1 # Microbial colonisation in diverse surface soil types in Surtsey and diversity
2 analysis of its subsurface microbiota

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10

11 **Abstract**

12 Colonisation of life on Surtsey has been observed systematically since the formation of the
13 island 50 years ago. Although the first colonisers were prokaryotes, such as bacteria and blue-
14 green algae, most studies have been focused on settlement of plants and animals but less on
15 microbial succession. To explore microbial colonization in diverse soils and the influence of
16 associate vegetation and birds on numbers of environmental bacteria, we collected 45 samples
17 from different soils types on the surface of the island. Total viable bacterial counts were
18 performed with plate count at 22°, 30° and 37°C for all soils samples and the amount of
19 organic matter and nitrogen (N) was measured. Selected samples were also tested for
20 coliforms, faecal coliforms aerobic and anaerobic bacteria. The subsurface biosphere was
21 investigated by collecting liquid subsurface samples from a 181 meters borehole with a
22 special sampler. Diversity analysis of uncultivated biota in samples was performed by 16S
23 rRNA gene sequences analysis and cultivation. Correlation was observed between nutrient
24 deficits and the number of microorganisms in surface soils samples. The lowest number of
25 bacteria ($1x10^4$-$1x10^5$/g) was detected in almost pure pumice but the count was significant
26 higher ($1x10^6$-$1x10^9$/g) in vegetated soil or pumice with bird droppings. The number of faecal
27 bacteria correlated also to the total number of bacteria and type of soil. Bacteria belonging to
28 *Enterobacteriaceae* were only detected in vegetated and samples containing bird droppings.
29 The human pathogens *Salmonella*, *Campylobacter* and *Listeria* were not in any sample. Both
30 thermophilic bacteria and archaea 16S rDNA sequences were found in the subsurface samples

collected at 145 m and 172 m depth at 80°C and 54°C, respectively, but no growth was observed in enrichments. The microbiota sequences generally showed low affiliation to any known 16S rRNA gene sequences.

1 Introduction

Microorganisms are typically in a great abundance and high diversity in common soil and their integrated activity drives nutrient cycling on the ecosystem scale. Organic matter (OM) inputs from plant production support microbial heterotrophic soil microbial communities that drive also processes that make nutrients available in the system. This, in turn, supports plant primary productivity and basic food webs on the ground and in the subsurface (Fenchel et al., 2012; Roesch et al., 2007; Schlesinger, 1997; Whitman et al., 1998). Moreover, as soil develops, soil geochemistry and OM availability changes (Vitousek and Farrington, 1997) due to mineral-OM interactions and geochemical constraints on biological activity (Kleber et al., 2007; Sinsabaugh et al., 2008). Nutrient limitations can constrain plant and food web development, thus shaping the rate of succession of plant and animal life within the ecosystem (Odum, 1969; Walker and del Moral, 2003).

Subsequent of a volcanic eruption, lava flow and ash deposition, new surfaces are created where both organismal growth and weathering processes are effectively reset. Microbial cells colonizing new volcanic deposits must be successful in either growing autotrophically, by fixing C and N using light or inorganic energy sources for growth, e.g. *Cyanobacteria* and sulfate-reducing bacteria (Edwards et al., 2003; Ernst, 1908; Konhauser et al., 2002) or using carbon monoxide as a C and energy source (Dunfield and King, 2004; King and Weber, 2008) or by growing heterotrophically using trace amounts of organic carbon (Cockell et al., 2009; Wu et al., 2007). Studies on the microbiota of volcanic terrains have only emerged within the past few years, revealing that such habitats are capable of harbouring significant microbial diversity, despite their extreme nature (Gomez-Alvarez et al., 2007; Kelly et al., 2010). However, completely isolated volcanic terrains, such as islands, are extremely rare. One of few such places is Surtsey, a neo-volcanic island, created by a series of volcanic eruption that started in 1963 and ended in 1967 (Þórarinsson, 1967, 1968; Þórarinsson, 1965). The eruption was thoroughly documented from the first plume of ash until the end of the lava flow in June 1967. In 1979 ~~and then~~ a 181 m deep hole was drilled ~~in 1979~~ to investigate the substructure of the volcano as well as the nature of the hydrothermal system (Jakobsson & Moore 1979). Consequently, the island of Surtsey provides a unique laboratory for the investigation of

64 biological establishment and succession on relatively newly deposited volcanic substrata, on
65 the surface and in the subsurface, with its drill hole. The first reports of living forms in
66 Surtsey were from 1964 - 1966 (Brock, 1966; Friðriksson, 1965), when the first cyanobacteria
67 were observed, even before the end of the eruption. Phototrophs were further investigated in
68 1968 (Schwabe, 1970) and with subsequent investigations in following years (Brock, 1973;
69 Schwabe and Behre, 1972). However, despite such remarkable habitat, very little research on
70 the microbiology has been performed since the first years of the island formation despite
71 frequent research expeditions and the most recent report on microbes in Surtsey is only from
72 the end of last century (Frederiksen et al., 2000). Besides, no reports or data exist on
73 heterotrophic growth or distribution of such bacteria in the surface soils of the island and
74 nothing is known about distribution of faecal bacteria or pathogens possibly brought by bird
75 inputs of organic matter, such as faeces. Additionally, even less is known about the island
76 subsurface life, but such life is well known in subseafloor sediments and within the deep
77 biosphere where high number of microbes are present and active (Kallmeyer et al., 2012).
78 The overall aim of this study was to explore microbial colonization in different surface soil
79 types and in the subsurface below 160 m depth in a drill hole in Surtsey. That was done by
80 obtaining viable count and distribution of heterotrophic microbes on the island surface and by
81 obtaining the correlation of nutrients and other environmental measurements to different soil
82 types and determine how that affects microbial communities in Surtsey. By investigating
83 presence, survival and possible dissemination routes of pathogenic bacteria into such remote
84 environments and by investigating the existence and diversity of subsurface microbial
85 biosphere and their possible dissemination routes.
86

87 ## 2 Material and methods

88 ### 2.1 Surface sampling and study sites

89 Samples were collected during a sample expedition at Surtsey in July 2009. About 1.0 cm
90 thick layer was retrieved inside a frame of 20 x 20 cm ($0.04m^2$) with spoon that was washed
91 with 70% ethanol between samples. Samples were collected into sterile plastic box and stored
92 outside in the shade and then at 4°C when arrival to the laboratory in Reykjavík until they
93 were processed. Samples were retrieved all around the island and the GPS location of each
94 sample taken (Figure 1). The samples can be divided into three types of samples (Figure 2),
95 SS samples (sand or pumice with bird droppings), SJ samples (pure sand or pumice) and SR

samples (vegetated, partly vegetated and none vegetated area) that were taken at permanent study plots in Surtsey (Magnusson et al., 2014).

2.1.1 Media and cultural conditions

The basic methodology used at the Laboratory for media and culturing were NMKL methods (Nordisk Metodikkomité for Næringsmidler) and methods from the Compendium of Methods for the Microbiological Examination of Foods published by the American Public Health Association (APHA-2001). About 25g of each surface samples was weighed and 225ml Peptone water was mixed in before using a stomacher for blending the soil for 1 min. The supernatant of each sample was used and analysed with different methods.

2.1.2 Total viable count of environmental bacteria

The conventional "pour-plate" method was used on Plate Count Agar. Briefly, 1 ml of homogenate sample was used with 20 ml melted and cooled culture medium. Incubation temperatures was at 22°C for 72 hours and 30°C for xx days in aerobic an anaerobic conditions (FDA, 2001, chapter 3 (pour plate), NMKL 86, 4th ed., 2006, NMKL 74, 3rd ed., 2000). Total viable count was also estimated by filtering 0.1, 1, 10 and 100 ml samples through a sterile 0.22 µm cellulose membrane filter (Millipore Corporation, MA, USA) to capture microbial cells and placed onto Reasoner's 2A (R2A) agar (Difco, Kansas, USA) and incubated in 22°C for 4-5 days for evaluation of total viable count. For better results, 20 ml of sterile phosphate buffer (FB) was used with the 0.1 and 1 ml samples to increase the volume filtered, allowing better dispersion of cells to be grown on the filter paper. All 44 surface samples were tested with these methods and the results expressed as cfu/g.

2.1.3 Total coliforms, faecal coliforms and *Escherichia coli*.

A reference method based on most probable number (MPN) from NMKL (NMKL 96, 4th ed., 2009, Compendium 4th ed., 2001, chapter 8 (8.71, 8.72, 8.81) was used to estimate total coliforms, faecal coliforms and *Escherichia coli.* Pre-enrichment was in LST broth (37°C for 48 hours) and confirmation tests were done in BGLB broth for total coliforms (37°C for 48 hours) and in EC broth for faecal coliforms (44°C for 24 hours). *Escherichia coli* was confirmed by the testing of indol production. The expression of results are in cfu/g.

126 ### 2.1.4 Total viable count of *Enterobacteriaceae.*

127 A reference method from NMKL (Reference: NMKL 144, 3rd ed., 2005.) was used to
128 estimate total *Enterobacteriaceae* in all 44 surface samples. The medium Violet Red Bile
129 Glucose Agar (VRBGA) was used (pour-plate method with overlay). Plates were incubated
130 for 24 hours at 37°C and typical colonies counted. Oxidase test was used for confirmation.
131 The expression of results is cfu/g.

132 ### 2.1.5 Detection of pathogens

133 Reference methods from NMKL was used to estimate total number of pathogens or for
134 *Salmonella, Campylobacter* and *Listeria.* The following NMKL method (Reference: NMKL
135 71, 5th ed., 1999, ISO 6579:2002, Wellcolex- serogroup identification) was used for
136 Salmonella detection. Briefly, pre-enrichment was in BPW broth (37°C for 24 hours), 25 g
137 into 225 of enrichment broth. Second enrichment was in RV broth (41.5°C for 24 hours) and
138 tetrathionate broth (41.5°C for 24 hours). Broths from these enrichments were streaked onto
139 two solid media: XLD and BG (37°C for 24 hours). Typical colonies (2-4 or as needed) were
140 inoculated into TSI- and LI-agarslants (37°C for 24 hours). Confirmation was done by testing
141 for flagellar (H) and somatic (O) antigens. The expression of results was pos/neg in 25 g and
142 17 selected surface samples were tested.

143 The following NMKL method (NMKL 119, 3rd ed., 2007) was used for *Campylobacter*
144 *jejuni/coli* detection. Briefly, enrichment was in Bolton broth, 11 g of sample into 100 ml of
145 enrichment broth. (41.5°C for 48 hours). Broths from these enrichments were streaked onto
146 mCCDA agar and incubated at 41.5°C for 48 hours in an anaerobic jar with microaerobic
147 atmosphere. The expression of results is pos/neg in 11 g and 17 selected surface samples were
148 tested (NMKL 119, 3rd ed., 2007).

149 The following NMKL method (Reference: NMKL 136, 5th ed., 2010) was used for *Listeria*
150 *monocytogenes* detection Briefly, pre-enrichment in *Listeria* broth, 25 g into 225 ml of
151 enrichment broth (30°C for 24 hours). Then further inoculation was in Fraser broth (37°C for
152 up to 48 hours). Both primary and secondary enrichment cultures were streaked onto Oxford
153 and OCLA agar (37°C for 24 and 48 hours). Confirmation tests were done on 5 colonies from
154 each plate and include Gram-staining, catalase and motility. Species identification includes
155 haemolysis on Blood agar and testing on API Listeria (System for the identification of
156 *Listeria*, bioMérieux SA/France). The expression of results was pos/neg in 25 g and 17
157 selected surface samples were tested.

158 2.1.6 Soil geochemistry, chemical and soil moisture analysis

159 The total amount of nitrogen (totN%) was measured on a nitrogen analyzer (2400 Kjeltec
160 Analyzer unit Foss Tecator). About 3 g of soil was analyzed at 420°C for 2.5 hours according
161 to the method ISO 5983-2:2005. The total amount of carbon (totC%) was calculated from loss
162 on ignition after heating at 550°C for 4 hours, assuming the organic matter contained 50%
163 carbon according to the method ISO 5984-2002 (E). Soil gravimetric water content (GWC)
164 was measured as the mass lost from soil after drying 5 g soil for 24 hours at 103°C

165 2.1.7 CO_2 flux measurements

166 The measurement of Net Ecosystem Exchange (NEE, µmol CO_2 m^{-2} s^{-1}) and ecosystem
167 Respiration (Re, µmol CO_2 m^{-2} s^{-1}) were performed as described by Sigurdsson and
168 Magnusson (2010) on top of microbial samples market as SR-samples that were collected
169 from permanent vegetation survey plots, which are 10×10 m in area (Magnusson et al., 2014;
170 Sigurdsson and Magnusson, 2010). Briefly, an EGM-4 infrared gas analyzer and a CPY-2
171 transparent respiration chamber (PP Systems, UK) were first used to measure NEE in light,
172 which was measured as Photosynthetically Active Radiation (PAR) inside the chamber. Then
173 Re was subsequently measured in dark by covering the chamber. The total gross ecosystem
174 CO_2 uptake rate (GPP), was then calculated by the difference between Re and NEE.
175 Vegetation surface cover (Cov., %) was also recorded under the flux chamber at each
176 measurement point and soil temperature was recorded at 5 and 10 cm depth (Ts05 and Ts10,
177 °C) with a temperature probe placed adjacent to the respiration chamber.

178 2.1.8 Multivariate analysis of measured parameters

179 Multivariate analysis was performed on the environmental parameters collected in order to
180 visualise environmental similarities between sample sites. The parameters were: temperature,
181 total carbon, total nitrogen, water content, total microbial count on PCA and counts of
182 *Enterobacteriaceae*. Samples containing missing values were excluded in the analysis except
183 in in six occasions were total nitrogen values were not available. In these cases the values
184 were estimated based on other similar samples in the dataset. The other option would have
185 been to exclude those samples from the analysis. Data were normalised with ln (x+0.1) or ln
186 (x+1), latter for bacterial counts and standardised with (x-mean)/stdev. Non-metric
187 multidimensional scaling (NMDS) using Euclidean similarity measures were performed
188 (Ramette, 2007) using the environmental statistical analysis program PAST.

189 **2.2 Subsurface sampling**

190 2.2.1 Sampling and temperature data

191 The subsurface was sampled through continuously cored drill hole SE-1 (Moore, 1982;
192 Ólafsson and Jakobsson, 2009). The temperature was measured along the drill hole at 1 m
193 interval from the surface down to the bottom at 180 m with a borehole temperature meter. A
194 temperature logger (DST milli-PU logger from StarOddi, Reykjavík, Iceland, was placed for
195 approximately 21 hours at 168 m depth in the borehole and the temperature was recorded
196 every 15 minutes with SeaStar software. Samples were collected in a "homemade" downhole
197 water sampler made of stainless steel. The total capacity of the sampler is about 1.3 L that was
198 kept open (flow through) to the sampling depth and closed with a messenger. Contamination
199 of samples were avoided washing the sampler with several equivalent volumes of 70%
200 ethanol before operation. Samples SB1, 2, 4, 5 and SB6 were retrieved from 57, 58, 145, 168
201 and 170 m respectively. Samples SB4, 5 and 6 were sampled below the see level (58 m).
202 Samples were reduced by Na_2S solution (0.05% w/v final concentration) and kept under
203 anaerobic conditions at low temperature during the field trip and at 4°C in the laboratory.

204 2.2.2 Enrichment cultures of subsurface samples

205 Media for enrichments of chemolithotrophic and chemoorganotrophic organisms were
206 prepared by using 0.5 ml sample and 4.5 ml 0.2 μm-filtered water from the subsurface
207 sample. Cultures were incubated under aerobic (ambient headspace) and anaerobic conditions
208 at 40°C, 60°C and 80°C. Each enrichment was prepared in Hungate culture tubes with 0.01%
209 yeast extract, vitamins solution, Balch element solution (Balch et al., 1979), S^0 and resazurin
210 and incubated under pure N_2 and 0.025% final wt/v $Na_2S•9H_2O$, same but aerobically with
211 ambient headspace and incubation with 80%/20% H_2/CO_2 and 0.025% final wt/v $Na_2S•9H_2O$
212 Additional enrichments used R_2A medium and 162 *Thermus* medium (Degryse et al., 1978),
213 both aerobically with ambient headspace; and *Thermotoga* ("Toga") medium (Marteinsson et
214 al., 1997) and YPS medium (Marteinsson et al., 2001a) under pure N_2 headspace. Growth in
215 enrichments was examined with phase-contrast microscopy (Olympus BX51).

216 2.2.3 DNA Extraction and PCR reactions subsurface samples

217 To capture microbial cells for DNA extraction and analysis, 250 ml of sample was filtered
218 through a 47 mm, 0.22 μm-pore size cellulose membrane filter (Millipore Corporation,
219 Bedford, MA, USA) in our laboratory in Reykjavik. Isolation of chromosomal DNA

220 extraction from and obtained biomass from filter was performed as described by Marteinsson
221 et al., 2001a.

222 2.2.4 Clone library construction and sequencing

223 PCR amplification was performed according to the protocol in Skírnisdóttir *et al.* (2001) with
224 primers 9F (`5-GAGTTTGATCCTGGCTCAG-3`) and 805R (`5-
225 GACTACCAGGGTATCTAATCC-3`) (Skírnisdóttir et al., 2001). PCR product was cloned
226 by the TA method using a TOPO TA cloning kit (Invitrogen). Plasmid DNA from single
227 colonies was isolated and sequenced using a BigDye Terminator Cycle Sequencing Ready
228 Reaction Kit on an ABI sequencer (PE Applied Biosystems). Clones were sequenced using
229 the reverse primer 805R. Cloned sequences were analysed and edited by using the program
230 Sequencer 4.8 from ABI. A total of 41 clones sequences were grouped into operational
231 taxonomic units (OTUs) at a threshold of 98% sequence identity and then aligned by using
232 ClustalW within the MEGA package, version 5.1 (Thompson et al., 1994). In order to check
233 for species identification, sequences were searched against those deposited in GenBank,
234 through the NCBI BLAST (Altschul et al., 1990). Neighbour-joining phylogenetic trees were
235 constructed with MEGA 5.1 (Tamura et al., 2011) using a representative sequence from each
236 OTU and related GenBank sequences.

237 2.2.5 Pyrosequencing and analysis

238 Two sets of reactions targeting the v4-v6 regions of the archeal 16S rRNA gene were
239 performed using the VAMPS primers (Sogin et al., 2006). First, pyrosequencing of short
240 reads, 70-100 nt of the archeal v6 variable region (primers 958F and 1048R; ´5-
241 AATTGGANTCAACGCCGG-3` and ´5-CGRCGGCCATGCACCWC-3`) in the 16S
242 ribosomal gene was performed with a 454 GS-FLX (Roche) on sample SB4. Cycling
243 conditions included an initial denaturation at 94°C for 3 minutes; 30 cycles of 94°C for 30
244 seconds, 57-60°C for 45 seconds, and 72°C for 1 minute; and a final extension at 72°C for 2
245 minutes. Tags shorter than 60 nt were discarded when trimmed by the GS FLX software.
246 Second, a 454 GS-FLX with Titanium chemistry on samples SB-4, SB-5 and SB-6 for longer
247 reads using v4-v6 Vamps primers (5´YCTACGGRNGGCWGCAG-3´and 5´-
248 CGACRRCCATGCANCACCT-3´). Titanium adaptors A and B were attached to the forward
249 and reverse primers respectively along with multiplex identifier (MID) adaptors
250 recommended by Roche to be used in the FLX pyrosequencing. The PCR was performed in a
251 25 uL reaction volume using FastStart High Fidelity polymerase system (Roche, Madison,

252 WI). The PCR program was as follows: 94°C 10 min, 35 cycles of 94°C for 40 s, 52°C for 40
253 s and 72°C for 60 s and a final extension step at 72°C for 7 min. After the recovery of a PCR
254 product from the DNA the rest of the workflow prior to sequencing was done according to
255 manufacturer instructions for FLX amplicon sequencing using the GS Titanium SV emPCR
256 Lib-A kit (Roche, Madison, WI). With both short and long amplicons, the raw sequences
257 were filtered, trimmed and processed through the Qiime pipeline using the Greenegene
258 database (version 12.1). The first steps included various quality processing including filtering
259 sequences which were under 200 bp and over 1000 bp, contained incorrect primer sequences
260 (>1 mismatch) and removal of chimera using Decipher (Wright et al., 2011). Sequences were
261 assigned to samples through the MID sequences and clustered into Operational Taxonomic
262 Units based on 97% similarity in the 16S rRNA sequences using Uclust and then assigned
263 phylogenetic taxonomy through RDP classifier. OTU sequences where then aligned with
264 PyNast.

265 **3 Results**

266 **3.1 Surface sampling and study sites**

267 At total 44 surface samples were collected around the island. An overview of the sampling
268 site is shown in Figure 1. Most of the samples were collected on the South site of the island
269 were the soil was highly variable ranging from sand to completely vegetated environment
270 with significant interactive effects of bird association including nesting seabirds.

271 3.1.1 Viable count of total environmental bacteria and *Enterobacteriaceae.*

272 A good visual correlation was found between total bacterial counts with plate count agar
273 method and growth on R2A media from all samples incubated at 22°C (Figure 3). Positive
274 relationship was also observed between the reduced vegetation or nutrient deficits soils and
275 the number of microorganisms in the samples. The lowest number of bacteria ($1x10^4$-$1x10^5$/g)
276 was detected in almost pure sand or pumice but the count was significantly higher ($1x10^6$-
277 $1x10^9$/g) in vegetated soil, sand or pumice with bird drop (Figure 3). The number or detection
278 of *Enterobacteriaceae* in the soil samples showed similar correlation to the viable count and
279 no growth was observed in samples with low numbers of bacteria or < $1x10^6$cfu/g except in
280 one sample (SS10).

281 ### 3.1.2 Counts of total coliforms, faecal coliforms *Escherichia coli*, aerobic and anaerobic
282 bacteria growing at 30°C and pathogen detection.

283 A total of 12 soil samples that showed significantly high numbers of environmental bacteria
284 or > $1x10^6$cfu/g were selected for further testing of viable count of: total coliforms, faecal
285 coliforms, *Escherichia coli*, aerobic and anaerobic bacteria growing at 30°C and detection of
286 pathogens. Additionally samples containing various soil types and with low viable count of
287 total environmental bacteria < $1x10^6$ cfu/g were also tested as controls. The results are
288 summarized in Table 1. *Listeria, Campylobacter* or *Salmonella* were not detected in any of
289 the selected samples.

290 ### 3.1.3 Soil environment and biogeochemical variables

291 Soil nitrogen, carbon and moisture measurements were performed for all samples with
292 sufficient soil quantity for analysis. Measurements of total nitrogen, carbon and water content
293 was performed in 37 samples except in 6 samples that lacks totN% measurements. Seven
294 samples could not be measured (Table 1). Average totN% measurements were similar in SJ
295 and SR samples, 0.01 and 0.02 respectively but SS samples containing bird droppings was at
296 least 60 times higher or 0.68. Average totC% was also highest in SS samples or 4.68 and SJ
297 and SR samples were 1.17 and 2.74 respectively. Average water content in SJ, SR and SS
298 samples were 0.34, 0.91 and 0.61 respectively.

299 ### 3.1.4 Multivariate analysis of environmental parameters

300 In order to capture the niche similarities between sampling sites multivariate NMDS analysis
301 was performed based on measurements environmental parameters. The analysis showed that
302 the SS samples are separated from other samples while the SR and SJ samples overlap.
303 Samples SR15-17 are well separated from all other samples which is due to it higher load of
304 *Enterobacteriaceae*, total viable counts and higher water content compared to other sampling
305 sites (Figure 4). For selected samples, more environmental data was recorded (NEE, Re.,
306 GPP, PAR, Ts05, Ts10, Cov.) and was used as a base for another sub-NMDS analysis which
307 confirmed previous analysis and clustered the most vegetated samples together (data not
308 shown). Table 2 shows the CO" flux measurements. Other SR samples were gathered together
309 except for SR-3 which is also in a great distance geographically from the other SR samples.
310 Subsurface sampling

311

312 ### 3.1.5 Sampling and temperature data

313 The temperature was measured along the drill hole at 1 m interval from the surface down to
314 the bottom at 178 m with a borehole temperature meter. The temperature measurements are
315 showed in figure 5 in correlation to the depth in the drill hole. The maximum temperature was
316 130°C at 95 m depth and the bottom temperature was 40°C at 178 m depth. The temperature
317 was 54.8±0.1°C at 168 m depth and remained stable over 21 hours -. About 250 ml were
318 sampled at every depth, 57 m (SB-1) and 58 m depth (SB-2), both samples at 100°C, at 145 m
319 depth (SB-4) at 80°C, at 168 m (SB-5) and 170 m depth (SB-6) both samples at 54-55°C. The
320 pH was little above 8.0 in the samples and the salinity was above sea salinity or around 3.7%.

321 ### 3.1.6 Enrichment cultures of subsurface samples

322 No growth could be observed after about 6 weeks of incubation in any of the enrichments
323 incubated at 40°C, 60°C and 80°C.

324 ### 3.1.7 DNA extraction, PCR reactions and clone library construction

325 Very small pellets of unsure biomass was obtained from all SB samples and DNA
326 concentration was extremely low. PCR amplification products were achieved from SB-4, SB-
327 5 and SB-6 with both universal bacterial and archaeal primers. Libraries construction was
328 successful with clones containing bacterial 16S rRNA genes that were amplified in samples
329 SB-5 and SB-6 and with archaeal genes in sample SB-6.

330 ### 3.1.8 Subsurface diversity analysis, clonal and next generation sequencing

331 Three approaches were used assess the bacterial and archeal taxa composition in the samples;
332 partial sequencing of cloned 16S rRNA fragments, pyrosequencing of short fragment of the
333 v6 region and pyrosequencing of a longer fragment of v4-v6 region. Clone libraries of the 40
334 archaeal 16S rRNA genes (500 bp) in sample SB-6 showed high homology (99%) to
335 uncultured subsurface archaeon related sequences (Genbank accession DQ354739.1) from
336 subsurface water of the Kalahari Shield, South Africa by BLAST method. All the clones were
337 dominated by this one sequence except two clones which showed high homology to
338 uncultured subsurface archaeon related sequences (DQ988142) (AB301979.1), from methane
339 cycling in subsurface marine sediments and from in hydrothermal sediments at the Yonaguni
340 Knoll IV hydrothermal field in the Southern Okinawa trough, respectively. Clone libraries of
341 the bacterial 16S rRNA genes in sample SB-5 and SB-6 and their closest known relatives are
342 presented in a neighbour-joining trees of sequences which is summarized in figure 6. The SB-

343 4 v6 library consists mostly or 94.5% of a single taxon affiliated with genus *Archeoglobus*
344 from the Phylum *Euryarchaeota* (18.08724.000 short sequences), 0.1% was affiliated to
345 *Methanomicrobia*, 3.5% to unassignable *Euryarchaeota*, 0.1% to *Crenarchaeota* and 1.8% to
346 undefined Archaea. The longer reads of the v4-v6 regions with Titanium chemistry on
347 samples SB-5 and SB-6 showed the vast majority of pyrosequencing reads taxonomically
348 affiliated with one taxa *Methanobacteriales*, SB-6 76.5% (5121 sequences) and SB-5 84.2%
349 (8307 sequences).The results are summarized in figure 7.
350

351 **4 Discussion**

352 4.1 Surface soil samples

353 Before sampling, surface samples were classified into three sample types according to their
354 visual appearance in the field; pumice soil with bird droppings (10 SS samples), pure pumice
355 soil (15 SJ samples) and mixed samples (19 SR samples). The SR samples were soil that was
356 totally or partly vegetated or pure pumice. They were all collected inside of a defined area
357 used for activity measurements of the soil (Magnusson et al., 2014; Sigurdsson and
358 Magnusson, 2010). Ecosystem respiration (Re) was measured inside these zones in order to
359 investigate soil properties and surface cover of vascular plants. These zones were distributed
360 among the juvenile communities of the island, inside and outside a seagull colony established
361 on the island (Sigurdsson, 2009). As shown with an overview of the sampling sites on the
362 Surtsey island (Figure 1), most of the surface samples were collected on the South site of the
363 island, at the same area as seagull (*Larus* spp.) colony has been established and consequently
364 with high vegetation, but also outside that area that contained less vegetation. The content of
365 organic matter such as carbon and nitrogen is low in the soil of Surtsey where there is no
366 vegetation but normally high in vegetated soils with bird droppings. In the early stage of
367 primary succession on the island, the plant nutrients are retained within the soil system and
368 within microorganisms. We observed a significant correlation between the amount of organic
369 matter in soils and the number of heterotrophic environmental microorganisms grown on two
370 different media at 22°C, and the lowest number of bacteria ($1x10^4$-$1x10^5$/g) was measured in
371 pure pumice but the count was significant higher ($1x10^6$-$1x10^9$/g) in vegetated soil or pumice
372 with bird droppings (Figure 3). Moreover, the number of bacteria belonging to
373 *Enterobacteriaceae* in all the soils samples showed strong correlation to higher counts of total
374 environmental bacteria in samples containing high organic matter. Samples (SS samples)
375 encompassing bird droppings enclosed also *Enterobacteriaceae* but in low numbers (10^1/g).

376 Correlation of high numbers of microbes to organic matter can be anticipated as input of
377 organic matter from plant production support growth of microbial heterotrophic soil microbial
378 communities (Fenchel et al., 2012; Roesch et al., 2007; Schlesinger, 1997; Whitman et al.,
379 1998). It has also been demonstrated that high number of viable counts of environmental
380 bacteria and high organic matter could designate to more diverse groups of bacteria such as
381 coliforms, faecal coliforms, *Escherichia coli*, aerobic and anaerobic bacteria growing at 30°C
382 and even pathogenic bacteria (Girdwood et al., 1985; Pommepuy et al., 1992). Consequently,
383 samples in this study, that showed significantly high number of environmental bacteria and
384 few other samples with low bacterial counts, were selected for further investigation to
385 measure coliforms and the presence of pathogenic microbes such as *Listeria, Campylobacter*
386 or *Salmonella*.

387 Interestingly, the results in this study showed relatively little variance among soils types. The
388 controls or pure pumice samples showed little growth by any culturing method tested as
389 expected but soils with some vegetation and bird droppings revealed also low cell counts
390 apart from aerobic bacteria growing at 30°C. Noteworthy, despite high vegetation and seagull
391 activity, we measured low number of faecal coliforms except in one sample (SR-17) and few
392 other samples with bird droppings but none of the samples contained any of the pathogens
393 that were tested for. This suggests that the coliforms and faecal coliforms cannot survive long
394 in the soil (Avery et al., 2004; Sun et al., 2006).
395 Our classification of sample types by using multivariate NMDS analysis based on our results
396 is in agreement with the visual classification of sample types to certain extend. The analysis
397 showed that the all SS samples were clearly separated from the other samples while the other
398 two types of soil samples, SR and SJ were gathered into one big group, that could be divided
399 into two smaller sub groups and one small group completely apart. This unique group (SR
400 samples) contained samples that were vastly vegetated. The vegetated samples i.e. SR-14 to
401 SR-19 were particular and different from all other samples due to higher load of
402 *Enterobacteriaceae*, total viable counts, higher % of carbon, nitrogen and water content
403 compared to other sampling sites (Figure 4, Table 1). Moreover, by taking into account data
404 only from samples (all SR samples) collected for ecosystem respiration (Re), they could be
405 divided mainly into two groups reflecting the soil properties or vegetation, inside and outside
406 the seagull colony. SR16, 15, and 19 were clustered inside the main seagull colony on the
407 Southern part of the island were SR7, 11, 5 and 9 are clustered just beside the main seagull

408 colony or South-east part of the island, while the two most dissimilar samples SR-3 and SR-1
409 were collected far away from the seagull colony or on the Northern part of the island.

410

411 4.2 Subsurface samples

412 An access to the deep biosphere in a remote neo volcanic island is extremely unique. We were
413 able for the first time to collect hot subsurface samples deep in the centre of a volcanic island,
414 created by a series of volcanic eruption only 42 years after the eruption break. Equally, as
415 reported for geothermal boreholes in Reykjavík, the surface of the drill hole in Surtsey can be
416 regarded as a window to the deep subsurface biosphere of the island (Marteinsson et al.,
417 2001a). This window has been open for 30 years before our sampling in 2009 as the borehole
418 was finished in August 1979 (Jakobsson and Moore, 1982). The purpose of the drill hole was
419 to obtain a core for studying the structure of the island and the hydrothermal alteration of the
420 tephra formed during the Surtsey eruption (Ólafsson and Jakobsson, 2009). The drill site is
421 located on the edge of the Surtur tephra crater at 58 m above sea level with a total depth of
422 181 m. Several temperature measurements have been taken along the depth of the drill hole
423 since the drilling and it appears that the hole has cooled since 1980 (Ólafsson and Jakobsson,
424 2009). Our temperature measurements along the drill hole at 1 m interval from the surface
425 down to the bottom at 180 m showed drastic temperature changes compared to previously
426 measurements. Our highest temperature measurement was 126.5°C which is about 14°C
427 lower than maximal heat reported in 1980 and 3.5 °C lower than in 2004 (Ólafsson and
428 Jakobsson, 2009). In our study we were able to record with 15 min interval the temperature
429 with a temperature logger for 21 hours at 168 m depth in the borehole and the temperature
430 showed to be remarkable stable at this depth at 54.8±0.1°C. This could indicate very little
431 cooling effect of the cold seawater into the system. To our knowledge, this is the first long
432 term temperature measurements in the borehole of Surtsey. Such deep environment with
433 temperature below 100°C and high temperature barrier (130°C) atop, are ideal conditions for
434 the growth of extreme microorganisms. The high temperature and the casing of the borehole
435 down to 165 m isolates the bottom environment from the upper layers or surface
436 microorganisms (Ólafsson and Jakobsson, 2009). The high sterilizing temperature atop of the
437 borehole suggest indigenous subterrestrial microbiota that has probably disseminated from the
438 below faults and cracks of the seafloor in a similar manner as has been reported in other
439 various subterrestrial environments, geothermal boreholes in Reykjavik (Marteinsson et al.,
440 2001a), fresh water hydrothermal vent cones in Eyjafjörður (Marteinsson et al., 2001b) and in

441 subglacial lakes on Vatnajökull (Marteinsson et al., 2013). Furthermore, our results on the
442 microbial diversity supports such deep indigenous subterrestrial microbiota speculations as
443 our 16S rRNA gene sequence showed only similarity to uncultivated taxon originated from
444 the deep biosphere. Our archaeal clone libraries of the 40 archaeal 16S rRNA genes in sample
445 SB-6 at 172 m depth showed high homology (99%) to uncultured subsurface archaeon related
446 sequences from subsurface water of the Kalahari Shield, South Africa by BLAST method
447 (Genbank accession DQ354739.1). All the clones were dominated by this one sequence
448 except two clones which showed high homology to uncultured subsurface archaeon related
449 sequences from methane cycling in subsurface marine sediments and from a hydrothermal
450 sediments at the Yonaguni Knoll IV hydrothermal field in the Southern Okinawa trough,
451 (DQ988142) and (AB301979.1) respectively. The bacterial clone libraries obtained from
452 samples SB-5 and SB-6 showed high diversity as is presented in a neighbour-joining tree of
453 sequences in Figure 6. All these clones could not be affiliated with high homology to any
454 cultivated bacteria and their closest relatives were uncultivated bacterium clones from various
455 subsurfaces or sediments. Interestingly, few clones (12 clones) showed homology to clone
456 SUBT-5 from geothermal boreholes in Reykjavik (Marteinsson et al., 2001a). Similar, with
457 our deep pyrosequencing results, the SB-4 v6 library consists mostly or 94.5% of a single
458 taxon affiliated with genus *Archeoglobus* from the phylum *Euryarchaeota*, 0.1% was
459 affiliated to *Methanomicrobia*, 3,5% to unassignable *Euryarchaeota*, 0,1% to *Crenarchaeota*
460 and 1,8% to Archaea (Figure 7). It is noteworthy that *Archeoglobus* species has been isolated
461 from various marine environments and has optimum growth temperature at 80°C or at the
462 same temperature measured at 145 m depth of the borehole (SB-4) (Huber et al., 1995; Stetter
463 et al., 1993; Stetter et al., 1987). The longer reads of the v4-v6 regions with Titanium
464 chemistry on samples collected at 172 m depth at 55°C (SB-5 and SB-6) showed the vast
465 majority of pyrosequencing reads taxonomically affiliated with one taxa *Methanobacteriales*,
466 84.2% and 76.5% respectively (Figure 7). Interestingly, many methanogens grow at similar
467 temperatures as found in these sample depths. Finally, despite various enrichment conditions
468 and media, we were not able to enrich any microbes with our culture techniques. This may
469 suggest that we have not been able to create the right physical growth conditions and/or to use
470 the right media composition for developing growth.

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Conclusion:

We have explored for the first time microbial colonization in diverse surface soils and the influence of associate vegetation and birds on viable counts of environmental bacteria at the surface of the Surtsey. The number of faecal bacteria correlated to the higher total number of environmental bacteria and type of soil but no pathogenic microbes were detected in any sample tested. We were able for the first time to collect hot subsurface samples deep in the centre of this volcanic island and record the temperature for 21 hours at 168 m depth. Both uncultivated bacteria and archaea were found in the subsurface samples collected below 145 m. The microbial community at 54°C and 172m depth was dominating with diverse bacteria and a homogeny archaeal community of *Methanobacteriales* while the archaeal community at 145 m depth and 80°C was dominated by *Archaeoglobus* like sequences. The subsurface microbial community in Surtsey may be regarded as indigenous subterrestrial microbiota as both bacteria and archaea showed low affiliation to any known microbiota and there is a high temperature barrier (130°C) atop

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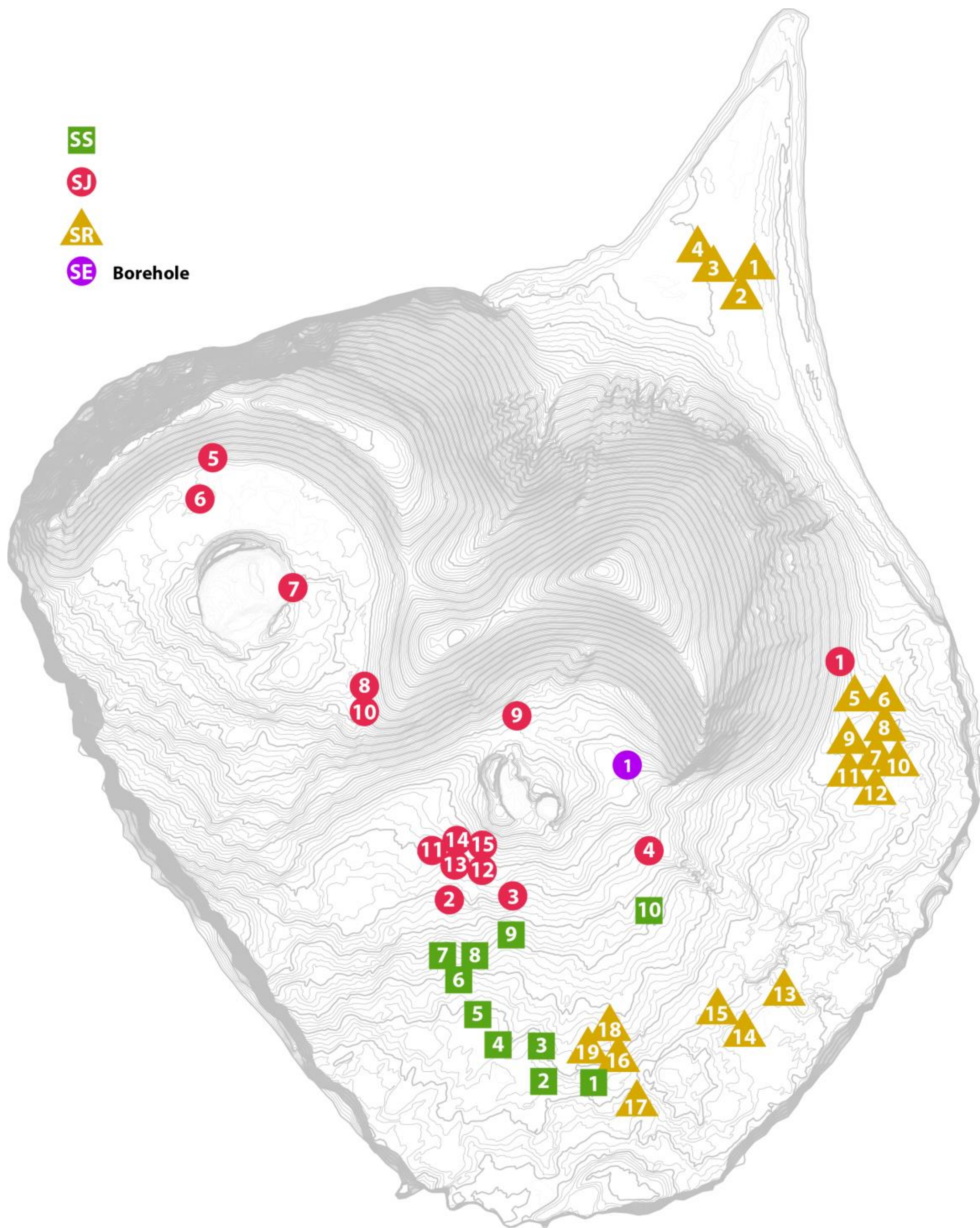
657 **Figures**



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660 Figure 1. An overview of the sampling site. The sites are marked with green squares for sand
661 or pumice with bird droppings samples (SS), pink circles for pure sand or pumice and
662 vegetated samples (SJ), yellow triangles for partly vegetated and none vegetated area samples
663 (SR) collected inside squares for activity measurements and purple circle for the drill hole site
664 (SE) and are distributed with GPS points.

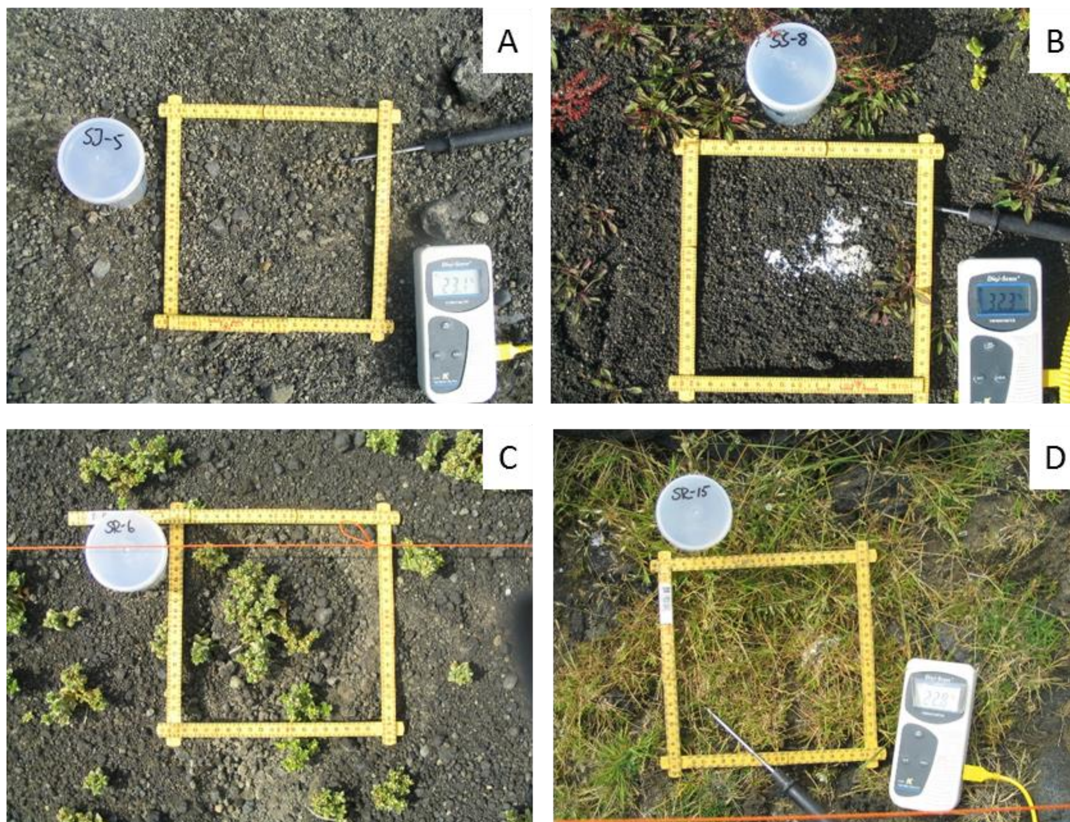


Figure 2. Pictures of the sample types. Samples were divided into three types: a) SJ samples (Barren sand or pumice without bird droppings, see SJ-5), b) SS samples (Barren sand or pumice with bird droppings, see SS-8) and SR samples c) (partly vegetated surfaces, see SR6) and d) (totally vegetated surfaces, see SR-15).

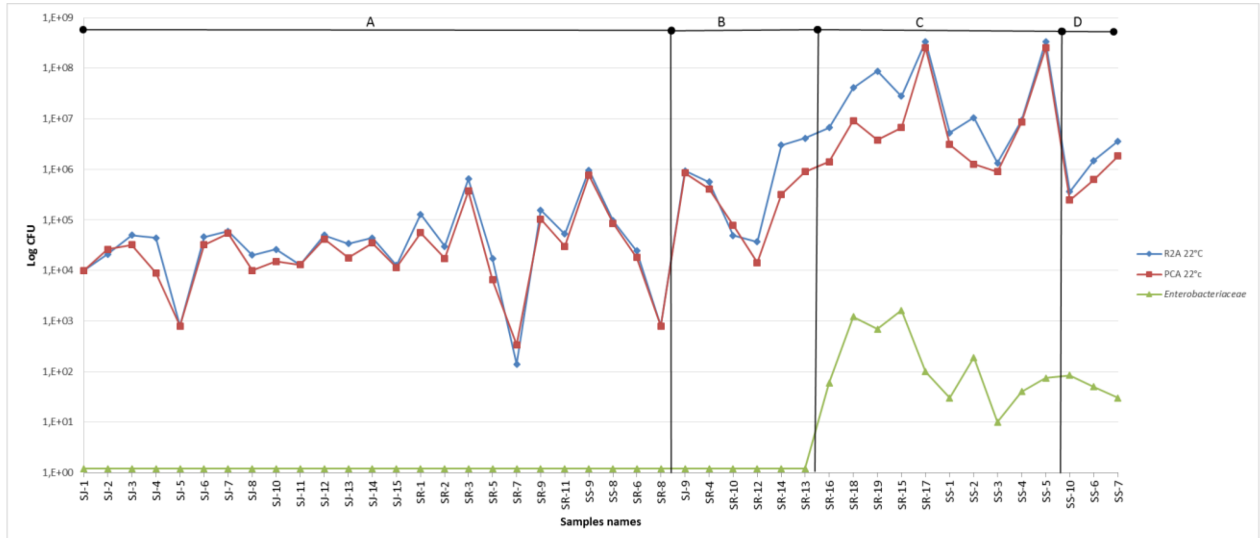


Figure 3. Total bacterial counts with plate count agar method at 22°C. The blue diamond shaped dots shows the total environmental bacterial counts with plate count agar method and the red squares shaped dots the numbers grown on R2A media at 22°C. The number of *Enterobacteriaceae* in the soil samples are showed with green triangle shaped dots. The samples order is oriented according to the appearance of the surface soil, starting with pure sand or pumice and in some cases with tiny vegetation (SR9, SR6) or bird droppings (SS9, SS8, SR8) including sample SR8 (A), partly vegetation including sample SR13 (B), total vegetation with bird droppings including sample SS5 (C) and sand with bird droppings including sample SS10 (D). See Figure 1 for locations.

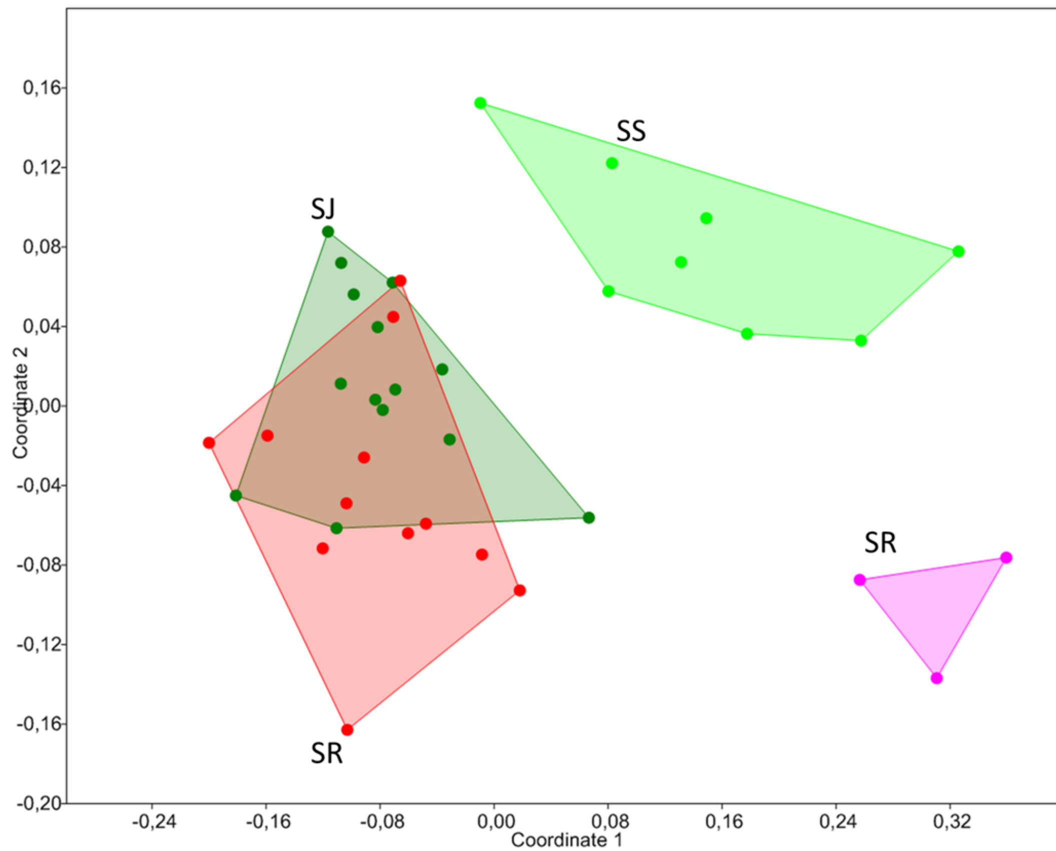


Figure 4. Non-metric multidimensional scaling, Euclidean distances. Environmental parameters included in analysis: temperature, total carbon, total nitrogen, water content, total microbial count on PCA and counts of *Enterobacteriaceae*. SS samples (3-10) in light green (sand or pumice with bird droppings), SJ samples (1-7 & 10-15) in dark green (pure sand or pumice) and SR (1-12) samples in red (partly vegetated and none vegetated area) and SR (15-17) in purple (vegetated).

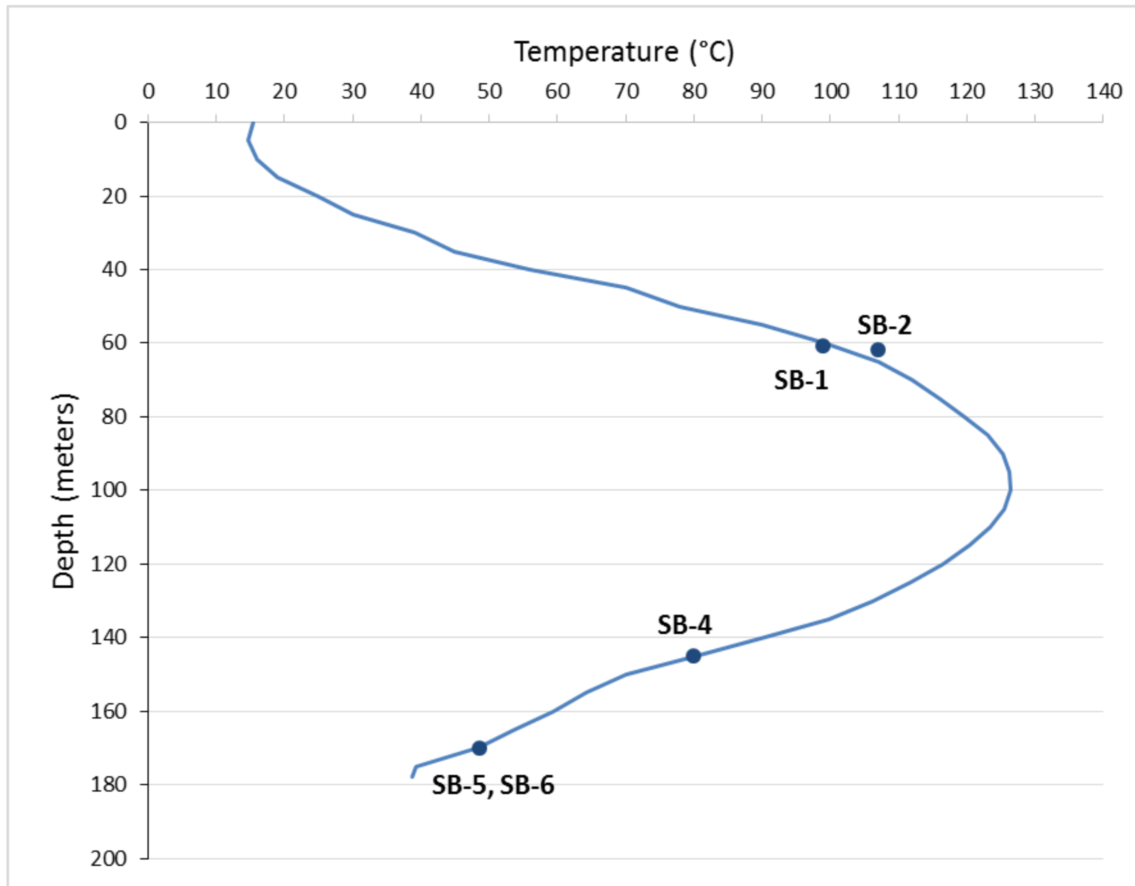


Figure 5. ~~Temperature measurements along the drill hole.~~ Temperatures ~~were measured along the drill hole~~ at 5 m interval along the drill hole, from the surface down to the bottom at 178 m ~~with a borehole temperature meter~~. The circles show the depth and temperature of the SB samples.

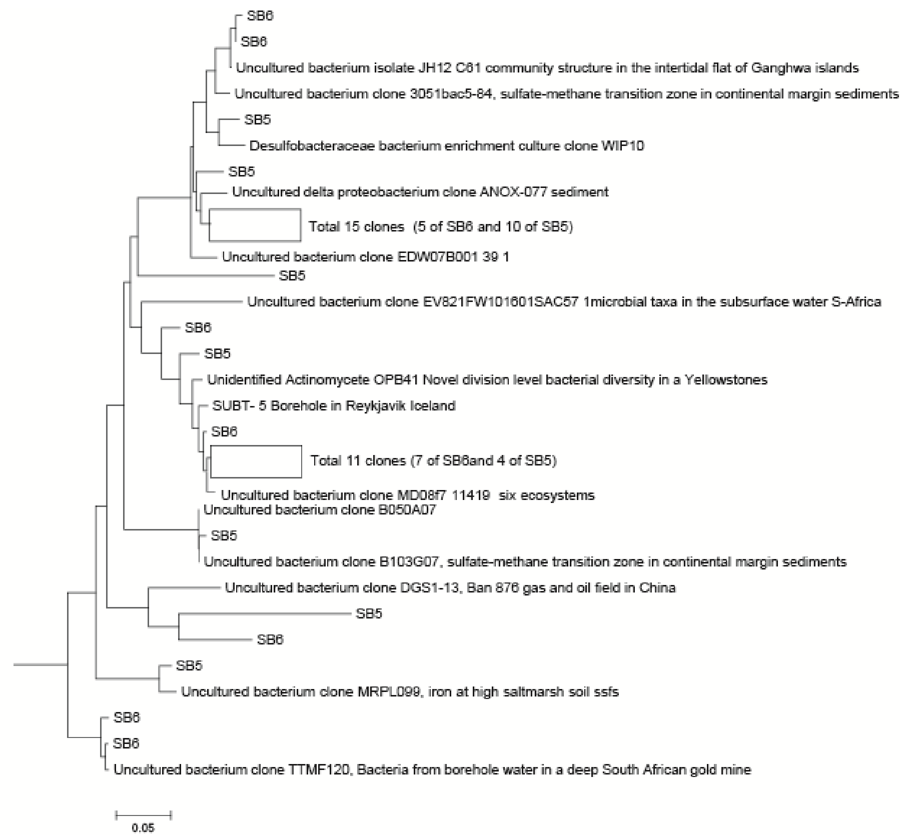
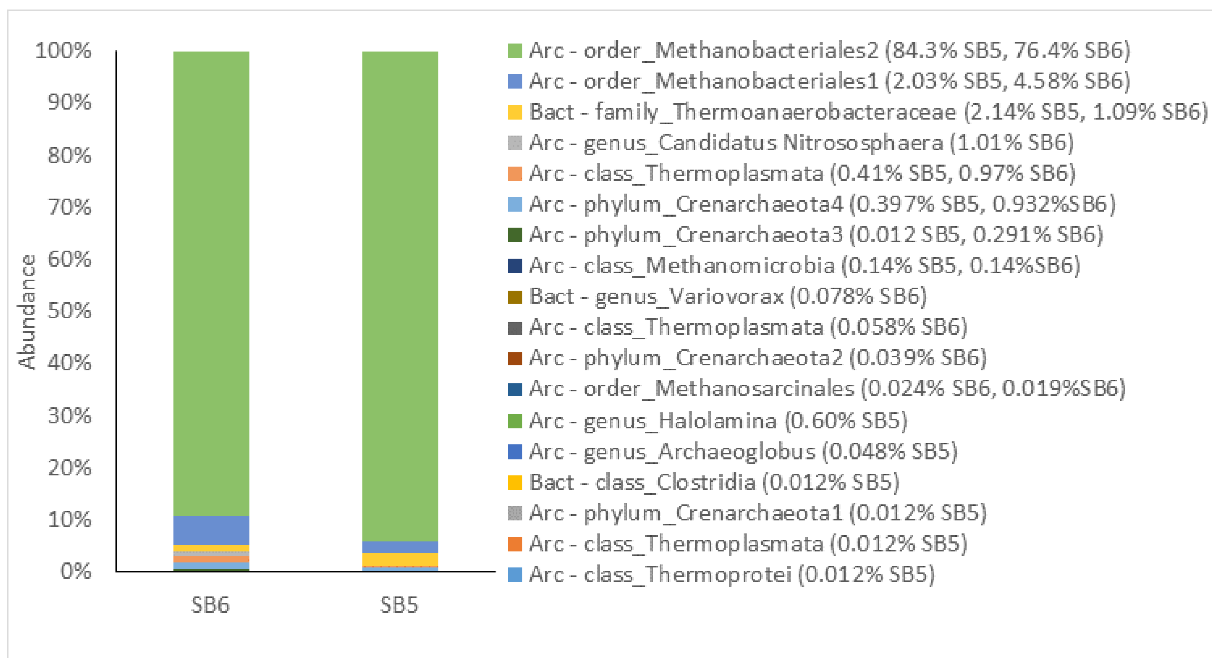


Figure 6. Neighbour-joining trees of sequences from the 16S rRNA clone libraries, databases showing phylogenetic relationships. The scale bar represents the expected % of substitutions per nucleotide position and a marine *Crenarchaeon* was used as outgroup. The cluster in uncultured delta *proteobacterium* clone ANOX-077 represents 11 clones with 99% sequence similarity (5 SB-6 and 10 SB-5 from the borehole).The cluster in uncultured bacterium clone MD08f7 11 clones with 99% sequence similarity (7 SB-6 and 5 SB-5 from the borehole).

a



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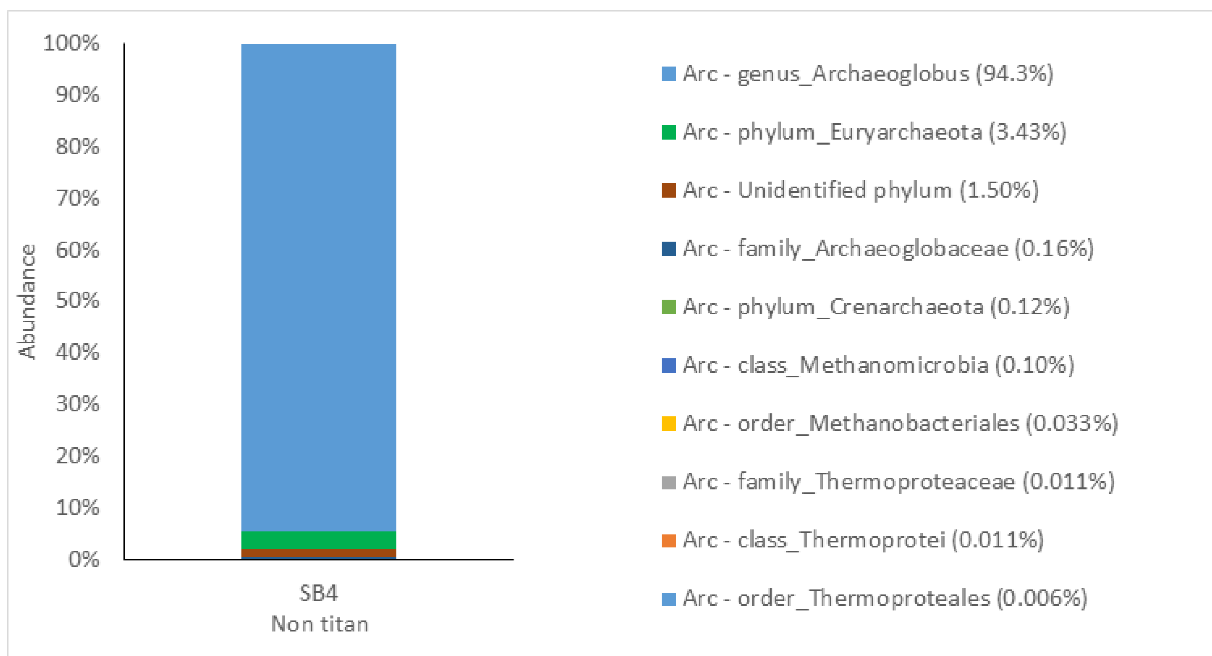

Figure 7. Sequencing results of the 16S rRNA gene with next generation sequencing method. a) The longer reads of the v4-v6 regions with Titanium pyrosequencing on samples SB-5 and SB-6 (from the borehole). b) pyrosequencing of short fragment of the v6 region of the SB-4 (from the borehole) v6 library. The columns and the colours show the % of each major taxon (see text and % of each major and minor taxon on the right side).

Table

Table 1 : Viable count of total coliforms, faecal coliforms, *Escherichia coli*, aerobic and anaerobic bacteria growing at 30°C and detection of pathogens on different media (PCA, R2A, VRBGA).

Soil #	T°(C)	PCA 22°C (CFU/g)	R2A 22°C (CFU/g)	VRBGA 37°C (CFU/g)	CFU 30°C		Coliforms /MPN	Faecal *coli* /MPN	*E. coli* /MPN	totN (% of dw)	totC (% of dw)	GWC
					Aerobic	Anaerobic						
SJ-1	21.2	$1.0x10^4$	$1.0x10^4$	0	nd	nd	nd	nd	nd	0.0095	0.24	0.20
SJ-2	30.6	$2.6x10^4$	$2.1x10^4$	0	nd	nd	nd	nd	nd	0.0110	0.28	0.18
SJ-3	25.7	$3.2x10^4$	$5.0x10^4$	0	nd	nd	nd	nd	nd	0.0280	0.43	0.15
SJ-4	27.0	$9.0x10^3$	$4.4x10^4$	0	nd	nd	nd	nd	nd	0.0020	0.18	0.14
SJ-5	23.1	$8.0x10^2$	$8.0x10^2$	0	nd	nd	nd	nd	nd	0.0010	0.09	0.09
SJ-6	26.5	$3.2x10^4$	$4.6x10^4$	0	nd	nd	nd	nd	nd	0.0030	0.20	0.30
SJ-7	27.0	$5.4x10^4$	$6.0x10^4$	0	nd	nd	nd	nd	nd	nd	2.49	0.13
SJ-8	26.8	$1.0x10^4$	$2.0x10^4$	0	nd	nd	nd	nd	nd	nd	9.06	1.37
SJ-9	25.9	$8.5x10^5$	$9.3x10^5$	0	nd	nd	nd	nd	nd	nd	nd	nd
SJ-10	26.0	$1.5x10^4$	$2.6x10^4$	0	nd	nd	nd	nd	nd	0.0180	0.99	0.84
SJ-11	33.0	$1.3x10^4$	$1.3x10^4$	0	nd	nd	nd	nd	nd	nd	0.38	0.17
SJ-12	27.0	$4.2x10^4$	$5.0x10^4$	0	$3.1x10^2$	$1.0x10^1$	$3.0x10^0$	$3.0x10^0$	$3.0x10^0$	0.0090	0.36	0.34
SJ-13	31.0	$1.79x10^4$	$3.4x10^4$	0	$8.2x10^2$	$1.0x10^1$	$3.0x10^0$	$3.0x10^0$	$3.0x10^0$	0.0165	1.00	0.30
SJ-14	29.5	$3.5x10^4$	$4.4x10^4$	0	nd	nd	nd	nd	nd	0.0030	0.33	0.28

SJ-15	32.0	1.14×10^{4}	1.26×10^{4}	0	nd	nd	nd	nd	nd	nd	0.41	0.29
SR-1	15.5	5.6×10^{4}	1.29×10^{5}	0	nd	nd	nd	nd	nd	0.0060	0.21	0.14
SR-2	21.0	1.73×10^{4}	3.0×10^{4}	0	nd	nd	nd	nd	nd	0.0060	0.08	0.14
SR-3	21.0	3.7×10^{5}	6.4×10^{5}	0	nd	nd	nd	nd	nd	0.0030	1.28	0.99
SR-4	21.0	4.1×10^{5}	5.6×10^{5}	0	2.2×10^{3}	1.5×10^{2}	3.0×10^{0}	3.0×10^{0}	3.0×10^{0}	0.0120	0.73	0.49
SR-5	22.4	6.5×10^{3}	1.71×10^{4}	0	nd	nd	nd	nd	nd	0.0210	2.42	0.23
SR-6	22.3	1.8×10^{4}	2.43×10^{4}	0	nd	nd	nd	nd	nd	0.0030	0.18	0.18
SR-7	24.7	3.4×10^{2}	1.4×10^{2}	0	nd	nd	nd	nd	nd	0.0340	0.18	0.18
SR-8	25.3	8.0×10^{2}	8.0×10^{2}	0	nd	nd	nd	nd	nd	0.0015	0.24	0.24
SR-9	30.7	1.04×10^{5}	1.57×10^{5}	0	1.2×10^{2}	1.0×10^{1}	3.0×10^{0}	3.0×10^{0}	3.0×10^{0}	nd	0.35	0.22
SR-10	29.7	7.9×10^{4}	4.9×10^{4}	0	nd	nd	nd	nd	nd	0.0165	0.32	0.30
SR-11	22.0	3.0×10^{4}	5.3×10^{4}	0	nd	nd	nd	nd	nd	0.0030	0.61	0.59
SR-12	24.0	1.43×10^{4}	3.7×10^{4}	0	nd	nd	nd	nd	nd	nd	0.25	0.24
SR-13	26.3	9.0×10^{5}	4.1×10^{6}	2.2×10^{2}	nd	nd	nd	nd	nd	nd	nd	nd
SR-14	23.7	3.2×10^{5}	3.0×10^{6}	0	nd	nd	nd	nd	nd	nd	nd	nd
SR-15	22.7	6.7×10^{6}	2.8×10^{7}	1.63×10^{3}	5.8×10^{4}	3.0×10^{1}	1.5×10^{2}	9.3×10^{1}	9.3×10^{1}	0.0210	6.14	2.82
SR-16	25.0	1.42×10^{6}	6.7×10^{6}	1.2×10^{2}	8.8×10^{4}	1.9×10^{3}	3.0×10^{0}	3.0×10^{0}	3.0×10^{0}	0.0690	14.22	3.99
SR-17	23.0	1.0×10^{8}	1.0×10^{8}	2.0×10^{2}	1.4×10^{4}	1.0×10^{1}	9.3×10^{2}	9.3×10^{2}	9.3×10^{2}	0.0420	13.87	2.85
SR-18	nd	9.1×10^{6}	4.1×10^{7}	2.42×10^{3}	7.6×10^{4}	4.5×10^{2}	3.0×10^{0}	3.0×10^{0}	3.0×10^{0}	nd	nd	nd
SR-19	27.9	3.8×10^{6}	8.7×10^{7}	6.92×10^{2}	4.1×10^{3}	5.0×10^{1}	3.0×10^{0}	3.0×10^{0}	3.0×10^{0}	nd	nd	nd
SS-1	27.1	3.1×10^{6}	5.3×10^{6}	6.0×10^{1}	3.7×10^{4}	1.3×10^{2}	2.3×10^{1}	9.0×10^{0}	9.0×10^{0}	nd	nd	nd

SS-2	26.0	$1.26x10^{6}$	$1.05x10^{7}$	$3.8x10^{2}$	$2.4x10^{4}$	$6.0x10^{1}$	$4.0x10^{0}$	$3.0x10^{0}$	$3.0x10^{0}$	nd	nd	nd
SS-3	26.7	$9.0x10^{5}$	$1.34x10^{6}$	$2.0x10^{1}$	$1.4x10^{3}$	$4.0x10^{1}$	$9.3x10^{2}$	$9.3x10^{2}$	$9.3x10^{2}$	0.6785	7.30	0.98
SS-4	25.7	$8.5x10^{6}$	$9.3x10^{6}$	$4.0x10^{1}$	$1.0x10^{4}$	$5.0x10^{1}$	$4.3x10^{1}$	$4.3x10^{1}$	$4.3x10^{1}$	0.6785	14.90	1.56
SS-5	28.4	$2.52x10^{8}$	$3.37x10^{8}$	$7.5x10^{1}$	$1.4x10^{4}$	$1.0x10^{1}$	$9.3x10^{2}$	$9.3x10^{2}$	$9.3x10^{2}$	0.6785	8.10	1.00
SS-6	26.5	$6.3x10^{5}$	$1.49x10^{6}$	$5.0x10^{1}$	$4.6x10^{3}$	$3.0x10^{2}$	$3.0x10^{0}$	$3.0x10^{0}$	$3.0x10^{0}$	0.6785	1.70	0.45
SS-7	26.5	$1.82x10^{6}$	$3.6x10^{6}$	$3.0x10^{1}$	$3.0x10^{4}$	$2.0x10^{4}$	$9.3x10^{2}$	$9.3x10^{2}$	$9.3x10^{2}$	0.6785	2.30	0.28
SS-8	32.3	$8.5x10^{4}$	$9.6x10^{4}$	0	nd	nd	nd	nd	nd	0.6785	0.60	0.19
SS-9	25.5	$7.6x10^{5}$	$9.6x10^{5}$	0	nd	nd	nd	nd	nd	0.6785	2.10	0.29
SS-10	25.3	$2.47x10^{5}$	$3.6x10^{5}$	$8.4x10^{1}$	$3.3x10^{3}$	$6.2x10^{2}$	$4.6x10^{2}$	$4.6x10^{2}$	$4.6x10^{2}$	0.6785	0.50	0.15

nd : not determinated

CFU : Colony-forming unit

MPN : Most Probable Number

totN (% of dw) : percentage of nitrogen

totC (% of dw) : percentage of carbon

GWC : Soil gravimetric water content

T°(C): Surface temperature

Table 2. CO_2 flux measurements from selected samples.

	NEE (umol CO2 m-2 s-1)	Respiration (umol CO2 m-2 s-1)	GPP (umol CO2 m-2 s-1)	PAR (umol photons m-2 s-1)	Vegetation cover (%)
SR-1	-1.59	0.26	-1.85	761	90
SR-3	0.06	0.1	-0.04	802	1
SR-5	0.05	0.05	0	1270	0
SR-7	0.15	0.15	0	870	0
SR-9	0.01	0.01	0	1170	0
SR-11	0.02	0.02	0	800	0
SR-15	-0.02	0.02	-0.04	854	60
SR-16	-0.18	0.4	-0.58	687	100
SR-19	-0.07	0.27	-0.34	1209	100