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1 **Marine bacterial communities are resistant to elevated carbon dioxide levels**

2 Running title: Marine bacterial communities and elevated CO<sub>2</sub>

3

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13

14 **Keywords:** Ocean acidification; Taxa-time relationships; Distance-decay relationships; taxa turnover;

15 **Bacterial resistance**

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31 **Summary**

32 It is well established that the release of anthropogenic derived CO<sub>2</sub> into the atmosphere will be mainly  
33 absorbed by the oceans, with a concomitant drop in pH; a process termed ocean acidification. As  
34 such, there is considerable interest in how changes in increased CO<sub>2</sub> and lower pH will affect marine  
35 biota, such as bacteria, which play central roles in oceanic biogeochemical processes. Set within an  
36 ecological framework, we investigated the direct effects of elevated CO<sub>2</sub>, contrasted with ambient  
37 conditions, on the resistance and resilience of marine bacterial communities in a replicated temporal  
38 seawater mesocosm experiment. The results of the study strongly indicate that marine bacterial  
39 communities are highly resistant to the elevated CO<sub>2</sub> and lower pH conditions imposed, as  
40 demonstrated from measures of turnover using taxa-time relationships and distance-decay-  
41 relationships. In addition, no significant differences in community abundance, structure or composition  
42 were observed. Our results suggest that there are no direct effects on marine bacterial communities  
43 and that the bacterial fraction of microbial plankton holds enough flexibility and evolutionary capacity  
44 to withstand predicted future changes from elevated CO<sub>2</sub> and subsequent ocean acidification.

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## 61 Introduction

62 It is well established that most anthropogenically derived carbon dioxide that is released into the  
63 atmosphere, as a result of burning fossil fuels and cement production over the past 200 years, will  
64 eventually be absorbed by the oceans (Caldeira and Wickett, 2003; Raven et al., 2005). This process  
65 of absorption of atmospheric carbon dioxide ( $p\text{CO}_2$ ) is changing the chemistry of the oceans and in  
66 particular is decreasing pH, making seawater more acidic (Caldeira and Wickett, 2003; Raven et al.,  
67 2005; Joint et al., 2011). Joint and colleagues (2011) succinctly described the chemical absorption  
68 process; stating that as anthropogenic  $\text{CO}_2$  increases in the atmosphere, it dissolves in the surface  
69 ocean, aqueous  $\text{CO}_2$  then reacts with water to form a weak acid (carbonic acid,  $\text{H}_2\text{CO}_3$ ), the  
70 dissociation of which forms hydrogen ( $\text{H}^+$ ) and bicarbonate ions ( $\text{HCO}_3^-$ ). The increase in the  
71 concentration of hydrogen ions then results in an inevitable drop in oceanic pH: a process which is  
72 commonly termed ocean acidification (OA), since the ocean's buffering capacity is only able to  
73 neutralize some of this additional  $\text{CO}_2$  (Sabine et al., 2004; Raven et al., 2005). The present average  
74 surface ocean pH is approximately 8.1, being 0.1 units lower than pre-industrial revolution levels  
75 (Caldeira and Wickett, 2003). Atmospheric  $\text{CO}_2$  is predicted to reach between 550 and 1000  $\mu\text{atm}$  by  
76 the year 2100, with a concurrent decline in surface ocean pH of between 0.2 and 0.5 units, for which  
77 there is no known analogue from the past 300 million years (Wolf-Gladrow et al., 1999; Nakicenovic et  
78 al., 2000).

79

80 There is significant interest in how changes in  $p\text{CO}_2$  levels and subsequent ocean acidification will  
81 affect the oceans biota and integral processes (Orr et al., 2005; Fabry et al., 2008; Guinotte and  
82 Fabry, 2008; Doney et al., 2009; Kerr, 2010; Sabine and Tanhua, 2010). The marine ecosystem  
83 contributes over 90% of the Earth's biosphere and marine microbes play an essential role in marine  
84 biogeochemical cycles central to the biological chemistry of the Earth with around 50% of global  
85 primary production attributed to phytoplanktonic bacteria, and protists (Field et al., 1998). Further to  
86 this, over half of autotrophically fixed oceanic  $\text{CO}_2$  is reprocessed or turned over by heterotrophic  
87 bacteria and archaea through processes such as the microbial loop and carbon pump (Azam, 1998;  
88 Jiao et al., 2010). An increasing number of studies have reshaped our understanding of the extent  
89 and importance of marine bacterial diversity (Giovannoni et al., 1990; Britschgi and Giovannoni, 1991;  
90 Schmidt et al., 1991; Fuhrman et al., 1992; Fuhrman et al., 1993; Rappe et al., 2000), with more

91 recent additional insights into the functional and phylogenetic diversity of the Earth's oceans,  
92 reinforcing the perceived importance of marine microbial communities to the biogeochemical cycles  
93 present globally (Kannan et al., 2007; Rusch et al., 2007; Yooseph et al., 2007).

94

95 The application of basic ecological principals has proven to be a powerful tool in explaining the  
96 community distribution and abundance patterns of macro-organisms in response to environmental  
97 change, yet these ideas have only been applied to microbiology recently (Prosser et al., 2007). An  
98 important aspect of community analysis in an environmentally disturbed system (such as CO<sub>2</sub>  
99 perturbation) is the accurate evaluation of biological integrity and recovery following such an event  
100 (Ager et al., 2010) - how will a community respond to change and will it recover? Previous mesocosm  
101 studies investigating community response to OA suggested that the total abundance of bacteria did  
102 not significantly differ between CO<sub>2</sub> perturbation treatments, although changes in free living bacterial  
103 community composition did, likely leading to no loss of function (Grossart et al., 2006; Allgaier et al.,  
104 2008). Most recently the European project on ocean acidification (EPOCA) found free living bacterial  
105 community structure was not majorly affected by degree of ocean acidification, but by variations in  
106 productivity and nutrient availability (Roy et al., 2013; Sperling et al., 2013; Zhang et al., 2013). This  
107 highlights not only the often conflicting results found in such studies but also the difficulty in  
108 distinguishing direct effects upon bacteria from indirect effects relating to phytoplankton assemblages.

109

110 When discussing ocean acidification Joint and colleagues (2011) proposed the null hypothesis that  
111 'marine microbes possess the flexibility to accommodate pH change and there will be no catastrophic  
112 changes in marine biogeochemical processes that are driven by phytoplankton, bacteria and archaea'  
113 a view supported by some studies (Allgaier et al., 2008; Newbold et al., 2012; Roy et al., 2013;  
114 Sperling et al., 2013), but not all (Grossart et al., 2006; Liu, 2010; Lidbury et al., 2012). In our previous  
115 work we demonstrated that in 5 out of 6 key bacterial groups no significant response to CO<sub>2</sub>  
116 perturbation was observed, yet this work reflected only a small proportion of the total community and  
117 therefore an in depth study of the direct changes in total bacterial community response is warranted  
118 (Newbold et al., 2012).

119

120 Here, we test null hypothesis of Joint and colleagues (2011), focusing on direct bacterial community  
121 responses to elevated CO<sub>2</sub> in a replicated temporal seawater mesocosm experiment. Specifically,  
122 using culture independent methods, we examined bacterial community turnover, composition,  
123 structure, and abundance under elevated CO<sub>2</sub> and ambient conditions.

124

## 125 **Results and Discussion**

### 126 *pH and abundance*

127 Seawater samples were collected daily over an 18 day study period from six mesocosms each with a  
128 working volume of ~11000 L. Three mesocosms were enriched with carbon dioxide (elevated CO<sub>2</sub>),  
129 while the remaining three were used as control (ambient condition) mesocosms. A consequence of  
130 increased dissolved carbon dioxide in seawater will be a decrease in pH and subsequent ocean  
131 acidification (Joint et al., 2011). This was the case in the experimental mesocosms where an inverse  
132 relationship was observed between pH and pCO<sub>2</sub>, being autocorrelated as expected ( $pH = a - b \log$   
133  $pCO_2$  [ $r^2 = 0.99$ ;  $F_{1,100} = 2560.2$ ;  $P < 0.0001$ ]). Measurement and analyses of the physical and  
134 chemical parameters within the mesocosms revealed that only pCO<sub>2</sub>, pH and total inorganic dissolved  
135 (TID) carbon were significantly different between treatments (Table S1); where pCO<sub>2</sub> and TID carbon  
136 were significantly higher and, conversely, pH was significantly lower in mesocosms under elevated  
137 CO<sub>2</sub> conditions compared to the ambient control mesocosms (Table S1; Fig. S1a&b).

138

139 The mean bacterial abundance within the elevated CO<sub>2</sub> mesocosms was  $4.5 \times 10^6 \pm 1.03 \times 10^6$  cells  
140 ml<sup>-1</sup> and was not significantly different (ANOVA:  $F_{1,4} = 2.05$ ;  $P = 0.23$ ) from the mean abundance within  
141 the mesocosms under ambient conditions;  $5.74 \times 10^6 \pm 9.79 \times 10^5$  cells ml<sup>-1</sup>. The temporal patterns of  
142 mean bacterial abundance mirrored each other until day 14, thereafter mean cell counts under  
143 elevated CO<sub>2</sub> conditions declined, albeit with high variance, in contrast to mean cell counts under  
144 ambient conditions (Fig. S1c). To determine whether the mean abundance distributions over time  
145 were significantly different we applied the two-sample Kolmogorov-Smirnov distribution fitting test,  
146 which indicated that there was no statistical difference in the bacterial abundance dynamics between  
147 treatments ( $D = 0.353$ ;  $P = 0.245$ ). Furthermore, no significant relationships were observed between  
148 bacterial cell counts and pCO<sub>2</sub> concentrations or pH in any of the mesocosms ( $P > 0.05$  in all cases).

149 This finding is in line with other studies where bacterial abundance was largely unaffected by CO<sub>2</sub>  
150 perturbation (Grossart et al., 2006; Allgaier et al., 2008; Liu, 2010; Krause et al., 2012; Newbold et al.,  
151 2012; Lindh et al., 2013).

### 152 *Temporal turnover in acidified bacterial communities*

153 The bacterial communities within each mesocosm, over the 18 day study period, were analysed by  
154 16S rRNA terminal restriction fragment length polymorphism (T-RFLP). In this study, TRF peak  
155 richness and intensity were used to infer the richness and relative abundance of bacterial taxa within  
156 each mesocosm. Taxa-time relationships (TTR) were used to investigate the effect of elevated CO<sub>2</sub>  
157 levels on bacterial diversity (Fig. 1); specifically, to assess temporal taxa turnover of bacterial taxa  
158 across the two treatments. The TTR describes how the observed taxa richness of a community in a  
159 habitat of fixed size increases with the length of time over which the community is monitored (van der  
160 Gast et al., 2008). The TTR was modelled with the power law equation,  $S = cT^w$ . Where  $S$  is the  
161 cumulative number of observed taxa over time  $T$ ,  $c$  is the intercept and  $w$  is the temporal scaling  
162 exponent and therefore increasing values of  $w$  can be taken as greater rates of taxa turnover. The  
163 mean  $w$ -value within the elevated CO<sub>2</sub> mesocosms was  $0.145 \pm 0.018$ , whereas  $w$  was significantly  
164 higher (ANOVA:  $F_{1,4} = 63.21$ ;  $P < 0.001$ ) within the ambient mesocosms,  $w = 0.240 \pm 0.011$  (Fig. 1).  
165 As slopes, the values of  $w$  for each mesocosm between treatments, using the  $t$ -distribution method  
166 (Fowler et al., 1998), were found to be significantly different (Table 1a); that is the rate of turnover  
167 within the elevated CO<sub>2</sub> mesocosms produced a significant decrease in cumulative taxa richness and  
168 therefore taxa turnover, when compared to the ambient mesocosms.

169

170 In addition to the TTR analyses, distance-decay relationships were employed to measure bacterial  
171 community turnover rates within the mesocosms (Fig. 2). The distance-decay relationship essentially  
172 allows an analysis of how similarity in community composition between sites changes with the  
173 geographic distance separating those sites (van der Gast et al., 2011). For the current study,  
174 geographical distance was substituted for temporal distance (days) and the rate of decay in  
175 community similarity through time was assessed and compared amongst the experimental  
176 mesocosms. The distance-decay relationship was modelled with the power law equation,  $S_{\text{SOR}} = cD^d$ ,  
177 where  $S_{\text{SOR}}$  is the pair-wise similarity between any two samples using the Sørensen index,  $c$  is a  
178 constant,  $D$  is temporal distance between pair-wise samples and  $d$  is the rate of decay in similarity or

179 community turnover rate. The mean rate of decay within the elevated CO<sub>2</sub> mesocosms was  $d = -$   
180  $0.030 \pm 0.007$ , however,  $d$  was significantly higher (ANOVA:  $F_{1,4} = 36.07$ ;  $P < 0.004$ ) within the  
181 ambient mesocosms;  $d = -0.167 \pm 0.039$  (Fig. 2). Using the  $t$ -distribution method, the slopes for each  
182 mesocosm distance-decay relationship when compared between treatments were found to be  
183 significantly different (Table 1b). This indicated that the rate of decay, and therefore turnover, was  
184 significantly dampened within the elevated CO<sub>2</sub> mesocosms, selecting for a more conserved  
185 community composition through time when compared to the more dynamic communities within the  
186 ambient mesocosms. Although the distance-decay relationships significantly differed by the  
187 overarching treatment, the temporal scaling of bacterial taxa within the mesocosms was driven by time  
188 (temporal distance) and not day-to-day differences in pH or pCO<sub>2</sub> concentrations (Table 2).

189

190 As expected, Mantel tests demonstrated pCO<sub>2</sub> and pH were significantly autocorrelated in all  
191 mesocosms: Mantel statistic  $r =$  (Mesocosm 1) 0.949, (2) 0.966, (3) 0.966, (4) 0.950, (5) 0.943, and (6)  
192 0.942 ( $P < 0.0001$  in all cases). In addition, Mantel and partial Mantel tests revealed that other  
193 environmental variables (including, temperature, salinity, nitrate, phosphate, etc.) did not have  
194 significant relationships with similarity in any of the experimental mesocosms. Based on a direct  
195 ordination approach, the bacterial community composition was significantly influenced by time, the  
196 best explanatory variable in all mesocosms, and phosphate and / or nitrate (Table 3). In agreement  
197 with the Mantel based tests, pH or pCO<sub>2</sub> did not significantly explain any of the variance in the  
198 mesocosms communities.

199

200 Bacterial taxa abundance distributions for mesocosms under different treatments were plotted as rank-  
201 abundance curves to examine differences in evenness and dominance over the course of the study  
202 and specifically to determine what impact elevated CO<sub>2</sub> levels had on community structure (Fig. S2).  
203 It is generally accepted that a reduction of taxa richness will occur in an ecological community as a  
204 consequence of an environmental perturbation (Magurran and Phillip, 2001). In addition, the loss of  
205 species is accompanied by a change in community structure (Ager et al., 2010). Whereby,  
206 unperturbed species-rich assemblages are typically evenly distributed and following a perturbation are  
207 replaced by species-poor assemblages with high dominance (Magurran and Phillip, 2001; Ager et al.,  
208 2010). To more clearly visualise changes in community structure, the mean slope values ( $b$ ) from the



209 rank-abundance plots were used as a descriptive statistic of evenness and plotted over time for each  
210 treatment (Fig. S3). When the mean slope values were compared ( $\text{CO}_2$   $b = -0.077 \pm 0.026$ , and  
211 Ambient  $b = -0.080 \pm 0.014$ ) no significant differences in community structure were observed by  
212 treatment (ANOVA:  $F_{1,4} = 0.51$ ;  $P = 0.514$ ).

213

214 Allison and Martiny (2008) defined resistance as 'the degree to which microbial composition remains  
215 unchanged in the face of a disturbance' and resilience as 'the rate at which microbial composition  
216 returns to its original composition after being disturbed' regardless of the system studied. The EPOCA  
217 studies of Roy et al. (2013); Sperling et al. (2013) and Zhang et al. (2013) suggested that variations in  
218 nutrients and productivity were the dominant drivers of free living bacterial community change, not  
219 increased  $\text{CO}_2$ . In contrast, we found evidence that species turnover was significantly dampened  
220 within the elevated  $\text{CO}_2$  mesocosms, selecting for a more conserved community composition through  
221 time, giving clear evidence that the bacteria constituted a community resistant to  $\text{CO}_2$  perturbation.  
222 Further to this, distance decay measures demonstrated that community composition changes little with  
223  $\text{CO}_2$  perturbation, indicating that the elevated  $\text{CO}_2$  likely had no direct effect upon the mesocosm  
224 community. Other's have demonstrated that microbial communities are 'resistant' to perturbation  
225 (Klamer et al., 2002; Chung et al., 2005; Horz et al., 2005; Kasurinen et al., 2005; Gruter et al., 2006;  
226 Bowen et al., 2011). However before generalising it's important to consider that bacterial communities  
227 don't all respond in the same way (Bissett et al., 2013).

228

## 229 *Conclusions*

230 Our findings suggest that the bacterioplankton communities studied were resistant to short term  
231 catastrophic  $\text{pCO}_2$  perturbation. This study corroborates the emerging perception that bacteria are  
232 able to withstand much environmental change (Liu, 2010; Joint et al., 2011). We cannot however rule  
233 out the effect of OA upon the long term resilience of communities. For example Newbold and  
234 colleagues (2012) found significant differences in key members of the picoeukaryote community  
235 assemblage, a finding also evident in the study of Brussaard and colleagues (2013). Any changes in  
236 the pelagic food web are likely to have an effect upon the bacterioplankton as much of bacterial  
237 community structure is determined by 'top down' pressures (Bell et al., 2010; Martinez-Garcia et al.,  
238 2012). To our knowledge recovery has not been measured in a similar mesocosm experiment greater

239 than 30 days (the EPOCA arctic campaign 2010). The changes imposed in our study are meant to  
240 simulate conditions faced in 100 years time, 100 years represents millions of bacterial generations and  
241 therefore the scope for evolutionary adaption is huge. This study highlights the need for long term  
242 naturalistic studies, which would examine the effects of ocean acidification upon bacterioplankton in a  
243 biologically relevant setting and time scale.

244

245

## 246 **Experimental procedures**

### 247 *Experimental set up and sampling regime*

248 The complete experimental set up has been outlined previously (Gilbert et al., 2008; Hopkins et al.,  
249 2010; Meakin and Wyman, 2011). We present the data for 3 elevated CO<sub>2</sub> (experimental) and 3  
250 ambient CO<sub>2</sub> control mesocosms (2m diameter, 3.5m deep, ~11,000L). Experimental mesocosm  
251 enclosures were gently sparged with CO<sub>2</sub> (750 µatm) for 2 days until a pH ~ 7.8 was established. To  
252 control for sparging effects ambient condition mesocosm enclosures were sparged with air. In order to  
253 simulate natural conditions more closely, a phytoplankton bloom was induced through the addition of  
254 nitrate and phosphate in all mesocosms (initial concentrations: 1 µmol l<sup>-1</sup> phosphate; 17 µmol l<sup>-1</sup>  
255 nitrate). Blooming phytoplankton growth reduced CO<sub>2</sub> concentrations in the elevated CO<sub>2</sub>  
256 mesocosms, therefore 2 of the experimental mesocosm enclosures were re-acidified 11 days after  
257 mesocosm establishment (16/5/2006), and 2 ambient condition enclosures again sparged with air (the  
258 remaining 2 mesocosm bags left unsparged). To isolate picoplankton daily samples of ~2 L of water  
259 were pre-filtered through Whatman GF/A filters to remove large eukaryote cells and filtrate collected  
260 onto 0.2 µm Durapore membranes. These were stored at -80 °C prior to molecular analysis. Note  
261 that samples for molecular analysis were not taken on day 12 of the study. Physical and chemical  
262 parameters of the water samples (including; atmospheric carbon dioxide (pCO<sub>2</sub>), pH, temperature, and  
263 salinity) were taken and analysed as described previously (Hopkins et al., 2010), and the summary  
264 measurements are presented in Table S1.

265

### 266 *Enumeration of bacterial cells using flow cytometry*

267 Daily flow cytometric counts of absolute concentrations of bacterioplankton were performed using a  
268 Becton Dickinson FACSort™ flow cytometer equipped with an air-cooled blue light laser at 488nm

269 according to protocols outlined elsewhere (Gasol et al., 1999; Zubkov et al., 2001; Tarran et al., 2006;  
270 Zubkov et al., 2008).

271

### 272 *Terminal restriction fragment length polymorphism (T-RFLP)*

273 Full experimental procedures have been described previously (Newbold et al., 2012). In summary,  
274 total nucleic acids were extracted as previously described (Huang et al., 2009). Approximately 20-30  
275 ng of purified template was used per 50  $\mu$ L PCR reaction. A ~500 bp region of the 16S small subunit  
276 ribosomal RNA (SSU rRNA) was amplified using fluorescently labelled forward primer (6FAM) 27F and  
277 536R reverse primer (Suzuki et al., 1998). Amplification conditions were as follows; 2 minute pre-  
278 denaturation phase at 94 °C followed by 30 cycles of 94 °C for 1 minute, 52 °C for 1 minute, and 72 °C  
279 for 3 minutes and a final extension phase of 10 minutes at 72 °C. 20  $\mu$ L of gel purified PCR product  
280 was digested for 4 hours at 37 °C in a 30  $\mu$ L total reaction volume using 20 units restriction enzyme  
281 *MspI* (Promega, UK) and buffers. Digestion product (0.5  $\mu$ L) was combined with 0.5  $\mu$ L denatured  
282 LIZ600 size standard (Applied Biosystems) and 9  $\mu$ L Hi-Di formamide (Applied Biosystems), and run  
283 on an Applied Biosystems 3730 DNA sequencer. The sizes of restriction fragments were calculated  
284 and binned using Genemarker<sup>TM</sup> (Softgenetics) and restriction fragments crossed correlated to specific  
285 cloned sequences (see Newbold et al, 2012). Bin widths were checked and manually adjusted to  
286 encompass all detected peaks. To differentiate signal from background, a Fluorescence Unit (FU)  
287 threshold of 40 units was used for a presence/absence binary matrix. All peaks were manually  
288 checked for inclusion in analysis. Relative abundance measures were calculated by dividing individual  
289 peak height by total peak height spanning all valid peaks within the analysis. Resultant data were  
290 analysed for community richness, composition, and structure.

291

### 292 *Statistical analyses of data*

293 One-way ANOVA tests, regression analysis, coefficients of determination ( $r^2$ ), residuals and  
294 significance (P) were calculated using Minitab software (version 14.20; Minitab, University Park, PA,  
295 USA). The two-sample Kolmogorov-Smirnov test is used to compare empirical distribution fitting tests  
296 from a sample with a known distribution. It can be used, as was the case for the current study, for  
297 comparing two empirical distributions (Nikiforov, 1994). The test was performed using the XLSTAT  
298 program (version 2012; Addinsoft, France) and applied as previously described (Newbold et al., 2012).

299

300 Taxa-time relationships (TTR) were used as one method to visualise and statistically compare  
301 differences in marine bacterial temporal scaling between elevated CO<sub>2</sub> and ambient mesocosms as  
302 previously described (van der Gast et al., 2008). In addition to the TTR, we employed a second  
303 method, the distance-decay relationship (DDR), to also examine differences in marine bacterial beta  
304 diversity. The DDR describes how similarity in taxa composition between two communities varies with  
305 the geographical distance that separates them (Green et al., 2004). In addition, it also allows us to go  
306 onto determine how patterns of beta diversity are influenced by environmental factors (Green et al.,  
307 2004). In the current study, the DDR has been modified from the power law described previously (van  
308 der Gast et al., 2011), to incorporate temporal distance in place of geographic distance. The  
309 Sørensen index of community similarity and subsequent average linkage clustering of community  
310 profiles were performed using PAST (Paleontological Statistics program, version 2.16), available from  
311 the University of Oslo website link (<http://folk.uio.no/ohammer/past>) run by Øyvind Hammer. The *t*-  
312 distribution method was used to compare the regression line slopes generated from the taxa-time and  
313 distance-decay relationship analyses as described previously (Fowler et al., 1998).

314

315 Two complementary approaches, direct ordination and Mantel test (Tuomisto and Ruokolainen, 2006),  
316 were used to relate variability in the distribution of bacteria to environmental factors (pCO<sub>2</sub>,  
317 temperature, salinity, nitrate, phosphate, particulate organic nitrogen, particulate organic carbon, and  
318 total inorganic carbon) and temporal distance (days). For the direct ordination approach, temporal  
319 distance and environmental variables that significantly explained variation in bacterial communities  
320 were determined with forward selection (999 Monte Carlo permutations;  $\alpha < 0.05$ ) and used in  
321 canonical correspondence analysis (Peros-Neto et al., 2006). Partial canonical correspondence  
322 analysis was performed when both time and environmental variables were significant. Analyses were  
323 performed in the ECOMII software package (version 2.1.3.137; Pisces Conservation Ltd., Lymington,  
324 UK). For the Mantel approach (Mantel, 1967; Green et al., 2004; van der Gast et al., 2011a), bacterial  
325 similarity matrices for each mesocosm, using raw presence/absence T-RF data, were calculated using  
326 the Sørensen index of similarity. Similarity matrices for environmental factors were generated by  
327 calculating the absolute difference of values between each pair wise time point. Lower tailed partial  
328 Mantel tests were conducted in the XLSTAT program.

329

330 Rank-abundance plots were used to determine differences in bacterial community structure (Ager et  
331 al., 2010). For each sample the relative abundance of each taxon (TRF) was standardized to per cent  
332 values before construction of the rank-abundance plots. The rank-abundance plots were visualized by  
333 plotting the taxa rank order on the x-axis against relative abundance ( $\log_{10}$  transformed) on the y-axis.  
334 For each plot a linear regression model was fitted, represented by the equation,  $\log_{10}y = a + bx$ , where  
335  $a$  is the intercept and  $b$  is the slope of the plot. The slope ( $b$ ) was subsequently used as a descriptive  
336 statistic for changes in community structure as previously described (Ager et al., 2010).

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## 575 **Figure and Table legends**

576

577 **Fig. 1.** The taxa-time relationships (TTRs) for bacterial communities in mesocosms under elevated  
578 CO<sub>2</sub> and ambient conditions. Given are the TTR for each mesocosm (1 to 6) and the mean TTR by  
579 treatment. Error bars represent the standard deviation of the mean ( $n = 3$ ). Also given are the taxa-  
580 time power law equation  $S = cT^w$ : (1)  $r^2 = 0.94$ ,  $F_{1,15} = 253.2$ ; (2)  $r^2 = 0.70$ ,  $F_{1,15} = 34.4$ ; (3)  $r^2 = 0.76$ ,  
581  $F_{1,15} = 46.4$ ; (4)  $r^2 = 0.94$ ,  $F_{1,15} = 230.5$ ; (5)  $r^2 = 0.96$ ,  $F_{1,15} = 391.4$ ; (6)  $r^2 = 0.84$ ,  $F_{1,15} = 79.6$ ; (elevated  
582 CO<sub>2</sub> mean)  $r^2 = 0.89$ ,  $F_{1,15} = 117.7$ ; and (ambient mean)  $r^2 = 0.98$ ,  $F_{1,15} = 748.7$ . All regression  
583 coefficients were significant ( $P < 0.0001$ ).

584

585 **Fig. 2.** The distance-decay of bacterial community similarity (Sørensen index ( $S_s$ )) in mesocosms  
586 under elevated CO<sub>2</sub> and ambient conditions over time. Given are the distance-decay relationships  
587 (DDR) for each mesocosm (1 to 6) and the mean DDR by treatment. Error bars represent the  
588 standard deviation of the mean ( $n = 3$ ). Given are the distance-decay power law equation  $S_s = cD^d$ :  
589 (1)  $r^2 = 0.05$ ,  $F_{1,134} = 6.5$ ; (2)  $r^2 = 0.02$ ,  $F_{1,134} = 6.4$ ; (3)  $r^2 = 0.09$ ,  $F_{1,134} = 13.9$ ; (4)  $r^2 = 0.28$ ,  $F_{1,134} =$   
590  $52.8$ ; (5)  $r^2 = 0.40$ ,  $F_{1,134} = 90.5$ ; (6)  $r^2 = 0.43$ ,  $F_{1,134} = 101.6$ ; (elevated CO<sub>2</sub> mean)  $r^2 = 0.16$ ,  $F_{1,134} =$

591 24.6; and (ambient mean)  $r^2 = 0.56$ ,  $F_{1,134} = 168.8$ . All regression coefficients were significant ( $P <$   
592 0.05). Partial Mantel summary statistics are listed in Table 2.

593

594 **Table 1.** Comparison of power regression slopes between all mesocosms for (A) taxa-time  
595 relationships (TTR) and (B) distance-decay relationships. In each case, the  $t$ -distribution method test  
596 statistic ( $t$ ) is given in the lower triangle and significance ( $P$ ) is given in the upper triangle for each  
597 comparison. For the taxa-time relationships the degrees of freedom ( $df$ ) = 1,30, and for the distance-  
598 decay-relationships,  $df = 1, 268$ . Asterisks denote those slopes that were significantly different at the  
599  $P < 0.05$  level.

600

601 **Table 2.** Summary statistics for partial Mantel tests. The partial Mantel statistic  $r(AB.C)$  estimates the  
602 correlation between two proximity matrices,  $A$  and  $B$ , whilst controlling for the effects of  $C$ . Given are  
603 bacterial community similarity  $S$  (Sørensen index) and also  $C$  and  $p$  which are differences in  $pCO_2$  and  
604 pH, respectively. Also given is  $P$  to ascertain whether the partial Mantel regression coefficients were  
605 significantly different from zero following 9,999 permutations.  $P$ -values significant after Bonferroni  
606 correction for multiple comparisons ( $0.05/18 = 0.003$ ) are denoted with asterisks.

607

608 **Table 3.** Canonical correspondence analyses for determination of percent variation in bacterial  
609 communities in mesocosms under elevated  $CO_2$  or ambient conditions by environmental variables and  
610 time.

611

612

613 **Table 1**

A	Treatment	Elevated CO <sub>2</sub>			Ambient		
	Mesocosm	1	2	3	4	5	6
Elevated CO <sub>2</sub>	1		0.72	0.25	<0.0001*	<0.0001*	0.002*
	2	0.36		0.30	0.03*	0.01*	0.03*
	3	1.18	1.06		0.0002*	<0.0001*	<0.0001*
Ambient	4	4.48	2.22	4.37		0.55	0.51
	5	5.94	2.71	5.25	0.60		0.76
	6	3.41	2.31	3.73	0.67	0.31	1

B	Treatment	Elevated CO <sub>2</sub>			Ambient		
	Mesocosm	1	2	3	4	5	6
Elevated CO <sub>2</sub>	1		0.49	0.98	<0.00001*	<0.00001*	<0.00001*
	2	0.69		0.43	<0.00001*	<0.00001*	<0.00001*
	3	0.02*	0.80		<0.00001*	<0.00001*	<0.00001*
Ambient	4	5.34	5.86	5.67		0.02*	0.67
	5	4.74	5.65	5.61	2.41		0.01
	6	6.53	7.30	7.29	0.43	2.63	

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619 **Table 2**

Treatment	Mesocosm	<i>r</i> ( <i>SD.C</i> )	<i>P</i>	<i>r</i> ( <i>SC.D</i> )	<i>P</i>	<i>r</i> ( <i>SD.p</i> )	<i>P</i>	<i>r</i> ( <i>Sp.D</i> )	<i>P</i>
Elevated CO <sub>2</sub>	1	-0.293	0.001*	0.226	0.995	-0.279	<0.0001*	0.217	0.994
	2	-0.199	0.002*	-0.007	0.473	-0.200	0.001*	0.003	0.515
	3	-0.325	0.001*	0.115	0.089	-0.333	<0.0001*	0.160	0.968
Ambient	4	-0.472	<0.0001*	0.331	0.999	-0.643	<0.0001*	0.241	0.997
	5	-0.421	<0.0001*	0.275	0.998	-0.538	<0.0001*	-0.052	0.273
	6	-0.510	<0.0001*	0.074	0.202	-0.769	<0.0001*	0.247	0.998

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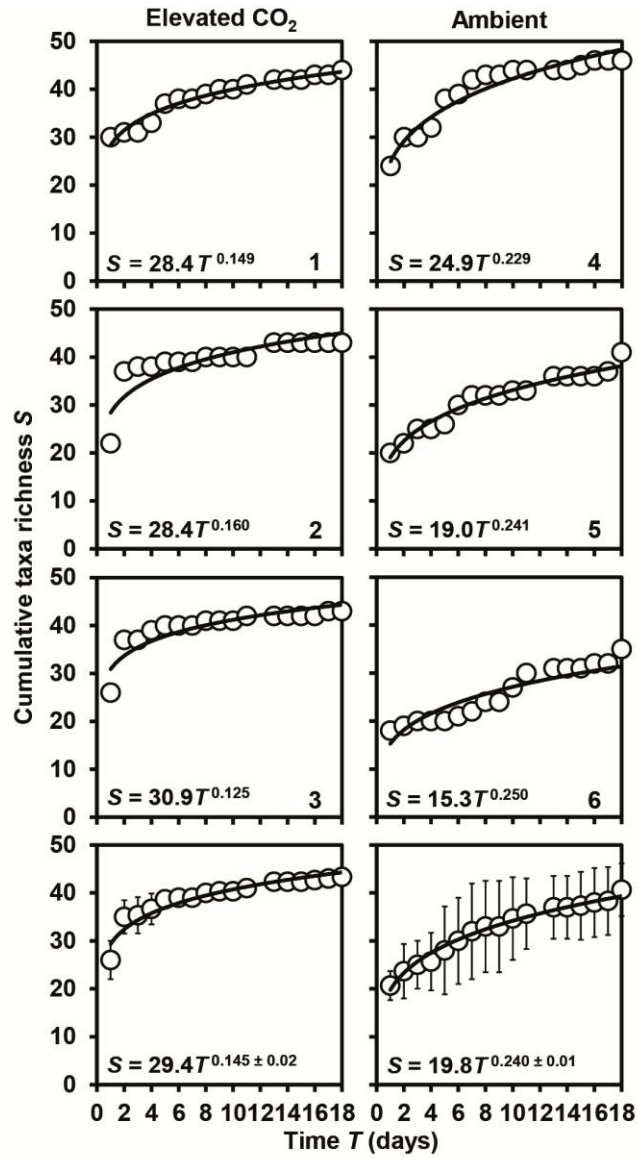
624 **Table 3**

Mesocosm	Elevated CO <sub>2</sub>			Ambient		
	1	2	3	4	5	6
Time	34.71	30.32	23.48	34.00	49.51	40.54
Phosphate	20.76	17.41	19.92	22.39	30.89	24.02
Nitrate	16.71	-	-	17.70	-	19.47
Undetermined	27.82	52.26	56.60	25.91	19.61	15.97

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