



**Manchester
Metropolitan
University**

Oliver, AE and Newbold, LK and Whiteley, AS and van der Gast, CJ (2014)
Marine bacterial communities are resistant to elevated carbon dioxide levels.
Environmental Microbiology Reports, 6 (6). pp. 574-582. ISSN 1758-2229

Downloaded from: <https://e-space.mmu.ac.uk/621057/>

Publisher: Wiley

DOI: <https://doi.org/10.1111/1758-2229.12159>

Please cite the published version

<https://e-space.mmu.ac.uk>

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

2 Running title: Marine bacterial communities and elevated CO₂

3

4 Anna E. Oliver^{1†}, Lindsay K. Newbold^{1,2†}, Andrew S. Whiteley³, and Christopher J. van der Gast^{1*}

5

6 ¹*NERC Centre for Ecology and Hydrology, Wallingford, OX10 8BB, UK*

7 ²*School of Civil Engineering and Geosciences, University of Newcastle upon Tyne, Newcastle, NE1*

8 *7RU, UK.*

9 ³*School of Earth and Environment, University of Western Australia, Crawley, WA 6009, Australia*

10

11 **For correspondence E-mail cjvdg@ceh.ac.uk; Tel. (+44) 1491 692647; Fax (+44) 1491 692424.*

12 *† Contributed equally to this work.*

13

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

15 **Bacterial resistance**

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31 **Summary**

32 It is well established that the release of anthropogenic derived CO₂ 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 CO₂ 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 CO₂, 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 CO₂ 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 CO₂ and subsequent ocean acidification.

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

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 CO_2 increases in the atmosphere, it dissolves in the surface
69 ocean, aqueous CO_2 then reacts with water to form a weak acid (carbonic acid, H_2CO_3), the
70 dissociation of which forms hydrogen (H^+) and bicarbonate ions (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 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 CO_2 is predicted to reach between 550 and 1000 μatm 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 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 CO₂
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 CO₂ 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 CO₂
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 CO₂ 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 CO₂ 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 CO₂),
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 pCO₂, 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 pCO₂, pH and total inorganic dissolved
135 (TID) carbon were significantly different between treatments (Table S1); where pCO₂ and TID carbon
136 were significantly higher and, conversely, pH was significantly lower in mesocosms under elevated
137 CO₂ conditions compared to the ambient control mesocosms (Table S1; Fig. S1a&b).

138

139 The mean bacterial abundance within the elevated CO₂ mesocosms was $4.5 \times 10^6 \pm 1.03 \times 10^6$ cells
140 ml⁻¹ 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⁻¹. The temporal patterns of
142 mean bacterial abundance mirrored each other until day 14, thereafter mean cell counts under
143 elevated CO₂ 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 pCO₂ 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 CO₂
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 CO₂
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 CO₂ mesocosms was 0.145 ± 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 CO₂ 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_{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 CO₂ mesocosms was $d = -$
180 0.030 ± 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 CO₂ 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 pCO₂ concentrations (Table 2).

189

190 As expected, Mantel tests demonstrated pCO₂ 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 pCO₂ 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 CO₂ 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 (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 CO_2 . In contrast, we found evidence that species turnover was significantly dampened
220 within the elevated CO_2 mesocosms, selecting for a more conserved community composition through
221 time, giving clear evidence that the bacteria constituted a community resistant to CO_2 perturbation.
222 Further to this, distance decay measures demonstrated that community composition changes little with
223 CO_2 perturbation, indicating that the elevated 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 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 CO₂ (experimental) and 3
250 ambient CO₂ control mesocosms (2m diameter, 3.5m deep, ~11,000L). Experimental mesocosm
251 enclosures were gently sparged with CO₂ (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⁻¹ phosphate; 17 µmol l⁻¹
255 nitrate). Blooming phytoplankton growth reduced CO₂ concentrations in the elevated CO₂
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 (pCO₂), 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 μ 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 μ L of gel purified PCR product
280 was digested for 4 hours at 37 °C in a 30 μ L total reaction volume using 20 units restriction enzyme
281 *MspI* (Promega, UK) and buffers. Digestion product (0.5 μ L) was combined with 0.5 μ L denatured
282 LIZ600 size standard (Applied Biosystems) and 9 μ 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 GenemarkerTM (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 CO₂ 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 (pCO₂,
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).

337

338

339

340

341

342 **Acknowledgments**

343 The authors would like to thank all of the members of the 2006 Bergen Mesocosm Experiment for their
344 invaluable help and data acquisition, especially, Ian Joint, Dorothee Bakker and Isabelle Mary. This
345 work was funded by the UK Natural Environment Research Council (NERC) Grant Number
346 NE/C507937/1 as part of the post-genomics and proteomics programme.

347

348 **References**

349 Ager, D., Evans, S., Li, H., and van der Gast, C.J. (2010) Anthropogenic disturbance affects the
350 structure of bacterial communities. *Environ Microbiol* **12**: 670-678.

351

352 Allgaier, M., Riebesell, U., Vogt, M., Thyrraug, R., and Grossart, H.P. (2008) Coupling of heterotrophic
353 bacteria to phytoplankton bloom development at different $p\text{CO}_2$ levels: a mesocosm study.
354 *Biogeosciences* **5**: 1007-1022.

355

356 Allison, S.D., and Martiny, J.B.H. (2008) Resistance, resilience, and redundancy in microbial
357 communities. *Proc Natl Acad Sci USA* **105**: 11512-11519.

358

359 Azam, F. (1998) Oceanography: Microbial Control of Oceanic Carbon Flux: The Plot Thickens.
360 *Science* **280**: 694-696.
361

362 Bell, T., Bonsall, M.B., Buckling, A., Whiteley, A.S., Goodall, T., and Griffiths, R.I. (2010) Protists have
363 divergent effects on bacterial diversity along a productivity gradient. *Biol Lett* **6**: 639-642.
364

365 Bissett, A., Brown, M.V., Siciliano, S.D., and Thrall, P.H. (2013) Microbial community responses to
366 anthropogenically induced environmental change: towards a systems approach. *Ecol Lett* **16**: 128-
367 139.
368

369 Bowen, J.L., Ward, B.B., Morrison, H.G., Hobbie, J.E., Valiela, I., Deegan, L.A., and Sogin, M.L.
370 (2011) Microbial community composition in sediments resists perturbation by nutrient enrichment.
371 *ISME J* **5**: 1540-1548.
372

373 Britschgi, T.B., and Giovannoni, S.J. (1991) Phylogenetic analysis of a natural marine
374 bacterioplankton population by rRNA gene cloning and sequencing. *Appl Environ Microbiol* **57**: 1707-
375 1713.
376

377 Brussaard, C.P.D., Noordeloos, A.A.M., Witte, H., Collenteur, M.C.J., Schulz, K., Ludwig, A., and
378 Riebesell, U. (2013) Arctic microbial community dynamics influenced by elevated CO₂ levels.
379 *Biogeosciences* **10**: 719-731.
380

381 Caldeira, K., and Wickett, M.E. (2003) Anthropogenic carbon and ocean pH. *Nature* **425**: 365.
382

383 Chung, H., R. Zak, D.R., and Lilleskov, E.A. (2005) Fungal community composition and metabolism
384 under elevated CO₂ and O₃. *Oecologia* **147**: 143-154.
385

386 Doney, S.C., Fabry, V.J., Feely, R.A., and Kleypas, J.A. (2009) Ocean acidification: The other CO₂
387 problem. *Ann Rev Mar Sci* **1**: 169-192.
388

389 EPOCA Artic Campaign 2010. URL <http://epocaarctic2010.wordpress.com/>
390

391 Fabry, V.J., Seibel, B.A., Feely, R.A., and Orr, J.C. (2008) Impacts of ocean acidification on marine
392 fauna and ecosystem processes. *ICES J Mar Sci* **65**: 414-432.
393

394 Field, C. B., Behrenfeld, M. J., Randerson, J. T. and Falkowski, P. (1998) Primary production of the
395 biosphere: integrating terrestrial and oceanic components. *Science*, **281**: 237-240.
396

397 Fowler, J., Cohen, L., and Jarvis, P. (1998) *Practical Statistics for Field Biologists*. Chichester, UK:
398 John Wiley and Sons.
399

400 Fuhrman, J.A., McCallum, K., and Davis, A.A. (1992) Novel major archaeobacterial group from marine
401 plankton. *Nature* **356**: 148-149.
402

403 Fuhrman, J.A., McCallum, K., and Davis, A.A. (1993) Phylogenetic diversity of subsurface marine
404 microbial communities from the Atlantic and Pacific Oceans. *Appl Environ Microbiol* **59**: 1294-1302.
405

406 Gasol, J.M., Zweifel, U.L., Peters, F., Fuhrman, J.A., and Hagstrom, Å. (1999) Significance of size and
407 nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Appl*
408 *Environ Microbiol* **65**: 4475-4483.
409

410 Gilbert, J.A., Field, D., Huang, Y., Edwards, R., Li, W., Gilna, P., and Joint, I. (2008) Detection of
411 Large Numbers of Novel Sequences in the Metatranscriptomes of Complex Marine Microbial
412 Communities. *PLoS ONE* **3**: e3042.
413

414 Giovannoni, S.J., Britschgi, T.B., Moyer, C.L., and Field, K.G. (1990) Genetic diversity in Sargasso
415 Sea bacterioplankton. *Nature* **345**: 60-63.
416

417 Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M. et al. (2004) Spatial
418 scaling of microbial eukaryote diversity. *Nature* **432**: 747-750.

419

420 Grossart, H.-P., Allgaier, M., Passow, U., and Riebesell, U. (2006) Testing the effect of CO₂
421 concentration on the dynamics of marine heterotrophic bacterioplankton. *Limnol Oceanogr* **51**: 1-11.
422

423 Gruter, D., Schmid, B., and Brandl, H. (2006) Influence of plant diversity and elevated atmospheric
424 carbon dioxide levels on belowground bacterial diversity. *BMC Microbiology* **6**: 68.
425

426 Guinotte, J.M., and Fabry, V.J. (2008) Ocean acidification and its potential effects on marine
427 ecosystems. *Ann N Y Acad Sci* **1134**: 320-342.
428

429 Hopkins, F.E., Turner, S.M., Nightingale, P.D., Steinke, M., Bakker, D., and Liss, P.S. (2010) Ocean
430 acidification and marine trace gas emissions. *Proc Natl Acad Sci USA* **107**: 760-765.
431

432 Horz, H.-P., Rich, V., Avrahami, S., and Bohannan, B.J.M. (2005) Methane-Oxidizing Bacteria in a
433 California Upland Grassland Soil: Diversity and Response to Simulated Global Change. *Appl Environ*
434 *Microbiol* **71**: 2642-2652.
435

436 Huang, W.E., Ferguson, A., Singer, A.C., Lawson, K., Thompson, I.P., Kalin, R.M. et al. (2009)
437 Resolving genetic functions within microbial populations: *In situ* analyses using rRNA and mRNA
438 stable isotope probing coupled with single-cell raman-fluorescence *in situ* hybridization. *Appl Environ*
439 *Microbiol* **75**: 234-241.
440

441 Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W. et al. (2010) Microbial
442 production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nat*
443 *Rev Microbiol* **8**: 593-599.
444

445 Joint, I., Doney, S.C., and Karl, D.M. (2011) Will ocean acidification affect marine microbes? *ISME J* **5**:
446 1-7.
447

448 Kannan, N., Taylor, S.S., Zhai, Y., Venter, J.C., and Manning, G. (2007) Structural and Functional
449 Diversity of the Microbial Kinome. *PLoS Biol* **5**: e17.

450

451 Kasurinen, A., Keinänen, M.M., Kaipainen, S., Nilsson, L.-O., Vapaavuori, E., Kontro, M.H., and
452 Holopainen, T. (2005) Below-ground responses of silver birch trees exposed to elevated CO₂ and O₃
453 levels during three growing seasons. *Glob Chang Biol* **11**: 1167-1179.

454

455 Kerr, R.A. (2010) Ocean acidification unprecedented, unsettling. *Science* **328**: 1500-1501.

456

457 Klamer, M., Roberts, M.S., Levine, L.H., Drake, B.G., and Garland, J.L. (2002) Influence of Elevated
458 CO₂ on the Fungal Community in a Coastal Scrub Oak Forest Soil Investigated with Terminal-
459 Restriction Fragment Length Polymorphism Analysis. *Appl Environ Microbiol* **68**: 4370-4376.

460

461 Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M.B., and Gerdtts, G. (2012) Small
462 Changes in pH Have Direct Effects on Marine Bacterial Community Composition: A Microcosm
463 Approach. *PLoS ONE* **7**: e47035.

464

465 Lidbury, I., Johnson, V., Hall-Spencer, J.M., Munn, C.B., and Cunliffe, M. (2012) Community-level
466 response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent
467 ecosystem. *Mar Poll Bull* **64**: 1063-1066.

468

469 Lindh, M.V., Riemann, L., Baltar, F., Romero-Oliva, C., Salomon, P.S., Granéli, E., and Pinhassi, J.
470 (2013) Consequences of increased temperature and acidification on bacterioplankton community
471 composition during a mesocosm spring bloom in the Baltic Sea. *Environ Microbiol Rep* **5**: 252-262.

472

473 Liu, J., Weinbauer, M. G., Maier, C., Dai, M. and Gattuso, J-P. (2010) Effect of ocean acidification on
474 microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning *Aquat Microb*
475 *Ecol* AME SPECIAL 4: PP4.

476

477 Magurran, A.E., and Phillip, D.A.T. (2001) Implications of species loss in freshwater fish assemblages.
478 *Ecography* **24**: 645-650.
479

480 Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer*
481 *Res* **27**: 209-220.
482

483 Martinez-Garcia, M., Brazel, D., Poulton, N.J., Swan, B.K., Gomez, M.L., Masland, D. et al. (2012)
484 Unveiling in situ interactions between marine protists and bacteria through single cell sequencing.
485 *ISME J* **6**: 703-707.
486

487 Meakin, N.G., and Wyman, M. (2011) Rapid shifts in picoeukaryote community structure in response
488 to ocean acidification. *ISME J* **5**: 1397–1405.
489

490 Nakicenovic, N., Alcamo, J., Davis, G., de Vries, B., Fenhann, J., Gaffin, S. et al. (2000) *Special*
491 *Report on Emissions Scenarios (Intergovernmental Panel on Climate Change)*. Cambridge, UK and
492 New York, USA: Cambridge University Press.
493

494 Newbold, L.K., Oliver, A.E., Booth, T., Tiwari, B., DeSantis, T., Maguire, M. et al. (2012) The response
495 of marine picoplankton to ocean acidification. *Environ Microbiol* **14**: 2293-2307.
496

497 Nikiforov, A.M. (1994) Algorithm AS 288: exact smirnov two-sample test for arbitrary distributions. *Appl*
498 *Stat* **43**: 265-270.
499

500 Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A. et al. (2005) Anthropogenic
501 ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**:
502 681-686.
503

504 Peros-Neto, P.R., Legendre, P., Dray, S., and Borcard, D. (2006) Variation partitioning of species data
505 matrices: Estimation and comparison of fractions. *Ecology* **87**: 2614-2625.
506

507 Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P. et al. (2007)
508 The role of ecological theory in microbial ecology. *Nat Rev Micro* **5**: 384-392.
509

510 Rappe, M.S., Vergin, K., and Giovannoni, S.J. (2000) Phylogenetic comparisons of a coastal
511 bacterioplankton community with its counterparts in open ocean and freshwater systems. *FEMS*
512 *Microbiol Ecol* **33**: 219-232.
513

514 Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U. et al. (2005) Ocean
515 acidification due to increasing atmospheric carbon dioxide Policy document. In. London, UK: The
516 Royal Society, pp. 1-60.
517

518 Roy, A.S., Gibbons, S.M., Schunck, H., Owens, S., Caporaso, J.G., Sperling, M. et al. (2013) Ocean
519 acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic
520 mesocosms. *Biogeosciences* **10**: 555-566.
521

522 Rusch, D.B., Halpern, A.L., Sutton, G., Heidelberg, K.B., Williamson, S., Yooseph, S. et al. (2007) The
523 Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific.
524 *PLoS Biol* **5**: e77.
525

526 Sabine, C.L., and Tanhua, T. (2010) Estimation of anthropogenic CO₂ inventories in the ocean. *Ann*
527 *Rev Mar Sci* **2**: 175-198.
528

529 Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L. et al. (2004) The oceanic sink
530 for anthropogenic CO₂. *Science* **305**: 367-371.
531

532 Schmidt, T.M., DeLong, E.F., and Pace, N.R. (1991) Analysis of a marine picoplankton community by
533 16S rRNA gene cloning and sequencing. *J Bacteriol* **173**: 4371-4378.
534

535 Sperling, M., Piontek, J., Gerdt, G., Wichels, A., Schunck, H., Roy, A.S. et al. (2013) Effect of
536 elevated CO₂ on the dynamics of particle-attached and free-living bacterioplankton communities in an
537 Arctic fjord. *Biogeosciences* **10**: 181-191.

538

539 Suzuki, M., Rappe, M.S., and Giovannoni, S.J. (1998) Kinetic Bias in Estimates of Coastal
540 Picoplankton Community Structure Obtained by Measurements of Small-Subunit rRNA Gene PCR
541 Amplicon Length Heterogeneity. *Appl Environ Microbiol* **64**: 4522-4529.

542

543 Tarran, G.A., Heywood, J.L., and Zubkov, M.V. (2006) Latitudinal changes in the standing stocks of
544 nano- and picoeukaryotic phytoplankton in the Atlantic Ocean. *Deep Sea Res Part 2 Top Stud*
545 *Oceanogr* **53**: 1516-1529.

546

547 Tuomisto, H., and Ruokolainen, K. (2006) Analyzing or explaining beta diversity? Understanding the
548 targets of different methods of analysis. *Ecology* **87**: 2697-2708.

549

550 van der Gast, C.J., Ager, D., and Lilley, A.K. (2008) Temporal scaling of bacterial taxa is influenced by
551 both stochastic and deterministic ecological factors. *Environ Microbiol* **10**: 1411-1418.

552

553 van der Gast, C.J., Gosling, P., Tiwari, B., and Bending, G.D. (2011) Spatial scaling of arbuscular
554 mycorrhizal fungal diversity is affected by farming practice. *Environ Microbiol* **13**: 241-249.

555

556 Wolf-Gladrow, D.U., Riebesell, U., Burkhardt, S., and Bijma, J. (1999) Direct effect of CO₂
557 concentration on growth and isotopic composition of marine plankton. *Tellus* **51B**: 461-476.

558

559 Yooseph, S., Sutton, G., Rusch, D.B., Halpern, A.L., Williamson, S.J., Remington, K. et al. (2007) The
560 Sorcerer II Global Ocean Sampling Expedition: Expanding the Universe of Protein Families. *PLoS Biol*
561 **5**: e16.

562

563 Zhang, R., Xia, X., Lau, S.C.K., Motegi, C., Weinbauer, M.G., and Jiao, N. (2013) Response of
564 bacterioplankton community structure to an artificial gradient of pCO₂ in the Arctic Ocean.
565 *Biogeosciences* **10**: 3679-3689.

566

567 Zubkov, M.V., Fuchs, B.M., Burkill, P.H., and Amann, R. (2001) Comparison of cellular and biomass
568 specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. *Appl*
569 *Environ Microbiol* **67**: 5210-5218.

570

571 Zubkov, M.V., Tarran, G.A., Mary, I., and Fuchs, B.M. (2008) Differential microbial uptake of dissolved
572 amino acids and amino sugars in surface waters of the Atlantic Ocean. *J Plankton Res* **30**: 211-220.

573

574

575 **Figure and Table legends**

576

577 **Fig. 1.** The taxa-time relationships (TTRs) for bacterial communities in mesocosms under elevated
578 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 ₂			Ambient		
	Mesocosm	1	2	3	4	5	6
Elevated CO ₂	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 ₂			Ambient		
	Mesocosm	1	2	3	4	5	6
Elevated CO ₂	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	

614

615

616

617

618

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 ₂	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

620

621

622

623

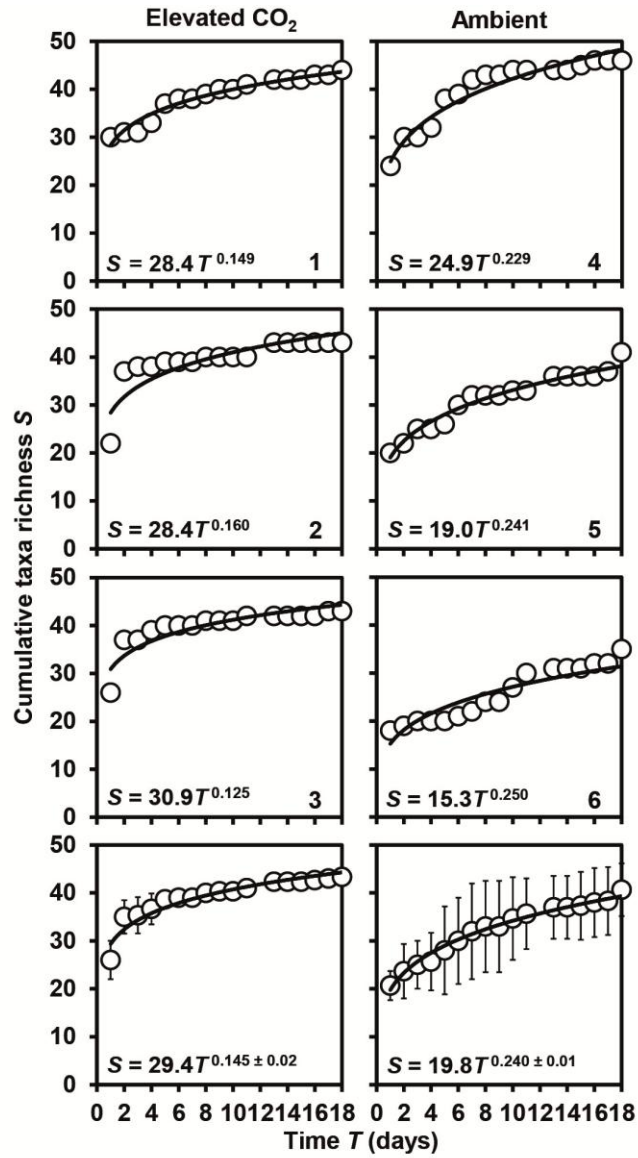
624 **Table 3**

Mesocosm	Elevated CO ₂			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

625

626

627



652

653

654

655

656

657

