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Baseline

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 $p\text{CO}_2$ 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 $p\text{CO}_2$. Uronic acids are a component of biofilms and increased significantly with high $p\text{CO}_2$. *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 $p\text{CO}_2$ compared with ambient conditions. This study characterises biofilm response to natural seabed CO_2 seeps and provides a baseline understanding of how coastal ecosystems may respond to increased $p\text{CO}_2$ levels.

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The ocean's absorption of anthropogenic CO_2 is resulting in a reduction of seawater pH, increased dissolved inorganic carbon and changes to carbonate chemistry (Raven et al., 2005; Riebesell et al., 2009). By the end of this century, under 'business as usual' 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 experienced for at least 300 million years (Caldeira and Wickett, 2003). This process is commonly referred to as 'ocean acidification'. A similar and more localised process is also possible from the leakage of CO_2 capture and storage sites (Blackford et al., 2009).

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

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

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 macroalgal 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 (~98% light reduction, low light, LL). After 16 d the slides were collected, rapidly frozen and stored at -20°C .

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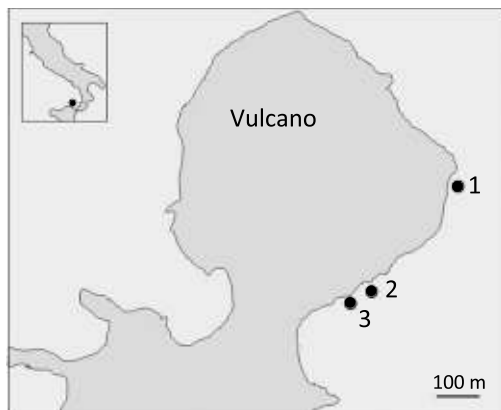


Fig. 1. Location of sample sites off Vulcano Island, part of the Aeolian Island chain, North East Sicily (38°25' N, 14°57' E).

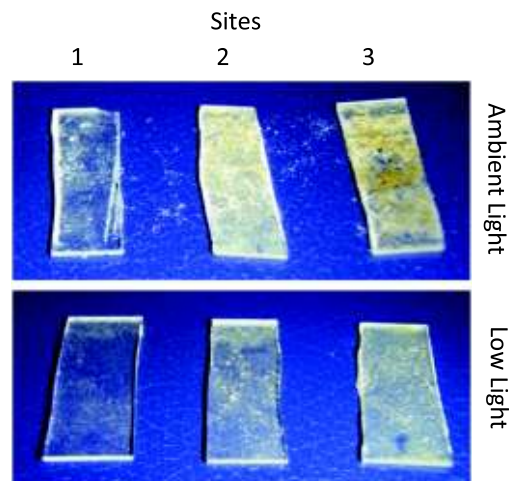


Fig. 2. Glass slides used to grow biofilm after submersion below the sea surface (0.5 m) for 16 d at each site.

85 During the experiment in May 2011 water conditions were typ-
86 ical for this location, with values within the range of the long term
87 data collected at the sample sites (Table 1) (Johnson et al., 2011).
88 Seawater temperature and salinity remained constant between
89 the sites (temperature = 19–21 °C, salinity = 38). Midday underwa-
90 ter light intensity was determined using a HOBO® light logger
91 (Onset, USA) and was also found to not vary significantly between
92 the sites (site one mean lux 36,935 ± 3641 and site three mean lux
93 38,895 ± 4234).

94 Uronic acids can be a significant component of biofilm exopoly-
95 saccharide (EPS) and were quantified with minor modification of
96 an existing protocol (Mojica et al., 2007). Briefly, slides were cut
97 using a glass cutter and placed into 2 mL tubes containing 0.5 mL
98 distilled water, vortexed for 2.5 min and left to stand for a further
99 2 min. The protocol of Mojica et al. (2007) was then followed ex-
100 actly. An uronic acid standard curve was made using glucuronic
101 acid (Sigma Aldrich, UK).

102 Light regime had the greatest overall affect on biofilm produc-
103 tion, however biofilm visibly increased at sites two and three com-
104 pared to site one (Fig. 2), and was more pronounced for the AL
105 biofilms. The concentration of uronic acids was higher in the AL
106 biofilms, and increased significantly with higher pCO₂ between
107 the three sites (Fig. 3). A similar increase occurred in the LL
108 biofilms, but was only significant between site one and the other sites
109 (Fig. 3).

110 Johnson et al. (2011) showed increased biofilm chlorophyll-*a*
111 concentrations and diatom abundance on acrylic slides in response
112 to elevated pCO₂ at the same sites. Diatoms are important primary
113 producers and are largely responsible for EPS production in
114 biofilms (Wolfstein and Stal, 2002). Increased pCO₂ can improve
115 photosynthesis efficiency by uncoupling energy demanding carbon
116 concentrating mechanisms (Raven, 1991; Riebesell et al., 2009)
117 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 pCO₂ 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	8.10 (8.08)	2.893	611	2.594
Site 3	7.72 (7.71)	2.869	1645	2.769

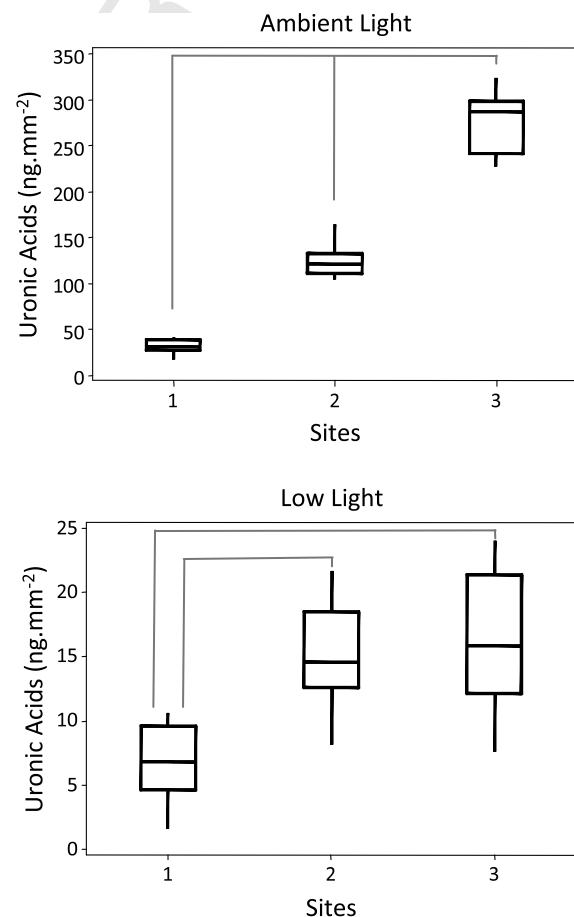


Fig. 3. Uronic acids quantified as glucuronic acid equivalents from biofilms attached to glass slides at each site (Ambient Light *n* = 8; Low Light *n* = 14). Lines show significant differences between sites (ANOVA, Tukey's pairwise, *P* < 0.05).

could account for the increased biofilm production in higher pCO₂ 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 pCO₂ areas (Rodolfo-Metalpa et al., 2011).

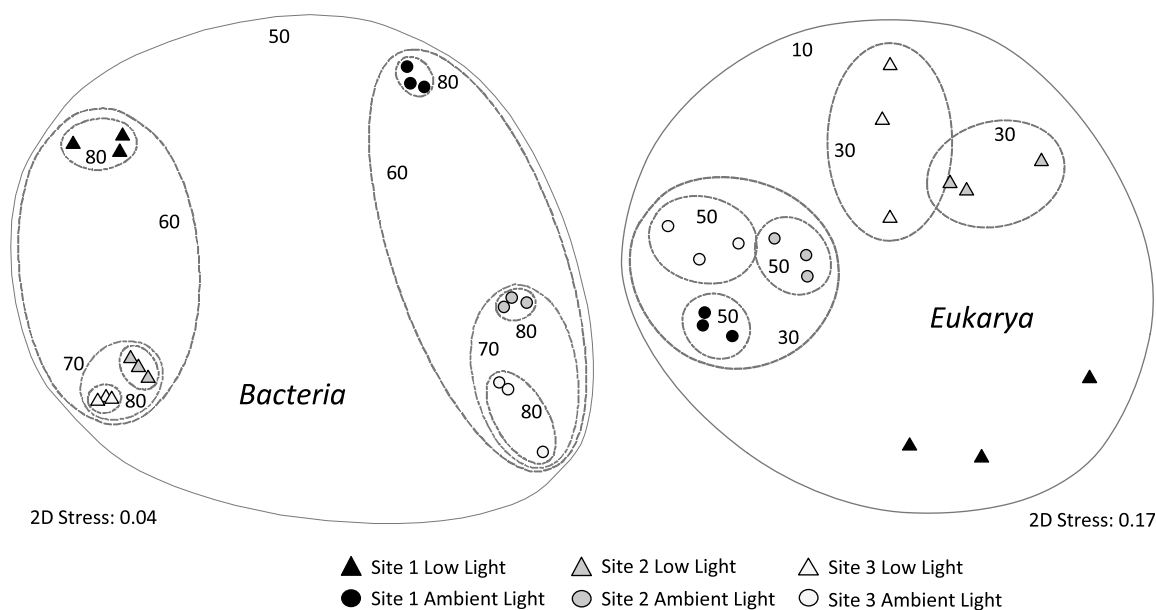


Fig. 4. Bray-Curtis similarity multidimensional scaling plots generated from *Bacteria* and *Eukarya* DGGE profiles with percentage similarity contours.

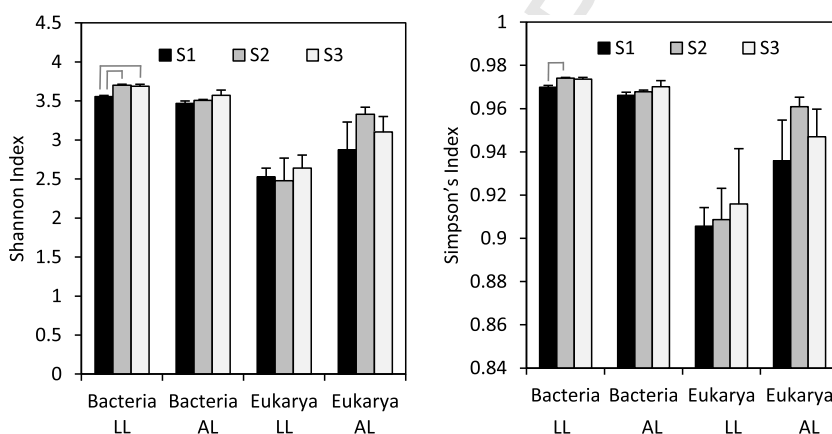


Fig. 5. Shannon and Simpson's index calculated from *Bacteria* and *Eukarya* DGGE profiles for ambient light (AL) and low light (LL) biofilms. Error bars show standard deviations. Lines show significant differences between sites (ANOVA, Tukey's pairwise, $P < 0.05$).

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

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

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 pCO_2 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

environmental factors, such as light levels, could have disproportionate effects on different species if other factors, such as $p\text{CO}_2$, change. For example, the ecological effects of light on two subtidal algal species was modified by increased $p\text{CO}_2$ (Russell et al., 2011). Changes in biofilm microbial diversity could also lead to more ecosystem-wide changes, including the subsequent settlement of 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 $p\text{CO}_2$ 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.

In summary, under natural high $p\text{CO}_2$ conditions that emulate future ocean acidification conditions, biofilm production significantly increases. This coincides with changes in the general structures of resident microbial communities. These results provide evidence for the modification of coastal ecosystems as a result of elevated $p\text{CO}_2$ and associated ocean acidification. The response of microbial biofilms to high $p\text{CO}_2$ conditions could be used a biological indicator of localised CO_2 release from carbon capture and storage leakage.

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