Interplay between eutrophication and climate warming on bacterial communities in coastal sediments differs depending on water depth and oxygen history

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Coastal aquatic systems suffer from nutrient enrichment, which results in accelerated eutrophication effects due to increased microbial metabolic rates. Climate change related prolonged warming will likely accelerate existing eutrophication effects, including low oxygen concentrations. However, how the interplay between these environmental changes will alter coastal ecosystems is poorly understood. In this study, we compared 16S rRNA gene amplicon based bacterial communities in coastal sediments of a Baltic Sea basin in November 2013 and 2017 at three sites along a water depth gradient with varying bottom water oxygen histories. The shallow site showed changes of only 1.1% in relative abundance of bacterial populations in 2017 compared to 2013, while the deep oxygen-deficient site showed up to 11% changes in relative abundance including an increase of sulfate-reducing bacteria along with a 36% increase in organic matter content. The data suggested that bacterial communities in shallow sediments were more resilient to seasonal oxygen decline, while bacterial communities in sediments subjected to long-term hypoxia seemed to be sensitive to oxygen changes and were likely to be under hypoxic/anoxic conditions in the future. Our data demonstrate that future climate changes will likely fuel eutrophication related spread of low oxygen zones.

The world's coastal oceans are important ecosystems that are increasingly degraded through human influence1. Two of the greatest threats to worldwide coastal marine health are accelerated eutrophication caused by anthropogenic nutrient inputs2 and anthropogenic induced climate change, including for example increased temperature of the water column, stratification, oxygen decline, and acidification3–5. However, to what extent many years of eutrophication and ongoing climate change will alter coastal oceans globally is poorly understood.

The Baltic Sea in northern Europe is heavily stressed by human activities including increased CO2 levels, elevated temperature, nutrient input, change in the distribution and abundance of marine species, as well as deoxygenation4,6. For example, warming of the Baltic Sea has resulted in shorter sea-ice seasons and reduced ice-thickness within the last century7. Modelling of Baltic Sea surface temperature predicts a likely rise of 2–4 °C by the end of this century8, resulting in an approximate reduction of sea-ice by 50–80%7. Warming of northern hemisphere water bodies, like the Baltic Sea, has accelerated two to four times greater than the global mean rate9 and how the seas respond is strongly influenced by interactions with other human-driven changes on the marine system, including overfishing and industrial plus household discharges10,11. These effects might result in a more fragile marine ecosystem, although to what extent this will happen remains uncertain.

Anthropogenic influenced increased nutrient concentrations have led to accelerated eutrophication in the Baltic Sea12 while climate warming within the last two decades has worsened the effects13,14. Prolonged higher temperatures will likely further accelerate these eutrophication effects such as hypoxic (O2 < 2 mg/L) or anoxic (O2 = 0 mg/L) conditions in coastal bottom waters, which are derived from organic matter decomposition1,15. These so called 'dead zones' have increased ten-fold compared to 100 years ago in the Baltic Sea13. In addition, hypoxia/anoxia at the water–sediment interface leads to release of hydrogen sulfide from the sediment that is toxic for benthic life15. The Helsinki Commission (HELCOM) proposed routines to reduce nutrient intake that has led to a

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The shallow site bottom water oxygen concentration in November in 2013 was 11.3 mg/L and was lower in November 2017 (pore water, 2013, 2.47 ± 2.18 µM). Pore water sulfate concentrations were lower in 2017 with 4.33 ± 0.16 mM compared to 2013, 4.72 ± 0.11 mM while organic matter sediment content was significantly higher (2013, n = 3, 33.73 ± 8.08 µM compared to 2017, n = 1 or 3 as indicated) ± standard deviation. *n = 1.

Table 1. Oxygen, temperature, and pH in 2013 and 2017. Overview of in situ oxygen and temperature in bottom waters in 2013 and 2017 at each sampling site as well as pH measured for bottom waters for both years and all sites in triplicates; mean (n = 1 or 3 as indicated) ± standard deviation. *n = 1.

| Sample  | Year | Oxygen (mg/L) n = 1 | Temperature (°C) n = 1 | pH n = 3 | References |
|---------|------|---------------------|------------------------|----------|------------|
| Shallow | 2013 | 11.3                | 6.5                    | 7.85     | Broman et al. 14 |
| Intermediate | 2013 | 0.8                 | 2.8                    | 7.95     | Broman et al. 14 |
| Deep    | 2013 | 0.85                | 2.6                    | 8.1 ± 0.19 | Broman et al. 14 |
| Shallow | 2017 | 5.88                | 8.5                    | 7.04 ± 0.03 | This study |
| Intermediate | 2017 | 2.55                | 2.5                    | 6.75 ± 0.04 | This study |
| Deep    | 2017 | 0.49                | 5.5                    | 7.29 ± 0.03 | This study |

Results

Sediment and bottom water chemistry. Within the last two decades data collected by HELCOM and ICES (International Council for the Exploration of the Sea)25 showed trends of increasing temperatures on coastal bottom waters (1–30 m below sea surface (mbs)) across the Baltic Sea (Western Gotland-, Eastern Gotland-, and Bornholm-Basins). The temperature increased by several degrees independent of the depth of the coastal bottom waters (Supplemental Fig. S1) along with a trend in oxygen decline (Supplemental Fig. S1). The data within our study showed similar trends (Table 1). The observation station closest to the investigated sampling sites showed a general temperature increase with increasing phosphate and decreasing nitrate concentrations between the years 2001 and 2012 (Supplemental information). Geochemical data from the three sites in 2013 and 2017 in this study are provided in Fig. 1 and Table 1 (statistical support in Supplemental Tables S1; S2) and support the HELCOM data temperature and oxygen concentration trends for the Baltic Sea as a whole.

The shallow site bottom water oxygen concentration in November in 2013 was 11.3 mg/L and was lower in 2017 by approximately 50% with 5.88 mg/L (Table 1). At the same time, the bottom water pH dropped from 7.85 to 7.04 (Table 1). The 6.5 m deep shallow site showed a strong geochemical response within the 0–1 cm sediment layer with significantly lower nitrate + nitrite (in combination) concentrations measured in November 2017 (pore water, 2013, n = 3, 33.73 ± 8.08 µM compared to 2017, n = 3, 19.19 ± 5.81 µM, ANOVA, pairwise comparison, p < 0.01) and high variation in total iron as measured in 2017 (pore water, 2013, 0.55 ± 0.21 µM and 2017, 2.47 ± 2.18 µM). Pore water sulfate concentrations were lower in 2017 with 4.33 ± 0.16 mM compared to 4.72 ± 0.11 mM in 2013 while organic matter sediment content was significantly higher (2013, n = 3, 11.7 ± 0.95% and 2017, n = 3, 13.83 ± 0.45%, p < 0.05). The low concentration of pore water phosphate stored in the shallow site had a small increase in 2017 (2013, 2.39 µM ± 0.8 and 2017, 7.86 µM ± 1.20).
The intermediate site showed the smallest changes over time. Contrary to the shallow and deep sites, the intermediate site showed a higher bottom water oxygen concentration in November 2017 with 2.25 mg/L (i.e. above the > 2 mg/L hypoxic cut-off) while being below the cut-off in November 2013 with 0.8 mg/L (Table 1). In addition, the intermediate site showed a lower bottom water pH in 2017 with 6.75 and 7.95 in 2013 (2017, 6.75 ± 0.04, 2013, 7.95; Table 1). Analysis of pore water within 0–1 cm showed nitrate + nitrite concentrations were slightly higher in 2017 (pore water, 2013, 3.78 ± 0.81 µM and 2017, 7.21 ± 3.14 µM) while the pore water total iron concentrations were lower in 2017 with 5.38 ± 4.66 µM compared to 6.65 ± 1.2 µM in 2013. Sulfate concentrations were lower in 2017 (pore water, 4.76 ± 0.15 mM and 4.67 ± 0.27 mM) while organic matter content was slightly increased from 18.54 ± 0.64% in 2013 to 19.00 ± 0.59% in 2017. The strongest response was observed in phosphate concentrations that were significantly lower in 2017 (pore water, 2013, n = 3, 65.13 ± 45.62 µM and 2017, n = 3, 5.74 ± 2.29 µM, p < 0.05).

In contrast to the intermediate site, the deep site sediments had strong responses. Despite that the site was partially oxic in 2017 (Supplemental Fig. S2), it has previously showed long-term hypoxic conditions. In November 2013 the bottom water oxygen concentration was hypoxic with 0.85 mg/L and decreased to 0.49 mg/L (Table 1) in November 2017. The bottom water pH was 8.1 in 2013 and was lower in 2017 with 7.27 (2013, 8.1 ± 0.19, 2017, 7.29 ± 0.03; Table 1). The pore water (0–1 cm) nitrate + nitrite concentration remained low with 3.19 ± 0.3 µM in 2013 compared to 3.54 ± 1.57 µM in 2017 as it was likely continually reduced in this long-term hypoxic site. Pore water total iron was 4.23 ± 0.72 µM in 2013 and showed lower concentrations with 2.85 ± 2.47 µM in 2017. In addition, no significant changes were observed in sulfate concentrations with 4.03 ± 0.27 mM in 2013 and slightly higher concentrations in 2017 (pore water, 4.26 ± 0.27 mM). In contrast, the organic matter content was significantly higher in 2017 (2013, n = 3, 18.38 ± 0.47% and 2017, n = 3, 30.24 ± 1.79%, p < 0.001). Finally, the deep site showed an approximately three-fold higher pore water phosphate concentration in 2017 (2013, n = 3, 35.8 ± 11.65 µM and 2017, n = 3, 110.59 ± 23.62 µM, p < 0.05).

In summary, a general trend of decreasing oxygen concentrations with decreasing depth was observed in 2013 and 2017. Furthermore, while phosphate concentrations peaked within the intermediate site in 2013 they shifted and peaked in 2017 in the deep site (Fig. 1). Similar trends of iron concentrations were observed along the depth gradient in both years, showing highest concentrations at the intermediate site, while organic matter...
increased with increasing depth (Fig. 1). High nitrate + nitrite concentrations were observed close to the coast and decreased with increasing water depth (Fig. 1).

**Microbial diversity, community composition, and taxonomy.** Rarefying the data to the lowest sample size (Supplemental Table S3 for comparison with rarefied data) confirmed that the main bacterial diversity was covered and therefore, to retain as much data as possible the unrarefied data was used. The unrarefied average sample size was 31,474 reads ($n=18$; Supplemental Table S4 for filtering results in DADA2). Changes in Shannon's H diversity occurred in all three sites with a general decrease of diversity with water depth (Supplemental Fig. S3), while the diversity slightly but significantly increased in the intermediate site in 2017 compared to 2013 (ANOVA, posthoc pairwise comparison, $n=6$, $p<0.05$; Supplemental Fig. S3 and Supplemental Table S1 for statistical support). The shallow site community composition did not change significantly (PERMANOVA, $n=6$, $p>0.05$) from 2013 to 2017 and showed small shifts in the community (Fig. 2, Supplemental Fig. S4, and Supplemental Table S1). The intermediate site community differed from 2013 to 2017 (Fig. 2, Supplemental Fig. S4) although the changes were insignificant ($n=6$, $p>0.05$; Supplemental Table S1). The bacterial community composition in the deep site significantly ($n=6$, $p<0.05$; Supplemental Table S1) changed comparing 2013 to 2017 (Supplemental Fig. S4), showing shifts and moved towards communities of the two shallower sites (Fig. 2). Finally, the communities in 2017 appeared more similar to each other than was observed in 2013 (Fig. 2 and Supplemental Fig. S4). In line with these results, similarity of percentages analysis (SIMPER) showed high dissimilarities along the depth gradient in 2013 (shallow-intermediate, 71.57%; shallow-deep, 81.25%; deep-intermediate, 74.61%) that decreased in 2017 (shallow-intermediate, 47.72%; shallow-deep, 73.22%; deep-intermediate, 70.76%) (Fig. 2).

The bacterial communities between the different sites along the depth gradient have been shown to be significantly different in a previous study\(^\text{14}\) that was confirmed for the year 2017 (PERMANOVA, $p<0.01$, Supplemental Table S1). Based on SIMPER analysis, the major contributors to the differences between the sampling sites in 2013 were the CG2-30-66-27, UBA6092, and Desulfobacula; while in 2017 the top contributors between sites shifted (Supplementary Tables S4 and Table S5). The top contributors between the shallow and intermediate stayed the same with the MBNT15 genera CG2-30-66-27 (6.61%, average contribution of dissimilarity) and UBA6092 (2.72%), while between the shallow and deep site the main contributor now included the Cyanobacteria Nodularia (7.73%). The differences between the deep and intermediate site were CG2-30-66-27 (10.85%) and Nodularia (7.76%) (Supplementary Tables S4 and Table S5).

A general trend could be observed when comparing the bacterial community composition of different sites between the years 2013 and 2017 where the highest dissimilarity were in the deep site (79.29%) with increasing similarity with decreasing depth (intermediate, 60.8%; shallow, 56.66%). Additional constrained ordination analysis (RDA) showed the environmental variables most explaining the changes between the sampling sites and years were oxygen (ANOVA, $p<0.05$, Supplemental Table S1 and Supplemental Fig. S5), temperature ($p<0.05$), and organic matter ($p<0.01$).

The number of detected phyla within each site differed between years, showing a generally higher diversity among the total sum of amplicon sequence variants (ASVs) (Supplemental Fig. S3) but a lower number of abundant ASVs (>0.5% relative abundance) from 2013 to 2017. The most abundant phyla within each site were the Proteobacteria, Desulfo bacterota, Bacteroidetes, and Cyanobacteria.

The relative abundance of Proteobacteria in the shallow site was higher during 2013 (mean ± SD, 33.2% ± 0.02) when compared to 2017 (24.8% ± 0.01) and had a 6.1% higher relative abundance of Desulfo bacterota (2013,
11.3% ± 0.002, 2017, 10.6% ± 0.02; Supplemental Fig. S6). Differential abundance analysis on the ASV level showed that 67% of the ASVs differed significantly between 2013 and 2017 (Fig. 3 and Supplemental Table S5 for differential abundance analysis) and that 98.9% of those significantly changed ASVs had a relative abundance below 0.5% each (Fig. 3). Of the 1.1% of significantly different ASVs with a relative abundance over 0.5%, the Proteobacteria and Campylobactera increased while Chloroflexota and Cyanobacteria increased (Fig. 4, Figs. S7 and Fig. S8 with summarized stacked bars of the replicates plus SD as errors bars, and Supplemental Table S5). In 2017, the number of abundant genera (i.e. above 0.5% relative abundance) decreased that included CG2-30-66-27 (2013, 4.6% ± 0.01 relative abundance & 2017, 3.1% ± 0.002) belonging to the MBNT15 phylum, Sulfurovum (0.4% ± 0.002 and 0.002% ± 0.0001), Gallionella (0.9% ± 0.002 and 0.4% ± 0.001), and Thauera (0.04% ± 0.001 and 0.01% ± 0.001) while genera including UBA6092 (2.2% ± 0.003 and 3.6% ± 0.003) increased (Fig. 4). SIMPER analysis within the shallow site between the years 2013 and 2017 showed that the average contribution of genera responsible for the differences between communities were rather small compared to the deeper sites, confirming the low significant differential abundant ASVs between the years. Most likely the genera UBA6092 (3.72% average contribution of dissimilarity), Caldilinea (2.56%), and CG2-30-66-27 (2.47%) contributed to the differences between the years (Supplemental Table S4).

Figure 3. Bar plot of relative abundance on phylum and genus levels. Bar plots of significantly differential abundant taxa over 0.5% relative abundance based on ASVs (the cutoff was based on an ASV having > 0.5% in at least one sample). Phyla < 0.5% and non-significant phyla > 0.5% are summarized. (a) Bar plot on phylum level showing years 2013 and 2017 in November at the three different sites (n = 3), R1-3 are the different replicates of each site. (b) Bar plot on genus level showing years 2013 and 2017 in November at the three different sites (n = 3), R1-3 are the different replicates of each site.
The intermediate site showed a 44% increase in Proteobacteria in 2017 (mean ± SD, 2013, 8.3% ± 0.05, 2017, 15.1% ± 0.05; Supplemental Fig. S6) that replaced the shallow site. A decline of the abundance of significant relative abundant ASVs (> 0.5%) on genera level by approximately 90% was observed from 2013 to 2017, with a significant decrease in e.g. Algoriphagus (2013, 2.3% ± 0.009, 2017, 0.03% ± 0.0001), Arcobacter (1.7% ± 0.01 and 0.3% ± 0.0001), and Desulfatiglans (4.2% ± 0.01 and 0.3% ± 0.001) while uncultured bacteria belonging to e.g. CG2-30-66-27 (2.2% ± 0.008 and 11.0% ± 0.008) and UBA1847 (0.6% and 4.3% ± 0.007) genera increased (Fig. 4). The increased relative abundance of these genera were most likely also the main contributor between the differences of the communities from 2013 to 2017 with 11.23% of dissimilarity contribution for CG2-30-66-27 and 4.78% for UBA1847 (Supplemental Table S4). The deep site had the strongest shift in the bacterial community between 2013 and 2017 with an increase of 44% in Proteobacteria (mean ± SD, 2013, 8.3% ± 0.03, 2017, 15.1% ± 0.03; Supplemental Fig. S6) that replaced...
*Desulfo bacterota* as the most abundant phylum. Compared to the other two sites, the deepest site had the fewest number of significantly changed ASVs with 63% and concomitantly a larger number of ASVs with a relative abundance of >0.5% with 11.1% (Fig. 3). Phyla including *Saprochaeta*, *Firmicutes*, and *Bacteroidetes* significantly decreased while *Cyanobacteria* increased in 2017 (Fig. 4, Figs. S7 and S8 with standard error as error bars of the replicates plus SD as errors bars). The genus *Nodularia* (2013, not detected and 2017, 14.4% ± 0.01) was most responsible for the significant increase in *Cyanobacteria* in 2017 while the genera *Desulfatiglans* (2.3% ± 0.009 and 6.1% ± 0.009) and *Desulfobacula* (7.3% ± 0.002 and 8.2% ± 0.001) also significantly increased. In contrast, e.g. *Dracinibacterium* (4.5% ± 0.004 and 1.5% ± 0.001) and *Algoriphagus* (2.4% ± 0.01 and 0.2% ± 0.001) decreased (Fig. 4). Finally, the diversity of abundant genera significantly decreased from 2013 to 2017 (Fig. 4). These results also confirmed that the main contributors for the differences between the years were most likely the *Nodularia* (20.73% average contribution of dissimilarity) followed by *Desulfobacula* (8.34%) and *Desulfatiglans* (6.21%) (Supplementary Table S4).

**Discussion**

Global coastal oceans are continuously influenced by anthropogenic induced changes such as accelerated eutrophication that can result in decreased oxygen concentrations in bottom waters4 and the steady influence of ongoing climate change related effects such as rising temperatures that exacerbate existing eutrophication effects5. In situ bottom water temperature and oxygen data from March 2017 to December 2017 showed a decline in oxygen concentrations along with a temperature increase in November in coastal bottom waters at all the three sampling sites. These data match that collected by HELCOM and ICES25 showing trends of increasing temperatures and declining oxygen concentrations in coastal bottom waters (1–30 mbs) independent of the depth across the Western Gotland-, Eastern Gotland-, and Bornholm Basins (Supplemental Fig. S1). A previous study showed significantly different microbial communities along this coastal water depth gradient in 201314, which could also be shown for the communities of the same gradient in the year 2017. The focus of this study was to investigate Baltic Sea sediment changes between 2013 and 2017 at different coastal water depths to study oxygen deficient zones common in the Baltic Sea22.

The deepest investigated coastal sediment (~ 30 mbs) was previously shown to be under consistent oxygen deficient conditions while the area around the bay has a history of long-term oxygen deficiency41. However, this area was found to have changed to seasonal varying hypoxic-oxic conditions in 2017 that was potentially due to the reduction of nutrient intake into a close-by bay (~ 4 km) from a nearby sewage-treatment plant49. The data suggested that most changes within the bacterial communities happened at the deep oxygen deficient site (11% significant different ASVs comparing 2013 and 2017). *Cyanobacteria*, especially *Nodularia*, went from being undetectable in 2013 to being the most abundant genus in 2017. This increase in *Nodularia* may also have caused the significant increase of organic matter due to deposition of pelagic *Cyanobacteria* on the sediment29. A further potential cause of increase in OM could have been due to primary production sources such as green algae or dinoflagellates that bloom in spring along with Diatoms that bloom in spring and autumn. This coupled to the generally higher OM content within the deep site may also have due to decreased OM degradation rates in deeper, less well mixed, and low oxygen conditions53. This increase in organic matter likely led to an increase in sulfate reducing bacteria like *Desulfobacula* and *Desulfatiglans* in 2017. Such bacteria have been shown to participate in the mineralization of organic matter58, and sulfate reduction can account for as much as up to half of organic matter mineralization53. Furthermore, the abundance of sulfate reducers has been shown to be positively correlated with organic nitrogen and phosphorus in the surface layer of sediments5, and *Desulfo bacula* has been found to be associated with Baltic Sea organic-rich sediments50. The higher relative abundance of sulfate reducers may also have caused the significant increase of phosphate as sulfate-reducing bacteria may indirectly contribute to dissolved phosphate release during organic matter mineralization59 by the production of sulfide that binds ferric iron and hinders the formation of phosphate binding ferric oxyhydroxides63. Another potential contribution to the increased pore water phosphate concentrations in November 2017 was the raised oxygen concentration from March to July 2017 leading to iron oxide (Fe–P) that binds phosphate37. As the oxygen concentrations became hypoxic in November, Fe–P dissolution may have increased the release of phosphate into the pore water58. The general increase in temperature within the last years could have led to a shift within seasonal changing oxygen states, with possible higher regional river-runoff in spring feeding even long-term hypoxic deeper coastal sediment with oxygen-rich water. This can lead to greater bacterial metabolism rates, followed by lower oxygen concentrations. Similar results have also been reported from arctic lakes with increasing runoff60. The data confirmed that with increasing temperature, an increase of cyanobacterial deposition in already hypoxic sediments is likely expected62. In addition, more organic matter leads to an increased sulfur cycling close to the sediment surface64,65, that might accelerate oxygen decline with subsequent increased release of phosphates62. Due to prolonged seasonal stagnant water with less mixing of the bottom water, deeper sites are more affected by eutrophication and can turn long-term hypoxic66, and an increase in temperature is likely to accelerate eutrophication induced hypoxia of benthic waters.

In comparison, intermediate coastal sediments (intermediate site, ~ 20 mbs) exposed to already long-term fluctuating hypoxic-oxic conditions may already have had a bacterial community that was adapted to oxygen fluctuations. Compared to 2013 less dominant bacteria taxa were found in 2017. These few dominant taxa include CG2-30-66-27 from the candidate phylum *MBNT15*, which might be obligate anaerobes that couple H₂ and acetate oxidation to nitrate reduction62. The genus *UBA1847* from the family *Woeseicaceae* within the *Proteobacteria* that are among the most abundant microorganism in coastal sediments. They cover a broad spectrum ranging from facultative sulfur- and hydrogen-based chemolithoautotrophic to obligate chemooorganoheterotrophic bacteria60 (Fig. 4). The significantly increased relative abundance of ASVs aligning within the candidate phylum *MBNT15* that thrives in anoxic deep sediments45 could be the result of several factors. Either an
environmental variation between 2013 and 2017 occurred or the proportion of anoxic sediment within the sliced 0–1 cm increased compared to 2013. In general, the data based on environmental variables in 2017 could suggest a potential reversal of eutrophication effects when compared to 2013, with for example an increase in oxygen concentrations and a decline in phosphorus. In addition to the minor changes in the microbial communities, the data suggested that oxygen histories may play an important role in the extent microbial communities react to environmental changes. This importance was already shown in previous studies, where long-term anoxic coastal Baltic Sea sediment has been re-oxygenated.

Shallow coastal areas are in general more exposed to anthropogenic influenced changes related to climate change or eutrophication, such as input of nutrients from land, increased acidification, and faster warming of the water column reaching the sediments. However, these sediments have due to their nature a constant oxygen input from e.g. mixing of the water column by the wind and previous studies show that the shallow area has been under long-term oxic conditions. Despite that the studied coastal bay has not undergone any artificial changes, beside decreased nutrient intake of a wastewater treatment plant of a close-by bay (~ 4 km) in the intervening years, seasonally changing oxygen conditions were observed between March and December 2017 (Supplemental Fig. S2). In addition, a previous study shows significant differences between bacterial communities at all three sites with the shallow site being most distinct from the other two. Within the shallow site, only 1.1% of the dominant taxa (i.e. comprising > 0.5% relative abundance) were significantly different comparing 2013 and 2017 indicating the highly abundant bacteria were still present (Fig. 3).

No simple, single response within bacterial communities of changes within coastal sediments was discerned and how bacterial communities react to environmental changes like eutrophication and/or climate change are an interplay of different spatiotemporal factors including water depth and oxygen supply. Sediments exposed to long-term hypoxic conditions were sensitive to even small changes in e.g. oxygen concentration and temperature increase, probably resulting in accelerated bacterial activity in combination with increased organic matter leading to potential future expansion of already existing hypoxia zones. Bacterial communities of oxygen rich shallow coastal sediments did not show strong responses to environmental changes, showing that these communities were most likely stable under potential transitory oxygen depletion. Continuous exposure to eutrophication, warming, and other climate change related effects has the potential to expand and aggravate already existing hypoxic zones.

Conclusions

No simple, single response within bacterial communities of changes within coastal sediments was discerned and how bacterial communities react to environmental changes like eutrophication and/or climate change are an interplay of different spatiotemporal factors including water depth and oxygen supply. Sediments exposed to long-term hypoxic conditions were sensitive to even small changes in e.g. oxygen concentration and temperature increase, probably resulting in accelerated bacterial activity in combination with increased organic matter leading to potential future expansion of already existing hypoxia zones. Bacterial communities of oxygen rich shallow coastal sediments did not show strong responses to environmental changes, showing that these communities were most likely stable under potential transitory oxygen depletion. Continuous exposure to eutrophication, warming, and other climate change related effects has the potential to expand and aggravate already existing hypoxic zones.

Materials and methods

Study site. The sampling location was a coastal Baltic Sea bay near the town of Loftahamraer, Sweden. The three sampling sites (Supplemental Fig. S9 and Supplemental Table S2) have different oxygenation histories and were designated as ‘shallow’ (6.5 mbs; WGS 57 53.214, 16 35.934), ‘intermediate’ (20.5 mbs; WGS 57 53.545, 16 35.476), and ‘deep’ (30.1 mbs; WGS 57 53.531, 16 35.165). Detailed descriptions of the sediments are published in previous studies.

Sampling. Sampling was conducted at each site on 2 November 2017 (n = 3). In addition, published data from the same sites (also n = 3 per site) from 2013 were used for comparison. Sampling was conducted as previously described using acrylic cores (internal diameter 7 cm, length 60 cm) to sample the sediment surface with a Kajak gravity corer at each location. Oxygen and temperature were measured in situ (Multiline sensor, WTW) in the bottom waters (0–10 cm above sediment; Supplemental Table S2). The sediment surface (0–1 cm) was sliced and transferred into a sterile 50 mL polypropylene centrifuge tube (Thermo Scientific) and mixed until homogenized. A subsample of 15 mL homogenized sediment was transferred into an acid washed 15 mL polypropylene centrifuge tube (Thermo Scientific) for pore water analysis as well as an additional subsample of 2 mL for organic matter analysis. The sediment remaining in the initial tube was used for DNA extraction. Samples for chemistry analysis and DNA extraction were stored cooled until transported back to the laboratory the same day and then stored until further analysis at −20 °C and −80 °C, respectively. Additionally, oxygen data were measured on bottom water in situ from March 2017 to December 2017 (n = 9) on all three sites (Supplemental Fig. S2, Table S2).

Chemistry analysis. Chemistry analyses were conducted on pore waters and the sediment itself. Pore waters were sampled by centrifuging the 15 mL sediment tubes at 2200 x g for 15 min and the supernatant transferred into a new 15 mL acid washed centrifuge tube (Thermo Scientific). The pore water was filtered.
through a 0.7 μm Target2™ GMF Syringe Filter (Thermo Scientific™) and pH was measured with a pH electrode (pHEnomenal, VWR™). Sulfate, dissolved inorganic phosphate, total iron, nitrate + nitrite (in combination), and organic matter were measured as previously described except for organic matter samples that were dried for a total of six days at 40 °C instead of 3 days at 80 °C.

DNA extraction. For extraction of sediment DNA, the samples were homogenized and the DNeasy® PowerSoil Extraction Kit (QIAGEN) was used according to the manufacturer’s guidelines. The extracted DNA was measured using Qubit® 2.0 (Invitrogen™, Life Technologies Corporation) and stored at ~ 20 °C until 16S rRNA gene amplification and Illumina library preparation. DNA was amplified with 16S rRNA gene PCR primers 341f and 805r53 (mainly targeting Bacteria) using a modified PCR program and libraries prepared with the Illumina Nextera kit according to Lindh et al.54.

Sequencing and bioinformatics analysis. The DNA samples were sequenced at the Science for Life Laboratory (SciLifeLab) in Stockholm on the MiSeq platform with 2 × 301 bp pair-end setup. The sequences were analyzed using the DADA2 pipeline on the UPPMAX cluster (Uppsala Multidisciplinary Center for Advanced Computational Science). Parameters for the quality trimming of the sequences were set to 290 (forward read) and 230 (reverse read) with left trimmed at 21 bp. The error model was run with a MAX_CONSIST of 30 for reads, and the R1 and R2 pairs were merged with minimum overlap of 10 bp and zero mismatch allowance. The average sequence count after quality trimming was 144,259 (min. 9330 and max. 428,077; Supplemental Table S6). Chimeras were removed and the taxonomy was assigned against the Genome Taxonomy Database (GTDB version 86) using default parameters with DADA2, before the final data were analyzed using R version 3.5.257.

Statistical approaches. To determine the sequencing depth, rarefaction curves were calculated with the ‘vegan’ package version 2.5-625 based on the number of ASVs and final read counts after DADA2 analysis. The dataset showed differences in sequencing depth between the years. To confirm our results based on scaling with ranked subsampling and relative abundance normalization, the data have been compared to results using rarefied samples that showed the methods were suitable (Supplemental Table S3).

Diversity of the microbial communities was estimated by calculating the alpha diversity (Shannon’s H index) based on counts normalized using scaling with ranked subsampling25. To test for significant differences in diversity between years and sites a linear regression model was used due to the nested nature of the data with three replicates per site and the interaction between the sites and years. Different models were fitted and the model that showed the best fit for the data structure was chosen based on Akaike information criterion (AIC). All factors (years and sampling sites as interaction and replicates nested within sites) were used as fixed effects in the final model (‘lme’ function, ‘stats’ package)72 due to the small sample size. The statistical differences between the factors were tested using the ‘anova’ function from the ‘stats’ package in R53 with pairwise comparison for years at each site using the ‘emmeans’ package (version 1.5.4)56. For testing on differential abundances between the years at each sampling site, taxa not seen in at least 20% of the samples were removed and a zero-inflated negative binomial model was used to reduce the excess of zeros in the dataset. Then, differential abundance analysis on counts was done with the ‘DESeq2’ package to analyze statistical differences between communities based on all ASVs. The cutoff of 0.5% was based on an ASV having > 0.5% relative abundance in at least one sample. For further analysis (if not otherwise stated), relative abundances were calculated from all ASV count data. Bray–Curtis distances. Variance inflation factor analysis showed multi-collinearity between oxygen and nitrate (Supplemental Table S1). The focus of this study was to investigate changes based on oxygen and temperature. Therefore, the variable nitrate has been removed for the redundancy analysis (RDA), which resulted in VIF < 5 for the remaining variables. The RDA analysis was performed using the ‘rDA()’ function within the ‘vegan’ package plus an ANOVA permutation test, with a direct model using marginal terms to investigate if significant environmental variables influenced the differences between the bacterial communities. Bray–Curtis dissimilarities were calculated using the ‘vegdist’ function within the ‘vegan’ package. To check if microbial community composition in 2013 and 2017 was significantly different, a PERMANOVA within the ‘adonis’ function in R52 was carried out using the ‘vegdist’ function within the ‘vegan’ package. Additionally, PERMANOVA testing was used to compare the microbial communities along the depth gradient (comparing the different sites) for the years 2013 and 2017. Similarity of percentages (SIMPER) analysis from the ‘vegan’ package was used to discriminate species responsible for dissimilarities between the year 2013 and 2017 at each site, as well as between sites within each year (Supplemental Table S4). To determine whether differences in environmental variables between the years were significant, the same model that was used for diversity testing was constructed and tested. The best fit (best AIC) for the data structure was reached using a linear regression model (‘lme’ function, ‘stats’ package) with years and sampling sites as interaction and replicate nested within site as fixed effects.

Data availability
16S rRNA gene sequencing data are available on the NCBI database under Bioproject PRJNA721576 and PRJNA322450.

Code availability
The code used is available at Supplemental Methods S1.
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Author contributions

M.D. and E.B. developed the experimental/methodological approach. L.S., E.B., and M.S. collected the data. L.S. and S.T. carried out the statistical approaches. L.S., M.D., and E.B. evaluated the data. L.S. and M.D. wrote the manuscript. All authors approved the manuscript for publication.

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Competing interests

The authors declare no competing interests.

Additional information

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