2014-05

Shallow Water Marine Sediment Bacterial Community Shifts Along a Natural CO₂ Gradient in the Mediterranean Sea Off Vulcano, Italy

Kerfahi, D

<http://hdl.handle.net/10026.1/2886>

10.1007/s00248-014-0368-7

Microbial Ecology

Springer Science and Business Media LLC

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Shallow Water Marine Sediment Bacterial Community Shifts Along a Natural CO₂ Gradient in the Mediterranean Sea Off Vulcano, Italy

Dorsaf Kerfahi · Jason M. Hall-Spencer ·
Binu M. Tripathi · Marco Milazzo · Junghoon Lee ·
Jonathan M. Adams

Received: 12 September 2013 / Accepted: 10 January 2014
© Springer Science+Business Media New York 2014

Abstract The effects of increasing atmospheric CO₂ 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 CO₂ 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 $p\text{CO}_2$ 419 μatm , minimum Ω_{arag} 3.77), moderately CO₂-enriched (median $p\text{CO}_2$ 592 μatm , minimum Ω_{arag} 2.96), and highly CO₂-enriched (median $p\text{CO}_2$ 1611 μatm , minimum Ω_{arag} 0.35). We tested the hypothesis that increasing levels of seawater $p\text{CO}_2$ 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 $p\text{CO}_2$. The relative abundances of most of the dominant genera were unaffected by the $p\text{CO}_2$ 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 $p\text{CO}_2$ 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.

Electronic supplementary material The online version of this article (doi:10.1007/s00248-014-0368-7) contains supplementary material, which is available to authorized users.

D. Kerfahi · B. M. Tripathi · J. M. Adams (✉)
Department of Biological Sciences, Seoul National University,
Gwanak-Gu, Seoul 151-747, Republic of Korea
e-mail: foundinkualalumpur@yahoo.com

J. M. Hall-Spencer
Marine Biology and Ecology Research Centre, Plymouth University,
Plymouth PL4 8AA, UK

M. Milazzo
Dipartimento di Scienze della Terra e del Mare, University of
Palermo, via Archirafi 28, 90123 Palermo, Italy

D. Kerfahi
School of Chemical and Biological Engineering, Seoul National
University, Gwanak-Gu, Seoul 151-747, Republic of Korea

J. Lee (✉)
School of Mechanical and Aerospace Engineering, Seoul National
University, Gwanak-Gu, Seoul 151-747, Republic of Korea
e-mail: jleenano@snu.ac.kr

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 CO₂ emissions have lowered the pH of surface waters of the world by about 0.1 pH unit, 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

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-CO₂ 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 CO₂ 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 $p\text{CO}_2$ on sediment bacterial communities off Vulcano in the Mediterranean. Our hypothesis was that a $p\text{CO}_2$ 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 CO₂

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

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.

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 CO₂ gradient on Vulcano island [20]

Station		pH range (NBS scale)	pCO ₂ (μ atm)	TA (mmol kg ⁻¹)	DIC (mmol kg ⁻¹)	CO ₃ ²⁻ (mmol kg ⁻¹)	HCO ₃ ⁻ (mmol kg ⁻¹)	Ω_{calcite}	$\Omega_{\text{aragonite}}$
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 CO₂ SYS program (using the constants of Roy et al. 1993 and Dickson for KSO₄)

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- μ l 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. Kruskal-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. Kruskal-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 $p\text{CO}_2$ 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 $p\text{CO}_2$ 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- $F_{2,18}=1.9635$, $P(\text{perm})=0.0001$) with significance between the pair-wise comparisons of the three different pH levels considered (high, low: $t=1.6374$, $P(\text{perm})=0.001$; high, medium: $t=1.1605$, $P(\text{perm})=0.025$; low, medium: $t=1.3539$, $P(\text{perm})=0.0045$).

Effect on Bacterial Community Diversity

Permutational univariate analysis of variance showed that pH change affected neither the OTU (species) richness (Pseudo- $F_{2,18}=1.9109$, $P(\text{perm})=0.1672$) nor the phylogenetic diversity (Pseudo- $F_{2,18}=2.9526$, $P(\text{perm})=0.0684$). However, the Shannon Index (Pseudo- $F_{2,18}=3.9597$, $P(\text{perm})=0.031$) revealed that pH significantly affected bacterial diversity, with the lower level of bacterial diversity observed at high-pH (i.e., ambient $p\text{H}/p\text{CO}_2$) than the low-pH (high, low: $t=2.3479$, $P(\text{perm})=0.0433$) sampling site (Fig. 5).

Effect of $p\text{CO}_2$ 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 $p\text{CO}_2$ 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 $p\text{CO}_2$ gradient. However, five genera (Table 2) within the bacterial community were significantly affected by the $p\text{CO}_2$ gradient. These were *Georgenia* ($\chi^2(2)=9.63$, $P=0.008$), *Lutibacter* ($\chi^2(2)=7.23$, $P=0.02$), *Photobacterium* ($\chi^2(2)=14.26$, $P=0.0008$), *Acinetobacter* ($\chi^2(2)=6.47$, $P=0.03$), and *Paenibacillus* ($\chi^2(2)=6.81$, $P=0.03$). Together, these genera accounted for less than 5 % of the bacterial community sampled.

Relationship Between Seawater $p\text{CO}_2$ and Sediment Properties

There was no correlation between seawater $p\text{CO}_2$ 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 $p\text{CO}_2$ 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** CO₂ 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 CO₂ vents off Vulcano, May 2011

Fig. 4 NMDS ordination, the low-pH (high CO₂) 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 $p\text{CO}_2$ 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 CO₂ vents themselves where *Beggiatoa*-like mats carpeted the sediment. In the present study, we chose a gradient of $p\text{CO}_2$ that was

Fig. 5 Marine sediment microbial Shannon Diversity (mean $\pm$ SE) at different seawater pH levels, with high pH being normal reference conditions (median $p\text{CO}_2$ 419) and low pH corresponding to median $p\text{CO}_2$ 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 geo-thermal 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
<i>Georgenia</i>	Actinobacteria	Actinobacteria	Actinomycetales	Bogoriellaceae
<i>Lutibacter</i>	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae
<i>Photobacterium</i>	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae
<i>Acinetobacter</i>	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae
<i>Paenibacillus</i>	Firmicutes	Bacilli	Bacillales	Peenibacillaceae

affected by seawater CO₂ concentration [21]. Johnson et al. [20] observed a change of both the grazing sea urchin and the calcifying macroalgae *Padina* along increasing CO₂ gradients in Vulcano, Italy. Moreover, a significant alteration of periphyton communities was detected as CO₂ concentrations increased; the periphyton community increased at the CO₂-enriched sites. This indicates an increase in primary productivity in the CO₂-enriched area. The reasons for the somewhat muted effects of *p*CO₂ 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 *p*CO₂ 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 *p*CO₂ gradient [47, 48].

Effects on Diversity

Although ordination analyses showed less profound effects of *p*CO₂ levels on sediment bacterial community composition

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

than those revealed in microcosm work by Krause et al. [12], we found that enriched *p*CO₂ 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 CO₂. Higher bacterial diversity at lower pH sites has also been reported for coastal microbial biofilms exposed to lower-than-ambient natural CO₂, although not under present ambient CO₂ conditions [21]. It is unclear why higher CO₂ 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 *p*CO₂ increase. Other systems might be considerably above neutral pH, in which case

lowering pH though higher $p\text{CO}_2$ 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 $p\text{CO}_2$, slowing down changes. However, all of these perspectives remain purely hypothetical at present: detailed experimental and observational evidence on sediment changes under increased $p\text{CO}_2$ 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 $p\text{CO}_2$ 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 $p\text{CO}_2$, and bacterial diversity increased toward higher $p\text{CO}_2$. 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 $p\text{CO}_2$ -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.

Acknowledgments This work was partly supported by a grant from the National Research Foundation (NRF) grant funded by the Korean government, Ministry of Education, Science and Technology (MEST) (NRF-2013-031400). This work was also partly supported by the Global Frontier Project, Centre of Integrated Smart Sensors funded by Ministry of Education Science and Technology, Korea (2012M3A6A6054201). DK is supported by the Korean Government Scholarship Program, Ministry of Education, Science, and Technology, South Korea. This work contributes to the EU FP7 project “Mediterranean Sea Acidification under a changing climate” (grant agreement no. 265103), with additional funding from Save Our Seas Foundation.

References

1. Widdicombe S, Spicer JJ, Kitidis V (2011) Effects of ocean acidification on sediment fauna. In: Gattuso JP, Hansson L (eds) Ocean acidification. Oxford University Press, Oxford, pp 176–191
2. Munn CB (2011) Marine microbiology: ecology and applications, 2nd edn. Garland Science, New York
3. Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature* 425(6956):365–365
4. Olafsson J, Olafsdottir SR, Benoit-Cattin A, Danielsen M, Arnarson TS, Takahashi T (2009) Rate of Iceland Sea acidification from time series measurements. *Biogeosciences* 6(11):2661–2668
5. Liu JW, Weinbauer MG, Maier C, Dai MH, Gattuso JP (2010) Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. *Aquat Microb Ecol* 61(3):291–305. doi:10.3354/Ame01446
6. Allgaier M, Riebesell U, Vogt M, Thyrhaug R, Grossart HP (2008) Coupling of heterotrophic bacteria to phytoplankton bloom development at different $p\text{CO}_2$ levels: a mesocosm study. *Biogeosciences* 5(4):1007–1022. doi:10.5194/bg-5-1007-2008
7. Newbold LK, Oliver AE, Booth T, Tiwari B, DeSantis T, Maguire M, Andersen G, van der Gast CJ, Whiteley AS (2012) The response of marine picoplankton to ocean acidification. *Environ Microbiol* 14(9):2293–2307
8. Ray JL, Topper B, An S, Silyakova A, Spindelbock J, Thyrhaug R, DuBow MS, Thingstad TF, Sandaa RA (2012) Effect of increased $p\text{CO}_2$ on bacterial assemblage shifts in response to glucose addition in Fram Strait seawater mesocosms. *Fems Microbiol Ecol* 82(3):713–723. doi:10.1111/j.1574-6941.2012.01443.x
9. Lindh MV, Riemann L, Baltar F, Romero-Oliva C, Salomon PS, Graneli E, Pinhassi J (2013) Consequences of increased temperature and acidification on bacterioplankton community composition during a mesocosm spring bloom in the Baltic Sea. *Environ Microbiol Rep* 5(2):252–262. doi:10.1111/1758-2229.12009
10. Sperling M, Piontek J, Gerdtts G, Wichels A, Schunck H, Roy AS, La Roche J, Gilbert J, Nissimov JJ, Bittner L, Romac S, Riebesell U, Engel A (2013) Effect of elevated CO_2 on the dynamics of particle-attached and free-living bacterioplankton communities in an Arctic fjord. *Biogeosciences* 10(1):181–191. doi:10.5194/bg-10-181-2013
11. Roy AS, Gibbons SM, Schunck H, Owens S, Caporaso JG, Sperling M, Nissimov JJ, Romac S, Bittner L, Muhling M, Riebesell U, LaRoche J, Gilbert JA (2013) Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms. *Biogeosciences* 10(1):555–566. doi:10.5194/bg-10-555-2013
12. Krause E, Wichels A, Gimenez L, Lunau M, Schilhabel MB, Gerdtts G (2012) Small changes in pH have direct effects on marine bacterial community composition: a microcosm approach. *Plos One* 7 (10)
13. Kroeker KJ, Micheli F, Gambi MC (2013) Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nat Clim Change* 3(2):156–159. doi:10.1038/Nclimate1680
14. Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, Death G, Okazaki R, Muehllehner N, Glas MS, Lough JM (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nat Clim Change* 1(3):165–169. doi:10.1038/Nclimate1122
15. Rodolfo-Metalpa R, Houlbreque F, Tambutte E, Boisson F, Baggini C, Patti FP, Jeffree R, Fine M, Foggo A, Gattuso JP, Hall-Spencer JM (2011) Coral and mollusc resistance to ocean acidification adversely affected by warming. *Nat Clim Change* 1(6):308–312
16. Kitidis V, Laverock B, McNeill LC, Beesley A, Cummings D, Tait K, Osborn MA, Widdicombe S (2011) Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophys Res Lett* 38: Art. L21603. doi:10.1029/2011gl049095

17. Ghosh A, Dey N, Bera A, Tiwari A, Sathyaniranjan K, Chakrabarti K, Chattopadhyay D (2010) Culture independent molecular analysis of bacterial communities in the mangrove sediment of Sundarban, India. *Saline Syst* 6(1):1. doi:[10.1186/1746-1448-6-1](https://doi.org/10.1186/1746-1448-6-1)
18. Hongxiang X, Min W, Xiaogu W, Junyi Y, Chunsheng W (2008) Bacterial diversity in deep-sea sediment from northeastern Pacific Ocean. *Acta Ecol Sin* 28(2):479–485. doi:[10.1016/S1872-2032\(08\)60026-8](https://doi.org/10.1016/S1872-2032(08)60026-8)
19. Hendriks IE, Duarte CM, Alvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. *Estuar Coast Shelf Sci* 86(2):157–164
20. Johnson VR, Brownlee C, Rickaby REM, Graziano M, Milazzo M, Hall-Spencer JM (2011) Responses of marine benthic microalgae to elevated CO₂. *Mar Biol*:1–12. doi:[10.1007/s00227-011-1840-2](https://doi.org/10.1007/s00227-011-1840-2)
21. Lidbury I, Johnson V, Hall-Spencer JM, Munn CB, Cunliffe M (2012) Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. *Mar Pollut Bull* 64(5):1063–1066. doi:[10.1016/j.marpolbul.2012.02.011](https://doi.org/10.1016/j.marpolbul.2012.02.011)
22. Boatta F, D'Alessandro W, Gagliano AL, Liotta M, Milazzo M, Rodolfo-Metalpa R, Hall-Spencer JM, Parello F (2013) Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. *Mar Pollut Bull*: 485–494. doi:[10.1016/j.marpolbul.2013.01.029](https://doi.org/10.1016/j.marpolbul.2013.01.029)
23. Kerrison P, Hall-Spencer JM, Suggett DJ, Hepburn LJ, Steinke M (2011) Assessment of pH variability at a coastal CO₂ vent for ocean acidification studies. *Estuar Coast Shelf Sci* 94(2):129–137. doi:[10.1016/j.ecss.2011.05.025](https://doi.org/10.1016/j.ecss.2011.05.025)
24. Unno T, Jang J, Han D, Kim JH, Sadowsky MJ, Kim OS, Chun J, Hur HG (2010) Use of barcoded pyrosequencing and shared OTUs to determine sources of fecal bacteria in watersheds. *Environ Sci Technol* 44(20):7777–7782. doi:[10.1021/Es101500z](https://doi.org/10.1021/Es101500z)
25. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microb* 75(23):7537–7541. doi:[10.1128/Aem.01541-09](https://doi.org/10.1128/Aem.01541-09)
26. Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ Microbiol* 12(7):1889–1898. doi:[10.1111/j.1462-2920.2010.02193.x](https://doi.org/10.1111/j.1462-2920.2010.02193.x)
27. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27(16):2194–2200. doi:[10.1093/bioinformatics/btr381](https://doi.org/10.1093/bioinformatics/btr381)
28. Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. *Plos One* 5(3):Art E9490. doi:[10.1371/Journal.Pone.0009490](https://doi.org/10.1371/Journal.Pone.0009490)
29. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M (2007) The vegan package. Community ecology package Disponível em: <http://www.R-project.org> Acesso em 10 (01):2008
30. Simmons S, Norris PR (2002) Acidophiles of saline water at thermal vents of Vulcano, Italy. *Extremophiles* 6(3):201–207. doi:[10.1007/s007920100242](https://doi.org/10.1007/s007920100242)
31. Rusch A, Amend JP (2008) Functional characterization of the microbial community in geothermally heated marine sediments. *Microb Ecol* 55(4):723–736. doi:[10.1007/s00248-007-9315-1](https://doi.org/10.1007/s00248-007-9315-1)
32. Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl Environ Microbiol* 69(12):7224–7235
33. Bowman JP, McCuaig RD (2003) Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Appl Environ Microb* 69(5):2463–2483
34. Ravensschlag K, Sahn K, Amann R (2001) Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard). *Appl Environ Microbiol* 67(1):387–395. doi:[10.1128/AEM.67.1.387-395.2001](https://doi.org/10.1128/AEM.67.1.387-395.2001)
35. Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Micr* 57:1424–1428. doi:[10.1099/ij.s.0.64749-0](https://doi.org/10.1099/ij.s.0.64749-0)
36. Altenburger P, Kämpfer P, Schumann P, Vybiral D, Lubitz W, Busse HJ (2002) *Georgenia muralis* gen. nov., sp. nov., a novel actinobacterium isolated from a medieval wall painting. *Int J Syst Evol Micr* 52(Pt 3):875–881
37. Park S, Kang SJ, Oh TK, Yoon JH (2010) *Lutibacter maritimus* sp. nov., isolated from a tidal flat sediment. *Int J Syst Evol Micr* 60:610–614. doi:[10.1099/Ijs.0.012401-0](https://doi.org/10.1099/Ijs.0.012401-0)
38. Choi DH, Cho BC (2006) *Lutibacter litoralis* gen. nov., sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from tidal flat sediment. *Int J Syst Evol Micr* 56:771–776. doi:[10.1099/ij.s.0.64146-0](https://doi.org/10.1099/ij.s.0.64146-0)
39. Farmer JJ III, Hickman-Brenner FW (2006) The genera *vibrio* and *photobacterium*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) *The prokaryotes*. Springer, New York, pp 508–563
40. Nogi Y, Masui N, Kato C (1998) *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment. *Extremophiles* 2(1):1–7. doi:[10.1007/s007920050036](https://doi.org/10.1007/s007920050036)
41. Wagner M, Erhart R, Manz W, Amann R, Lemmer H, Wedi D, Schleifer KH (1994) Development of an rRNA-targeted oligonucleotide probe specific for the genus *Acinetobacter* and its application for in situ monitoring in activated sludge. *Appl Environ Microbiol* 60(3):792–800
42. Heyndrickx M, Vandemeulebroecke K, Hoste B, Janssen P, Kersters K, De Vos P, Logan NA, Ali N, Berkeley RC (1996) Reclassification of *Paenibacillus* (formerly *Bacillus*) *pulvificiens* (Nakamura 1984) Ash et al. 1994, a later subjective synonym of *Paenibacillus* (formerly *Bacillus*) *larvae* (White 1906) Ash et al. 1994, as a subspecies of *P. larvae*, with emended descriptions of *P. larvae* as *P. larvae* subsp. *larvae* and *P. larvae* subsp. *pulvificiens*. *Int J Sys Bacteriol* 46(1):270–279
43. Dias BB, Hart B, Smart CW, Hall-Spencer JM (2010) Modern seawater acidification: the response of foraminifera to high-CO₂ conditions in the Mediterranean Sea. *J Geol Soc London* 167(5):843–846. doi:[10.1144/0016-76492010-050](https://doi.org/10.1144/0016-76492010-050)
44. Uthicke S, Momigliano P, Fabricius KE (2013) High risk of extinction of benthic foraminifera in this century due to ocean acidification. *Sci Rep* 3:1769. doi:[10.1038/srep01769](https://doi.org/10.1038/srep01769)
45. Pettit LR, Hart MB, Medina-Sanchez AN, Smart CW, Rodolfo-Metalpa R, Hall-Spencer JM, Prol-Ledesma RM (2013) Benthic foraminifera show some resilience to ocean acidification in the northern Gulf of California, Mexico. *Mar Pollut Bull*: 452–462. doi:[10.1016/j.marpolbul.2013.02.011](https://doi.org/10.1016/j.marpolbul.2013.02.011)
46. Moy AD, Howard WR, Bray SG, Trull TW (2009) Reduced calcification in modern Southern Ocean planktonic foraminifera. *Nat Geosci* 2(4):276–280
47. Meron D, Buia MC, Fine M, Banin E (2013) Changes in microbial communities associated with the sea anemone *Anemonia viridis* in a natural pH gradient. *Microb Ecol* 65(2):269–276. doi:[10.1007/s00248-012-0127-6](https://doi.org/10.1007/s00248-012-0127-6)
48. Meron D, Rodolfo-Metalpa R, Cunnig R, Baker AC, Fine M, Banin E (2012) Changes in coral microbial communities in response to a natural pH gradient. *Isme J* 6(9):1775–1785
49. Shannon CE, Weaver W (1948) A mathematical theory of communication. vol 27. American Telephone and Telegraph Company

50. Piontek J, Lunau M, Handel N, Borchard C, Wurst M, Engel A (2010) Acidification increases microbial polysaccharide degradation in the ocean. *Biogeosciences* 7(5):1615–1624. doi:[10.5194/bg-7-1615-2010](https://doi.org/10.5194/bg-7-1615-2010)
51. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88(6):1354–1364
52. Tripathi BM, Kim M, Singh D, Lee-Cruz L, Lai-Hoe A, Ainuddin AN, Go R, Rahim RA, Husni MH, Chun J, Adams JM (2012) Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. *Microb Ecol* 64(2):474–484. doi:[10.1007/s00248-012-0028-8](https://doi.org/10.1007/s00248-012-0028-8)
53. Chapin FS, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D (1997) Biotic control over the functioning of ecosystems. *Science* 277(5325):500–504. doi:[10.1126/science.277.5325.500](https://doi.org/10.1126/science.277.5325.500)