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CONNECTIONS BETWEEN HYDROTHERMAL SYSTEM GEOCHEMISTRY AND
MICROBIOLOGY: TRAVERSING TECTONIC BOUNDARIES IN THE SOUTH-
CENTRAL PERUVIAN ANDES

by

Heather Upin

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Geosciences

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2020

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ABSTRACT

Connections between hydrothermal systems
geochemistry and microbiology: traversing tectonic
boundaries in the south-central Peruvian Andes

by

Heather Upin, Master of Science

Utah State University, 2020

Major Professor: Dr. Dennis Newell
Department: Geosciences

Hot springs in continental arcs exhibit varied geochemistry, reflecting tectono-magmatic influences, fluid-rock interactions, and inputs from deeply-derived volatiles, that provide compositionally diverse niches for microbial life. Here we report new water, gas, and molecular microbiology data from 14 thermal springs along a transect from the amagmatic flat-slab region to the active magmatic arc in southern Peru. The springs examined are slightly acidic (pH 5.3–6.8), exhibit a wide range in surface temperature (17–81 °C), and have variable subsurface reservoir temperature estimates (RTEs) of 40–240 °C based on geothermometry. Springs contain a variety of redox sensitive species including Fe^{2+} (0.54–28 ppm) and As (0.01–23 ppm), and dissolved O_2 (0.002–0.253 mM) and H_2 (0.075–1.612 μM). There are geochemical and microbial differences between springs in the flat-slab (flat-slab springs, FSS) and back-arc (back-arc springs, BAS) regions. A principle component analysis of physiochemical parameters indicates four distinct

geochemical groups of springs. There are two FSS groups that are characterized by major ions and atmospheric gas signatures, and two BAS groups characterized by volatile signatures indicative of volcanic influence. Analysis of Similarities (ANOSIM) identified statistically significant relationships between spring geochemistry, microbial community composition, and geologic setting. FSS are chemically heterogeneous, have modest surface temperatures (20–45 °C) and RTEs (40–193 °C), and are dominated by members of the metabolically diverse phylum Proteobacteria (42.2% of sequences). BAS are more geochemically homogeneous, exhibit high concentrations of metals and dissolved gas, have generally elevated surface temperatures (43–81 °C, with one spring 17 °C) and RTEs (117–240 °C), and host a diverse group of putatively thermophilic microorganisms including *Deinococcus-Thermus* (18.4%), *Thaumarchaeota* (5.6%), and *Crenarchaeota* (4.4%) phyla. Flat-slab spring characteristics and microbial communities are impacted by near-surface water-rock re-equilibration and groundwater mixing; whereas, higher heat flow and increased contribution of volatiles shows influence from young magmatism in backarc springs. Our data suggest that transitions in tectonic setting influence the interplay of geochemistry and the resulting microbiology by altering the availability of nutrients through flow path processes such as circulation depth and extent of water-rock interaction.

(82 pages)

PUBLIC ABSTRACT

Connections between hydrothermal systems
geochemistry and microbiology: traversing tectonic
boundaries in the south-central Peruvian Andes

Heather Upin

Geochemistry and microbiology are inherently tied in the natural world. The study of geomicrobiology has historically taken place in extreme systems, like hot springs of Yellowstone National Park and deep-sea hydrothermal vents, because the organisms that exist there have deep lineages on the tree of life and provide insight into early life on Earth. These microbes use chemical energy from nutrients available in their environment rather than relying on photosynthesis, energy obtained from the sun, to support their metabolism. The goal of this study is to improve our understanding of geological controls (for example the tectonic setting) on hot spring geomicrobiology. Fourteen thermal springs were sampled for aqueous and gas geochemistry and molecular microbiology along a transect from the Peruvian flat-slab subduction segment southward to the back-arc setting with steep-dip subduction in the Altiplano. The flat-slab region currently lacks active magmatic activity in contrast to southern Peru that is characterized by active arc volcanoes and behind the arc volcanism. Flat-slab springs (FSS) are cooler, more geochemically varied, and are supported by cooler subsurface reservoir temperatures compared to back-arc springs (BAS). Conversely, BAS have higher temperatures, exclusively Na-Cl rich waters, and are supported by hot subsurface temperatures. Statistical analyses were employed to meaningfully interpret and link the geochemistry and microbial community datasets.

Significant relationships between spring geochemistry, spring temperature, microbial community composition, and geologic setting show that the FSS and BAS contain different phyla based on geologically influenced characteristics (i.e. temperature, chemistry). Our findings show that geological processes deep in the subsurface impart a primary control on surface geomicrobiological diversity.

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CONTENTS

	Page
ABSTRACT.....	iii
PUBLIC ABSTRACT	v
ACKNOWLEDGMENTS	vii
LIST OF TABLES.....	x
LIST OF FIGURES	xi
INTRODUCTION	1
GEOLOGIC SETTING AND HISTORY	4
METHODS	7
Field methods and sampling.....	7
Spring locations	7
Aqueous and gas samples and field measurements	8
Spectrophotometer measurements	8
Microbiology sample collection	9
Water and gas geochemical analysis	9
DNA extraction and 16s rRNA gene sequencing.....	10
Statistical methods.....	11
Geochemical modeling.....	12
RESULTS	13
Aqueous and gas geochemical patterns.....	13
Geothermometry.....	17
Molecular microbiology of spring systems	18
DISCUSSION.....	22
Geochemistry of spring systems	22
Microbial community ecology and connections to tectonic setting	28
SUMMARY AND CONCLUSIONS	32
REFERENCES	35
APPENDICES	46
Representative spring photo compilation.....	47

Aqueous and gas geochemistry and geothermometry data tables	49
Taxonomic information for $\geq 1\%$ abundant OTUs	52
Additional microbiology donut charts	61
Additional NMDS ordinations and statistical summary	64
R code	70

LIST OF TABLES

Table	Page
1. Spring identification, location, and field parameters	14
2. Major ion concentrations and ion balance	15
3. Notable microbiology sequences	30
A1. Trace element and gas chemistry	50
A2. Geothermometry temperature estimates	51
A3. Taxonomy for OTUs $\geq 1\%$ abundance (n = 210)	53
A4. Statistical summary	69

LIST OF FIGURES

Figure	Page
1. Spring location and tectonic setting map	3
2. Major element Piper diagram and principle component analysis	15
3. Microbial community class-level taxonomic data	20
4. Non-metric multidimensional scaling (NMDS) ordination	22
5. Geothermometry ternary diagram	24
6. Tectonic setting cross sections with microbiology donut charts.....	26
A1. Representative spring photos	48
A2. Microbial community taxonomic donut charts (3 levels)	62
A3. Novel versus characterized sequences donut charts.....	63
A4. Subsampled dataset NMDS ordinations	65

INTRODUCTION

Hot springs are geochemically heterogeneous environments that create ecological niches capable of supporting diverse populations of microorganisms (e.g., Macur et al., 2004; Inskeep et al., 2005; Huang et al., 2011; Hurwitz and Lowenstern, 2014; Wang et al., 2014; Colman et al., 2016). The biodiversity that inhabits these environments includes those microorganisms that use light for energy and those that are dependent on chemical energy. Photosynthetic processes convert light into chemical energy and have a functional upper limit of $\sim 73^{\circ}\text{C}$ (Brock, 1967; Boyd et al., 2012), limiting their existence to lower temperature hot spring systems. Chemosynthetic-dependent organisms can live above or below this temperature and are thus less limited by the physiochemical properties of the fluid environment. Non-photosynthetic microorganisms exploit disequilibrium in electron donor and acceptor pairs to generate energy for macromolecular biosynthesis and growth (Shock et al., 2010; Colman et al., 2016; Jelen et al., 2016; Merino et al., 2019). At hot springs, reduced subsurface thermal fluids mix with more oxidized near-surface groundwater to generate redox disequilibrium capable of providing a continual source of potential energy to support microbial life.

Thermal spring geochemistry is controlled by the depth of fluid circulation, the types of rocks encountered along fluid flow paths, water-rock interactions along these pathways, and the degree of mixing with shallow meteoric groundwater (e.g., Fournier, 1989; Arnold et al., 2017). These factors are closely tied to the overall tectono-magmatic setting, and spring chemical and isotopic composition (e.g., He, C, O, S) can inform the relative contribution from magmatic, crustal, and meteoric sources (Hilton, 1996; van Soest et al., 1998; de Hoog et al., 2001; Hilton et al., 2002; Zimmer et al., 2004; Newell et al.,

2008; Sano et al., 2009; Scott et al., 2020). Given the linkages between spring chemistry and microbiology it is likely that the types of microorganisms inhabiting hot springs may also be influenced by tectonic setting.

The link between geomicrobiology and host environment has been investigated at many continental hydrothermal systems (Shock et al., 2010; Colman et al., 2014; Colman et al., 2016), marine deep sea vents (Amend et al., 2011; Reveillaud et al., 2016), in deep wells and pore fracture fluids (Kieft et al., 2005; Moser et al., 2005; Rempfert et al., 2017), and at convergent margins associated with volcanic arcs (Burgess et al., 2012; Ward et al., 2017; Barry et al., 2019). Recent work at the magmatically active Costa Rican continental arc suggests that microbial respiration plays an important role in volatile cycling (Barry et al., 2019; Fullerton et al., 2019). Collectively, existing geomicrobiological research in tectonically active systems supports relationships between hot spring environment and microbiology, and hints at a connection to the tectonic setting that can be quantified. Continental arc settings exhibit distinct variations and transitions in tectonic style (e.g., subduction angle, magmatic gaps) and provide an opportunity to make direct comparisons between tectonic setting, geochemistry, and geomicrobiology.

Peru hosts an excellent example of a shift in subduction style (i.e., tectonic setting) along a continental arc, where a gap in magmatism associated with flat-slab subduction transitions southward to steeper subduction and an active magmatic arc (Fig.1). Here, we explore the interplay between tectonic setting in the Peruvian Andes, its influence on the geochemistry of hot spring emanations, and how these factors shape the composition of hot spring microbial communities. We investigate thermal springs in both the amagmatic flat-slab subduction and magmatically-active back-arc settings via a 1000 km, broadly arc-

parallel sampling transect (Fig. 1). We report new major and trace element chemistry, gas composition, and molecular microbiological results from 14 thermal spring systems to characterize relationships between tectonic setting, spring geochemistry, and microbial diversity. We suggest that differences in subduction style and magmatic activity exert strong controls on the diversity and putative function of microorganisms observed at thermal springs. In particular, we hypothesize that the composition of microbial communities reflects their environment and the availability of nutrients provided by geofluid-rock interaction along flow paths, which is ultimately influenced by the underlying tectonic setting.

Figure 1. Thermal springs (1–14) investigated along a NW-SE transect from the Peruvian flat-slab subduction segment into the backarc of the active subduction segment. Springs 1–5 are within the flat-slab region, and springs 6–14 are located in the backarc. The subducting Nazca slab contours (km) are from the Slab2 model (Hayes, 2018) and offer a schematic of slab depth and geometry.

GEOLOGIC SETTING AND HISTORY

Flat-slab subduction ($\sim < 10^\circ$ slab angle) is observed at $\sim 10\%$ of continental arcs and places the down-going oceanic plate into contact with the overriding lithosphere (e.g., James, 1978; Gutscher et al., 2000). A flat slab geometry eliminates the asthenospheric wedge and ceases the generation of melts, resulting in prominent gaps in magmatism along continental arcs. Today, most of the western South American margin is characterized by normal ($\sim 30^\circ$) subduction and active arc volcanism (Horton, 2018). These volcanically active sections of the Andean arc are separated by three prominent magmatic gaps associated with flat-slab subduction (Gutscher et al., 2000; Ramos and Folguera, 2009). The longest of these flat-slab segments (both in the Andes and worldwide) underlies most of Peru. This flat slab is coincident with the subducting Nazca ridge; however, the triggers and controls on flat-slab subduction are complicated and debated (e.g., Abbot et al., 1994; Ramos and Folguera, 2009; Antonijevic et al., 2015; Manea et al., 2017). South of the Nazca ridge, the flat slab steepens to normal subduction and an active arc (Fig. 1). Here, the back-arc region is geographically part of the Altiplano Plateau, the second highest continental plateau in the world and the highest at a non-collisional convergent margin (Lamb and Hoke, 1997; Allmendinger et al., 1997).

Prior to ~ 11 Ma, most of the present-day flat-slab segment was an active arc with normal-dip subduction as evidenced by widespread Cretaceous–Neogene plutons and volcanic rocks (Megard, 1984; Cobbing, 1999; Gutscher et al., 2000; Ramos and Folguera, 2009). A magmatic lull at the end of the Miocene signals the establishment of the Peruvian flat slab, concurrent with the Nazca Ridge subducting beneath the South American plate (Ramos and Folguera, 2009). The flat slab has been migrating southeastward due to the

oblique subduction of the Nazca plate, and presently the widest part of the flat-slab region is located above the Nazca Ridge (Gutscher et al., 2000; Hampel, 2002; Scire et al., 2016). Southeast of this area, the Altiplano Plateau preserves a prior geological record of the flat-to-steep subduction cycle (Lamb and Hoke, 1997; Ramos and Folguera, 2009; Mamani et al., 2010). Slab shallowing, evidenced by an eastward migration of volcanism, occurred between ~45 and 30 Ma, ultimately leading to the termination of arc magmatism (Mamani et al., 2010). A flat slab existed from ~30 to 25 Ma, which correlates with a lack of igneous rocks of this age (Ramos and Folguera, 2009). Widespread volcanism returned to this area ~26 to 22 Ma coincident with slab rollback and re-steepening (Sandeman et al., 1995; Allmendinger et al., 1997; Hoke and Lamb, 2007; Mamani et al., 2010; Horton et al., 2014).

The south-central Peruvian Andes are characterized geologically by three prominent morphostructural features: the Western Cordillera, the Altiplano Plateau, and the Eastern Cordillera. In the study area, the Western Cordillera defines the active arc, the Altiplano Plateau defines the high-elevation back-arc region, and the Eastern Cordillera is characterized by the thin-skinned Marañon fold and thrust belt (Megard, 1984; Lamb and Hoke, 1997; Scherrenberg et al., 2016). Generally, the Western Cordillera is composed of sedimentary strata with a continental history and plutonic and volcanic igneous rocks, including several granitoid batholiths along the coast (McKee and Noble, 1982; Cobbing, 1999). The Altiplano Plateau is characterized by Mesozoic marine and continental sedimentary rocks with some Cenozoic volcanoclastic units, and the Eastern Cordillera is characterized by upper Paleozoic volcano-sedimentary and plutonic rocks that post-date deformed lower Paleozoic plutonic and shallow marine sedimentary series (Suárez et al., 1983; Cabrera et al., 1991). Recent back-arc magmatism in the Altiplano is exemplified by

Cretaceous-Tertiary volcanic rocks. The youngest magmatic activity in the backarc and northern Altiplano is represented by a relatively small volume of Pliocene-Quaternary ultra-potassic intermediate intrusive and volcanic rocks, that host mantle and crustal xenoliths (Chapman et al., 2015). These recent magmatic rocks include 11–6 ka flows from the Quimsachata volcano southwest of Cusco (Global Volcanism Program, 2013). This activity is relevant to this study because young magmatism injects volatiles into the lithosphere, affecting the geochemical composition of hot springs in the surrounding region (e.g., Hilton et al., 1996).

The structural geology of south-central Peru is dominated by the thin-skinned deformation of the Marañon fold and thrust belt (Megard, 1984; Scherrenberg et al., 2016) near Cusco. Specifically, the Cusco region contains many large normal faults and older thrust faults (Megard, 1984; Cabrera et al., 1991); whereas, the Altiplano region is characterized by synsedimentary deformation including tight folding and faulting (Perello et al., 2003). Two generally NW-SE trending fault zones, Limatambo-Ayaviri and Abancay-Yauri faults, dominate the Cusco region and include both reverse and normal faulting (Cabrera et al., 1991; Perello et al., 2003). Regionally, the NW-trending Cusco-Vilcanota fault system dominates the structure of south-central Peru, separating the Altiplano into two structure blocks (Carlier et al., 2005).

The Peruvian Instituto Geológico Minero y Metalúrgico (INGEMMET) has performed surveys of Peru's hot spring systems to inventory thermal and mineral resources across the country. Two reports encompassing the transition from flat-slab subduction to the active backarc of the Altiplano Plateau provide the preliminary geochemical framework for this study (Steinmüller and Huamaní Huaccán, 1999; Huamaní Huaccán, 2001). In

addition to INGEMMET reports, geochemical studies on springs in Peru have focused on magmatically active hydrothermal systems (e.g., Pajuelo et al., 2020) and hot springs of the Cordillera Blanca and Cordillera Huayhuash regions (Newell et al., 2015; Scott et al., 2020), providing key data for comparison.

METHODS

Field methods and sampling

Spring locations

This study investigates fourteen hot spring systems located along a roughly NW-SE transect of the Peruvian Andes (Fig. 1). These springs were selected based on existing geochemical data sets, geographic location, accessibility, and tectonic setting (Steinmüller and Huamaní Huaccán, 1999; Huamaní Huaccán, 2001). The 14 springs are located in the Peruvian provinces of Ayacucho, Apurímac, Cusco, Puno, and Moquegua. Our aim was not to simply target extreme conditions, but rather to capture a range of aqueous and gas chemistries, temperatures, and geological settings. Many spring locations are actually a cluster of individual emanations. In those cases, sampling was based on temperature and specific conductance measurements so that the spring with the least amount of near-surface mixing with dilute and low-temperature groundwater could be targeted (e.g., highest temperature and highest specific conductance).

Aqueous and gas samples and field measurements

Spring sampling and field measurements were conducted as close to the spring source as possible while maintaining safe distance from hot fluids and unstable ground. Water and gas samples from large high temperature springs were collected using a funnel and tubing attached to a 2.5 m pole. Specific conductance, pH, and temperature measurements were collected in the field using a portable meter. Water samples for cations were field filtered into 60 mL wide-mouth Nalgene® HPDE bottles using 0.45 µm syringe filters (Thermo Scientific, surfactant-free cellulose acetate). Filtered cation samples were later acidified using 0.5 mL concentrated trace-metal grade nitric acid. Samples for anions and alkalinity determinations were collected unfiltered into 125 mL wide-mouth Nalgene® bottles with no head space. In cases where waters were particularly turbid, these samples were also filtered. Gasses from bubbling springs were collected into 12 in. by 3/8 in. ID copper tubes via polyethylene tubing and an inverted funnel, and cold sealed using metal clamps following methods adopted from Hilton et al. (2002). The funnel and tubing were flushed with spring water and then gasses before sampling to prevent atmospheric contamination. Samples were kept cool and dark prior to analyses.

Spectrophotometer measurements

Field spectrophotometer measurements of ferrous iron and sulfide were adapted from the HACH 8146 1,10-Phenanthroline Method and the HACH 8131 USEPA Methylene Blue Method, respectively (HACH 2014; 2015). Using these methods, the dynamic range for ferrous iron (Fe^{2+}) is 0.02 to 3.00 mg/L and 5 to 800 µg/L for sulfide (S^{2-}). Samples were collected as close to the source as possible, and readings were taken

immediately after collection. Samples were initially analyzed undiluted. If concentrations exceeded the maximum range for the method, the samples were diluted as necessary to within the detectable range. Field dilutions were accomplished using bottled water and a 50 ± 0.5 mL graduated cylinder.

Microbiology sample collection

Water (planktonic cells) and sediments (sediment-associated cells) from springs were collected for use in DNA extraction and sequencing. For planktonic cells, 1–3 L of water were passed through 0.2 μ m Sterivex® filters using a 140 mL syringe that was flushed with spring water prior to use. After filtration, a volume of air was passed through the Sterivex filter to remove any remaining water from the filter, which was then stored in a sterile Whirl Pak® bag. Microbial mat and sediment samples were collected using a flame sterilized metal spoon and placed into 15 mL centrifuge tubes. An approximately equal part of sucrose lysis buffer (Mitchell and Takacs-Vesbach, 2008) was added to the sediment and the contents were shaken to coat the entire sample (Colman et al., 2016). All microbiology samples were collected in either duplicate or triplicate and stored at room temperature for 5–15 days until shipment back to Utah State University (USU). Once back in the lab, samples were frozen to -80 °C.

Water and gas geochemical analyses

Dissolved gas composition was measured in 10 of the 14 springs using gas chromatography quadrupole mass spectrometry (MS) at the University of New Mexico Fluid and Volatiles Lab. Gas composition was reported as a mole percent and converted to

dissolved molar concentrations using published Henry's Law constants (Sander, 2005). Major ions and trace elements were measured in 14 springs by inductively coupled plasma MS (ICP-MS) and ion chromatography (IC) respectively at the USU Water Research Lab. Cation and anion samples were volumetrically diluted, as needed, for measurement on the ICP-MS and IC. Alkalinity was measured as total carbonate alkalinity in the USU Geochemistry Lab using colorimetric titration (USGS, 2006).

DNA extraction and 16S rRNA gene sequencing

Methods for genomic DNA extraction, polymerase chain reaction (PCR) amplification of 16S rRNA genes, sequencing, and informatics were conducted according to the methods outlined in Colman et al. (2016). Briefly, filters were cut open using a sterilized jewelry saw, removed from their housing, and peeled from the filter cartridges using sterilized tweezers. Filters or ~0.35 g of sediment were placed into FastDNA SPIN kit bead-beating tubes (MP Biomedicals) with sterilized tweezers or spatulas, respectively. DNA was extracted from each using the FastDNA SPIN Kit for Soil using previously described methods in Boyd et al. (2007) and yielded a total of 29 microbiology samples (one filter was split into two tubes due to a large amount of visible biomass). Genomic DNA was quantified using a Qubit fluorescence assay and fluorometer and roughly 200 ng of DNA were subject to PCR using universal primers (515F and 806R) as previously described in Colman et al. (2016). 16S rRNA gene amplicons were sent to the Molecular Research DNA Lab in Shallowater, Texas, for sequencing utilizing the paired end (2x300 bp) Illumina MiSeq platform.

16S rRNA genes were subjected to analysis using the software program Mothur (Schloss et al., 2009) as previously described by Hamilton et al. (2013), Colman et al. (2016), and Lindsay et al. (2018). In short, sequences were filtered, aligned, and trimmed using defined start and end sites based on 85% inclusion of total sequences. Redundant sequences and sequencing errors were detected and removed. Chimeras were identified and removed using UCHIME (Edgar et al., 2011). Operational taxonomic units (OTUs) were assigned at a sequence similarity of $\geq 97\%$ using the nearest-neighbor method. The sequence dataset was randomly subsampled to generate 45,226 gene sequences per sample. High abundance OTUs ($\geq 1\%$ relative abundance) were subjected to BLASTn analysis to identify the most closely related taxon with a characterized physiology. Adopting this information, a prediction of the metabolism for 16S rRNA gene phylotypes was made for each high abundance OTU.

Statistical methods

Statistical analyses of geochemical and microbiological data were conducted in RStudio (version 1.1.463). Multivariate analyses, including non-metric multidimensional scaling ordination (NMDS), were accomplished using the vegan package version 2.5-4 (Oksanen, 2015; Oksanen et al., 2019). The NMDS ordinations were made with the metaMDS function and a calculated Bray-Curtis distance matrix with three dimensions for combined sediment and planktonic 16S rRNA gene data. We used the envifit function within metaMDS to assess the correlation between environmental parameters and the community distance matrix. NMDS ordination provides a visualization of community similarity between sites. The function ANOSIM, a program in the vegan package, was used

to determine the statistical significance of chemical, temperature, and geographic spring groupings. ANOSIM provides a way to test whether different treatments are statistically significant to microbial community similarity, and returns a *P* value (considered significant if < 0.001 ; 99.9% confidence) and an *R* statistic (a metric specific to this program). The *R* statistic can vary between -1 and 1 and is based on the dissimilarities between and within groups. If the dissimilarity between groups is high but within groups is low, the *R* statistic will approach 1, indicating the communities within treatment groups are similar but different from one another. An *R* statistic of 0 implies random grouping and a negative *R* statistic indicates that the dissimilarity within groups is greater than between groups. A principle component analysis (PCA) of standardized temperature, pH, gas, and water composition data was created to address linear relationships between samples and geochemical variables. The PCA was made with the `prcomp` function in the `stats` package and graphically displayed with the `ggplot2` package. The plot is considered good if the first two principle components explain the majority of the variance in the dataset ($\geq 50\%$).

Geochemical modeling

Ion balances were calculated with the major ion and trace element chemistry for the 14 springs. The total concentration (in equivalents/L) of cations (*c*) and anions (*a*) were used to calculate a percent imbalance using the equation: $([c]-[a])/([c]+[a]) * 100$. Geochemists Workbench version 12.0.4 (Bethke, 2008) was used to generate a Piper diagram with the measured major ion chemistry (Piper, 1944), and calculate saturation indices for spring components.

Reservoir temperature estimates (RTEs) were obtained using empirical and thermodynamically based geothermometers. Multiple thermometers were considered but we focus on the Na-K-Ca, Na-K, and K-Mg cation geothermometers. We do not report silica thermometers because silica measurements were not made for the 14 springs. Calculations are based on original computations and measurements from Fournier and Truesdell (1973) and Giggenbach (1988) and as summarized in Baioumy et al. (2015). The geothermometry spreadsheet calculator from Powell and Cumming (2010) was used to calculate RTEs using the measured water chemistry.

RESULTS

Aqueous and gas geochemical patterns

For this study, the spring (Sp.) locations are numbered 1–14 along the sampling transect (Fig. 1; Table 1), where Sp. 1–5 are located above the flat slab (flat-slab springs, FSS) and Sp. 6–14 are located in the backarc (back-arc springs, BAS). Table 1 provides the local names of these springs. Springs range in temperature and pH from 16.6–80.1 °C and 5.31–6.84, respectively (Table 1). In general, BAS are hotter (> 40 °C) and have slightly more acidic fluids (5.3–6.5 pH) than FSS (< 45 °C and 6.0–6.8 pH). In 13 of the 14 springs, specific conductance varies from 1.31 to 18.76 mS/cm. Agua Termal Saucedá (Sp. 4) is a distinct outlier, issuing from evaporite-bearing strata that are locally mined for salt. Sp. 4 has a specific conductance of 85.80 mS/cm (Table 1). All 14 springs are associated with surface mineralization, such as travertine and iron oxide precipitates (Fig. A1).

Table 1. Spring and sample identification, location, and field parameters.

Sp.	Name	Elevation (m)	Longitude	Latitude	T (°C)	pH	Cond. (mS/cm)
1	Licapa	4103	-74.87776	-13.36887	20.2	6.53	1.31
2	Santo Tomás	1693	-72.94484	-13.65254	39.9	6.12	11.31
3	Ccónocc	1850	-72.63866	-13.54313	31.8	6.84	3.26
4	Agua termal Saucedá	2308	-72.49839	-13.50098	27.8	6.16	85.80
5	Baños termales Lares	3281	-72.05453	-13.11067	45.4	5.96	7.17
6	Baños de Upis	4418	-71.2752	-13.75014	71.0	6.54	9.01
7	Baños Pacchanta	4308	-71.24201	-13.71736	54.2	5.96	3.27
8	Aguas Calientes La Raya	4054	-71.07299	-14.45071	56.4	6.09	7.02
9	Pichacani-Santa Rosa	3931	-70.75443	-14.70899	16.6	6.15	6.76
10	Baños Termales Collpa Apacheta	4106	-70.14219	-16.26762	54.0	5.98	4.82
11	Aguas Calientes-Pinaya	4386	-70.88657	-15.56674	80.5	6.15	15.16
12	Aguas Calientes-Pinaya	4379	-70.88768	-15.56689	68.5	6.09	14.61
13	Aguas termales Crucero	4572	-70.11648	-16.74175	62.0	5.98	18.76
14	Punta Perdida- Pastogrande	4614	-70.08931	-16.76263	43.2	5.31	6.29

Major ion chemistry varies substantially in the 14 springs (Table 2); however, some distinct patterns emerge among FSS and BAS. Consistent with its high specific conductance, Sp. 4 exhibits very high concentrations of several geochemical constituents (Table 2; Table A1) and is omitted from the following comparisons. Overall the BAS are characterized by high salinity waters ($\text{Na}^+ = 422\text{--}4616$ ppm; $\text{Cl}^- = 490\text{--}6931$ ppm), whereas the FSS are generally more dilute. However Sp. 2, located above the flat-slab segment, has the highest HCO_3^- and Ca^{2+} concentrations reported in this study (1751 ppm and 674 ppm respectively). Concentrations of SO_4^{2-} vary widely between the 14 springs with no distinction between FSS and BAS (19.5–975 ppm). Patterns and trends in major ion chemistry are visualized using a Piper diagram (Fig. 2A), modified to include symbols scaled to the concentration of total dissolved solids. As outlined above, the majority of BAS classify as Na-Cl type waters, whereas FSS range from Na-Cl type waters to Ca- HCO_3 and Ca- SO_4 water types.

Table 2. Major ion concentrations (ppm) for the 14 spring. Ion balance was calculated with all relevant major and trace elements.

Sp.	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cl ⁻	SO ₄ ²⁻	NO ₃ ⁻	HCO ₃ ⁻	F ⁻	Ion Balance (%)
MRL	0.50	0.05	0.40	0.13	0.1	0.25	0.05			
1	225	24.5	70.0	8.15	89.7	77.6	< 0.10	848	1.49	-5
2	674	96.3	1923	296	3096	354	0.98	1751	2.26	4
3	295	88.0	381	9.95	614	912	0.24	223	1.70	-2
4	1649	182	16284	< 130	30023	1954	< 3.5	68	0.00	-5
5	585	138	894	100	1915	367	< 0.10	1330	2.10	-1
6	266	23.7	1498	263	2671	563	< 0.20	594	4.76	-5
7	263	19.5	422	54.0	490	832	< 0.10	532	3.93	-7
8	396	42.1	1256	123	1782	623	< 0.10	1015	3.56	1
9	434	115	1158	69.5	1938	247	< 0.20	1454	2.57	0
10	269	43.0	845	44.5	1159	561	< 0.10	643	2.13	0
11	365	33.5	3350	183	5034	900	< 0.50	247	8.34	2
12	357	33.4	3240	178	5014	975	3.50	229	8.76	0
13	153	26.4	4616	386	6931	70.4	< 0.50	637	6.34	3
14	73.1	5.73	1297	144	2174	19.5	< 0.20	198	3.03	0

Figure 2. Geochemical trends for the thermal springs investigated. (A) Piper diagram with major element chemistry (Piper, 1944). Anions and cations come together in the central diamond to showcase dominant geochemical trends and ring size is correlated to total dissolved solids concentration (TDS, mg/kg). Note that Sp. 4 has significantly higher TDS than the other springs (see text for further explanation). (B) Principle component analysis (PCA) with geochemical parameters and spring temperature. Each vector represents a geochemical parameter and the vector direction is the relationship to the two principle components. Longer vector length indicates a stronger correlation and two vectors are correlated if the angle between them is $< 90^\circ$.

Concentrations of trace elements and dissolved gases vary among the 14 springs. Trace elements are more pronounced in BAS; specifically, As, Mn, and Ba concentrations are all notably higher in BAS than in FSS (Table A1). Dissolved gas chemistry (CO_2 , CH_4 , H_2 , N_2 , O_2 , He, Ar) is reported for 10 of 14 springs (Table A1), including two FSS (Sp. 2 and Sp. 5) and 8 BAS (Sp. 6–9 and Sp. 11–14). FSS gas chemistry is characterized by elevated Ar (0.0063–0.0066 mM) and O_2 (0.0826–0.0896 mM) concentrations. In BAS higher concentrations of CO_2 (6.1–19.5 mM), He (0.0058–0.6474 μM), and CH_4 (0.0003–0.1374 mM) are found, and O_2 is much lower (as low as 0.0020 mM), compared to FSS. There is no distinction in H_2 concentrations between FSS and BAS (0.035–3.8374 μM).

PCA is used to identify geochemical groupings based on a more holistic composition by taking major ions, trace elements, and dissolved gases into consideration (Fig. 2B). The trace elements Pb, Ni, Zn, Cr, Co, Cu, Se, Sb were detected in only one spring each, and are thus excluded from the PCA. The axes on the PCA represent the first two principle components, with principle component 1 explaining 28% of the variance in the dataset and principle component 2 explaining 18% of the variance. A PCA is considered good if the first two principle components explain the majority of the dataset's variance. For this PCA they explain 46%. Vectors (arrows, Fig. 2B) represent each geochemical parameter and its relationship to principle components 1 and 2. Two vectors are positively correlated if the angle between them is $< 90^\circ$.

Four chemical groups emerged from the PCA analysis (colored groups in Fig. 2B) and follow a similar pattern to those outlined above. Groups A and B include the FSS, and Groups C and D comprise the BAS. Group A is characterized by the strong correlation between dissolved N_2 , O_2 , and Ar, and weaker correlation with total Fe. Group B is

characterized by the correlation of major ions Na^+ , Cl^- , and Ca^{2+} . Group C is characterized by the correlation of CO_2 and trace elements (i.e., As, Tl, and Ba). Group D is characterized by the correlation of dissolved gas, such as H_2 and He.

Geothermometry

We use multiple geothermometers, specifically the Na-K-Ca, Na-K, and K-Mg cation geothermometers, to estimate the temperature of subsurface geothermal fluids (RTEs) (Table A2). These estimates are based on the assumption of thermodynamic equilibrium at depth and the preservation of this equilibrium during flow to the surface (Fournier and Truesdell, 1973; Giggenbach, 1988). The Na-K-Ca geothermometer is an empirical thermometer and is most appropriate for surface fluids that contain high concentrations of Ca, controlled by the solubility of carbonate in the spring water, and low concentrations of dissolved CO_2 (Fournier and Truesdell, 1973; Giggenbach, 1988). The Na-K geothermometer is based on empirical and thermodynamic data and is best suited for reservoir temperatures $> 100^\circ\text{C}$. Cation concentrations impact these two geothermometers. The Na-K-Ca geothermometer is most useful for surface fluids that satisfy the equation: $\log(\text{Ca}^{1/2}/\text{Na}) + 2.06 > 1$; whereas the Na-K geothermometer is best suited for concentrations such that $\log(\text{Ca}^{1/2}/\text{Na}) + 2.06 < 1$ (Fournier and Truesdell, 1973). The K-Mg geothermometer can accurately estimate reservoir temperatures for mature water chemistries; however, it re-equilibrates rapidly and can thus reflect cooling and mixing during fluid ascent through the crust (Fournier and Truesdell, 1973; Giggenbach, 1988).

In this study, the Na-K-Ca geothermometer returns the widest range of RTEs (40–218 $^\circ\text{C}$), whereas the Na-K thermometer estimates are hotter (144–282 $^\circ\text{C}$), and the K-Mg

geothermometer gives, on average, cooler RTEs (44–159 °C) (Table A2). In general, the Na-K-Ca and K-Mg geothermometers provide similar temperatures for cooler FSS, and Na-K-Ca and Na-K are in close agreement for higher temperature BAS. Based on these observations and spring cation concentrations, the Na-K-Ca and Na-K geothermometers are the most useful to estimate maximum subsurface geothermal fluid temperatures. The maximum RTEs for Sp. 1–10 and Sp. 11–14 are estimated with the Na-K-Ca and the Na-K geothermometers, respectively. With respect to tectonic setting, RTEs, on average, yield lower temperatures for FSS (40–193°C) compared to the BAS (117–240°C). We note that springs located in the transition from the flat slab to backarc region (Sp. 6–9, Fig. 1) yield intermediate RTEs (117–218°C).

Molecular microbiology of spring systems

A total of 53,479 unique 16S rRNA gene OTUs were identified in planktonic and sediment communities sampled. This sequence dataset was subsampled for OTUs $\geq 1\%$ abundance in at least one spring, resulting in 210 distinct OTUs (Table A3). These were described with respect to their homology to characterized taxa, and each comparison includes a calculated percent match to the known cultivar. In this way, each OTU was classified as novel ($< 97\%$ match to described sequences) or previously characterized ($\geq 97\%$ match) (Fig. A2). Using this framework, 55% of the high abundance ($\geq 1\%$ of total sequences in a given spring) OTUs are considered previously characterized and 45% are considered novel. Within the 14 springs, the abundances of dominant OTUs vary markedly, with some genera comprising up to 50% of the spring microbial community.

Bacterial OTUs dominate the 14 springs (89.5% abundance), followed by Archaeal OTUs (10% abundance), and one unclassifiable OTU (Fig. A3A). These OTUs comprise 12 Bacteria phyla and two Archaea phyla (Fig. A3B). The three dominant bacterial phyla include Bacteroidetes, Firmicutes, and Proteobacteria, representing 46% of the high abundance OTUs. Proteobacteria is the dominant Bacteria phyla (24%) regardless of any further subsampling (i.e., planktonic versus sediment community), and includes Alpha-, Beta-, Gamma-, Delta-, and Zeta-proteobacteria classes. Two archaeal classes were identified, Nitrososphaerales and Thermoprotei (Fig. A3C), and are only present in BAS. Thermoprotei represents the majority of the OTUs in Sp. 11 (63%) and are only identified in this spring. OTUs affiliated with phototrophic microbes were identified in each of the 14 springs and include bacterial (Cyanobacteria) and algal (Chloroplast) populations. Putatively phototrophic OTUs comprise 12% of the dataset and are represented by as much as 28% of the high abundance OTUs in these springs.

To identify factors that may explain differences in the composition of microbial communities across the studied springs, class-level abundances of communities were compared to spring temperature (Fig. 3). There are five microbial classes that only appear in springs $> 45^{\circ}\text{C}$: Anaerolineae, Ignavibacteria (with the exception of 1.5% of the sediment fraction of Sp. 2), Deinococci, Nitrososphaerales (with the exception of 1% of the sediment fraction of Sp. 14), and Thermoprotei. The classes Zetaproteobacteria and Spirochaetia are not present above 25°C . With the exception of 2% of the planktonic fraction of Sp. 6, algal chloroplasts are also not detected in springs with temperatures above 25°C .

Figure 3. Microbial community taxonomic data at the class level for operational taxonomic units (OTUs) that represent $\geq 1\%$ relative abundance in at least one of the 14 springs. (A) Bar chart showing relative abundance of classes in each spring, including the sediment (S) and planktonic (P) communities. The springs are arranged from low to high temperature. (B) Donut chart displaying the composite distribution of Classes within all 14 springs. The key and color-coding applies to both charts and is rudimentarily based on inferred metabolism or another key characteristic of the Class. Purple represents dominantly chemotrophic species, orange signifies thermophiles, green shows dominantly phototrophs, blue represents general Bacteria, yellow represents Archaea, and grey signifies unknown or unclassified sequences.

NMDS ordinations for A) the entire dataset ($n = 53,479$), B) sequences $\geq 0.1\%$ abundance ($n = 765$), and C) sequences $\geq 1\%$ abundance ($n = 210$) return a stress of 0.14 (ideal fit if ≤ 0.05 , poor fit if ≥ 0.2 ; Buttigieg and Ramette, 2014), which is considered good fit. For the purpose of this analysis, we will use the NMDS ordination with all sequences (Fig. 4A). Springs that plot close together on the NMDS have more sequences in common than springs that plot farther apart. The tightest cluster on the NMDS is a group of BAS

(Sp. 6, 7, 8, 10, 13, 14), which corresponds to PCA Group C, indicating that these springs also have similar chemistries. The program ANOSIM statistically determines the similarity of sample communities in pre-defined groups, here outlined by observations of spring characteristics and geographic location (groups as polygons in Fig. 4B–D). Microbial communities cluster around temperature ($P < 0.001$ and R statistic = 0.5), chemistry ($P < 0.001$ and R statistic = 0.6), and the two tectonic setting groups (flat slab versus backarc) ($P < 0.001$ and R statistic = 0.5). These general patterns withstand regardless of which groups of springs (i.e. subsampled springs) are included in the NMDS analysis (Fig A4, Table A4).

Figure 4. (A) Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity for all OTUs in the 14 springs ($n = 53,489$), including both the sediment (S) and planktonic (P) microbial communities. The stress for this ordination is 0.14. Springs that plot close together have more species in common than those that plot far apart. Three observational treatments are overlain on the ordination and are supported by ANOSIM (see text for details): (B) microbial temperature classifications (R statistic = 0.5), (C) PCA geochemistry groups (R statistic = 0.6), and (D) tectonic setting (R statistic = 0.5).

DISCUSSION

Geochemistry of spring systems

Our data spans the transition from an amagmatic flat-slab subduction setting to an active backarc. Spring geochemical and statistical trends highlight cooler and chemically heterogeneous FSS, and metal-rich, gas-rich, and more geochemically variable BAS (Table 2; Table A1). Springs in both settings are carbonic with f_{CO_2} ($\sim P_{\text{CO}_2}$) that ranges from $10^{-1.8}$ to $10^{-0.3}$ atm. We note that all but one BAS issue gas bubbles at their source and are

primarily CO₂ (1.4–47.9%) based on dry gas data (Table A1). High volatile content in the backarc is consistent with influences from recent magmatism (11–6 ka flows) and possibly high heat flow (Henry and Pollack, 1988; Global Volcanism Program, 2013). The subsurface geology provides a source for metals (e.g., As and Fe) and the deep circulation of hot, CO₂-rich, likely reducing groundwater aids in chemical alteration and mobilization of these ions. High subsurface temperatures and a long flow path will increase the concentrations of ions at the surface. BAS generally have higher concentrations of Na⁺, K⁺, total Fe, Ba, and As, indicative of water-rock interaction during fluid flow and interaction with relatively young volcanic rocks (Hilton et al., 1996; Arnold et al., 2017). Conversely, FSS show a wider variety of dominant chemistry types (including Ca, HCO₃, and Na-Cl waters types) (Fig. 2A), reflecting shallow groundwater mixing, subsequently less high temperature water-rock interaction at greater depth, and partial re-equilibration with the variable near surface rock types.

Geochemically diverse FSS and saline-rich BAS are consistent with RTE patterns, geothermometry estimates, and flow path depth interpretations. RTEs inform fluid circulation depths, fluid maturity, and water-rock interaction (Fournier and Truesdel, 1973). The degree of agreement between the different geothermometers helps inform the extent of fluid-rock interaction. Disparity between temperature estimates implies less water-rock interaction at depth and fluid “immaturity” (Fig. 5). Springs with this signature likely cooled and re-equilibrated since traveling from the reservoir to the surface, and have since mixed with shallow, cooler groundwater (Fournier and Truesdell, 1973). Agreement or partial agreement between geothermometers is generally associated with higher RTEs. These fluids are termed “mature” and suggest a higher degree of water-rock interaction due

to hotter fluids. These fluids also reflect preservation of the primary geothermal fluid and the RTEs are likely more representative of fluid temperatures at depth (Giggenbach, 1988).

Figure 5. Ternary diagram for the Na-K and K-Mg geothermometers, modified from Giggenbach (1988). This diagram reflects the extent of water-rock interaction at depth and “immature” fluid signatures indicate re-equilibration since travel to the surface and groundwater mixing. Sp. 1–10 plot as “immature” waters. Sp. 11–14 are in partial equilibrium suggesting they have experienced less mixing and the geothermometry estimates for these springs are more representative of temperatures at depth.

Our RTEs support the observation that the transition from amagmatic flat-slab subduction into the active backarc is expressed geochemically in crustal fluids accessed at host springs. For example, Sp. 1–10 are “immature” waters (and include the transitional BAS), whereas Sp. 11–14 show partial equilibration between geothermometers and host more “mature” waters (Fig. 5), suggesting RTEs for these springs may be representative of maximum temperatures at depth. Using the average RTEs for FSS (40–193 °C) and BAS (117–240 °C), an average geothermal gradient of 25 °C/km, and surface temperature of 0 °C, we estimate circulation depths of ~4.2 km in the flat-slab region, and ~7.2 km in the backarc. Broadly, this shows the BAS likely have deeper flow paths and a deeper water-rock interaction signal is preserved compared to FSS (Fig. 6). We note that constraints on

the geothermal gradient are not available, and it is possible that recent magmatism in the backarc requires the use of a higher gradient (30 to 35 °C/km), which will lower the fluid circulation depth estimates (5.8 and 4.9 km, respectively).

Pichacani-Santa Rosa (Sp. 9) is a distinct outlier among BAS (Fig. 3 and Fig. 4D), exhibiting temperature and chemical similarities to some of the FSS. This spring is the coolest (16.6 °C) of the 14 investigated, vigorously bubbles CO₂-rich gases, emanates from a travertine spring mound near the Rio Santa Rosa, and lies along a large inferred fault (De La Cruz, 1995). The spring exhibits a regular fluctuation of water level and bubbling intensity (~20-minute cycles) reminiscent of other documented CO₂-geysers (Keating et al., 2010; Han et al., 2013). The CO₂ flux at this spring likely reflects a deep source that could be related to magmatism or a crustal origin (e.g., natural gas, carbonate decarbonation). However, compared to other BAS, the flow path for Sp. 9 is either shallower or significantly modified by groundwater mixing as evidenced by cooler RTEs (Na-K-Ca: 127 °C; K-Mg: 85 °C). This spring illustrates that in addition to the subduction style, local structural and geological controls are important when addressing factors affecting the overall system.

Figure 6. Schematic cross-sections and microbial breakdown for the flat-slab (left) and backarc (right) settings. (A) Key characteristics and differences between the two tectonic settings are highlighted, such as fluid circulation depth and volatile contributions impacting fluid-rock interaction and microbial respiration. Notably for the flat slab, there are shallower flow paths and influence from slab-derived fluids. In the backarc, fluid circulation depth is deeper and there is more intense water-rock interaction due to recent magmatism providing heat and volatiles. Each cross-section contains information on representative springs. Spring surface and subsurface temperature is highlighted to show generally lower temperatures in the flat slab compared to the backarc. The indicated ion concentrations are some of the highest in the study area and are possible energy sources used for microbial metabolism. (B) Schematic of the slab angle associated with each setting (after Hoke and Lamb, 2007). (C) Zoomed view of subduction style. Average surface water temperatures, RTEs, and deep subsurface temperatures are shown. (D) The donut charts (made with the same rudimentary color-coding as Fig. 3) indicate that the backarc is more microbially diverse than the flat slab which is dominated by Proteobacteria (42%). The phylum "Cyanobacteria/ Chloroplast" contains 2.8% Eukaryal chloroplast. Cross-sections (A & B) are not to scale.

We speculate that thermal springs in the back-arc region are fed through deeper, warmer flow paths than in the flat-slab region (Fig. 6). This is due, in part, to the tectonic history of southern Peru and the Altiplano, which has experienced recent and ongoing magmatism since ~22 Ma and includes Pliocene-Quaternary activity (Hoke and Lamb, 2007; Ramos and Folguera, 2009; Chapman et al., 2015). The magmatic influences in the backarc cause higher heat flow, at least locally, that result in deeper fluid circulation and the advection of hot, saline fluids towards the surface. These processes are associated with higher temperature subsurface fluids (estimated by RTEs), higher observed spring temperatures, and the relatively uniform Na-Cl water type. The younger magmatism also adds volatiles and metals (e.g., CO₂, S species, As) to the system (Arnold et al., 2017), reflected in the surface gas and water geochemistry.

In contrast, the flat-slab region has lower RTEs and spring temperatures that imply less heat flow and/or more shallowly circulated fluids than in the backarc. This results in a wide range of water types and salinities that are indicative of near-surface water-rock re-equilibration and groundwater mixing. The flat-slab geometry has ultimately shut off the magmatic volatiles and heat from melt, but fluids are still circulating (Fig. 6), albeit at shallower depths and cooler temperatures. However, FSS do contain some volatiles (e.g., CO₂, H₂), which could be associated with deep-seated decarbonation reactions and possibly mobilized by the migration of slab-derived fluids, which are consistent with observations elsewhere along the Peruvian flat slab (Newell et al., 2015; Scott et al., 2020).

Microbial community ecology and connections to tectonic setting

We propose that subduction style and magmatic activity influence the geochemistry and temperature of thermal springs which, in turn, shape the diversity of nutrients available to cultivate various communities of microorganisms in these environments. Sustained disequilibrium of electron donor and acceptor pairs, resulting in part from the flux of deeply-circulated reducing fluids mixing with more oxic surface waters, supports microbial metabolism in the 14 springs. NMDS ordination (Fig. 4) reflects the link between microbiology and environment, exhibiting clusters of springs that correspond to physiochemical groups. For instance, a NMDS cluster of BAS correlates to Group C from the PCA, suggesting a correlation between chemistry and community diversity for these specific springs. Our ANOSIM results give high R statistics for groups based on the flat-slab and back-arc tectonic settings ($R = 0.5$), the PCA analysis ($R = 0.6$), and spring temperature ($R = 0.5$). There is minimal overlap between tectonic style groups on the NMDS (colored polygons in Fig. 4D), suggesting that the microbial communities are distinctly different between but similar within these groups. Minimal overlap is observed for chemistry and temperature groups as well (Fig. 4B-C). Thus, the FSS and BAS contain entirely different phyla, despite the 14 springs containing a range of phototrophic and chemotrophic taxa.

We speculate the putative function of OTUs ($\geq 1\%$ abundance, in at least one spring) based on similarity to known cultivars and use spring inorganic geochemistry to discuss whether the microbial function reflects the observed chemistry of the environment (Colman et al., 2016; Rempfert et al., 2017). Microbial community differences between the FSS and BAS can be explained, in part, by the metabolic attributes of the organisms

responding to the given chemical components and temperatures of each habitat. Mesophilic springs located in the flat-slab region are dominated by Proteobacteria (52% of identified OTUs) with Alpha-, Beta-, Delta-, Gamma-, and Zeta- proteobacteria represented, highlighting the metabolic diversity of the phylum. This also reflects the variable spring chemistry in the flat slab where groundwater mixing results in water types that are Na-Cl, Ca-HCO₃, and Ca-SO₄ type waters (Fig. 2A). In contrast, the microbial composition of BAS are dominantly controlled by temperature and to a lesser extent geochemistry. In these springs SO₄-reducing organisms and high temperature tolerant microorganisms dominate (29% of OTUs) and reflect an increase of volatiles and heat to the backarc spring systems. There is also a wider variety of phyla in the communities of BAS compared to those of the FSS (Fig. 6C). Notable OTUs found in BAS are affiliated with Thermaceae, Desulfurococcaceae, Rhodothermaceae, Nitrososphaera, Ignavibacteriaceae, and Thermodesulfobacteriaceae. In this study, the family Thermaceae is the most prevalent family, and is found in abundance (11–50% abundant) in 5 of the 6 hottest springs (54–81 °C) in the backarc (Table 3).

The diverse chemistry of FSS is reflected in their microbial communities. For example, genera of Comamonadaceae (i.e., *Hydrogenophaga*) are present in, and dominate the community of, Sp. 3, 5, and 9 (up to 22% abundant). The presence of Comamonadaceae in these springs is consistent with the demonstrated ability of *Hydrogenophaga* to oxidize H₂ (Willems and Gillis, 2005). Sp. 5 and 9 contain appreciable amounts of dissolved H₂ (1.17 and 0.075 μ M, respectively), and although H₂ was not detected in Sp. 3, it is likely still present at very low levels and is used as a nutrient. Genera of Desulfomonadales (i.e., *Desulfuromonas*) are characterized by facultative Fe and S respiring microbes (Kuever et al., 2005). The presence of *Desulfuromonas carbonis* in the sediment fraction of Sp. 2 is consistent with the spring's elevated concentration of reduced Fe (4.95 ppm Fe²⁺). The halophilic microbial strain *Sediminimonas qiaohouensis* strain YIM B025 (phylum Proteobacteria) was isolated from a salt mine and is present in the planktonic fraction of Sp. 4 (100% ID match; Table 3). Sp. 4 has a very high concentration of Cl⁻ (> 30,000 ppm), reflecting the circulation of spring fluids through evaporite deposits, suggesting that local bedrock geology also contributes to community diversity.

The phylum Thermodesulfobacteria represents a group of hyperthermophilic, sulfate-reducing microorganisms generally isolated from Yellowstone National Park (YNP) and other hydrothermal systems (Garrity et al., 2001). An OTU most closely related to *Caldimicrobium rimae* (99% ID match) was found in Sp. 11, the BAS with the highest measured surface temperature (80.5 °C) and one of the highest SO₄²⁻ concentrations (900 ppm), a characteristic component of magmatically controlled hydrothermal systems. Sp. 11 also contains an OTU belonging to the sulfur-reducing family Desulfurococcaceae (phylum Crenarchaeota; Huber and Stetter, 2001) and is most closely related to

Thermophilum (99% ID match). This is the most abundant OTU in any one spring (51% of Sp. 11) of the study area.

We suggest that the distinct differences in geomicrobiology between FSS and BAS fundamentally reflect the subduction style and associated processes. The dominant factors impacting spring geomicrobiology are the flux of deeply circulated fluids to the surface, the extent of water-rock interaction along flow-paths, and ultimately the system temperature. Although local and regional rock types can impact nutrient availability (Rempfert et al., 2017), it is unlikely to be the dominant controlling factor here. It is well documented that microbial communities respond to gradients in temperature, chemistry, and acidity in places such as YNP (e.g., Brock, 1967; Shock et al., 2010; Inskeep et al., 2015; Colman et al., 2018) and along the Costa Rican convergent margin (Fullerton et al., 2019). These systems are influenced by a nearby volcanic presence that dominates the spring chemistry and thus microbial community ecology. Here, we show that despite a lack of a volcanic influence diverse niches of microbial communities still develop and likely give a clearer picture of the underlying system controls. The distinct geomicrobiological differences between the FSS and BAS indicate the importance of the subducting slab angle at convergent margins on the regulation of temperature and chemistry that ultimately shapes surface environments.

SUMMARY AND CONCLUSIONS

To evaluate the tectonic controls on thermal system microbial diversity, we quantify the linkages between geochemistry and microbial ecology in continental thermal springs along a traverse from the amagmatic flat-slab subduction segment to the active

back-arc region in south-central Peru. Aqueous and gas geochemistry and molecular microbiological sequence data was collected from 14 thermal springs. General trends show that FSS geochemistry is more variable with lower total dissolved solids (with some exceptions) than those in the backarc. BAS chemistry shows exclusively higher salinity, Na-Cl type waters with higher concentrations of volatiles such as CO₂ and S species. This is reflective of a magmatic influence, imparting more heat to the backarc. The microbial communities that develop in the FSS and BAS are reflective of the dominant chemistry and temperature signatures of each environment. The dominant phyla in FSS microbial communities is Proteobacteria (46% of sequences), including two springs that contain iron-oxidizing Zetaproteobacteria. There is a wider variety of phyla in BAS, however microbes in these environments respond to temperature more dominantly than chemistry, reflected by thermophilic taxa including Crenarchaeota (4%) and Deinococcus-Thermus (18%).

The geochemical differences between the two settings suggest different flow paths, flux of volatiles from depth, and degrees of water-rock interaction that we argue are ultimately controlled by the subducting slab angle, and the presence or absence of asthenosphere in contact with the continental lithosphere. Recent magmatism in the backarc fosters deeper and higher temperature flow paths, a greater amount of water-rock interaction, higher spring temperatures, and an influx of volatiles. In contrast, the flat slab region has limited heat flow and no present-day magmatism; as such, fluids spend less time in the subsurface along shallower flow-paths with more near-surface water-rock-aquifer interaction. There is likely still mantle to crust fluid transfer in the flat-slab region (e.g., Newell et al., 2015), which may help explain the high CO₂ content of some springs over the flat slab.

Statistically significant relationships between spring chemistry and temperature and microbial communities suggest a strong connection of spring physicochemical parameters to diversity. The relationship between spring microbial community and tectonic setting (flat slab versus backarc) is also significantly different, where FSS contain a different set of phyla than BAS. We suggest tectonic setting regulates the amount and variety of nutrients available for microbial metabolism by controlling subsurface processes (i.e., extent of water-rock interaction and temperatures of fluids at depth). Redox disequilibrium, created when deep reducing fluids mix with oxidizing groundwater, fosters diverse and geochemically reflective communities at the surface. We note that the springs southwest of Cusco (Sp. 6–9) are located where the slab angle is transitioning from flat to steep (Hayes, 2018). Based on observed and statistical overlaps in the geochemistry and microbiology of these springs, we suggest that gradients in tectonic setting also strongly influence the geomicrobiology of continental thermal springs.

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APPENDICES

Appendix A. Representative spring photo compilation

Figure A1. Representative thermal spring photos from the flat slab (Sp. 2, 5) and the backarc (Sp. 6, 10, 11, 13). These springs have a wide range of temperatures and compositions. They exhibit common features of the 14 springs, such as iron oxide and travertine deposits. Temperature and spring name are shown, and arrows denote the spring location.

Appendix B. Aqueous and gas geochemistry and geothermometry data tables

Table A1. Trace element concentration (in ppb) for investigated thermal springs and gas composition for 10 of the 14 springs. *Measured in the field using HACH portable spectrophotometer. “<” denotes the value was below the limit of detection but within range when dilution factor was added.

Sp. MRL	I ⁻	Fe ²⁺ *	S ²⁻ *	Fe	As	Mn	Ba	Be	Ni	Zn	Cr	Co	Cu	Se	Sb	Tl	Pb
1	-	0.00	-	< 50	47.6	< 5	105	< 0.625	< 10	52.0	< 1	< 1.6	< 6.3	< 1.3	< 30	1.42	22.68
2	115	4.95	-	3140	4720	561	123	< 3.125	< 50	173	< 5	< 8	< 31.5	< 6.5	< 150	24.1	< 12.5
3	-	0.01	-	80	10.1	< 5	19.7	< 0.625	< 10	263	< 1	< 1.6	< 6.3	1.90	< 30	3.73	< 2.5
4	-	0.54	-	< 5000	< 250	827	< 1250	< 62.5	< 1000	< 2000	< 100	< 160	< 630	< 130	< 3000	< 100	< 250
5	63.1	5.83	-	7261	710	618	89.0	5.85	< 10	20.3	< 1	< 1.6	< 6.3	< 1.3	< 30	13.7	< 2.5
6	-	2.44	93	479	24955	54.5	108	4.83	< 20	< 40	< 2	< 3.2	< 12.6	< 2.6	< 60	2.00	< 5
7	-	0.79	-	705	8154	104	37.9	10.1	< 10	< 20	< 1	< 1.6	< 6.3	< 1.3	30.0	4.58	< 2.5
8	-	4.18	-	3548	668	320	58.9	8.66	< 10	< 20	< 1	< 1.6	< 6.3	< 1.3	45.6	8.26	< 2.5
9	-	5.60	-	20782	227	3246	43.7	1.30	18.9	45.5	< 1	26.2	21.9	< 1.3	< 30	1.10	< 2.5
10	-	1.87	-	2029	38.0	1394	54.3	< 0.625	< 10	< 20	1.17	< 1.6	< 6.3	< 1.3	< 30	< 0.45	< 2.5
11	52.4	0.95	-	354	3587	461	115	< 3.125	< 50	< 100	< 5	< 8	< 31.5	< 6.5	< 150	12.2	< 12.5
12	-	0.64	-	252	3252	522	99.7	< 3.125	< 50	< 100	< 5	< 8	< 31.5	< 6.5	< 150	12.4	< 12.5
13	516	0.63	-	501	22907	322	1142	< 1.25	< 20	< 40	< 2	< 3.2	< 12.6	< 2.6	1331	52.6	< 5
14	-	5.94	-	9506	8387	4307	649	< 0.50	< 20	< 40	< 2	< 3.2	< 12.6	< 2.6	< 60	7.20	< 5

Table A1 Continued. Trace element concentration (in ppb) for investigated thermal springs and gas composition for 10 of the 14 springs.

Sp.	CO ₂ mol %; mM	He mol %; μ M	H ₂ mol %; μ M	Ar mol %; mM	O ₂ mol %; mM	N ₂ mol %; mM	CH ₄ mol %; mM	CO mol %; mM
2	51.16; 12.0	0.0386; 0.1522	0.1419; 1.0309	0.6099; 0.0066	8.917; 0.0826	39.14; 0.1966	0.0002; 0.0002	< 0.0002
5	44.07; 9.1	0.0401; 0.1579	0.1659; 1.1703	0.6381; 0.0063	10.62; 0.0896	44.46; 0.2045	0.0002; 0.0002	< 0.0002
6	66.59; 7.8	0.0508; 0.1994	0.1578; 0.9832	0.3155; 0.0021	5.040; 0.0286	27.18; 0.0860	0.6664; 0.4029	< 0.0002
7	89.73; 15.1	0.0074; 0.0289	0.5689; 3.8374	0.1361; 0.0012	0.5348; 0.0039	8.840; 0.0355	0.1410; 0.1132	0.0450; 0.0003
8	96.95; 15.5	0.0028; 0.0111	0.0201; 0.035	0.0033; 0.000	0.4806; 0.0034	2.494; 0.0097	0.0453; 0.0350	< 0.0002
9	20.88; 9.1	0.0015; 0.0058	0.0090; 0.0749	0.7372; 0.0123	17.60; 0.2525	60.77; 0.4604	0.0002; 0.0003	< 0.0002
11	66.46; 6.5	0.1651; 0.6474	0.2486; 1.4864	0.2218; 0.0013	0.4391; 0.0022	32.39; 0.0905	0.0739; 0.0385	< 0.0002
12	49.11; 6.1	0.1034; 0.4060	0.1838; 1.1585	0.4503; 0.0031	4.453; 0.0262	45.65; 0.1495	0.0539; 0.0340	< 0.0002
13	78.59; 11.1	0.0067; 0.0263	0.0630; 0.4091	0.1771; 0.0013	0.6448; 0.0042	20.32; 0.0729	0.1960; 0.1374	< 0.0002
14	90.06; 19.5	0.0093; 0.0368	0.2260; 1.6124	0.0896; 0.0009	0.2334; 0.0020	9.325; 0.0444	0.0600; 0.0589	< 0.0002
Henry's Law Constant	3.4 x 10 ⁻⁴	1.4 x 10 ⁻⁵	7.8 x 10 ⁻⁶	3.9 x 10 ⁻⁶	1.4 x 10 ⁻⁵	1.2 x 10 ⁻⁵	6.4 x 10 ⁻⁶	9.7 x 10 ⁻⁶

Table A2. Reservoir temperature estimates (°C) from cation geothermometers. Sp. 4 was excluded from the analysis due to exceptionally high cation values that precluded the model constraints. The geothermometers with an * are used in this study.

Sp.	Na-K-Ca*	Na/K Fournier 1979	Na/K Truesdell 1976	Na/K Giggenbach 1988*	Na/K Tonani 1980	Na/K Nieva & Nieva 1987	Na/K Arnorsson 1983	K/Mg Giggenbach 1986*
1	40	230	205	245	242	217	211	53
2	193	257	239	269	281	243	244	128
3	56	124	77	144	100	113	89	44
4	-	-	-	-	-	-	-	-
5	131	227	200	242	237	214	207	92
6	218	270	257	282	302	256	260	147
7	117	239	216	253	255	225	222	101
8	157	215	185	231	221	202	193	114
9	127	177	138	195	168	165	148	85
10	118	168	128	186	156	156	138	86
11	175	170	131	188	159	158	141	130
12	175	171	131	189	160	158	141	129
13	214	202	169	218	202	189	177	159
14	213	226	199	240	235	212	206	151

Appendix C. Taxonomic information for $\geq 1\%$ abundant OTUs

Table A3. Taxonomy for OTUs greater than $\geq 1\%$ relative abundance in at least one of the 14 springs (n = 210).

OTU	Taxonomy	Phylum	Class	Order	Family	BLASTn Genus species	Accession
Otu00001	Bacteria(100)	Deinococcus-Thermus(100)	Deinococci(100)	Thermales(100)	Thermaceae(100)	Thermus sp. Tok20 A.1	L09665
Otu00002	Bacteria(100)	Deinococcus-Thermus(100)	Deinococci(100)	Thermales(100)	Thermaceae(100)	Meiothermus sp. Strain CFH 78305	MN826747
Otu00003	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Cyanobacteria(100)	Family_I(100)	GpI(100)	Mastigocladus sp. CHP1	KX035101
Otu00004	Bacteria(100)	Chloroflexi(100)	Chloroflexia(100)	Chloroflexales(100)	Chloroflexaceae(100)	Chloroflexus aurantiacus partial	AJ308501
Otu00005	Bacteria(100)	Proteobacteria(100)	Zetaproteobacteria(100)	Mariprofundales(100)	Mariprofundaceae(100)	Mariprofundus ferrinatatus strain CP-8	CP018800
Otu00006	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Cytophaga sp. T2/21.8	HQ658664
Otu00007	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillaceae 1(95)	Bacillus thioparans strain 1B12	MH929667
Otu00008	Archaea(100)	Crenarchaeota(100)	Thermoprotei(100)	Desulfurococcales(100)	Desulfurococaceae(100)	Thermodiscus maritimus strain S2	NR_044907
Otu00009	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Clostridiaceae 1(100)	Clostridium favosporum	JQ897403
Otu00010	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Cyclobacteriaceae(100)	Algoriphagus taiwanensis strain CC-PR-82	NR_148276
Otu00011	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Chloroplast(100)	Chloroplast(100)	Bacillariophyta(99)	Vaucheria litorea chloroplast	EU912438
Otu00012	Archaea(100)	Thaumarchaeota(100)	Nitrososphaerales(100)	Nitrososphaeraeae(100)	Nitrososphaera(100)	Candidatus Nitrososomicus sp. Kfb	KX863712
Otu00013	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Thermodesulfobivibrio yellowstonii DSM 11347	CP001147
Otu00014	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Vibrionales(100)	Vibrionaceae(100)	Vibrio sp. strain 201707CJOP-Y195	MG593759
Otu00015	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Planococcaceae(83)	Bacillus halmopalus strain DSM 8723	NR_026144
Otu00016	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Filamentous bacterium EU25	DQ232757
Otu00017	Bacteria(100)	Cyanobacteria/Chloroplast(99)	Cyanobacteria(99)	Cyanobacteria_unclassified(99)	Cyanobacteria_unclassified(99)	Cyanobacterium apominum Kh.A	MH179053
Otu00018	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	Pseudoxanthomonas mexicana strain EGE-B-3	KP050791
Otu00019	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Methanogenic archaeon CH50	DQ513419
Otu00020	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Aureisphaera salina strain A6D-50	NR_151885
Otu00021	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillaceae 1(97)	Bacillus sp. Y1 chromosome	CP030028
Otu00022	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Chloroplast(100)	Chloroplast(100)	Bacillariophyta(100)	Odontella sinensis	Z67753
Otu00023	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Cyanobacteria(100)	Cyanobacteria_unclassified(100)	Cyanobacteria_unclassified(100)	Pseudanabaenaceae cyanobacterium CENA319	KT731140
Otu00024	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Methanogenic archaeon CH50	DQ513419
Otu00025	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(99)	Hydrogenophaga aquatica strain CC-KL-3	NR_158120
Otu00026	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Clostridiaceae 1(100)	Clostridium sp. BG-C66	FJ384378

Otu00027	Bacteria(100)	Chloroflexi(100)	Chloroflexia(100)	Chloroflexales(100)	Oscillochloridaceae(95)	Candidatus Chloranaerofilum corporosum clone JG124185J35167_10218671	KY937209
Otu00028	Bacteria(100)	Chloroflexi(100)	Chloroflexia(100)	Chloroflexales(100)	Chloroflexaceae(100)	Roseiflexus castenholzii strain HL08	NR_112114
Otu00029	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillales_Incertae_Sedis_ XII(100)	Exiguobacterium mexicanum strain 0A641	MH929903
Otu00030	Bacteria(100)	Deinococcus- Thermus(100)	Deinococci(100)	Thermales(100)	Thermaceae(100)	Thermus kawayensis strain KW11	NR_112160
Otu00031	Bacteria(100)	Deinococcus-Thermus(99)	Deinococci(99)	Thermales(99)	Thermaceae(99)	Thermus Tok3 A.1	L10069
Otu00032	Bacteria(100)	Chloroflexi(100)	Chloroflexi_unclassified(97)	Chloroflexi_unclassified(97)	Chloroflexi_unclassified(97)	Longilinea arvoryzae strain KOME- 1	NR_041355
Otu00033	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Sediminimonas qiaohouensis strain YIM B025	EU878004
Otu00034	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Chromatiales(100)	Chromatiaceae(100)	Rheinheimera sp. F8	CP013656
Otu00035	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Rhodocyclales(99)	Rhodocyclaceae(99)	Methyloversatilis discipulorum strain B4	MG757549
Otu00036	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Gallionellales(99)	Gallionellaceae(99)	Sideroxydans paludicola strain BrT	DQ386858
Otu00037	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillales_unclassified(100)	Paenibacillus sp. D-1	KF020729
Otu00038	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Clostridiaceae_1(100)	Clostridium quinii strain DSM 6736	NR_026149
Otu00039	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Cytophaga sp. T2/21.8	HQ658664
Otu00040	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Alteromonadales(100)	Shewanellaceae(100)	Shewanella sp. strain L8-6-3	MG561188
Otu00041	Archaea(100)	Thaumarchaeota(100)	Nitrososphaerales(100)	Nitrososphaeraeae(100)	Nitrososphaera(100)	Octopus Spring nitrifying crenarchaeote OS70	EU239962
Otu00042	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Chromatiales(100)	Chromatiaceae(100)	Rheinheimera japonica	LC011485
Otu00043	Bacteria(100)	Spirochaetes(100)	Spirochaetia(100)	Spirochaetales(100)	Spirochaetaceae(100)	Oceanispirochaeta sediminicola strain SY2	NR_158023
Otu00044	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Rhodobacter sp. KT6	FJ439600
Otu00045	Bacteria(100)	Spirochaetes(100)	Spirochaetia(100)	Spirochaetales(100)	Spirochaetaceae(100)	Spirochaeta perfliivii strain P	NR_115202
Otu00046	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Rhodothermaceae(100)	Rhodothermus marinus strain R-10	NR_029282
Otu00047	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Candidatus Magnetococcus mohavensis strain LO-1	GU979422
Otu00048	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Betaproteobacteria_unclassified(100)	Betaproteobacteria_unclassified(100)	Bacterium SCGC AAA018-M4	HQ290506
Otu00049	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Vibrionales(100)	Vibrionaceae(100)	Vibrio ruber strain HMF8004	KY047409
Otu00050	Bacteria(100)	Bacteroidetes(93)	Bacteroidetes_unclassified(93)	Bacteroidetes_unclassified(93)	Bacteroidetes_unclassified(93)	Pedobacter quisiiliarum strain C62-2	KU973598
Otu00051	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Nitrospira sp. SRI-9	AF255603
Otu00052	Bacteria(100)	Chloroflexi(100)	Anaerolineae(100)	Anaerolineales(100)	Anaerolineaceae(100)	Thermomarinilinea lacunifontana strain SW7	NR_132293
Otu00053	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Burkholderiaceae(95)	Limnobacter thiooxidans strain AD12	KY284082
Otu00054	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Pseudomonas sp. R-52653	KT185147

Otu000055	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Betaproteobacteria_unclassified(100)	Betaproteobacteria_unclassified(100)	Candidatus Nitrotoza sp. AM1	LC190436
Otu000056	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Chitinophagaceae(100)	Sediminibacterium goheungense strain HME7863	NR_133854
Otu000057	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillales_unclassified(91)	Bacillus luteolus strain YIM 93174	NR_108638
Otu000058	Bacteria(99)	Bacteria_unclassified(99)	Bacteria_unclassified(99)	Bacteria_unclassified(99)	Bacteria_unclassified(99)	Limibaculum halophilum strain CAU 1123	NR_158129
Otu000059	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacterales(100)	Haematobacter massiliensis strain KC2145	NR_115743
Otu000060	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Chloroflexi bacterium Ver9Iso1	DQ812549
Otu000061	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Candidatus Roseilinea gracile clone JGI24185135167_10016968	KY937207
Otu000062	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Gammaproteobacteria_incertae sedis(100)	Solimonas(100)	Sinimarinibacterium flocculans strain NH6-24	NR_137419
Otu000063	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Methanogenic archaeon CH50	DQ513419
Otu000064	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Rhodothermaceae(100)	Rhodothermus marinus strain R-10	NR_029282
Otu000065	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacterales(100)	Phaeobacter sp. URN3	AB916871
Otu000066	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Betaproteobacteria_unclassified(100)	Betaproteobacteria_unclassified(100)	Bacterium B10D1	JX869434
Otu000067	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Lentimicrobium saccharophilum strain TBC1	NR_149795
Otu000068	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Cyanobacteria(100)	Cyanobacteria_unclassified(100)	Cyanobacteria_unclassified(100)	Oscillatoria amphigranulata strain 11-3	AF317503
Otu000069	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Candidatus Acetothermus autotrophicum	AP011801
Otu000070	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Methanospirillum sp.	L48407
Otu000071	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Alteromonadales(100)	Alteromonas aestuarii strain JDTF-113	NR_157790
Otu000072	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Ornatilinea apprima strain P3M-1	NR_109544
Otu000073	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Thermoanaerobaculum aquaticum strain MP-01	NR_109681
Otu000074	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Pseudomonadales(100)	Pseudomonadales(100)	Pseudomonas sp. strain B-BETUL-M	MG664256
Otu000075	Bacteria(100)	Ignavibacteriia(100)	Ignavibacteriia(100)	Ignavibacteriales(100)	Ignavibacteriales(100)	Ignavibacterium album strain JCM 16511	NR_074698
Otu000076	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Rhodocyclales(98)	Rhodocyclales(98)	Thauera sp. MZ1T	CP001281
Otu000077	Archaea(100)	Crenarchaeota(98)	Thermoprotei(98)	Thermoprotei_unclassified(98)	Thermoprotei_unclassified(98)	Thermophilum uzonense strain 1807-2	NR_146002
Otu000078	unknown(100)	unknown_unclassified(100)	unknown_unclassified(100)	unknown_unclassified(100)	unknown_unclassified(100)	unknown_unclassified(100)	
Otu000079	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Alteromonadales(100)	Aeromonas caviae strain R25-2	CP025777
Otu000080	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Lewinella xylanilytica strain 13-9-B8	NR_137393
Otu000081	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacterium SCGC AAA018-K8	HQ290503

Otu00082	Bacteria(100)	Proteobacteria(100)	Zetaproteobacteria(100)	Mariprofundales(100)	Mariprofundaceae(100)	Mariprofundus micogutta	LC107871
Otu00083	Bacteria(100)	candidate_division_WPS-2(100)	candidate_division_WPS-2_unclassified(100)	candidate_division_WPS-2_unclassified(100)	candidate_division_WPS-2_unclassified(100)	Tepidisphaera mucosa strain 2813	KM052380
Otu00084	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Sphingomonadales(100)	Sphingomonadaceae(100)	Alpha proteobacterium Mz1	AF531016
Otu00085	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Alteromonadales(100)	Alteromonadaceae(100)	Agarivorans gilvus strain WH0801	NR_117238
Otu00086	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(98)	Comamonadaceae bacterium strain hainich_106	MG980500
Otu00087	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Rhodoferrax sp. strain BDP12	MG712812
Otu00088	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Fluviicola hefeinensis strain MYL-8	NR_133750
Otu00089	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Candidatus Kaiserbacteria bacterium GW2011_GWA2_52_12	KX123441
Otu00090	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Chromatiales(100)	Chromatiaceae(100)	Rheinheimera sp. WMF-1	AM690025
Otu00091	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Chitinophagaceae(100)	Sediminibacterium salmoneum strain NJ-44	NR_044197
Otu00092	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(100)	Hydrogenophaga taeniospiralis strain C2pO1	JQ689193
Otu00093	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	Lysobacter hankyongensis strain KTee-2	NR_147746
Otu00094	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Aureisphaera salina strain A6D-50	NR_151885
Otu00095	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Pseudomonadales(100)	Pseudomonadaceae(100)	Pseudomonadaceae bacterium strain hainich_182	MG980551
Otu00096	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Chromatiales(100)	Chromatiaceae(100)	Rheinheimera sp. A1a-3	HG738873
Otu00097	Bacteria(100)	Acidobacteria(100)	Acidobacteria_Gp3(100)	Gp3(95)	Gp3_unclassified(95)	Paludibaculum fermentans strain P105	NR_134120
Otu00098	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Flavobacterium gelidilacus	AJ871468
Otu00099	Bacteria(100)	Chloroflexi(100)	Anaerolineae(100)	Anaerolineales(100)	Anaerolineaceae(100)	Anaerolinea thermophila strain UN1-	NR_036818
Otu00100	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Chromatiales(100)	Chromatiaceae(100)	Rheinheimera sp. 235	JQ012975
Otu00101	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Sphingomonadales(100)	Sphingomonadaceae(100)	Sphingomonas asaccharolytica strain MadaFrogSkinBac-DB-4184	MF526480
Otu00102	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Cryomorphaceae(100)	Bacterium SCGC_AA166-P03	JF488636
Otu00103	Archaea(100)	Thaumarchaeota(100)	Nitrososphaerales(100)	Nitrososphaeraceae(100)	Nitrososphaera(100)	Thaumarchaeota archaeon C13	KU290365
Otu00104	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Gram-positive bacterial sp. (strain Ok9_BI)	L09186
Otu00105	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Burkholderiales_unclassified(100)	Comamonadaceae bacterium PIV-3D	AJ505855
Otu00106	Bacteria(100)	Proteobacteria(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Candidatus Combothrix italica clone BIO53	AY590699
Otu00107	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Chloroplast(100)	Chloroplast(100)	Bacillariophyta(100)	Synedra acus chloroplast	JQ088178
Otu00108	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Clostridiales_unclassified(98)	Anaerosolibacter carboniphilus strain IRF19	KF601947
Otu00109	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Cyanobacteria(100)	Cyanobacteria_unclassified(99)	Cyanobacteria_unclassified(99)	Plankthothrix sp. CYN61	GQ853411

Otu00110	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodospirillales(100)	Rhodospirillaceae(100)	Azospirillum sp. NS33	AB480702
Otu00111	Bacteria(100)	Cyanobacteria/Chloroplast (99)	Cyanobacteria(89)	Cyanobacteria_unclassified(89)	Cyanobacteria_unclassified(89)	Geminocystis sp. NIES-3709	AP014821
Otu00112	Bacteria(100)	Chloroflexi(100)	Chloroflexi_unclassified(100)	Chloroflexi_unclassified(100)	Chloroflexi_unclassified(100)	Bellilinea caldifistulae strain GOMI-1	NR_041354
Otu00113	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Maledivibacter halophilus strain DSM 5387	NR_125713
Otu00114	Bacteria(100)	Cyanobacteria/Chloroplast (100)	Cyanobacteria(100)	Cyanobacteria_unclassified(100)	Cyanobacteria_unclassified(100)	Geitlerinema sp. Pond 63 16S	KJ140095
Otu00115	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Methanospirillum sp.	L48407
Otu00116	Bacteria(100)	Proteobacteria(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Anaeromyxobacter dehalogenans strain FRC-W	FJ190062
Otu00117	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Candidatus Roseilinea gracile clone JG124185J35167 10016968	KY937207
Otu00118	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Psychroflexus saliphilus strain WDS4A13	NR_153694
Otu00119	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Filamentous bacterium EU25	DQ232757
Otu00120	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Burkholderiales(100)	Comamonadaceae(99)	Hydrogenophaga sp. PBC	CP017311
Otu00121	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Thaumarchaeota_unclassified(100)	Candidatus Nitrosotalea devanaterri isolate Nd1	JN227488
Otu00122	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Lutibacter maritimus strain S7-2	NR_116738
Otu00123	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Pseudomonadales(100)	Pseudomonadaceae(100)	Cellvibrio fontiphilus strain MVW-40	NR_157755
Otu00124	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Lentimicrobium saccharophilum strain TBC1	NR_149795
Otu00125	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	Desulfuromonadaceae(100)	Desulfuromusa ferrireducens strain 102	NR_043214
Otu00126	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Methanogenic archaeon CH50	DQ513419
Otu00127	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Alphaproteobacteria_unclassified(98)	Vibrionaceae(100)	Vibrio anguillarum strain AL8-6W	MH881409
Otu00128	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Kroppenstedtia sanguinis strain X0394	NR_146671
Otu00129	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	Desulfuromonadales_unclassified(97)	Delta Proteobacterium G50V1	AJ786070
Otu00130	Bacteria(100)	Cyanobacteria/Chloroplast (100)	Cyanobacteria(100)	Cyanobacteria_unclassified(100)	Cyanobacteria_unclassified(100)	Microcoleus chthonoplastes CCY9603	GQ402020
Otu00131	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Paracoccus koreensis strain Ch05	NR_041238
Otu00132	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(96)	Bdellovibrionales(96)	Bacteriovoracaceae(96)	Bacteriovorax sp. MER21	DO631740
Otu00133	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Flavobacterium sp. strain N72	MH714638
Otu00134	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Lachnospiraceae(100)	Cellulosilyticum lentoceillum strain DSM 5427	NR_119067
Otu00135	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodospirillales(100)	Rhodospirillaceae(98)	Elstera cyanobacteriorum strain TH019	NR_158149
Otu00136	Bacteria(100)	Bacteria_unclassified(99)	Bacteria_unclassified(99)	Bacteria_unclassified(99)	Bacteria_unclassified(99)	Olavius ilvae Delta 1 endosymbiont	AJ620500
Otu00137	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Meliobacter roseus strain P3M-2	NR_074796

Otu00138	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	Xanthomonadaceae bacterium strain hainich_160	MG980541
Otu00139	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Coxiella endosymbiont of Rhizopneustes microplus isolate Rhmicro2	KP994840
Otu00141	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Cyanobacteria(100)	Cyanobacteria_unclassified(100)	Cyanobacteria_unclassified(100)	Desulfotomaculum d'azianense PMC 909.15	MF579907
Otu00144	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacterium strain BB12	KX815444
Otu00145	Bacteria(100)	Armatimonadetes(100)	Armatimonadetes_gp7(100)	Armatimonadetes_gp7_unclassified(100)	Armatimonadetes_gp7_unclassified(100)	Eubacterium sp. (OS type L)	L04707
Otu00146	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Clostridiales_unclassified(100)	Abyssivirga alkaniphila strain L81	NR_148837
Otu00147	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Gemmobacter sp. strain 3AM-11	KT992325
Otu00149	Archaea(100)	Thaumarchaeota(100)	Thaumarchaeota_unclassified(100)	Thaumarchaeota_unclassified(100)	Bacteria_unclassified(100)	Ignavibacterium album strain JCM 16511	NR_074698
Otu00150	Bacteria(100)	Bacteroidetes(99)	Bacteroidetes_unclassified(99)	Bacteroidetes_unclassified(99)	Bacteroidetes_unclassified(99)	Bacteroides sp. ECP-C1	AF529225
Otu00151	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Palaeococcus helgesonii strain P11	NR_029059
Otu00152	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Gammaproteobacteria_incertae_sedis(100)	Solimonas(100)	Sinimarinibacterium flocculans strain NH6-24	NR_137419
Otu00153	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroides bacterium PP50E2	AY548787
Otu00155	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Pseudomonadales(100)	Moraxellaceae(100)	Perluclidibaca aquatica strain BK296	NR_157660
Otu00158	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	F.aquatile	M62797 M28236
Otu00159	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Alteromonadaceae(100)	Marinobacter hydrocarbonoclasticus strain E13	MH746060
Otu00160	Archaea(100)	Thaumarchaeota(100)	Nitrososphaerales(100)	Nitrososphaerales(100)	Nitrososphaera(100)	Thaumarchaeota archaeon C13	KU290365
Otu00161	Bacteria(100)	Proteobacteria(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Desulfomicrobium macestii strain M-9	NR_025349
Otu00162	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_incertae_sedis(100)	Ohtaekwangia(100)	Ohtaekwangia_unclassified(100)	Bacteroides bacterium KJY	AB540000
Otu00163	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Rubrimonas shengliensis strain SL014B-80A	GU125652
Otu00164	Bacteria(100)	Bacteroidetes(99)	Bacteroidetes_unclassified(99)	Bacteroidetes_unclassified(99)	Bacteroidetes_unclassified(99)	Mucilaginibacter pedocola strain TBZ30	NR_152040
Otu00165	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Desulfonatronum thioautotrophicum strain ASO4-1	NR_116693
Otu00166	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Mariniflexile jejuense strain SSK2-3	NR_109619
Otu00168	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Vibrionales(100)	Alphaproteobacteria_unclassified(98)	Pannonibacter sp. VTT E-073067	EU438959
Otu00169	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadaceae(100)	Arenimonas daejeonensis strain T7-07	NR_108139

Otu00170	Archaea(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Archaea_unclassified(100)	Candidatus Caldiarchaeum subterraneum	AP011878
Otu00173	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Gammaproteobacteria(100)	Gammaproteobacteria(100)	Gammaproteobacteria_incertae sedis(100)	Solimonas flava strain CW-KD 4	NR_044123
Otu00174	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(94)	Sphingobacteriales(94)	Sphingobacteriales(94)	Sphingobacteriales(94)	Candidatus Aquirestis calciphila	AJ786331
Otu00175	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriales(100)	Flavobacteriales(100)	Flavobacterium inkyongense strain IMCC27201	NR_156036
Otu00177	Bacteria(100)	Chloroflexi(96)	Chloroflexi_unclassified(96)	Chloroflexi_unclassified(96)	Chloroflexi_unclassified(96)	Chloroflexi_unclassified(96)	Thermomarinilinea lacunifontana strain SW7	NR_132293
Otu00179	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Pseudomonadales(100)	Pseudomonadales(100)	Pseudomonadales(100)	Cellvibrio fulvus strain NCIMB 8634	NR_025210
Otu00180	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	Desulfuromonadales(100)	Desulfuromonadales(100)	Delta proteobacterium EtOHpelo	AY771935
Otu00181	Bacteria(100)	Proteobacteria(99)	Deltaproteobacteria(92)	Bdellovibrionales(90)	Bdellovibrionales(90)	Bacteriovoracaceae(90)	Bacteriovorax sp. MER21	DQ631740
Otu00182	Archaea(100)	Thaumarchaeota(100)	Nitrososphaerales(96)	Nitrososphaerales(96)	Nitrososphaerales(96)	Gp23_unclassified(100)	Eubacterium sp. (OS type K)	L04711
Otu00183	Bacteria(100)	Bacteroidetes(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes_unclassified(100)	Bacteroidetes bacterium SCGC AAA487-A14	HQ663650
Otu00184	Bacteria(100)	Proteobacteria(100)	Proteobacteria_unclassified(95)	Proteobacteria_unclassified(95)	Proteobacteria_unclassified(95)	Proteobacteria_unclassified(95)	Marine alpha proteobacterium RS.Sph.017	DQ097291
Otu00185	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Xanthomonadales(100)	Xanthomonadales(100)	Xanthomonadales(100)	Thermomonas sp. ROi19	EF219041
Otu00186	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Gammaproteobacteria_unclassified(100)	Gammaproteobacteria_unclassified(100)	Gammaproteobacteria_unclassified(100)	Wenzhouxiangella marina strain 4S-CH-S3-s2	MG264256
Otu00187	Bacteria(100)	Bacteroidetes(98)	Bacteroidetes_unclassified(98)	Bacteroidetes_unclassified(98)	Bacteroidetes_unclassified(98)	Bacteroidetes_unclassified(98)	Hydrobacter penzbergensis strain EM 4	NR_134746
Otu00189	Bacteria(100)	Deinococcus-Thermus(100)	Deinococci(100)	Thermales(100)	Thermales(100)	Thermales(100)	Thermus scotoductus SA-01	CP001962
Otu00190	Bacteria(100)	Ignavibacteriiae(100)	Ignavibacteriia(100)	Ignavibacteriales(100)	Ignavibacteriales(100)	Ignavibacteriales(100)	Ignavibacterium album strain JCM 16511	NR_074698
Otu00191	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Rhizobiales(100)	Rhizobiales(100)	Rhizobiales(100)	Aurantimonas aggregata strain ICBM	KY984095
Otu00193	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Deltaproteobacteria(100)	Deltaproteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonas carbonis strain ICBM	KJ776405
Otu00195	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Vibrionales(100)	Vibrionales(100)	Vibrionales(100)	Vibrio sp. ER-65	KT325172
Otu00197	Bacteria(100)	Proteobacteria(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Proteobacteria_unclassified(100)	Desulfobacca acetoxidans strain DSM 11109	NR_074955
Otu00198	Bacteria(100)	Proteobacteria(100)	Gammaproteobacteria(100)	Chromatiales(100)	Chromatiales(100)	Chromatiales(100)	Allochrochromium vinosum DSM 180	CP001896
Otu00199	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(92)	Deltaproteobacteria_unclassified(92)	Deltaproteobacteria_unclassified(92)	Deltaproteobacteria_unclassified(92)	Anaeromyxobacter sp. PSR-1	AB795400
Otu00200	Bacteria(100)	Firmicutes(100)	Bacilli(100)	Bacillales(100)	Bacillales(100)	Bacillales(100)	Paenibacillus solanacearum strain T16R-228	KU379666
Otu00201	Bacteria(100)	Acidobacteria(100)	Acidobacteria Gp23(99)	Gp23(99)	Gp23(99)	Gp23(99)	Thermoanaerobaculum aquaticum strain MP-01	NR_109681
Otu00202	Bacteria(100)	Acidobacteria(100)	Acidobacteria Gp23(100)	Gp23(100)	Gp23(100)	Gp23(100)	Candidatus Nitrosotalea devanattera isolate Nd1	JN227488
Otu00204	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Methylophilales(100)	Methylophilales(100)	Methylophilales(100)	Methylophilobacter mobilis	AB698738

Otu00205	Bacteria(100)	Firmicutes(100)	Clostridia(100)	Clostridiales(100)	Clostridiales_Incertae_Sedis_XII(100)	Fusibacter bizertensis strain LTF Kr01	KJ420408
Otu00206	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Parubacteria group bacterium GW2011_GWB1_45_9	KX123379
Otu00208	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Polycladomyces subterraneus strain KSR_13	NR_158012
Otu00214	Bacteria(100)	Cyanobacteria/Chloroplast(100)	Cyanobacteria(100)	Family_XIII(100)	GpXIII(100)	Arthrospira platensis strain PCC 7345	NR_125711
Otu00215	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Rhodothermaceae(100)	Rhodothermus marinus strain R-10	NR_029282
Otu00220	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Syntrophobacteriales(100)	Syntrophobacteraceae(100)	Desulfosoma profundum strain SPD X02-08	NR_117786
Otu00223	Bacteria(100)	Thermodesulfobacteria(100)	Thermodesulfobacteria(100)	Thermodesulfobacteriales(100)	Thermodesulfobacteriaceae(100)	Caldimicrobium rimae strain DS	NR_044283
Otu00224	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Chloroflexi bacterium Ver9Iso1	DQ812549
Otu00225	Bacteria(100)	Bacteroidetes(100)	Sphingobacteriia(100)	Sphingobacteriales(100)	Chitinophagaceae(100)	Terrimonas lutea strain DY	NR_041250
Otu00233	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Psychroflexus tropicus strain LA1	NR_028854
Otu00234	Bacteria(100)	Proteobacteria(100)	Gammaaproteobacteria(100)	Chromatiales(100)	Chromatiaceae(100)	Rheinheimera arenilitoris strain J-MS1	NR_134151
Otu00238	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Delta proteobacterium TP492	EF636113
Otu00239	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(95)	Burkholderiales(95)	Burkholderiales_unclassified(89)	Burkholderiales bacterium VC93	FM995606
Otu00246	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Candidatus Acetothermus autotrophicum	AP011801
Otu00247	Bacteria(100)	Proteobacteria(100)	Alphaproteobacteria(100)	Sphingomonadales(100)	Sphingomonadaceae(100)	Sphingomonadaceae bacterium W45	LC094487
Otu00248	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Planctomycete GMD14H10 small subunit	AY162122
Otu00252	Bacteria(100)	Acidobacteria(100)	Acidobacteria Gp16(100)	Gp16(100)	Gp16_unclassified(100)	Bacterium Ellin6529	HM748677
Otu00262	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Planctomycetes bacterium SCGC AAA001-J07	HQ675380
Otu00267	Bacteria(100)	Proteobacteria(100)	Betaproteobacteria(100)	Neisseriales(100)	Neisseriaceae(100)	Vogesella perlucida strain BN_2063	MG438520
Otu00268	Bacteria(100)	Bacteroidetes(100)	Cytophagia(100)	Cytophagales(100)	Flammeovirgaceae(100)	Roseivirga maritima strain GM-5	KU507204
Otu00271	Bacteria(100)	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Nanhaeicola litoreus strain DPG-25	NR_132282
Otu00282	Bacteria(100)	Proteobacteria(100)	Deltaproteobacteria(100)	Deltaproteobacteria_unclassified(100)	Deltaproteobacteria_unclassified(100)	Chondromyces pediculatus	AJ233940
Otu00285	Bacteria(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Bacteria_unclassified(100)	Ammoniphilus oxalivorans strain RAOx-FS	NR_026433

Appendix D. Additional microbiology donut charts

Figure A2. Donut charts for each spring show the amount of $\geq 1\%$ abundance sequences that are previously characterized ($\geq 97\%$ ID match to known cultivars) or considered novel ($< 97\%$ ID match). The novel fraction is generally higher in BAS compared to those for FSS, indicating there are more uncharacterized microbes in springs in the back-arc setting. This is due, in part, to the geology of the backarc, fostering hot and magmatically influenced environments that sustain uncharacterized archaeal and bacterial assemblages.

Figure A3. Donut charts at three levels of taxonomic classifications (A) Domain, (B) Phyla, (C) Class, for OTUs that are $\geq 1\%$ abundance in at least one spring. The donuts are rudimentarily color-coded based on inferred metabolism or another key characteristic, where purple represents dominantly chemotrophs, orange signifies thermophiles, green shows dominantly phototrophs, blue represents general Bacteria, yellow represents Archaea, and grey signifies unknown or unclassified sequences.

Appendix E. Additional NMDS ordinations and statistical summary

Figure A4. NMDS on subsampled datasets including: (A) OTUs $\geq 0.1\%$ abundance, Sp. 1–14; (B) OTUs $\geq 1\%$ abundance, Sp. 1–14; (C) Sediment OTUs, Sp. 1–14; (D) Planktonic OTUs, Sp. 1–14; (E) All OTUs, Sp. 1–14 excluding Sp. 4; (F) OTUs $\geq 1\%$ abundance, Sp. 1–14 excluding Sp. 4. Eliminating OTUs based on abundance to reduce noise, for instance in B and C, did not change the NMDS stress level. Likewise, for springs in the backarc (E and F), the stress changed very little when subsampling for $\geq 1\%$ abundance. The ANOSIM statistics are generally consistent across all subsampling.

Table A4. Statistical summary for NMDS and ANOSIM. Classification: A - chemistry groups based on PCA; B - chemistry groups based on Piper diagram; C - temperature groups based on microbial temperature preference (i.e. mesophile); D - temperature groups based on 15 °C increments; E - tectonic setting groups (i.e. FSS and BAS) with Sp. 9 separate; F - tectonic setting groups FSS and BAS.

Ordination	NMDS stress	ANOSIM		
		Classification	P value	R statistic
All OTUs	0.14	A	0.001	0.597
		B	0.343	0.039
		C	0.001	0.488
		D	0.001	0.386
		E	0.001	0.543
0.1% Abundant OTUs	0.14	A	0.001	0.584
		C	0.001	0.476
		E	0.001	0.533
1% Abundant OTUs	0.14	A	0.001	0.563
		C	0.001	0.468
		E	0.001	0.525
Planktonic Sample OTUs	0.09	A	0.002	0.510
		B	0.662	-0.094
		C	0.004	0.329
		D	0.054	0.229
		E	0.001	0.519
		F	0.001	0.483
Sediment Sample OTUs	0.05	A	0.001	0.694
		B	0.493	-0.017
		C	0.001	0.561
		D	0.003	0.402
		E	0.001	0.544
		F	0.003	0.534
All OTUs excluding Spring 4	0.13	A	0.001	0.268
		C	0.001	0.538
		E	0.001	0.574
1% Abundant OTUs excluding Spring 4	0.14	A	0.005	0.237
		C	0.001	0.516
		E	0.003	0.388
		F	0.001	0.553
1% Abundant OTUs for Springs 1-5	0.04	-	-	-
1% Abundant OTUs for Springs 1-3, 5	0.02	-	-	-
All OTUs for Springs 6-14	0.11	C	0.004	0.532
1% Abundant OTUs for Springs 6-14	0.12	C	0.001	0.533

Appendix D. R code

```

#non-metric multidimensional scaling (NMDS)
#load and prep the matrix
count2 <- read.delim("~FileOrigin")
count <- as.data.frame(count2)
countnames <- count[,-1]
rownames(countnames) <- count[,1]
count <- countnames
rm(countnames)

#calc NMDS, plot Shepard Plot, dim, and NMDS ~ from the tutorial (vegantutor)
count.dis <- vegdist(count)
count.mds0 <- isoMDS(count.dis)
count.mds <- metaMDS(count,trace=FALSE,k=3)
count.mds

#add environmental factors ~ from the tutorial (vegantutor)
chem <- read.csv("~FileOrigin")
ef <- envfit(count.mds,chem,permu=999) #strata=chem[-c(25)]
plot(count.mds,display="sites")
plot(ef,p.max=0.1)
with(count.mds,text(count.mds,labels=rownames(count),pos=3,cex=0.3))

#add ordi hulls (polygons) around the seperate groups
treatments0 <- read.csv("~FileOrigin")
treatments <- as.data.frame(treatments0)
treatments_rnames <- treatments[,-1]
rownames(treatments_rnames) <- treatments[,1]
treatments <- treatments_rnames
rm(treatments_rnames)

plot(count.mds,display="sites",type="p")
with(count.mds,text(count.mds,labels=rownames(count),pos=3,cex=0.3))
with(count.mds,mtext("All, thermos",side=3))
with(treatments,ordihull(count.mds,Thermos,col="blue",lty=2))

#analysis of similarities (ANOSIM) in vegan package to determine grouped significance
count.ano <- with(treatments,anosim(count.dis,Thermos))
summary(count.ano)
plot(count.ano)
with(count.ano,mtext("thermos",side=4))

#principle component analysis
chemPCA <- read.csv("~FileOrigin")
pca_chem <- prcomp(chemPCA,center=TRUE,scale.=TRUE)
ggbiplot(pca_chem,labels=rownames(chem4))
summary(pca_chem)
str(pca_chem)

```