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Marine bacterial communities are resistant to elevated carbon dioxide levels

Running title: Marine bacterial communities and elevated CO_2

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Summary

It is well established that the release of anthropogenic derived CO_2 into the atmosphere will be mainly absorbed by the oceans, with a concomitant drop in pH; a process termed ocean acidification. As such, there is considerable interest in how changes in increased CO_2 and lower pH will affect marine biota, such as bacteria, which play central roles in oceanic biogeochemical processes. Set within an ecological framework, we investigated the direct effects of elevated CO_2, contrasted with ambient conditions, on the resistance and resilience of marine bacterial communities in a replicated temporal seawater mesocosm experiment. The results of the study strongly indicate that marine bacterial communities are highly resistant to the elevated CO_2 and lower pH conditions imposed, as demonstrated from measures of turnover using taxa-time relationships and distance-decay-relationships. In addition, no significant differences in community abundance, structure or composition were observed. Our results suggest that there are no direct effects on marine bacterial communities and that the bacterial fraction of microbial plankton holds enough flexibility and evolutionary capacity to withstand predicted future changes from elevated CO_2 and subsequent ocean acidification.

Introduction

It is well established that most anthropogenically derived carbon dioxide that is released into the atmosphere, as a result of burning fossil fuels and cement production over the past 200 years, will eventually be absorbed by the oceans (Caldeira and Wickett, 2003; Raven et al., 2005). This process of absorption of atmospheric carbon dioxide (pCO_2) is changing the chemistry of the oceans and in particular is decreasing pH, making seawater more acidic (Caldeira and Wickett, 2003; Raven et al., 2005; Joint et al., 2011). Joint and colleagues (2011) succinctly described the chemical absorption process; stating that as anthropogenic CO_2 increases in the atmosphere, it dissolves in the surface ocean, aqueous CO_2 then reacts with water to form a weak acid (carbonic acid , H_2CO_3), the dissociation of which forms hydrogen (H^+) and bicarbonate ions (HCO_3^-). The increase in the concentration of hydrogen ions then results in an inevitable drop in oceanic pH: a process which is commonly termed ocean acidification (OA), since the ocean's buffering capacity is only able to neutralize some of this additional CO_2 (Sabine et al., 2004; Raven et al., 2005). The present average surface ocean pH is approximately 8.1, being 0.1 units lower than pre-industrial revolution levels (Caldeira and Wickett, 2003). Atmospheric CO_2 is predicted to reach between 550 and 1000 µatm by the year 2100, with a concurrent decline in surface ocean pH of between 0.2 and 0.5 units, for which there is no known analogue from the past 300 million years (Wolf-Gladrow et al., 1999; Nakicenovic et al., 2000).

There is significant interest in how changes in pCO_2 levels and subsequent ocean acidification will affect the oceans biota and integral processes (Orr et al., 2005; Fabry et al., 2008; Guinotte and Fabry, 2008; Doney et al., 2009; Kerr, 2010; Sabine and Tanhua, 2010). The marine ecosystem contributes over 90% of the Earth's biosphere and marine microbes play an essential role in marine biogeochemical cycles central to the biological chemistry of the Earth with around 50% of global primary production attributed to phytoplanktonic bacteria, and protists (Field et al., 1998). Further to this, over half of autotrophically fixed oceanic CO_2 is reprocessed or turned over by heterotrophic bacteria and archaea through processes such as the microbial loop and carbon pump (Azam, 1998; Jiao et al., 2010). An increasing number of studies have reshaped our understanding of the extent and importance of marine bacterial diversity (Giovannoni et al., 1990; Britschgi and Giovannoni, 1991; Schmidt et al., 1991; Fuhrman et al., 1992; Fuhrman et al., 1993; Rappe et al., 2000), with more

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

The application of basic ecological principals has proven to be a powerful tool in explaining the community distribution and abundance patterns of macro-organisms in response to environmental change, yet these ideas have only been applied to microbiology recently (Prosser et al., 2007). An important aspect of community analysis in an environmentally disturbed system (such as CO_2 perturbation) is the accurate evaluation of biological integrity and recovery following such an event (Ager et al., 2010) - how will a community respond to change and will it recover? Previous mesocosm studies investigating community response to OA suggested that the total abundance of bacteria did not significantly differ between CO_2 perturbation treatments, although changes in free living bacterial community composition did, likely leading to no loss of function (Grossart et al., 2006; Allgaier et al., 2008). Most recently the European project on ocean acidification (EPOCA) found free living bacterial community structure was not majorly affected by degree of ocean acidification, but by variations in productivity and nutrient availability (Roy et al., 2013; Sperling et al., 2013; Zhang et al., 2013). This highlights not only the often conflicting results found in such studies but also the difficulty in distinguishing direct effects upon bacteria from indirect effects relating to phytoplankton assemblages.

When discussing ocean acidification Joint and colleagues (2011) proposed the null hypothesis that 'marine microbes possess the flexibility to accommodate pH change and there will be no catastrophic changes in marine biogeochemical processes that are driven by phytoplankton, bacteria and archaea' a view supported by some studies (Allgaier et al., 2008; Newbold et al., 2012; Roy et al., 2013; Sperling et al., 2013), but not all (Grossart et al., 2006; Liu, 2010; Lidbury et al., 2012). In our previous work we demonstrated that in 5 out of 6 key bacterial groups no significant response to CO_2 perturbation was observed, yet this work reflected only a small proportion of the total community and therefore an in depth study of the direct changes in total bacterial community response is warranted (Newbold et al., 2012).

Here, we test null hypothesis of Joint and colleagues (2011), focusing on direct bacterial community responses to elevated CO_2 in a replicated temporal seawater mesocosm experiment. Specifically, using culture independent methods, we examined bacterial community turnover, composition, structure, and abundance under elevated CO_2 and ambient conditions.

Results and Discussion

pH and abundance

Seawater samples were collected daily over an 18 day study period from six mesocosms each with a working volume of ~11000 L. Three mesocosms were enriched with carbon dioxide (elevated CO_2), while the remaining three were used as control (ambient condition) mesocosms. A consequence of increased dissolved carbon dioxide in seawater will be a decrease in pH and subsequent ocean acidification (Joint et al., 2011). This was the case in the experimental mesocosms where an inverse relationship was observed between pH and pCO_2, being autocorrelated as expected (pH = a – b log pCO_2 [r^2 = 0.99; $F_{1,100}$ = 2560.2; P < 0.0001]). Measurement and analyses of the physical and chemical parameters within the mesocosms revealed that only pCO_2, pH and total inorganic dissolved (TID) carbon were significantly different between treatments (Table S1); where pCO_2 and TID carbon were significantly higher and, conversely, pH was significantly lower in mesocosms under elevated CO_2 conditions compared to the ambient control mesocosms (Table S1; Fig. S1a&b).

The mean bacterial abundance within the elevated CO_2 mesocosms was $4.5 \times 10^6 \pm 1.03 \times 10^6$ cells ml^{-1} and was not significantly different (ANOVA: $F_{1,4}$ = 2.05; P = 0.23) from the mean abundance within the mesocosms under ambient conditions; $5.74 \times 10^6 \pm 9.79 \times 10^5$ cells ml^{-1}. The temporal patterns of mean bacterial abundance mirrored each other until day 14, thereafter mean cell counts under elevated CO_2 conditions declined, albeit with high variance, in contrast to mean cell counts under ambient conditions (Fig. S1c). To determine whether the mean abundance distributions over time were significantly different we applied the two-sample Kolmogorov-Smirnov distribution fitting test, which indicated that there was no statistical difference in the bacterial abundance dynamics between treatments (D = 0.353; P = 0.245). Furthermore, no significant relationships were observed between bacterial cell counts and pCO_2 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_2
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_2
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_2 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 CO_2 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, $S_{SOR} = cD^d$,
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

community turnover rate. The mean rate of decay within the elevated CO_2 mesocosms was d = -0.030 ± 0.007, however, d was significantly higher (ANOVA: $F_{1,4}$ = 36.07; P < 0.004) within the ambient mesocosms; d = -0.167 ± 0.039 (Fig. 2). Using the *t*-distribution method, the slopes for each mesocosm distance-decay relationship when compared between treatments were found to be significantly different (Table 1b). This indicated that the rate of decay, and therefore turnover, was significantly dampened within the elevated CO_2 mesocosms, selecting for a more conserved community composition through time when compared to the more dynamic communities within the ambient mesocosms. Although the distance-decay relationships significantly differed by the overarching treatment, the temporal scaling of bacterial taxa within the mesocosms was driven by time (temporal distance) and not day-to-day differences in pH or pCO_2 concentrations (Table 2).

As expected, Mantel tests demonstrated pCO_2 and pH were significantly autocorrelated in all mesocosms: Mantel statistic r = (Mesocosm 1) 0.949, (2) 0.966, (3) 0.966, (4) 0.950, (5) 0.943, and (6) 0.942 (P < 0.0001 in all cases). In addition, Mantel and partial Mantel tests revealed that other environmental variables (including, temperature, salinity, nitrate, phosphate, etc.) did not have significant relationships with similarity in any of the experimental mesocosms. Based on a direct ordination approach, the bacterial community composition was significantly influenced by time, the best explanatory variable in all mesocosms, and phosphate and / or nitrate (Table 3). In agreement with the Mantel based tests, pH or pCO_2 did not significantly explain any of the variance in the mesocosms communities.

Bacterial taxa abundance distributions for mesocosms under different treatments were plotted as rank-abundance curves to examine differences in evenness and dominance over the course of the study and specifically to determine what impact elevated CO_2 levels had on community structure (Fig. S2). It is generally accepted that a reduction of taxa richness will occur in an ecological community as a consequence of an environmental perturbation (Magurran and Phillip, 2001). In addition, the loss of species is accompanied by a change in community structure (Ager et al., 2010). Whereby, unperturbed species-rich assemblages are typically evenly distributed and following a perturbation are replaced by species-poor assemblages with high dominance (Magurran and Phillip, 2001; Ager et al., 2010). To more clearly visualise changes in community structure, the mean slope values (*b*) from the

rank-abundance plots were used as a descriptive statistic of evenness and plotted over time for each treatment (Fig. S3). When the mean slope values were compared (CO_2 b = -0.077 ± 0.026, and Ambient b = -0.080 ± 0.014) no significant differences in community structure were observed by treatment (ANOVA: $F_{1,4}$ = 0.51; P = 0.514).

Allison and Martiny (2008) defined resistance as 'the degree to which microbial composition remains unchanged in the face of a disturbance' and resilience as 'the rate at which microbial composition returns to its original composition after being disturbed' regardless of the system studied. The EPOCA studies of Roy et al. (2013); Sperling et al. (2013) and Zhang et al. (2013) suggested that variations in nutrients and productivity were the dominant drivers of free living bacterial community change, not increased CO_2. In contrast, we found evidence that species turnover was significantly dampened within the elevated CO_2 mesocosms, selecting for a more conserved community composition through time, giving clear evidence that the bacteria constituted a community resistant to CO_2 perturbation. Further to this, distance decay measures demonstrated that community composition changes little with CO_2 perturbation, indicating that the elevated CO_2 likely had no direct effect upon the mesocosm community. Other's have demonstrated that microbial communities are 'resistant' to perturbation (Klamer et al., 2002; Chung et al., 2005; Horz et al., 2005; Kasurinen et al., 2005; Gruter et al., 2006; Bowen et al., 2011). However before generalising it's important to consider that bacterial communities don't all respond in the same way (Bissett et al., 2013).

Conclusions

Our findings suggest that the bacterioplankton communities studied were resistant to short term catastrophic pCO_2 perturbation. This study corroborates the emerging perception that bacteria are able to withstand much environmental change (Liu, 2010; Joint et al., 2011). We cannot however rule out the effect of OA upon the long term resilience of communities. For example Newbold and colleagues (2012) found significant differences in key members of the picoeukaryote community assemblage, a finding also evident in the study of Brussaard and colleagues (2013). Any changes in the pelagic food web are likely to have an effect upon the bacterioplankton as much of bacterial community structure is determined by 'top down' pressures (Bell et al., 2010; Martinez-Garcia et al., 2012). To our knowledge recovery has not been measured in a similar mesocosm experiment greater

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

Experimental procedures

Experimental set up and sampling regime

The complete experimental set up has been outlined previously (Gilbert et al., 2008; Hopkins et al., 2010; Meakin and Wyman, 2011). We present the data for 3 elevated CO_2 (experimental) and 3 ambient CO_2 control mesocosms (2 m diameter, 3.5 m deep, ~11,000L). Experimental mesocosm enclosures were gently sparged with CO_2 (750 µatm) for 2 days until a pH ~ 7.8 was established. To control for sparging effects ambient condition mesocosm enclosures were sparged with air. In order to simulate natural conditions more closely, a phytoplankton bloom was induced through the addition of nitrate and phosphate in all mesocosms (initial concentrations: 1 µmol l^{-1} phosphate; 17 µmol l^{-1} nitrate). Blooming phytoplankton growth reduced CO_2 concentrations in the elevated CO_2 mesocosms, therefore 2 of the experimental mesocosm enclosures were re-acidified 11 days after mesocosm establishment (16/5/2006), and 2 ambient condition enclosures again sparged with air (the remaining 2 mesocosm bags left unsparged). To isolate picoplankton daily samples of ~2 L of water were pre-filtered through Whatman GF/A filters to remove large eukaryote cells and filtrate collected onto 0.2 µm Durapore membranes. These were stored at -80 ºC prior to molecular analysis. Note that samples for molecular analysis were not taken on day 12 of the study. Physical and chemical parameters of the water samples (including; atmospheric carbon dioxide (pCO_2), pH, temperature, and salinity) were taken and analysed as described previously (Hopkins et al., 2010), and the summary measurements are presented in Table S1.

Enumeration of bacterial cells using flow cytometry

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

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

Terminal restriction fragment length polymorphism (T-RFLP)

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

Statistical analyses of data

One-way ANOVA tests, regression analysis, coefficients of determination (r2), residuals and significance (P) were calculated using Minitab software (version 14.20; Minitab, University Park, PA, USA). The two-sample Kolmogorov-Smirnov test is used to compare empirical distribution fitting tests from a sample with a known distribution. It can be used, as was the case for the current study, for comparing two empirical distributions (Nikiforov, 1994). The test was performed using the XLSTAT program (version 2012; Addinsoft, France) and applied as previously described (Newbold et al., 2012).

Taxa-time relationships (TTR) were used as one method to visualise and statistically compare differences in marine bacterial temporal scaling between elevated CO_2 and ambient mesocosms as previously described (van der Gast et al., 2008). In addition to the TTR, we employed a second method, the distance-decay relationship (DDR), to also examine differences in marine bacterial beta diversity. The DDR describes how similarity in taxa composition between two communities varies with the geographical distance that separates them (Green et al., 2004). In addition, it also allows us to go onto determine how patterns of beta diversity are influenced by environmental factors (Green et al., 2004). In the current study, the DDR has been modified from the power law described previously (van der Gast et al., 2011), to incorporate temporal distance in place of geographic distance. The Sørensen index of community similarity and subsequent average linkage clustering of community profiles were performed using PAST (Paleontological Statistics program, version 2.16), available from the University of Oslo website link (http://folk.uio.no/ohammer/past) run by Øyvind Hammer. The *t*-distribution method was used to compare the regression line slopes generated from the taxa-time and distance-decay relationship analyses as described previously (Fowler et al., 1998).

Two complementary approaches, direct ordination and Mantel test (Tuomisto and Ruokolainen, 2006), were used to relate variability in the distribution of bacteria to environmental factors (pCO_2, temperature, salinity, nitrate, phosphate, particulate organic nitrogen, particulate organic carbon, and total inorganic carbon) and temporal distance (days). For the direct ordination approach, temporal distance and environmental variables that significantly explained variation in bacterial communities were determined with forward selection (999 Monte Carlo permutations; $\alpha < 0.05$) and used in canonical correspondence analysis (Peros-Neto et al., 2006). Partial canonical correspondence analysis was performed when both time and environmental variables were significant. Analyses were performed in the ECOMII software package (version 2.1.3.137; Pisces Conservation Ltd., Lymington, UK). For the Mantel approach (Mantel, 1967; Green et al., 2004; van der Gast et al., 2011a), bacterial similarity matrices for each mesocosm, using raw presence/absence T-RF data, were calculated using the Sørensen index of similarity. Similarity matrices for environmental factors were generated by calculating the absolute difference of values between each pair wise time point. Lower tailed partial Mantel tests were conducted in the XLSTAT program.

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

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Figure and Table legends

Fig. 1. The taxa-time relationships (TTRs) for bacterial communities in mesocosms under elevated CO_2 and ambient conditions. Given are the TTR for each mesocosm (1 to 6) and the mean TTR by treatment. Error bars represent the standard deviation of the mean (n = 3). Also given are the taxa-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, $F_{1,15}$ = 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 CO_2 mean) r^2 = 0.89, $F_{1,15}$ = 117.7; and (ambient mean) r^2 = 0.98, $F_{1,15}$ = 748.7. All regression coefficients were significant (P < 0.0001).

Fig. 2. The distance-decay of bacterial community similarity (Sørensen index (S_s)) in mesocosms under elevated CO_2 and ambient conditions over time. Given are the distance-decay relationships (DDR) for each mesocosm (1 to 6) and the mean DDR by treatment. Error bars represent the standard deviation of the mean (n = 3). Given are the distance-decay power law equation $S_s = cD^d$: (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, $F_{1,134}$ = 52.8; (5) r^2 = 0.40, $F_{1,134}$ = 90.5; (6) r^2 = 0.43, $F_{1,134}$ = 101.6; (elevated CO_2 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	

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618

Table 2

Treatment	Mesocosm	*r*(*SD.C*)	*P*	*r*(*SC.D*)	*P*	*r*(*SD.p*)	*P*	*r*(*Sp.D*)	*P*
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

623

624 **Table 3**

	Elevated CO_2			Ambient		
Mesocosm	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

628 **Figure 1**

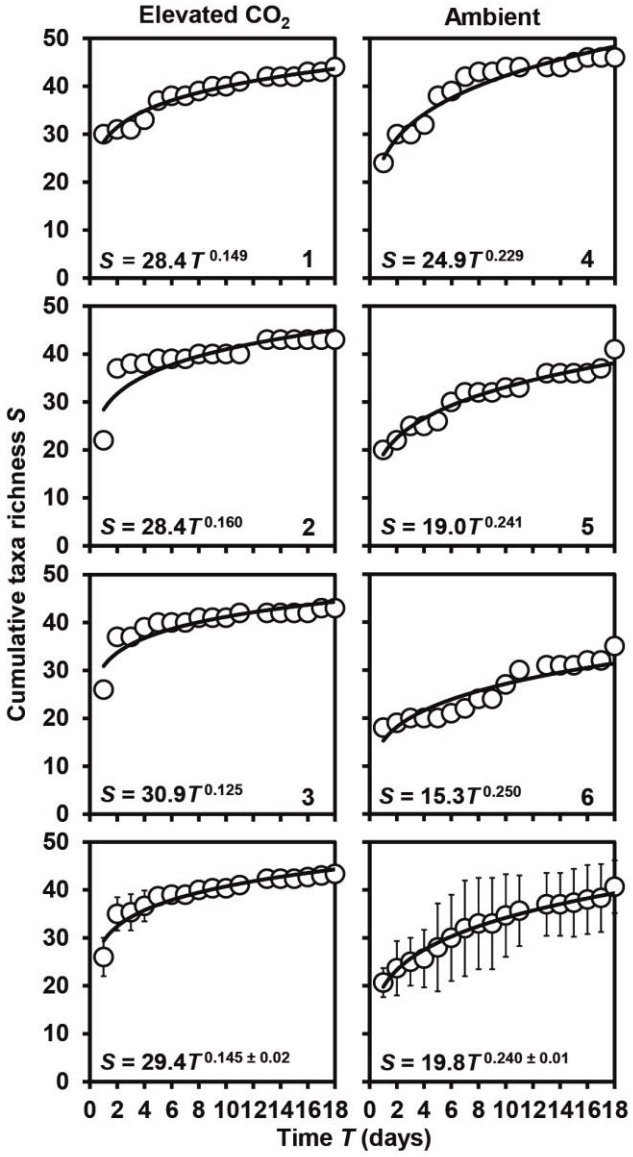

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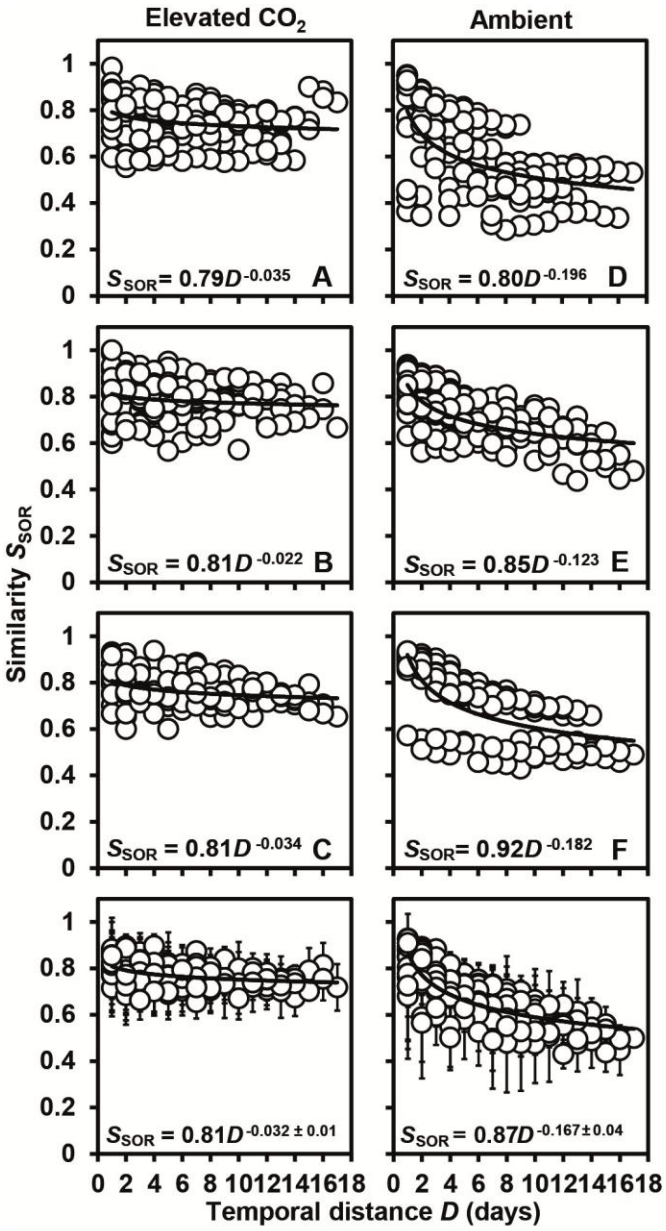
Figure 2


Elevated CO_2
Ambient
Similarity S_{SOR}
S_{SOR}= 0.79$D^{-0.035}$ A
S_{SOR} = 0.80$D^{-0.196}$ D
S_{SOR} = 0.81$D^{-0.022}$ B
S_{SOR} = 0.85$D^{-0.123}$ E
S_{SOR} = 0.81$D^{-0.034}$ C
S_{SOR}= 0.92$D^{-0.182}$ F
S_{SOR} = 0.81$D^{-0.032 \pm 0.01}$
S_{SOR}= 0.87$D^{-0.167 \pm 0.04}$
0 2 4 6 8 10 12 14 16 18
Temporal distance D (days)