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RESEARCH ARTICLE

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Key Points:

- Natural microboring communities actively dissolved aragonite and calcite substrates in temperate systems at Ischia (Italy) and Faial (Azores) even at pH 7.2
- Chemical dissolution of carbonates at the CO₂ vent sites (due to $\Omega \leq 1$) maintained microboring communities at a pioneer stage and thus limited their erosive activity
- Our results suggest that there is a pH threshold between 7.4 and 7.7 below which biogenic dissolution of carbonates by microborers is strongly limited

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Limited Carbonate Dissolution by Boring Microflora at Two Volcanically Acidified Temperate Sites: Ischia (Italy, Mediterranean Sea) and Faial (Azores, NE Atlantic Ocean)

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Abstract In situ effects of ocean acidification on carbonate dissolution by microboring flora, also called biogenic dissolution, have only been studied once in tropical environments. Naturally acidified seawaters due to CO₂ vents offer a perfect setting to study these effects in temperate systems. Three sites were selected at Ischia (Italy, Mediterranean Sea) with one experiencing ambient pH and the two others a mean pH_T of 7.2 and 7.5. At Faial (Azores, NE Atlantic), one site with ambient pH and one acidified site with a mean pH_T of 7.4 were selected. Experiments were carried out during 1.5 months and 6 months in Azores and Ischia, respectively, to determine the effects of OA on microboring communities in various carbonate substrates. Low pH influenced negatively boring microflora development by limiting their depth of penetration and abundance in substrates. Biogenic dissolution was thus reduced by a factor 3 to 7 depending on sites and substrate types. At sites with ambient pH in Faial, biogenic dissolution contributed up to 23% to the total weight loss, while it contributed less than 1% to the total weight loss of substrates at the acidified sites. Most of the dissolution at these sites was due to chemical dissolution (often $\Omega \leq 1$). Such conditions maintained microboring communities at a pioneer stage with a limited depth of penetration in substrates. Our results, together with previous findings that showed an increase of biogenic dissolution at pH > 7.7, suggest that there is a pH tipping point below which microborer development and thus carbonate biogenic dissolution is strongly limited.

1. Introduction

Dissolution of carbonates by microbial boring flora (or euendolithic flora) comprising cyanobacteria, algae, and fungi is one of the major processes involved in CaCO₃ erosion and sedimentation in coastal ecosystems (Schneider & Le Campion-Alsumard, 1999; Schneider & Torunski, 1983; Tribollet et al., 2011; Wisshak et al., 2011). This process, also called biogenic dissolution, is particularly important in coral reefs (Perry & Harborne, 2016; Perry & Hepburn, 2008; Schoenberg et al., 2017; Tribollet, 2008a; Tribollet & Golubic, 2011). Although it is known since geological time (Zhang & Golubic, 1987) and occurs in all kinds of carbonate substrates from cold to tropical marine systems (Tribollet et al., 2011), it has received little attention in comparison to the other processes involved in coastal carbonate biogeochemical cycles, that is, chemical dissolution of sediments, either thermodynamically driven or driven by bacteria (Andersson & Gledhill, 2013; Milliman, 1993; Morse & MacKenzie, 1990), and biogenic marine CaCO₃ precipitation (e.g., review by Guinotte & Fabry, 2008). Several studies have also investigated the effects of ocean acidification (OA) and other anthropogenic-related factors such as increased seawater temperature and eutrophication, on sediment chemical dissolution and biogenic CaCO₃ precipitation (Andersson et al., 2015, 2006; Chauvin et al., 2011; Comeau et al., 2014; Eyre et al., 2014; Haynert et al., 2014; Krumins et al., 2013; Langdon & Atkinson, 2005; Rodolfo-Metalpa et al., 2011; Stubler & Peterson, 2016; Trnovsky et al., 2016). In contrast, there has been limited research on the impacts of climatic and anthropogenic factors on biogenic dissolution of carbonates by boring microflora, and the vast majority of studies have been mostly restricted to tropical environments. Chazottes et al. (2002), Carreiro-Silva et al. (2012), Carreiro-Silva et al. (2005, 2009) showed, for instance, the positive effects of eutrophication on rates of biogenic dissolution, while Tribollet and Golubic (2005) highlighted the negative influence of a hypersedimentation on boring microflora development and thus on rates of biogenic dissolution in coral reef ecosystems. Tribollet et al. (2009) showed under controlled conditions

and over a 3 month period that the main agent of reef biogenic dissolution, the chlorophyte *Ostreobium* sp. (Tribollet, 2008b), responds positively to an elevated aqueous $p\text{CO}_2$ of 750 ppm ($p\text{CO}_2$ predicted for the end of the century (IPCC, 2013) and corresponding to a $\text{pH} = 7.9$). The growth stimulation of microboring communities dominated by *Ostreobium* increased rates of biogenic dissolution by 50%. This enhanced biogenic dissolution by elevated $p\text{CO}_2$ was recently confirmed by two studies, one conducted under different controlled conditions (Reyes-Nivia et al., 2013) and another one under naturally acidified tropical waters (Enochs et al., 2016). It was thus suggested that biogenic dissolution of carbonate substrates will increase significantly by the end of the century, potentially offsetting carbonate precipitation, together with bioeroding sponges and bacteria activity in reef ecosystems (Andersson et al., 2015; Reyes-Nivia et al., 2013; Schoenberg et al., 2017; Tribollet et al., 2009, 2014; Wisshak et al., 2013). In comparison, considerably less is known on the effects of OA on temperate environments.

At the ecosystem scale such as at Ischia (Italy), benthic communities shift from a dominance of calcifiers at ambient pH toward a dominant noncalcifying community at the CO_2 vent sites (Hall-Spencer et al., 2008). But live and dead carbonate substrates do not respond similarly to OA in such an ecosystem, with dead substrates being more susceptible to net dissolution than living ones, and various aragonite corals being impacted differently (Rodolfo-Metalpa et al., 2011). Rodolfo-Metalpa et al. suggested that these differences result most likely from the protective effect of organic layers when present, in shells and corals. But they ignored or overlooked the impact of biogenic dissolution of carbonates by microborers, which is known in tropical waters to be much more important in dead substrates than in live ones (e.g., Le Campion-Alsumard et al., 1995; Tribollet & Payri, 2001). In order to better understand the susceptibility of various carbonate substrates to dissolution under OA in temperate ecosystems and more generally, to better predict temperate coastal carbonate budgets in the future, it is thus essential to study the in situ effects of OA on microboring flora communities and their dissolution activity.

In this context, two experiments were run in parallel between 2009 and 2011 at Ischia (Italy) and Faial (Azores). These two locations were selected as they are under the influence of CO_2 vents driving seawater pH below 7.4 in some areas (Hall-Spencer et al., 2008; Hall-Spencer & Rodolfo-Metalpa, 2009), which is the level of ocean acidity predicted to occur in shallow coastal waters by the year 2300 (Caldeira & Wickett, 2003; IPCC, 2013). Carbonate substrates selected in this study were aragonite and calcite shells and skeletons of conspicuous calcifiers in temperate environments, that is, shells of clams, limpets and gastropods, and skeletons of solitary corals. Specific objectives of this study were to (1) determine the effects of decreased pH on microboring community development and CaCO_3 substrate dissolution in temperate settings, (2) compare the relative importance of biogenic dissolution versus abiotic processes (environmental dissolution + mechanical breakage), and (3) highlight differences in dissolution rates among carbonate substrates with different density and mineralogy.

2. Material and Methods

2.1. Studied Sites

2.1.1. Ischia (Italy)

The volcanic vent area south of Castello Aragonese is located on the northeastern side of Ischia Island ($40^\circ 43.84' \text{N}$, $13^\circ 57.08' \text{E}$) (Figure 1). Hall-Spencer et al. (2008) described this area for the first time, and later, Rodolfo-Metalpa et al. (2011) characterized seawater carbonate chemistry at the vents. Gas vents occurring at 3–6 m depth acidify seawater at Castello Aragonese on the coast of Ischia. Gas bubbles contain 90–95% CO_2 , 3–7% N_2 , 0.6–0.8% O_2 , 0.2–0.8% CH_4 (no sulfur), and 0.08–0.1% Ar. The three selected sites for the present work (Figure 1) are the same used by Rodolfo-Metalpa et al. (2011) and comprise station “C” as the ambient pH site with a mean pH_T of 8.0 for the length of the experiment ($p\text{CO}_2 = 477 \pm 58$, $\Omega_{\text{arag}} = 3.6 \pm 0.5$) and the stations “B1” and “B2” with acidified waters (B1: $\text{pH}_T = 7.2 \pm 0.4$, $p\text{CO}_2 = 5,662.5 \pm 5,607.6$, $\Omega_{\text{arag}} = 1.0 \pm 0.7$; B2: $\text{pH}_T = 7.5 \pm 0.4$, $p\text{CO}_2 = 3,619.4 \pm 4,240.1$, $\Omega_{\text{arag}} = 1.4 \pm 0.8$; see Table 1). Those two stations are located approximately 50 m away from the CO_2 vents (gas bubbles). Site C is in a shallow (<3 m depth) *Posidonia oceanica* meadow and presents a high species diversity (encrusting algae, urchins, limpets, barnacles, and macroalgae) (Hall-Spencer et al., 2008; Hall-Spencer & Rodolfo-Metalpa, 2009). B1 and B2 stations, located at 2–3 m depth as well, are in contrast characterized by various species of macroalgae and almost no calcifying organisms (Hall-Spencer et al., 2008).

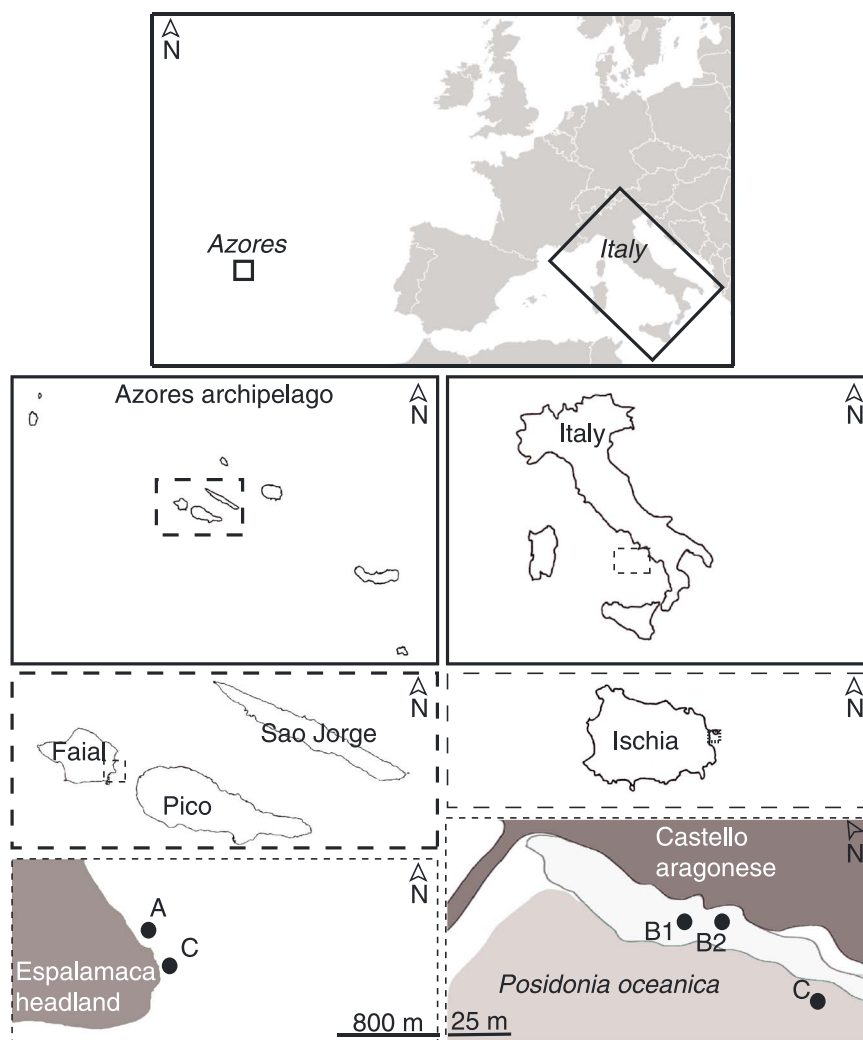


Figure 1. Study sites at Ischia (Italy) and Faial (Azores).

Table 1
Environmental Variables at Ischia and Faial During the Experiments

Parameters	Ischia (Italy)			Faial (Azores)	
	C	CO ₂ vents B1	CO ₂ vents B2	C	CO ₂ vents
Temperature (°C)	21.0 ± 4.2 (13.0–26.3)	20.2 ± 4.0 (13.0–26.1)	20.9 ± 4.2 (13.0–26.0)	22.3 ± 0.3 (22.0–22.9)	22.3 ± 0.3 (22.0–22.9)
A _t (μmol Kg ⁻¹)	2,578 ± 17 (2,552–2,606)	2,572 ± 27 (2,507–2,611)	2,573 ± 15 (2,545–2,596)	2,379 ± 8 (2,365–2,390)	2,374 ± 8 (2,356–2,389)
pH _t	8.04 ± 0.02 (7.94–8.09)	7.22 ± 0.42 (6.56–7.83)	7.45 ± 0.42 (6.66–7.89)	8.04 ± 0.03 (7.97–8.09)	7.44 ± 0.25 (7.00–7.82)
DIC (μmol Kg ⁻¹)	2,264 ± 44 (2,180–2,335)	2,669 ± 230 (2,366–3,131)	2,571 ± 225 (2,366–3,014)	2,091 ± 17 (2,061–2,124)	2,337 ± 90 (2,203–2,487)
pCO ₂	477 ± 58 (384–598)	5,663 ± 5,608 (804–17,378)	3,619 ± 4,240 (680–13,613)	417 ± 41 (366–502)	2,220 ± 1,235 (746–5,454)
CO ₃ ²⁻ (μmol Kg ⁻¹)	236 ± 36 (172–300)	60 ± 46 (9–166)	109 ± 77 (11–165)	207 ± 15 (178–229)	72 ± 38 (23–140)
HCO ₃ ⁻ (μmol Kg ⁻¹)	2,014 ± 75 (1,882–2,153)	2,431 ± 110 (2,176–2,552)	2,571 ± 225 (2,366–3,014)	1,877 ± 30 (1,823–1,927)	2,236 ± 87 (2,041–2,298)
Ω _{Calcite}	5.4 ± 0.8 (4.0–6.7)	1.3 ± 1.0 (0.2–2.9)	2.2 ± 1.2 (0.3–3.9)	4.9 ± 0.4 (4.2–5.4)	1.7 ± 0.9 (0.6–3.3)
Ω _{Aragonite}	3.6 ± 0.5 (2.6–4.1)	1.0 ± 0.7 (0.1–2.5)	1.4 ± 0.8 (0.2–2.5)	3.2 ± 0.2 (2.8–3.6)	1.1 ± 0.6 (0.4–2.2)

Note. Mean ± SD. Min and Max values in parentheses.

2.1.2. Faial (Azores)

The vent area is located in the Faial-Pico channel off the Espalamaca headland (38°32.586'N, 028°36.136'W), close to shore (~500 m), and thus exposed to occasional strong tidal currents and waves (Figure 1). The vent site, here named as the "Faial vent site," is present at 5 to 8 m depth and harbors dense bubble streams among large boulders and sparse bubble streams in sandy areas. Gas composition of bubbles comprises 96–99% CO₂, 0.04–1% N₂, 0.05–1% O₂, and 0–0.02% CH₄, without sulfur. Within the venting area, seawater pH steeply increased away from each bubble stream, reaching ambient values at less than 0.5 m from the bubble stream. No hypoxic conditions were thus observed at our studied acidified sites (similarly to Ischia stations). The vent area is therefore a mosaic of patches with reduced pH, with the local pH depending on the proximity of bubble streams. Minimum pH_T values in the bubble streams were 6.2. For the experiment we selected a site with an average pH_T of 7.4 ± 0.3 (pCO₂ = 2,220 ± 1,235, Ω_{cal} = 1.7 ± 0.9, Ω_{arag} = 1.1 ± 0.6). The ambient pH site with no degasification but with a similar topography to the vent site had a mean ambient pH of 8.04 (pCO₂ = 417 ± 14, Ω_{cal} = 4.94 ± 0.11, Ω_{arag} = 3.24 ± 0.07; see Table 1).

2.2. Carbonate System Characterization

Methods used in Ischia are reported in Rodolfo-Metalpa et al. (2011) and consisted of frequent in situ measurements of total alkalinity (A_T) using Gran titration, pH_T (in total scale), and temperature at each studied site over the experiment duration (see also Table S2 in Rodolfo-Metalpa et al., 2011). In Faial, discrete bottom seawater samples were taken once a week over a 5 week period between July and August 2011, for measurements of pH_T, salinity, temperature, and A_T. Water samples were collected with scuba, using 100 mL syringes from inside experimental cages. The syringes were equipped with gas-tight caps to avoid seawater-atmosphere exchange after collection. Water samples were immediately transported to the laboratory, and pH_T was measured using a SevenGo pro pH/ORP/Ion meter SG8 (accuracy = 0.002 pH units) calibrated with TRIS buffer solution (Dickson laboratory, batch #90). Salinity was measured using a Mettler Toledo SevenGo conductivity meter SG3 previously calibrated with a certified buffer solution.

Seawater samples for A_T measurement were filtered through 0.45 μm pore size syringe filters (Minisart, Sartorius), immediately treated with 0.1 mL saturated mercury chloride solution, sealed in boron glass bottles, and stored at 4°C in the dark until analysis. A_T was then measured using duplicate Gran titrations with 0.1 mol L⁻¹ HCl performed using a titration analyzer (Titro-Line, Si-Analytix GmbH) and a pH glass electrode (Crison +25) calibrated on the total H⁺ scale (with TRIS buffer). Titrations of A_T from standard seawater provided by A.G. Dickson (batch #90) were within 2.14 μmol kg⁻¹ of the nominal value (SD = 5.50 μmol kg⁻¹; n = 10).

Seawater carbonate chemistry parameters, pCO₂, aragonite (Ω_{Arag}) and calcite saturation states (Ω_{calc}), carbonate ion concentrations ([CO₃⁻²]), and dissolved inorganic carbon (DIC) were calculated from measured A_T, pH_T, salinity, and temperature using the program CO2SYS (Lewis & Wallace, 2006) with the thermodynamic constants of Mehrbach et al. (1973) as refitted by Dickson and Millero (1987).

2.3. Experimental Design

Slightly different experimental designs were developed in parallel at Ischia and Faial due to availability of experimental substrates, access to selected sites, and to study bioeroding microflora communities and their erosive activity at different early stages of development in various carbonates. Thus, only one type of aragonite substrate was deployed at Ischia for colonization by microborers over 6 months (March–September 2009), while various calcite and aragonite substrates were deployed at Faial over a period of 1.5 months (July–August 2011). At Ischia, experimental substrates were exposed to all erosion processes including abiotic/biogenic dissolution and grazing by herbivores, while substrates at Faial were put in cages to exclude grazing influence of large herbivores. Details on each experimental design are provided below. Despite those differences in experimental design, the results remain comparable as the experiments were both carried out in CO₂ vents in temperate carbonate and shallow systems with comparable gas composition, pH ranges, and temperatures and over a few months to focus on early colonization of carbonate substrates by boring microflora. Moreover, all results are expressed in the same unit allowing comparisons between the two experiments and with the literature following what is commonly done in bioerosion studies (see for instance the review by Schoenberg et al., 2017).

2.3.1. Ischia

Live shells of the bivalve *Callista chione*, which have a dense aragonite microstructure (shell microdensity (MD) = $2.75 \pm 0.18 \text{ g cm}^{-3}$, and porosity was negligible; parameters measured according to Bucher et al., 1998) were chosen for Ischia experiment as those shells are abundant in the Mediterranean Sea, are of commercial importance (Ezgeta-Balic et al., 2011), and have been used in other bioerosion studies in temperate waters allowing comparison of data (e.g., Wisshak et al., 2005, 2011). Nine shells were emptied and bleached during a few days to remove all organic matter. The shells were halfway embedded in epoxy resin to minimize the loss of substrate and to expose only the pristine inner side of the valves to boring microflora. Such design and treatment are commonly used in microbioerosion studies (e.g., Grange et al., 2015; Wisshak et al., 2011) so only the new colonization of substrates by microborers is studied allowing calculation of rates of biogenic dissolution over a certain time of exposure. Three PVC platforms with three shells each were laid at 3 m depth at each site for colonization by boring microflora in March 2009. After a 6 month exposure, the nine shells were retrieved and preserved in a buffered 3% solution of formaldehyde in seawater prior analysis.

2.3.2. Faial

The experimental substrates consisted of shells and skeletons of commonly seen calcifiers in Azores that differed in mineral composition (aragonite versus calcite). After a bleaching treatment, similarly to shells used at Ischia, substrate MD and porosity were measured using the buoyant weight technique according to Bucher et al. (1998) (accuracy of $\sim 0.1 \text{ mg}$ for MD). The skeleton of the coral *Caryophyllia cyathus* (aragonite) had MD = 2.78 g cm^{-3} and porosity = 55% ($n = 10$), shells of the gastropod mollusk *Charonia lampas* (aragonite) had MD = 2.81 g cm^{-3} and porosity = 43% ($n = 9$), the clam *Tapes decussatus* (aragonite) had MD = 2.85 g cm^{-3} and porosity = 32% ($n = 5$), and the limpet *Patella aspera* (layers of calcite/aragonite) had MD = 2.69 g cm^{-3} and porosity = 44% ($n = 6$). The dry weight of each substrate was also measured prior and after deployment on the field to allow determination of net carbonate loss (=gross accretion – gross dissolution (i.e., abiotic dissolution + biogenic dissolution)). Two pieces of each substrate type were placed inside plastic cages (two replicate cages per site, total of four replicate shells per site) constructed with 3 cm mesh plastic caging material and fully closed, at each site, to avoid grazing by large herbivores such as urchins. Cages were solidly attached to the sea bottom with the aid of cables and nails. Substrates were colonized by epilithic and microboring organisms during 45 days in summer 2011. Similarly to the Ischia experiment, the samples retrieved were placed in a buffered 3% solution of formaldehyde in seawater prior to analysis.

2.4. Substrate Analysis

Collected experimental substrates (shells and coral skeletons) from Ischia and Faial were analyzed to identify epiliths and microboring species after decalcification with 10% HCl of small pieces of substrates (observations done under light microscopes: Eclipse LV100, Nikon in France and Leica DM6000 in Azores).

At Ischia, the mean depth at which 80–85% of microboring filaments living inside shells were observed, bioeroded surface area at the surface of substrates, and rates of carbonate biogenic dissolution (i.e., gross biogenic dissolution) were quantified according to methods described in Tribollet et al. (2009) and equation (1) provided below. In summary, one-half of each of the substrates was cut in thin sections for determining the depth of penetration of microboring filaments and the relative abundance of the most commonly seen microboring species in communities. Fifteen to 20 measurements of depth of penetration of filaments were performed on each of the four thin sections cut per substrate type ($n > 60$ measurements per substrate type per studied site). The other half of substrates was used to estimate the surface area bioeroded on the top surface of substrates by microborers based on the analysis of 40 pictures taken per site using a scanning electron microscope (SEM) (see also Tribollet, 2008a). Although this methodology underestimates rates of biogenic dissolution as the branching pattern of microboring filaments is not fully taken into account (see Tribollet et al., 2009), it allows comparisons among relative rates quantified at different sites.

At Faial, rates of biogenic dissolution were estimated according to the method described in Carreiro-Silva et al. (2009) using a resin casting technique that results in 3-dimensional casts of the gallery systems produced by microborers within the shells. For this method, the surface area occupied by boring traces and their depth of penetration were measured on 80 SEM pictures of the substrate inner upper surface per substrate type and site. The volume of substrate removed by microborers in each sample was then calculated by multiplying the percent area colonized by the boring traces by their depth of penetration. Rates of biogenic

dissolution were then estimated by multiplying the volume of calcium carbonate removed by microborers by the substrate MD ($\text{g CaCO}_3 \text{ cm}^{-3}$), and divided by the surface area of the sample and the time of exposure, following equation (1).

Rates of biogenic dissolution were estimated based on measurements solely of the substrate upper surface, because this surface is free of preexisting microborers, which would otherwise confound the results. Moreover, biogenic dissolution rates have been shown to be 10 times greater on the illuminated upside versus the shaded underside of substrates in the photic zone of the Azores (Wisshak et al., 2011). Given the low exposure time of the experiment (1.5 months), carbonate biogenic dissolution on the downfacing and sides of the shells was assumed to be negligible.

All rates of biogenic dissolution are provided in the same unit, that is, g CaCO_3 removed per m^2 of exposed surface area of substrate and per year, permitting comparisons with those published in the literature.

Estimation of biogenic dissolution rates (BD):

$$\text{BD} = \frac{\text{Depth of penetration} \times \text{Bioeroded surface area} \times \text{MD}}{\text{Upper surface area of substrate} \times \text{Time of exposure.}} \quad (1)$$

where BD is expressed in g or kg of calcium carbonate dissolved by microborers per square meter of dead carbonate substrates exposed to colonization per year, "MD" is the microdensity of substrates expressed in $\text{gCaCO}_3 \text{ cm}^{-3}$, "Upper surface area of substrate" is the upper surface area of shell exposed to colonization (in cm^2) excluding the area occupied by voids (natural porosity of substrates) visible at the surface of skeletons of the coral *Caryophyllia cyathus*, and "Time of exposure" is the duration of the experiment.

In Faial, to make total weight loss of each substrate comparable to the rates of biogenic dissolution, the pre-deployment and postdeployment weights of samples were converted to sample volumes by using each substrate's density and obtain loss of weight per volume of sample in $\text{g CaCO}_3/\text{cm}^3$. The values of total weight loss and biogenic dissolution were then converted to relative percent weight loss for comparisons.

2.5. Statistical Analyses

All data were statistically tested using the software R 2.15.1 or PRIMER 6 & PERMANOVA (Anderson et al., 2008). Prior analysis, the normal distribution and homoscedasticity of data sets were tested. In the case of Ischia data sets, normal distribution (Shapiro test) and homoscedasticity (Bartlett test) conditions were not respected; thus, several Kruskal-Wallis tests were carried out to compare variables (depth of penetration, bioeroded surface area, and rates of biogenic dissolution) among the three studied sites. Post-hoc test (*U* Mann Whitney test with $\alpha = 0.05$) were then performed for pairwise multiple comparison. In the case of Faial data sets, normality and homoscedasticity conditions were also not respected; thus, one-way permutational multivariate analysis of variance (PERMANOVA) was used to examine differences in physical-chemical characteristics among sites (vent versus ambient pH sites) and two-way crossed PERMANOVA allowed determining the effects of sites and substrate types on microborer abundance, depth of penetration, bioeroded surface area, and abiogenic versus biogenic dissolution rates. All tests were run using a resemblance matrix based on Euclidean distance (using 9,999 permutations) to produce *P* values. Significant factors were further analyzed using a PERMANOVA pairwise comparison. The mean \pm SE (standard error) of each variable per site and per substrate is indicated.

3. Results

3.1. Environmental Parameters

Overall during each experiment, total alkalinity (A_T) and seawater temperature did not vary among sites (Kruskal-Wallis and one-way PERMANOVA gave $p > 0.05$; see also Rodolfo-Metalpa et al., 2011). At Ischia, the mean A_T was $2,578 \pm 17 \mu\text{mol kg}^{-1}$ and temperature-matched ambient seasonal fluctuation varied between 13°C and 26°C (Table 1). At Faial, A_T and temperature did not vary between sites and averaged $2,377 \pm 8 \mu\text{mol kg}^{-1}$ and 22.3°C . In contrast, for both Ischia and Faial islands, pH, $p\text{CO}_2$, DIC, and the saturation states of aragonite and calcite varied significantly among ambient pH and acidified sites (Kruskal-Wallis and one-way PERMANOVA, $p < 0,05$; Table 1). At Ischia, the environmental parameters did not differ significantly between the two "acidified sites" B1 and B2 (Wilcoxon, $p > 0.05$; Table 1).

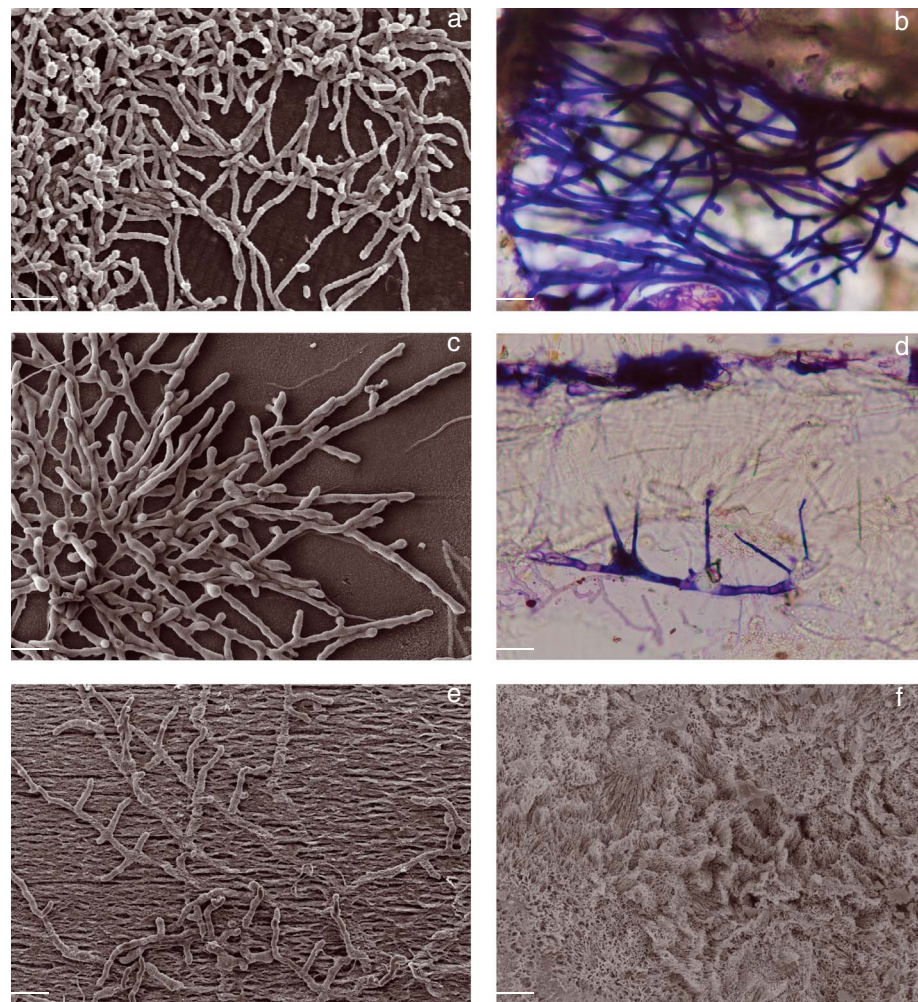


Figure 2. Traces produced by (a, c, and e) microboring organisms (resin casts) and (b and d) their producers, as well as example of a heavily chemically corroded surface of (e) the shell of the gastropod *Charonia lampas* and (f) skeleton of the coral *Caryophyllia* sp. Typical assemblage of microboring community in the ambient pH site at Faial dominated by the trace *Eurygonum nodosum* (Figure 2a) produced by the cyanobacterium *Mastigocoleus testarum* (Figure 2b); typical assemblage of the microboring community in the CO₂ vent site dominated by the trace *Rophalia catenata* (Figure 2c) produced by the chlorophyte *Phaeophila* sp (Figure 2d). Scale bar = 100 μm for all pictures except Figures 2b and 2d (scale bar = 20 μm).

3.2. Epilithic Communities

At the ambient pH sites at both locations, experimental substrates were mostly covered by turf algae, serpulids, bryozoans, and crustose coralline algae. Some foraminifera were also observed on substrates at Faial ambient site. In contrast, at the acidified sites (B1, B2, and Faial vent), only turf algae were growing on substrates. No calcifying organisms were observed while traces of chemical dissolution could be distinguished on substrate surfaces. At Ischia, evident traces of grazing were also observed at the ambient pH site, probably resulting from the activity of gastropods and urchins. No trace of grazing was observed at either site of Faial due to the design of the experiment.

3.3. Microboring Communities: Diversity, Abundance, and Distribution in Substrates

All substrates from all sites were colonized by microboring communities. The euendolithic cyanobacteria and chlorophytes that were seen both at Ischia and Faial were *Hyella* spp. and *Mastigocoleus testarum* and *Eugomontia* sp. and *Phaeophila* sp., respectively (Figure 2). The chlorophyte *Entocladia* sp. and other unidentified chlorophytes, as well as unidentified fungal filaments, were also observed on shells collected at Ischia.

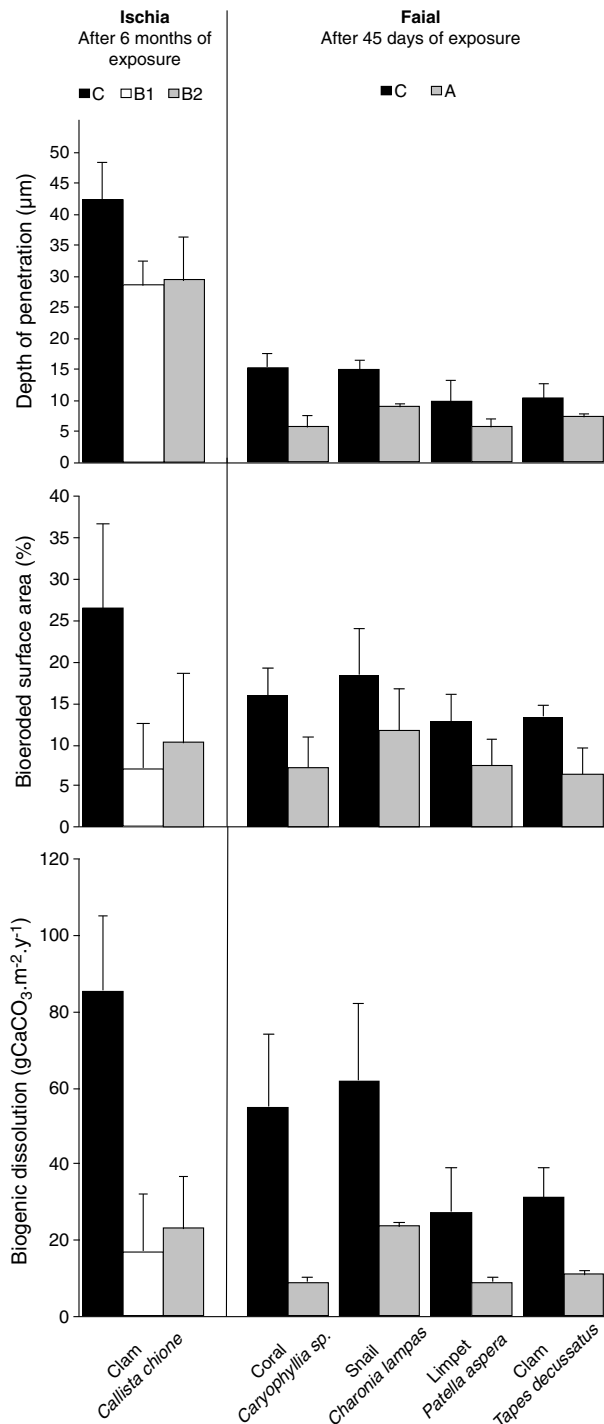


Figure 3. Depth of penetration of euendolithic filaments in the (a) various studied substrates, (b) bioeroded area at the substrate surface, and (c) rates of biogenic dissolution at the ambient pH site and the two acidified sites at Ischia after 6 months of exposure and at the ambient pH versus acidified site at Faial after 1.5 months of exposure. Mean ± SD are presented.

Caryophyllia cyathus, and between $4.7 \pm 1.6\%$ ($0.13 \pm 0.04 \text{ g of CaCO}_3 \text{ cm}^{-3} \text{ mo}^{-1}$) and $14.6 \pm 1.7\%$ ($0.41 \pm 0.05 \text{ g of CaCO}_3 \text{ cm}^{-3} \text{ mo}^{-1}$) for the same substrates at the Faial vent site. Under ambient pH conditions, coral skeletons lost 12 times more weight than other substrate types (two-way PERMANOVA substrate type effects, $p < 0.01$), while the amount of net carbonate loss was not significantly different among other

Two fungal species were identified in Azores substrates: *Ostracobable implexa* and *Lithopythium gangliiforme*. Community composition differed between ambient pH and acidified sites at both locations. Cyanobacteria such as *Hyella sp.* and *M. testarum* and fungi tended to dominate communities at the acidified sites, while the chlorophyte *Phaeophila sp.* dominated communities at the ambient pH sites. *Phaeophila* was, however, often observed at the acidified sites B1 and B2 at Ischia.

Microboring communities were always more abundant and penetrated deeper into substrates at both ambient pH sites than at the acidified sites (no significant differences between B1 and B2, Figure 3). Depths of penetration of euendolithic filaments were at least a factor 1.3 higher at the ambient pH sites than at the acidified sites (two-way PERMANOVA, interaction pH × substrate type $p < 0.05$). At Ischia, the mean depth of penetration of filaments at the ambient pH site was $42 \pm 6 \mu\text{m}$, while it was about $29 \pm 5 \mu\text{m}$ at B1 and B2. At Faial, the mean depth of penetration of euendolithic filaments in the various types of substrates varied between $10 \pm 3 \mu\text{m}$ and $15 \pm 2 \mu\text{m}$ at the ambient pH site and $5 \pm 2 \mu\text{m}$ and $9 \pm 3 \mu\text{m}$ at the Faial vent site. Under ambient pH conditions at Faial, depths of penetration of euendolithic filaments were significantly higher in the coral and snail substrates than in limpet and clam shells (pairwise comparisons $p < 0.05$; Figure 3). Under reduced pH conditions, depths of penetration were significantly higher in snail shells than in the other substrate types (pairwise comparisons $p < 0.05$; Figure 3).

Bioeroded surface areas followed the same trend as depths of penetration of euendolithic filaments and were at least 1.5 higher at the ambient pH sites than at the acidified sites (up to 2.5 times at Faial for some substrates; two-way PERMANOVA, pH effects, $p < 0.01$). The bioeroded surface area at the ambient pH site at Ischia was higher than the one measured at the ambient pH site at Faial ($27 \pm 10\%$ versus $18 \pm 3\%$; Figure 3). Bioeroded surface area at B1 and B2 and Faial vent sites were comparable (7–10% and 6–12% respectively; Figure 3). Differences of bioeroded surface area among substrates at each site of Faial were for the most part not significant due to a relatively high variability of bioeroded surface area within each substrate type (two-way PERMANOVA, pairwise comparisons, $p > 0.05$). The bioeroded surface area in the mollusk shell *C. lampas* was, however, significantly higher than that of clams and limpets whatever the seawater pH at Faial (ambient versus acidified waters) (pairwise comparisons, $p < 0.05$; Figure 3).

3.4. Net Carbonate Loss and Gross Biogenic Dissolution

Carbonate substrates placed at the Faial vent site lost weight 3 to 21 times faster than substrates at the ambient pH site (two-way PERMANOVA, $p < 0.001$ for pH effects, Figure 4). Experimental substrates at the ambient pH site lost between $0.4 \pm 0.05\%$ of initial weight ($0.01 \pm 0.001 \text{ g of CaCO}_3 \text{ cm}^{-3} \text{ mo}^{-1}$) for the limpet *Patella aspera* and $4.9 \pm 1.5\%$ ($0.14 \pm 0.01 \text{ g of CaCO}_3 \text{ cm}^{-3} \text{ mo}^{-1}$) for the skeletons of the

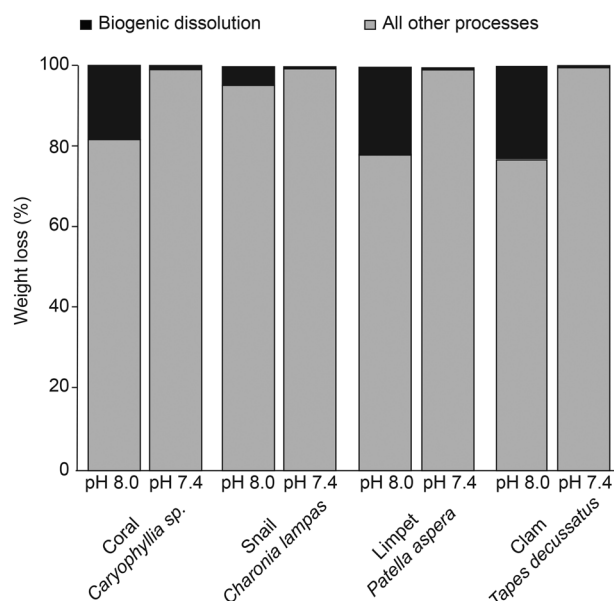


Figure 4. Contribution of biogenic dissolution to the total substrate weight loss at the ambient pH site at Faial and the vent site and its variation among substrate types, $n = 4$ substrates per substrate type and site for Faial. All processes = all other processes than biogenic dissolution.

types of substrates (pairwise comparisons, $p > 0.05$). Under acidic conditions, however, coral skeletons lost 1.5–3 times more weight than limpet or clam shells (two-way PERMANOVA substrate-type effects, $p < 0.01$), but there were no significant differences in the amount of weight loss between the coral and the gastropod *C. lampas* (pairwise comparisons, $p > 0.05$). The contribution of biogenic dissolution to net carbonate loss at the ambient pH site was 4–23% and less than 1% at the acidified site whatever the substrate type considered at Faial.

Gross biogenic dissolution of carbonate substrates due to boring microflora was always much higher at the ambient pH sites than at the acidified sites, that is, by a factor 4 to 7 at Ischia depending on site, and a factor 3 to 6 at Faial depending on substrate type (Wilcoxon, $p < 0.05$ and two-way ANOVA, pH effects, $p < 0.02$; Figures 3 and 4). Rates of gross biogenic dissolution were not significantly different between the two acidified sites (B1 and B2) at Ischia despite a factor of 2 between means (variance was quite high). Thus, the average rate for B1 and B2 was 20 ± 17 g CaCO_3 dissolved $\text{m}^{-2} \text{y}^{-1}$ compared with 86 ± 20 g CaCO_3 dissolved $\text{m}^{-2} \text{y}^{-1}$ at the ambient pH site. At Faial, the highest rate of biogenic dissolution was found in shells of the gastropod *C. lampas* whether it was at ambient pH or low pH (6.1 ± 2.0 and 2.3 ± 1.0 g CaCO_3 dissolved $\text{m}^{-2} \text{y}^{-1}$ respectively; pairwise comparisons, $p < 0.05$). High variability of biogenic dissolution rates rendered differences among other substrate types not statistically different (pairwise comparisons, $p > 0.05$).

4. Discussion

The present study took advantage of the existence of two CO_2 vent systems in different temperate regions but sharing common properties in terms of gas composition, bathymetry, pH ranges, and temperatures, which made it possible to undertake two separate experiments with the common objective of testing the effects of OA on biogenic dissolution of the shells and skeletons of common temperate calcifiers by microborers. This is important because, although CO_2 vents have been widely recognized as good analogies of future ocean acidification scenarios, each vent site has some unique biological or physical-chemical features making inference of single studies difficult to extrapolate to other regions. Our study shows, however, that despite differences in experimental design, vent systems, and methodologies, the obtained results and trends were very similar, with a significant negative influence of extremely low seawater saturation state of carbonate minerals ($\Omega \sim 1$) and pH (7.2–7.4) on the process of biogenic dissolution of carbonates by natural (in situ) pioneer microboring communities colonizing calcite and aragonite substrates in temperate carbonate systems.

4.1. Microboring Communities Under Naturally Acidified Waters

Surprisingly, microboring communities were able to colonize all experimental carbonate substrates despite extremely low seawater pH (7.2–7.4) and regular undersaturated carbonate conditions in the studied CO_2 venting areas in Ischia and Faial. Epilithic communities were also observed and were conformed to those reported at Ischia by Hall-Spencer et al. (2008) and Martin et al. (2008), with a general reduction of calcareous epiliths at low pH sites due to the low carbonate saturation state. Observed microboring communities at both ambient and acidified waters at Ischia and Faial comprised in abundance several species of cyanobacteria (including *Hyella* spp. and *Mastigocoleus testarum*) and chlorophytes (e.g., *Phaeophila* sp.), which are characteristic of pioneer microboring communities (Gektidis, 1999; Grange et al., 2015; Le Campion-Alsumard, 1975; Tribollet, 2008a). This was expected for substrates exposed to less than 2 months of colonization by microborers in Azores. In the Mediterranean Sea, microboring cyanobacteria such as *Hyella* spp. and *M. testarum* were reported as the early colonizers of calcite spar after 1 month of exposure (Le Campion-Alsumard, 1975). Similar communities were also observed in dead shells of *Callista* sp. in shallow waters of the Faial channel (Azores) after 3 months of exposure (Wisshak et al., 2011) and in coral skeletons and shells in

different coral reefs after 2–3 months (Carreiro-Silva et al., 2005; Chazottes et al., 1995; Enochs et al., 2016; Grange et al., 2015; Kiene et al., 1995). In contrast at Ischia, communities of microborers were expected to be mature as substrates were exposed during 6 months to colonization, but instead, they were immature (dominance of the chlorophyte *Phaeophila* sp. at the ambient pH site). Mature communities dominated by the chlorophyte *Ostreobium* sp., and to a less extent, the cyanobacterium *Plectonema terebrans* and fungal hyphae, are indeed generally established after more than 6 months of exposure as documented for tropical, warm-temperate, and cold carbonates (Chazottes et al., 1995; Gektidis, 1999; Grange et al., 2015; Tribollet, 2008a; Wisshak et al., 2005, 2011). The pattern observed at Ischia most probably resulted from (1) the grazing activity at the ambient pH site as deep grazing traces were observed at the surface of shells and (2) environmental conditions at the acidified sites as chemical dissolution occurred during a significant part of the experiment. Although a few grazers were observed under acidified waters (pers. observ.; Hall-Spencer et al., 2008), no trace of grazing was observed on shells maintained at low pH.

Both processes, that is, grazing and chemical dissolution, resulted therefore in the recurrent removal of substrate surfaces, which dramatically limited the development of microboring filaments and constantly created new surfaces available to colonization by microboring pioneer species. This was especially true at the acidified sites where a higher diversity of pioneer microboring species was observed (not reported in details here). The synergy between the process of grazing and microborer development has been well described by Schneider and Torunski (1983) in temperate-cold waters, and by Chazottes et al. (1995), Tribollet and Golubic (2005), and more recently, by Grange et al. (2015) in coral reefs. This is the first time, however, that the synergy over time between environmental dissolution (thermodynamically driven) and biogenic dissolution is reported. Interestingly, environmental (chemical) dissolution caused the same effects on carbonate substrate surfaces than an intense grazing pressure (e.g., by sea urchins; Chazottes et al., 1995) preventing microboring community successions, colonization, and penetration in depth into substrates. Measured depths of penetration in all substrates at the CO₂ venting areas in Ischia and Faial were particularly low (<30 μm after 6 months at Faial) and always lower than those found at ambient pH. Those depths, due mainly to *Phaeophila* sp., were similar to those reported by several authors for various carbonate substrates in warm temperate (Färber et al., 2015) and shallow tropical settings (Carreiro-Silva et al., 2009, 2012; Enochs et al., 2016; Kiene et al., 1995; Mao Che et al., 1996). *Phaeophila* sp. is known as a photophile microboring chlorophyte like the pioneer cyanobacteria *Hyella* sp. and *M. testarum* and is therefore not expected to penetrate really deep into substrates. A few studies reported, however, a maximal depth of penetration of ~100–200 μm for these photophile microborers in shells and coral skeletons after a long period of exposure (Grange et al., 2015; Kaehler, 1999; Tribollet, 2008b).

In terms of surface area colonized by microborers, 13–16% and 27% of substrate surfaces at Faial and Ischia, respectively, were colonized by microborers at ambient pH, while less than 10% of surfaces were colonized at low pH. Differences between Azores and Italy are due to the duration of the experiment and types of substrates used. Chazottes et al. (1995) reported that about 20% of coral skeleton surfaces are colonized by pioneer microborers after 2 months and that no more than 30% of surface areas are bioeroded by mature communities after 6–12 months of exposure in coral reef ecosystems under ambient pH (see also Tribollet, 2008a). Similarly, Enochs et al. (2016) found a percent of colonization of 20% in carbonates exposed at ambient pH in Maug reef (Mariana Islands) during 3 months. In naturally acidified seawater at Maug (pH ≥ 7.9), the percent of colonization was still mainly due to *Phaeophila* sp. but reached more than 30%–50% after 3 months of exposure depending on the level of acidity (mid-pCO₂ versus high-pCO₂). In this later study, the saturation state of carbonate minerals never reached 1 ($\Omega_{\text{arag}} > 3.4$). This alga seems thus to benefit from the rise of aqueous pCO₂, like it is suggested for *Ostreobium* in coral skeletons (Reyes-Nivia et al., 2013; Tribollet et al., 2009, 2014). In the light of the recent study published by Grange et al. (2015) on the dynamics of microboring community successions over a year period, Enochs et al.'s results strongly suggest that ocean acidification could accelerate microboring communities' shift from an immature stage dominated by *Phaeophila* sp. to a mature stage dominated by *Ostreobium* sp. and thus enhance carbonate biogenic dissolution as *Ostreobium* is the main agent of this process (Chazottes et al., 1995; Grange et al., 2015; Reyes-Nivia et al., 2013; Tribollet, 2008b; Tribollet et al., 2009). But this is probably true as long as the acidity is not too drastic as otherwise successions are delayed (present study). Further investigations are needed to better understand dynamics of microboring community successions in various naturally acidified waters.

4.2. Contribution of Biogenic Dissolution to Carbonate Total Weight Loss and pH Tipping Point

Environmental dissolution (chemical) and/or mechanical breakage were the main processes of carbonate loss due to seawater conditions and the relative low colonization by microboring flora at the acidified studied sites. Indeed, biogenic dissolution contributed to less than 1% to the total loss of carbonates at Faial under low pH. Emerging aragonite prisms were conspicuously visible on the degraded shell surfaces of the gastropod *C. lampas* and the bivalve *C. chione* for instance (Figure 2e) and were indicative of structural disintegration compromising both shell integrity and strength. Interestingly, the different studied carbonate substrates were not similarly affected by abiotic and biogenic dissolution processes. Both microdensity and mineral composition of substrates influenced rates of net carbonate loss and biogenic dissolution. Under low pH conditions, aragonite skeletons of the coral *C. cyathus* and the gastropod *C. lampas* lost about 2.5 times more carbonates than aragonite shells of *T. decussatus* and the calcitic shell of *P. aspera*. The lower net weight loss of *P. aspera* shells is not surprising as calcite is known to be less soluble than aragonite in undersaturated waters (Krumins et al., 2013; Morse et al., 2006). Contrastingly, the influence of substrate microdensity and mineralogy on biogenic dissolution was more pronounced under ambient pH conditions where colonization by microborers was highest. The contribution of biogenic dissolution to the total weight loss of substrates in this case varied between 4 and 23%. The less dense and more porous aragonite shells of *C. lampas* (porosity = 43%) were more colonized and thus more intensively dissolved by microborers than the denser and less porous shells of *T. decussates* (porosity = 32%). The shells of *P. aspera* (limpet), although having the lowest microdensity of all substrates, were not as eroded as the other substrates, likely because of its calcitic mineralogy. This suggests that the process of biogenic dissolution is facilitated in aragonite substrates, especially the most porous ones (see also Rodolfo-Metalpa et al., 2011). Very recently, Couradeau et al. (2017) showed the mineral substrate preference in microboring cyanobacteria and suggested the use of various mechanisms by microborers to dissolve carbonates (see also Alexandersson, 1975; Tribollet, 2008b; Garcia-Pichel et al., 2010; Ramirez-Reinat & Garcia-Pichel, 2012). It is also known that the density, porosity, and microstructure of carbonates allow more or less light to penetrate inside, influencing microboring phototroph abundance and thus biogenic dissolution rates. In coral reefs, for instance, the porous coral skeletons are intensively bioeroded compared to nonporous mollusk shells, coralline algal thalli, and limestone (e.g., Carreiro-Silva et al., 2005; Kiene et al., 1995; Mao Che et al., 1996; Perry, 1998; Tribollet & Payri, 2001).

While several studies showed that an elevated $p\text{CO}_2$ with a pH of 7.9 increases rates of biogenic dissolution by at least 50% (Enochs et al., 2016; Reyes-Nivia et al., 2013; Tribollet et al., 2009), our data show the opposite trend. Rates of biogenic dissolution were indeed reduced by a factor 4 to 7 and 3 to 6 depending on sites and substrate types at the acidified sites at Ischia and Faial, respectively, compared to ambient pH sites. This is due to the very low pH tested ($\text{pH} < 7.4$) in our experiments. We do not exclude that microboring phototrophs may have benefited from higher concentration of CO_2 at the CO_2 venting sites, but the potential positive effect was definitely masked by the intense removal of substrate surfaces due to chemical dissolution (and mechanical breakage in Azores). We cannot exclude that microboring communities may have been affected by lower concentrations of oxygen in venting gases at the acidified sites compared to ambient sites. We do not believe, however, that low oxygen levels influenced significantly microboring communities as no hypoxic conditions were observed (acidified sites harbored well-developed benthic macroalgal communities; Hall-Spencer et al., 2008). Rates of biogenic dissolution quantified at the acidified sites were highly “residual” (see definition in Chazottes et al., 1995) and never exceeded $20 \text{ g m}^{-2} \text{ y}^{-1}$ (Ischia). Based on our results and available literature, we thus suggest that there is a pH tipping point, probably between 7.4 (our study) and 7.7 (lowest pH tested with a positive effect on microborers; Reyes-Nivia et al., 2013). Below this threshold the benefits of increased CO_2 to microboring phototrophs are overcome by the instability of substrate surfaces and/or potentially the acidosis of phototroph physiology as shown for some macroalgae (Porzio et al., 2011).

4.3. Consequences to Coastal Carbonate Systems

Based on models of anthropogenic CO_2 emissions, the pH levels examined in the present study (< 7.4 and $\Omega \sim 1$) should occur in shallow cold-temperate waters by 2,300 (Caldeira & Wickett, 2003; IPCC, 2013). It is thus expected that in ~ 300 years, remaining skeletons of calcifiers as well as limestone and sediments, in shallow waters of temperate ecosystems will be intensively—if not completely—dissolved due to chemical conditions of seawater ($\Omega \leq 1$) and much less by the activity of microboring flora. The presence of microborers

in carbonates under such conditions may, however, enhance or facilitate the effects of chemical dissolution, similarly to the known synergy between grazers and microborers in coral reef systems (e.g., Tribollet & Golubic, 2005). Overall, biogenic dissolution appears so negligible at the low pH levels tested in our study that it does not need to be taken into account in biogeochemical cycle models. In contrast, benthic carbonate temperate ecosystems at ambient pH today, where important commercial calcifiers are present (Harley et al., 2006), may greatly and increasingly be affected by biogenic dissolution by 2100 as the acidification of temperate waters will be faster than that of warm-tropical waters (IPCC, 2013). Moreover, based on our results and previous studies (Reyes-Nivia et al., 2013), we suggest that some critical pH between 7.4 and 7.7 (with $\Omega > 1$) could allow a maximal biogenic dissolution. Indeed as long as the saturation state of carbonate minerals is above 1, the process of biogenic dissolution by microborers, together with the metabolic activity of boring sponges, dominates processes of carbonate dissolution in hard substrates—at least in shallow coral reef systems—(Andersson et al., 2015; Schoenberg et al., 2017; Tribollet et al., 2009, 2014; Wisshak et al., 2013). Under such conditions, the lifespan of biogenic structures made for instance of mollusks that are major components of benthic communities in shallow temperate coastal areas, providing important habitat even after their death and contribute greatly to sedimentation, bioturbation, and community trophodynamics (Widdicombe et al., 2009), could greatly be reduced. This may be especially true for ecosystems with organisms with aragonite shells, such as the gastropod *C. lampas* dominate, as they are more sensitive to microborer infestation (present results). On the other hand, the infesting microborers may have benefits to their mollusk host under certain circumstances and despite their dissolution activity. Indeed, Zardi et al. (2016) showed that phototrophic microborers, which are sometimes lethal to some bivalves (Kaehler & McQuaid, 1999), can become beneficial under heat stress conditions. In the light of all those results, more research is definitely needed to (i) determine the benefit and deleterious effects of microborers upon calcifying organisms under various environmental conditions and (ii) improve forecasts of the coastal carbon cycle as the process of biogenic dissolution is not yet taken into account in models (Krumins et al., 2013).

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5. Conclusions

Here we show for the first time that several microboring organisms comprising cyanobacteria, algae, and fungi are able to grow in aragonite and calcite carbonate substrates exposed to very low pH (<7.4) and undersaturated conditions in natural shallow temperate waters. We also show that carbonate biogenic dissolution resulting from the action of these microorganisms is considerably reduced when seawater pH is below 7.4, especially in calcite substrates, while at a pH about 7.9, microborers' growth is stimulated, increasing rates of biogenic dissolution. We thus stress the necessity of studying further the process of biogenic dissolution under a wider range of pH and saturation states of carbonates in different substrates to determine the threshold below which biogenic dissolution starts to be reduced due to chemical conditions. This will allow better understanding biogenic dissolution dynamics and its contribution to the biogeochemical cycle of CaCO_3 in temperate coastal systems.

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