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Modified niche optima and breadths explain the historical contingency of bacterial community responses to eutrophication in coastal sediments

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Abstract

Previous studies have shown that the response of bacterial communities to disturbances depends on their environmental history. Historically fluctuating habitats host communities that respond better to disturbance than communities of historically stable habitats. However, the exact ecological mechanism that drives this dependency remains unknown. Here, we experimentally demonstrate that modifications of niche optima and niche breadths of the community members are driving this dependency of bacterial responses to past environmental conditions. First, we develop a novel, simple method to calculate the niche optima and breadths of bacterial taxa regarding single environmental gradients. Then, we test this method on sediment bacterial communities of three habitats, one historically stable and less loaded and two historically more variable and more loaded habitats in terms of historical chlorophyll-α water concentration, that we subject to hypoxia via organic matter addition ex situ. We find that communities containing bacterial taxa differently adapted to hypoxia show different structural and functional responses, depending on the sediment’s environmental history. Specifically, in the historically less fluctuating and loaded sediments where we find more taxa poorly adapted to hypoxic conditions, communities change a lot over time and organic matter is not degraded efficiently. The opposite is true for the historically more fluctuating and loaded sediments where we find more taxa well adapted to hypoxia. Based on the community responses observed here, we also propose an alternative calculation of community resistance that takes into account how rapidly the communities respond to disturbances and not just the initial and final states of the community.

Keywords: adaptation, bacterial communities, community resistance, disturbance, environmental history

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Introduction

With a total biomass similar to that of all the plants on earth combined, bacteria play a central role in the cycling of carbon and nutrients in the biosphere (Whitman et al. 1998). Examining how bacterial communities respond to environmental stress is integral to determine their contributions to the different ecosystem services in the global scale (Peralta et al. 2014b). Therefore, there is high scientific value in understanding what drives the structural and functional responses of bacterial communities.

Currently, empirical evidence suggests that past environmental fluctuations can constrain the responses of bacterial communities to environmental disturbance in soils and sediments (Bouskill et al. 2013; Peralta et al.)
2013, 2014a), but the underlying ecological mechanism is still unknown. Environmental history could modify the abiotic parameters of a soil or sediment system such as, among others, the grain size, the water holding capacity or the porosity of a soil matrix, for example, by the deposition of organic matter or inorganic minerals through time (Barbier et al. 2010). Thus, community responses could be constrained just because of the differences in the environmental parameters among systems with different environmental histories. Alternatively, environmental history could alter the preferences (niche optima) and the degree of specialization (niche breadths) of the bacterial taxa within a community, and that could also constrain community responses because communities from historically stable environments would be ‘less trained’ to disturbances. Recent results from an in silico modelling study support the latter, showing that when communities that contain taxa with random niche parameters are exposed to varying levels of historical environmental heterogeneity, functional constraints manifest (Hawkes & Keitt 2015). This process would result in communities with distinct niche parameters, as per their environmental history. However, this has not been examined experimentally for real bacterial communities yet.

Such a task is challenging because it requires the determination of the niche parameters of each taxon within bacterial communities, which are usually very diverse (Sogin et al. 2006). This can be done by examining the response of community members along environmental gradients, but in field studies many environmental variables become spatially autocorrelated, and therefore, the effect of a single gradient can be hard to measure (Langenheder & Ragnarsson 2007; Langenheder et al. 2011; Caporaso et al. 2012; Lindström & Langenheder 2012). Therefore, experiments that involve the quantification of the effects of single environmental gradients on bacterial communities can benefit from the more controlled environment of laboratory experiments (Vellend 2010; Hanson et al. 2012; Lindström & Langenheder 2012; Nemergut et al. 2013; Srivastava & Kratina 2013).

Here, we designed and performed a laboratory experiment to empirically examine how past environmental conditions drive the bacterial community’s structural and functional response to disturbances. We focused on bacterial communities of coastal sediments that we exposed to hypoxia, which was mediated by the addition of organic matter. Coastal sediments are buffer zones where organic matter and nutrient flow is regulated between terrestrial and marine environments (Barbier et al. 2010). As a consequence of human-related activities, coastal ecosystems are frequently subjected to organic enrichment. This often leads to eutrophication that is characterized by the increased chlorophyll-α (chl-α) concentration and oxygen depletion in the water column and in the top sediment (millimetres to centimetres, depending on the habitat) that is, otherwise, oxic (Diaz & Rosenberg 2008). Thus, organic matter loading, eutrophication of the water column and hypoxia at the top sediment are interconnected at coastal habitats. We hypothesized that sediment communities from historically more fluctuating environments in terms of eutrophication (in this study: the estuary and marina habitats, Fig. 1A) would be structurally and functionally more resistant to hypoxia (Fig. 1B) compared with communities from historically less fluctuating habitats (in this study: the beach habitat, Fig. 1A). With that in mind, we also sought to revise the way that community resistance is calculated so that the rate of the response is taken into account, along with the initial and final community states. Moreover, we hypothesized that the historically more fluctuating habitats would possibly contain more hypoxia-acclimated (in the ecological sense) bacterial taxa (Fig. 1C), because the latter could have likely encountered hypoxic conditions more often. Environmental history could be especially important in controlling the responses of coastal sedimentary habitats, because sediment bacteria experience temporal variability in a more static manner compared with water column bacteria, the latter being subjected to much greater environmental variability due to the current transport than due to seasonality (Doblin & van Sebille 2016). Moreover, because sediments are considerably isolated spatially (Zinger et al. 2014), environmental rescue by arrival of new community members is less frequent and the role of local interactions in shaping the resident bacterial communities is strengthened (Büchi & Vuilleumier 2014).

We added organic matter ex situ (Table 1) to induce hypoxia in bacterial communities from three coastal habitats originating from the same parent material (shale; Dornsiepen & Manutsoglu 1994; Zachariasse et al. 2008) but experiencing different chl-α historical fluctuations in the water column (Fig. 1A) and having different sediment characteristics (Table 2) and distinct communities (see Results). The eastern Mediterranean Sea is an ultra-oligotrophic basin (Krom et al. 2005). Therefore, although none of the examined sites can be considered historically eutrophic in a global scale, the historical differences in chl-α levels among the examined sites represent extremities on a local scale. We developed a novel, simple computational method by which we calculated the niche optima and breadths of the bacterial taxa in each community concerning a single parameter, in our case the sediment redox potential (Eh). Then, we assessed the communities’ structural and functional responses to organic enrichment through time. We observed that not only community functional and structural responses were constrained by the
environmental history, but also that the latter led to communities with different niche optima and breadths.

**Materials and methods**

**Study sites and sampling strategy**

Coastal sediment was collected in May 2011 from three sites along the North coast of the Greek Island, Crete: a beach (35°24′9.58″N, 24°57′12.82″E), an estuary (35°20′21.59″N, 25°6′44.74″E) and a marina (35°21′12.35″N, 25°2′34.10″E; see Fig. S8, Supporting information for details). Replicate cores at each site were collected following a 3 m × 4 m systematic unaligned grid sampling (grid cell dimensions were 1 m × 1 m; Wollenhaupt & Wolkowski 1994). Historical water column chl-α levels were significantly different between the beach and the estuary and between the beach and the marina (Fig. 1A, Supporting information).

At a water depth of ~1 m, we sampled the upper 15 cm of the sediment with sterilized cylindrical PVC corers (9.4 cm internal diameter). Twelve core samples were collected from each site, sealed with rubber stoppers and transferred to the laboratory in a vertical position within 1 h after sampling. Sediment samples were also collected in situ (t_{106}) for DNA extraction, granulometry analysis and determination of organic matter content. Sediment temperature and Eh at the water–sediment interface were also measured in situ.

**Ex situ organic enrichment experimental set-up**

In the laboratory, each core was placed in one of 12 microcosms (50-L capacity) in a controlled temperature
chamber. Three replicate cores from the same sampling site were placed vertically in each microcosm, each of which was filled with 35 L of sterile saline water (35 g/L NaCl) before removing the rubber stoppers. The water level within each chamber was at least 10 cm above the rim of the cores at all times throughout the experiment. The chamber was illuminated with cold white light (6500 K) with a photoperiod of 12 h and temperature was fixed to incubate the cores at the in situ temperature on the day of sampling (19 °C).

Cores were left to settle for 24 h and then (t_0) incubated for 32 days under four different treatments: two control treatments and two treatments provided with supplementary organic matter to represent different eutrophication scenarios. Samples in treatment 1 (Tr1) received no organic enrichment or water column aeration; those in treatment 2 (Tr2) received no organic enrichment, but did receive water column aeration; those in treatment 3 (Tr3) received organic enrichment in the absence of water column aeration; and those in treatment 4 (Tr4) received both organic enrichment and water column aeration (Table 1).

Three microcosms (one per site), each containing three cores from the same sampling site, were incubated under the conditions of each treatment (Fig. S9, Supporting information). We acknowledge that this experimental manipulation resulted in pseudoreplication of each [site × treatment] combination because the different cores within the same mesocosm cannot be considered entirely independent; they are subject to the effects of the same mesocosm.

Organic enrichment was achieved by adding 0.31 g of sterilized fish food pellets once every 2 days (starting on t_0) at the top sediment as described elsewhere (Valdemarsen et al. 2009). Aeration of the water column was performed with air pumps at a high input rate (~10 L/min). Salinity was measured and fixed on a daily basis with the addition of sterile distilled water. For each core, changes in bacterial community structure (through 16S rRNA gene Illumina amplicon sequencing), hydrogen sulphide, organic matter content, Eh and dissolved oxygen at the water column were monitored on t_0 and every 4 days throughout the experiment (i.e. eight samplings within 32 days). Sampling was always performed before organic matter addition on experimental days 0, 4, 8, 12, 16, 20, 24, 28 and 32. Sampling was performed on the top half centimetre of the sediment from each core, with all possible effort to minimize the external contamination.

**Environmental parameter monitoring**

Historical water column chl-α concentration data from the three sites were obtained from the NASA AQUA-MODIS website (http://oceancolor.gsfc.nasa.gov/cgi/13) and chl-α values were calculated with the use of the OCI algorithm (Hu et al. 2012) in the System for AUTOMATED GEOSCIENTIFIC ANALYSES software version 2.2.

The data for all sites fit a normal distribution (Table S1 and Fig. S9, Supporting information), but did not have homogenous variances, and therefore, Mann–Whitney pairwise tests were used to identify the differences in the median values between sites. Methodological details are given in Supporting Information.

We measured Eh with an electrode standardized with Zobell’s solution (ZoBell 1946) and sulphide concentration at the water–sediment interface using an electrode (Orion Sure-Flow™ Combination Ag+/S²⁻ Electrode), as described elsewhere (Wildish et al. 1999). Labile organic matter (LOM) and refractory organic matter (ROM) contents of each sample and of the homogenized fish food were distinguished by the ‘loss on ignition’ method (Loh et al. 2008). Total organic matter (TOM – used for in situ samples) content was defined as TOM = LOM + ROM. Sediment granulometry analysis to determine the median grain size (MD) and the sand and silt/clay fractions was performed through dry sieving. Temperature was measured with a standard mercury thermometer.

**Bacterial community structure monitoring**

DNA was extracted from the sediment using a combination of mechanical lysis and chemical lysis followed by isolation and purification (Roose-Amsaleg et al. 2001). Polymerase chain reaction of the V4 hypervariable region of the 16S rRNA gene using primers 515f (5’-GTGCCAGCMGCCGCGGTAA-3’) and 806r (5’-GGACTACHVGGGTWTCTAAT-3’) was performed with air pumps at a high input rate (~10 L/min). Salinity was measured and fixed on a daily basis with the addition of sterile distilled water. For each core, changes in bacterial community structure (through 16S rRNA gene Illumina amplicon sequencing), hydrogen sulphide, organic matter content, Eh and dissolved oxygen at the water column were monitored on t_0 and every 4 days throughout the experiment (i.e. eight samplings within 32 days). Sampling was always performed before organic matter addition on experimental days 0, 4, 8, 12, 16, 20, 24, 28 and 32. Sampling was performed on the top half centimetre of the sediment from each core, with all possible effort to minimize the external contamination.

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Statistical analyses

To measure community coverage, we calculated a Good’s coverage index for each sample in QIIME (Caporaso et al. 2010) version 1.8; any sample with an index below 0.9 was removed because such values indicate an inadequate community coverage (Good 1953). The Bray–Curtis (BC) similarity was calculated using the OTU table obtained from QIIME and the vegdist function of the vegan package (Oksanen et al. 2013) in R (R Development Core Team, 2014). Only OTUs that were taxonomically assigned to Bacteria were used for the BC calculation because Archaea are considerably underrepresented using primers 515f–806r (Klindworth et al. 2013). Prior to calculating the BC index, read counts, which conventionally represent the abundance of each taxon within each sample, were log(x + 1)-transformed (where x is the read count). To compare the environmental parameters across treatments, sites and trough times, Spearman rank correlations, parametric and nonparametric t-tests were performed in the PAST 3.04 software version for Mac OS (Hammer et al. 2001), and P-values were Bonferroni-corrected when applicable. The analysis of similarity (ANOSIM) tests, which were used to test for significant grouping among in situ samples or among differently treated samples (i.e. enriched–nonenriched, aerated–nonaerated samples) during the experiment, were performed in PRIMER 6 (Clarke & Warwick 2001).

Community similarity decay. To assess the community similarity decay over time at each site, we calculated the BC similarity at each time point between each control organically enriched sample pair (i.e. all sample pairs between treatment 1 and treatment 3, treatment 1–treatment 4, treatment 2–treatment 3 and treatment 2–treatment 4). First, we used a generalized linear model to test whether the BC decay rate was different among the three sites (for details, please refer to Table S5, Supporting information). Then, we plotted the BC values against the corresponding experimental time points separately for each site to reveal whether the linear, quadratic, exponential or logistic model best fit the decay over time; the models were selected based on the Akaike information criterion (AIC) in R (Table S3, Supporting information). Graphical representation of the modelled BC decay and the 95% confidence intervals through time were performed in the PAST 3.04 software (Hammer et al. 2001). Community resistance (RS) for each site was also calculated as RS = 1 – 2(BC<sub>10</sub> – BC<sub>18</sub>)/(BC<sub>10</sub> + |BC<sub>10</sub> – BC<sub>18</sub>|) (eqn 1; Shade et al. 2012), where BC<sub>10</sub> and BC<sub>18</sub> are the average BC values between each control organically enriched sample pair at the beginning and at the end of the experiment, respectively (n = 20–144 depending on the site and experimental time).

Ecosystem functioning. We assessed the organic matter dynamics over time as a means of ecosystem functioning at each site. To do this, we examined the ROM/LOM ratio over time for cores that received organic matter (treatments 3 and 4). We calculated the initial ROM/LOM ratio at each site (R<sub>0</sub>) and the expected ROM/LOM ratio if no degradation of LOM occurred (R<sub>e</sub>) (Supporting Information). The latter calculation was based on the consistency in organic matter of the fish food pellets that we added. Then, we examined the ROM/LOM values that we observed over the course of the experiment for the cores that received organic matter. In a well-functioning sedimentary bacterial community, LOM is degraded more rapidly than ROM because it is more bioavailable. Thus, in a well-functioning community, the ROM/LOM ratio should be greater than R<sub>e</sub>. Alternatively, ROM/LOM ratio values close to R<sub>e</sub> indicate that the bacterial community cannot effectively catabolize the available organic matter. To compare the ROM/LOM values against R<sub>e</sub>, we used Wilcoxon tests, because the ROM/LOM values were not normally distributed. We compared the median of the ROM/LOM values at each site across all experimental days against the R<sub>e</sub>. To correct for sampling errors imposed by the nature of the experiment, we chose to use the ROM/LOM ratio rather than the LOM percentage alone. For example, bias in the total organic matter content of a sample could arise from accidental ‘scooping’ of remaining fish food aggregates during sampling, despite thoroughly homogenizing the fish food. Although this potential error could create LOM estimation biases, it would not create a bias in the ROM/LOM ratio. We also plotted the ROM/LOM ratio at the different incubation times for nonorganically enriched samples (samples from treatments 1 and 2) of each different site, as an additional control. We assumed that the degradation of organic matter was mostly performed by bacteria, because we observed a rapid switch to anaerobic conditions (Figs S1–S6, Supporting information) where the activity of eukaryotic organisms such as macrofauna is limited (Furukawa et al. 2004).

Community acclimation to hypoxia. To examine whether the bacterial communities at the different sites contained bacteria with different environmental preferences (with respect to hypoxia), we developed a simple model by which we calculated the niche optimum and breadth of each bacterial taxon. Although here we applied the model along a redox potential (Eh) gradient at the water-sediment interface, the method is not specific for Eh and thus it can be used to calculate the niche parameters of bacterial taxa along other environmental gradients. The bacterial taxa correspond to operational
taxonomic units (OTUs), which are defined as groups of sequences with 97% similarity, or more, using 16S rRNA gene amplicon sequencing. OTUs with 97% sequence similarity are commonly used as an equivalent for bacterial species (Mariadassou et al. 2015). For each OTU, the model uses the OTU’s relative abundance profile along the environmental gradient (in our case Eh, Fig. 2) to calculate the following three parameters.

\[ \text{xmax} \]  

The value of the environmental gradient at the sample where the examined OTU’s relative abundance is the highest among all samples. This value is used to indicate the preferred environment, that is the niche optimum, of each OTU. Based on this value, OTUs can be categorized into ecologically meaningful fractions. For example, here it was used to determine whether an OTU is aerophilic or anaerophilic: if xmax > 0, the OTU was categorized as aerophilic (e.g. Fig. 2 – OTU 1), and if xmax ≤ 0, the OTU was categorized as anaerophilic (e.g. Fig. 2 – OTU 0). It has to be noted that this approach does not exclude the contribution of other correlated abiotic or biotic factors (e.g. grazing or interactions among bacterial taxa) to shaping niche optima.

\[ \text{ymax} \]  

The maximum relative abundance (% of total) of an OTU among all samples. High ymax values combined with small dσ values are indicative of specialist taxa, that is taxa that thrive in a narrow range of environmental conditions (Hawkes & Keitt 2015; e.g. OTU 0 in Fig. 2).

\[ \text{dσ} \]  

This parameter represents how rapidly the relative abundance of an OTU changes with respect to the environmental gradient. In other words, it describes how tolerant an OTU is to changes in the examined environmental parameter, that is, its niche breadth. If the distributions of the OTUs along the Eh gradient were normal, standard deviation would be an ideal indicator for this purpose; however, because the distributions of OTUs are largely heterogeneous, we needed a broader estimate of niche breadth. Therefore, we defined dσ as a broad analogue of the standard deviation (although qualitatively similar results were obtained independently of the choice of scaling factor): 34% of the maximum absolute difference between xmax – x1 and xmax – x2, where x1 and x2 are two environmental values of two samples, with x1 ≤ xmax ≤ x2 and with the relative abundance of an OTU at x1 and x2, y1 and y2, respectively, being equal to or greater than 5% of the ymax. We excluded any observation with relative abundance <5% of the ymax as it could artificially enlarge dσ. Mathematically expressed, \[ \text{dσ} = 0.68 \times \text{max}|x1 – x2|/2, \]  

for those xs where yi ≥ ymax/20 and x1 ≤ xmax ≤ x2. The more generalist an OTU is concerning a single environmental gradient, the higher the dσ.

We determined whether the niche parameters for Eh, namely xmax, ymax and dσ, were phylogenetically conserved among OTUs to ensure that they are ecologically important (see the Supporting Information) by means of the K-statistic (Blomberg et al. 2003) implemented in the picante package (Kembel et al. 2010) in R. We plotted the xmax, ymax and dσ for the aerophilic and anaerophilic community fractions at each site as normal distribution plots with mean = xmax, sd = dσ and f(xmax) = ymax to improve visualization and to facilitate the comparison among sites. And finally, we plotted the relative abundance of three example OTUs along the Eh gradient (Fig. 2).

**Results**

*The hypoxic gradient during the experiment was a major driver of bacterial dynamics*

Results from *in situ* sampling showed different sediment characteristics and distinct bacterial communities at each site. The sediment at the estuary contained the highest percentage of silt and clay, the smallest median...
grain size, the highest total organic matter content and the lowest redox potential (Table 2). The sediment at the beach was entirely coarse sand, which contained the lowest organic matter content and the highest redox potential, while the sediment at the marina showed intermediate values for these parameters (Table 2). The initial average Bray–Curtis similarity indices between sites were 25.08% (marina–estuary), 15.2% (marina–beach) and 10.9% (beach–estuary), with significant differences between communities at each site (ANOSIM test, n = 8, global R = 0.612, P = 0.004). The initial average within-site BC similarity indices were 54.43%, 46.12% and 44.29% for beach, marina and estuary samples, respectively.

The experimental manipulation resulted in a decrease in Eh during incubation followed by an increase in hydrogen sulphide emissions for all treatments and sediments (Figs S1–S6, Supporting information). The Eh at the water–sediment interface ranged between −235 and 375 mV during the experiment. Initially, Eh values were positive in all microcosms (337–375, 45–70 and 39–52 mV for beach, estuary and marina samples, respectively), but by the end of the experiment, Eh values were negative in all microcosms (−221 to −223, −220 to −235 and −224 to −235 mV for beach, estuary and marina samples, respectively). Organic matter addition had a strong and significant effect on community similarity (Bray–Curtis (BC) similarity) across all sites and experimental times (ANOSIM, global R = 0.224, P < 0.001). On the opposite, aeration had no significant effect on community similarity for any site or at any time (ANOSIM, global R = 0.001, P = 0.391). Collectively, these results suggest that oxygen availability in the sediments was limited by diffusion as reported elsewhere (Valdemarsen et al. 2009), irrespective of the treatment. However, the dissolved oxygen in the water column was lower in the microcosms that received organic matter compared with those that did not (Fig. S7, Supporting information), as typically occurs in the cases of eutrophication in nature (Diaz & Rosenberg 2008).

We used Eh as a descriptor of the eutrophication gradient because among all measured environmental variables (Eh, hydrogen sulphide emission; LOM, labile organic matter; and ROM, refractory organic matter), it explains most of the variance in bacterial communities at each site (12.8%–23%, P < 0.01, Table S6, Supporting information). Moreover, the niche optima (xmax) and breadths (δr) of the OTUs along the Eh gradient exerted a strong phylogenetic signal (K = 0.126, P = 0.001; and K = 0.142, P = 0.001; for xmax and δr, respectively). This indicates that niche parameters are phylogenetically conserved at the OTU level, meaning that phylogenetically more related OTUs have more similar niche parameters. However, the maximum relative abundance of an OTU (ymax) was not phylogenetically conserved (K = 0.470, P = 1).

The structural and functional community responses to eutrophication are linked to differently acclimated communities

The environmental preferences of OTUs regarding Eh were site dependent. Samples from the beach contained a total of 118 458 bacterial OTUs: 74 533 were anaerophilic; that is, their niche optimum was at hypoxic (Eh < 0) sediments, and 43 925 were aerophilic; that is, their niche optimum was at oxic (Eh > 0) sediments (−1.70 ratio). Samples from the other two sites contained a larger proportion of anaerophilic OTUs. Samples from the estuary contained a total of 110 572 bacterial OTUs: 92 893 were anaerophilic and 17 679 were aerophilic (−5.25 ratio). Samples from the marina contained a total of 100 275 bacterial OTUs: 75 001 were anaerophilic and 25 274 were aerophilic (−2.97 ratio). The niche parameters of the aerophilic and anaerophilic OTUs were also different among the different sites (Welch F tests, P < 0.0001 for all comparisons, Table S4, Supporting information), with the most profound differences being found among the aerophilic taxa of the different habitats (Fig. 3, dashed lines; Table 3). For example, communities from the beach contained aerophilic bacteria with little tolerance for decreased Eh and with a strong preference for oxic environments (Fig. 3, dashed blue line). On the contrary, the estuary and marina communities contained aerophilic bacteria with higher tolerance for decreased Eh and with less strong preference for oxic environments (Fig. 3, dashed green and orange lines).

The differences in the niche parameters of the bacterial OTUs among the sites were also concomitant with different structural and functional community responses depending on the sediment site of origin. The community similarity decreased exponentially among enriched/control samples at the beach (exponential decrease, a = 26.94, b = −0.372, c = 23.15, adjusted R² = 0.573, n = 327, P = 2.1E−16; Fig. 4A, left side), the site that historically had the lowest chl-a levels and variability in the water column among the three sites. The community resistance for the beach samples was measured at 0.233. In addition, the ROM/LOM ratio in the organically enriched samples from the beach did not differ significantly from the expected ratio if no LOM degradation occurred (Wilcoxon test, normal appr. z = 0.681, P = 0.495; Fig. 4B, left side). This indicates that the bacterial community could not effectively catabolize the added organic matter. The opposite was observed for the other two, historically more loaded and fluctuating, habitats. These communities changed
Fig. 3 The acclimation of the communities along the redox potential gradient at the water–sediment interface. Normal distribution plots of the niche parameters, xmax, ymax and dr for aerophilic (dashed lines, n = 74 533, 17 679 and 25 274 for beach, estuary and marina samples, respectively) and anaerophilic (solid lines, n = 43 925, 92 893 and 75 001 for beach, estuary and marina samples, respectively) bacterial operational taxonomic units (OTUs) from beach (blue), estuary (green) and marina (orange) samples. The mean of each distribution was set to equal the average niche optimum, that is the average Eh of the sample where the maximum relative abundance of the OTUs composing the fraction was observed (xmax). The standard deviation was set to equal the average niche breadth defined in this study (dr) for each community fraction. The height (on the y-axis) of each distribution was set to equal the average maximum relative abundance of the OTUs composing the fraction (ymax).

Table 3 The ratio and the mean niche optima (xmax) and breadths (dr) for the aerophilic and the anaerophilic community fractions of each site

<table>
<thead>
<tr>
<th></th>
<th>Beach</th>
<th>Estuary</th>
<th>Marina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerophilic/Aerophilic ratio</td>
<td>1.70</td>
<td>5.25</td>
<td>2.97</td>
</tr>
<tr>
<td>Mean anaerophilic xmax (mV)</td>
<td>−132.5 ± 0.2</td>
<td>−158.2 ± 0.2</td>
<td>−154.6 ± 0.2</td>
</tr>
<tr>
<td>Mean anaerophilic dr (mV)</td>
<td>71.8 ± 0.22</td>
<td>62.28 ± 0.18</td>
<td>65.7 ± 0.21</td>
</tr>
<tr>
<td>Mean aerophilic xmax (mV)</td>
<td>220 ± 0.7</td>
<td>130.4 ± 0.9</td>
<td>122.3 ± 0.8</td>
</tr>
<tr>
<td>Mean aerophilic dr (mV)</td>
<td>108 ± 0.3</td>
<td>136.3 ± 0.4</td>
<td>125.5 ± 0.4</td>
</tr>
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</table>

Standard deviation is given after the ± symbol.

less over time (quadratic community similarity decay, a = 0.011, b = −0.825, c = 35.09, $R^2 = 0.227$, $n = 184$, $P = 4.85E−12$ for estuary and $a = 0.033$, $b = −1.711$, $c = 38.986$, adjusted $R^2 = 0.646$, $n = 175$, $P = 2.2E−16$ for marina samples, respectively; Fig. 4A, middle and right for estuary and marina samples, respectively), had higher community resistance scores than the beach (0.586 and 0.388 respectively), and the ROM/LOM ratio in the organically enriched samples differed significantly from the expected ratio if no LOM degradation occurred (Wilcoxon tests, normal appr. $z = 4.32$ and 4.27, $P < 0.001$ for estuary and marina, respectively; Fig. 4B, middle and right for estuary and marina samples, respectively), suggesting an effective calabolism of the added organic matter.

An alternative way to measure community resistance

We initially measured community resistance using the equation presented by Shade et al. (2012):

$$RS = 1 - 2 | y_0 - y_L | / (y_0 + | y_0 - y_L |).$$

(1)

where $y_0$ is a relevant community parameter measured at a time $t_0$ (Shade et al. 2012). In our case, the measured parameter is the Bray–Curtis similarity, measured at the beginning and at the end of the incubation. Notably, eqn 1 only takes into account the initial and final averages of community similarity. Meanwhile our results suggest that community similarity may not always decrease at the same rate among different communities (generalized linear model, Supporting Information and Fig. 4A). That has implications regarding the sensitivity of a community to environmental selection; for example, even if the decrease in similarity during a given time period after a disturbance is the same for communities following either exponential or quadratic decay trends, the former trend is indicative of a community that changed faster. Therefore, we propose that the rate of the decay should be included in calculating community resistance: calculate the integral of the equation that best fits the similarity decay and divide it by the product of the initial fitted community similarity and time

$$R = \frac{\int_0^t f(t)dt}{f(0) \times t_f}$$

(2)

where $R$ is the community resistance, $t$ is the time, $t_f$ is the duration of the experiment and $f(t)$ is a function explaining a significant part of the variation of community similarity, or any other community metric, over time. If we apply our data to eqn 2, we calculate the community resistance of beach, marina and estuary samples as 0.507, 0.587 and 0.735, respectively. Overall, these results are qualitatively similar to those obtained using eqn 1; however, the community resistance value for marina samples is closer to that of the beach than of
the estuary, due to the rapid similarity decay for the marina during the first 20 experimental days (Fig. 4A, right side).

**Discussion**

*Modified niche optima and breadths can explain the historical contingency of bacterial structural and functional community responses*

Our results indicate that the communities at the estuary and marina are expected to change less and catabolize organic matter more efficiently, compared with the communities at the beach, in the case of future fluctuations in organic matter and/or oxygen availability within the sediment. This confirms our first hypothesis that environmental history can constrain the structural and functional responses to eutrophication in coastal sediments, because the estuary and the marina are historically more fluctuating and loaded than the beach in terms of chl-α in the water column. Our results widen the existing knowledge of historical environmental constraints on bacterial community responses in other habitats and disturbances (Bouskill et al. 2013; Peralta et al. 2013, 2014a), for coastal sediments and eutrophication. Nevertheless, future studies that include more sites could further verify our results, as our study included two high-disturbance sites (the estuary and the marina) but only one low-disturbance site (the beach).

Except from historical environmental constraints on community responses, we also found that communities from the beach contained aerophilic bacteria with little tolerance for decreased Eh and with a strong preference for oxic environments, while that was not the case for the marina and estuary communities. This indicates that the aerophilic bacteria in the estuary and the marina communities can better cope with the decreasing Eh in the sediment during a future eutrophication event,
compared with the respective bacteria in the beach community. Interestingly, the magnitude of organic matter addition in our experiment resembles sedimentation rates beneath fish farms or mussel rafts (Valdemarsen et al. 2010), which are usually very eutrophic throughout the year, representing a scenario of intense future anthropogenic disturbance. Thus, the estuary and marina communities are expected to be more functional and more stable than the beach community during future eutrophication events, until the sediment becomes highly hypoxic and the anaerophilic bacteria dominate the communities. This confirms our second hypothesis and implies the modification of the niche optima and breadths of the bacterial taxa as a possible mechanism behind the historical environmental contingency of the community responses to eutrophication.

Such mechanistic evidence that links the variability in community responses to environmental preferences of the community members is limited in the literature. Lennon et al. have mapped the environmental preferences of 23 bacterial and 22 fungal strains by cultivating them as pure cultures along a moisture gradient in artificial soil (Lennon et al. 2012). This method cannot be applied in complex communities de facto because of the enormous taxonomic diversity of bacteria within most environmental samples and the lack of cultivability of most community members. More recently, Hawkes and Keitt showed in silico that communities forming through arrival of taxa with random niche parameters and different environmental fluctuations had historically constrained functionalities (i.e. substrate utilization; Hawkes & Keitt 2015). Our results hint to the same conclusion for real communities, showing that historically constrained communities contain taxa with modified niche breadths and optima as per their environmental history.

Modelling the niche optima and breadths of bacterial taxa along environmental gradients

Using the parameter xmax of our model, the taxa within a community can be categorized into ecologically meaningful fractions, for example alkalinophilic, neutrophilic and acidophilic (Gubry-Rangin et al. 2011), if the distribution along a pH gradient is being examined. Then, the community-wise or fraction-wise niche parameters can be calculated and compared among the different sample categories or fractions.

Our model assumes that there is a meaningful link between the environmental gradient and the community. Therefore, additional tests should be applied to ensure the ecological importance of the environmental parameters on which the gradient is based on and of the calculated niche parameters. The importance of the environmental gradient could be examined by testing how much of the community variation is explained by the gradient, using, for example, distance-based redundancy or canonical correspondence analysis (Borcard et al. 2011). For the evaluation of the model results, a significant result in a phylogenetic signal test would indicate that the niche parameters are ecologically important because they are phylogenetically conserved; that is, phylogenetically close taxa have more similar niche parameters than expected by chance (Blomberg et al. 2003). In our case, the phylogenetic conservatism of the niche optima and breadths regarding Eh is not surprising; redox potential preferences are deeply conserved phylogenetically in microorganisms (Martiny et al. 2015). On the other hand, the nonconserved ymax suggests that bacterial niche specialization for oxygen availability does not necessarily correlate with high abundance at the environmental optimum, a fact opposite to the assumption made by many ecological models for resource utilization (Hawkes & Keitt 2015). However, this should be expected in our case, because there is no direct link between oxygen availability and abundance.

Like all the microbial community analyses that are based on 16S rRNA gene amplicon sequencing, the model uses the read counts of each OTU divided by the total sample reads as a proxy for each OTU’s relative abundance, and that number can be potentially biased (Schirmer et al. 2015). However, the model examines the change in each OTU’s relative abundance along the environmental gradient rather than the relative abundance per se. This is considered as a measure of beta diversity, and beta diversity measurements are known to be largely unaffected by biases related to 16S rRNA amplicon sequencing (Mariadassou et al. 2015). Nevertheless, the relative abundance can only indicate whether a taxon is dominant within a certain community, giving limited information about the ability of a taxon to proliferate in a specific environment. We cannot exclude the possibility that taxa with broad niches dominate a community, but perform poorly in the environment, for example having high relative abundance within a low bacterial density sample. Thus, our modelling approach would benefit from the incorporation of total bacterial abundance so that the niche parameters are based on the absolute, rather than the relative, abundance. A promising emerging technique for high-throughput cell enumeration in soils and sediments is counting by flow cytometry after detachment from the soil/sediment matrix and staining (Frossard et al. 2016). The relative abundance profiles obtained from high-throughput 16S amplicon sequencing can be transformed into the absolute abundance first by correcting with the (predicted) number of 16S copies for each OTU [e.g. using the normalize_by_copy_number.py
Implications for future studies

If historical environmental contingencies in bacterial communities are driven by the modification of niche optima and breadths of the bacterial taxa as our results suggest, microbiome engineering (Mueller & Sachs 2015) and artificial selection (Blouin et al. 2015) approaches could be used to modify the community-wise bacterial responses. For example, the introduction of pure cultures or mixed consortia of acclimated bacteria from historically fluctuating habitats could improve the community-wise responses of historically stable habitats, provided that the added microorganisms can proliferate in the new habitat. Moreover, experiments similar to ours could identify the responses of bacterial communities to other types of environmental disturbances such as temperature increases, salinity pulses or exposure to contaminants. That could be valuable considering, for example, the global warming effects on cold ecosystems that were not previously exposed to temperature increase (O’Gorman et al. 2014), their bacterial communities possibly being nonacclimated. The niche parameters of the bacterial taxa could be modelled in a similar manner and community resistance could be calculated by taking into account the rates of the structural and functional changes and not just the initial and final states of the community.

Finally, predicting future structural and functional community responses based on the environmental history and on the niche parameters of the individual taxa may be more complicated in the cases of severe environmental disturbances. These types of disturbances often cause regime shifts and lead to alternative stable states (Scheffer et al. 2001), such as at the increasingly frequent eutrophication and hypoxia events at the Chesapeake Bay (Kemp et al. 2005) and Kattegat (Karlson et al. 2002). In these cases where the community is dominated by anaerophiles that push the system further towards hypoxia/anoxia with various feedback mechanisms (Diaz & Rosenberg 2008), disproportionally large environmental changes may be required for the community to return back to its predisturbance state.

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Conflict of interest

The authors declare no conflict of interest.

References


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E.D.L., I.K. and S.F. designed the experiment; S.F., N.P., O.M. and I.T. performed the experiment and collected the raw data; S.F., A.M. and G.M. performed the analyses; A.M., G.M. and D.D. provided new methods and materials; S.F. wrote the first version of the manuscript; and all authors contributed to revisions.

**Data accessibility**

Raw sequence data were submitted to the Short Read Archive (SRA) section of the NCBI database under the alias SRA16686.

**Supporting information**

Additional supporting information may be found in the online version of this article.

**Fig. S1** Eh values (Y axis) over time (t1-t8, X axis) for samples originating from the beach.

**Fig. S2** Eh values (Y axis) over time (t1-t8, X axis) for samples originating from the marina.

**Fig. S3** Eh values (Y axis) over time (t1-t8, X axis) for samples originating from the estuary.

**Fig. S4** Sulphide emission (Y axis) over time (t1-t8, X axis) for samples originating from the beach.

**Fig. S5** Sulphide emission (Y axis) over time (t1-t8, X axis) for samples originating from the marina.

**Fig. S6** Sulphide emission (Y axis) over time (t1-t8, X axis) for samples originating from the estuary.

**Fig. S7** 25-75% percentile comparison boxplots of dissolved oxygen concentration, in microcosms with (Treatments 1/2 – grey boxes) and without (Treatments 3/4 – white boxes) organic enrichment.

**Fig. S8** The sampling map of the sites where sediment was originally collected from, with the island of Crete (bottom) and a focus to the specific area where the three different sites are located (above).

**Fig. S9** Schematic representation of the microcosm experimental design.

**Table S1** Normality tests for the satellite data values of chlorophyll-a.

**Table S2** The distribution of the samples that were included in the analysis based on the site of origin and incubation conditions.

**Table S3** Model fit (AIC) regarding the BC community similarity decay through time for the samples from each site.

**Table S4** Welch test comparisons of the niche parameters among the different community fractions (aerophilic and anaerophilic OTUs) from the different sites.

**Table S5** The results of the general additive model regarding the community similarity decay over time for the communities from each site.

**Table S6** Distance-based canonical correspondence analysis (db-cca) results.