

1 **Metabolic potential and survival strategies of microbial communities across**
2 **extreme temperature gradients on Deception Island volcano, Antarctica**

3
4 Amanda Gonçalves Bendia^{1*}, Leandro Nascimento Lemos², Lucas William Mendes², Camila
5 Negrão Signori¹, Brendan J. M. Bohannan³, Vivian Helena Pellizari¹

6
7 ¹Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São Paulo
8 (USP). Praça do Oceanográfico, 191. CEP: 05508-900 São Paulo, SP, Brazil.

9 ²Laboratório de Biologia Celular e Molecular, Centro de Energia Nuclear na Agricultura,
10 Universidade de São Paulo. Avenida Centenário 303, CEP 13416-00 Piracicaba, SP, Brazil.

11 ³Department of Biology, Institute of Ecology and Evolution, University of Oregon, Eugene, OR,
12 United States

13
14 *Corresponding author:

15 Amanda Gonçalves Bendia

16 Address: Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São
17 Paulo, São Paulo, Brazil. Praça do Oceanográfico, 191. CEP: 05508-900 São Paulo, SP, Brazil.

18 Email: amandagb@usp.br

19 Phone number: +55 (11) 3091-6557

20
21 **Running Title:** Community survival strategies on Deception Island

22
23
24
25
26
27
28
29
30
31

32 **Abstract**

33 Active volcanoes in Antarctica, in contrast to the rest of the icy landscape, have remarkable
34 temperature and geochemical gradients that could select for a wide variety of microbial adaptive
35 mechanisms and metabolic pathways. Deception Island is a stratovolcano flooded by the sea,
36 resulting in contrasting ecosystems such as permanent glaciers (<0 °C) and active fumaroles (up
37 to 100 °C). Steep gradients in temperature, salinity and geochemistry over very short distances
38 have been reported for Deception Island, and have been shown to effect microbial community
39 structure and diversity. However, little is known regarding how these gradients affect ecosystem
40 functioning, for example due to inhibition of key metabolic enzymes or pathways. In this study,
41 we used shotgun metagenomics and metagenome-assembled genomes to explore how microbial
42 functional diversity is shaped by extreme geochemical, salinity and temperature gradients in
43 fumarole and glacier sediments. We observed that microbial communities from a 98 °C fumarole
44 harbor specific hyperthermophilic molecular strategies, as well as reductive and autotrophic
45 pathways, while those from <80 °C fumaroles possess more diverse metabolic and survival
46 strategies capable of responding to fluctuating redox and temperature conditions. In contrast,
47 glacier communities showed less diverse metabolic potentials, comprising mainly heterotrophic
48 and carbon pathways. Through the reconstruction of genomes, we were able to clarify putative
49 novel lifestyles of underrepresented taxonomic groups, especially those related to Nanoarchaeota
50 and thermophilic ammonia-oxidizing archaeal lineages. Our results enhance understanding of the
51 metabolic and survival capabilities of different extremophilic lineages of Bacteria and Archaea.

52

53 **Key words:** Deception Island, Antarctica, Microbial ecology, Microbial processes, Metagenome-
54 assembled genomes, Extremophiles

55 **Introduction**

56 The study of life in extreme environments has long fascinated biologists. Understanding how life
57 persists at environmental extremes provides insight into how living systems function, as well as
58 providing a unique window into the evolutionary history of life itself (Merino et al., 2019). The
59 Deception Island volcano contains a unique combination of extreme temperatures and geochemical
60 energy sources that together have the potential for selecting a wide variety of microbial adaptive
61 mechanisms and metabolic pathways. Deception Island is located in the South Shetland Islands at
62 the spreading center of the Bransfield Strait marginal basin, which harbors contrasting ecosystems
63 of permanent glaciers and active fumaroles with continuous emissions of gases, mostly carbon
64 dioxide and hydrogen sulfide (Somoza et al., 2004). This combination of glaciers and fumaroles
65 is produced by the interaction between the cryosphere and water mass contact with hot ascending
66 magmas (Geyer et al., 2019). Unlike Antarctic continental volcanoes, Deception Island fumaroles
67 reach up to 100 °C and have direct marine influence, creating a remarkable combination of thermal,
68 geochemical and salinity gradients (Bartolini et al., 2014; Herbold et al., 2014; Muñoz-Martín et
69 al., 2005).

70

71 While early research carried out on Deception focused primarily on obtaining bacterial isolates
72 from hot or cold ecosystems (e.g. Carrión et al., 2011; Llarch et al., 1997; Stanley et al., 1967), a
73 more recent study was able to recover both psychrophilic and thermophilic isolates among the
74 steep temperature gradients (Bendia et al., 2018a). Previous molecular studies described microbial
75 diversity on Deception fumaroles using DGGE (Amenábar et al., 2013; Muñoz et al., 2011) and
76 shotgun metagenomics to characterize the resistome profiles in cold sediments from Whalers Bay

77 (Centurion et al., 2019). These previous studies were limited with respect to sampling depth and
78 extent since only fumaroles or cold sediments were analyzed.

79

80 Two previous studies have focused on understanding the effect of Deception temperature gradients
81 on microbial communities. The first, performed by our group, focused on determining taxonomic
82 diversity through 16S rRNA gene sequencing (Bendia et al., 2018b), and a second study applied
83 the Life Detector Chip (LDChip) to describe general functions of communities from Cerro Caliente
84 (Lezcano et al., 2019). Our previous study showed that the steep gradients on Deception were able
85 to select a unique combination of taxonomic groups found in deep and shallow hydrothermal vents
86 (including hyperthermophilic Archaea, such as *Pyrodictium spp.*), geothermal systems and those
87 typical from polar ecosystems (Bendia et al., 2018b). Also we reported that the bacterial
88 community structure on Deception Island is strongly niche driven by a variety of environmental
89 parameters (temperature, pH, salinity and volcanic geochemicals, such as sulfate), while archaeal
90 diversity is mainly shaped by temperature (Bendia et al., 2018b). These previous studies, however,
91 did not address the linkages between microbial structure and their adaptive and metabolic
92 strategies over the particular environmental gradients found on Deception Island.

93

94 Although several studies have demonstrated that temperature is a primary driver of microbial
95 taxonomic diversity in different geothermal and hydrothermal ecosystems (e.g. Antranikian et al.,
96 2017; Price and Giovannelli, 2017; Sharp et al., 2014; Ward et al., 2017), it is still unclear to what
97 extent temperature affects the functional processes of a microbial community, such as their
98 adaptive mechanisms and metabolic pathways. The majority of these previous studies have
99 focused on nonpolar thermal ecosystems, where the temperature range is narrower than in polar

100 volcanoes; the exception is a study of deep-sea hydrothermal vents, in which the contrasting
101 temperatures are created by the contact of heat with the surrounding cold seawater (with
102 temperatures 0-4 °C). Indeed, the Deception communities from fumaroles have similar members
103 (Bendia et al., 2018b) to those found in deep-sea hydrothermal vents (e.g. Dick, 2019; Nakagawa
104 et al., 2006; Takai et al., 2001), which suggests that, regarding differences in pressure (and other
105 environmental effects), the wide temperature range typical of polar volcanoes and deep-sea
106 hydrothermal vents can act as a strong selective pressure that favors (hyper)thermophilic
107 specialists capable of thriving in high temperatures but that can also tolerate the cold surroundings.

108

109 Furthermore, there is controversy about the impact of extreme environments on microbe-microbe
110 interactions. Although some studies have reported a decrease in the frequency of microbe-microbe
111 interactions inferred from co-occurrence patterns (Cole et al., 2013; Merino et al., 2019; Sharp et
112 al., 2014), others have observed the opposite trend (Lin et al., 2016; Mandakovic et al., 2018). The
113 analysis of co-occurrence patterns is useful for examining the nature of the ecological
114 rearrangements that take place in a microbial community facing contrasting environments (Freilich
115 et al., 2010; Mandakovic et al., 2018).

116

117 In the current study, we assessed the microbial functional profile in fumarole and glacier sediments
118 from two geothermal sites on the Deception Island volcano, Antarctica. For this, we performed
119 shotgun metagenomics to unveil functional diversity and reconstructed genomes to reveal the
120 microbes' putative lifestyles and survival capabilities, combining the genomic information with
121 community functional profiles. Here, we hypothesize that (i) similar to what has been reported for
122 community diversity, survival and metabolic strategies are also influenced by the combination of

123 geochemical, salinity and extreme temperature gradients; (ii) these communities follow the
124 redundancy of metabolic potential that was reported for deep-sea hydrothermal vent communities;
125 and (iii) microbe-microbe interactions decrease with increasing temperature. This study adds
126 important information regarding the ecological processes of microbial communities inhabiting a
127 steep gradient of temperature, and addresses central questions regarding the functional adaptability
128 of extremophiles in polar regions.

129

130

131 **Materials and methods**

132 **Study site and sampling strategy**

133 Deception Island (62°58' S, 60°39' W) is a complex stratovolcano located in the South Shetland
134 Islands, Bransfield Strait, near the Antarctic Peninsula. A past eruption occurring approximately
135 10,000 years ago collapsed the central part of the island giving rise to a flooded caldera called Port
136 Foster Bay, 9 km in diameter (Baker et al., 1975). Fumaroles are found mainly at Fumarole Bay
137 (FB), Whalers Bay (WB), and Pendulum Cove (Fermani et al., 2007; Geyer et al., 2019), and they
138 are distributed mostly in submerged and partially submerged regions (intertidal zones), with
139 temperatures varying from 40-60°C in WB and 80-100°C in FB (Rey et al., 1995; Somoza et al.,
140 2004). Carbon dioxide and hydrogen sulfide gases are emitted by fumaroles and are oxidized to
141 products such as sulfite and sulfate (Somoza et al., 2004; Zhang and Millero, 1993).

142

143 Sampling was performed during the XXXII Brazilian Antarctic Expedition (December 2013 to
144 January 2014), with logistical support from the polar vessel NPo. Almirante Maximiano. We
145 collected surface sediment samples (*ca.* 5 cm) in fumaroles and glaciers at geothermally active

146 sites in FB (62°58'02.7" S, 60°42' 36.4" W) and WB (62°58'45.1" S, 60°33'27.3" W) (Figure 1a
147 and 1b), with temperatures between 0 and 98 °C. At each site, we obtained samples from three
148 different points within the temperature gradient, and triplicates were performed for each collected
149 point, totaling 18 sediment samples. Points A and B were defined as samples collected in
150 fumaroles, while point C samples were collected from the glacier, few cm below the glacier's edge
151 (Figure 1c and 1d). The point FBA was the hottest fumarole, measuring 98 °C at the sediment
152 surface (*ca.* 20 cm). Distances between fumaroles and glaciers at each site were approximately 15
153 m, and the WB and FB transects were approximately 10 km apart. All fumaroles were in the
154 intertidal zone, except for point B from FB, which was in the subtidal zone (submerged at 50 cm
155 depth in the water column). Samples were stored at -20 °C until arrival at the University of São
156 Paulo, Brazil in April 2014.

157

158 **DNA Extraction and metagenomic sequencing**

159 To compare our results with the study performed by Bendia et al. (2018b), we used the same
160 dataset and DNA extractions as a template for the metagenomics. Due to low DNA mass even
161 after several concentration efforts, extracted DNA was subjected to multiple displacement
162 amplification (MDA) using the illustra GenomiPhi V2 DNA amplification kit (GE Healthcare,
163 Piscataway, NJ, USA), following the manufacturer's instructions. Three amplification reactions
164 per sample were pooled to obtain sufficient DNA for sequencing. DNA was then purified using
165 AMPure XP beads kit (Beckman Coulter) following the manufacturer's instructions. Library
166 constructions and shotgun metagenomic sequencing were conducted at "Laboratório Central de
167 Tecnologias de Alto Desempenho em Ciências da Vida" (LaCTAD), Universidade Estadual de

168 Campinas State (UNICAMP), on the Illumina Hiseq 2000 platform using 2x100 bp paired-end
169 system.

170

171 **Physical-chemical parameters**

172 To correlate biological and environmental data, we used the physical-chemical parameters for
173 sediments measured by Bendia et al. (2018b), which included granulometry, electrical
174 conductivity, humidity, micronutrients (B, Cu, Fe, Mn, and Zn), organic matter, organic carbon,
175 pH, P, Si, Na, K, Ca, Mg, Al, total nitrogen, nitrate, ammonia, and sulfate.

176

177 **Taxonomic and functional inference of metagenomic reads**

178 Reads were quality trimmed using Sickle (Joshi and Fass, 2011) with phred >30 and then uploaded
179 to MG-RAST (Keegan et al., 2016). Functional and taxonomic profiles of reads were generated
180 through subsystem and best hit classifications using the SEED subsystem, M5NR (non-redundant
181 protein database) and KEGG, available in MG-RAST (Aziz et al., 2008; Kanehisa and Goto, 2000;
182 Keegan et al., 2016; Wilke et al., 2012), with the following parameters: 1×10^{-5} e-value, minimum
183 50 bp alignment, and 60% identity. Data generated by MG-RAST were statistically analyzed using
184 Statistical Analysis of Metagenomic Profiles (STAMP) software (Parks et al., 2014) and R
185 software (R Development Core Team), using the packages *vegan* (Oksanen, 2007) and *ggplot*
186 (Wickham, 2011). The *p* values were calculated using Fisher's exact two-sided test and the
187 confidence intervals were calculated using the method of Newcombe-Wilson. Statistical
188 comparisons were performed by grouping the samples according to environmental temperatures:
189 glaciers, fumaroles up to 80 °C and fumarole at 98 °C. Principal component analysis (PCA)
190 ordination was performed by using level 3 functions of SEED subsystems and then visualized in

191 STAMP software. Values were normalized to relative abundance for comparison of taxonomic
192 composition across samples. In addition, Spearman correlations were performed to determine
193 relationships between taxonomic and functional profiles and the environmental parameters.
194 Genetic data are available in MG-RAST under the project ID mgp15628. MG-RAST IDs for each
195 sample are described in Supplementary Table 2.

196
197 To investigate the complexity of community interactions at each sampling site, we used co-
198 occurrence network analysis. For this, non-random co-occurrence analyses were performed using
199 the Python module ‘SparCC’ (Friedman and Alm, 2012). A table of frequency of hits affiliated to
200 the genus level was used for analysis. For each network, we considered only strong (SparCC > 0.9
201 or < -0.9) and highly significant ($p < 0.01$) correlations between microbial taxa. The nodes in the
202 reconstructed network represent taxa at the genus level, whereas the edges represent significantly
203 positive or negative correlation between nodes. The analysis of network complexity was based on
204 a set of measures, such as the number of nodes and edges, modularity, the number of communities,
205 average node connectivity, average path length, diameter, and cumulative degree distribution
206 (Newman, 2003). Network visualization and property measurements were calculated with the
207 software Gephi (Bastian et al., 2009).

208

209

210 **Metagenomic assembly and genome reconstruction**

211 We used two different strategies for metagenomic assembly and genomic binning of the eighteen
212 metagenomic datasets from Deception Island volcano. First, reads were assembled using IDBA-
213 ud (Peng et al., 2012) (-mink 50, -maxk 92, -tep 4, -min_contig 1000) and then genomic binning
214 was performed through MaxBin 2.0 (Wu et al., 2016). Contigs were annotated using the Integrated

215 Microbial Genomes & Microbiomes (IMG/M) system (Markowitz et al., 2009) and archived on
216 the JGI/IMG server under Project ID Gs0141992. IMG accession numbers for each sample are
217 described in Supplementary Table 2.

218

219 Furthermore, reads were co-assembled using MEGAHIT v. 1.0.2. (Li et al., 2015), discarding
220 contigs smaller than 1000 bp. Then contigs were binned using anvi'o v. 5 following the workflow
221 described by Eren et al. (2015). Reads for each metagenome were mapped to the co-assembly
222 using bowtie2 with default parameters (Langmead and Salzberg, 2012). A contig database was
223 generated using the 'anvi-gen-contigs-database'. Prodigal (Hyatt et al., 2010) was used to predict
224 open reading frames (ORFs). Single-copy bacterial and archaeal genes were identified using
225 HMMER v. 3.1b2 (Finn et al., 2011). The program 'anvi-run-ncbi-cogs' was used to annotate
226 genes with functions by searching for them against the December 2014 release of the Clusters of
227 Orthologous Groups (COGs) database (Galperin et al., 2015) using blastp v2.10.0+ (Altschul et
228 al., 1990). Predicted protein sequences were functionally and taxonomically annotated against
229 KEGG with GhostKOALA (genus_prokaryotes) (Kanehisa et al., 2016). Individual BAM files
230 were profiled using the program 'anvi-profile' with a minimum contig length of 4 kbp. Genome
231 binning was performed using CONCOCT (Alneberg et al., 2013) through the 'anvi-merge'
232 program with default parameters. We used 'anvi-interactive' to visualize the merged data and
233 identify genome bins. Bins were then manually refined using 'anvi-refine', and completeness and
234 contamination were estimated using 'anvi-summarize'.

235

236 Bins generated by the assembly and co-assembly approaches were quality checked through
237 CheckM v. 1.0.7 (Parks et al., 2015), which is based on the representation of lineage-specific

238 marker gene sets. Bins were taxonomically classified based on genome phylogeny using GTDB-
239 Tk (Chaumeil et al., 2020).

240

241 **Taxonomic and functional annotation of metagenome-assembled genomes (MAGs)**

242 Bins were defined as a high-quality draft (>90% complete, <5% contamination), medium-quality
243 draft (>50% complete, <10% contamination) or low-quality draft (<50% complete, <10%
244 contamination) metagenome assembled-genome (MAG), according to genome quality standards
245 suggested by Bowers et al. (2017). We selected 11 MAGs based on their medium or high-quality
246 and taxonomy, preferably selecting groups related to extremophiles or associated to sulfur and
247 nitrogen metabolisms. Annotation of all predicted ORFs in MAGs was performed using prokka
248 v.14.5 (Seemann, 2014). Further, proteins were compared to sequences in the KEGG Database
249 through GhostKOALA (genus_prokaryotes) (Kanehisa et al., 2016) and in the SEED Subsystem
250 through RASTtk (Brettin et al., 2015). Phenotypes were predicted using the PICA framework
251 (Feldbauer et al., 2015) and PhenDB (<https://phendb.csb.univie.ac.at/>).

252

253 **Results**

254 To investigate links between metabolic potential and genes associated with survival strategies
255 across extreme temperature and geochemical gradients of the Deception Island volcano, we
256 analyzed the metagenomes of a total of eighteen samples, comprising fumaroles with temperatures
257 of 98 °C, 80 °C, 50 °C, and 10 °C, and glaciers with temperatures around 0 °C. Shotgun sequencing
258 of community genomic DNA on 3 lanes of Illumina HiSeq2000 produced a total of 567,410,264
259 paired-end reads, within which 475,895,996 were filtered by quality (Q>30) for further analyses.
260 A total of 162,755,88 reads were taxonomically annotated as Bacteria, 3,680,020 as Archaea,

261 2,094,916 as Eukarya and 79,111 as viruses (Supplementary Table 2). The total number of proteins
262 predicted in reads were 296,818,692 (62.3%). Relative abundances of the detected genes were
263 used to compare the metabolic potential and genes related to survival strategies under
264 environmental extremes among fumaroles and glaciers samples.

265
266 *De novo* assemblies of the quality-filtered reads generated a total of 543,945 contigs. The
267 prediction of ORFs resulted in 1,396,820 putative genes, 353,731 assigned within Bacteria, 12,034
268 within Archaea, and 1,557 and 1,534 within Eukarya and viruses, respectively. We used different
269 databases for assembly annotation through JGI/IMG that resulted in 487 putative 16S rRNA genes,
270 842,798 genes based on the COG database and 304,173 genes based on KEGG (Supplementary
271 Table 2).

272

273 **Taxonomic profile of microbial communities on the Deception Island volcano**

274 Through the annotation of reads, we observed that the taxonomic composition in the 98 °C
275 fumarole was distinct in comparison with other fumaroles and glaciers. Archaea were dominant in
276 samples from the 98 °C fumarole (relative abundance between 31.5 and 87.3%), with the most
277 abundant archaeal phyla classified as Crenarchaeota (23.8-79.3%), followed by Euryarchaeota
278 (2.5-7.5%) and Korarchaeota (0.1-0.4%). Firmicutes (3.1-22.4%), Bacteroidetes (0.6-15.3%),
279 Aquificae (0.3-4.6%), and Thermotogae (0.3-1.0%) were also detected in minor proportions in the
280 98 °C fumarole. Looking at the class level, Thermoprotei, Thermococci, Methanococci,
281 Archaeoglobi, Methanobacteria, Methanopyri, and Methanomicrobia represented the most
282 abundant archaeal classes (>0.1%) in the 98 °C site, and Bacilli, Gammaproteobacteria,

283 Betaproteobacteria, Fusobacteria, Flavobacteria, Aquificae (order Aquificales) and Thermotogae
284 (order Thermotogales) were the dominant classes within Bacteria (Figure 2a).

285
286 Archaea were less dominant in the other samples, with a relative abundance of 0.7-2% in fumaroles
287 <80 °C and 0.4-0.6% in glaciers. Although some dominant phyla were common between <80 °C
288 fumaroles and glaciers (e.g. Bacteroidetes, Proteobacteria, and Firmicutes), less dominant phyla
289 were uniquely distributed according to temperature. For example, Thaumarchaeota was
290 predominantly found in <80 °C fumaroles (0.8-1% for Whalers Bay and 0.2-0.3% in Fumarole
291 Bay). Verrucomicrobia and Acidobacteria were only detected in glaciers (1.2-3.1% and 1-1.6%,
292 respectively) (Figure 2a). The main classes affiliated within the Bacteroidetes phylum were
293 Cytophagia, Flavobacteria and Sphingobacteria, whereas Gamma- and Alphaproteobacteria were
294 the most represented classes within Proteobacteria, followed by Beta-, Delta- and Epsilonbacteria
295 (Figure 2b). Solibacteres was the abundant class within Acidobacteria, and Verrucumicrobiaea
296 within Verrucomicrobia. Thaumarchaeota assignments were not classified at the class level using
297 reads annotation in MG-RAST. The taxonomic annotation of contigs through the IMG/M system
298 showed similar patterns when compared to reads annotation (Supplementary Figure 1).

299
300 We then used co-occurrence network analysis to explore the complexity of interactions within the
301 microbial communities in each treatment (Figure 2c). For this, we calculated SparCC correlations
302 between microbial taxa at the genus level based on metagenome reads annotated in MG-RAST. In
303 general, the complexity of the community increased with the temperature. We also noted that
304 communities of Fumarole Bay were more complex than Whalers Bay. The FBA (98 °C) site
305 showed the highest level of complexity and a modular structure, whereas the WBC (0 °C) site had

306 the least complex network. Interestingly, the proportion of positive/negative correlations also
307 changed according to the temperature; at higher temperatures, the proportion is even, while in
308 lower temperatures there was an increase in the number of positive correlations.

309

310 **Comparative functional profile of microbial communities on Deception Island volcano**

311 Functional profiles of metagenomes were compared using a multivariate method and hypothesis
312 test, and significant variations in all functional levels were observed between the 98 °C fumarole,
313 <80 °C fumaroles and glaciers. Clear differences between these three distinct sample groups were
314 observed through both the SEED Level 1 profile (Figure 3a) and SEED functional level through
315 PCA ordination (Figure 3b). Further, a distinct pattern between samples from the highest
316 temperature was observed. The prevalent core of functions among Deception samples were
317 “clustering-based subsystems”, “carbohydrates”, “amino acids and derivatives”, “protein
318 metabolism”, “RNA metabolism”, “DNA metabolism” and “cofactors, vitamins, prosthetic groups
319 and pigments”. Significant differences between sample groups and functions from level 1 of
320 SEED, calculated using Fisher's exact two-sided test and the Newcombe-Wilson method, showed
321 the highest abundance of genes belonging to the categories “DNA metabolism” ($p = 0.046$),
322 “protein metabolism” ($p = 0.049$), and “phages, prophages, transposable elements and plasmids”
323 ($p = 3.97e-3$) in the 98 °C fumarole in comparison with other fumaroles and glaciers. The
324 categories “nitrogen metabolism” ($p = 1.07e-4$), “photosynthesis” ($p = 5.07e-3$), “sulfur
325 metabolism” ($p = 0.015$), and “metabolism of aromatic compounds” ($p = 0.036$) exhibited the
326 highest significant values in <80 °C fumaroles when compared to the 98 °C fumarole, and “motility
327 and chemotaxis” ($p = 2.71e-8$), “RNA metabolism” ($p = 0.01$), and “protein metabolism” ($p =$
328 0.023) when compared to glaciers. In contrast, observations for glaciers showed more genes

329 associated with “carbohydrates” and “virulence, disease and defense” categories in comparison
330 with the 98 °C fumarole ($p = 0.049$ and $p = 0.036$, respectively) and <80 °C fumaroles ($p = 1.68e-$
331 6 and $p = 0.012$, respectively) (Figure 3c).

332

333 **Patterns of metabolic partitioning among extreme temperatures**

334 We observed different partitioning patterns of metabolic diversity according to environmental
335 temperatures (Figure 4). The fumarole with the highest temperature (98 °C) exhibited metabolic
336 potential significantly higher for functions associated with sulfate reduction ($p < 0.001$),
337 dissimilatory nitrite reduction ($p < 0.001$) and carbon dioxide fixation ($p < 0.001$) when compared
338 to other fumaroles and glaciers. Although sulfur metabolism was abundant among all
339 temperatures, different metabolic pathways related to sulfur were observed according to the
340 temperature. While sulfate reduction was prevalent in the highest temperature fumarole, a high
341 number of genes related to inorganic sulfur assimilation ($p < 0.001$) and sulfur oxidation (p value
342 was not significant, $p < 0.1$) were detected in <80 °C fumaroles. In general, nitrogen metabolism
343 was dominant in <80 °C fumaroles when compared to other samples, with nitrate and nitrite
344 ammonification ($p < 0.02$), denitrification ($p < 0.01$), nitrogen fixation ($p < 0.05$) and ammonia
345 assimilation ($p < 0.001$) as the prevalent metabolic nitrogen pathways. All fumaroles showed a
346 similar abundance of genes belonging to sulfur oxidation, nitrate and nitrite ammonification, and
347 dissimilatory nitrite reduction. The genetic potential for carbon fixation was much higher in the 98
348 °C fumarole ($p < 0.01$), whereas photosynthesis was mainly detected in the <80 °C fumaroles and
349 glaciers. In glaciers, the genes identified within carbon metabolism were mainly associated with
350 heterotrophy and central carbon pathways, such as the pentose phosphate pathway and glycolysis,
351 as were respiration and fermentation. The function of carbon storage regulators was significantly

352 higher in <80 °C fumaroles, in addition to the observation of other carbon-related processes, such
353 as photosynthesis, fermentation, and carbon fixation (Figure 4).

354

355 **Community survival strategies under environmental extremes**

356 To understand community survival strategies under extreme temperature and geochemical
357 gradients, we selected genes in our metagenomes that are involved with stress response, DNA
358 repair, protein biosynthesis, and transport and chemotaxis. Although communities from all
359 samples were equally abundant in genes related to stress response, very distinct patterns of specific
360 responses were observed accordingly environmental temperature (Figure 4). The oxidative stress
361 response was markedly higher in the fumarole at 98 °C, mainly represented by glutaredoxins,
362 glutathione (redox cycle) and rubrerythrin functions (all with $p < 0.001$ in comparison with other
363 samples). Contrastingly, osmotic stress genes were prevalent in glaciers samples, represented
364 mainly by functions such as osmoregulation ($p < 0.001$), osmoprotectant (*yehX*) ($p = 0.01$),
365 aquaporin Z ($p = 0.05$) and synthesis of osmoregulated periplasmatic glucans ($p < 0.001$). The
366 abundance of genes related to heat and cold responses (thermal response) was distinctly distributed
367 among fumaroles and glaciers. General function of heat-shock proteins (including *hsp70/dnaK*)
368 were prevalent in glaciers and <80 °C fumaroles ($p < 0.001$), whereas specific archaeal thermal
369 responses dominated the 98 °C fumarole, such as thermosome chaperonin ($p < 0.001$, 0.7% of total
370 relative abundance), as were bacterial and archaeal heat-shocks *groEL/groES* ($p < 0.001$). The
371 relative abundance of cold shock *cspA* was higher in <80 °C fumaroles, followed by glaciers ($p <$
372 0.001). Glaciers and <80 °C fumaroles exhibited the highest abundance of dormancy and
373 sporulation function ($p < 0.01$) and all fumaroles had a prevalence of the universal stress protein
374 family ($p < 0.01$) (Figure 4).

375

376 Differences in abundance patterns of DNA repair, protein biosynthesis, transport and chemotaxis
377 were also observed across environmental temperatures (Supplementary Figure 2). Base excision
378 repair, recombination through *recU* and reverse gyrase (all with $p < 0.01$) were the main strategies
379 of DNA positive supercoiling and repair notably found in communities of the highest temperature
380 fumarole (98 °C). Strategies of DNA repair using *uvrABC* complex, recombination through *recA*
381 and photolyase were dominant in <80 °C fumaroles and glaciers (all with $p < 0.001$). Protein
382 biosynthesis genes were dominant in the highest temperature fumarole (98 °C); functions such as
383 universal GTPases ($p < 0.01$) and translation elongation factors in Archaea ($p < 0.001$) were
384 significantly higher when compared to the other samples. Chemotaxis genes were also prevalent
385 in the highest temperature fumarole (98 °C) ($p < 0.001$), as were several transport systems
386 (transport of Ni, Co, and Zn) and ABC transporters (e.g. branched-chain amino acid, oligopeptide
387 and tungstate) (all with at least $p < 0.05$). Mn transport and the ABC transporters of iron and
388 peptides were significantly higher in <80 °C fumaroles (all with at least $p < 0.05$) (Supplementary
389 Figure 2).

390

391 **Physical-chemical influence on taxonomic and functional diversity**

392 To identify key environmental drivers of community taxonomy (at phylum level) and function
393 (SEED level 1), Spearman correlations were calculated; then only significant ($p < 0.05$) and strong
394 correlations ($r > -0.6$ or 0.6) were considered. In general, the phyla that positively correlated with
395 temperature were Euryarchaeota, Crenarchaeota, Korarchaeota, Nanoarchaeota, Thermotogae, and
396 Aquificae, whereas several phyla were negatively correlated with temperature (e.g. Acidobacteria,
397 Bacteroidetes, Spirochaetes, Actinobacteria, Verrucumicrobia, Nitrospirae, Deinococcus-

398 Thermus, and Gemmatimonadetes, among others) (Figure 5a). The phyla that negatively correlated
399 with ammonia were Proteobacteria, Thaumarchaeota, Euryarchaeota, and Deferribacteres; those
400 positively correlated were Firmicutes, Acidobacteria, Cyanobacteria, and Spirochaetes, among
401 others. All significant nitrate correlations were positive, including phyla such as Firmicutes,
402 Nitrospirae, Thermotogae, etc. Sulfate showed significant positive correlations with
403 Proteobacteria, Thaumarchaeota, Euryarchaeota, Deferribacteres and Crenarchaeota, and negative
404 correlations with phyla such as Acidobacteria, Cyanobacteria, Actinobacteria, and
405 Verrucomicrobia, among others. Other parameters exhibited positive and negative correlations
406 with several phyla, such as organic matter, organic carbon, B, Cu (uniquely negative correlations),
407 Fe (uniquely negative correlations), Na, K, Ca, Mg and Al (Figure 5a, Supplementary Table 3).

408
409 The functional categories (SEED level 1) which presented positive correlations with temperature
410 were “DNA metabolism”, “nucleosides and nucleotides” and “RNA metabolism”, and those which
411 exhibited negative correlations were “fatty acids, lipids and isoprenoids”, “iron acquisition”,
412 “metabolism of aromatic compounds”, “phosphorus metabolism”, “photosynthesis”, “secondary
413 metabolism”, “stress response”, and “sulfur metabolism” (Figure 5b). Ammonia was negatively
414 correlated with functions such as “carbohydrates”, “motility and chemotaxis”, “respiration” and
415 “RNA metabolism”, whereas positive correlations comprised functions as “amino acids and
416 derivatives” and “cofactors, vitamins, prosthetic groups and pigments”. Nitrate also presented
417 negative correlations with “carbohydrates”, “dormancy and sporulation” and “phages, prophages,
418 transposable elements and plasmids”. In contrast, sulfate was positively correlated with
419 “carbohydrates”, “DNA metabolism”, “motility and chemotaxis”, “respiration” and “RNA
420 metabolism”. Other parameters exhibited positive and negative correlations with several functions,

421 such as organic matter, organic carbon, B, Cu, Fe, Si, Na, K, Ca, Mg and Al (Figure 5b,
422 Supplementary Table 3).

423

424 **Metabolic potential and survival strategies in MAGs**

425 In general, the anvi'o pipeline using co-assembly showed the best binning results for our eighteen
426 metagenomes, generating a total of 158 MAGs. We included in our analyses only 1 MAG produced
427 through the idba-ud assembler and MaxBin binning since this MAG belonged to a taxon
428 (*Calditrichia*) which was not achieved through the anvi'o pipeline (Supplementary Figure 3,
429 Supplementary Table 4). From the 159 MAGs, 12 were assigned as Archaea through GTDB-Tk
430 and GhostKoala, belonging to Nitrososphaerales (*Candidatus Nitrosocaldus* according with
431 GhostKoala taxonomy) (2), *Nitrosoarchaeum* (1), *Nitrosotenus* (1), *Nitrospumilus* (1),
432 Desulfurococcales (*Aeropyrum* according with GhostKoala taxonomy) (1), Acidilobaceae (1),
433 Pyrodictiaceae (2) and Woesearchaeia (Nanoarchaeota) (3). The bacterial MAGs were classified
434 through GTDB-Tk and GhostKoala as the following phyla: Acidobacteriota (1), Aquificota (2),
435 Bacteroidota (92), Calditrichota (5), Campylobacterota (1), Chloroflexota (3), Cyanobacteriota
436 (1), Firmicutes (1), Nitrospirota (2), Patescibacteria (4) and Proteobacteria (35) (Supplementary
437 Figure 4, Supplementary Table 4). A total of 13 MAGs were considered as high quality and 82 as
438 medium quality drafts.

439

440 The MAGs were selected for functional annotation by their quality and based on groups related to
441 extremophiles and associated to sulfur and nitrogen metabolisms. These 11 selected MAGs were
442 assigned as DI_MAG_00003 (*Sulfurimonas*), DI_MAG_00004
443 (*Hydrogenothermaceae/Persephonella*), DI_MAG_00006 (*Promineofilaceae/Candidatus*

444 *Promineofilum*), DI_MAG_00010 (Caldilineaceae/*Caldilinea*), DI_MAG_00011
445 (Thermonemataceae), DI_MAG_00019 (Chitinophagaceae), DI_MAG_00020
446 (Pyrodictiaceae/*Pyrodictium*), DI_MAG_00021 (Dojkabacteria), DI_MAG_00022
447 (Woesearchaeia/archaeon GW2011_AR20), DI_MAG_00049 (Nitrososphaerales/*Candidatus*
448 *Nitrosocaldus*) and DI_MAG_FBB2_12 (Calditrichia) (Table 1).

449

450 We identified in the high-quality DI_MAG_00004 (Hydrogenothermaceae/*Persephonella*, ~ 97%
451 completeness) genes for nitrate reduction (*narGHI* and *nirA*), denitrification (*narGHI*),
452 nitrification (*narGH*), sulfate reduction (*sat*, *cysH*, *sir*), sulfur and thiosulfate oxidation
453 (*soxAXBYZ*), and incomplete pathways for carbon fixation (Figure 6a). This MAG had several
454 genes associated with stress response, especially oxidative stress (e.g. superoxide reductase and
455 dismutase, rubrerythrin and rubredoxin) and thermal response (e.g. *groES*, *hsp20* and *hspR*), as
456 different DNA repair mechanisms, including photolyase repair (Figure 6b). In general, genes
457 involved with the nitrogen cycle were identified in almost all selected MAGs, except for MAGs
458 DI_MAG_00020, DI_MAG_00021, and DI_MAG_00022. Sulfate reduction genes were also
459 detected in different selected MAGs, except for MAGs DI_MAG_00020, DI_MAG_00021,
460 DI_MAG_00022 and DI_MAG_FBB2_12. All MAGs had incomplete pathways for carbon
461 fixation, except for DI_MAG_00004 and DI_MAG_00021 (Figure 6a).

462

463 Different cold-shock genes were detected among MAGs; DI_MAG_00006 was the one which
464 presented more *csp* genes. We did not find any *csp* genes in archaeal MAGs (DI_MAG_00020,
465 DI_MAG_00022, and DI_MAG_00049). However, we observed genes in all selected MAGs that
466 were related to different heat-shock responses, including *groEL/groES* genes in DI_MAG_00004,

467 DI_MAG_00020, and DI_MAG_00021. Thermosome (*thsA*) and reverse gyrase genes were
468 identified in all the selected MAGs assigned as Archaea (DI_MAG_00020, DI_MAG_00022, and
469 DI_MAG_00049). Although all MAGs showed the potential presence of oxidative stress response
470 (except DI_MAG_00049), rubrerythrin and rubredoxin genes were only observed in
471 DI_MAG_00004 and DI_MAG_00003. Different DNA repair mechanisms were identified in
472 selected MAGs, such as several recombination genes (*rec* genes), DNA mismatch repair (*mut*
473 genes), nucleotide excision repair (*uvr* genes), double-strand break repair (*herA*, only in archaeal-
474 selected MAGs) and photolyase repair (only in DI_MAG_00004) (Figure 6b).

475

476 **Discussion**

477 The primary goal of our study was to unveil how communities functionally respond to the
478 combination of environmental factors typical of polar marine volcanoes. Our results show that
479 regardless of proximity between fumaroles and glaciers on Deception Island, the community
480 function is strongly driven by the combination of contrasting environmental factors, as occurred
481 similar to what we previously observed for community composition and diversity (Bendia et al.,
482 2018b). We detected some bacterial groups present in both glacier and fumarole sediments (most
483 notably the phyla Proteobacteria, Firmicutes, and Bacteroidetes), despite the strong gradients in
484 temperature, geochemistry and salinity. In addition, we observed specific groups that varied
485 according to the environmental temperature: the hyperthermophilic members belonging to
486 Crenarchaeota/Thermoprotei, Aquificae and Thermotoga phyla in the 98 °C fumarole,
487 Thaumarchaeota in <80 °C fumaroles, and Acidobacteria and Verrucomicrobia in glaciers. These
488 patterns are consistent with previous work carried out on Deception Island using the same sample
489 set for diversity analysis (16S rRNA gene sequencing) (Bendia et al., 2018b), except for the

490 Aquificae and Thermotogae phyla, which were not detected by that method. Furthermore, our
491 taxonomic patterns were also consistent with a previous report that observed similar members
492 along a temperature gradient ranging from 7.5 to 99 °C in geothermal areas in Canada and New
493 Zealand (Sharp et al., 2014).

494

495 Surprisingly, our network analysis showed that the community interaction in the hottest fumarole
496 (98 °C) was more complex and presented fewer positive interactions when compared to the lowest
497 temperatures, in contrast to previous studies that showed that community complexity decreases
498 with temperature increase (Cole et al., 2013; Merino et al., 2019; Sharp et al., 2014). Our results
499 suggest that hyperthermophilic temperatures on Deception probably trigger ecological interactions
500 between community members to modulate their resistance and resilience when facing strong
501 environmental stressors. Similar patterns of community interaction have been previously observed
502 in stressful conditions in the Atacama Desert (Mandakovic et al., 2018) and with increasing
503 temperature in anaerobic digestion (Lin et al., 2016), although these environmental conditions are
504 different from those found on Deception Island.

505

506 Correlation with environmental drivers varied among both taxonomic and functional groups. For
507 example, groups positively influenced by temperature, sulfate, and sodium were those mainly
508 abundant in fumaroles, while groups and functions prevalent in glaciers were positively correlated
509 with ammonia. These results indicate that the mosaic of environmental parameters shapes both
510 taxonomic and functional diversity of microbial communities. Indeed, we observed a partition of
511 metabolic diversity among the steep environmental gradients on Deception Island. Unlike previous
512 studies carried out at hydrothermal vents which pointed to metabolic functional redundancy at the

513 community level (Galambos et al., 2019; Reveillaud et al., 2016), Deception communities showed
514 metabolic heterogeneity across the sharp temperature gradient. The observation of functional
515 redundancy despite the taxonomic variation has been observed in several environments such as
516 venting fluids from the Mariana back-arc, cold seafloor ecosystems, freshwater and gut
517 microbiomes (Louca et al., 2016; Trembath-Reichert et al., 2019; Tully et al., 2018; Turnbaugh
518 and Gordon, 2009; Várbró et al., 2017). The metabolic heterogeneity observed in our results
519 indicates that microbial communities on Deception harbor a remarkably diverse genetic content
520 that reflects the strong selective pressures caused by a remarkable interaction between the volcanic
521 activity, the marine environment, and the cryosphere.

522

523 The functional pattern clustered the samples by temperature, rather than by geographic location,
524 and showed that microbial communities on Deception Island are grouped by 98 °C fumarole, <80
525 °C fumaroles and glaciers. The predominant metabolic potential in the hottest fumarole (98 °C)
526 was mostly associated with reductive pathways, such as sulfate reduction, ammonification, and
527 dissimilatory nitrite reduction, and carbon fixation. We suggest that the hydrogen sulfide emissions
528 and hyperthermophilic conditions of this fumarole (98 °C) (Somoza et al., 2004) may decrease the
529 dissolved oxygen even in the superficial sediment layers, creating a steep redox gradient and
530 preferably selecting microorganisms with reductive and autotrophic pathways. In addition,
531 communities from the hottest fumarole (98 °C) exhibited several genes related to different adaptive
532 strategies, such as those associated with oxidative stress, specific archaeal heat-shock responses,
533 base excision repair, recombination (*recU*), reverse gyrase, protein biosynthesis, chemotaxis, and
534 ABC transporters. This reflects its primary stress factors, including the fumarolic production of
535 hydrogen sulfide, which has a strong reductive power capable of causing oxidative stress, and

536 hyperthermophilic temperature that induces disturbance to metabolic processes and cell-
537 component denaturation (Hedlund et al., 2015; Merino et al., 2019). Enrichment in genes involved
538 with chemotaxis was also observed in metagenomes from hydrothermal vents at Juan de Fuca
539 Ridge (Xie et al., 2011), but different DNA repair mechanisms were found when compared to
540 Deception metagenomes. Different types of ABC transporters were also detected in Ilheya
541 hydrothermal fields (Wang and Sun, 2017); reverse gyrase and thermosome mechanisms have
542 often been described in several groups of hyper(thermophilic) Archaea (Forterre et al., 2000;
543 Lemmens et al., 2018; Lulchev and Klostermeier, 2014).

544

545 In contrast, <80 °C fumaroles were dominated by genes involved with different energetic and
546 chemolithotrophic pathways: sulfur oxidation, ammonification, denitrification, nitrogen fixation,
547 and dissimilatory nitrite reduction. This suggests a trend for both reductive and oxidative
548 pathways, as well as metabolic versatility and complex biogeochemical processes at the local
549 community level. Although genes related to sulfur and nitrogen pathways were detected in
550 glaciers, the majority of potential pathways for glacier communities were related to carbon
551 metabolism and heterotrophy. This lowest metabolic diversity can be explained by the decrease of
552 marine and volcanic geochemicals (e.g. sulfate) towards glaciers (Supplementary Table 1), making
553 these substrates unavailable for exploiting different energy sources, as occurs in fumaroles. The
554 <80 °C fumaroles and glacier communities harbored mechanisms for both heat and cold-shock
555 genes, dormancy and sporulation functions, and DNA repair mechanisms through *uvrABC*
556 complex, *recA*, and photolyase. Diverse survival strategies in <80 °C fumaroles and glaciers might
557 be explained by community exposure to fluctuating temperatures and redox conditions that are
558 more variable when compared to the stability of hottest fumarole, which maintains the

559 hyperthermophilic temperatures and hydrogen sulfide emissions for long periods. Further, glacier
560 communities exhibited more genes associated with osmotic stress, which reflects the low liquid
561 water availability due to the predominant freezing conditions of the Antarctic ecosystems (Wei et
562 al., 2016).

563

564 Although several studies have shown a quantitative decrease in microbial diversity as temperature
565 increases in both geothermal and hydrothermal ecosystems (Cole et al., 2013; Sharp et al., 2014),
566 little is known about how temperature affects ecosystem functioning due to inhibition of key
567 metabolic enzymes or pathways (Hedlund et al., 2015). Despite the limitation of metagenomics in
568 revealing the truly active microbial metabolic pathways, our results increase understanding of the
569 potential temperature limits on different microbial metabolism at the community level and
570 encourage more studies to elucidate the direct effect of temperature extremes on specific
571 biogeochemical processes in Antarctic volcanic ecosystems.

572

573 The 159 MAGs recovered from the Deception Island volcano comprised a broad phylogenetic
574 range of archaeal and bacterial phyla. The 11 MAGs selected for annotation included
575 hyperthermophilic and thermophilic lineages, as well as lineages containing homologs of different
576 predicted sulfur and nitrogen pathways, and archaeal groups underrepresented in genome data,
577 such as *Ca. Nitrosocaldus* and Nanoarchaeota/Woesearchaeia. Since *Ca. Nitrosocaldus* was
578 previously reported only in terrestrial geothermal environments (Abby et al., 2018; Daebeler et al.,
579 2018; Torre et al., 2008), their presence on Deception fumaroles represents a novel outcome for
580 the ecological distribution of thermophilic ammonia-oxidizing Archaea and encourages further
581 investigation to better understand their role in marine volcanic ecosystems. Furthermore, the

582 majority of our selected MAGs are equipped with gene-encoding proteins that protect cells against
583 several stressful conditions, including cold and heat-shock, carbon starvation, oxidative and
584 periplasmic stress, and DNA damage, likely enabling survival and adaptation of these
585 microorganisms to a broad combination of extreme parameters. One of our MAGs was closely
586 related to archaeon GW2011_AR20, which is an uncultivated and underrepresented
587 Nanoarchaeota/Woearchaeia member described previously in aquifer samples and appears to
588 have a symbiotic or pathogenic lifestyle due to the small genome size and lack of some
589 biosynthesis pathways (Castelle et al., 2015). The genome analysis of our Woearchaeia MAG
590 (archaeon GW2011_AR20, DI_MAG_00022) suggests a novel putative thermophilic lifestyle or
591 at least a potential heat tolerance for this lineage due to the (i) lack of cold-shock genes, (these
592 genes are mostly absent in the genomes of thermophilic archaea, while usually present in
593 psychrophilic/mesophilic archaeal members (Cavicchioli, 2006; Giaquinto et al., 2007), and (ii)
594 the presence of reverse gyrase, thermosome and other heat-shock genes (e.g. *groES*) that are
595 essentially related to (hyper)thermophiles and heat response. Although these heat-shock genes
596 were also detected in some mesophilic archaeal lineages within Halobacteria, Thaumarchaeota,
597 and *Methanosarcina* spp. (Lemmens et al., 2018), reverse gyrase is the only protein found
598 ubiquitously in hyperthermophilic organisms, but absent in mesophiles (Catchpole and Forterre,
599 2019), pointing to this Woearchaeia member as a likely thermophile or hyperthermophile.

600

601 **Conclusion**

602 By combining the annotation of reads and contigs together with genome reconstruction from
603 metagenomic data, we provide the first genetic and genomic evidence that microorganisms
604 inhabiting the Deception Island volcano possess a variety of adaptive strategies and metabolic

605 processes that are shaped by steep environmental gradients. We observed that hyperthermophilic
606 temperatures (98 °C) preferably select microorganisms with reductive and autotrophic pathways,
607 while communities from fumaroles <80 °C show a high metabolic versatility with both reductive
608 and oxidative pathways, and glaciers harbor communities with metabolic processes especially
609 related to carbon metabolism and heterotrophy. Survival strategies of microorganisms from the
610 hottest fumarole are very specialized in responding to the hyperthermophilic temperatures and
611 oxidative stress, while <80 °C fumaroles and glacier communities possess a variety of strategies
612 that are capable of responding to fluctuating redox and temperature conditions. We found more
613 complex and negative interactions among the communities from the hottest fumarole (98 °C),
614 which indicate that the strong environmental stressors probably trigger competitive associations
615 among community members. Furthermore, through the reconstruction of MAGs, we were able to
616 clarify a putative novel thermophilic lifestyle for a *Woesearchaeia* member and a marine lifestyle
617 for a *Ca. Nitrosocaldus* lineage. Our work represents, as far as we know, the first study to reveal
618 through shotgun metagenomics the response of microbial functional diversity to the extreme
619 temperature gradient (0 to 98°C) of an Antarctic volcano. Furthermore, our study was one of the
620 first to recover MAGs from these ecosystems and it provides new insights regarding the metabolic
621 and survival capabilities of different extremophiles inhabiting the Antarctic volcanoes.

622

623

624

625

626

627

628 **Author Contributions**

629 AB collected the samples, conceived and designed the experiments, performed the experiments,
630 analyzed the data, performed bioinformatic analysis, wrote the paper, and prepared figures and
631 tables. LL contributed to discussion of metagenome-assembled genome analysis, discussed the
632 data, wrote the paper, and reviewed drafts of the paper. LM performed network analysis, wrote the
633 paper, and reviewed drafts of the paper. CS discussed the data, wrote the paper, and reviewed
634 drafts of the paper. BB discussed the data, wrote and reviewed drafts of the paper. VP conceived
635 and designed the experiments, contributed reagents, materials, and analysis tools, and wrote and
636 reviewed drafts of the paper.

637

638 **Funding**

639 This study was part of the projects Microsfera (CNPq 407816/2013-5) and INCT-Criosfera (CNPq
640 028306/2009) and supported by the Brazilian National Council of Technological and Scientific
641 Development (CNPq) and the Brazilian Antarctic Program (ProAntar). The São Paulo Research
642 Foundation – FAPESP supported ABs Doctoral fellowship (2012/23241-0).

643

644 **Conflict of Interest Statement**

645 The authors declare that the research was conducted in the absence of any commercial or financial
646 relationships that could be construed as a potential conflict of interest.

647

648

649

650

651 **Acknowledgments**

652 We thank the captain and the crew of the research polar vessel Almirante Maximiano, Dr. Luiz
653 Henrique Rosa, Dr. Wânia Duleba, and Dr. Antônio Carlos Rocha Campos for their support in
654 sampling. We are very thankful to LECOM's research team and Rosa C. Gamba for their scientific
655 support.

656

657

658 References

- 659
660
- 661 Abby, S.S., Melcher, M., Kerou, M., Krupovic, M., Stieglmeier, M., Rossel, C., Pfeifer, K.,
662 Schleper, C., 2018. Candidatus Nitrosocaldus cavascurensis, an Ammonia Oxidizing,
663 Extremely Thermophilic Archaeon with a Highly Mobile Genome. *Front. Microbiol.* 9.
664 <https://doi.org/10.3389/fmicb.2018.00028>
- 665 Alneberg, J., Bjarnason, B.S., de Bruijn, I., Schirmer, M., Quick, J., Ijaz, U.Z., Loman, N.J.,
666 Andersson, A.F., Quince, C., 2013. CONCOCT: Clustering cONTigs on Coverage and
667 ComposiTiOn. *ArXiv13124038 Q-Bio*.
- 668 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment
669 search tool. *J. Mol. Biol.* 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- 670 Amenábar, M.J., Flores, P.A., Pugin, B., Boehmwald, F.A., Blamey, J.M., 2013. Archaeal
671 diversity from hydrothermal systems of Deception Island, Antarctica. *Polar Biol.* 36, 373–
672 380. <https://doi.org/10.1007/s00300-012-1267-3>
- 673 Antranikian, G., Suleiman, M., Schäfers, C., Adams, M.W.W., Bartolucci, S., Blamey, J.M.,
674 Birkeland, N.-K., Bonch-Osmolovskaya, E., da Costa, M.S., Cowan, D., Danson, M.,
675 Forterre, P., Kelly, R., Ishino, Y., Littlechild, J., Moracci, M., Noll, K., Oshima, T., Robb,
676 F., Rossi, M., Santos, H., Schönheit, P., Sterner, R., Thauer, R., Thomm, M., Wiegel, J.,
677 Stetter, K.O., 2017. Diversity of bacteria and archaea from two shallow marine
678 hydrothermal vents from Vulcano Island. *Extremophiles* 21, 733–742.
679 <https://doi.org/10.1007/s00792-017-0938-y>
- 680 Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes,
681 S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek,
682 R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C.,
683 Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O., 2008. The RAST Server:
684 Rapid Annotations using Subsystems Technology. *BMC Genomics* 9, 75.
685 <https://doi.org/10.1186/1471-2164-9-75>
- 686 Baker, P.E., McReath, I., Harvey, M.R., Roobol, M.J., Davies, T.G., 1975. The geology of the
687 South Shetland Islands: V. Volcanic evolution of Deception Island. *British Antarctic*
688 *Survey*, Cambridge.
- 689 Bartolini, S., Geyer, A., Martí, J., Pedrazzi, D., Aguirre-Díaz, G., 2014. Volcanic hazard on
690 Deception Island (South Shetland Islands, Antarctica). *J. Volcanol. Geotherm. Res.* 285,
691 150–168. <https://doi.org/10.1016/j.jvolgeores.2014.08.009>
- 692 Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: An Open Source Software for Exploring and
693 Manipulating Networks, in: *Third International AAAI Conference on Weblogs and Social*
694 *Media*. Presented at the Third International AAAI Conference on Weblogs and Social
695 *Media*.
- 696 Bendia, A.G., Araujo, G.G., Pulschen, A.A., Contro, B., Duarte, R.T.D., Rodrigues, F., Galante,
697 D., Pellizari, V.H., 2018a. Surviving in hot and cold: psychrophiles and thermophiles from
698 Deception Island volcano, Antarctica. *Extremophiles* 22, 917–929.
699 <https://doi.org/10.1007/s00792-018-1048-1>
- 700 Bendia, A.G., Signori, C.N., Franco, D.C., Duarte, R.T.D., Bohannon, B.J.M., Pellizari, V.H.,
701 2018b. A Mosaic of Geothermal and Marine Features Shapes Microbial Community

- 702 Structure on Deception Island Volcano, Antarctica. *Front. Microbiol.* 9.
703 <https://doi.org/10.3389/fmicb.2018.00899>
- 704 Bowers, R.M., Kyrpidis, N.C., Stepanauskas, R., Harmon-Smith, M., Doud, D., Reddy, T.B.K.,
705 Schulz, F., Jarett, J., Rivers, A.R., Eloie-Fadrosch, E.A., Tringe, S.G., Ivanova, N.N.,
706 Copeland, A., Clum, A., Becraft, E.D., Malmstrom, R.R., Birren, B., Podar, M., Bork, P.,
707 Weinstock, G.M., Garrity, G.M., Dodsworth, J.A., Yooseph, S., Sutton, G., Glöckner, F.O.,
708 Gilbert, J.A., Nelson, W.C., Hallam, S.J., Jungbluth, S.P., Ettema, T.J.G., Tighe, S.,
709 Konstantinidis, K.T., Liu, W.-T., Baker, B.J., Rattei, T., Eisen, J.A., Hedlund, B.,
710 McMahon, K.D., Fierer, N., Knight, R., Finn, R., Cochrane, G., Karsch-Mizrachi, I.,
711 Tyson, G.W., Rinke, C., Lapidus, A., Meyer, F., Yilmaz, P., Parks, D.H., Eren, A.M.,
712 Schriml, L., Banfield, J.F., Hugenholtz, P., Woyke, T., 2017. Minimum information about
713 a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of
714 bacteria and archaea. *Nat. Biotechnol.* 35, 725–731. <https://doi.org/10.1038/nbt.3893>
- 715 Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R.,
716 Parrello, B., Pusch, G.D., Shukla, M., Thomason, J.A., Stevens, R., Vonstein, V., Wattam,
717 A.R., Xia, F., 2015. RASTtk: A modular and extensible implementation of the RAST
718 algorithm for building custom annotation pipelines and annotating batches of genomes.
719 *Sci. Rep.* 5, 8365. <https://doi.org/10.1038/srep08365>
- 720 Carrión, O., Miñana-Galbis, D., Montes, M.J., Mercadé, E., 2011. *Pseudomonas deceptionensis*
721 sp. nov., a psychrotolerant bacterium from the Antarctic. *Int. J. Syst. Evol. Microbiol.* 61,
722 2401–2405. <https://doi.org/10.1099/ijs.0.024919-0>
- 723 Castelle, C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins, M.J., Frischkorn,
724 K.R., Tringe, S.G., Singh, A., Markillie, L.M., Taylor, R.C., Williams, K.H., Banfield, J.F.,
725 2015. Genomic Expansion of Domain Archaea Highlights Roles for Organisms from New
726 Phyla in Anaerobic Carbon Cycling. *Curr. Biol.* 25, 690–701.
727 <https://doi.org/10.1016/j.cub.2015.01.014>
- 728 Catchpole, R.J., Forterre, P., 2019. The Evolution of Reverse Gyrase Suggests a
729 Nonhyperthermophilic Last Universal Common Ancestor. *Mol. Biol. Evol.* 36, 2737–
730 2747. <https://doi.org/10.1093/molbev/msz180>
- 731 Cavicchioli, R., 2006. Cold-adapted archaea. *Nat. Rev. Microbiol.* 4, 331–343.
732 <https://doi.org/10.1038/nrmicro1390>
- 733 Centurion, V.B., Delforno, T.P., Lacerda-Júnior, G.V., Duarte, A.W.F., Silva, L.J., Bellini, G.B.,
734 Rosa, L.H., Oliveira, V.M., 2019. Unveiling resistome profiles in the sediments of an
735 Antarctic volcanic island. *Environ. Pollut.* 255, 113240.
736 <https://doi.org/10.1016/j.envpol.2019.113240>
- 737 Chaumeil, P.-A., Mussig, A.J., Hugenholtz, P., Parks, D.H., 2020. GTDB-Tk: a toolkit to classify
738 genomes with the Genome Taxonomy Database. *Bioinformatics* 36, 1925–1927.
739 <https://doi.org/10.1093/bioinformatics/btz848>
- 740 Cole, J.K., Peacock, J.P., Dodsworth, J.A., Williams, A.J., Thompson, D.B., Dong, H., Wu, G.,
741 Hedlund, B.P., 2013. Sediment microbial communities in Great Boiling Spring are
742 controlled by temperature and distinct from water communities. *ISME J.* 7, 718–729.
743 <https://doi.org/10.1038/ismej.2012.157>
- 744 Daebeler, A., Herbold, C.W., Vierheilig, J., Sedlacek, C.J., Pjevac, P., Albertsen, M., Kirkegaard,
745 R.H., de la Torre, J.R., Daims, H., Wagner, M., 2018. Cultivation and Genomic Analysis
746 of “*Candidatus Nitrosocaldus islandicus*,” an Obligately Thermophilic, Ammonia-

- 747 Oxidizing Thaumarchaeon from a Hot Spring Biofilm in Graendalur Valley, Iceland. *Front.*
748 *Microbiol.* 9. <https://doi.org/10.3389/fmicb.2018.00193>
- 749 Dick, G.J., 2019. The microbiomes of deep-sea hydrothermal vents: distributed globally, shaped
750 locally. *Nat. Rev. Microbiol.* 17, 271–283. <https://doi.org/10.1038/s41579-019-0160-2>
- 751 Eren, A.M., Esen, Ö.C., Quince, C., Vineis, J.H., Morrison, H.G., Sogin, M.L., Delmont, T.O.,
752 2015. Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* 3,
753 e1319. <https://doi.org/10.7717/peerj.1319>
- 754 Feldbauer, R., Schulz, F., Horn, M., Rattei, T., 2015. Prediction of microbial phenotypes based on
755 comparative genomics. *BMC Bioinformatics* 16, S1. [https://doi.org/10.1186/1471-2105-](https://doi.org/10.1186/1471-2105-16-S14-S1)
756 [16-S14-S1](https://doi.org/10.1186/1471-2105-16-S14-S1)
- 757 Fermani, P., Mataloni, G., Van de Vijver, B., 2007. Soil microalgal communities on an antarctic
758 active volcano (Deception Island, South Shetlands). *Polar Biol.* 30, 1381–1393.
759 <https://doi.org/10.1007/s00300-007-0299-6>
- 760 Finn, R.D., Clements, J., Eddy, S.R., 2011. HMMER web server: interactive sequence similarity
761 searching. *Nucleic Acids Res.* 39, W29–W37. <https://doi.org/10.1093/nar/gkr367>
- 762 Forterre, P., Tour, C.B. de la, Philippe, H., Duguet, M., 2000. Reverse gyrase from
763 hyperthermophiles: probable transfer of a thermoadaptation trait from Archaea to Bacteria.
764 *Trends Genet.* 16, 152–154. [https://doi.org/10.1016/S0168-9525\(00\)01980-6](https://doi.org/10.1016/S0168-9525(00)01980-6)
- 765 Freilich, S., Kreimer, A., Meilijson, I., Gophna, U., Sharan, R., Ruppín, E., 2010. The large-scale
766 organization of the bacterial network of ecological co-occurrence interactions. *Nucleic*
767 *Acids Res.* 38, 3857–3868. <https://doi.org/10.1093/nar/gkq118>
- 768 Friedman, J., Alm, E.J., 2012. Inferring Correlation Networks from Genomic Survey Data. *PLoS*
769 *Comput. Biol.* 8. <https://doi.org/10.1371/journal.pcbi.1002687>
- 770 Galambos, D., Anderson, R.E., Reveillaud, J., Huber, J.A., 2019. Genome-resolved metagenomics
771 and metatranscriptomics reveal niche differentiation in functionally redundant microbial
772 communities at deep-sea hydrothermal vents. *Environ. Microbiol.* 21, 4395–4410.
773 <https://doi.org/10.1111/1462-2920.14806>
- 774 Galperin, M.Y., Makarova, K.S., Wolf, Y.I., Koonin, E.V., 2015. Expanded microbial genome
775 coverage and improved protein family annotation in the COG database. *Nucleic Acids Res.*
776 43, D261–D269. <https://doi.org/10.1093/nar/gku1223>
- 777 Geyer, A., Álvarez-Valero, A.M., Gisbert, G., Aulinas, M., Hernández-Barreña, D., Lobo, A.,
778 Martí, J., 2019. Deciphering the evolution of Deception Island's magmatic system. *Sci.*
779 *Rep.* 9, 373. <https://doi.org/10.1038/s41598-018-36188-4>
- 780 Giaquinto, L., Curmi, P.M.G., Siddiqui, K.S., Poljak, A., DeLong, E., DasSarma, S., Cavicchioli,
781 R., 2007. Structure and Function of Cold Shock Proteins in Archaea. *J. Bacteriol.* 189,
782 5738–5748. <https://doi.org/10.1128/JB.00395-07>
- 783 Hedlund, B.P., Thomas, S.C., Dodsworth, J.A., Zhang, C.L., 2015. Life in High-Temperature
784 Environments, in: *Manual of Environmental Microbiology*. John Wiley & Sons, Ltd, pp.
785 4.3.4-1-4.3.4-15. <https://doi.org/10.1128/9781555818821.ch4.3.4>
- 786 Herbold, C.W., McDonald, I.R., Cary, S.C., 2014. Microbial Ecology of Geothermal Habitats in
787 Antarctica, in: Cowan, D.A. (Ed.), *Antarctic Terrestrial Microbiology: Physical and*
788 *Biological Properties of Antarctic Soils*. Springer, Berlin, Heidelberg, pp. 181–215.
789 https://doi.org/10.1007/978-3-642-45213-0_10
- 790 Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal:
791 prokaryotic gene recognition and translation initiation site identification. *BMC*
792 *Bioinformatics* 11, 119. <https://doi.org/10.1186/1471-2105-11-119>

- 793 Joshi, N., Fass, J., 2011. Sicklet: A sliding-window, adaptive, quality-based trimming tool for
794 FastQ files (Version 1.33) [Software].
- 795 Kanehisa, M., Goto, S., 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids*
796 *Res.* 28, 27–30. <https://doi.org/10.1093/nar/28.1.27>
- 797 Kanehisa, M., Sato, Y., Morishima, K., 2016. BlastKOALA and GhostKOALA: KEGG Tools for
798 Functional Characterization of Genome and Metagenome Sequences. *J. Mol. Biol.,*
799 *Computation Resources for Molecular Biology* 428, 726–731.
800 <https://doi.org/10.1016/j.jmb.2015.11.006>
- 801 Keegan, K.P., Glass, E.M., Meyer, F., 2016. MG-RAST, a Metagenomics Service for Analysis of
802 Microbial Community Structure and Function, in: Martin, F., Uroz, S. (Eds.), *Microbial*
803 *Environmental Genomics (MEG), Methods in Molecular Biology.* Springer, New York,
804 NY, pp. 207–233. https://doi.org/10.1007/978-1-4939-3369-3_13
- 805 Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9,
806 357–359. <https://doi.org/10.1038/nmeth.1923>
- 807 Lemmens, L., Baes, R., Peeters, E., 2018. Heat shock response in archaea. *Emerg. Top. Life Sci.*
808 2, 581–593. <https://doi.org/10.1042/ETLS20180024>
- 809 Lezcano, M.Á., Moreno-Paz, M., Carrizo, D., Prieto-Ballesteros, O., Fernández-Martínez, M.Á.,
810 Sánchez-García, L., Blanco, Y., Puente-Sánchez, F., de Diego-Castilla, G., García-
811 Villadangos, M., Fairén, A.G., Parro, V., 2019. Biomarker Profiling of Microbial Mats in
812 the Geothermal Band of Cerro Caliente, Deception Island (Antarctica): Life at the Edge of
813 Heat and Cold. *Astrobiology* 19, 1490–1504. <https://doi.org/10.1089/ast.2018.2004>
- 814 Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W., 2015. MEGAHIT: an ultra-fast single-node
815 solution for large and complex metagenomics assembly via succinct de Bruijn graph.
816 *Bioinformatics* 31, 1674–1676. <https://doi.org/10.1093/bioinformatics/btv033>
- 817 Lin, Q., De Vrieze, J., Li, J., Li, X., 2016. Temperature affects microbial abundance, activity and
818 interactions in anaerobic digestion. *Bioresour. Technol.* 209, 228–236.
819 <https://doi.org/10.1016/j.biortech.2016.02.132>
- 820 Llarch, À., Logan, N.A., Castellví, J., Prieto, M.J., Guinea, J., 1997. Isolation and Characterization
821 of Thermophilic *Bacillus* spp. from Geothermal Environments on Deception Island, South
822 Shetland Archipelago. *Microb. Ecol.* 34, 58–65. <https://doi.org/10.1007/s002489900034>
- 823 Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global
824 ocean microbiome. *Science* 353, 1272–1277. <https://doi.org/10.1126/science.aaf4507>
- 825 Lulchev, P., Klostermeier, D., 2014. Reverse gyrase—recent advances and current mechanistic
826 understanding of positive DNA supercoiling. *Nucleic Acids Res.* 42, 8200–8213.
827 <https://doi.org/10.1093/nar/gku589>
- 828 Mandakovic, D., Rojas, C., Maldonado, J., Latorre, M., Travisany, D., Delage, E., Bihouée, A.,
829 Jean, G., Díaz, F.P., Fernández-Gómez, B., Cabrera, P., Gaete, A., Latorre, C., Gutiérrez,
830 R.A., Maass, A., Cambiazo, V., Navarrete, S.A., Eveillard, D., González, M., 2018.
831 Structure and co-occurrence patterns in microbial communities under acute environmental
832 stress reveal ecological factors fostering resilience. *Sci. Rep.* 8, 5875.
833 <https://doi.org/10.1038/s41598-018-23931-0>
- 834 Markowitz, V.M., Mavromatis, K., Ivanova, N.N., Chen, I.-M.A., Chu, K., Kyrpides, N.C., 2009.
835 IMG ER: a system for microbial genome annotation expert review and curation.
836 *Bioinformatics* 25, 2271–2278. <https://doi.org/10.1093/bioinformatics/btp393>

- 837 Merino, N., Aronson, H.S., Bojanova, D.P., Feyhl-Buska, J., Wong, M.L., Zhang, S., Giovannelli,
838 D., 2019. Living at the Extremes: Extremophiles and the Limits of Life in a Planetary
839 Context. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.00780>
- 840 Muñoz, P.A., Flores, P.A., Boehmwald, F.A., Blamey, J.M., 2011. Thermophilic bacteria present
841 in a sample from Fumarole Bay, Deception Island. *Antarct. Sci.* 23, 549–555.
842 <https://doi.org/10.1017/S0954102011000393>
- 843 Muñoz-Martín, A., Catalán, M., Martín-Dávila, J., Carbó, A., 2005. Upper crustal structure of
844 Deception Island area (Bransfield Strait, Antarctica) from gravity and magnetic modelling.
845 *Antarct. Sci.* 17, 213–224. <https://doi.org/10.1017/S0954102005002622>
- 846 Nakagawa, T., Takai, K., Suzuki, Y., Hirayama, H., Konno, U., Tsunogai, U., Horikoshi, K., 2006.
847 Geomicrobiological exploration and characterization of a novel deep-sea hydrothermal
848 system at the TOTO caldera in the Mariana Volcanic Arc. *Environ. Microbiol.* 8, 37–49.
849 <https://doi.org/10.1111/j.1462-2920.2005.00884.x>
- 850 Newman, M., 2003. The Structure and Function of Complex Networks. *Struct. Funct. Complex*
851 *Netw.* 45, 167–256. <https://doi.org/10.1137/S003614450342480>
- 852 Oksanen, J., 2007. *vegan: Community Ecology Package*. R package version 1.8-5 [WWW
853 Document]. URL [/paper/vegan-%3A-Community-Ecology-Package.-R-package-1.8-5-](http://paper/vegan-%3A-Community-Ecology-Package.-R-package-1.8-5-Oksanen/ce62be133614e05a8a63c39743e42a43765a5db0)
854 [Oksanen/ce62be133614e05a8a63c39743e42a43765a5db0](http://paper/vegan-%3A-Community-Ecology-Package.-R-package-1.8-5-Oksanen/ce62be133614e05a8a63c39743e42a43765a5db0) (accessed 6.3.20).
- 855 Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM:
856 assessing the quality of microbial genomes recovered from isolates, single cells, and
857 metagenomes. *Genome Res.* 25, 1043–1055. <https://doi.org/10.1101/gr.186072.114>
- 858 Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of
859 taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124.
860 <https://doi.org/10.1093/bioinformatics/btu494>
- 861 Peng, Y., Leung, H.C.M., Yiu, S.M., Chin, F.Y.L., 2012. IDBA-UD: a de novo assembler for
862 single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics*
863 28, 1420–1428. <https://doi.org/10.1093/bioinformatics/bts174>
- 864 Price, R.E., Giovannelli, D., 2017. A Review of the Geochemistry and Microbiology of Marine
865 Shallow-Water Hydrothermal Vents, in: *Reference Module in Earth Systems and*
866 *Environmental Sciences*. Elsevier. <https://doi.org/10.1016/B978-0-12-409548-9.09523-3>
- 867 Reveillaud, J., Reddington, E., McDermott, J., Algar, C., Meyer, J.L., Sylva, S., Seewald, J.,
868 German, C.R., Huber, J.A., 2016. Subseafloor microbial communities in hydrogen-rich
869 vent fluids from hydrothermal systems along the Mid-Cayman Rise. *Environ. Microbiol.*
870 18, 1970–1987. <https://doi.org/10.1111/1462-2920.13173>
- 871 Rey, J., Somoza, L., Martínez-Frías, J., 1995. Tectonic, volcanic, and hydrothermal event sequence
872 on Deception Island (Antarctica). *Geo-Mar. Lett.* 15, 1–8.
873 <https://doi.org/10.1007/BF01204491>
- 874 Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069.
875 <https://doi.org/10.1093/bioinformatics/btu153>
- 876 Sharp, C.E., Brady, A.L., Sharp, G.H., Grasby, S.E., Stott, M.B., Dunfield, P.F., 2014. Humboldt's
877 spa: microbial diversity is controlled by temperature in geothermal environments. *ISME J.*
878 8, 1166–1174. <https://doi.org/10.1038/ismej.2013.237>
- 879 Somoza, L., Martínez-Frías, J., Smellie, J.L., Rey, J., Maestro, A., 2004. Evidence for
880 hydrothermal venting and sediment volcanism discharged after recent short-lived volcanic
881 eruptions at Deception Island, Bransfield Strait, Antarctica. *Mar. Geol.* 203, 119–140.
882 [https://doi.org/10.1016/S0025-3227\(03\)00285-8](https://doi.org/10.1016/S0025-3227(03)00285-8)

- 883 Stanley, S.O., Rose, A.H., Smith, J.E., 1967. Bacteria and yeasts from lakes on Deception Island.
884 *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 252, 199–207.
885 <https://doi.org/10.1098/rstb.1967.0012>
- 886 Takai, K., Komatsu, T., Inagaki, F., Horikoshi, K., 2001. Distribution of Archaea in a Black
887 Smoker Chimney Structure. *Appl. Environ. Microbiol.* 67, 3618–3629.
888 <https://doi.org/10.1128/AEM.67.8.3618-3629.2001>
- 889 Torre, J.R.D.L., Walker, C.B., Ingalls, A.E., Könneke, M., Stahl, D.A., 2008. Cultivation of a
890 thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. *Environ. Microbiol.*
891 10, 810–818. <https://doi.org/10.1111/j.1462-2920.2007.01506.x>
- 892 Trembath-Reichert, E., Butterfield, D.A., Huber, J.A., 2019. Active seafloor microbial
893 communities from Mariana back-arc venting fluids share metabolic strategies across
894 different thermal niches and taxa. *ISME J.* 13, 2264–2279. <https://doi.org/10.1038/s41396-019-0431-y>
- 896 Tully, B.J., Wheat, C.G., Glazer, B.T., Huber, J.A., 2018. A dynamic microbial community with
897 high functional redundancy inhabits the cold, oxic seafloor aquifer. *ISME J.* 12, 1–16.
898 <https://doi.org/10.1038/ismej.2017.187>
- 899 Turnbaugh, P.J., Gordon, J.I., 2009. The core gut microbiome, energy balance and obesity. *J.*
900 *Physiol.* 587, 4153–4158. <https://doi.org/10.1113/jphysiol.2009.174136>
- 901 Várbiro, G., Görgényi, J., Tóthmérész, B., Padisák, J., Hajnal, É., Borics, G., 2017. Functional
902 redundancy modifies species–area relationship for freshwater phytoplankton. *Ecol. Evol.*
903 7, 9905–9913. <https://doi.org/10.1002/ece3.3512>
- 904 Wang, H., Sun, L., 2017. Comparative metagenomics reveals insights into the deep-sea adaptation
905 mechanism of the microorganisms in Iheya hydrothermal fields. *World J. Microbiol.*
906 *Biotechnol.* 33, 86. <https://doi.org/10.1007/s11274-017-2255-0>
- 907 Ward, L., Taylor, M.W., Power, J.F., Scott, B.J., McDonald, I.R., Stott, M.B., 2017. Microbial
908 community dynamics in Inferno Crater Lake, a thermally fluctuating geothermal spring.
909 *ISME J.* 11, 1158–1167. <https://doi.org/10.1038/ismej.2016.193>
- 910 Wei, S.T.S., Lacap-Bugler, D.C., Lau, M.C.Y., Caruso, T., Rao, S., de los Rios, A., Archer, S.K.,
911 Chiu, J.M.Y., Higgins, C., Van Nostrand, J.D., Zhou, J., Hopkins, D.W., Pointing, S.B.,
912 2016. Taxonomic and Functional Diversity of Soil and Hypolithic Microbial Communities
913 in Miers Valley, McMurdo Dry Valleys, Antarctica. *Front. Microbiol.* 7.
914 <https://doi.org/10.3389/fmicb.2016.01642>
- 915 Wickham, H., 2011. ggplot2. *WIREs Comput. Stat.* 3, 180–185. <https://doi.org/10.1002/wics.147>
- 916 Wilke, A., Harrison, T., Wilkening, J., Field, D., Glass, E.M., Kyrpides, N., Mavrommatis, K.,
917 Meyer, F., 2012. The M5nr: a novel non-redundant database containing protein sequences
918 and annotations from multiple sources and associated tools. *BMC Bioinformatics* 13, 141.
919 <https://doi.org/10.1186/1471-2105-13-141>
- 920 Wu, Y.-W., Simmons, B.A., Singer, S.W., 2016. MaxBin 2.0: an automated binning algorithm to
921 recover genomes from multiple metagenomic datasets. *Bioinformatics* 32, 605–607.
922 <https://doi.org/10.1093/bioinformatics/btv638>
- 923 Xie, W., Wang, F., Guo, L., Chen, Z., Sievert, S.M., Meng, J., Huang, G., Li, Y., Yan, Q., Wu, S.,
924 Wang, X., Chen, S., He, G., Xiao, X., Xu, A., 2011. Comparative metagenomics of
925 microbial communities inhabiting deep-sea hydrothermal vent chimneys with contrasting
926 chemistries. *ISME J.* 5, 414–426. <https://doi.org/10.1038/ismej.2010.144>
- 927 Zhang, J.-Z., Millero, F.J., 1993. The products from the oxidation of H₂S in seawater. *Geochim.*
928 *Cosmochim. Acta* 57, 1705–1718. [https://doi.org/10.1016/0016-7037\(93\)90108-9](https://doi.org/10.1016/0016-7037(93)90108-9)
929

930

931 **Figures legends**

932

933

934 Figure 1. The sampling map with the location of Antarctic Peninsula (A) and Deception Island,
935 with Fumarole Bay and Whalers Bay geothermal sites highlighted (B). Distribution of collected
936 samples across environmental gradients at studied geothermal sites are described in C for Fumarole
937 Bay and D for Whalers Bay. *In situ* temperatures are represented in blue (glaciers) and orange
938 (fumaroles). The arrow indicates the direction of low and high values of temperature, salinity and
939 volcanic compounds, such as sulfate. Figure was retrieved from Bendia et al., 2018b.

940

941 Figure 2. Relative abundances of microbial community taxonomy based on annotation of reads
942 from shotgun metagenomics, represented at phylum (A) and class (B) levels. Environmental
943 temperatures and geothermal sites of each sample are represented. Taxonomy assignments were
944 performed based on best hit classifications and M5NR (non-redundant protein database), with an
945 e-value of $<1 \times 10^{-5}$, minimum 50 bp alignment, and 60% identity. Co-occurrence network analysis
946 at the genus level are represented in (C), grouping triplicates of each sampling point and
947 highlighting the increases of environmental temperatures and complexity. Complexity was
948 calculated based on a set of measures, such as the number of nodes and edges, modularity, the
949 number of communities, average node connectivity, average path length, diameter, and cumulative
950 degree distribution.

951

952 Figure 3. Extended error plots for functional general profiles of microbial communities generated
953 through annotation of metagenomic reads, visualized through STAMP based on SEED
954 subsystems, are represented in (A). The *p* values were calculated using Fisher's exact two-sided
955 test and the confidence intervals were calculated by the method from Newcombe-Wilson.
956 Differences were considered significant at $p < 0.05$. PCA ordination was performed based on
957 functions at level 3 of the SEED subsystem (B). Heatmap is representing relative abundances of
958 level 1 functions (C). Samples are clustered and colored according to environmental temperature,
959 following the three different groups: 98 °C fumarole, <80 °C fumaroles and glaciers.

960

961 Figure 4. Extended error plots for functional profiles regarding metabolic pathways, including
962 sulfur, nitrogen and carbon metabolisms, and stress response, including oxidative and osmotic,
963 and heat/cold shock responses. Profiles were visualized through STAMP based on annotation of
964 metagenomic reads using SEED subsystems. The p values are represented and were calculated
965 using Fisher's exact two-sided test, with the confidence intervals calculated by the method from
966 Newcombe-Wilson. Samples are clustered and colored according to environmental temperature,
967 following the three different groups: 98 °C fumaroles, <80 °C fumaroles and glaciers.

968
969 Figure 5. Spearman correlation between taxonomic profile (A) (phylum level) and functional
970 profile (level 1 SEED subsystem) (B) and environmental parameters. Only parameters that
971 exhibited $p < 0.05$ in a correlation analysis are represented. The environmental parameters are:
972 Temp (temperature), pH, EC (electrical conductivity), B, Cu, Fe, Zn, OM (organic matter), OC
973 (organic carbon), P, Si, Na, K, Ca, Mg, sulfate, nitrogen, ammonia, nitrate, sand, silt, and clay.

974
975 Figure 6. Functional annotation of the 11 selected metagenome-assembled genomes (MAGs),
976 including metabolic potential (A) and adaptive strategies (B). A black circle represents the
977 presence of genes or complete gene cluster/pathway, and a yellow circle represents incomplete
978 gene cluster or pathway. MAGs codes are represented on the upper side of figures, whereas their
979 taxonomic classification based on GTDB-Tk and GhostKoala are at the bottom. Genes are
980 presented here with identifiers of KEGG Orthology (KO), Clusters of Orthologous Groups (COG)
981 or Enzyme Commission numbers (EC).

982
983 **Table legends**

984
985 Table 1. List of the 11 selected MAGs and their taxonomic classification based on GTDB-Tk and
986 GhostKoala. Characteristics of total genome length, N50, GC content, redundancy and
987 completeness (based on CheckM), and the genome quality status, are described.

988
989
990
991

992 **Supplementary Figures and Tables**

993

994 Figure S1. Relative abundances of microbial community taxonomy based on annotation of contigs,
995 represented at the phylum level. Contigs were constructed through IDBA-ud and annotated using
996 the Integrated Microbial Genomes & Microbiomes (IMG/M) system.

997

998 Figure S2. Extended error plots for functional profiles regarding DNA repair, helicase and
999 topoisomerase, protein biosynthesis, and transport and chemotaxis. Profiles were visualized
1000 through STAMP based on annotation of metagenomic reads using SEED subsystems. The *p* values
1001 are represented and were calculated using Fisher's exact two-sided test, with the confidence
1002 intervals calculated by the method from Newcombe-Wilson. Samples are clustered and colored
1003 according to environmental temperature, following the three different groups: 98 °C fumarole, <80
1004 °C fumaroles and glaciers.

1005

1006 Figure S3. A circular view of the 158 metagenome-assembled genomes (MAGs) that were
1007 recovered through anvi'o v. 5 pipeline and are represented with the mean coverage of contigs, and
1008 the MAGs redundancy, completeness, GC content, total reads mapped and number of SNVs
1009 reported. The clustering dendrogram in the center shows the hierarchical clustering of contigs
1010 based on their sequence composition, and their distribution across samples.

1011

1012 Figure S4. Heatmap representing the 13 high quality and 82 medium quality MAGs based on read
1013 mapping per sample with Z-score. Samples are clustered and colored according to environmental
1014 temperature, following the three different groups: 98 °C fumarole, <80 °C fumaroles and glaciers.

1015

1016

1017 Table S1. Physical-chemical parameters data per sample including temperature, pH, EC (electrical
1018 conductivity), B, Cu, Fe, Zn, OM (organic matter), OC (organic carbon), P, Si, Na, K, Ca, Mg,
1019 sulfate, nitrogen, ammonia, nitrate, sand, silt, and clay.

1020

1021 Table S2. General information about reads and contigs annotation.

1022

1023 Table S3. *P*-values of Spearman correlations comparing taxonomic and functional profiles with
1024 the environmental data.

1025

1026 Table S4. A complete list of all reconstructed MAGs with their characteristics: taxonomic
1027 classification based on GTDB-Tk and GhostKoala, the total genome length, N50, GC content,
1028 redundancy and completeness, based on anvi'o and CheckM, and the genome quality status.

FIGURE 1

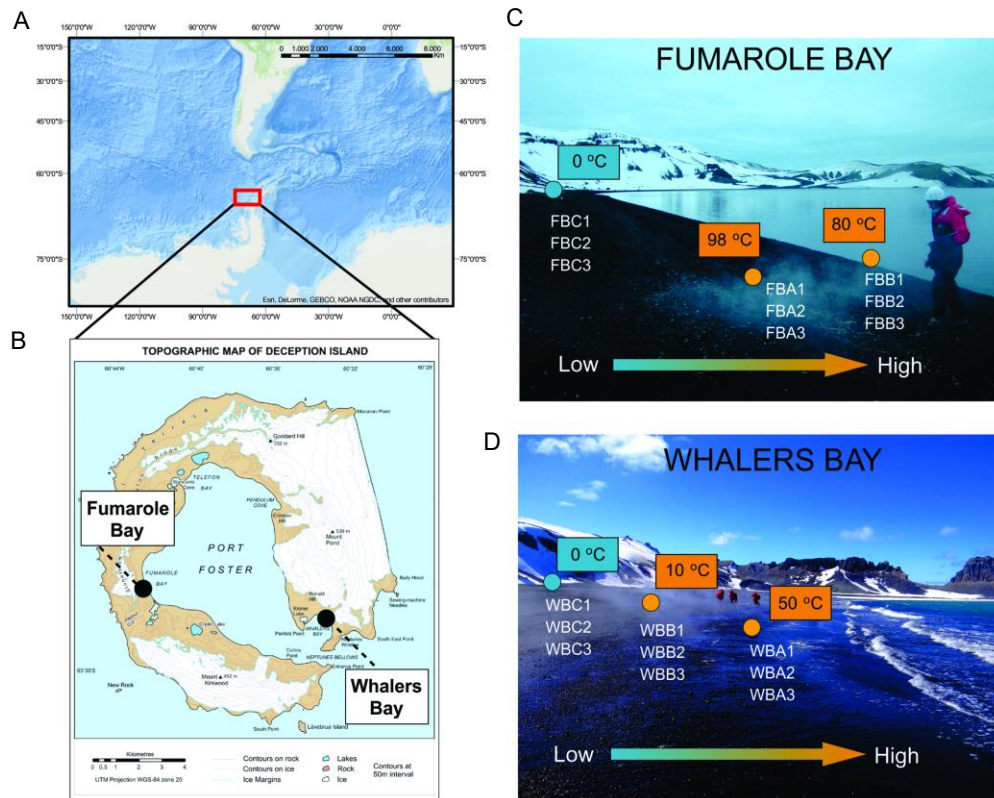


FIGURE 2

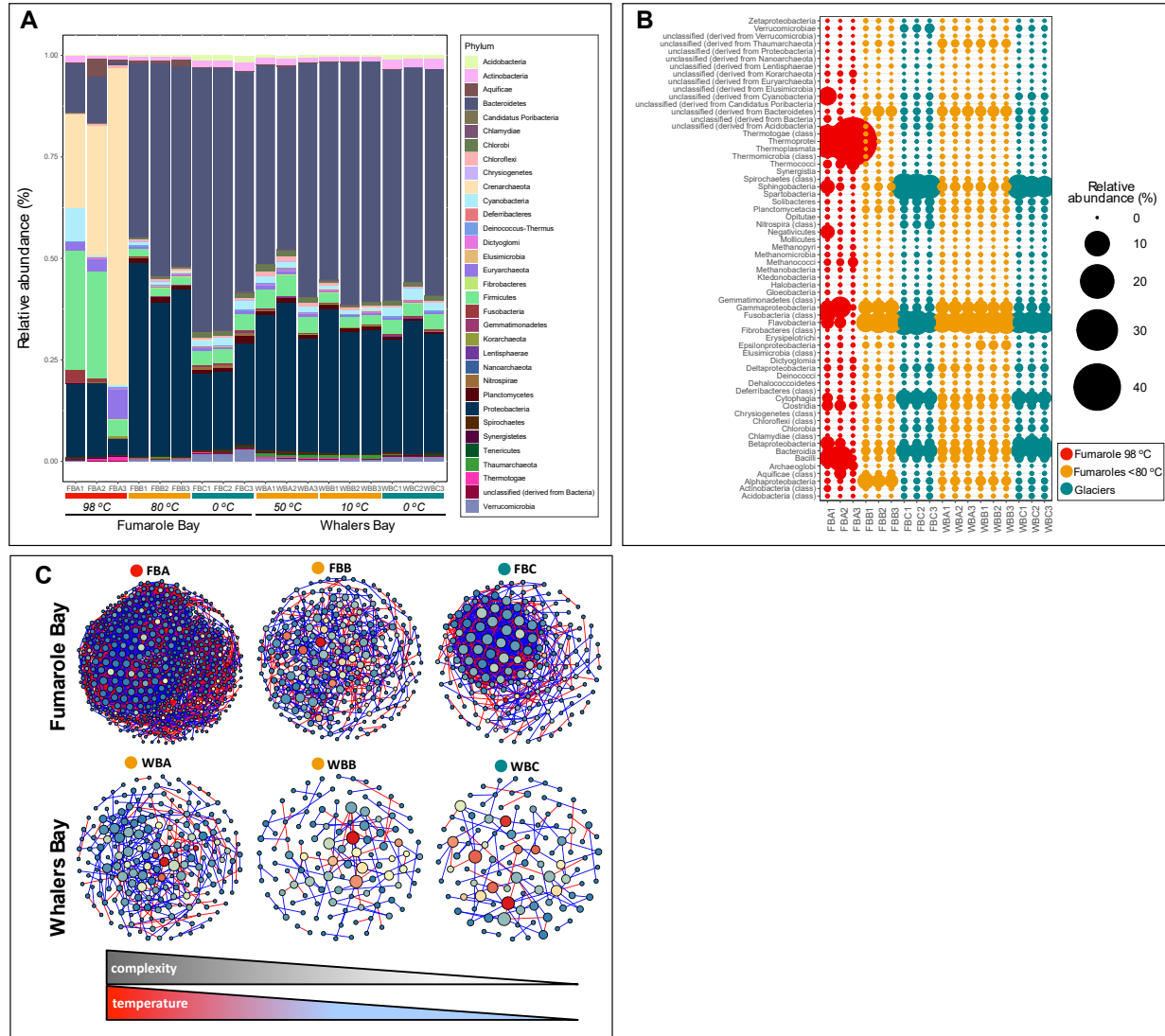


FIGURE 3

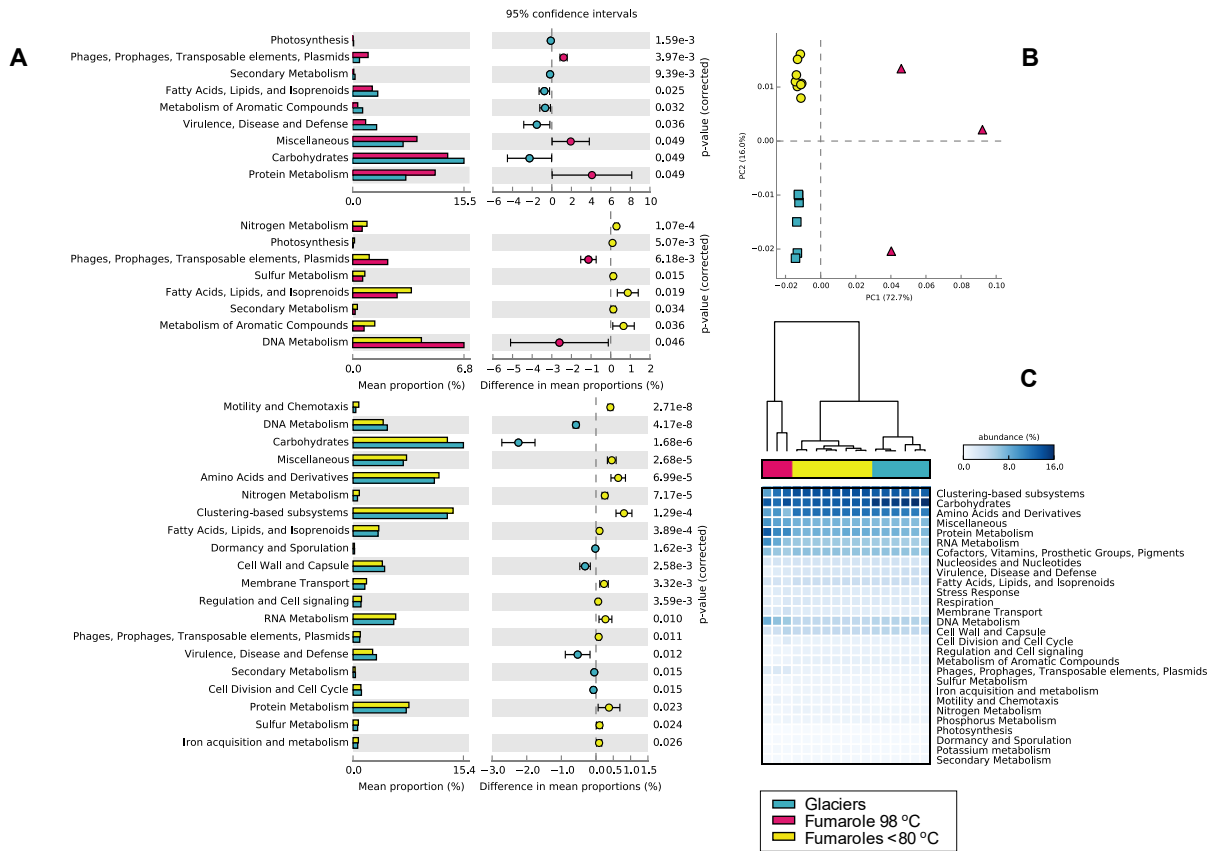


FIGURE 4

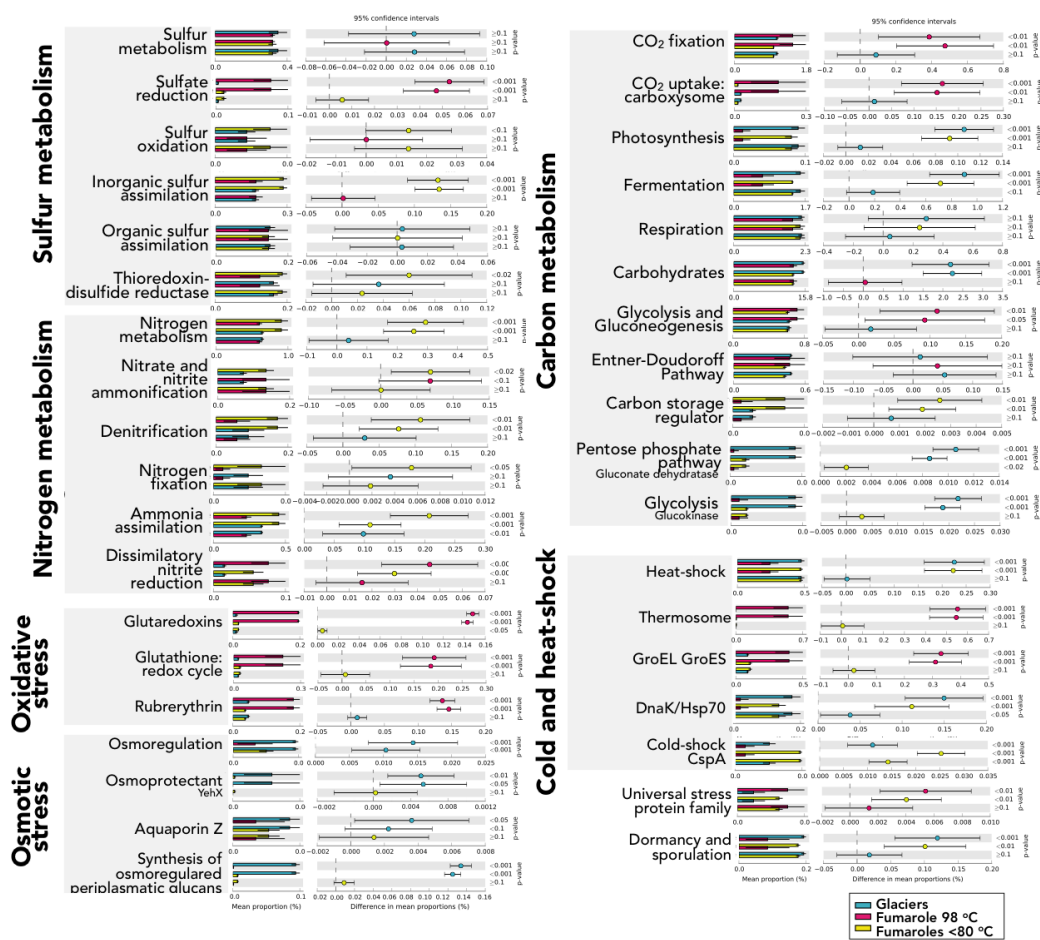


FIGURE 5

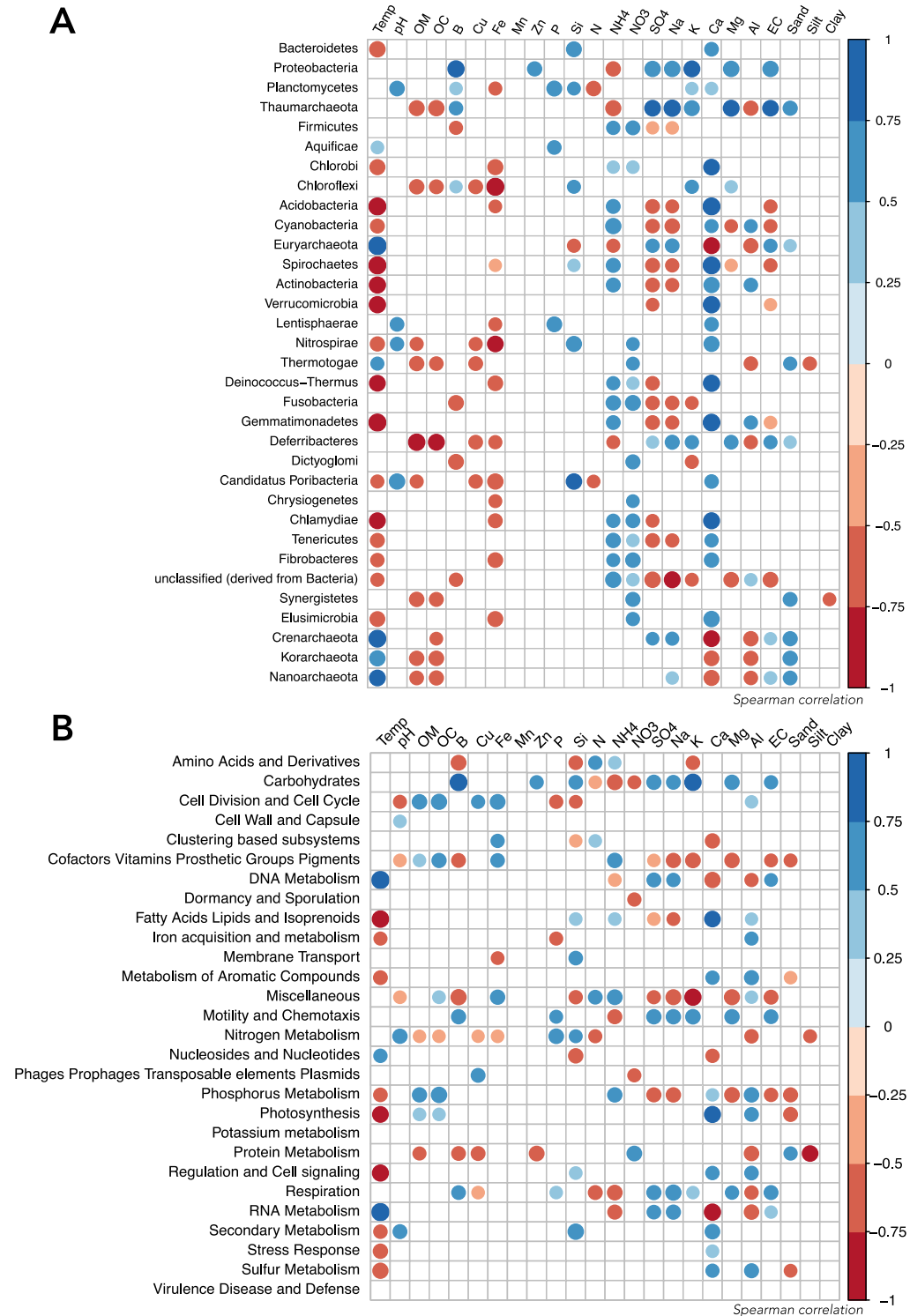


FIGURE 6

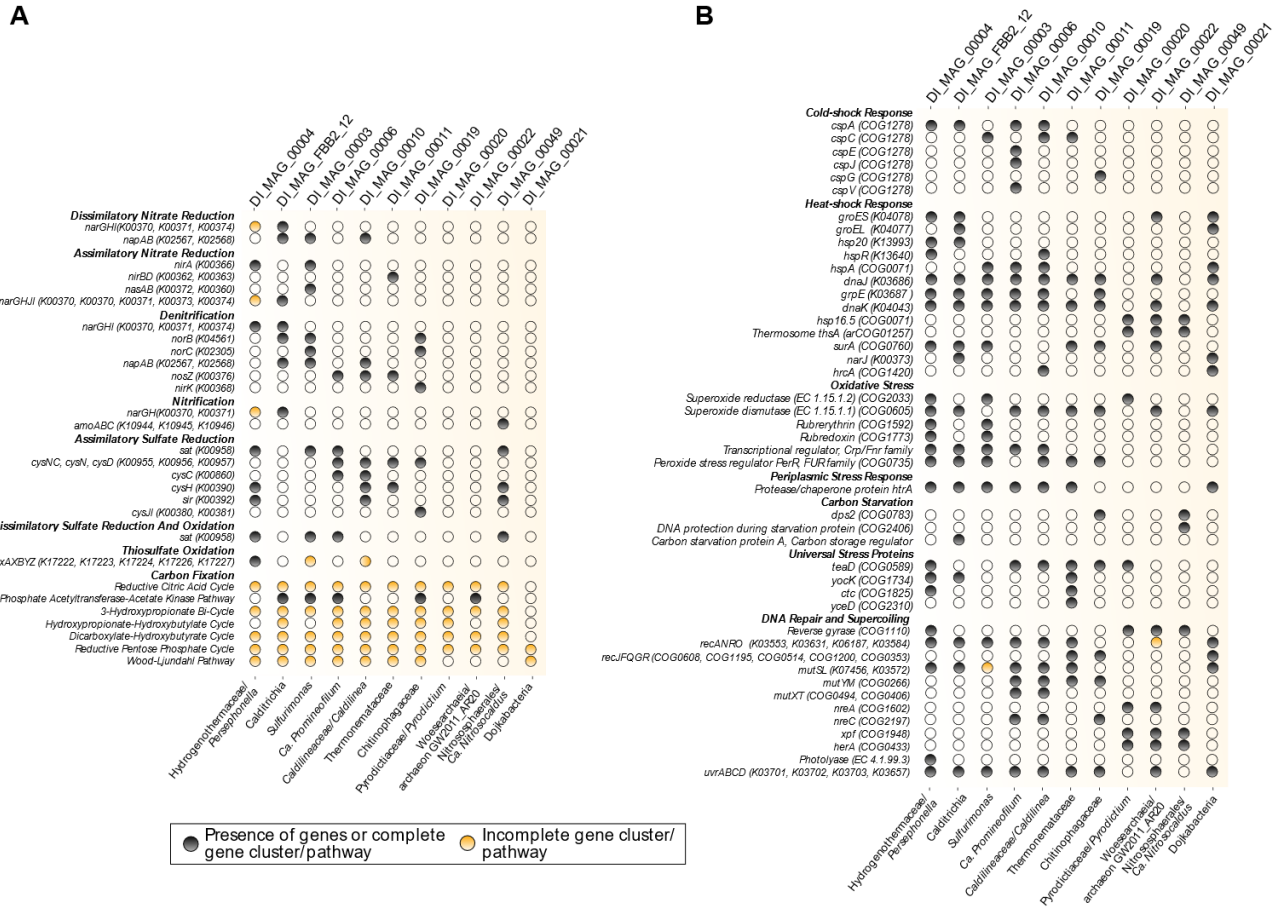


TABLE 1

MAG Code	KEGG/Ghost Koala taxonomy	GTDB-Tk taxonomy	Total Length	Contigs number	N50	GC content	Completeness	Redundancy	Draft Quality
DI_MAG_00003	<i>Sulfurimonas</i>	Bacteria; Campylobacterota; Campylobacteria; Campylobacterales; Thiovulaceae; <i>Sulfurimonas</i>	1,912,170	94	32,276	39.42	97.95	0.41	High
DI_MAG_00004	<i>Persephonella</i>	Bacteria; Aquificota; Aquificae; Hydrogenothermales; Hydrogenothermaceae	1,682,998	67	44,923	30.32	98.37	0.22	High
DI_MAG_00006	<i>Candidatus Promineofilum</i>	Bacteria; Chloroflexota; Anaerolineae; Promineofilales; Promineofilaceae	4,660,983	56	102,288	52.27	92.73	2.00	High
DI_MAG_00010	<i>Caldilinea</i>	Bacteria; Chloroflexota; Anaerolineae; Caldilineales; Caldilineaceae	4,473,267	92	90,197	57.82	96.36	0.91	High
DI_MAG_00011	Unknown	Bacteria; Bacteroidota; Bacteroidia; Cytophagales; Thermonemataceae	2,518,210	152	22,003	49.00	88.43	0.55	High
DI_MAG_00019	Unknown	Bacteria; Bacteroidota; Bacteroidia; Chitinophagales; Chitinophagaceae	3,082,789	324	10,795	37.53	62.85	7.88	Medium
DI_MAG_00020	<i>Pyrodictium</i>	Archaea; Crenarchaeota; Thermoprotei; Desulfurococcales; Pyrodictiaceae	1,071,537	28	68,816	47.76	75.27	0.47	Medium
DI_MAG_00021	Unknown	Bacteria; Patescibacteria; Dojkabacteria	734,971	8	176,471	31.53	77.27	1.72	Medium
DI_MAG_00022	archaeon GW2011_AR20	Archaea; Nanoarchaeota; Woesearchaeia	1,155,662	40	83,443	44.63	79.44	0.00	Medium
DI_MAG_00049	<i>Candidatus Nitrosocaldus</i>	Archaea; Crenarchaeota; Nitrososphaeria; Nitrososphaerales	982,006	145	6,877	32.09	60.52	1.94	Medium
DI_MAG_FBB2_12	<i>Caldithrix</i>	Bacteria; Caldithrichota; Caldithrichia	2,931,548	176	21,244	39.10	95.54	0.00	High