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Shallow Water Marine Sediment Bacterial Community Shifts Along a Natural CO_2 Gradient in the Mediterranean Sea Off Vulcano, Italy

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Abstract The effects of increasing atmospheric CO_2 on ocean ecosystems are a major environmental concern, as rapid shoaling of the carbonate saturation horizon is exposing vast areas of marine sediments to corrosive waters worldwide. Natural CO_2 gradients off Vulcano, Italy, have revealed profound ecosystem changes along rocky shore habitats as carbonate saturation levels decrease, but no investigations have yet been made of the sedimentary habitat. Here, we sampled the upper 2 cm of volcanic sand in three zones, ambient (median pCO_2 419 μatm, minimum Ω_{arag} 3.77), moderately CO_2-enriched (median pCO_2 592 μatm, minimum Ω_{arag} 2.96), and highly CO_2-enriched (median pCO_2 1611 μatm, minimum Ω_{arag} 0.35). We tested the hypothesis that increasing levels of seawater pCO_2 would cause significant shifts in sediment bacterial community composition, as shown recently in epilithic biofilms at the study site. In this study, 454 pyrosequencing of the V1 to V3 region of the *16S rRNA* gene revealed a shift in community composition with increasing pCO_2. The relative abundances of most of the dominant genera were unaffected by the pCO_2 gradient, although there were significant differences for some 5 % of the genera present (viz. *Georgenia*, *Lutibacter*, *Photobacterium*, *Acinetobacter*, and *Paenibacillus*), and Shannon Diversity was greatest in sediments subject to long-term acidification (>100 years). Overall, this supports the view that globally increased ocean pCO_2 will be associated with changes in sediment bacterial community composition but that most of these organisms are resilient. However, further work is required to assess whether these results apply to other types of coastal sediments and whether the changes in relative abundance of bacterial taxa that we observed can significantly alter the biogeochemical functions of marine sediments.

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Introduction

Covering around 70 % of the Earth, marine sediments play a major role in ecosystem processes and underpin carbon and nutrient cycling [1]. They are colonized by a vast, but unknown, diversity of microorganisms living in a variety of habitats [2]. Anthropogenic CO_2 emissions have lowered the pH of surface waters of the world by about 0.1 pH uni,t and a further global reduction of 0.2–0.4 pH units is expected by 2100 [3]. This rate of ocean acidification is, as far as we know, unprecedented and is rapidly exposing vast areas of seabed habitats to waters that are corrosive to carbonate [4].

Despite the importance of benthic microbial communities in global biogeochemical processes, our knowledge of their community dynamics is rudimentary, and their response to

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ocean acidification remains largely unknown [5–10], although mesocosm work is broadening our understanding of effects on pelagic microbial communities [11]. Studies on the effects of ocean acidification on marine pelagic microbes have mainly been in vitro experiments which have limitations when attempting to scale-up to effects on seawater [12]. Recently, volcanic vents have started to be used as natural laboratories to evaluate the consequences of increase in seawater acidity on marine ecosystems in situ, revealing tipping points beyond which seagrass, coral reef, and rocky shore systems are radically altered due to adverse effects on calcified organisms [13–15]. Kitidis et al. [16] performed the first field investigation of the impact of ocean acidification on sediment bacteria and found that although microbial ammonia oxidation was affected by ocean acidification in the water column, it was not affected in sediments, perhaps due to buffering within the sediments or adaptation of the ammonia-oxidizing microbes to high-CO_2 conditions.

In recent years, major advances in our understanding of marine microbial diversity and community structure have been driven by advances in molecular techniques [2]. For marine sediments, *16S rRNA* genes are now routinely amplified using PCR from nucleic acids extracted from the sediment; the PCR products are then cloned and sequenced [17, 18]. A meta-analysis by Liu et al. [19] concluded that it is likely that microbes will adapt to ocean acidification by genetic modification at the species level as well as by replacement of sensitive species by nonsensitive or less sensitive ones at the community level. Studies of microbes along marine CO_2 gradients have so far confirmed that the community compositions of resident marine microbiota alter as a result of acidification [20, 21].

Here, we used 454 pyrosequencing of *16S rRNA* gene to investigate the effect of a natural gradient in seawater pCO_2 on sediment bacterial communities off Vulcano in the Mediterranean. Our hypothesis was that a pCO_2 gradient would cause similar community shifts in sedimentary bacterial communities as those recently observed in biofilm grown on slides at the same field site [21]. We predicted that significant changes in sedimentary bacterial communities would occur due to the direct effects of acidified seawater on sediment biota, as well as indirect effects due to documented changes in the biota of surrounding habitats [20] which contribute to the supply of organic material to the sediment.

Materials and Methods

Study Site

Sampling took place on Vulcano Island 25 km off the northeast coast of Sicily (Fig. 1). Samples were collected from Levante Bay, on the northeast side of Vulcano where CO_2 from volcanic activity is released through vents at 0- to 10-m depth creating a pH gradient from 5.6 to >8.0 along the bay [22]. The sediment along this pH gradient was deposited during a volcanic eruption in 1888–1890 [22]. Samples were taken at 1-m depth from three sites which had the same salinity, temperature, and alkalinity (Fig. 1). Site 1 (S1) was located outside an area influenced by the vents and had diurnal variations in pH that are typical for shallow vegetated habitats [23], with a minimum pH of 8.08 at dawn and a maximum pH of 8.29 at dusk. At site 2 (S2), plumes of acidified water occasionally drove the pH down to 7.76, although this site was regularly flushed with ambient normal pH seawater. Site 3 (S3) was closest to the vents and had the widest pH variation; point measurements by Johnson et al. [20] had minimum pH 7.07 although long-term monitoring revealed an unusual brief minimum of pH 6.6 at this station (see Fig. 7 in [22]). Table 1 summarizes the seawater carbonate chemistry of these stations.

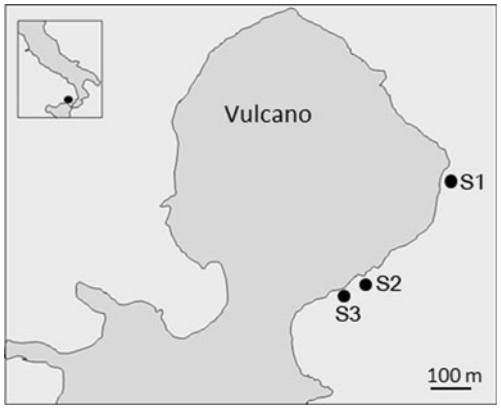

Fig. 1 Location of sample sites off Vulcano Island, part of the Aeolian island chain, northeast Sicily (38°25′N, 14°57′E)

Marine Sediment Sampling and DNA Extraction

At each sampling site, seven surface sediment samples were taken on May 2011. Each sample was taken using a 20-cm-diameter cylindrical corer: the top 2 cm of sediment in each core was kept for analysis. At each site, samples were taken at approximately 0.5 m apart along a transect running parallel to the shore at 1-m depth. One sample was retained for granulometry; the remainder were stored in sterile tubes at −20 °C until DNA extraction and carbon and nitrogen analyses.

The marine sediment genomic DNA was extracted from 0.3 g of sediment by using the PowerSoil DNA Extraction Kit (MO BIO Laboratories, Carlsbad, CA, USA) following the protocol described by the manufacturer. Due to an error in labeling, the small subsamples that were sent from UK to South Korea for DNA extraction were not labeled identically to the original sediment sample bags that they were taken from and from which sediment parameters were taken.

Table 1 Maximum, median, and minimum seawater pH with corresponding carbonate chemistry measurements along a CO_2 gradient on Vulcano island [20]

Station		pH range (NBS scale)	pCO_2 (μatm)	TA (mmol kg^{-1})	DIC (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	HCO_3^- (mmol kg^{-1})	$\Omega_{calcite}$	$\Omega_{aragonite}$
S1	Max	8.29	331	2.625	2.197	0.32	1.871	7.54	7.97
	Median	8.21	419		2.233	0.29	1.929	7	4.65
	Min	8.08	603		2.339	0.22	2.101	5.26	3.77
S2	Max	8.22	410	2.642	2.23	0.31	1.912	7.38	4.91
	Median	8.08	592		2.401	0.19	2.193	4.45	2.89
	Min	7.76	1429		2.512	0.12	2.349	2.96	1.96
S3	Max	8.1	599	2.736	2.409	0.25	2.14	6.05	4.02
	Median	7.71	1611		2.656	0.1	2.508	2.28	1.49
	Min	7.07	7454		2.95	0.02	2.682	0.54	0.35

Temperature (range 18.6–27.7 °C), pH (NBS scale), and salinity (38) were measured on several occasions between September 2009 and October 2010 (n=18). Total alkalinity (TA) is point measurement taken on 2 October 2010. The remaining parameters were calculated using CO_2 SYS program (using the constants of Roy et al. 1993 and Dickson for KSO_4)

Consequently, although pH zone is known for each DNA and sediment sample, the correspondence to the exact individual sample for sediment analysis is not known. This precludes relating each sample individually to the sediment parameters in that exact sample. Instead, we analyzed three sediment samples at random from each pH zone.

PCR Amplification and Pyrosequencing

The sediment sample DNA was amplified using primers targeting the V1 to V3 hypervariable regions of the bacterial *16S rRNA* gene [24]. Polymerase chain reactions (PCRs) were performed in 50-μl reactions under the following conditions: initial denaturation at 94 °C for 5 min, followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C to 55 °C with a touchdown program for 45 s, and elongation at 72 °C for 90 s. This was followed by an additional 20 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and elongation at 72 °C for 90 s. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and quantified using PicoGreen (Invitrogen) spectrofluorometrically (TBS-380, Turner Biosystems, Inc., Sunnyvale, CA, USA). Fifty nanograms of purified PCR product for each sample were combined in a single tube and sent to Macrogen Incorporation (Seoul, Korea) for sequencing using 454/Roche GS FLX Titanium Instrument (Roche, NJ, USA).

Analysis of Pyrosequencing Data

The sequence data obtained after pyrosequencing were processed using mothur [25]. Sequences shorter than 200 nt with homopolymers longer than 8 nt and, all reads containing ambiguous base calls or incorrect primer sequences were removed. Next, the sequences were aligned using mothur (default settings: kmer searching with 8mers and the Needleman–Wunsch pairwise alignment method) and the SILVA database (available at http://www.mothur.org/wiki/Alignment_database) and further filtered to remove gaps. The sequences were then preclustered using the mothur implementation of pseudo-single linkage preclustering algorithm from Huse and colleagues [26]. Putative chimeric sequences were detected and removed via the Chimera Uchime algorithm contained within mothur [27] in de novo mode, which first splits sequences into groups and then checks each sequence within a group using the more abundant groups as reference. A distance matrix was constructed using the average neighbor algorithm at phylogenetic distances of 0.03 (equivalent to species) in mothur. Pairwise distances between aligned sequences were calculated at a 0.97 % similarity cutoff and were then clustered into unique operational taxonomic units (OTUs).

All taxonomic classifications were performed using mothur's version of the Ribosomal Database Project (RDP) Bayesian classifier, using a RDP training dataset number 9 (available at http://www.mothur.org/wiki/RDP_reference_files) normalized to contain six taxonomic levels for each sequence at 80 % Naïve Bayesian bootstrap cutoff with 1,000 iterations. All sequence data are available under the following NCBI SRA092138.

Statistical Processing and Analysis of Results

All samples were standardized by random subsampling to 676 sequences per sample using the sub.sample command (http://www.mothur.org/wiki/Sub.sample) in mothur. Phylogenetic diversity was calculated as Faith's phylogenetic diversity in mothur by using a maximum-likelihood (ML) tree inferred from partial *16S rRNA* gene sequences of representative OTUs using FastTree2 with default settings [28]. OTUs (at 97 % similarity), Shannon Diversity Index, and rarefaction values

were also calculated using the mothur. OUT-based abundance data were first square root transformed to build the Bray-Curtis distance matrix using the vegdist function in the vegan package of R (Table S1). We performed a nonmetric multidimensional scaling (NMDS) plot using the metaMDS function in the vegan package of R[29]. This used the Bray-Curtis distance matrix to assess whether bacterial community composition clustered according to different pH levels. PERMANOVA analyses and post hoc *t* tests were based on 9,999 random permutations, using type III sums of squares and unrestricted permutation of raw data. Kruskall-Wallis tests were performed to assess the effect of pH on relative abundance of major phyla. ANOVA test was performed to check the relationship between pH and soil properties. PERMANOVA analyses were conducted in Primer 6.1.10. Kruskall-Wallis and rarefaction curve analysis were performed using R.

Results

Where gas bubbling was strongest, the benthic microbial community was clearly affected, as the seabed was coated with bacterial mats (Fig. 2a). However, we chose sampling sites with less extreme levels of pCO_2 where bacterial mats were absent and the black volcanic sand habitat appeared to be homogenous (Fig. 2b).

We obtained 62,238 quality sequences in total, which were classified into 1,726 operational taxonomic units (OTUs) at 97 % similarity level. On average, each individual sample was represented by 2,973 classifiable sequences, with a range of 676 to 4,255 sequences per sample. Most samples showed no sign of reaching an asymptote in OTU richness among the total number of reads available in the rarefaction analysis. This means that to cover the full taxonomic diversity, more sequences would be required (Fig. 3).

Effect of Seawater pCO_2 on Bacterial Community Composition

An NMDS plot reveals a shift in benthic sediment bacterial communities at the three different seawater CO_2 levels (Fig. 4). Most of the samples from each pH zone cluster separately. The PERMANOVA test results show that the observed overall community differences among pH levels were highly significant (Pseudo-$F_{2,18}$=1.9635, P(perm)=0.0001) with significance between the pair-wise comparisons of the three different pH levels considered (high, low: t=1.6374, P(perm)=0.001; high, medium: t=1.1605, P(perm)=0.025; low, medium: t=1.3539, P(perm)=0.0045).

Effect on Bacterial Community Diversity

Permutational univariate analysis of variance showed that pH change affected neither the OTU (species) richness (Pseudo-$F_{2,18}$=1.9109, P(perm)=0.1672) nor the phylogenetic diversity (Pseudo-$F_{2,18}$=2.9526, P(perm)=0.0684). However, the Shannon Index (Pseudo-$F_{2,18}$=3.9597, P(perm)=0.031) revealed that pH significantly affected bacterial diversity, with the lower level of bacterial diversity observed at high-pH (i.e., ambient pH/pCO_2) than the low-pH (high, low: t=2.3479, P(perm)=0.0433) sampling site (Fig. 5).

Effect of pCO_2 on Relative Abundance of Dominant Taxa

The dominant taxon across all sediment samples was Gammaproteobacteria (56 %), followed by Firmicutes (14 %), Bacteroidetes (12 %), Alphaproteobacteria (11 %), Actinobacteria (4 %), and Betaproteobacteria (1.3 %), respectively (Fig. 6, Fig.S1 and Table S2). They were, statistically, nonsignificant along the pCO_2 gradient (all $P>0.1$).

We found 21 genera of Gammaproteobacteria, of which *Pseudomonas* and *Vibrio* were predominant (Fig. 7 and Fig. S2), and we found no significant shift in the dominance of these bacteria along the pCO_2 gradient. However, five genera (Table 2) within the bacterial community were significantly affected by the pCO_2 gradient. These were *Georgenia* ($x^2(2)=9.63$, $P=0.008$), *Lutibacter* ($x^2(2)=7.23$, $P=0.02$), *Photobacterium* ($x^2(2)=14.26$, $P=0.0008$), *Acinetobacter* ($x^2(2)=6.47$, $P=0.03$), and *Paenibacillus* ($x^2(2)=6.81$, $P=0.03$). Together, these genera accounted for less than 5 % of the bacterial community sampled.

Relationship Between Seawater pCO_2 and Sediment Properties

There was no correlation between seawater pCO_2 and the total nitrogen (TN, $R^2=-0.16$, $P=0.67$) and total organic carbon (TOC, $R^2=-0.2$, $P=0.73$) content of the sediment (Table 3). There was also no correlation between pH zone and sediment granulometry; all samples were medium sand on the Wentworth scale (site 1 mean Φ 1.497, site 2 mean Φ 1.097, and site 3 mean Φ 1.210) and were unusual, for coastal sediments, in that carbonates were absent.

Discussion

Our analyses of sediments along a natural pCO_2 gradient (from median 419 to 1,611 μatm) revealed that the volcanic sand habitat of each of the three pH zones sampled was closely similar in composition with no differences in granulometry, carbonate, carbon, or nitrogen content. In addition, the overlying water column in each of the three zones had no differences in salinity,

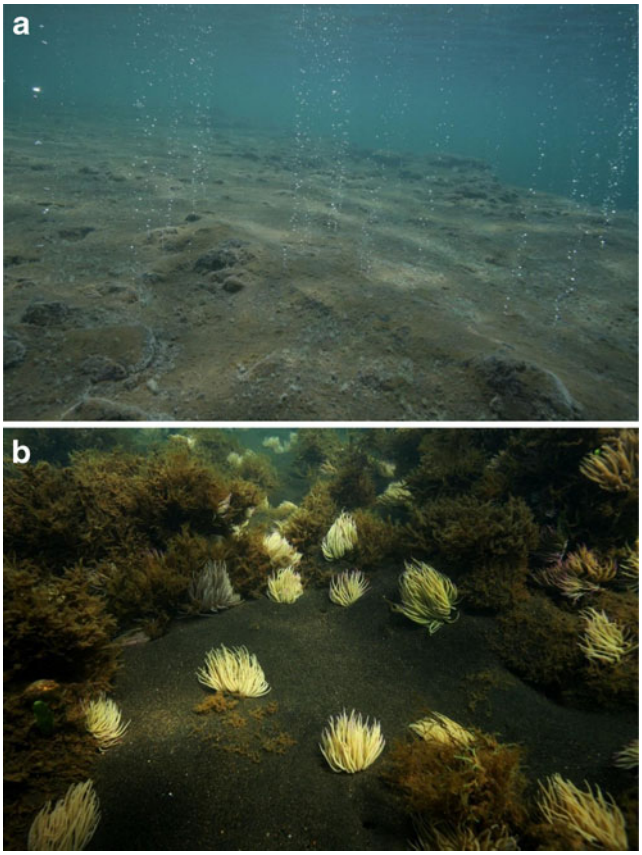

Fig. 2 Sediment habitat at 1-m depth in Baia di Levante, Vulcano, May 2011. **a** CO_2 venting area (pH 5.6) showing bacterial mats on the sediment surface. **b** Patch of black volcanic sand typical of sites 1–3, surrounded by rock outcrops, anemones, and macroalgae

temperature, or alkalinity, providing a consistent basis for comparison of the effects of seawater acidification.

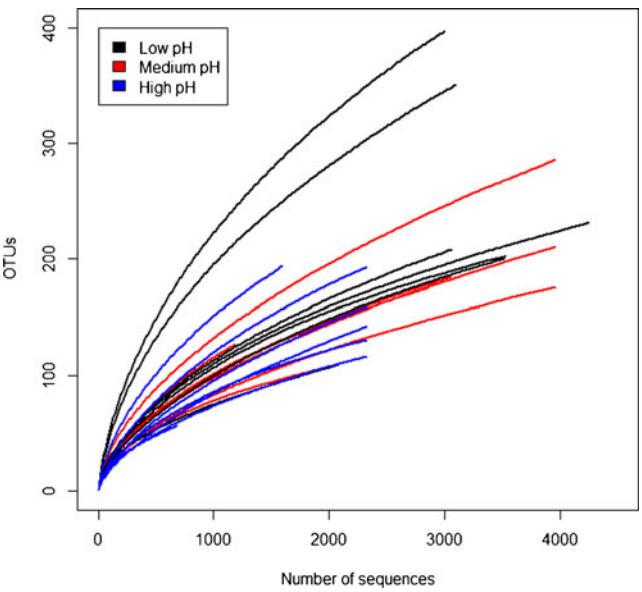

Fig. 3 Rarefaction curves comparing surface sediment bacterial communities at 1-m depth along a seawater pH gradient caused by volcanic CO_2 vents off Vulcano, May 2011

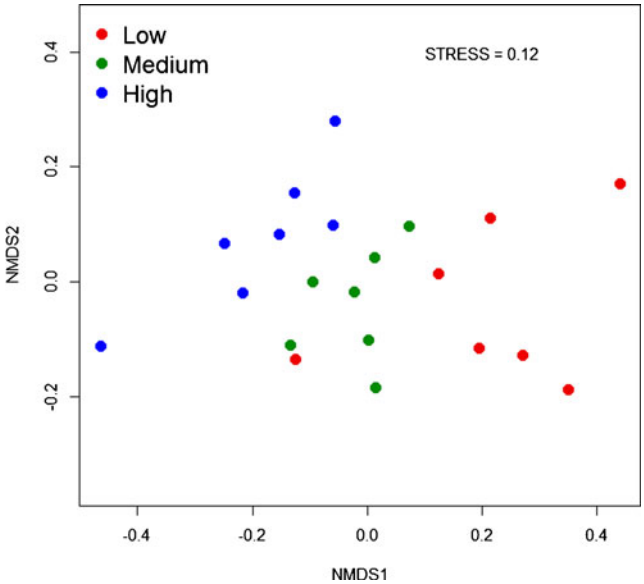

Fig. 4 NMDS ordination, the low-pH (high CO_2) marine sediment bacterial communities cluster separately from the communities sampled at medium and high (i.e., normal) pH

Although significant changes in sediment bacterial community were observed along the chosen pCO_2 gradient, as revealed by the NMDS plot and Shannon Diversity, the majority of major taxa of sediment-dwelling bacteria were not significantly affected by the steep gradients in seawater pH and carbonate saturation. Changes in sediment bacterial communities were obvious to the naked eye at the CO_2 vents themselves where *Beggiatoa*-like mats carpeted the sediment. In the present study, we chose a gradient of pCO_2 that was

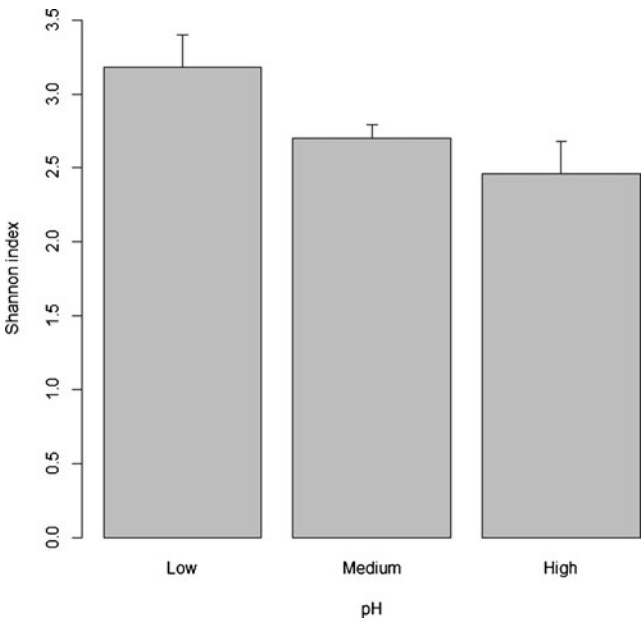

Fig. 5 Marine sediment microbial Shannon Diversity (mean±SE) at different seawater pH levels, with high pH being normal reference conditions (median pCO_2 419) and low pH corresponding to median pCO_2 1611 μatm

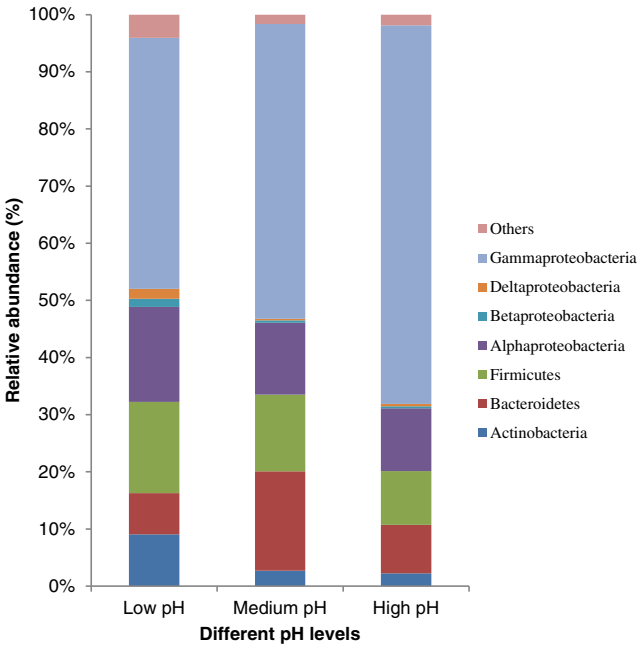

Fig. 6 Relative abundance of sediment bacterial classes along a pH gradient in the overlying water column, Vulcano, May 2011

representative of the changes that are predicted to occur due to ocean acidification and lacks confounding factors such as geothermal heating and the presence of H_2S that profoundly affect microbial communities [22, 30, 31]. Gammaproteobacteria were the dominant bacterial type at all the pCO_2 levels we studied and were also the most abundant bacterial group in a

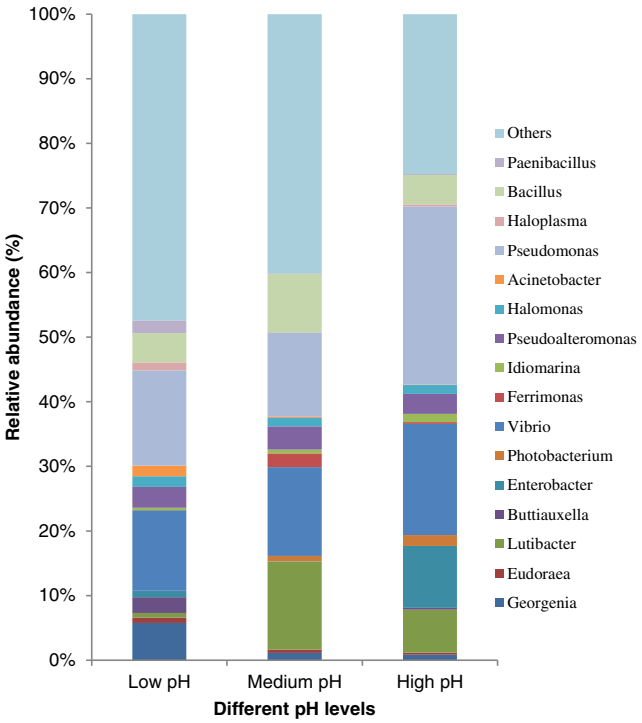

Fig. 7 Relative abundance of dominant marine sediment bacterial taxa at different pH levels

marine volcanic ash layer in the Sea of Okhotsk [32]. Gammaproteobacteria are usually the most abundant type of bacteria found in marine sediments, often comprising >50 % of the microbial community and the bulk of the marine sediment bacterial biomass [33, 34]. Of the Gammaproteobacteria at the study site, *Pseudomonas* was the most common and is generally abundant in marine sediments where they are able to use diverse organic compounds as a carbon source and electron donor [18]. The genera *Georgenia*, *Lutibacter*, *Photobacterium*, *Acinetobacter*, and *Paenibacillus* were less abundant at our study sites, and all of these genera exhibited significant shifts in their abundance along the pCO_2 gradient. *Georgenia* is a Gram-positive, nonsporulating and motile, or nonmotile aerobic bacterium, but the growth under anaerobic conditions can also occur. Temperature range for growth is 10–37 °C, with optimum growth at 28–30 °C. The pH range for growth is between 6.5 and 10, with optimum growth at pH 7[35, 36]. *Lutibacter* is a Gram-negative and rod-shaped bacteria. They can grow in heterotrophic and aerobic conditions. The growth occurs at 5–30 °C (optimum 25–30 °C) and at pH values of between 7 and 8 [37, 38]. *Photobacterium* is a Gram-negative bacteria. They are widely distributed in the marine environment and are facultative anaerobes. The pH range for the optimal growth is between 5 and 9 [39, 40]. *Acinetobacter* is a nonmotile, Gram-negative, and strictly aerobic bacteria. They are ubiquitous organisms [41]. *Paenibacillus* is a Gram-positive, facultative anaerobic bacterium. The optimum growth temperature is 28–30 °C and grows also at 20 °C [42].

Other laboratory and field-based studies have shown detectable effects of CO_2 gradients on sediment bacterial community composition or activity. Krause et al. [12] found, using seawater acidification studies on sediment in microcosms, that a reduction in pH caused major shifts in bacterial community composition that had direct effects on nutrient availability and use within the sediment although Kitidis et al. [16] found that natural gradients of CO_2 had no significant effect on sediment microbial ammonia oxidation activity. Kitidis et al. [16] suggested that this could be explained by adaptation of the microbial communities to high pCO_2 or due to interstitial pH buffering that may lessen the impact of ocean acidification on life within sediments [1].

The relatively subtle shifts we observed in bacterial sediment communities contrast with the major shifts seen in benthic foraminifera and biofilms at similar pCO_2 levels. Dias et al. [43] and Uthicke et al. [44] noted remarkable reductions in foraminifera diversity and abundance. A significant increase in foraminifera dissolution along gradients of decreasing seawater carbonate saturation state at CO_2 vents in Italy, Mexico, and Papua New Guinea was recorded by Dias et al. [43], Pettit et al. [45], and Uthicke et al. [44]. Also, Moy et al. [46] document reduced calcification in Southern Ocean planktonic foraminifera and a decline in their abundance that correlates with declining carbonate saturation states. Successional processes in bacteria and Eukarya communities forming biofilms were also strongly

Table 2 Classification of the five taxa affected by pH change

Taxon	Phylum	Class	Order	Family
Georgenia	Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae
Lutibacter	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobactereaceae
Photobacterium	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae
Acinetobacter	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae
Paenibacillus	Firmicutes	Bacilli	Bacillales	Peanibacillaceae

affected by seawater CO_2 concentration [21]. Johnson et al.[20] observed a change of both the grazing sea urchin and the calcifying macroalgae *Padina* along increasing CO_2 gradients in Vulcano, Italy. Moreover, a significant alteration of periphyton communities was detected as CO_2 concentrations increased; the periphyton community increased at the CO_2-enriched sites. This indicates an increase in primary productivity in the CO_2-enriched area. The reasons for the somewhat muted effects of pCO_2 gradients on sediment dwelling bacteria in our study, compared with the study of Krause et al. [12], may lie in the differences in the composition of sediments tested. Furthermore, they used microcosms, whereas we examined natural sediment affected by waves, currents, and macrobenthic organisms; bioturbation, for example, will hinder the formation of a surface biofilm. The system used in the present study was open offering ample opportunity for the colonization by bacterial strains that are tolerant of acidified conditions. In addition, it has been present for decades providing more time for bacteria to evolve than has been available in manipulative experiments. A study of the microbial communities associated with anemones and corals along a volcanic pCO_2 gradient did not pick up any significant changes, which may be due to acclimation to pH variations, although the host organisms will modify the seawater chemistry of their surfaces that might mask any effects of the overlying seawater pCO_2 gradient [47, 48].

Effects on Diversity

Although ordination analyses showed less profound effects of pCO_2 levels on sediment bacterial community composition than those revealed in microcosm work by Krause et al. [12], we found that enriched pCO_2 sites had a higher Shannon Index which takes into account both the "richness" and "evenness" aspects of diversity [49]. This is in fact the opposite of the findings of Krause et al. [12] and the predictions by Unno et al. [24] that ocean acidification might cause changes in bacterial community of the world's ocean sediments with a shift toward communities more heavily dominated by a few species. It is also similar to that observed by Roy et al. [11] on pelagic microbes, whereby most taxa were unaffected, and a few rare taxa increased in abundance with higher CO_2. Higher bacterial diversity at lower pH sites has also been reported for coastal microbial biofilms exposed to lower-than-ambient natural CO_2, although not under present ambient CO_2 conditions [21]. It is unclear why higher CO_2 might have increased diversity in our study but have the opposite effect in others. No detailed studies have yet been done on the changing ecology and physiology of bacteria in such systems. There are certainly some chemical processes in sediment that may be expected to change as the pH shifts. For example, accelerated bacterial degradation of polysaccharides by extracellular enzymes may occur in lower pH conditions, and the changes in resource availability may bring about community change [50]. Increased biofilm EPS (extracellular polymeric substances) production with increased polysaccharide degradation in reduced pH could also be a cause of the change of community structure and the adjustment of available niches [21]. How such changes should produce a shift in diversity, however, are presently unclear.

Studies on terrestrial soils generally show lower bacterial diversity as pH declines or increases away from neutral [51, 52]. The explanation favored in these studies [51, 52] is that the internal pH of bacterial cells is always near neutral, and at higher and lower ambient pH values, high physiological demands are placed on bacterial cells which are maintaining their intracellular pH. This may limit niche viability, or the possibilities of evolutionary origin of new lineages, into these more physiologically demanding environments. It is challenging to measure the pH of pore water within the upper layer of a marine sediment, as the process of measuring pH strongly perturbs the system. Hypothetically, some of these sediment systems could be near neutral pH—in which case as in soils, diversity could decline with pCO_2 increase. Other systems might be considerably above neutral pH, in which case

Table 3 Marine sediment properties: total organic carbon (TOC) and total nitrogen (TN) contents

Sample	TOC (%)	TN (%)
Low pH1	0.29	0.018
Low pH2	0.16	0.016
Low pH3	0.28	0.025
Medium pH1	0.21	0.018
Medium pH2	0.17	0.012
Medium pH3	0.25	0.021
High pH1	0.16	0.014
High pH2	0.25	0.019
High pH3	0.37	0.018

lowering pH though higher pCO_2 may bring the sediment pH closer to neutral, and thereby increase diversity.

The effects of ocean acidification on sediment microbes are likely to differ between different sedimentary environments: we studied sediment that was somewhat unusual in that it lacked carbonates and was surrounded by dense stands of macroalgae. The microbes of carbonate sediments may be affected differently as corrosive waters begin to dissolve the carbonate. Sediment carbonate may act as a buffer, but also its physical loss may profoundly change the microscopic pore structure of the sediment. Such buffering effects of sedimentary carbonates seem most likely to affect time transgressive responses to a recent increase in pCO_2, slowing down changes. However, all of these perspectives remain purely hypothetical at present: detailed experimental and observational evidence on sediment changes under increased pCO_2 will be necessary for an improved understanding of bacterial community changes.

It is unclear what functional implications shifts in sediment microbial diversity might have for the overall biogeochemical functioning of benthic communities. Some authors have claimed that increasing diversity makes ecosystems more resilient to changes [53], although beyond a minimal low level of diversity, the biogeochemical functions of communities may well be left unchanged by shifts in diversity, if multiple species can fulfill the same ecological role.

Conclusions

From a global change viewpoint, the response of benthic bacterial diversity to pCO_2 enrichment provides a warning that ocean acidification has the potential to significantly alter marine sediment bacterial communities worldwide. Bacterial community composition showed detectable shifts with increasing pCO_2, and bacterial diversity increased toward higher pCO_2. Given that these shifts occur, it is possible that the biogeochemical functions of marine sediments will also be significantly affected by ocean acidification.

Firmer conclusions will only be possible after the roles of pCO_2-sensitive marine sediment bacteria are known, following direct in situ analyses of sediment biogeochemical processes have been conducted, and a range of sediment types has been tested at a variety of locations.

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