. Only the OTUs that contributed to 70% of the cumulative overall variation between regional communities are shown (n=40). Samples are shown as columns and are arranged in order of ascending pH for Icelandic communities (left) and YNP communities (right). The relative abundances are log10 transformed to facilitate comparison and visualization, as indicated by the scale at the bottom left.

Fig. 6. Network visualization based on correlations in the abundances of 16S rRNA gene amplicon sequence variants (ASVs) in hot spring communities from Iceland, Yellowstone National Park (YNP, U.S.A.), and Japan. The network was constructed from significant correlations in abundance among ASVs in 87 total hot spring communities. Only ASVs occurring in >5% relative abundance in at least one community were included (n=125). The number of hot springs an ASV was detected in were summed for each region and these totals were used to identify the ASV as only abundant in hot springs in Iceland (black nodes), YNP (blue nodes), or Japan (orange nodes), in addition to those that were abundant in springs from multiple regions (grey nodes). ASVs abundant in >1 region are identified with letters that correspond to detailed abundance information in **Supplementary Fig. 8**. The asterisk indicates *Thermocrinis* ASV 4648 that was the only ASV with negative correlations of abundance to other ASVs (four negative correlations to largely YNP-specific ASVs).


A
● Iceland ● YNP ● Japan
Temperature °C
100
95
90
85
80
75
70
65
2
4
6
8
10
pH
B
log10 SO₄²⁻ (ppm or mg/L)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
log10 Cl⁻ (ppm or mg/L)
C
log10 SO₄²⁻ (mg l⁻¹)
2.5
2.0
1.5
5-7
>7
< 5
5-7
>7
< 5
5-7
Iceland
YNP
Japan


0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
NMDS Axis 2
-0.5
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
NMDS Axis 1
Iceland
pH < 5
pH 5-7
pH >7
Japan
pH < 5
pH 5-7
Yellowstone
pH < 5
pH 5-7
pH >7


F^- (mg/L; 0.73***)
20
15
10
5
0
pH (0.66***)
8
6
4
2
Si (mg/L; 0.45***)
400
300
200
100
log SO_4^{2-} (mg/L; 0.45***)
2.5
2
1.5
log Mg^{2+} (mg/L; 0.39***)
0
-1
-2
log K^+ (mg/L; 0.37***)
2
1.5
1
0.5
0
Temp (°C; 0.34***)
100
90
80
70
log Fe (mg/L; 0.24***)
1
0
-1
-2
log Al (mg/L; 0.23**)
0
-1
-2
log Ca^{2+} (mg/L; 0.23**)
2
1.5
1
0.5
0
log Cl^- (mg/L; 0.22**)
3
2
1
0
log Na^+ (mg/L; 0.19**)
3
2.5
2
1.5
1
log As (mg/L; 0.18*)
1
0
-1
-2



The relative abundances of 16S rRNA gene amplified sequence variants (ASVs) in communities from hot springs (pH 5 – 7 only) in Iceland and Yellowstone National Park (YNP; U.S.A. that most contributed to region-based differences. The taxonomic classification of each ASV is given on the left (to the highest level of classification) and the ASVs are ordered based on taxonomic classification, with Archaea shown in tan on the top and Bacteria in green at the bottom. The SIMPER contribution scores, as calculated from Bray-Curtis community differences, are shown to the right for each ASV. Only the OTUs that contributed to 70% of the cumulative overall variation between regional communities are shown (n=39). Samples are shown as columns and are arranged in order of ascending pH for communities from Iceland (left) and YNP (right). The relative abundances are log10 transformed to facilitate comparison and visualization, as indicated by the scale at the bottom left.

258x168mm (600 x 600 DPI)

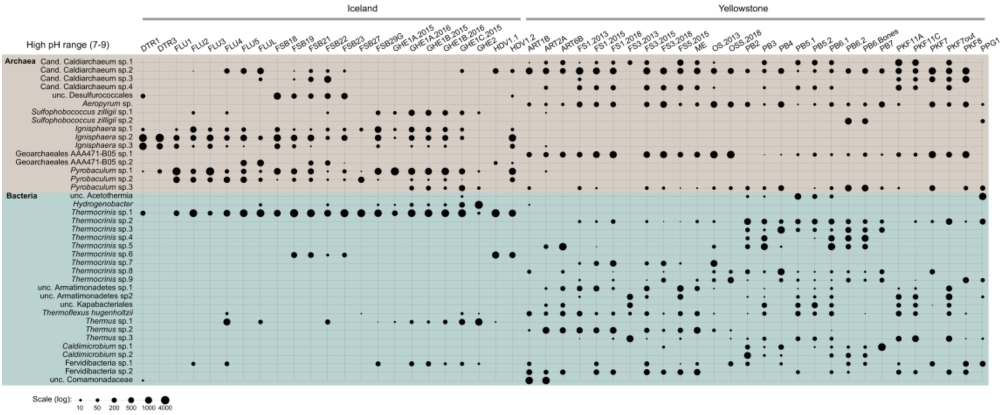
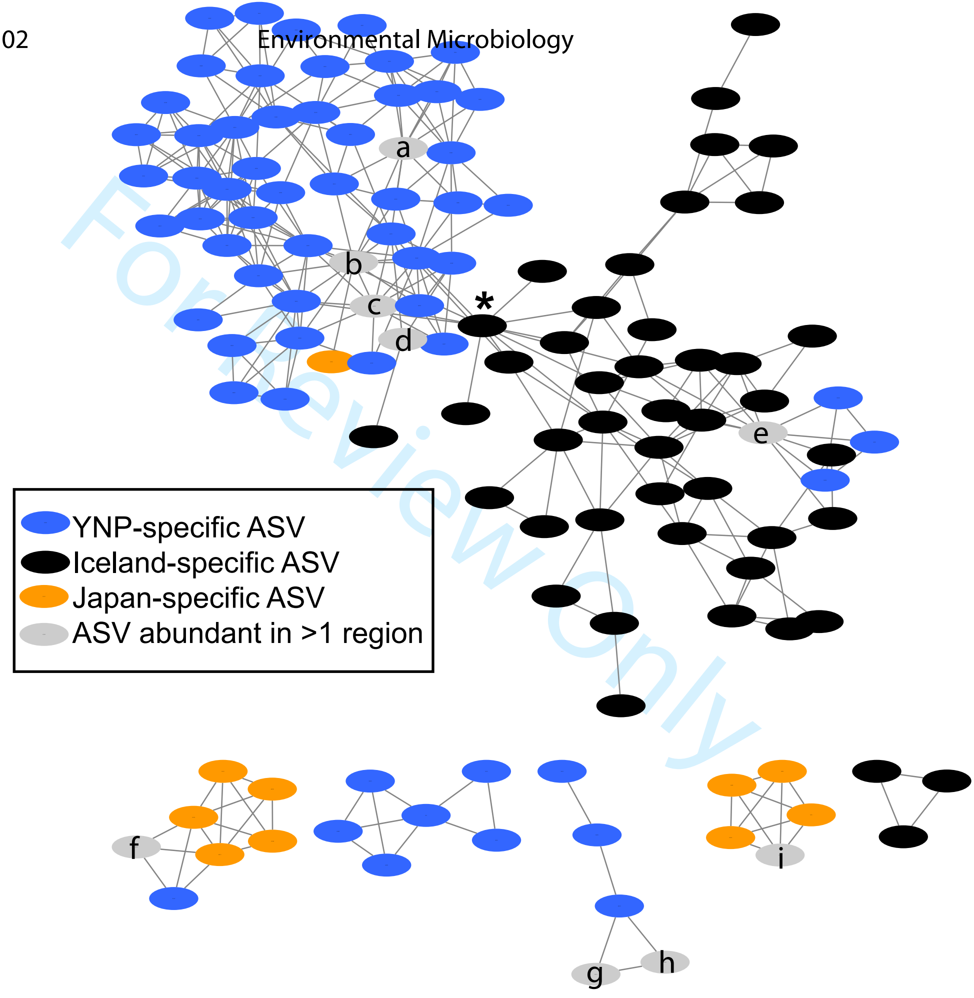


Fig. 5. The relative abundances of 16S rRNA gene amplified sequence variants (ASVs) in communities from hot springs (pH >7 only) in Iceland and Yellowstone National Park (YNP, U.S.A.) that most contributed to region-based differences. The taxonomic classification of each ASV is given on the left (to the highest level of classification) and the ASVs are ordered based on taxonomic classification, with Archaea shown in tan on the top and Bacteria in green at the bottom. The SIMPER contribution scores, as calculated from Bray-Curtis community differences, are shown to the right for each ASV. Only the OTUs that contributed to 70% of the cumulative overall variation between regional communities are shown (n=40). Samples are shown as columns and are arranged in order of ascending pH for Icelandic communities (left) and YNP communities (right). The relative abundances are log10 transformed to facilitate comparison and visualization, as indicated by the scale at the bottom left.

451x185mm (600 x 600 DPI)


YNP-specific ASV
Iceland-specific ASV
Japan-specific ASV
ASV abundant in >1 region
a
b
c
d
*
e
f
g
h
i