Characterization of bacterioplankton communities and quantification of organic carbon pools off the Galapagos Archipelago under contrasting environmental conditions

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Bacteria play a crucial role in the marine carbon cycle, contributing to the production and degradation of organic carbon. Here, we investigated organic carbon pools, aggregate formation, and bacterioplankton communities in three contrasting oceanographic settings in the Galapagos Archipelago. We studied a submarine CO_2 vent at Roca Redonda (RoR), an upwelling site at Bolivar Channel (BoC) subjected to a weak El Niño event at the time of sampling in October 2014, as well as a site without volcanic or upwelling influence at Cowley Islet (CoI). We recorded physico-chemical parameters, and quantified particulate and dissolved organic carbon, transparent exopolymeric particles, and the potential of the water to form larger marine aggregates. Free-living and particle-attached bacterial communities were assessed via 16S rRNA gene sequencing. Both RoR and BoC exhibited temperatures elevated by 1 - 1.5°C compared to CoI. RoR further experienced reduced pH between 6.8 and 7.4. We observed pronounced differences in organic carbon pools at each of the three sites, with highest dissolved organic carbon concentrations at BoC and RoR, and highest particulate organic carbon concentrations and aggregate formation at BoC. Bacterioplankton communities at BoC were dominated by opportunistic copiotrophic taxa, such as *Alteromonas* and *Roseobacter*, known to thrive in phytoplankton blooms, as opposed to oligotrophic taxa dominating at CoI, such as members of the SAR11 clade. Therefore, we propose that bacterial communities were mainly influenced by the availability of organic carbon at the investigated sites. Our study provides a comprehensive characterization of organic carbon pools and bacterioplankton communities, highlighting the high heterogeneity of various components of the marine carbon cycle around the Galapagos Archipelago.

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22 **Abstract**

23 Bacteria play a crucial role in the marine carbon cycle, contributing to the production and

24 degradation of organic carbon. Here, we investigated organic carbon pools, aggregate formation,

25 and bacterioplankton communities in three contrasting oceanographic settings in the Galapagos

26 Archipelago. We studied a submarine CO_2 vent at Roca Redonda (RoR), an upwelling site at

27 Bolivar Channel (BoC) subjected to a weak El Niño event at the time of sampling in October

28 2014, as well as a site without volcanic or upwelling influence at Cowley Islet (CoI). We

29 recorded physico-chemical parameters, and quantified particulate and dissolved organic carbon,

30 transparent exopolymeric particles, and the potential of the water to form larger marine

31 aggregates. Free-living and particle-attached bacterial communities were assessed via 16S rRNA

32 gene sequencing. Both RoR and BoC exhibited temperatures elevated by 1 - 1.5°C compared to

33 CoI. RoR further experienced reduced pH between 6.8 and 7.4. We observed pronounced

34 differences in organic carbon pools at each of the three sites, with highest dissolved organic

35 carbon concentrations at BoC and RoR, and highest particulate organic carbon concentrations

36 and aggregate formation at BoC. Bacterioplankton communities at BoC were dominated by

37 opportunistic copiotrophic taxa, such as *Alteromonas* and *Roseobacter*, known to thrive in

38 phytoplankton blooms, as opposed to oligotrophic taxa dominating at CoI, such as members of

39 the SAR11 clade. Therefore, we propose that bacterial communities were mainly influenced by

40 the availability of organic carbon at the investigated sites. Our study provides a comprehensive

41 characterization of organic carbon pools and bacterioplankton communities, highlighting the

42 high heterogeneity of various components of the marine carbon cycle around the Galapagos

43 Archipelago.

1. Introduction

Bacteria play a major role in the marine carbon cycle as primary producers, as well as organic matter degraders. In surface waters, photosynthetic microorganisms convert CO_2 into organic carbon, effectively reducing the pCO_2 concentration in the upper ocean. While the majority of the organic carbon produced by photosynthesis is respired by heterotrophic organisms and released back into the atmosphere as CO_2 (Hutchins et al., 2017), a small portion escapes remineralization and sinks to deeper waters as particulate organic carbon (POC) in the form of marine aggregates (Alldredge & Silver, 1988; Ducklow et al., 2001; Giering et al., 2014). The formation of these marine aggregates may be enhanced by the presence of transparent exopolymeric particles (TEP; Passow, 2002), which can be formed by cellular exudation from living or lysed cells (Passow et al., 2001; Bhaskar et al., 2005), and which, due to their sticky nature, function as biological glue facilitating aggregation (Jackson, 1995; Engel, 2000). Marine aggregates are heavily colonized by microorganisms and constitute a hotspot of bacterial activity since they contain inorganic and organic nutrients in abundance (Azam and Malfatti, 2007). Particle-attached (PA) bacteria are able to hydrolyze and resuspend the POC of marine aggregates (Azam and Malfatti, 2007; Giering et al. 2014), thereby reducing the vertical flux of organic matter (Grossart & Ploug 2001). Conversely, free-living (FL) bacteria, which often constitute taxonomically and functionally distinct communities (Rieck et al., 2015), thrive on dissolved organic carbon (DOC) in the ocean (Azam & Malfatti, 2007). As such, the fate of organic carbon pools in the ocean is tightly coupled to the composition and activity of the FL and PA bacterioplankton community.

The Galapagos Archipelago consists of a group of volcanic islands, located in a complex system of tropical and temperate ocean currents, and intense upwelling zones. Seasonal upwelling occurs on the western side of the Galapagos Archipelago, where the Equatorial Undercurrent (EUC) collides with the iron-rich island platform and is diverted upwards, bringing colder (18°C) and inorganic nutrient-rich waters to the surface (Houvenaghel, 1978; Palacios, 2002). The increased nutrient supply during the upwelling season is creating a productive area where massive phytoplankton blooms occur and large amount of carbon are converted into organic matter (Jimenez, 1981; Feldman, 1986). Every few years, this seasonal upwelling is suppressed, and surface waters become warmer (26°C) and depleted of their nutrients. This anomaly, known as El Niño, is thereby preventing the formation of phytoplankton blooms

78   (Houvenaghel, 1978; Sweet et al. 2007; Liu et al. 2014). The eastern side of the Galapagos

79   Archipelago is governed by the South Equatorial Current (SEC) and does not experience

80   upwelling (Schaeffer et al., 2008). Here, although inorganic nutrient concentrations, specifically

81   nitrate, may reach higher concentrations, primary production is generally limited by the

82   availability of iron, resulting in conditions comparable to the high-nitrate low-chlorophyll

83   (HNLC) areas that characterize most of the eastern and central equatorial Pacific in the absence

84   of an upwelling system (Schaeffer et al., 2008). The Galapagos Archipelago is furthermore

85   among the world's most active volcanic areas today. As a consequence, many islands are

86   characterized by submarine gas seepage through fumaroles consisting mostly of CO_2 (Standish et

87   al., 1998), thereby locally reducing seawater pH and altering the chemical composition of the

88   seawater. Given these contrasting environmental conditions throughout the Galapagos

89   Archipelago, distinct FL and PA bacterioplankton communities as well as organic carbon pools

90   and aggregation processes are expected to occur in different regions of the Galapagos

91   Archipelago.

92   Previous studies documenting aspects of the marine carbon cycle off the Galapagos

93   Archipelago focused mostly on upwelling and El Niño events, using satellite imagery in

94   combination with *in situ* measurements to quantify physical water properties, inorganic nutrients

95   and chlorophyll a as proxy for primary production (Palacios, 2004; Sweet et al., 2007; Schaeffer

96   et al., 2008). Data on organic carbon pools around the Galapagos Archipelago are scarce and so

97   far restricted to POC inferred from remote sensing information (Gardner, Mishonov &

98   Richardson, 2006; Kislik et al., 2017). A direct quantification of DOC and POC, as well as TEP

99   and aggregation processes, is still lacking for this region. Furthermore, apart from isolated

100 observations during global ocean sampling campaigns (Rusch et al., 2007; Yilmaz et al., 2012),

101 no studies have as yet reported on bacterioplankton communities and their potential role in the

102 marine carbon cycle under the varied environmental settings of the Galapagos Archipelago.

103   The objective of the current study was to investigate various components of the marine

104 carbon cycle by jointly exploring organic carbon pools, aggregate formation, and bacterial

105 community composition under three contrasting environmental conditions around the Galapagos

106 Archipelago. The study sites were selected based on expected temperature and pH differences:

107 seasonal warming in an area otherwise influenced by upwelling, localized pH reduction, and an

108 area outside the upwelling or pH reduction zones. We aimed (i) to compare the physico-chemical

109 conditions, (ii) to quantify changes in organic carbon pools (POC, DOC, TEP), including the

110 potential for marine aggregate formation, and (iii) to assess differences in the diversity and

111 composition of FL and PA bacterial communities in the water column among these sites.

112 Thereby, we identified bacterial taxa that displayed strong changes in their contribution to

113 bacterioplankton communities at each of the sites, discussing their importance in the marine

114 carbon cycle under the selected environmental conditions.

115

116 **2. Materials and methods**

117 **2.1 Study site**

118 The sampling was conducted at three sites around the Galapagos Archipelago during a

119 week-long cruise from October 20 to October 27, 2014 (research permit: PNG Permiso de

120 investigacion científica PC-86-14; Fig. 1). The Bolivar Channel (BoC) west of Isabela Island

121 (latitude: -0.2560667°, longitude: -91.41733611°) is located in an upwelling region, but was

122 exhibiting elevated temperatures caused by the occurrence of a weak El Niño event during the

123 sampling period. Roca Redonda (RoR), located about 25 km north of Isabela Island (latitude:

124 0.2817528°, longitude: -91.61177222°), is the nascent peak of a submarine volcano (Standish et

125 al., 1998). CO_2 is released through numerous shallow (10 – 18 m) vents on the south-eastern side

126 of the peak, locally acidifying the water column. Cowley Islet (CoI), located east of Isabela

127 Island (latitude: -0.3861444°, longitude: -90.96356667°) showed similar bathymetry to RoR and

128 BoC, while lacking detectable influences from upwelling or geothermal processes.

129

130 **2.2 Water sampling**

131 At each site, spatially separated ($\geq$ 20 m) sub-sites with similar characteristics were selected

132 to replicate the conditions of each site across independent water samples. At CoI and BoC five

133 sub-sites were selected, while at RoR only four sub-sites were sampled due to the limited

134 locations offering similar conditions at accessible depth. At each sub-site, seawater was collected

135 at a depth of approximately 15 m using a 3 L Niskin bottle. At RoR this sampling depth

136 corresponded to 50 cm above the vent. Seawater samples were exported to the Centre for

137 Tropical Marine Research (ZMT), Bremen, Germany, with the approval of the Environmental

138 Ministry of Ecuador (export clearance number 2419373).

139   Temperature, pH, salinity, dissolved oxygen and chlorophyll a concentration of the water

140 column were measured with a Eureka MANTA 2 multi-probe (Eureka Environmental

141 Engineering, Texas, USA), at the same depth the seawater samples were taken. The

142 concentrations of inorganic nutrients (nitrate/nitrite and phosphate) were measured using a

143 continuous flow injection analyzer (FIAstar 5000, Foss Tecator, Denmark).

144   To investigate different components of the organic carbon pool in the water column, we

145 quantified DOC, TEP, and POC. DOC concentrations were measured by the high-temperature

146 combustion (HTC) technique (Wangersky, 1978; Dafner & Wangersky, 2002), using a TOC-

147 VCPH analyzer (Shimadzu, Mandel, Canada). For the determination of TEP concentrations, 300

148 mL water samples were filtered through 0.45 µm polycarbonate filters at a constant vacuum of

149 0.160 bar. TEP was then measured semi-quantitatively using the Xanthan equivalent method

150 (Engel, 2009; Passow & Alldredge, 1995). To quantify POC, we filtered 2 L of seawater through

151 a pre-combusted (at 400°C) and pre-weighed 47 mm glass microfiber filter (GF/F) in the field

152 using a Millipore® all-glass filtration apparatus. After filtration, we stored the filters in plastic

153 Petri dishes at room temperature (22°C) until they were dried overnight at 40°C in the laboratory

154 and weighed again. We determined the concentration of POC per litre as the difference in filter

155 weight divided by the filtered seawater volume. Data on measured water and carbon pool

156 parameters are available on Pangaea (https://doi.pangaea.de/10.1594/PANGAEA.890858).

157

158 **2.3 Rolling tank experiments**

159   To induce the natural formation of marine aggregates and simulate the continuous sinking of

160 the particles, we performed rolling tank experiments (Shanks and Edmondson, 1989). One

161 cylindrical Plexiglas rolling tank was filled with 1.5 L of the seawater collected at each sub-site.

162 The tanks were rotated for 48 hours at a constant speed of 3 turns per minute. Every 24 hours, we

163 measured aggregate formation by counting aggregates and classifying them into 5 size categories

164 (<1 mm, 1-3 mm, 3-5 mm, 5-10 mm and >10 mm). The rotation of the tanks was briefly

165   interrupted to count and measure the aggregates non-invasively. Based on the number of
166   aggregates in each category, we calculated the total volume of aggregates per tank, assuming
167   spherical shapes (Cárdenas et al., 2015). Data on aggregation potential are available on Pangaea
168   (https://doi.pangaea.de/10.1594/PANGAEA.890858).

169

170   **2.4 Bacterial community composition**

171   The FL and PA fractions of the bacterial community were separated via serial filtration. 1 L
172   of seawater was first filtered through a 3.0 μm polycarbonate filter to collect the PA fraction,
173   followed by filtration through a 0.2 μm polycarbonate filter to collect the FL fraction of the
174   bacterial community. Two to four technical replicates were taken at each sub-site. The
175   polycarbonate filters were folded into 1.5 ml Eppendorf tubes with 1 mL of RNAlater solution
176   (Ambion) and immediately stored at -20 °C until DNA extraction. DNA was extracted
177   individually from each technical replicate for both size fractions following the protocol
178   developed by Boström et al. (2004) for low biomass samples. The DNA was quantified using a
179   Bio Photometer Plus spectrophotometer (Eppendorf, Hamburg, Germany). To assess the
180   diversity and composition of the bacterial community, the V3-V4 hypervariable region of the
181   bacterial 16S rRNA gene was sequenced on the Illumina MiSeq PE platform at LGC Genomics
182   GmbH (Berlin, Germany) using a 2x300bp paired-end approach with universal bacterial primers
183   (Klindworth et al. 2013). Separate libraries were prepared for technical replicates, which were
184   then individually sequenced.

185   The primer-clipped sequences provided by LGC Genomics were further processed
186   according to (Hassenrück et al., 2016). Sequences were quality trimmed with a sliding window
187   of 4 bases and a minimum average quality score of 15 using trimmomatic version 0.32 (Bolger,
188   Lohse and Usadel 2014). Paired-end reads were merged with PEAR version 0.9.5 (Zhang et al.
189   2014) using a minimum overlap of 10 base pairs, retaining only merged reads with a minimum
190   and maximum sequence length of 350 and 500 base pairs, respectively. OTU clustering was
191   performed with swarm version 2.0 using the fastidious algorithm with default parameters (Mahé
192   et al. 2014). OTUs consisting of only 1 sequence (singletons) were removed from the data set.
193   As representative sequence per OTU, the seed sequence of each swarm was taxonomically
194   classified with silvangs with default settings (https://www.arb-silva.de/ngs/; date accessed

195 02.08.2017) using the SILVA ribosomal RNA gene database version 128 as reference (Quast et

196 al., 2013). Only OTUs that occurred in at least 2 of the technical replicates per sub-site and size

197 fraction were included in the final data set. OTUs of technical replicates were then merged into

198 one community profile per sub-site by taking the sum of the sequence counts per OTU.

199 Furthermore, OTUs with a sequence similarity of less than 93% to the reference database, OTUs

200 unclassified at phylum level, and OTUs matching chloroplast and mitochondrial sequences were

201 excluded from the analysis. The primer-clipped sequences were deposited in the European

202 Nucleotide Archive (ENA, Toribio et al. 2017) using the data brokerage service of the German

203 Federation for Biological Data (GFBio; Diepenbroek et al. 2014), in compliance with the

204 Minimal Information about any (X) Sequence (MIxS) standard (Yilmaz et al. 2011) and are

205 available with the accession PRJEB27168. The final OTU tables are accessible at Pangaea

206 (https://doi.pangaea.de/10.1594/PANGAEA.890858).

207

208 **2.5 Statistical analysis**

209   We performed a principal component analysis (PCA) on the measured water and carbon

210 pool parameters after standardizing each variable using z-scoring (Legendre & Legendre, 1998;

211 Ramette, 2007). Kruskal-Wallis tests were used to detect differences in water and carbon pool

212 parameters between study sites, followed by pairwise comparisons based on Wilcoxon tests with

213 False Discovery Rate (FDR)-corrected p-values (Benjamini and Hochberg, 1995). Aggregation

214 potential was assessed based on the increase in aggregate volume over time during the rolling

215 tank experiments. Differences in aggregate volume between the study sites were tested using

216 general linear mixed models (GLMMs) with sub-site, i.e. rolling tank, as random factor.

217   Alpha diversity, specifically effective species richness, of the bacterial communities in the

218 FL and PA fraction was assessed with the inverse Simpson index (Chao et al. 2014). No

219 subsampling was required to account for unequal library sizes, since rarefaction curves based on

220 the inverse Simpson index, unlike OTU number, were saturated at observed sequencing depths

221 (SI Fig. 1). Differences in alpha diversity between FL and PA bacterial communities were tested

222 with a paired Wilcoxon test. Patterns in alpha diversity between sampling sites were then

223 analyzed separately within each size fraction using Kruskal-Wallis tests. P-values of pairwise

224 comparisons based on Wilcoxon tests were adjusted using FDR correction (Benjamini and

225   Hochberg 1995). The relationship between alpha diversity and temperature and pH was assessed

226   via Spearman rank correlations.

227   Differences in the composition of the bacterial communities (beta diversity) among samples

228   were explored by cluster analysis and tested by analysis of similarity (ANOSIM) using a Bray-

229   Curtis dissimilarity matrix calculated separately from the OTU data of the FL and PA fraction.

230   Similarity percentages (SIMPER) were used to identify the OTUs contributing most to the

231   dissimilarity between the FL and PA fraction within each water sample. The amount of variation

232   in bacterial community composition explained by sampling site as well as temperature and pH,

233   as parameters of specific interest, was assessed via redundancy analysis (RDA). Separate RDAs

234   were performed for FL and PA communities. Prior to RDA, the data set was reduced by

235   removing OTUs with low sample coverage and rare OTUs, i.e. OTUs occurring in less than 50%

236   of the samples from at least one site and those contributing less than 1% to the sequences in at

237   least one sample. There were four main reasons for this reduction step: (i) We were foremost

238   interested in patterns among dominant OTUs. (ii) We wanted to remove statistically

239   uninformative OTUs. OTUs with a low sample coverage are likely outliers and not

240   representative of the conditions in the sampled area, and may therefore bias statistical tests. (iii)

241   To perform further statistical analyses, a centered log ratio (clr)-transformation was applied to

242   the OTU matrix. To enable the log operation a pseudo-count of 0.5 was added to all sequence

243   counts in the OTU matrix (Fernandes et al. 2014). If an OTU matrix contains too many zeros,

244   this addition may bias the original data. Therefore, rare OTUs were excluded to reduce the

245   proportion of zero counts in the OTU matrix. (iv) By reducing the number of OTUs we were

246   further able to increase the sensitivity of statistical approaches requiring multiple testing by

247   applying a less severe p-value correction due to the reduced number of multiple tests. While this

248   removal affected 99% and 96% of the OTUs, and 33% and 25% of the sequences, in the FL and

249   PA fractions, respectively, we confirmed that it did not alter trends in beta diversity (Mantel test,

250    $r > 0.99$, $p < 0.001$). Sequence counts were further clr-transformed with the aldex.clr function of

251   the R package ALDEx2, using the median of 128 Monte Carlo Dirichlet instances (Fernandes et

252   al. 2014). ALDEx2 was also used to identify differentially abundant OTUs between sampling

253   sites in the reduced data set of each size fraction at a parametric FDR-adjusted and a non-

254   parametric unadjusted significance threshold of 0.05.

255   All statistical analyses were conducted in R, using the R core distribution (R Core Team,
256   2017) with the additional R packages lmerTest (Kuznetsova et al., 2916), vegan (Oksanen et al.,
257   2017), and ALDEx2 (Fernandes et al. 2014). All scripts (R and bash) for sequence processing
258   and statistical data analysis are available with the data set on Pangaea
259   (https://doi.pangaea.de/10.1594/PANGAEA.890858).

260

261   **3. Results**

262   **3.1 Water parameters and organic carbon pools**

263   We observed pronounced differences in measured water parameters and the components of
264   the organic carbon pool in the water column between the sampling sites at CoI, RoR and BoC.
265   Both RoR and BoC exhibited temperatures elevated by approximately 1 to 1.5°C compared to
266   CoI, where values of about 22°C were recorded (Table 1). Highest temperatures occurred at BoC
267   with maximum values of 23.8°C. pH ranged from 6.83 at RoR to 8.35 at BoC. Whereas at CoI
268   and BoC measured pH values were always above 8, observed pH at RoR did not exceed 7.35
269   (Table 1). Salinity only varied within a small range among all three sites from 34.38 to 34.72
270   psu. With the exception of one oxygen measurement of 8.19 mg L^{-1} at RoR, dissolved oxygen
271   concentrations were highest at CoI with median values of 6.78 mg L^{-1}, and lowest at BoC with
272   6.43 mg L^{-1}. Inorganic nutrients, chlorophyll a, and POC exhibited similar patterns between
273   sampling sites with values 1.5 to 2 times as high at CoI and BoC than at RoR (Table 1). For
274   instance, while median POC concentrations of 32.93 and 36.65 mg L^{-1} were measured at CoI and
275   BoC, respectively, POC concentrations at RoR did not exceed 22.37 mg L^{-1}. Conversely, we
276   observed highest DOC concentrations at RoR and BoC, with median values of 100.54 µmol L^{-1}
277   and 92.14 µmol L^{-1}, respectively, as opposed to 80.87 µmol L^{-1} at CoI. The amount of TEP in the
278   water column did not differ significantly between sampling sites (Table 1). Additional to these
279   patterns among sampling sites, most of the measured parameters, except pH, were quite
280   heterogeneous even at the same site, so that the within-site variation was sometimes as large as
281   the between-site variation (Table 1).

282   The trends in water and carbon pool parameters were summarized in a PCA, which showed
283   well-defined differences among sampling sites (Fig. 2). RoR was separated from CoI and BoC

284 along principal component (PC) 1, which captured 41.89% of the total variation in the data set.

285 This separation was driven mostly by pH, nitrate/nitrite, chlorophyll a, and POC. Additionally,

286 CoI was separated from RoR and BoC along PC2, which captured 27.59% of the total variation

287 in the data set and was mostly determined by temperature, salinity and DOC (Fig. 2).

288

3.2 Aggregate formation

290 After filling the rolling tanks, the total aggregate volume per rolling tank at CoI and BoC of 0.40

291 $\pm$ 0.19 and 0.44 $\pm$ 0.13 cm³ (mean $\pm$ standard deviation), respectively, was about one order of

292 magnitude higher than at RoR (Fig. 3). Over the course of the experiment, aggregate volume in

293 the rolling tanks increased at all sites (GLMM, $F_{2,37}$ = 6.26, p = 0.005). This potential for

294 aggregate formation differed significantly between sampling sites (GLMM, $F_{2,37}$ = 25.29, p <

295 0.001). The strongest increase was observed at BoC, where total aggregate volume had more

296 than tripled after 48 h (1.33 $\pm$ 0.21 cm³) compared to the beginning of the experiment (Fig. 3). At

297 CoI total aggregate volume increased only slightly to 0.64 $\pm$ 0.09 cm³, whereas at RoR even after

298 48h total aggregate volume (0.08 $\pm$ 0.06 cm³) did not reach initial mean values reported for the

299 other two sites (Fig. 3). Additional to the differences in total aggregate volume between the

300 sampling sites, the size distribution of aggregates was markedly different, with larger aggregates

301 in the range of 5 to 10 mm dominating the rolling tanks at CoI and BoC compared to RoR, where

302 the size of the majority of the aggregates did not exceed 3 mm (SI Fig. 2). Therefore, even

303 though there were more, but smaller, aggregates in the rolling tanks at RoR, their total volume

304 was not comparable to that of the fewer, but larger, aggregates at CoI and BoC.

305

3.3 Bacterial diversity and community composition

307 A total of 798,402 quality-filtered, non-singleton, and taxonomically classified sequences

308 were obtained, which clustered into 14,811 OTUs. After the merging of technical replicates and

309 removal of OTUs not occurring in at least 2 technical replicates per sub-site, 90% of the

310 sequences were retained while denoising the data set by excluding 60% of the OTUs, resulting in

311 a final number of 717,420 sequences and 5,970 OTUs.

312   Effective species richness (inverse Simpson index) ranged from a minimum of 2.31 in the

313   FL fraction at RoR to a maximum of 65.62 in the PA fraction at CoI. There was no consistent

314   difference between FL and PA effective species richness within the same water sample (paired

315   Wilcoxon test, V = 47, p = 0.761), although values varied considerably (Table 1). Within both

316   the FL and PA fraction, highest median inverse Simpson indices of 33.82 and 48.12,

317   respectively, were recorded at CoI, followed by 21.04 and 17.80 at RoR and 8.46 and 4.85 at

318   BoC, where effective species richness was within a similar range (Table 1).

319   At class-level, the bacterial communities around the Galapagos Archipelago were

320   dominated by *Gammaproteobacteria* (48% of the total number of sequences), *Flavobacteriia*

321   (17%), *Alphaproteobacteria* (16%), and *Cyanobacteria* (10%). Among these dominant bacterial

322   classes, we detected higher proportions of *Flavobacteriia*, *Alphaproteobacteria*, and

323   *Cyanobacteria* at CoI, whereas *Gammaproteobacteria* were predominantly found at RoR and

324   BoC (Fig. 4). At OTU level, pairwise Bray-Curtis dissimilarities within each water sample

325   between FL and PA bacterial communities ranged from 0.40 to 0.88, and were comparable to

326   Bray-Curtis dissimilarities among samples within the same size fraction. At CoI these

327   differences between FL and PA bacterial communities were attributed mostly to a lower

328   proportion of OTUs affiliated with the cyanobacterial genus *Synechococcus* and the OM60

329   (NOR5) clade of the *Gammaproteobacteria*, as well as a higher proportion of an OTU affiliated

330   with the verrucomicrobial genus *Roseibacillus* in the PA fraction. At RoR and BoC, other OTUs

331   contributed most to the differences between size fractions. There, the PA fraction was often

332   dominated by one OTU affiliated with the gammaproteobacterial genus *Alteromonas*, which

333   constituted 30 to 55% of the sequences in more than half of the samples at RoR and BoC.

334   Cluster analysis based on Bray-Curtis dissimilarities revealed prominent patterns in bacterial

335   community composition among size fractions and sampling sites (Fig. 4), which we further

336   explored using ANOSIM. In the FL fraction, bacterial communities from CoI were very similar

337   to each other with an average within-group dissimilarity of 0.26, and well-separated from those

338   at RoR and BoC, despite their comparatively low between-group dissimilarity (Table 2). FL

339   communities at RoR and BoC were more heterogeneous and did not show a strong separation.

340   Within the PA fraction, we observed average within-group Bray-Curtis dissimilarities of

341   approximately 0.50 at all sampling sites. Similar to the FL fraction, strongest differences in

342   bacterial community composition were detected between CoI and each of the other two sampling

343   sites. Additionally, the separation of bacterial communities from RoR and BoC was more

344   pronounced in the PA fraction (Table 2).

345   Focusing on common and dominant OTUs, RDA showed that sampling site was able to

346   explain 57% and 49% of the variation in bacterial community composition in the FL and PA

347   fraction, respectively (Table 3). The explanatory power of temperature and pH was not as high as

348   that of sampling site, although the resulting RDA ordinations were very similar (SI Fig. 3).

349   Among the two factors temperature and pH, temperature was able to explain a larger proportion

350   of the variation in community composition than pH. For FL communities, the amount of

351   variation explained by pH was negligible, whereas its contribution to explaining variation in PA

352   communities was more substantial (Table 3). Including additional water and carbon pool

353   parameters did not increase the model fit according to the Akaike Information Criterion (data not

354   shown).

355   To further identify, which OTUs were responsible for the patterns in community

356   composition, the differences in the proportion of individual OTUs between sampling sites were

357   tested with ALDEx2. In total, 36 and 30 OTUs were detected as differentially abundant between

358   sampling sites within the FL and PA fraction, respectively (Fig. 5). Of those OTUs, 17 showed

359   similar trends in both size fractions. The majority of differentially abundant OTUs in both size

360   fractions was affiliated with *Gammaproteobacteria*, *Flavobacteriia*, *Alphaproteobacteria*, and

361   *Verrucomicrobiae*, although within each size fraction often different OTUs of those classes

362   exhibited trends between sampling sites. In the FL fraction, differentially abundant OTUs were

363   further found among the *Cyanobacteria* and *Acidimicrobiia*, and in the PA fraction among the

364   *Cytophaga*. In the cases where more than one OTU per class was detected as differentially

365   abundant, those OTUs often exhibited divergent patterns between sampling sites (Fig. 5).

366   Among the *Gammaproteobacteria*, OTUs affiliated with the genera *Alteromonas*,

367   *Pseudoalteromonas* and *Oleibacter* were enriched in both size fractions at RoR and BoC,

368   whereas OTUs affiliated with *Balneatrix* and the OM60 (NOR5) clade were depleted at these

369   sites compared to CoI. Within the PA fraction, one OTU of the genus *Thiothrix* was further only

370   enriched at RoR. OTUs of the flavobacterial genera *Flavicella*, *Formosa*, and the NS4 marine

371   group were characteristic for CoI in both size fractions. OTUs of the genus *Polaribacter* showed

372 divergent trends among sampling sites with different OTUs enriched at either CoI or BoC. Other

373 OTUs of the *Flavobacteriia* that were strongly enriched at BoC were affiliated with *Nonlabens*,

374 *Mesoflavibacter*, and *Dokdonia*, although differences in the latter were only detected in the FL

375 fraction. Among the *Alphaproteobacteria*, more OTUs exhibited differences in their sequence

376 proportions between sampling sites in the FL than in the PA fraction. Especially OTUs of the

377 *Rhodobacterales*, i.e. *Candidatus* Pelagibacter and another representative of the *Surface 1* clade

378 of SAR11, as well as *Sulfitobacter* and other *Rhodobacteraceae*, tended to be higher enriched at

379 CoI than at RoR and BoC, although for some OTUs this trend was restricted to the FL fraction.

380 In the case of *Sulfitobacter*, divergent trends were observed with different OTUs enriched at

381 either CoI or BoC. OTUs predominantly found at RoR and BoC were affiliated with the genus

382 *Shimia* and *Ruegeria*, likewise members of the *Rhodobacteraceae*, although for the latter this

383 pattern was only detected in the PA fraction. Among the *Verrucomicrobiae*, OTUs of the genus

384 *Roseibacillus* were depleted at BoC in both site fractions. OTUs affiliated with *Cyanobacteria*

385 only differed among sampling sites in the FL fraction, with a decreasing trend of two OTUs of

386 the genera *Prochlorococcus* and *Synechococcus*, respectively, from CoI to RoR and BoC. In

387 general, 20 to 80% of the sequences per sample belonged to differentially abundant OTUs.

388 Especially in the PA fraction, there was an overall increase in the sequence proportion of

389 differentially abundant OTUs from CoI to RoR and BoC.

390

4. Discussion

4.1 Physico-chemical characteristics

393 We characterized the physico-chemical properties of the water column at each of the three

394 sites at BoC, RoR and CoI. As expected, the three sites exhibited different temperature and pH

395 conditions. Additionally, we observed marked differences in inorganic nutrients and chlorophyll

396 a concentrations. At CoI to the east of Isabela Island, temperature and pH were comparable to

397 previous observations at this site, and can be considered representative for the eastern region of

398 the Galapagos Archipelago during that time of the year (Sweet et al., 2007; Liu et al., 2014).

399 Chlorophyll a concentrations were comparatively low considering the amount of nitrate/nitrite in

400 the water column, resembling HNLC conditions common to the eastern and central equatorial

401 Pacific (Schaeffer et al., 2008).

402   BoC to the west of Isabela Island and CoI to the east, are influenced by different prevailing
403   ocean currents (Schaeffer et al., 2008; Liu et al., 2014). During upwelling, which constitutes
404   regular conditions at BoC, temperatures drop to about 18°C, and inorganic nutrient and
405   chlorophyll a concentrations are expected to be considerably higher than any observed in this
406   study (Liu et al., 2014). However, the El Niño event during the sampling period was not as
407   strong as previous events, where temperatures of approximately 26°C were reached (Chavez et
408   al., 1999; Palacios, 2004). This suggests that although the upwelling of the EUC was severely
409   reduced, it was not completely inhibited at the time of sampling, thereby limiting but not
410   eliminating the physical resupply of nutrients to surface waters. Probably as a consequence of the
411   weak El Niño, apart from temperature, most of the other observed water parameters were
412   comparable to CoI, and did not resemble the extremes recorded during strong El Niño or La Niña
413   events (Chavez et al., 1999; Palacios, 2004; Liu et al., 2014).

414   At the submarine volcano at RoR, pH values were markedly lower than what is considered
415   ambient surface ocean pH around the Galapagos Archipelago, or indeed most other oceanic
416   regions today (IPCC, 2013), and water temperatures were comparable to BoC. The current
417   system that is influencing BoC, also supplies the north of Isabela Island with nutrient-rich waters
418   under upwelling conditions (Palacios, 2002). However, the weak El Niño event at the time of
419   sampling seemed to strongly diminish this influence, thereby resulting in a stronger influence of
420   the SEC with reduced inorganic nutrients and chlorophyll a concentrations, as well as elevated
421   temperatures at RoR (Schaeffer et al., 2008; Liu et al. 2014).

422

423   **4.2 Organic carbon pools under differing environmental regimes**

424   The amount of organic carbon, partitioned into particulate and dissolved organic carbon that
425   we measured in the surface water off the Galapagos Archipelago was within the range of
426   previous estimates for the equatorial Pacific Ocean (Ogawa & Tanoue, 2003; Gardner, Mishonov
427   & Richardson, 2006). Additional to DOC and POC concentration, we evaluated TEP content and
428   aggregation potential to gain a better understanding of the processes that govern particle
429   formation and carbon export. Our results suggest marked differences in the physical and
430   biological processes that influence organic carbon pools at the three sampling sites at the time of
431   sampling, despite a pronounced local variability.

432 At CoI and BoC, congruently elevated inorganic nutrient, chlorophyll a and POC
433 concentrations may indicate increased phytoplankton abundance, with primary production
434 contributing to the generation of POC (Ducklow et al., 2001). However, despite these
435 similarities, DOC concentrations and aggregation potential were both considerably higher at
436 BoC. We observed a positive correlation between DOC concentrations and temperature,
437 suggesting that DOC accumulation may be stimulated by the elevated temperature at BoC, a
438 relationship, which has been previously experimentally documented, although for larger
439 temperature differences than those observed here (Wohlers et al., 2009; Kim et al., 2011;
440 Biermann, Engel & Riebesell, 2014). It has also been shown that increased temperatures promote
441 aggregation formation in phytoplankton blooms due to an increased release of TEP, which is
442 then quickly converted to aggregates (Claquin et al., 2008; Piontek et al., 2009; Biermann, Engel
443 & Riebesell, 2014), and which may explain the high aggregation potential at BoC. Despite the
444 elevated temperatures, the observed patterns in organic carbon pools and aggregate formation at
445 BoC therefore further corroborate the occurrence of a weak El Niño event at the time of
446 sampling, where some of the upwelling characteristics were still retained and lead to the
447 formation of a phytoplankton bloom.

448 DOC concentrations at RoR were similarly elevated as at BoC, presumably likewise related
449 to increased temperatures (Wohlers et al., 2009; Kim et al., 2011; Biermann, Engel & Riebesell,
450 2014) rather than reduced pH (Zark, Riebesell & Dittmar, 2015). Interestingly, the aggregation
451 potential at RoR was severely diminished compared to the other two sites despite comparable
452 TEP concentrations. Aggregate formation from TEP can be influenced by a decrease in pH,
453 which reduces the stickiness of TEP and thereby its potential to form aggregates (Mari, 2008;
454 Cárdenas et al., 2015). The increased CO_2 concentrations in the water column at RoR therefore
455 may have directly impacted aggregate formation. Theoretically, the increased availability of CO_2
456 at RoR can also fuel planktonic primary production (Riebesell et al., 2007). However,
457 chlorophyll a concentrations recorded at RoR suggest otherwise, indicating that other factors
458 may be limiting phytoplankton growth at this site. Indeed, we detected only low amounts of
459 nitrate and phosphate in the water column. Additionally, unobserved parameters, such as iron,
460 may further limit primary production (Palacios, 2002; Tyrrell et al., 2005). These circumstances
461 highlight the fact that the strength of CO_2 effects is strongly dependent on the environmental
462 setting (Fu et al., 2008; Sala et al., 2016).

463   There are several other factors, which may contribute to explaining the observed patterns in

464   organic carbon pools, and which were not investigated here. For instance, the source and

465   composition of the DOC in the water column may affect its fate in the marine carbon cycle

466   (Thornton, 2014). Apart from the recalcitrant DOC pool in the water column, processes such as

467   cell lysis, leakage, and exudation by planktonic and benthic organisms replenish the pool of

468   labile DOC (Haas & Wild, 2010; Moran et al., 2016). For instance, it is likely that at the time of

469   sampling distinct phytoplankton communities occurred at each of the sampling sites, with a more

470   diverse community at CoI than at the other two sites (SI Fig. 4), which could have influenced the

471   concentration and composition of the organic carbon pool (Thornton, 2014). Furthermore, the

472   composition of the benthic community most likely differed among the sampling sites, which fall

473   into separate biogeographic regions with distinct benthic communities (Edgar et al., 2004),

474   presumably resulting in differences in the amount and composition of the DOC being released

475   into the water column (Haas & Wild, 2010; Haas et al., 2011; Cárdenas et al., 2015).

476

477   4.3 Bacterioplankton communities under contrasting environmental conditions

478   Bacterial communities in the water column, either free-living or attached to particles, play

479   an important role in the marine carbon cycle, and are strongly interconnected with organic

480   carbon dynamics (Azam & Malfatti, 2007). This study is the first to describe the diversity and

481   composition of bacterial communities in the waters around the Galapagos Archipelago, apart

482   from isolated observations during the Global Ocean Sampling (GOS) Expedition (Rusch et al.,

483   2007; Yilmaz et al., 2012). In general and at a coarse taxonomic resolution, the composition of

484   the bacterial community described in this study is similar to previous observations from tropical

485   ocean surface waters, including those from the vicinity of the Galapagos Archipelago (Rusch et

486   al., 2007; Zinger et al., 2011; Yilmaz et al., 2012; Sunagawa et al., 2015; Suzuki et al., 2017).

487   However, at a higher taxonomic as well as spatial resolution, we observed pronounced

488   differences in bacterial community composition between size fractions as well as sampling sites.

489   A sizeable proportion of up to 80% of the sequences per sample were affiliated with taxa that

490   differed in their relative contribution to community composition between the sampling sites,

491   suggesting a major restructuring of bacterial communities with potential implications for

492   bacterial processes involved in carbon cycling in the water column.

493   Of the observed environmental parameters, temperature was the main determinant of

494 bacterial community composition in both the FL and PA fractions, whereas pH only played a

495 minor role for PA communities. Interestingly, given the correlation of temperature and DOC

496 concentrations in our study, the variation in bacterial community composition explained by

497 temperature, could also be related to changes in DOC concentrations (Borcard, Legendre &

498 Drapeau, 1992; Ramette, 2007). Changes in bacterial community composition related to

499 temperature and DOC concentrations, respectively, and to a lesser degree pH, further

500 corresponded well with the observed pattern between sampling sites, with communities from

501 RoR and BoC, i.e. the two sites with elevated temperature and DOC concentrations, being more

502 similar to each other than to communities from CoI. In general, these dissimilarities were

503 attributed to a decreased proportion of what could be considered typical oligotrophic

504 bacterioplankton taxa at RoR and BoC, which were strongly dominated by opportunistic

505 copiotrophic bacteria (SI table 1). These changes in community composition therefore also

506 affected community evenness, evident in the strongly diminished effective species richness at

507 RoR and BoC. In the remainder of this section we will explore the patterns in bacterioplankton

508 composition in more detail.

509   The only differentially abundant OTU that was enriched only at RoR was affiliated with the

510 gammaproteobacterial genus *Thiothrix* and occurred almost exclusively in the PA fraction.

511 *Thiothrix* is a filamentous bacterium capable of sulfur oxidation and usually associated with

512 sulfidic hydrothermal vents, where it exhibits an attached lifestyle (Tarasov et al., 2005;

513 Gugliandolo, Italiano & Maugeri, 2006; Bailey et al., 2011; Giovannelli et al., 2013; Zapata-

514 Hernández et al., 2014). Its occurrence at RoR may indicate the presence of sulfide in the area of

515 the hydrothermal gas venting at RoR. Although there were no obvious indications that sulfide

516 was present at the sampling site, i.e. we did not observe a sulfidic smell or white bacterial mats,

517 it is still possible that the community on the particles originated from sulfidic vents that exist in

518 the vicinity of RoR and host mats of bacterial sulfur oxidizers, or that low concentrations of

519 sulfide persisted (Gallardo & Espinoza, 2007). In the first case, the presence of an OTU may also

520 not necessarily implicate metabolic activity (Lanzen et al., 2011). Furthermore, we did not detect

521 any OTUs enriched exclusively at RoR in the FL fraction. Presumably, the exposure to the

522 acidified water at RoR was insufficient to trigger changes in the composition of the FL bacterial

523 community (Weinbauer et al., 2010).

524   *Synechococcus* and *Prochlorococcus* are two cyanobacterial genera, which are known to

525   comprise the majority of bacterioplankton primary producers in the oligotrophic eastern

526   equatorial Pacific, especially in HNLC areas (Partensky, Blanchot & Vaulot, 1999; Zubkov et

527   al., 2003; Alvain et al., 2008; Brown et al., 2014). Here, they were predominantly found in the

528   FL fraction at CoI, where they may contribute considerably to TEP production (Iuculano et al.,

529   2017). At BoC, and to some degree at RoR, they made up a smaller, although in the case of

530   *Synechococcus* still substantial, part of the FL bacterioplankton community. Assuming that a

531   diminished phytoplankton bloom developed at BoC under the weak El Niño conditions,

532   eukaryotic phytoplankton, such as representatives of the genera *Thalassiosira*, *Chaetoceros*,

533   *Emiliania*, which have been previously documented in the Galapagos upwelling system

534   (Jimenez, 1981), and which were presumably present at the sampling sites at the time of

535   sampling (SI Fig. 4), may have dominated primary production instead of taxa better adapted to

536   non-bloom conditions (Zubkov et al., 2003; Brown et al., 2014).

537   The remainder of differentially abundant OTUs were affiliated with heterotrophic bacteria

538   involved in the degradation of organic matter. We observed a replacement of organic matter

539   degrading bacterial taxa that may be indicative of functional redundancy, i.e. changing

540   community composition while function is retained (Allison & Martiny, 2008), but also of distinct

541   bacterial communities adapted to the degradation of different kinds of organic matter, i.e.

542   performing different functions depending on the concentration and composition of the available

543   organic matter (Giovannoni, 2005; Mou et al., 2008; Giebel et al., 2011; Moran et al., 2016). The

544   latter is supported by the differential abundance of mostly oligotrophic taxa at CoI and

545   copiotrophic taxa at RoR and BoC. Among the OTUs predominantly found at CoI, especially

546   members of the SAR11 clade are known to constitute the majority of heterotrophic

547   bacterioplankton in oligotrophic environments, where they are adapted to the degradation of

548   organic matter at low concentrations and predominantly of labile or semi-labile character

549   (Giovannoni, 2005; Moran et al., 2016; Bunse & Pinhassi, 2017). During periods of high organic

550   matter availability, such as phytoplankton blooms, these oligotrophic taxa are generally

551   outcompeted by copiotrophic organisms.

552   Bacterial communities at RoR and BoC were dominated by OTUs affiliated with

553   representatives of the alphaproteobacterial *Roseobacter* clade (e.g. *Shimia, Ruegeria*), several

554   flavobacterial groups, as well as *Alteromonas*, and *Pseudoalteromonas* (*Gammaproteobacteria*).

555   In general, taxa enriched at RoR and BoC have been previously associated with phytoplankton

556   blooms in several oceanic regions, where they contribute to the degradation of mostly labile

557   organic matter during the decline of the bloom (Buchan et al., 2014; Teeling et al., 2016). Their

558   opportunistic copiotrophic lifestyle is well-suited to exploit short-term organic matter pulses in

559   an otherwise nutrient-poor environment (López-Pérez et al., 2012). *Alteromonas* was foremost

560   enriched in PA fraction. Its genetic repertoire encodes for a wide variety of organic matter

561   degrading enzymes, making them highly adapted to utilize the rich availability of substrates on

562   marine aggregates (López-Pérez et al., 2012).

563   The high proportion of *Alteromonas* in the PA fraction also largely contributed to the

564   dissimilarity between size fractions at RoR and BoC, whereas at CoI the prevalence of

565   *Synechococcus* in the FL fraction was most responsible. Therefore, although FL and PA bacterial

566   communities were consistently different from each other (Rieck et al., 2015; Suzuki et al., 2017),

567   the taxa driving this separation were dependent on the environmental setting. The differences

568   between FL and PA communities were further determined by changes in OTU proportions rather

569   than by a replacement of dominant OTUs. This observation is consistent with Bižić-Ionescu et al.

570   (2015) and Mestre et al. (2017), who detected a considerable proportion of shared taxa between

571   different size fractions, which however differed drastically in their contribution to the

572   community composition of the respective size fractions. Barring technical biases, this situation

573   may indicate that many bacteria are able to switch between FL and PA lifestyles (Grossart, 2010;

574   Bižić-Ionescu et al., 2015; Mestre et al., 2017).

575   Interestingly, in several instances we observed divergent trends in OTU proportions between

576   the sampling sites within the same taxonomic group, specifically *Polaribacter* (*Flavobacteriia*),

577   *Sulfitobacter* and other *Rhodobacteraceae* (*Alphaproteobacteria*). These OTUs presumably

578   represent different ecotypes of closely related strains that are adapted to contrasting

579   environmental conditions. Such a phenomenon has been previously reported for the

580   aforementioned taxa, where both oligotrophic and copiotrophic strains with opposing genetic

581   potential were discovered within the same genus (Xing et al., 2015). Sub-genus and sub-species

582   diversity of ecotypes is widespread in microbial ecology, highlighting the importance of high

583   resolution methods, such as the approach employed here (Eren et al., 2014; Mahé et al., 2014).

584   Overall, the taxonomic profile of the bacterial communities at RoR and BoC closely
585   resembled a community that may be expected during and after phytoplankton blooms occurring
586   under upwelling conditions. We consider this further evidence that the west of Isabela Island was
587   experiencing a weak upwelling event, and associated phytoplankton bloom, during the time of
588   sampling. Indeed, considering the temperature and DOC preferences of the taxonomic groups
589   enriched at RoR and BoC, DOC concentrations, presumably together with DOC composition,
590   may constitute the more likely reason for the observed trends in community composition than
591   any of the other measured environmental parameters. Interestingly, bacterial community
592   composition seemed unrelated to POC concentrations, which differed significantly between RoR
593   and BoC. More extensive data on organic matter quality and composition, which may determine
594   community composition as strongly as quantity, as well as bacterial abundance, which may be
595   more strongly affected by organic matter quantity than community composition (Pinhassi et al.,
596   2004; Ortega-Retuerta et al., 2013, Buchan et al., 2014; López-Pérez et al., 2016; Osterholz et
597   al., 2016), will be necessary to further disentangle organic matter and bacterial community
598   dynamics around the Galapagos Archipelago.

599

600   **4.4 Conclusion**

601   Our study presents a detailed description of environmental conditions, organic carbon pools,
602   aggregation processes, and bacterioplankton composition, highlighting the high heterogeneity of
603   various components of the marine carbon cycle and bacterial communities around the Galapagos
604   Archipelago, and providing baseline data for future investigations. By employing a combination
605   of different analytical approaches, we were able to characterize the conditions at each of the
606   sampling sites more comprehensively than by any method on its own, and to demonstrate the
607   tight coupling between organic carbon dynamics and bacterioplankton communities. Still, our
608   findings only represent a snapshot in time in a temporally highly variable system. Further
609   research based on repeated observations, including qualitative data on carbon pools, quantitative
610   information on bacterial abundance, as well as estimates of bacterial metabolic activity will be
611   necessary to overcome the limitations and to answer the open questions of the current study, to
612   better understand organic carbon and bacterial community dynamics around the Galapagos
613   Archipelago.

614

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625

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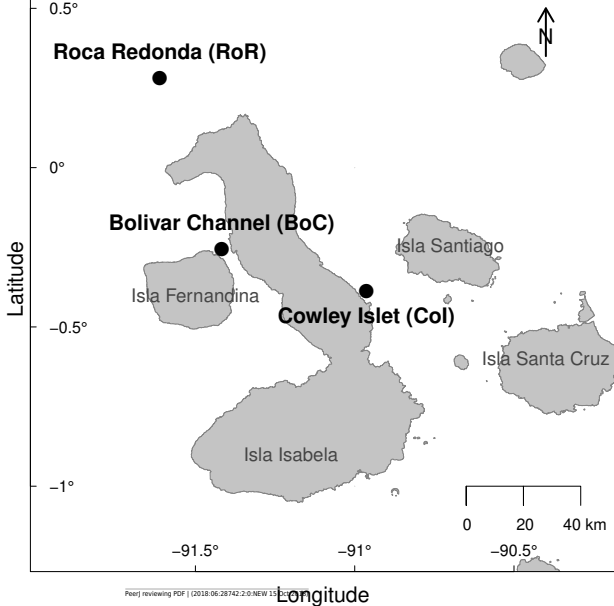
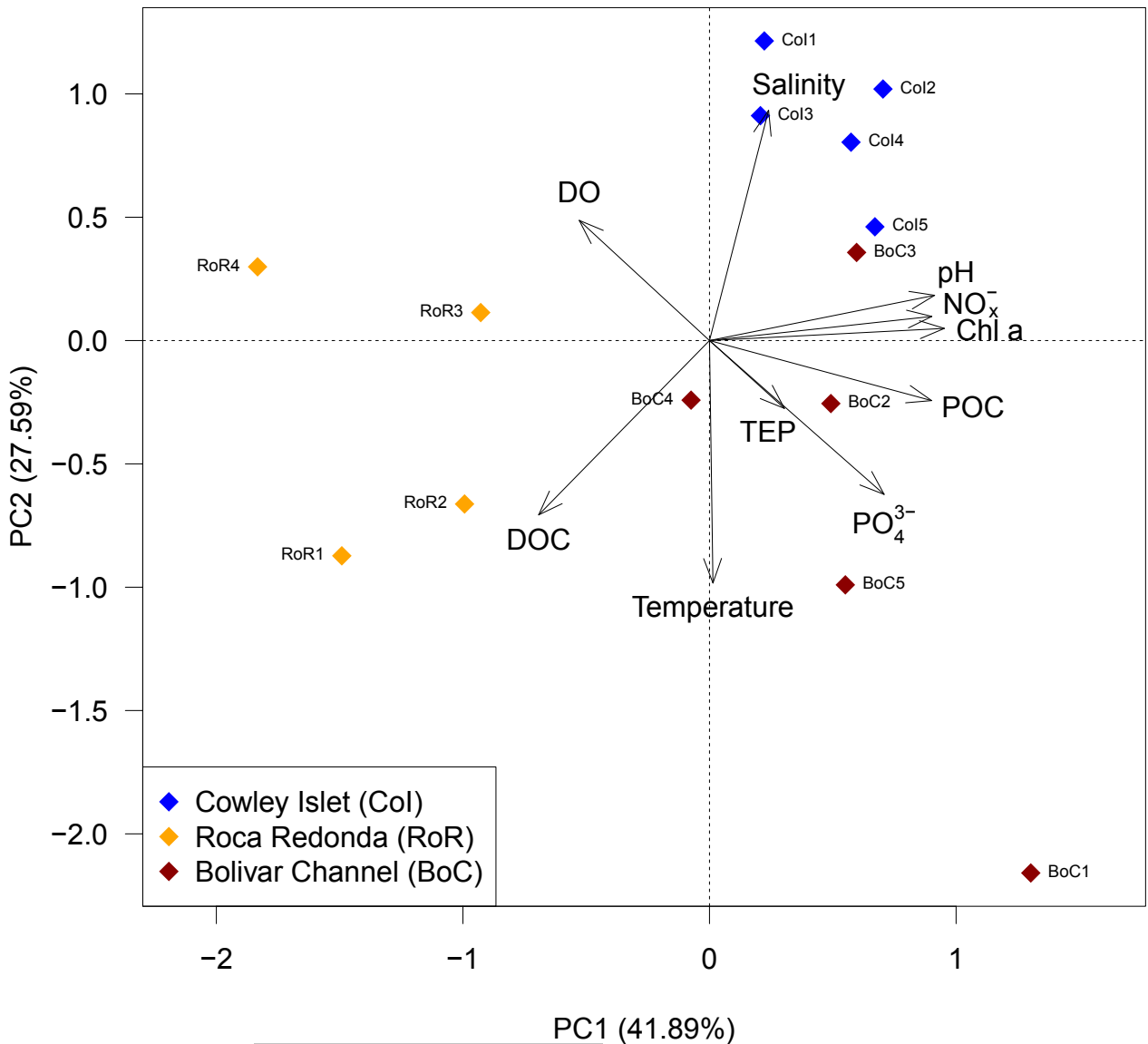
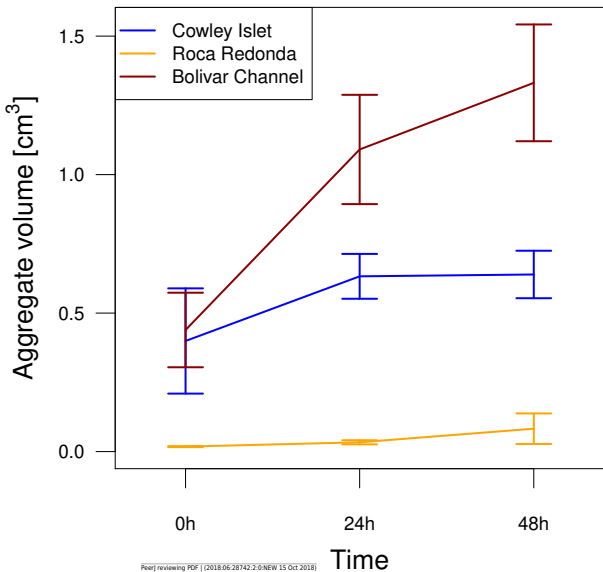
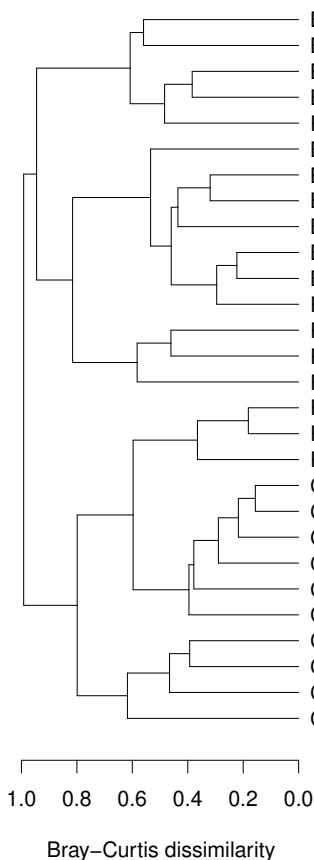
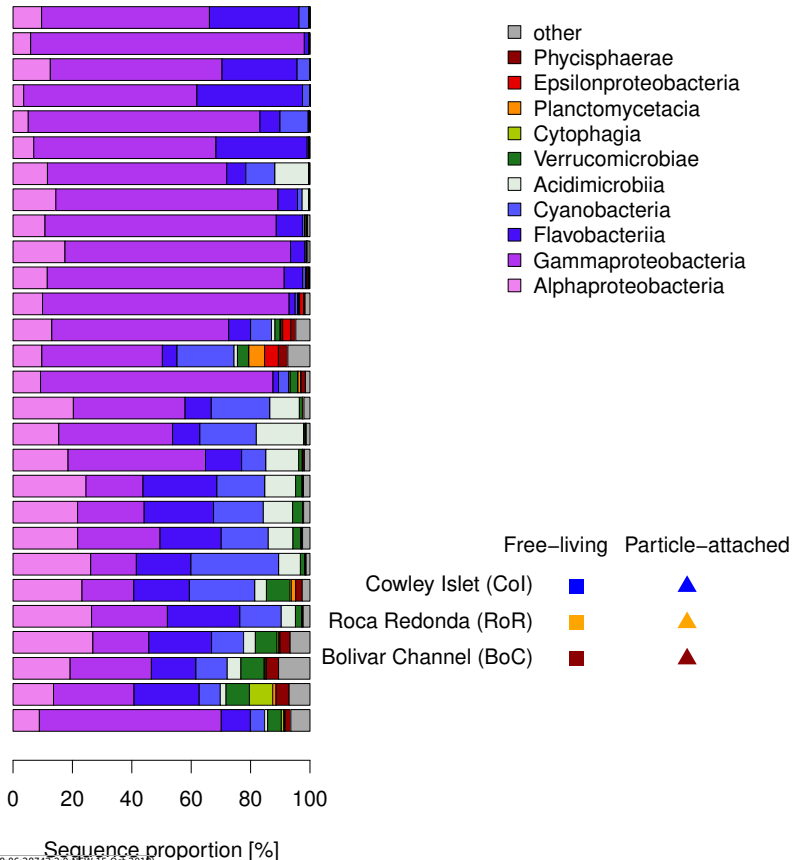
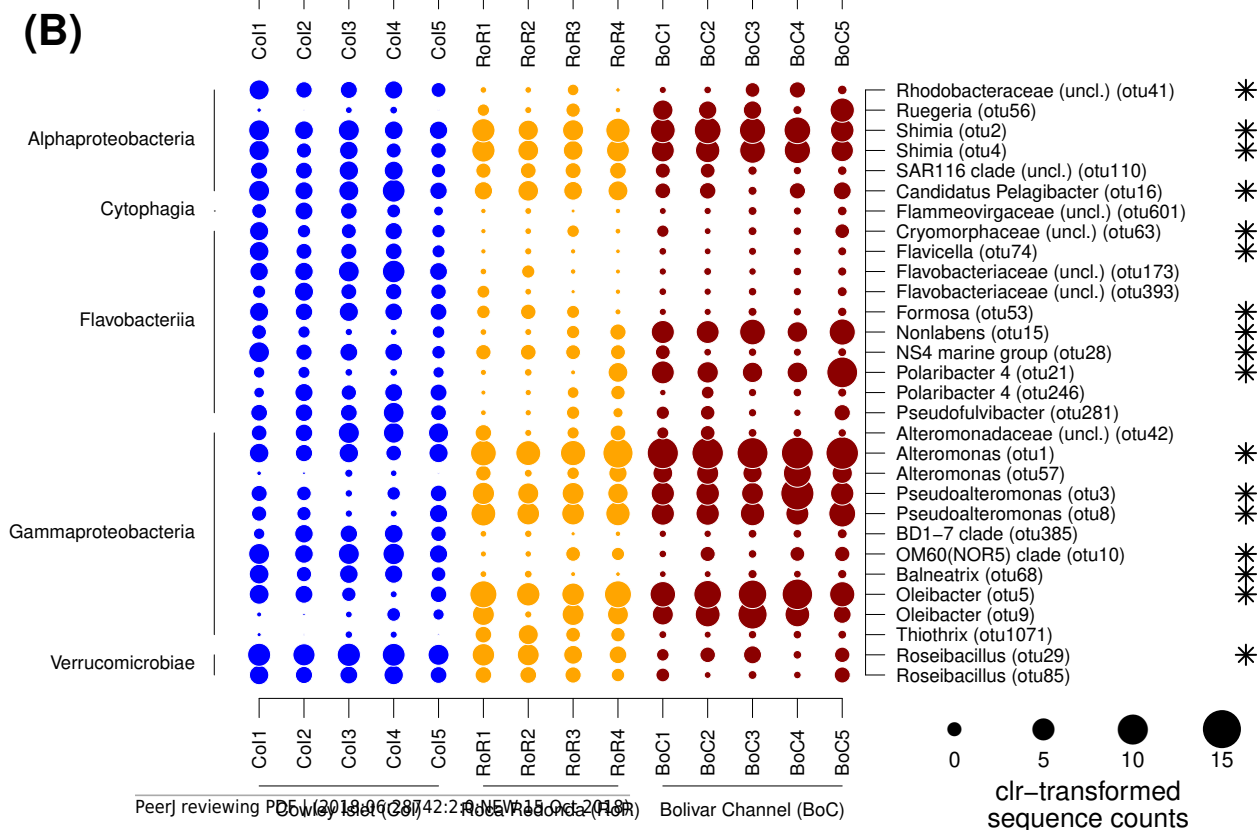
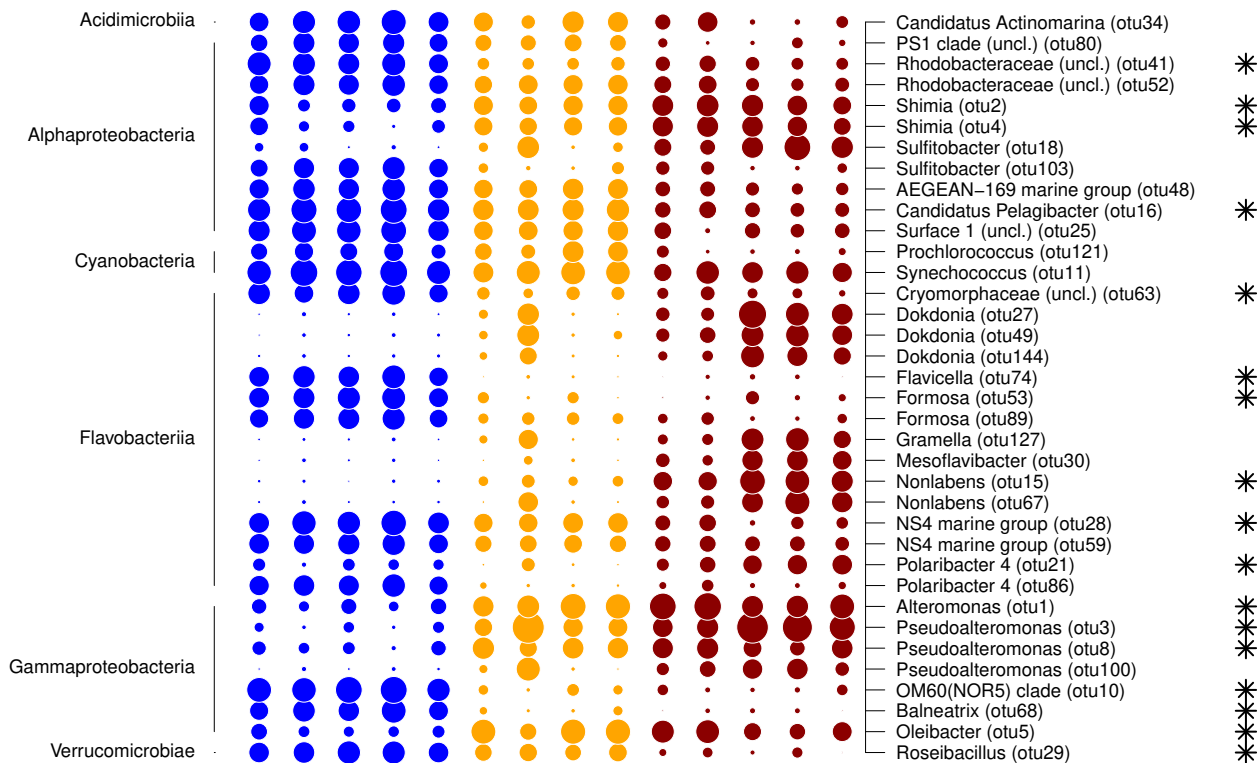
917

Map of the sampling sites at Cowley Islet (CoI), Roca Redonda (RoR), and Bolivar
Channel (BoC) around the Galapagos Islands

Principal component analysis (PCA) of observed water parameters at Cowley Islet (CoI), Roca Redonda (RoR), and Bolivar Channel (BoC)

DO: dissolved oxygen, Chl a: chlorophyll a, PO_4^{3-}: phosphate, NO_x^-: nitrate/nitrite, DOC: dissolved organic carbon, TEP: transparent exopolymers, POC: particulate organic carbon.

Aggregation potential at Cowley Islet (CoI), Roca Redonda (RoR), and Bolivar Channel (BoC) based on rolling tank experiment

The mean of total aggregate volume per rolling tank +/- standard error is shown for each day during the experiment. Sample sizes: 5 (CoI), 4 (RoR), 5 (BoC).

Composition of the free-living and particle-attached bacterial communities in the water column

A: Cluster diagram based on complete linkage hierarchical clustering of Bray-Curtis dissimilarity coefficients. Sample names specify replicate number at each of the three sampling sites: Cowley Islet (CoI1-5), Roca Redonda (RoR1-4), and Bolivar Channel (BoC1-5), and size fraction: free-living (>0.2μm), particle-attached (>3.0μm). B: Taxonomic composition of the bacterial communities at class level.

Dotplot of differentially abundant OTUs among sampling sites

A: Free-living bacterial communities. B: particle-attached bacterial communities. Sample names specify replicate number at each of the three sampling sites: Cowley Islet (CoI1-5), Roca Redonda (RoR1-4), and Bolivar Channel (BoC1-5). The size of each dot represents centered log ratio (clr)-transformed sequences counts. Values higher than zero indicate enrichment compared to the other OTUs per sample. The taxonomic affiliation of each OTUs is provided on class (left side) and genus level (right side). Asterisks indicate OTUs detected as differentially abundant in both size fractions.

Summary of observed water parameters and alpha diversity of free-living and particle-attached bacterial communities in the water column

1 Table 1: **Summary of observed water parameters and alpha diversity of free-living and**
2 **particle-attached bacterial communities in the water column.**

Parameter[1]	T [°C]	pH	Salinity [psu]	DO [mg L^{-1}]	NO$_x^-$ [μmol L^{-1}]	PO$_4^{3-}$ [μmol L^{-1}]
Cowley Islet (CoI)						
Min	21.35	8.14	34.67	6.65	4.65	0.55
Median	21.96	8.16	34.69	6.78	5.19	0.59
Max	22.13	8.25	34.72	6.83	5.80	0.68
Group[2]	a	a	a	a	a	a
Roca Redonda (RoR)						
Min	22.14	6.83	34.43	6.23	2.60	0.43
Median	22.80	7.22	34.53	6.60	3.59	0.49
Max	23.32	7.35	34.64	8.19	4.49	0.52
Group[2]	b	b	b	a,b	b	b
Bolivar Channel (BoC)						
Min	22.39	8.25	34.38	6.09	4.76	0.60
Median	23.24	8.32	34.62	6.43	5.12	0.64
Max	23.80	8.35	34.67	6.46	5.92	1.26
Group[2]	b	c	b	b	a	a
Parameter[1]	Chl a [μg L^{-1}]	DOC [μmol L^{-1}]	TEP [μg X$_{eq}$ L^{-1}]	POC [mg L^{-1}]	InvS (FL)	InvS (PA)
Cowley Islet (CoI)						
Min	0.23	75.65	184.23	23.45	19.55	28.66
Median	0.35	80.87	235.40	32.93	33.82	48.12
Max	0.38	88.57	346.76	37.03	40.50	65.62
Group[2]	a	a	ns	a	a	a
Roca Redonda (RoR)						
Min	0.09	88.96	142.57	18.46	2.31	4.41
Median	0.13	100.54	293.20	20.64	21.04	17.80
Max	0.19	112.73	323.67	22.37	24.99	20.29
Group[2]	b	b	ns	b	a,b	b
Bolivar Channel (BoC)						
Min	0.15	86.76	243.33	25.38	4.19	3.16
Median	0.33	92.14	303.36	36.65	8.46	4.85
Max	0.40	97.99	459.05	44.09	14.75	7.67
Group[2]	a	b	ns	a	b	b

3 [1] T: temperature, DO: dissolved oxygen, NO$_x^-$: nitrate/nitrite, PO$_4^{3-}$: phosphate, Chl a:
4 chlorophyll a, DOC: dissolved organic carbon, TEP: transparent exopolymers, POC: particulate
5 organic carbon, InvS (FL): Inverse Simpson index of free-living bacterial communities, InvS
6 (PA): Inverse Simpson index of particle-attached bacterial communities.

7   ² Lower case letters indicate membership to significantly different groups based on pairwise
8   Wilcoxon posthoc tests between sampling sites (ns: no significant differences among sites based
9   on Kruskal Wallis tests).

Bray Curtis dissimilarity (BC) of bacterioplankton communities among sampling sites

Average within and between-group BC and analysis of similarity (ANOSIM) between sampling sites of free-living and particle-attached bacterial communities in the water column.

1 Table 2: **Bray Curtis dissimilarity (BC) of bacterioplankton communities among sampling**
2 **sites.** Average within and between-group BC and analysis of similarity (ANOSIM) between
3 sampling sites of free-living and particle-attached bacterial communities in the water column.

	Within-group BC	Comparison	Between-group BC	ANOSIM R	P-value[1]
Free-living	0.67			0.63	**0.001**
CoI	0.26	CoI – RoR	0.61	0.72	**0.017**
RoR	0.55	CoI – BoC	0.89	0.89	**0.017**
BoC	0.64	RoR – BoC	0.71	0.29	0.108
Particle-attached	0.70			0.83	**0.001**
CoI	0.50	CoI – RoR	0.75	0.96	**0.015**
RoR	0.55	CoI – BoC	0.91	1.00	**0.015**
BoC	0.49	RoR – BoC	0.61	0.48	**0.019**

4 [1] For pairwise comparisons between the individual sampling sites, Cowley Islet (CoI), Roca
5 Redonda (RoR), and Bolivar Channel (BoC), false discovery rate (FDR)-adjusted p-values are
6 shown. P-values defined as significant at a threshold of 0.05 are highlighted in bold.

Contribution of sampling site or temperature and pH to explaining variation in the composition of free-living and particle-attached bacterioplankton communities based on redundancy analysis (RDA)

Variation partitioning was used to test pure effects accounting for the variation explained by the other factors in the model. Akaike Information Criterion (AIC) and adjusted R squared are provided as goodness-of-fit metrics.

1 Table 3: **Contribution of sampling site or temperature and pH to explaining variation in the**
2 **composition of free-living and particle-attached bacterioplankton communities based on**
3 **redundancy analysis (RDA).** Variation partitioning was used to test pure effects accounting for
4 the variation explained by the other factors in the model. Akaike Information Criterion (AIC) and
5 adjusted R squared are provided as goodness-of-fit metrics.

Explanatory variable	AIC	Adjusted R squared	F	df[1]	P-value[2]
Free-living					
Sampling site	85.31	0.571	9.65	2,11	**0.001**
Temperature + pH	91.20	0.347	4.45	2,11	**0.006**
Temperature (pure)		0.348	7.40	1,11	**0.004**
pH (pure)		0.032	1.58	1,11	0.187
Particle-attached					
Sampling site	84.82	0.489	7.21	2,11	**0.001**
Temperature + pH	89.37	0.292	3.69	2,11	**0.003**
Temperature (pure)		0.213	4.61	1,11	**0.008**
pH (pure)		0.100	2.70	1,11	**0.015**

6 [1] degrees of freedom (numerator, denominator).

7 [2] P-values defined as significant at a threshold of 0.05 are highlighted in bold.