environmental microbiology

Environmental Microbiology (2011) **13**(11), 2976–2989 doi:10.1111/j.1462-2920.2011.02571.x

Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef

Verena Witt,[1,2]* Christian Wild,[2] Kenneth R. N. Anthony,[1] Guillermo Diaz-Pulido[3] and Sven Uthicke[1]

[1]*Australian Institute of Marine Science, PMB 3, Townsville MC, Qld. 4810, Australia.*

[2]*Coral Reef Ecology Group (CORE), Leibniz Center for Tropical Marine Ecology (ZMT), Fahrenheitstr. 6, 28359 Bremen, Germany.*

[3]*Griffith School of Environment and Australian Rivers Institute – Coast & Estuaries, 170 Kessels Road, Nathan Campus, Griffith University, Nathan Qld. 4111, Australia.*

Summary

Rising anthropogenic CO_2 emissions acidify the oceans, and cause changes to seawater carbon chemistry. Bacterial biofilm communities reflect environmental disturbances and may rapidly respond to ocean acidification. This study investigates community composition and activity responses to experimental ocean acidification in biofilms from the Australian Great Barrier Reef. Natural biofilms grown on glass slides were exposed for 11 d to four controlled pCO_2 concentrations representing the following scenarios: A) pre-industrial (~300 ppm), B) present-day (~400 ppm), C) mid century (~560 ppm) and D) late century (~1140 ppm). Terminal restriction fragment length polymorphism and clone library analyses of 16S rRNA genes revealed CO_2-correlated bacterial community shifts between treatments A, B and D. Observed bacterial community shifts were driven by decreases in the relative abundance of *Alphaproteobacteria* and increases of *Flavobacteriales* (*Bacteroidetes*) at increased CO_2 concentrations, indicating pH sensitivity of specific bacterial groups. Elevated pCO_2 (C + D) shifted biofilm algal communities and significantly increased C and N contents, yet O_2 fluxes, measured using in light and dark incubations, remained unchanged. Our findings suggest that bacterial biofilm communities rapidly adapt and reorganize in response to high pCO_2 to maintain activity such as oxygen production.

Received 7 April, 2011; accepted 18 July, 2011. *For correspondence. E-mail v.witt@aims.gov.au; Tel. +61-7-47534483; Fax +61-7-4772 5852.

Introduction

The world's oceans have absorbed 30–40% of anthropogenically emitted CO_2 from the atmosphere (Feely *et al*., 2004; Sabine *et al*., 2004) since the beginning of the industrial era. The uptake of CO_2 alters the seawater carbonate chemistry including a reduction in pH (Caldeira and Wickett, 2003; Raven *et al*., 2005). Such ocean acidification has already been detected in the Australian Great Barrier Reef (Wei *et al*., 2009). Ocean acidification may erode away the structural foundation for coral reef growth (Kleypas *et al*., 1999; Langdon *et al*., 2000; De'ath *et al*., 2009; Ries *et al*., 2009; Silverman *et al*., 2009), inducing future shifts from coral- to algal-dominated reefs (Hoegh-Guldberg, 1999; Hughes *et al*., 2003; Pandolfi *et al*., 2005; Anthony *et al*., 2011).

Bacterial communities play a critical role in the health of coral reef ecosystems (Ritchie, 2006; Mouchka *et al*., 2010; Meron *et al*., 2011). However, little is known about how microorganisms such as bacteria respond to changing ocean carbon chemistry (reviewed in (Liu *et al*., 2010; Joint *et al*., 2011). A recent study on the effects of pH on bacterial communities associated with corals has revealed community shifts and increased bacterial diversity with decreasing pH (Meron *et al*., 2011). Further, previous mesocosm experiments in Norway suggest that heterotrophic planktonic free-living bacterial communities shifted in response to high CO_2 levels, while the communities of particle-attached bacteria, bacterial abundance and activity remained unaffected by high CO_2, and were rather linked to a phytoplankton bloom (Allgaier *et al*., 2008). In addition to planktonic life styles, bacteria within marine environments often exist as surface-attached biofilm communities (Costerton *et al*., 1995; Crump and Baross, 1996; Crump *et al*., 1998; Teske and Wooldridge, 2001; Thornton, 2002), which are defined as complex surface-attached microbial communities comprised of photo- and heterotrophic microorganisms embedded in an extracellular polymeric matrix (Mihm *et al*., 1981). Biofilms contribute significantly to primary production, rapid

Table 1. pH, pCO_2 (partial pressure CO_2), TA (total alkalinity), DIC (dissolved inorganic carbon) and Ω_{Arag} (aragonite saturation state) values are means of eight replicates (SE).

Treatments	pH	pCO_2 (μatm)	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	Ω_{Arag}
Pre-industrial	8.12 (0.02)	305 (11)	2193 (13)	1882 (16)	3.37 (0.06)
Today	8.02 (0.02)	402 (20)	2170 (27)	1918 (29)	2.78 (0.07)
Projected mid century under A1Fi scenario	7.85 (0.01)	564 (12)	2208 (22)	2012 (17)	2.26 (0.07)
Projected late century under A1Fi scenario	7.63 (0.02)	1140 (52)	2212 (20)	2123 (24)	1.32 (0.04)

pH and TA were measured while *p*CO2, DIC and Ω_{Arag} were calculated for 24–25°C. Samples collected in May 2009.

nutrient recycling and the efficient degradation of organic matter (Lock *et al*., 1984; Battin *et al*., 2003), and are hence essential components of oligotrophic coral reef ecosystems. Bacterial biofilm communities have the ability to structurally self-organize and respond rapidly to changing environmental conditions (Tolker-Nielsen and Molin, 2000). By providing surfaces for larval settlement of marine invertebrates, microbial biofilms also influence settlement cues and the induction of metamorphosis of important reef building organisms, such as corals, and therefore affect coral reef establishment, recovery and resilience (Wieczorek and Todd, 1998; Webster *et al*., 2004). Further, coral reefs provide an immense surface area for biofilm colonization and development and therefore, shifts in biofilm communities may therefore substantially affect reef productivity, biomass, composition and ecosystem functioning.

Studies have shown effects of high CO_2 concentrations on the photosynthetic productivity of microalgal and bacterial communities, but results are conflicting or scarce, precluding predictions of general patterns about possible future ecological impacts (Rost *et al*., 2008; Doney *et al*., 2009; Ries *et al*., 2009; Hendriks *et al*., 2010; Liu *et al*., 2010). As ocean acidification poses potential threats to coral reefs, understanding how primary reef colonizing biofilms may respond to future ocean acidification is essential. Acidification may impact biofilm communities by shifting towards algal dominance, affecting productivity and nutrient cycling (reviewed in Rost *et al*., 2008) and an increased occurrence of disease causing bacteria (Meron *et al*., 2011). Ocean acidification has been investigated on various invertebrates and plankton; however, despite the importance of biofilm communities, impacts of ocean acidification on these communities have not been studied in biofilms. This study therefore explores the potential effects of ocean acidification on the activity (expressed as O_2 fluxes) and community composition of tropical coral reef-associated biofilms from the Great Barrier Reef, Australia.

Results

Seawater chemistry

As expected when using CO_2 bubbling, the total alkalinity only varied slightly between treatments (Table 1). In contrast, dissolved inorganic carbon concentrations increased markedly from 1882 μmol kg^{-1} in the pre-industrial treatment to 2123 μmol kg^{-1} in the late century treatment due to the CO_2 additions. Based on these measurements actual pCO_2 concentrations in the treatments were calculated as 305, 402, 564 and 1140 ppm. (Table 1)

Changes in the macro community composition in biofilms

The frequency of phototrophic flora components (e.g. diatoms and algae) of the biofilm macro communities at 305 ppm significantly differed from the groups found at 402 ppm, and both were significantly different compared with 1140 ppm (Wilcoxon test, $P < 0.05$) (Tables 2 and S1). At 1140 ppm the phototrophic community members exclusively comprised of diatoms (Bacillariophyceae), green filamentous and green algae (Chlorophyta), and the

Table 2. Macro community analysis of the algal component of biofilm replicates ($n = 6$) after 11 d for different pCO_2 treatments.

Treatment (pCO_2)	305 ppm	402 ppm	564 ppm	1140 ppm
Algae specimen				
Diatoms	23	24	24	22
Filamentous green algae	15	15	15	15
Green algae	18	18	9	2
Filamentous red algae	6	6	2	0
Calcareous red algae	6	6	1	0
Fleshy red algae	6	6	1	0

The sum of rank numbers: (4) dominant (3) frequent (2) occasional (1) rare (0) absent, are listed for each algae category in each pCO_2 treatment.

Table 3. Total organic carbon, total inorganic carbon and total nitrogen are shown with error bars representing one standard deviation of the mean (mean ± SD) at each pCO_2 treatment at the end of the experiment (11 d).

Treatment (pCO_2)	Total inorganic carbon (mg^{-1} cm^{-2})	Total organic carbon (mg^{-1} cm^{-2})	Total nitrogen (mg^{-1} cm^{-2})	C : N ratio (mol)
305 ppm	0.084 (0.026)	0.137 (0.019)	0.018 (0.003)	7.767 (3.588)
402 ppm	0.128 (0.238)	0.132 (0.009)	0.023 (0.065)	7.172 (1.816)
564 ppm	0.157 (0.295)	0.157 (0.050)	0.020 (0.096)	7.987 (2.225)
1140 ppm	0.189 (0.476)	0.175 (0.022)	0.025 (0.086)	8.245 (0.556)

green algae decreased with rising CO_2. Further, compared with the biofilms in the other treatments, red algae, filamentous red algae and calcareous red algae (Rhodophyta), were completely absent at 1140 ppm (Table 2).

Metabolic activity of biofilm communities

During the period under high light conditions all biofilms were net O_2 producers and oxygen production (average = 2.270 μmol^{-1} cm^{-2} h^{-1} ± 0.009) and consumption rates (average = 0.069 μmol^{-1} O_2 cm^{-2} h^{-1} ± 0.0361) of biofilms between the four pCO_2 treatments were statistically indistinguishable (one-way ANOVA production $F_{3,60} = 1.35$, $P = 0.2668$; consumption $F_{3,40} = 1.11$, $P = 0.3561$).

Carbon and nitrogen in biofilms

Total organic carbon and inorganic carbon in biofilms after 11 d were significantly higher at 1140 ppm pCO_2 compared with 305 ppm (ANOVA $F_{3,20} = 5.50$, $P = 0.0006$; $F_{3,20} = 4.70$, $P = 0.0121$, respectively). Nitrogen content increased significantly (ANOVA $F_{3,20} = 3.32$, $P = 0.0407$) (Table 2) with increasing pCO_2 levels between 305 and 1140 ppm (Tukey–Kramer test). C : N molar ratios (8.196 ± 2.499) remained statistically indistinguishable between pCO_2 treatments (ANOVA $F_{3,20} = 2.14$, $P = 0.4644$) (Table 3).

Terminal restriction fragment length polymorphism (T-RFLP) of bacterial biofilm communities

A total of 37 peaks have been identified using T-RFLP. Thereof, 91.9% could be successfully assigned to a clone from the clone libraries (within ± 0.5 bp) (Table S2). The most T-RFs were affiliated with the *Alphaproteobacteria* (35.1%), *Bacteroidetes* (27%) and *Gammaproteobacteria* (21.6%). The family *Rhodobacteraceae* (16%) was dominant in the *Alphaproteobacterial* T-RFs, and families *Flavobacteriaceae* (13.5%) and *Flexibacteraceae* (8.1%) were dominant in *Bacteroidetes* T-RFs in all pCO_2 treatments. Profiles of initial communities were significantly different from those at the end of the experiment (data not shown). At 1140 ppm more T-RFs belonging to the *Flavobacteriaceae* were identified than at 305 ppm. Pooling the relative abundances of the T-RFs (bacterial taxa) for each phylum in each treatment revealed that *Bacteroidetes* increased with rising pCO_2, being significantly more abundant (ANOVA $F_{3,230} = 4.40$, $P = 0.0048$) at 1140 ppm compared with the other treatments (Fig. 1). Conversely, the *Alphaproteobacteria* showed a decreasing trend, as their relative abundance was significantly lower (ANOVA $F_{3,230} = 4.43$, $P = 0.0093$) at 1140 ppm. *Cyanobacteria* showed a significantly lower relative abundance at 402 than 305 ppm ($F_{3,81} = 4.15$, $P = 0.0124$). Relative abundances of other groups including *Gammaproteobacteria*, Diatom plastids and *Deltaproteobacteria* were statistically indistinguishable. A principle component analysis (PCA) of T-RFLP data revealed that distinct bacterial community assemblages were present in both high pCO_2 treatments compared to both lower pCO_2 treatments (Fig. 2). PCA showed that the bacterial biofilm community assemblages at 305 ppm CO_2 had the highest variability between replicate samples within a treatment, followed by 402 ppm CO_2, while the bacterial community assemblages within both high CO_2 treatments (especially the highest 1140 ppm) showed much less community variability (Fig. 2). This same pattern was revealed by re-analysis using nMDS (not shown). One-way ANOSIM of T-RFLP data revealed global dissimilarities ($P < 0.0001$) of the treatments and *post hoc* tests indicated significant differences between both 305 ppm ($P = 0.0035$) and 402 ppm ($P = 0.0106$) compared with 1140 ppm. A SIMPER analysis of T-RFLP data showed an overall average dissimilarity of 40% between treatments. In agreement with the PCA biplot, T-RFs contributing the most to the dissimilarities between 305 and 402 ppm to 1140 ppm were Diatom plastids, *Silicibacter*, *Tenacibaculum* and *Roseobacter* (Fig. 3 and Table 4). The average relative abundance of Diatom plastids, *Roseobacter* and *Silicibacter* T-RFs showed a decreasing trend with rising pCO_2, while the *Tenacibaculum* T-RF increased notably (one-way ANOVA $F_{3,40} = 6.62$, $P = 0.0009$) in both elevated pCO_2 treatments compared with 305 ppm (Fig. 3). Two further taxa contributing to the dissimilarities in the bacterial assemblages of 305 and 402 ppm compared with the other treatments were T-RFs from the family *Rhodobacteraceae*, the order *Flavobacteriales* and the genus *Synechococcus*.

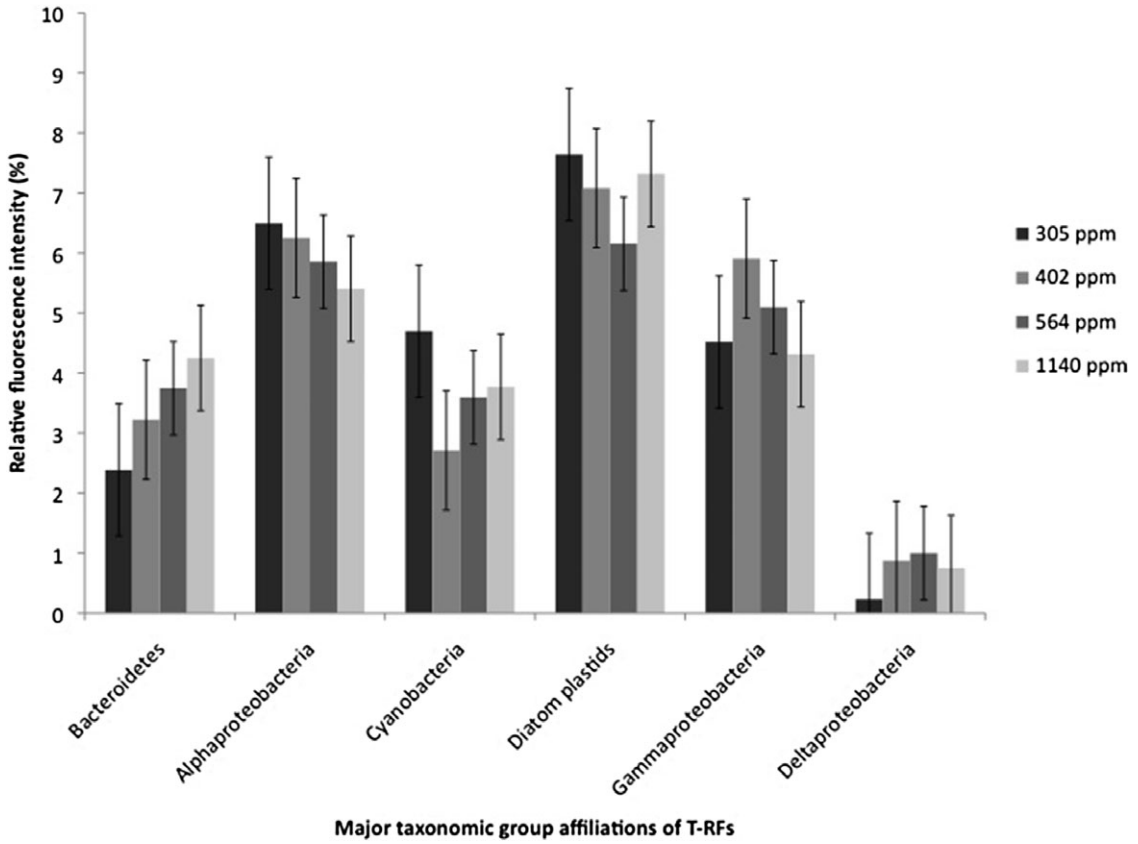

Fig. 1. Average relative abundance of T-RFs of the major phylogenetic groups revealed for each pCO_2 treatment determined by T-RFLP with standard error of the mean (mean ± SE).

Clone libraries of bacterial biofilm communities

Analysis of sequences obtained from the 16S rRNA gene libraries from biofilms demonstrated that sequences affiliated with the *Alphaproteobacteria* were most abundant in all pCO_2 treatments, except in the control (402 ppm), where *Bacteroidetes* affiliated sequences were most frequent (Fig. 4). Diatom plastid affiliated sequences (~10%) was the only group showing a treatment related trend in the clone library analysis, and increased with rising pCO_2. The cyanobacterial sequences (~10%) belonged to three different orders, *Chroococcales*, *Oscillatoriales* and *Nostocales* at 305 ppm, while at 1140 ppm *Chroococcales* sequences were found exclusively (data not shown). The LIBshuff test revealed statistically significant differences in the bacterial community compositions between the libraries derived from 305 and 402 ppm, and 305 and 564 ppm (Table S3). Further, statistically significant differences were detected between 305 and 402 ppm when compared with 1140 ppm, while the bacterial communities from 564 and 1140 ppm were statistically indistinguishable. All libraries significantly differed from the initial community ($t = 0$).

Discussion

The present study is the first to investigate the effects of ocean acidification on oxygen production and microbial community composition in marine biofilms associated with tropical coral reefs. The microbial community in the investigated biofilms was rapidly (after 11 d) and significantly affected by simulated ocean acidification. Despite an increase of carbon and nitrogen content and a significant change in the algal component of the biofilm community, photosynthetic and respiration activity remained on a similar level. Analysis of T-RFLP and clone libraries illustrated that high CO_2 concentrations caused significant shifts in the microbial community composition. These findings add to the growing evidence that microbes are sensitive to disturbance by high CO_2 and are able to adapt on short-term scales to maintain a constant level of activity.

Macro community changes and oxygen fluxes in biofilms

Changes in the macro communities of investigated biofilms imply that exposure during the early stages of biofilm

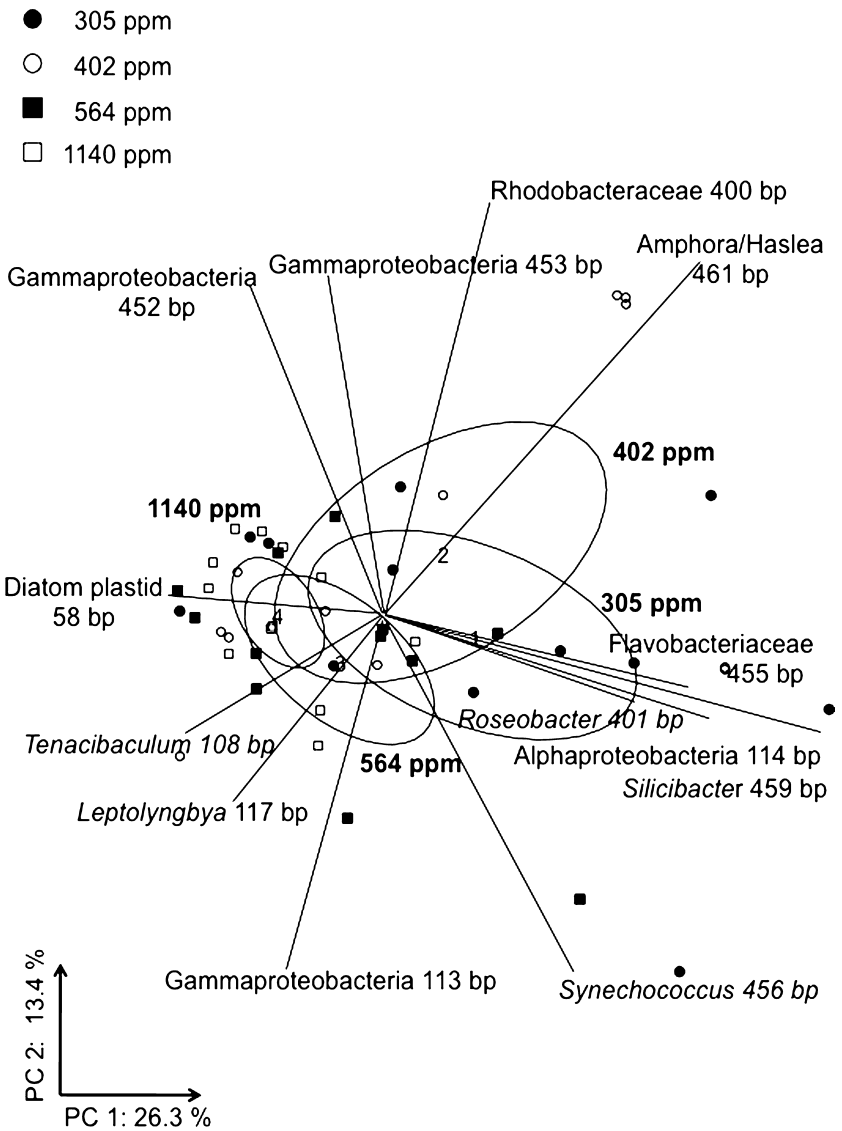

Fig. 2. Principal component analysis incorporating relative abundances of T-RFs (using the relative fluorescence peak intensity matrix) showing microbial assemblages for different pCO_2 treatments. Vectors show 40% of the species contributing the most to the differences between pCO_2 treatments and ellipses represent 95% confidence around the centroid of each treatment (305, 402, 564 and 1140 ppm).

development to short-term high CO_2 levels significantly decreases algal diversity and promotes a shift towards diatom and filamentous green algae dominated biofilm communities. Similarly, previous studies under elevated CO_2 showed that phytoplankton communities became diatom-dominated (Tortell *et al*., 2002) and promoted an increase in filamentous algae (Kuffner *et al*., 2008). Further, high CO_2 has shown to promote non-calcareous turf algal growth with a significant increase in biomass (Russell *et al*., 2009; Connell and Russell, 2010) and such an increase in biomass was also observed in the present study. The abundance of red algae, especially calcareous red algae, was significantly decreased at high CO_2, which agrees with similar studies indicating a decrease in production, biomass or calcification rates of calcareous red algae under elevated pCO_2 conditions (Anthony *et al*., 2008; Kuffner *et al*., 2008; Russell *et al*., 2009). In addition, negative effects on photosynthesis and growth in red seaweeds in response to CO_2 enrichment have been observed (Zou and Gao, 2009).

Although only hourly production rates under full sunlight were measured, the average respiration rates in the dark were about one order of magnitude lower than the average production rates. It is therefore conservative to assume that the investigated biofilm communities were net autotrophic over a 24 h period, indicating a dominant phototrophic component in the biofilms. Despite alterations in the algal community, no significant changes in metabolic activity could be detected, indicating adaption mechanisms of phototrophic members of the macro community in biofilms to high CO_2 conditions.

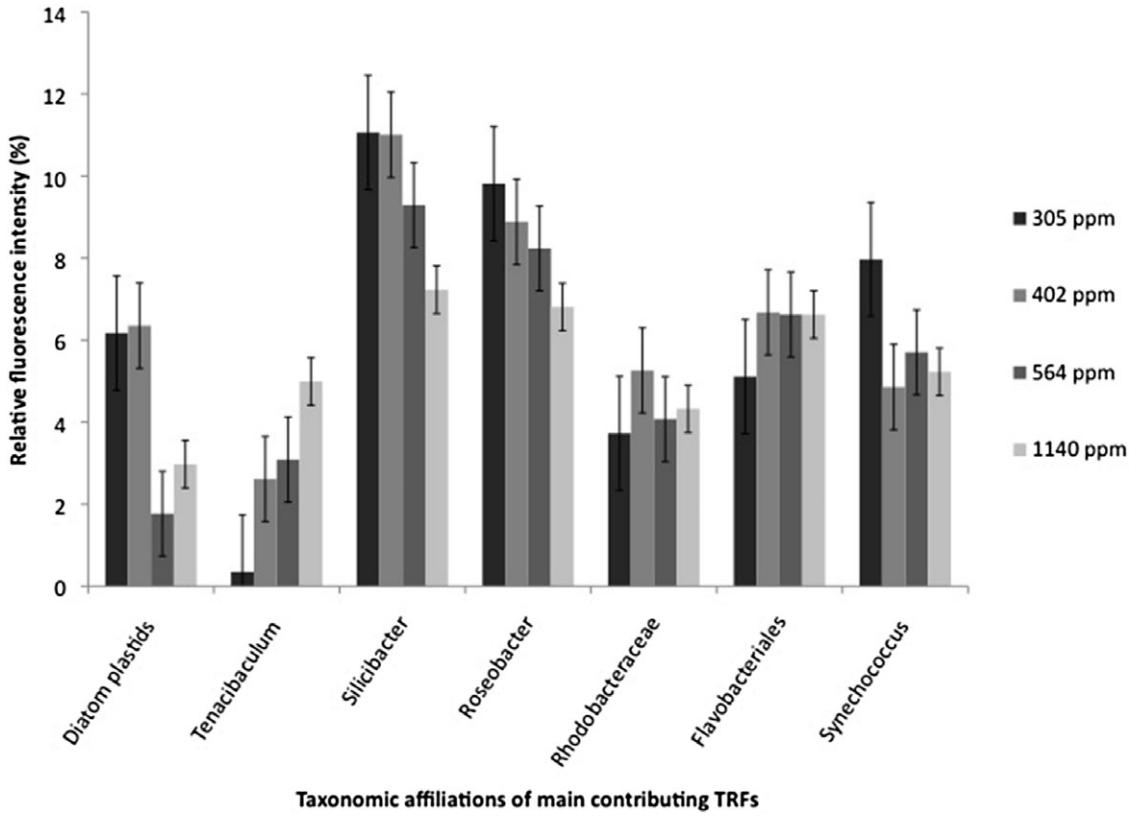

Fig. 3. Average relative abundance with standard error of the mean (mean ± SE) of T-RFs determined by T-RFLP contributing the most to the differences between pCO_2 treatments as revealed by SIMPER analysis.

Carbon and nitrogen contents in biofilms

Although not linear, organic nitrogen and carbon contents in the investigated biofilms generally increased under higher pCO_2 (564 and 1140 ppm). In the present study, C : N ratios did not vary with pCO_2 treatment, but were slightly higher (~8) than the Redfield ratio (6.6), supporting the assumption of algal dominated communities. Values exceeding the Redfield ratio may indicate nitrogen limitation (Healey and Hendzel, 1979), which is typical for primary producers in oligotrophic environments such as the GBR. Increased organic C and N may be due to higher production rates of extracellular polymeric substances (EPS), as often seen in bacteria [reviewed in (Sutherland, 2001)] and diatoms [reviewed in (Thornton, 2002)] under stress due to nutrient limitation. Thus, we hypothesize that the release from potential CO_2 limitation may have increased N limitation, thus leading to enhanced EPS production binding more calcareous reef sediment particles in the biofilm matrix, hence also elevating the inorganic C. Further, generally elevated C : N ratios may also be explained by enhanced EPS production as a protection against harmful UV-radiation (Elasri and Miller, 1999), which is an important factor to consider in Australia and hence should be tested in future studies.

Table 4. Similarity percentage (SIMPER) analysis (overall average dissimilarity of 40%) showing the contribution (%) of the bacterial taxa most responsible for the overall dissimilarities and dissimilarities between the significantly different (ANOSIM) microbial assemblages at the respective pCO_2 treatment.

	Contribution (%)			
Taxon	Overall	305 vs. 402	305 vs. 1140	402 vs. 1140
Diatom plastids	2.4	2.5	2.4	2.5
Silicibacter	2.3	2.6	2.2	2.2
Roseobacter	2.2	2.4	2.6	2.3
Synechococcus	1.9	2.2	2.1	2.0
Tenacibaculum	1.8	>1	2.8	1.9

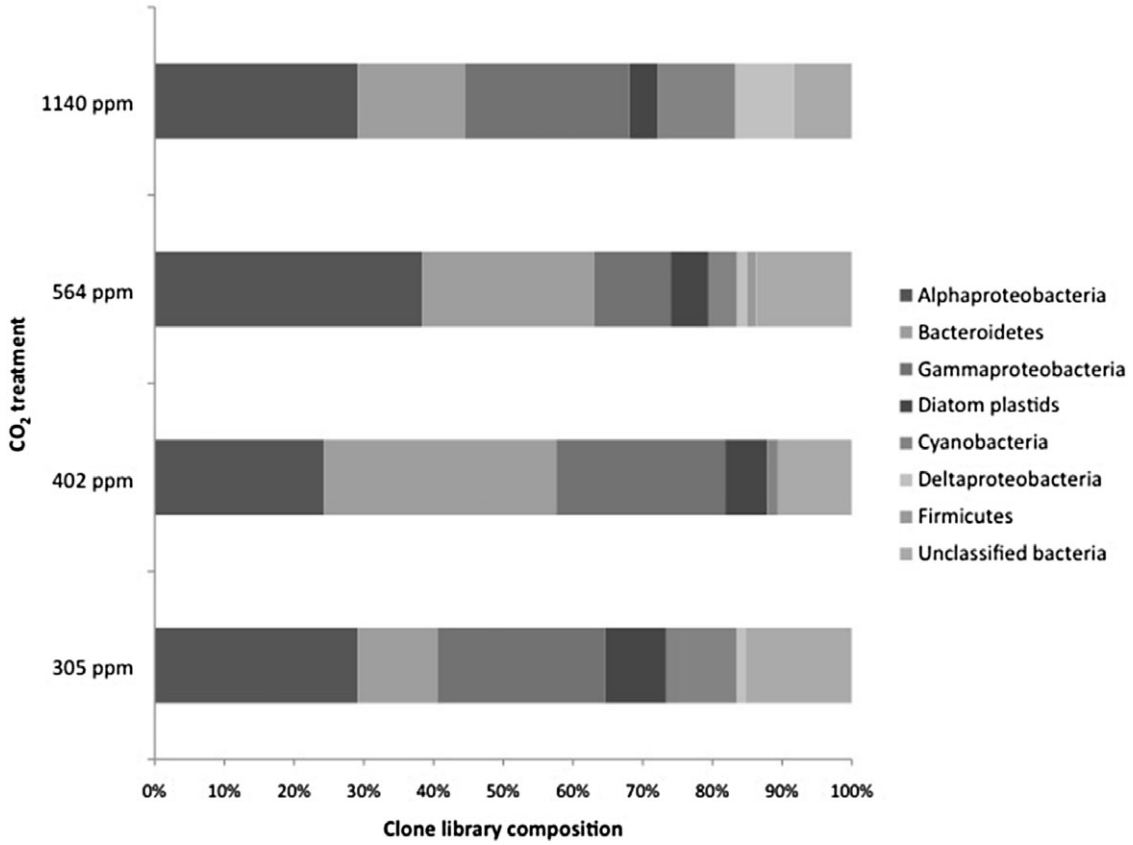

Fig. 4. Affiliations of 16S rRNA gene sequences retrieved from clone libraries from the different pCO_2 treatments (305 ppm $n = 79$, 402 ppm $n = 66$, 564 ppm $n = 75$, 1140 ppm $n = 74$). Bacterial sequence affiliations were grouped into dominant ribotype at phylum and phylum subdivisions. Only groups representing 5% or more of the clone library are represented in the 'other' shows minor groups showing < 5% of each library.

On the one hand, elevated C : N ratios exceeding the Redfield ratio (~8) were also detected in phytoplankton in response to high CO_2, and were speculated to lead to an excess CO_2 sequestration potential through the biological carbon pump in future oceans (Riebesell *et al*., 2007). Further, a study combining phytoplankton growth with TEPC formation has proposed that constant C : N ratios are no longer appropriate for estimating new production of POC from DIN uptake due to carbon overconsumption (Schartau *et al*., 2007), which might be applicable in a high CO_2 environment. Alternatively, CO_2 bubbling in the treatments may have formed transparent exopolymer particles from dissolved matter (Zhou *et al*., 1998), which may have also potentially contributed to the increased C : N contents of biofilms within high pCO_2 treatments compared with the remaining treatments without or little bubbling. However, if increased C : N contents in biofilms are in fact linked to high CO_2, increased EPS production may therefore lead to a change in dissolved organic matter bioavailability with elevated pCO_2 that may change particle aggregation and substrate availability for marine microbes. Although this mechanism is plausible and may have far-reaching impacts for biofilm functioning, no direct measurements were conducted and further research is required to investigate this hypothesis.

Bacterial community composition

Statistical tests (ANOSIM and LIBshuff) of fingerprinting and clone library data suggest that rising pCO_2 levels significantly change community composition in biofilms. Sequences affiliated with the *Rhodobacteraceae* (*Alphaproteobacteria*) and *Cytophaga-Flavobacterium-Bacteroides* (*CFB*) (*Bacteroidetes*) contributed most importantly to the differences between bacterial communities from the different treatments. The frequent detection of these two groups was not surprising as *CFB* are primarily found on surfaces (McBride, 2001; Nocker *et al*., 2004; Webster and Negri, 2006), as well as members of the *Roseobacter* that are ubiquitous and rapid colonizers of surfaces, and both are therefore commonly found in marine biofilms (Dang and Lovell, 2000; Dang *et al*., 2008). Overall, the relative abundance of *Bacteroidetes*, in particular the genus *Tenacibaculum* of the *CFB*,

increased with rising pCO_2, while *Alphaproteobacteria*, specifically members of the *Roseobacter* clade, appeared to show the inverse trend. Similar trends of *Flavobacteria* and *Alphaproteobacteria* were also observed in crustose coralline algae associated biofilms in response to other climate change factors such as elevated sea surface temperatures of 32°C (Webster *et al*., 2010). Further, a substantial increase in *Bacteroidetes*, predominantly *Flavobacteria*, was observed in corals in response to low pH (Vega Thurber *et al*., 2009). This community shift may therefore be a response to high CO_2. Further, the high relative abundance of *Bacteroidetes* in the biofilms may be due to the fact that many members of the *CFB* cluster excrete exoenzymes to decompose high molecular weight organic material from detritus (Reichenbach, 1991; Keil and Kirchman, 1999; Cottrell and Kirchman, 2000; Kirchman *et al*., 2000), which may be an advantageous trait in oligotrophic waters as found on the GBR. The degradation rate of marine organic matter by bacterial extracellular enzymes is accelerated by high CO_2 (Piontek *et al*., 2010), which may be a possible reason for the increase in *CFB* with increasing pCO_2. A potentially higher EPS production under higher pCO_2 (see above) may give members of the *CFB* group a selective advantage due to the more effective acquisition of catabolic substrates.

Furthermore, little changes in the apparent relative abundances of diatoms as revealed by both T-RFLP and clone library analysis suggested little impact of pCO_2 levels on the frequency of diatoms. Previous findings showed that diatoms appear to be insensitive to pCO_2 regarding silification processes (i.e. building of frustules) (Milligan *et al*., 2009), and that pCO_2 only caused small changes in diatom populations (Kim *et al*., 2006). Further, Cyanobacterial sequences of the order *Chroococcales*, such as *Synechococcocus*, were detected exclusively in the 1140 ppm treatment in clone libraries and appeared to be more abundant in comparison with other *Cyanobacteria* in this treatment as determined by T-RFLP. *Synechococcus* strains have higher growth rates at elevated pCO_2 levels (Fu *et al*., 2007), suggesting that this group may also obtain a selective advantage over other *Cyanobacteria* in high CO_2 conditions.

Interestingly, T-RFLP showed a low community variability among replicates at high pCO_2 (1140 ppm) compared with 305 and 402 ppm (Fig. 4) that may therefore suggest that communities become more specialized and adapted to lower seawater pH. A study by Takeuchi and colleagues on the effects of seawater acidification on the growth rates of cultured marine microorganisms found that bacteria were relatively resistant to high concentrations of CO_2. Impacts on bacterial growth were observed only at pH values as low as 5.5 to 6.0 (Takeuchi *et al*., 1997). However, Takeuchi's study solely focused on bacterial growth rates and not community composition or diversity and artificial cultivation may cause a different response.

In the current study, both molecular techniques were in agreement and suggested that rising pCO_2 significantly changed community composition of biofilms. Low variability may indicate that there is less competition among bacterial groups. Taken together, the detected shifts in marine bacterial biofilm communities in this study may suggest the ability of these communities to structurally reorganize in response to increased levels of pCO_2.

Further, these community shifts in response to short-term exposure may also be stress-related. We expected to observe adaption via physiological adjustment such as increased O_2 production rates as previously observed in, e.g. diatoms (Tortell *et al*., 2008) to compensate increased pCO_2 availability, but this was not the case. Community changes involved elevated C : N ratios, possibly due to enhanced EPS production as part of a stress response to short-term (11 d) high pCO_2 exposure as proposed above. However, Allgaier and colleagues (2008) also observed C : N ratios of ~8 after 24 d exposure to high pCO_2 with unaltered bacterial abundance and activity. As no further long-term exposure results are available, the assumption persists that community shifts display adaption mechanisms rather than short-term stress responses.

Concluding remarks

Here, we propose that overall, short-term exposure (11 d) to rising pCO_2 levels was sufficient to significantly alter the algal community and the bacterial community composition of biofilms adjacent of tropical coral reefs. These findings indicate sensitivity, but at the same time the adaptive ability of such communities. This study aimed to provide insights into the impacts of elevated pCO_2 in bacterial biofilms and revealed possible target groups, such as *CFB*, diatoms, *Cyanobacteria* and the *Roseobacter* clade for future studies investigating the emerging field of ocean acidification on marine biofilm microbes. Future work should also focus on microbial processes and interactive effects of acidification and other direct or indirect changes of global change (such as temperature and nutrients), as well as exposure to these parameters for longer periods. The sensitivity of some bacterial biofilm species in response to altered ocean chemistry may lead to shifts in their abundance and/or function. As biofilms affect biogeochemical cycling the potential effects on future reef ecology needs further investigation.

In recent publications, the sensitivity of marine organisms to ocean acidification has been questioned (Hendriks and Duarte, 2010; Hendriks *et al*., 2010) and a null hypothesis has been put forward that responses of marine microbes are negligible and that acidification will have

little effect on biogeochemical processes other than calcification (Joint *et al*., 2011). In contrast, a meta-analysis by (Liu *et al*., 2010) rejected this null hypothesis, but pointed out that microbes in response to ocean acidification have been investigated far too little to draw conclusions for future ecological scenarios. Ocean acidification is suggested to have worse effects on the development of larval stages of marine organisms (Dupont *et al*., 2010) and was shown to affect juvenile corals (Albright *et al*., 2010; Suwa *et al*., 2010). As biofilms are primary reef colonizers, facilitating invertebrate larval settlement and development, changes in these communities may have detrimental effects on future coral reefs.

Experimental procedures

Experimental design

We conducted an experiment simulating ocean acidification at the Heron Island Research station, located in the Southern Great Barrier Reef, Australia (23°27′S, 151°55′E), during May 2009 (Austral autumn) using an outdoor flow-through aquarium system. The aquaria set-up used a computer-controlled CO_2 dosing system (Aquatronica-AEB Technologies, Italy). Target pCO_2 values were set and the corresponding pH values were continuously monitored in the CO_2 200 l mixing tanks, which supply individual tanks, using pH probes (Mettler-Toledo polarographic sensors), which automatically logged pH values. The pH probes used were of high precision and were routinely calibrated to the total seawater scale throughout the experiment at temperatures of 24–25°C, providing high confidence in the target values. The pH readings controlled the open/close status of a solenoid valve (Dupla Australia, Littlehampton, Australia) to regulate the pure CO_2 (analytical grade) supply (bubbling) in each mixing tank of each treatment [set up as in (Diaz-Pulido *et al*., 2011)]. The experimental design consisted of three CO_2 dosing regimes and a control treatment, representing A: pre-industrial levels of 300 ppm (pH 8.1–8.2) (CO_2 scrubbing using soda lime as described in Reynaud *et al*., 2003; IPCC, 2007); B: present-day control 400 ppm (pH 8.0) (reflecting the diurnal variability of the intake water from the reef ranging from pH 7.9 to 8.2); C: projected mid century 560 ppm (pH 7.9) and D: projected late century under the A1FI scenario 1140 ppm (pH 7.6) by the Intergovernmental Panel on Climate Change (IPCC, 2007). Each treatment was replicated by six individual tanks (total of 24 tanks) with a volume of 10 l each at a flow-rate of 2 l^{-1} min^{-1} over 11 d. The aquaria were organized randomly, and shade screens were used to reduce the natural sunlight by 30% to average noon levels of about 1200 (max: 1700) μmol photons m^{-2} s^{-1}. Additionally, the pH of individual tanks was monitored daily using a hand-held pH electrode (HQ10-HQ20 Meters, Hach, USA). Samples for dissolved inorganic carbon and total alkalinity (TA) were taken throughout the course of the experiment (reported in Diaz-Pulido *et al*., 2011). The calculated pCO_2 values were very close to the target values (Table 1). Light (Odyssey, Christchurch, New Zealand) and temperature (UA-001, Onset, USA) loggers were used over the duration of the experiment. As a suitable substrate type for colonization, glass microscope slides (as proposed by Witt *et al*., 2011) were fixed vertically in custom-built PVC holders (20 × 11 cm) holding six slides each. For preconditioning, pre-cleaned (washed in ethanol 70% and rinsed in sterile water) glass slides were immersed into a flow-through tank with natural seawater from the lagoon for 24 d. Subsequently, established biofilm slides were introduced to the experimental tanks and exposed to four different CO_2 treatments for 11 d. Biofilm slides were sampled on the initial day ($t = 0$) and thereafter every third day for oxygen production measurements (see section below). For sample collection, as much as possible of the biofilm material was carefully scraped off the substrates into cryovials using sterile No. 11 scalpel blades (material yield was usually > 2 g, sufficient to extract a high yield of high-quality DNA for PCR amplification see section below) and snap-frozen in liquid nitrogen and stored at –80°C until further processing.

Determination of macro communities in biofilms

The algal component of the macro communities of biofilm replicates from six replicate tanks were examined every 3 days after the production measurements, under a binocular microscope (Olympus, Japan). Visualized organisms were photographed and then assigned to a category for each specimen (diatoms, filamentous green algae, green algae, filamentous red algae, calcareous red algae, fleshy red algae) and their frequency of occurrence (% coverage) was determined. Five categories were scored with the following cut-off values: dominant (90%), frequent (70%), occasional (50%), rare (20%) and absent (0%). The categories were given a rank number in decreasing value: (4) dominant (3) frequent (2) occasional (1) rare and (0) absent. A Wilcoxon Mann–Whitney rank sum test (*U*-test) was used to test for significant differences in average ranks between pCO_2 treatments.

Determination of oxygen fluxes in biofilm communities

Oxygen production by the biofilm slides was measured in a time series every 3 days under both light and dark conditions for biofilm slides. For the light incubation, replicate ($n = 6$) biofilm slides were enclosed with water of the corresponding treatment in custom-made airtight glass vials (50 ml), with glass lids that were fixed with plastic clips. The vials were secured horizontally in custom-made holders to enable equal light distribution onto individual slides that were incubated in a transparent outdoor flow-through seawater tank at *in situ* seawater temperature of *c.* 23°C and natural light conditions at noon for 30 min. The dark incubations occurred correspondingly in an opaque tank for 1 h. Test experiments revealed that the chosen incubation times were sufficient to measure a clear response (at least a change of 10% in dissolved O_2 concentrations). Vials were gently mixed before measuring dissolved oxygen concentrations using a hand-held luminescent dissolved oxygen optode (HQ10-HQ20 Meters HACH, Hydrolab oxygenmeter, USA) at start and end of incubations. Triplicate blank controls (seawater only) were run simultaneously with all incubations. Changes in O_2 con-

centration in the blanks were small (< 1%) compared with the biofilm incubations and were subtracted from the biofilm measurements. Oxygen production and consumption rates were calculated in μmol O_2 cm^{-2} h^{-1}. As this was an outdoor experiment with natural photon flux density variability due to cloud cover, average values during measurements on all sampling days (every 3 days) at noon of 1406 at initial measurements, 1301 on 3 d, 982 on 7 d and 1054 μmol photons m^{-2} s^{-1} at final measurements were used.

Carbon and nitrogen measurements

For the measurement of total carbon and nitrogen concentrations in the samples, one half of each biofilm on the microscope slide (8.125 cm^2) was scraped off and transferred onto pre combusted GF/F filters (Whatman, 25 mm in diameter). Filters were dried for 48 h at 40°C and analysed as described in Wild and colleagues (2008). Measurements were performed with a THERMO NA 2500 elemental analyser (standard deviations of C and N concentration measurements of replicates of the laboratory standard peptone were < 3%) to derive total carbon and nitrogen concentrations. Biofilms were rinsed with freshwater to remove salts, then dried at 60°C and homogenized using mortar and pestle. The percentage of organic carbon (treated with 200 μl of 1 M HCl) of total carbon was determined on a parallel sample for each slide on a Shimadzu elemental analyser (TOC5000A) using standard reference material (MESS-1 and Round 40).

Genomic DNA extraction

Total DNA was extracted from 0.5 g of the total biofilm (wet weight) sample using the MoBio UltraClean Soil Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's protocol with the following modifications. Bead-beating (Mini-Bead-Beater, Biospec Products, Bartleville, OK, USA) (2 × 30 s) cycles were performed and DNA was eluted with 2 × 50 μl of 1 × TE buffer. DNA extracts were examined by standard 1% agarose gel electrophoresis and quantified using a Nanodrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

PCR amplification, cloning and sequencing

Bacterial 16S rRNA genes were amplified by PCR using the general bacterial 16S rRNA gene primers 63F (5′-CAGG CCTAACACATGCAAGTC-3′) and 1389R (5′-ACGGGCG GTGTGTACAAG-3′) (Sigma-Proligo, The Woodlands, TX, USA) (Marchesi *et al*., 1998). Each biofilm sample was amplified in triplicate 25 μl reactions containing 2.5 μM non-acetylated bovine serum albumin (New England Biolabs, USA), 2 μM (2 mM each) dNTP (Astral Scientific, Australia), 2.5 μM forward primer 63F, 1.25 μM reverse primer 1389R, 1 μM $MgCl_2$ (Qiagen), 1.25 U HotStar Taq (Qiagen), 2.5 μl HotStar Buffer (Qiagen, Germany) and ~2 ng of template DNA. Amplification was performed with an initial incubation at 95°C for 15 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 90 sec, and a final extension at 72°C for 10 min.

Five clone libraries of bacterial 16S rRNA genes amplified from DNA extracted from biofilms grown on glass substrate were constructed, and represent one library for each treatment ($t = 0$, 305, 402, 564 and 1140 ppm). DNA extracted from each biofilm sample from all six replicate tanks of each treatment was then subject to PCR in each triplicate PCR reactions to avoid PCR bias. Triplicate amplicons of each of the six samples per treatment were then individually pooled back to the original six replicates. Each of the six replicates from each treatment was then pooled according to treatment for construction of the five clone libraries. Pooled samples were purified using the MinELUTE PCR Clean-Up Kit (Qiagen) and cloned using a TOPO-TA Cloning Kit (Invitrogen, USA) according to the manufacturer's instructions. After blue-white screening, colonies were checked for correct insert size using a colony PCR method with the specific sequencing primer 63F. Randomly picked clones were dispersed in LB media and 10% glycerol in 96-well plate format and sent to the Australian Genome Research Facility (Brisbane, Australia) for purification and sequencing by an ABI3730 XL Automatic DNA Sequencer.

Phylogenetic analysis of clone sequences

Retrieved sequences were edited using Chromas Lite 2.33 (Technelysium Pty, Australia), saved as fasta files and submitted to the Greengenes NAST Aligner (DeSantis *et al*., 2006) (for alignment of sequences to the Greengenes database). Greengenes NAST-aligned 16S rRNA gene sequences were checked for chimeras using Bellerophon Version 3 (Huber *et al*., 2004), and identified chimeras were excluded from further analysis. The NAST-aligned 16S rRNA gene sequences were submitted to the Greengenes batch sequence classifier [http://greengenes.lbl.gov/cgi-bin/nph-classify.cgi], and taxonomic assignments for each sequence were recorded using the NCBI taxonomy system.

T-RFLP analysis

Bacterial 16S rRNA genes were PCR amplified using the same reaction mixture and conditions outlined for clone libraries, except that fluorescently labelled 5′Cy-5 63F (Sigma-Aldrich) and 1389R primers were used. Each of the six duplicate biofilm samples per treatment (12) (in triplicate PCR) was purified using the MinElute PCR Purification Kit (Qiagen). PCR products were quantified using the NanoDrop Spectrophotometer (Thermo Fisher Scientific), and 150 ng of each purified product was digested with the restriction enzyme MspI (New England Biolabs) according to the manufacturer's instructions. Digested fragments were desalted using the DyeEx 2.0 Spin Kit (Qiagen) and vacuum dried for 40 min at low temperature in the dark. Terminal restriction fragments (T-RFs) were resolved and visualized using the CEQ 8800 Genetic Analysis System (Beckman-Coulter, Fullerton, CA, USA) with a 600 bp size standard (Beckman-Coulter). The threshold for relative peak height was set at 20% of the height of the second highest peak to remove any spurious artefact peaks from the analysis. Replicate samples were compared using the software T-align (Smith *et al*., 2005) with a range of 0.5 bp peak area to determine the consensus

peaks between duplicates. The relative fluorescence intensity of the peak area of T-RFs was used as a relative abundance measure of dominant T-RFs in further statistical analyses detailed below. For verification and identification of taxonomic identity of T-RFs, purified DNA from individual clones (provided by AGRF), taxonomically identified as above, were subject to PCR. The samples were analysed by T-RFLP using the same protocol as for environmental samples, except that 75 ng of digested PCR products generated from each clone was used. Each clone produced a single peak (T-RF), which was then manually assigned to T-RFs identified from whole community T-RFLP profile analyses.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to determine significant differences between pCO_2 treatments for the response parameters: relative abundance of T-RFs, total organic and inorganic carbon, total nitrogen and carbon/nitrogen ratio. Two-way ANOVA was performed on production and respiration data to determine effects of the fixed factors time and treatment. Homogeneity of variances was tested using the Levene's Test, and the Tukey–Kramer Test was used as *post hoc* tests to investigate the differences between the individual time points and treatments. These analyses were performed using the NCSS 2007 (NCSS, USA) statistical software.

LIBshuff in MOTHUR was used to determine the percentage coverage of the populations and whether there were significant differences in bacterial community composition between clone libraries. All sequences were submitted to the GenBank Database (Accession numbers: HQ601614-HQ601616, HQ601619- HQ601701, HQ601703- HQ601707, HQ601709- HQ601744, HQ601746- HQ601786, HQ601788-HQ601791, HQ601793- HQ601797, HQ601799-601814, HQ601816- HQ601900).

T-RF values were third root transformed and standardized before analysis. This is a standard transformation for Principal Component Analysis (PCA), to remove variance–mean relationships and remove over-emphasis of extremely rare or extremely abundant taxa (peaks). PCA and non-metric multidimensional scaling (nMDS) analysis using the Bray-Curtis distance measure was applied to determine whether bacterial assemblage in samples grouped by treatment. The significance of assemblage dissimilarities between pCO_2 treatments was tested by one-way analysis of similarity (ANOSIM) based on permutation procedures also using the Bray-Curtis distance measure. The contributions of each taxon to the total dissimilarities of treatments were analysed using the Similarity Percentage (SIMPER) routine. All analyses were performed using PAST statistical software (Hammer *et al*., 2001).

Acknowledgements

We thank M. Guezo for helping with the experiment and the Heron Island Research Station Staff, in particular A. Bryant, for great help. We also thank O. Hoegh-Guldberg for supporting this study and N.S. Webster for comments on the manuscript. This research was supported by German Research Foundation (DFG) Grant Wi 2677/2–1 to C. Wild, the Global Environment Facility (GEF) and the Marine and Tropical Sciences Research Facility (MTSRF).

References

Albright, R., Mason, B., Miller, M., and Langdon, C. (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. *Proc Nat Acad Sci USA* **107:** 20400–20404.

Allgaier, M., Riebesell, U., Vogt, M., Thyrhaug, R., and Grossart, H.P. (2008) Coupling of heterotrophic bacteria to phytoplankton bloom development at different *p*CO(2) levels: a mesocosm study. *Biogeosciences* **5:** 1007–1022.

Anthony, K.R.N., Kline, D.I., Diaz-Pulido, G., Dove, S., and Hoegh-Guldberg, O. (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc Nat Acad Sci USA* **105:** 17442–17446.

Anthony, K.R.N., Maynard, J.A., Diaz-Pulido, G., Mumby, P., Marshall, P., Cao, L., and Hoegh-Guldberg, O. (2011) Ocean acidification and warming will lower coral reef resilience. *Glob Change Biol* **7:** 1798–1808.

Battin, T.J., Kaplan, L.A., Denis Newbold, J., and Hansen, C.M. (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426:** 439–442.

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

Connell, S.D., and Russell, B.D. (2010) The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proc Royal Soc B-Biol Sci* **277:** 1409–1415.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., and Lappin-Scott, H.M. (1995) Microbial biofilms. *Annu Rev Microbiol* **49:** 711–745.

Cottrell, M.T., and Kirchman, D.L. (2000) Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66:** 1692–1697.

Crump, B., and Baross, J.A. (1996) Particle-attached bacteria and heterotrophic plankton in the Columbia River estuary. *Mar Ecol Prog Ser* **138:** 265–273.

Crump, B., Simenstad, C.A., and Baross, J.A. (1998) Particle-attached bacteria dominate the Columbia River estuary. *Aquat Microb Ecol* **14:** 7–18.

Dang, H.Y., and Lovell, C.R. (2000) Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Appl Environ Microbiol* **66:** 467–475.

Dang, H.Y., Li, T.G., Chen, M.N., and Huang, G.Q. (2008) Cross-Ocean distribution of *Rhodobacterales* bacteria as primary surface colonizers in temperate coastal marine waters. *Appl Environ Microbiol* **74:** 52–60.

De'ath, G., Lough, J.M., and Fabricius, K.E. (2009) Declining Coral Calcification on the Great Barrier Reef. *Science* **323:** 116–119.

DeSantis, T.Z., Hugenholtz, P., Keller, K., Brodie, E.L., Larsen, N., Piceno, Y.M., *et al*. (2006) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Res* **34:** W394–W399.

Diaz-Pulido, G., Gouezo, M., Tilbrook, B., Dove, S., and Anthony, K.R.N. (2011) High CO(2) enhances the competitive strength of seaweeds over corals. *Ecol Lett* **14:** 156–162.

Doney, S.C., Fabry, V.J., Feely, R.A., and Kleypas, J.A. (2009) Ocean acidification: the other CO_2 problem. *Annu Rev Mar Sci* **1:** 169–192.

Dupont, S., Dorey, N., and Thorndyke, M. (2010) What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? *Estuar Coast Shelf* **89:** 182–185.

Elasri, M.O., and Miller, R.V. (1999) Study of the response of a biofilm bacterial community to UV radiation. *Appl Environ Microbiol* **65:** 2025–2031.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J., and Millero, F.J. (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **305:** 362–366.

Fu, F.X., Warner, M.E., Zhang, Y.H., Feng, Y.Y., and Hutchins, D.A. (2007) Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Prochlorococcus (Cyanobacteria). *J Phycol* **43:** 485–496.

Hammer, O., Harper, D.A.T., and Ryan, P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Paleontol Electron* **4:** 1–9.

Healey, F., and Hendzel, L.L. (1979) Indicators of phosphorus and nitrogen deficiency in five algae cultures. *J Fish Res Board Canada* **36:** 1364–1369.

Hendriks, I.E., and Duarte, C.M. (2010) Ocean acidification: separating evidence from judgment – a reply to Dupont *et al*. Discussion. *Estuar Coast Shelf* **89:** 186–190.

Hendriks, I.E., Duarte, C.M., and Alvarez, M. (2010) Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. *Estuar Coast Shelf* **86:** 157–164.

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshw Res* **50:** 839–866.

Huber, T., Faulkner, G., and Hugenholtz, P. (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20:** 2317–2319.

Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C., *et al*. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science* **301:** 929–933.

IPCC (2007) *The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*. Cambridge, UK: Cambridge University Press.

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

Keil, R.G., and Kirchman, D.L. (1999) Utilization of dissolved protein and amino acids in the northern Sargasso Sea. *Aquat Microb Ecol* **18:** 293–300.

Kim, J.M., Lee, K., Shin, K., Kang, J.H., Lee, H.W., Kim, M., *et al*. (2006) The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. *Limnol Oceanogr* **51:** 1629–1636.

Kirchman, D.L., Meon, B., Cottrell, M.T., Hutchins, D.A., Weeks, D., and Bruland, K.W. (2000) Carbon versus iron limitation of bacterial growth in the California upwelling regime. *Limnol Oceanogr* **45:** 1681–1688.

Kleypas, J.A., Buddemeier, R.W., Archer, D., Gattuso, J.P., Langdon, C., and Opdyke, B.N. (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284:** 118–120.

Kuffner, I.B., Andersson, A.J., Jokiel, P.L., Rodgers, K.S., and Mackenzie, F.T. (2008) Decreased abundance of crustose coralline algae due to ocean acidification. *Nat Geosci* **1:** 114–117.

Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard, J., Marubini, F., *et al*. (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochem Cycles* **14:** 639–654.

Liu, J.W., Weinbauer, M.G., Maier, C., Dai, M.H., and Gattuso, J.P. (2010) Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquat Microb Ecol* **61:** 291–305.

Lock, M.A., Wallace, R.R., Costerton, J.W., Ventullo, R.M., and Charlton, S.E. (1984) River epilithon: toward a structural-functional model. *Oikos* **42:** 10–22.

McBride, M.J. (2001) Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu Rev Microbiol* **55:** 49–75.

Marchesi, J.R., Sato, T., Weightman, A.J., Martin, T.A., Fry, J.C., Hiom, S.J., and Wade, W.G. (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol* **64:** 795–799.

Meron, D., Atias, E., Iasur Kruh, L., Elifantz, H., Minz, D., Fine, M., and Banin, E. (2011) The impact of reduced pH on the microbial community of the coral *Acropora eurystoma*. *ISME J* **5:** 51–60.

Mihm, J.W., Banta, W.C., and Loeb, G.I. (1981) Effects of adsorbed organic and primary fouling films on bryozoan settlement. *J Exp Mar Bio Ecol* **54:** 167–169.

Milligan, A.J., Mioni, C.E., and Morel, F.M.M. (2009) Response of cell surface pH to pCO_2 and iron limitation in the marine diatom *Thalassiosira weissflogii*. *Mar Chem* **114:** 31–36.

Mouchka, M.E., Hewson, I., and Harvell, C.D. (2010) Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. *Integr Comp Biol* **50:** 662–674.

Nocker, A., Lepo, J.E., and Snyder, R.A. (2004) Influence of an oyster reef on development of the microbial heterotrophic community of an estuarine biofilm. *Appl Environ Microbiol* **70:** 6834–6845.

Pandolfi, J.M., Jackson, J.B.C., Baron, N., Bradbury, R.H., Guzman, H.M., Hughes, T.P., *et al*. (2005) Ecology – are US coral reefs on the slippery slope to slime? *Science* **307:** 1725–1726.

Piontek, J., Lunau, M., Handel, N., Borchard, C., Wurst, M., and Engel, A. (2010) Acidification increases microbial polysaccharide degradation in the ocean. *Biogeosciences* **7:** 1615–1624.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., *et al*. (2005) Ocean acidification due to increasing atmospheric carbon dioxide. *Policy Document 12/05, The Royal*

Society, London. [WWW document]. URL http://www.royalsoc.ac.uk.
Reichenbach, H. (1991) The order Cytophagales. 3631–3675.
Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pages, C., Jaubert, J., and Gattuso, J.P. (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Glob Change Biol* **9:** 1660–1668.
Riebesell, U., Schulz, K.G., Bellerby, R.G.J., Botros, M., Fritsche, P., Meyerhofer, M., *et al*. (2007) Enhanced biological carbon consumption in a high CO_2 ocean. *Nature* **450:** 545–548.
Ries, J.B., Cohen, A.L., and McCorkle, D.C. (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology* **37:** 1131–1134.
Ritchie, K.B. (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* **322:** 1–14.
Rost, B., Zondervan, I., and Wolf-Gladrow, D. (2008) Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. *Mar Ecol Prog Ser* **373:** 227–237.
Russell, B.D., Thompson, J.A.I., Falkenberg, L.J., and Connell, S.D. (2009) Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats. *Glob Change Biol* **15:** 2153–2162.
Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., *et al*. (2004) The oceanic sink for anthropogenic CO_2. *Science* **305:** 367–371.
Schartau, M., Engel, A., Schroter, J., Thoms, S., Volker, C., and Wolf-Gladrow, D. (2007) Modelling carbon overconsumption and the formation of extracellular particulate organic carbon. *Biogeosciences* **4:** 433–454.
Silverman, J., Lazar, B., Cao, L., Caldeira, K., and Erez, J. (2009) Coral reefs may start dissolving when atmospheric CO2 doubles. *Geophys Res Lett* **36:** L05606. doi:10.1029/2008GL036282.
Smith, C.J., Danilowicz, B.S., Clear, A.K., Costello, F.J., Wilson, B., and Meijer, W.G. (2005) T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. *FEMS Microbiol Ecol* **54:** 375–380.
Sutherland, I.W. (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology-Uk* **147:** 3–9.
Suwa, R., Nakamura, M., Morita, M., Shimada, K., Iguchi, A., Sakai, K., and Suzuki, A. (2010) Effects of acidified seawater on early life stages of scleractinian corals (Genus *Acropora*). *Fish Sci* **76:** 93–99.
Takeuchi, K., Fujioka, Y., Kawasaki, Y., and Shirayama, Y. (1997) Impacts of High Concentration of CO_2 on Marine Organisms; a Modification of CO2 Ocean Sequestration. *Energy Convers Manag* **38:** 337–341.
Teske, P.R., and Wooldridge, T. (2001) A comparison of the macrobenthic faunas of permanently open and temporarily open/closed South African estuaries. *Hydrobiologia* **464:** 227–243.
Thornton, D.C.O. (2002) Diatom aggregation in the sea: mechanisms and ecological implications. *Eur J Protistol* **37:** 149–161.
Tolker-Nielsen, T., and Molin, S. (2000) Spatial organization of microbial biofilm communities. *Microb Ecol* **40:** 75–84.
Tortell, P.D., DiTullio, G.R., Sigman, D.M., and Morel, F.M.M. (2002) CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. *Mar Ecol Prog Ser* **236:** 37–43.
Tortell, P.D., Payne, C.D., Li, Y.Y., Trimborn, S., Rost, B., Smith, W.O., *et al*. (2008) CO_2 sensitivity of Southern Ocean phytoplankton. *Geophys Res Lett* **35:** L04605. doi: 04610.01029/02007GL032583.
Vega Thurber, R., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R.A., Angly, F., *et al*. (2009) Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* **11:** 2148–2163.
Webster, N.S., and Negri, A.P. (2006) Site-specific variation in Antarctic marine biofilms established on artificial surfaces. *Environ Microbiol* **8:** 1177–1190.
Webster, N.S., Smith, L.D., Heyward, A.J., Watts, J.E.M., Webb, R.I., Blackall, L.L., and Negri, A.P. (2004) Metamorphosis of a scleractinian coral in response to microbial biofilms. *Appl Environ Microbiol* **70:** 1213–1221.
Webster, N.S., Soo, R., Cobb, R., and Negri, A.P. (2010) Elevated seawater temperature causes a microbial shift on crustose coralline algae with implications for the recruitment of coral larvae. *ISME J* **5:** 759–770. DOI10.1038/ismej.2010.1152.
Wei, G.J., McCulloch, M.T., Mortimer, G., Deng, W.F., and Xie, L.H. (2009) Evidence for ocean acidification in the Great Barrier Reef of Australia. *Geochim Cosmochim Acta* **73:** 2332–2346.
Wieczorek, S.K., and Todd, C.D. (1998) Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling* **12:** 81–118.
Wild, C., Jantzen, C., Struck, U., Hoegh-Guldberg, O., and Huettel, M. (2008) Biogeochemical responses following coral mass spawning on the Great Barrier Reef: pelagic-benthic coupling. *Coral Reefs* **27:** 123–132.
Witt, V., Wild, C., and Uthicke, S. (2011) Effect of substrate type on bacterial community composition in biofilms from the Great Barrier Reef. *FEMS Microbiol Lett* DOI: 10.1111/1574-6968.2011.02374.x
Zhou, J., Mopper, K., and Passow, U. (1998) The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnol Oceanogr* **43:** 1860–1871.
Zou, D.H., and Gao, K.S. (2009) Effects of elevated CO2 on the red seaweed Gracilaria lemaneiformis (Gigartinales, Rhodophyta) grown at different irradiance levels. *Phycologia* **48:** 510–517.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Wilcoxon-Mann-Whitney rank sum test (*U*-test) of the macro community analysis of the algal component of six replicates after 11 d for different pCO_2 treatments. Different algae groups were observed and grouped into categories (diatoms, red algae, filamentous green algae, etc.). The

frequency of each algae category was assigned with an index: (4) dominant (3) frequent (2) occasional (1) rare (0) absent. Significances are shown as *P*-values and $P < 0.05$ are highlighted for clarity.

Table S2. Terminal restriction fragments (T-RFs) are shown with their phylogenetic affiliation (as classified using the greengenes batch sequence classifier [http://greengenes.lbl.gov/cgi-bin/nph-classify.cgi] using the NCBI taxonomy) and accession number as derived from the 16S rRNA gene clone libraries from each treatment. 16S rRNA identification of the nearest phylogenetic neighbour with accession number and sequence similarity (%) to each corresponding T-RF as determined by BLAST from the Greengenes database are shown.

Table S3. LIBshuff statistical analysis for differences between 16S rRNA gene sequences in clone libraries and clone library coverage at different pCO_2 treatments (305, 402, 564 and 1140 ppm), generated using the program MOTHUR are shown. Coverage (C) values of libraries are given in ΔC_{AB} (top diagonal) and ΔC_{BA} (lower diagonal) scores and significances are given in *P*-values Significant values for this analysis are those when $P < 0.05$. C_A and C_{AB} represent the coverage within community A and the coverage of community A onto community B. *P*-values for the observed ΔC_{AB} and ΔC_{BA} values are determined by determining the fraction of 10 000 matrix permutations resulting in ΔC_{AB} and ΔC_{BA} values, e.g. AB is significantly different, but BA is insignificant this means that A is a subset of B.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.