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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 pCO_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 pCO_2. Uronic acids are a component of biofilms and increased significantly with high pCO_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 pCO_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 pCO_2 levels.

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 Stűben, 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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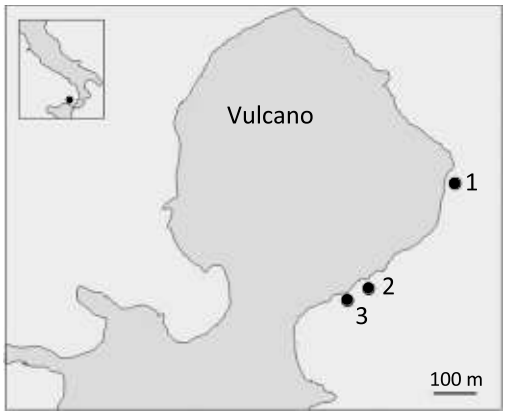

Fig. 1. Location of sample sites off Vulcano Island, part of the Aeolian Island chain, North East Sicily (38°25′ N, 14°57′ E).

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 pCO_2 at the same sites. Diatoms are important primary producers and are largely responsible for EPS production in biofilms (Wolfstein and Stal, 2002). Increased pCO_2 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 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).

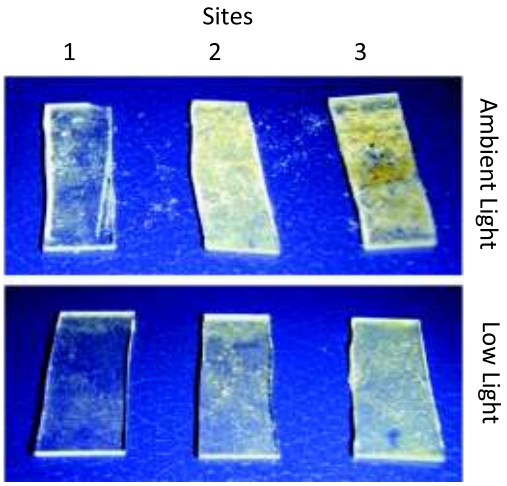

Fig. 2. Glass slides used to grow biofilm after submersion below the sea surface (0.5 m) for 16 d at each site.

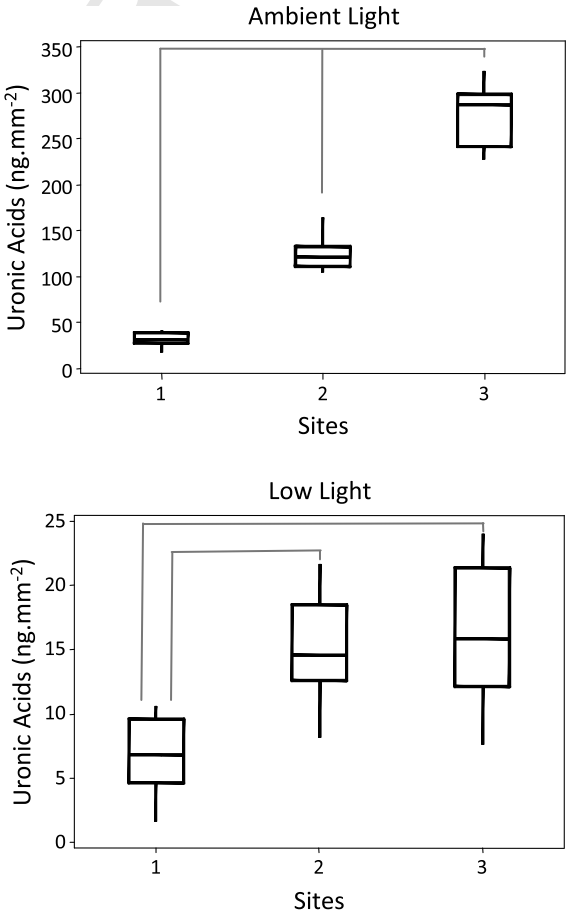

Fig. 3. Uronic acids quantified as glucuronic acid equivalents from biofilms attached to glass slides at each site (Ambient Light $n = 8$; Low Light $n = 14$). Lines shows significant differences between sites (ANOVA, Tukey's pairwise, $P = <0.05$).

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 CO_2SYS (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

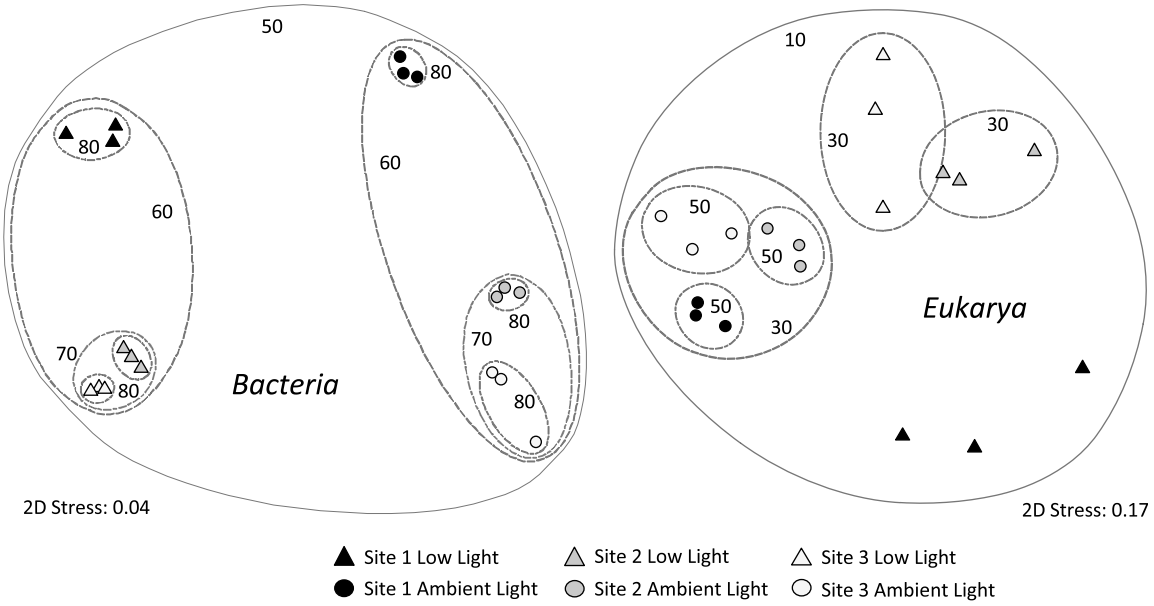

Fig. 4. Bray-Curtis similarity multidimensional scaling plots generated from *Bacteria* and *Eukarya* DGGE profiles with percentage similarity contours.

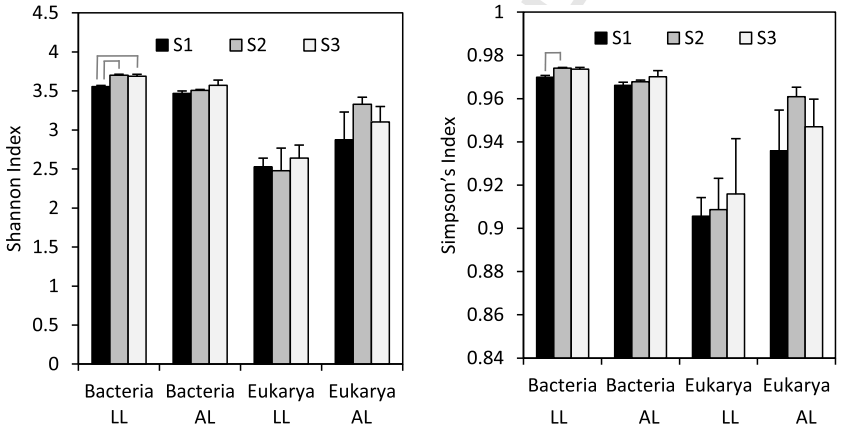

Fig. 5. Shannon and Simpson's index calculated from *Bacteria* and *Eukarya* DGGE profiles for ambient light (AL) and low light (LL) biofilms. Error bars show standard deviations. Lines show significant differences between sites (ANOVA, Tukey's pairwise, $P = <0.05$).

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

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environmental factors, such as light levels, could have disproportionate effects on different species if other factors, such as pCO_2, change. For example, the ecological effects of light on two subtidal algal species was modified by increased pCO_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 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.

In summary, under natural high pCO_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 pCO_2 and associated ocean acidification. The response of microbial biofilms to high pCO_2 conditions could be used a biological indicator of localised CO_2 release from carbon capture and storage leakage.

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References

Allgaier, M., Riebesell, U., Vogt, M., Thyrhaug, R., Grossart, H.P., 2008. Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO_2 levels: a mesocosm study. Biogeosciences 5, 1007–1022.

Baubron, J.C., Allard, P., Toutain, J.P., 1990. Diffuse volcanic emissions of carbon dioxide from Vulcano Island, Italy. Nature 344, 51–53.

Blackford, J. et al., 2009. An initial assessment of the potential environmental impact of CO_2 escape from marine carbon capture and storage systems. Proceedings of the Institution of Mechanical Engineers Part A-Journal of Power and Energy 223, 269–280.

Caldeira, K., Wickett, M.E., 2003. Oceanography: anthropogenic carbon and ocean pH. Nature 425, 365.

Cunliffe, M., Murrell, J.C., 2010. *Eukarya* 18S rRNA gene diversity in the sea surface microlayer: insights on the structure of the neustonic microbial loop. The ISME Journal 4, 455–458.

Cunliffe, M., Whiteley, A.S., Schäfer, H., Newbold, L., Oliver, A., Murrell, J.C., 2009. Comparison of bacterioneuston and bacterioplankton dynamics during a phytoplankton bloom in a fjord mesocosm. Applied and Environmental Microbiology 75 (22), 7173–7181.

Decho, A.W., 2000. Microbial biofilms in intertidal systems: an overview. Continental Shelf Research 20 (10–11), 1257–1273.

Dickson, A.G., 1990. Standard potential of the (AgCl + 1/2 H_2 = Ag + HCl(aq)) cell and the association of bisulfate ion in synthetic sea water from 273.15 to 318.15 K. The Journal of Chemical Thermodynamics 22, 113–127.

Engel, A., 2002. Direct relationship between CO_2 uptake and transparent exopolymer particles production in natural phytoplankton. Journal of Plankton Research 24 (1), 49–53.

Fabricius, K.E. et al., 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nature Climate Change 1, 165–169.

Hall-Spencer, J.M. et al., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454, 96–99.

Harley, C.D. et al., 2006. The impacts of climate change in coastal marine systems. Ecology Letters 9, 228–241.

Haynes, K., Hofmann, T.A., Smith, C.J., Ball, A.S., Underwood, G.J., Osborn, A.M., 2007. Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. Applied and Environmental Microbiology 73 (19), 6112–6124.

Johnson, V.R., Brownlee, C., Rickaby, R.E.M., Hall-Spencer, J.M., 2011. Responses of marine benthic microalgae to ocean acidification. Marine Biology. doi:10.1007/s00227-011-1840-2.

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

Lau, S.C.K., Thiyagarajan, V., Cheung, S.C.K., Qian, P.-Y., 2005. Roles of bacterial community composition in biofilms as a mediator for larval settlement of three marine invertebrates. Aquatic Microbial Ecology 38, 41–51.

Lewis E., Wallace W.R., 1998. Program developed for CO_2 system calculations. Oak Ridge National Laboratory, US Department of Energy.

Liu, J., Weinbauer, M.G., Maier, C., Dai, M., Gattuso, J.-P., 2010. Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. Aquatic Microbial Ecology 61, 291–305.

Mojica, K., Elsey, D., Cooney, M.J., 2007. Quantitative analysis of biofilm EPS uronic acid content. Journal of Microbiological Methods 71 (1), 61–65.

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

Qian, P.Y., Lau, S.C., Dahms, H.U., Dobretsov, S., Harder, T., 2007. Marine biofilms as mediators of colonization by marine macroorganisms: implications for antifouling and aquaculture. Marine Biotechnology 9 (4), 399–410.

Raven, J.A., 1991. Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature. Plant, Cell & Environment 14 (8), 779–794.

Raven, J.A. et al., 2005. Ocean acidification due to increasing atmospheric carbon dioxide, London: Policy Document 12/05 Royal Society.

Riebesell, U., Körtzinger, A., Oschlies, A., 2009. Sensitivities of marine carbon fluxes to ocean change. Proceedings of National Academic Science USA 106 (49), 20602–20609.

Rodolfo-Metalpa, R. et al., 2011. Coral and mollusc resistance to ocean acidification adversely affected by warming. Nature Climate Change 1, 308–312.

Roy, R.N. et al., 1993. The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 deg°C. Marine Chemistry 44, 249–267.

Russell, B.D., Passarelli, C.A., Connell, S.D., 2011. Forecasted CO_2 modifies the influence of light in shaping subtidal habitat. Journal of Phycology 47, 744–752.

Sedwick, S., Stüben, D., 1996. Chemistry of shallow submarine warm springs in an arc-volcanic setting: Vulcano Island, Aeolian Archipelago, Italy. Marine Chemistry 53, 147–161.

Thompson, R.C., Norton, T.A., Hawkins, S.J., 2004. Physical stress and biological control regulate the producer-consumer balance in intertidal biofilms. Ecology 85 (5), 1372–1382.

Tortell, P.D., DiTullio, G.R., Sigman, D.M., Morel, F.M.M., 2002. CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. Marine Ecology Progress Series 236, 37–43.

Witt, V., Wild, C., Anthony, K.R., Diaz-Pulido, G., Uthicke, S., 2011. Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef. Environmental Microbiology. doi:10.1111/j.1462-2920.2011.02571.x.

Wolfstein, K., Stal, L.J., 2002. Production of extracellular polymeric substances (EPS) by benthic diatoms: effect of irradiance and temperature. Marine Ecology Progress Series 236, 13–22.