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Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem

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ABSTRACT

The impacts of ocean acidification on coastal biofilms are poorly understood. Carbon dioxide vent areas provide an opportunity to make predictions about the impacts of ocean acidification. We compared biofilms that colonised glass slides in areas exposed to ambient and elevated levels of *p*CO₂ along a coastal pH gradient, with biofilms grown at ambient and reduced light levels. Biofilm production was highest under ambient light levels, but under both light regimes biofilm production was enhanced in seawater with high *p*CO₂. Uronic acids are a component of biofilms and increased significantly with high *p*CO₂. *Bacteria* and *Eukarya* denaturing gradient gel electrophoresis profile analysis showed clear differences in the structures of ambient and reduced light biofilm communities, and biofilms grown at high *p*CO₂ compared with ambient conditions. This study characterises biofilm response to natural seabed CO₂ seeps and provides a baseline understanding of how coastal ecosystems may respond to increased *p*CO₂ levels.

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35 The ocean's absorption of anthropogenic CO₂ is resulting in a reduction of seawater pH, increased dissolved inorganic carbon 36 37 and changes to carbonate chemistry (Raven et al., 2005; Riebesell et al., 2009). By the end of this century, under 'business as usual' 38 39 fossil fuel usage scenarios, oceans are set to globally experience a pH decrease of 0.3–0.4 pH units, a rate faster than the oceans have 40 experienced for at least 300 million years (Caldeira and Wickett, 41 2003). This process is commonly referred to as 'ocean acidification'. 42 A similar and more localised process is also possible from the leak-43 age of CO₂ capture and storage sites (Blackford et al., 2009). 44

45 Establishing the effects of ocean acidification on marine microorganisms is challenging (Joint et al., 2011; Liu et al., 2010). Joint 46 et al. (2011) proposed that because marine ecosystems already 47 48 experience natural pH variations, processes other than calcification will not be fundamentally affected. Conversely, Liu et al. (2010) ar-49 50 gued that, based on meta-analysis, the rate of several microbially driven processes will be affected. The possible effects of ocean 51 52 acidification on the links between microbial community structure 53 and ecosystem function are unknown.

Coastal marine ecosystems are both ecologically and socio-eco nomically important, with their 'value' under direct threat from cli mate change (Harley et al., 2006). Biofilms are an underpinning

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component of coastal ecosystems, creating new organic matter, cycling nutrients and providing grazing for marine invertebrates (Decho, 2000; Thompson et al., 2004). Biofilms also condition surfaces for further settlement of marine invertebrates and macroal-gal propagules (Qian et al., 2007). For example, there is evidence that biofilm bacterial community structure can influence invertebrate settlement (Lau et al., 2005).

Naturally occurring areas of elevated CO_2 are starting to be used to study macroorganism community responses to ocean acidification (Fabricius et al., 2011; Hall-Spencer et al., 2008; Rodolfo-Metalpa et al., 2011), and could help to determine how microbial communities respond to ocean acidification (Liu et al., 2010). The response of benthic diatom assemblages was recently investigated at Vulcano, an island in the Tyrrhenian Sea, where CO_2 vents acidify the seawater producing a pH gradient (Johnson et al., 2011). The Vulcano Island vents are formed from fumarolic degassing of nearly pure CO_2 (Baubron et al., 1990). Whilst hydrogen sulfide has been recorded at the vent sources (Sedwick and Stüben, 1996), it is undetectable (<2 ppm) at the sampling stations used in this study (Parello pers. comm.).

In order to determine microbial biofilm community responses to natural ocean acidification, glass slides were attached to floats held 0.5 m below the water surface at three sites around Vulcano (Fig. 1). In order to compare biofilms exposed to different light levels the glass slides were either held freely in open water (ambient light, AL) or within a light-reducing cover (\sim 98% light reduction, low light, LL). After 16 d the slides were collected, rapidly frozen and stored at -20 °C.

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Fig. 1. Location of sample sites off Vulcano Island, part of the Aeolian Island chain, North East Sicily (38°25' N, 14°57' E).

During the experiment in May 2011 water conditions were typical for this location, with values within the range of the long term data collected at the sample sites (Table 1) (Johnson et al., 2011). Seawater temperature and salinity remained constant between the sites (temperature = 19-21 °C, salinity = 38). Midday underwater light intensity was determined using a HOBO[®] light logger (Onset, USA) and was also found to not vary significantly between the sites (site one mean lux 36,935 ± 3641 and site three mean lux 38,895 ± 4234).

Uronic acids can be a significant component of biofilm exopolysaccharide (EPS) and were quantified with minor modification of an existing protocol (Mojica et al., 2007). Briefly, slides were cut using a glass cutter and placed into 2 mL tubes containing 0.5 mL distilled water, vortexed for 2.5 min and left to stand for a further 2 min. The protocol of Mojica et al. (2007) was then followed exactly. An uronic acid standard curve was made using glucuronic acid (Sigma Aldrich, UK).

Light regime had the greatest overall affect on biofilm production, however biofilm visibly increased at sites two and three compared to site one (Fig. 2), and was more pronounced for the AL biofilms. The concentration of uronic acids was higher in the AL biofilms, and increased significantly with higher pCO_2 between the three sites (Fig. 3). A similar increase occurred in the LL biofilms, but was only significant between site one and the other sites (Fig. 3).

Johnson et al. (2011) showed increased biofilm chlorophyll-*a* concentrations and diatom abundance on acrylic slides in response to elevated *p*CO₂ at the same sites. Diatoms are important primary producers and are largely responsible for EPS production in biofilms (Wolfstein and Stal, 2002). Increased *p*CO₂ can improve photosynthesis efficiency by uncoupling energy demanding carbon concentrating mechanisms (Raven, 1991; Riebesell et al., 2009) and subsequently enhance EPS production (Engel, 2002). This

Table 1

Seawater conditions during the experiment in May 2011 at the sampling sites off Vulcano Island. Annual average pH measurements for the sites are in brackets and were taken at multiple time points between September 2009 and October 2010 (n = 18) (Johnson et al., 2011). Total Alkalinity (TA) was measured on a AS-Alk 2 Total Alkalinity Titrator (Apollo SciTech Inc, Georgia, USA), dissolved inorganic carbon (DIC) and pCO_2 were calculated using CO₂SYS (Dickson, 1990; Lewis and Wallace, 1998; Roy et al., 1993).

	pH (NBS scale)	$TA \ (mmol \ kg^{-1})$	pCO_2 (μ atm)	DIC (mmol kg ⁻¹)
Site 1	8.26 (8.21)	2.563	342	2.198
Site 2 Site 3	8.10 (8.08) 7.72 (7.71)	2.893	1645	2.594 2.769



Fig. 2. Glass slides used to grow biofilm after submersion below the sea surface (0.5 m) for 16 d at each site.



Fig. 3. Uronic acids quantified as glucuronic acid equivalents from biofilms attached to glass slides at each site (Ambient Light n = 8; Low Light n = 14). Lines shows significant differences between sites (ANOVA, Tukey's pairwise, P = <0.05).

could account for the increased biofilm production in higher pCO_2 areas reported here, particularly in the photoautotrophic dominated AL biofilms. More biofilm biomass has wider ecosystem significance because grazing marine molluscs, such as limpets, require more energy to sustain increased calcification rates in higher pCO_2 areas (Rodolfo-Metalpa et al., 2011).

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Fig. 4. Bray-Curtis similarity multidimensional scaling plots generated from Bacteria and Eukarya DGGE profiles with percentage similarity contours.



Fig. 5. Shannon and Simpson's index calculated from *Bacteria* and *Eukarya* DGGE profiles for ambient light (AL) and low light (LL) biofilms. Error bars show standard deviations. Lines show significant differences between sites (ANOVA, Tukey's pairwise, *P* = <0.05).

124 In order to make a rapid general assessment of biofilm microbial community structure biofilm was removed from the slides 125 using a sterile scalpel and DNA was extracted from the biofilm 126 using the DNeasy Kit (Qiagen, UK). PCR of Bacteria 16S rRNA genes 127 (primers 341F GC/518R), Eukarya 18S rRNA genes (primers EUK 128 1209^{GC}/UNI1392R) and denaturing gradient gel electrophoresis 129 130 (DGGE) were performed as previously described (Cunliffe and Mur-131 rell, 2010; Cunliffe et al., 2009). DGGE profiles were analysed using ImageJ and PRIMER 6 (PRIMER-E Ltd., Plymouth, UK). 132

Bacteria and Eukarya communities showed clear differentiation 133 134 between sample sites and light exposure (Fig. 4). Similarity of all 135 bacterial communities was >50%, but AL and LL biofilms formed 136 two separate clusters that were both >60% similar. Within both AL and LL clusters there was a clear effect of sample site, with sites 137 two and three being >70% similar and distinct from site one (Fig. 4). 138 139 Similarity between Eukarya communities was lower than that of the Bacteria communities. The AL Eukarya communities formed a 140 141 cluster that was >30% similar, within this cluster the three samples sites were distinct with >50% similarity. The LL Eukarya communi-142 143 ties were more disparate, with sites two and three being only >30% similar (Fig. 4). Shannon diversity index increased significantly 144

from site one to sites two and three in the LL *Bacteria* communities (Fig. 5). Simpson's Index also increased significantly from site one to site two in the LL *Bacteria* communities (Fig. 5).

Simulated ocean acidification mesocosm studies have also shown that phytoplankton and bacterioplankton communities can change in response to increased pCO_2 (Allgaier et al., 2008; Liu et al., 2010; Tortell et al., 2002). Allgaier et al. (2008) showed that free-living bacterioplankton community structure changes with increased pCO_2 , however attached bacterioplankton communities are linked to phytoplankton community development. Biofilms studied in a simulated mesocosm experiment using water collected from the Great Barrier Reef also showed that bacterial communities change with high pCO_2 (Witt et al., 2011).

Reasons for community change are yet to be elucidated and clearly warrant future study. Bacterial degradation of polysaccharides by extracellular enzymes accelerates at lower pH (Piontek et al., 2010). Increased biofilm EPS production in high *p*CO₂ coupled with increased polysaccharide degradation could lead to the adjustment of available niches and alter community structure, similar processes have been reported for estuarine sediment diatom-bacterial communities (Haynes et al., 2007). Existing

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166 environmental factors, such as light levels, could have dispropor-167 tionate effects on different species if other factors, such as pCO_2 , 168 change. For example, the ecological effects of light on two subtidal 169 algal species was modified by increased pCO_2 (Russell et al., 2011). 170 Changes in biofilm microbial diversity could also lead to more eco-171 system-wide changes, including the subsequent settlement of 172 macroorganism such as marine invertebrates (Lau et al., 2005).

Inherent in studying a natural system, it is not possible to separate the effects of high pCO_2 and reduced pH on the biofilms in this study. Future experiments using a meso- or microcosm based approach could aim to test both parameters independently.

177 In summary, under natural high pCO_2 conditions that emulate 178 future ocean acidification conditions, biofilm production signifi-179 cantly increases. This coincides with changes in the general structures of resident microbial communities. These results provide 180 181 evidence for the modification of coastal ecosystems as a result of 182 elevated pCO_2 and associated ocean acidification. The response of microbial biofilms to high pCO₂ conditions could be used a biolog-183 184 ical indicator of localised CO2 release from carbon capture and 185 storage leakage.

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