Bioturbation as a key driver behind the dominance of Bacteria over Archaea in near-surface sediment

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The factors controlling the relative abundances of Archaea and Bacteria in marine sediments are poorly understood. We determined depth distributions of archaeal and bacterial 16S rRNA genes by quantitative PCR at eight stations in Aarhus Bay, Denmark. Bacterial outnumber archaeal genes 10–60-fold in uppermost sediments that are irrigated and mixed by macrofauna. This bioturbation is indicated by visual observations of sediment color and faunal tracks, by porewater profiles of dissolved inorganic carbon and sulfate, and by distributions of unsupported 210Pb and 137Cs. Below the depth of bioturbation, the relative abundances of archaeal genes increase, accounting for one third of 16S rRNA genes in the sulfate zone, and half of 16S rRNA genes in the sulfate-methane transition zone and methane zone. Phylogenetic analyses reveal a strong shift in bacterial and archaeal community structure from bioturbated sediments to underlying layers. Stable isotopic analyses on organic matter and porewater geochemical gradients suggest that macrofauna mediate bacterial dominance and affect microbial community structure in bioturbated sediment by introducing fresh organic matter and high-energy electron acceptors from overlying seawater. Below the zone of bioturbation, organic matter content and the presence of sulfate exert key influences on bacterial and archaeal abundances and overall microbial community structure.

Archaeal and bacterial populations in marine sediments account for a significant fraction of global living biomass1,2. Members of both domains drive globally important biogeochemical processes, e.g. Archaea perform methanogenesis and Bacteria perform sulfate reduction. In recent years, novel insights into the metabolism of uncultivated marine sediment Archaea and Bacteria have emerged from studies involving fluorescence-in-situ-hybridization (FISH), single-cell genomics, and metagenomics3–5. Yet, much remains to be learned6, and it is currently not even known which variables drive the relative abundances of Archaea and Bacteria in marine sediments.

Depth profiles of archaeal and bacterial populations frequently show a large scatter7–9, possibly due to vertical heterogeneity in sediment geochemistry and lithology10. Methods for quantification of microbes, e.g. FISH, quantitative PCR (qPCR), or lipid biomarker analyses, often show limited agreement between each other7,11–13, suggesting inherent methodological biases. Limited agreement is also common when results from different FISH protocols, nucleic acid extraction methods, and lipid biomarker analytical methods are compared amongst each other, presumably due to variations in extraction efficiency14–16, cell labeling efficiency7, PCR primer biases14,17, and instrument sensitivities18,19. In addition, many standard DNA and lipid analyses rely on bulk extraction methods that include intra- and extracellular DNA and lipid pools. DNA outside living cells accounts for ≥10–90% of total DNA14, and extracellular lipids may have long half-lives, in particular those of archaeal origin20. Significant quantitative biases due to inclusion of extracellular DNA and lipids from outside of living cells are thus likely.

Despite uncertainties associated with each quantification method, almost all studies suggest that both archaeal and bacterial abundances decrease with depth in marine sediments7–9,14,15. Most studies, moreover, indicate that Bacteria dominate8,21–23 or occur in equal proportions to Archaea in marine subsurface sediments7,9,12, and...
that Bacteria clearly dominate over Archaea in surface sediments\(^ {22,24-26} \). Reasons for the dominance of Bacteria in surface sediments are not known, but vertical changes in relative abundances might provide clues. Lipp et al.\(^ {15} \) measured a dramatic shift in dominance from bacterial to archaeal intact polar lipids (IPLs) in the top 10 cm of sediments on the Peru Margin, and interpreted this shift to confirm the notion of Valentine\(^ {27} \), who had proposed that Archaea are better adapted to long-term low-energy stress than Bacteria. Based on Catalyzed-Reporter deposition-FISH (CARD-FISH), Molari et al.\(^ {28} \) documented a clear decrease in Bacteria-to-Archaea Ratios (BAR) in the top 10–15 cm of sediment in the southern Adriatic Sea, and attributed this shift to vertical changes in sediment water content and grain size.

Here we examine the relative abundances of archaeal and bacterial 16S rRNA genes (abbreviated as “16S genes” hereafter in the text) from the seafloor down to 11 meters below seafloor (mbsf) at eight stations (stations M1, M5, M21, M22A, M24, M26, M27A and M29A; Supplementary Fig. 1) in Aarhus Bay, Denmark. Intact surface sediments were recovered using 1-m Rumohr Lot cores, while deeper layers were sampled by 6–12 m long gravity cores. With the exception of a freshwater peat layer (M1) and a glacial sand layer (M21) at the bottom of cores from two stations, all sediments consisted of Holocene marine mud. We extracted DNA using a method that includes an initial wash step to separate soluble DNA (sDNA) that is dissolved in sediment porewater or adsorbed to sediment matrices from non-soluble (nsDNA) particulate pools that include DNA from living cells\(^ {14} \). Archaeal and bacterial 16S gene abundances were then quantified in nsDNA extracts by qPCR using domain-specific primers. Variations in the depth of the sulfate-methane transition zone (SMTZ) and the presence of free methane gas between stations allowed us to investigate links between archaeal and bacterial abundance and the distribution of sulfate-reducing and methanogenic activity and free methane gas.

Initial results showed that the biggest shifts in archaeal and bacterial 16S gene abundances occurred in the top 20 cm of sediment rather than across the SMTZs or due to presence of free methane gas. We hypothesized these shifts to be linked to macrofaunal activity, and thus compared trends in archaeal and bacterial 16S gene abundances in surface sediments to patterns in macrofaunal activity. We inferred the vertical extent of bioturbation, i.e. the movement of overlying seawater into sediment by macrofauna during feeding and respiratory activities\(^ {29} \), from porewater concentration profiles of sulfate, dissolved inorganic carbon (DIC), and \( \delta ^{13} C-\text{DIC} \). We calculated the vertical extent of sediment reworking, i.e. the vertical mixing of sediment due to macrofaunal feeding and burrowing activities\(^ {28} \), based on sedimentation rates and depth profiles of radionuclides (unsupported \( ^{210} \text{Pb} \) (\( ^{210} \text{Pb}_{\text{unsupp}} \)), \( ^{137} \text{Cs} \))\(^ {19,30} \). We, moreover, compared vertical profiles of 16S gene abundances with those of solid-phase total organic carbon (TOC) and total nitrogen (consisting of \( >95\% \) of organic N), ratios of TOC:TN (called C:N ratios from now on), \( ^{13} \text{C}-\text{TOC} \), and \( ^{15} \text{N}-\text{TN} \). Hereby TOC and TN served as proxies for bulk organic matter (OM) content, whereas C:N, \( ^{13} \text{C}-\text{TOC} \), and \( ^{15} \text{N}-\text{TN} \) were used as proxies for bulk OM composition.

Our results indicate that the activities of benthic macrofauna are a critical, yet widely overlooked determinant of archaeal and bacterial abundances in marine surface sediments. Mechanisms by which sediment reworking and bioturbation may affect abundances of Archaea and Bacteria and overall microbial community structure are discussed, in addition to likely drivers behind microbial community structure in sediments below the bioturbation zone.

**Results**

**Trends in archaeal and bacterial 16S gene abundances vs. sediment depth.** Depth distributions of archaeal and bacterial 16S gene abundances show consistent trends across all eight stations (Fig. 1). Bacterial greatly outnumber archaeal 16S genes in the top 10–20 cm. BARs are typically highest in the top 0–4 cm, where they range from 10:1 to 60:1, and then decrease due to a decrease in bacterial and concomitant increase in archaeal gene abundance down to \( \sim 20 \) cm. Below 20 cm, both archaeal and bacterial 16S gene abundances gradually decrease with depth. Bacterial decreases in 16S gene abundances are slightly steeper than those for Archaea, as is evident from mean BARs of \( \sim 2:1 \) at 20 cm depth which gradually decrease to \( \sim 1:1 \) at 3 mbsf. These overall trends are consistent throughout the Holocene mud layers across all stations, and suggest that the biggest changes in BARs occur within a small, uppermost part of the sulfate reduction zone, and are thus not driven by sulfate concentrations, or the presence of free methane gas (Supplementary Table 1). BARs are also similar between Holocene mud (0–7.4 mbsf) and an underlying peat layer (below 10.5 mbsf) at M1, but are interestingly lowest in a glacial sand layer at M21, where archaeal 16S genes conspicuously outnumber bacterial 16S genes by factors of 2 to 4.

When the 8 Aarhus Bay stations are examined individually, the above trends are even more clear, due to very low data scatter between adjacent depths from the same stations (Supplementary Fig. 2 and next section). Bacterial outnumber archaeal 16S genes by at least one order of magnitude at the sediment surface. Archaeal 16S gene abundances increase and bacterial 16S gene abundances decrease downward from the sediment surface to \( \sim 20 \) cmbsf. Below \( \sim 20 \) cmbsf, both archaeal and bacterial 16S gene abundances decrease, with bacterial typically exceeding archaeal 16S genes down to the SMTZ, and reaching similar values in bacterial and archaeal gene copy numbers below. The exception is again M21, where archaeal outnumber bacterial 16S genes in sulfate-rich sediment associated with organic-poor glacial sand. Moreover, small local increases in 16S gene abundances of Archaea and/or Bacteria are present in the SMTZ at five out of the six stations where the SMTZ was reached by coring.

**Fine-scale depth profiles in surface sediments.** Given that sedimentation rates vary between stations in Aarhus Bay, e.g. M1 is in an area of Aarhus Bay with net erosion\(^ {21} \), whereas stations M24, M26, M27A, M29A, and M5 are subjected to variable but significant rates of net sedimentation (0.86 to 1.11 mm yr\(^ {\sim 12} \); note: no data are available for M21 or M22A), the high similarity in BAR depth profiles is unlikely to be driven by sedimentation alone or sedimentation-related rates of OM deposition. The rates and depth profiles of microbial respiration in...
surface sediments are also too different33, and seasonal changes in microbial respiration too high34 to explain the observed congruence in BAR depth profiles across sites and sampling dates. We hypothesize that the activity of macrofauna inhabiting the top layer of sediment at all Aarhus Bay sites has a ‘homogenizing’ effect that causes similar depth profiles in bacterial and archaeal abundances across sites. To assess this hypothesis, we compared the high depth-resolution qPCR data on Rumohr Lot cores from M1 and M5 (labeled M1-RL and M5-RL in Fig. 1) to a range of geochemical data that provide clues to the depths of macrofaunal reworking, OM input, and bioirrigation (Fig. 2, Supplementary Fig. 4).

Both Rumohr cores show the characteristic decrease in bacterial and concomitant increase in archaeal 16S genes in the top 20 cmbsf (Fig. 2a,b). Similarly, the BARs show a clear and consistent decrease, from ~20:1 down to ~1:1 at M1, and from ~15:1 down to ~2:1 at M5 (Fig. 2c,d). Closer inspection suggests that the stabilization of BARs occurs slightly above 20 cmbsf. At M1, BARs stabilize at ~1:1 at ~16 cmbsf, whereas BARs at M5 stabilize at ~2:1 at ~12 cmbsf.

Highly similar radionuclide profiles indicate similar depth distributions of macrofaunal sediment reworking at M1 and M5. $^{210}$Pb$_{supp}$ concentrations are uniformly high from the sediment surface to 6 cm depth (Figs 2e,f and 3), indicating high rates of vertical sediment mixing over time scales that are much shorter than the half-life of the tracer. Below 6 cmbsf, $^{210}$Pb$_{supp}$ values drop considerably but remain above the detection limit down to 14 cmbsf, indicating continued albeit reduced vertical sediment mixing. Calculated particle mixing coefficients ($D_p$) for the interval from 6 to 14 cm are 0.53 cm yr$^{-1}$ at M1 and 0.42 cm yr$^{-1}$ at M5 (Fig. 3). Below 14 cmbsf, $^{210}$Pb$_{supp}$ is below detection, indicating low or no vertical sediment mixing. The $^{210}$Pb$_{supp}$ values strongly co-vary with $^{137}$Cs values, which are lower than the $^{210}$Pb$_{supp}$ at the sediment surface but higher at depth, remaining above detection to ~18 cmbsf (Fig. 2e,f), in accordance with the longer half-life (22.3 years for $^{210}$Pb and 30.2 years for $^{137}$Cs). Overall, the radionuclide data are consistent with visual observations of bioturbation made on Rumohr cores at M1 and M5, which indicate the presence of a heavily mixed and oxidized ‘surface mixed layer’ (SML; to 4 ± 1 cmbsf), and a deep mixed layer’ (DML) that is dark grey but contains light brown streaks to ~10 cmbsf at M1, and to 12–20 cmbsf at M5; these streaks indicate occasional transport of oxidized surface sediment to deeper layers that are otherwise grey due to the production of iron(II) sulfides under reducing conditions (more info under ‘Core Descriptions (2012)’ in Supplementary Methods).

Porewater concentrations of DIC and sulfate at M1 and M5 indicate strong bioirrigation of surface sediment (Fig. 2g,h). DIC concentrations remain around seawater values (~2 mM) in the SML, consistent with high rates of porewater exchange between sediments and overlying seawater, and then increase, reaching 8.6 and 16 mM at 20 cm depth at M1 and M5, respectively. These increases in DIC concentrations indicate that, unlike in the SML, macrofaunal bioirrigation rates in the DML are insufficient to dilute out the DIC signal produced by microbial mineralization of OM. DIC concentration increases are steepest from 4 to 8 cmbsf at M1 and from 4 to 10 cmbsf at M5 and level off below 8 and 10 cmbsf, respectively, indicating a depth-related decrease in microbial CO$_2$ production. The steeper increase in DIC concentrations with depth at M5 reflects the higher rates of microbial mineralization of OM at this station compared to M1.
Sulfate concentrations at M1 and M5 decrease with sediment depth, indicating microbial sulfate reduction. At M1, sulfate concentrations show a steady decrease from the seafloor downward, with a slight inflection in the

Figure 2. Fine-scale microbial abundance and geochemical profiles of surface sediments at M1 (M1-RL, top row of panels) and M5 (M5-RL, bottom row of panels). (a,b), abundances of archaeal and bacterial 16S genes; (c,d), BARs; (e,f) mean \( ^{210}\text{Pb}_{\text{unsupp}} \) and \( ^{137}\text{Cs} \) values ± standard deviation (SD) - the detection limit of \( ^{210}\text{Pb}_{\text{unsupp}} \) and \( ^{137}\text{Cs} \) is indicated by the dashed black line; (g,h), concentrations of sulfate and DIC; (i,j), \( \delta^{13}\text{C-DIC} \) and \( \delta^{13}\text{C-TOC} \). The horizontal grey dashed lines indicate 0.14 mbsf, which is the minimum depth of macrofaunal sediment reworking based on \( ^{210}\text{Pb}_{\text{unsupp}} \). The shaded grey area at M5 marks the interval of the SMTZ.

Figure 3. Modeled sediment mixing coefficients, \( D_b \), based on depth profiles of \( ^{210}\text{Pb}_{\text{unsupp}} \) in the depth interval from 4–14 cmbsf at M1 and M5. Error bars indicate the standard deviation of \( ^{210}\text{Pb}_{\text{unsupp}} \) within each depth sampled. The horizontal grey dashed lines indicate the minimum depth of macrofaunal sediment reworking (0.14 mbsf).

Sulfate concentrations at M1 and M5 decrease with sediment depth, indicating microbial sulfate reduction. At M1, sulfate concentrations show a steady decrease from the seafloor downward, with a slight inflection in the
sulfate profile at ~18 cmbsf. This inflection suggests input of sulfate-rich seawater by bioirrigation or significant production of sulfate by re-oxidation of reduced sulfur compounds with seawater-derived electron acceptors, e.g. O₂ or nitrate, to this depth. This interpretation is supported by the concentration gradient of sulfate in the SML and DML. Despite sulfate reduction rates being the highest in the DML and DML33,34, the sulfite concentration gradient is less steep than in the underlying sediment, where sulfate reduction rates are orders of magnitude lower33 and only a low sulfate flux is required to account for the entire depth-integrated reduction of sulfate32. At M5, sulfate concentrations are nearly constant in the SML, suggesting very high rates of bioirrigation that replace nearly all sulfate that is consumed by sulfate reduction. Below 4 cmbsf, sulfate concentrations at M5 decrease more steeply than at M1, consistent with higher microbial respiration rates at M5. The sulfate concentration profile below 4 cmbsf is near-linear, in spite of the highest measured sulfate reduction rates at M5 occurring in the top 10 cmbsf33; as at M1, the sulfate concentration profile in the SML and DML of M5 thus indicates a significant input of sulfate by bioirrigation. Reflecting the higher rates of OM remineralization at M5, sulfate is depleted at 56 cmbsf, while at M1 sulfate concentrations are still >10 mM at this depth. Methane concentrations increase slightly but remain in the micromolar range throughout the bioturbated surface interval and sulfate zones at both stations, and only show strong increases below the SMTZ (Supplementary Fig. 3a,b).

The δ13C-isotopic compositions of DIC and TOC show clear depth-related trends that are consistent with the radionuclide and porewater concentration data (Fig. 2f). The δ13C-DIC at M1 shows a diagenetic gradient from δ13C-DIC of ~−12‰ at the sediment surface and decreases with depth, suggesting an increasing proportion of DIC produced by organic carbon diagenesis. The δ13C-DIC at M1 is approximately ~6.9‰ lower than at M5, and decreases steeply to ~11.6‰ at 10 cmbsf, and then decreases only slightly to ~12‰ below 20 cmbsf. Apart from an abnormal δ13C-DIC of ~−13.5‰ in the top 2 cm of M5, which can be considered an outlier, the δ13C-DIC depth profile at M5 shows high similarity to that at M1. Values decrease steeply from ~12‰ to ~18‰ below 2 cmbsf, and then continue to decrease more gradually, stabilizing around ~16‰ at 20 cmbsf and below. Consistent with the formation of 12C-enriched methane by CO₂ reduction at M5, DIC progressively becomes 13C-enriched in the methane zone.

The δ13C-TOC shows a similar trend at both stations. The δ13C-TOC is lowest in surface sediments, ranging from ~22 to ~21‰ in the top 8 cm of M1 and from ~23 to ~22.5‰ in the top 4 cm of M5. Below, the δ13C-TOC increases, and then stabilizes around ~18.5 to ~17‰ below 16 cmbsf at M1 and below 20 cmbsf at M5. These changes in δ13C-TOC indicate a possible shift in the predominant OM source from surface sediment to the bottom of the bioturbation zone (also see next section). By comparison, the TOC contents in surface sediments do not show consistent trends across stations (Supplementary Fig. 3c,d). At M1, TOC decreases from ~3.8‰ in surface sediments to ~3‰ at 20 cmbsf, and fluctuates between 2.5 to 3.7‰ below. At M5, TOC increases from ~2.5‰ at the seafloor to ~4‰ at 20 cmbsf, and fluctuates between ~2.9 and 4.7‰ below.

Comparison to Non-Bioturbated Station in Aarhus Harbor. Surface sediments at a non-bioturbated station in Aarhus Harbor (AHB) display a different trend in bacterial and archaeal abundances compared to the stations in Aarhus Bay (Supplementary Fig. 4). Here the abundances of archaeal and bacterial 16S gene co-vary in surface sediment, with both showing overall decreases with depth. The BAR profile fluctuates between 3:1 and 9:1 and decreases only slightly with depth. Compared to the stations in Aarhus Bay, BARs at AHB are consistently lower than at M21, M22A, M24, M26, M27A, M29A, and M1. Compared to M1-2012 and M5-2012, BARs at AHB are clearly lower in the top 6 cmbsf, but display similar values below 6 cmbsf.

Relationships between OM content and composition and microbial abundances. If BARs in surface sediments are influenced by macrofaunal activity, then macrofaunal influences on the supply and quality of OM are a logical driver of the observed bacterial and archaeal abundance trends, given that the majority of sediment microorganisms are organoheterotrophs. Similarly, the amount and composition of OM below the bioturbation zone would likely have an effect on the abundances of Bacteria and Archaea in deeper non-bioturbated layers. We expanded our OM data set and compared bacterial and archaeal abundances to proxies for OM content (TOC, TN) and OM quality (C:N, δ13C-TOC, and δ15N-TN) at all Aarhus Bay sites. As already seen for M1-2012 and M5-2012, TOC values in surface sediment are scattered showing no clear relationship with bacterial or archaeal abundances (Fig. 4a; range: 1–5 wt%). Yet, the general decrease in TOC with depth below the bioturbated surface layer (range: ~1.5 to 2.8 wt% below 4 mbsf) falls within the same depth interval over which a gradual decrease in bacterial and archaeal abundances and BARs was observed. The same trend, i.e. scatter with no clear relationship to gene abundances or BARs in surface sediment, and a possible relationship in deeper layers can be seen for TN (Fig. 4b). The glacial till layer at M21 remains an exception, with TOC (0.4 to 0.6 wt%) and TN (~0.02 wt%) values that are lower and far outside the range of all other samples. C:N ratios show an overall increase throughout the top ~1 m of sediment, with the steepest increase in the bioturbated layer, and decrease below (Fig. 4c). The range varies from ~8.8 to 11.6 in surface sediment, to ~10.7 to 13.0 at 1 mbsf, and ~10.0 to 12.3 below 4 mbsf, and is consistent with a predominantly marine origin of the OM. Exceptions are the glacial till layer at M21 and the deep terrestrial soil layer at M1; here, consistent with the terrestrial origin of the OM, C:N values are higher and range from ~17 to 18.

The strongest similarity to BAR trends in surface layers occurs in the δ13C-TOC values (Fig. 4d). An increase, from values of ~24 to ~21‰ to values of ~21 to ~18‰, occurs from surface sediment to sediment immediately underlying the bioturbated sediment layer, indicating a change in OM composition. Below the depth of
bioturbation, $\delta^{13}C$-TOC values decrease gradually to values that are mostly between $-23$ and $-20\%$ below 4 mbsf. Exceptions are the deep terrestrial layers at M21 and M1 (M21: $-23.7$ to $-22.9\%$; M1: $-27.2\%$). The $\delta^{15}N$-TN also show a decrease in surface sediments (Fig. 4e); however, this decrease matches the BAR changes to a lesser extent than the $\delta^{13}C$-TOC data, as it occurs more steadily throughout the top 50 cmbsf, from $+5.3$ to $+6.2\%$ at the sediment surface to $+2.0$ to $+4.2\%$ at 50 cmbsf. Below 50 cmbsf, the $\delta^{15}N$-TN increases slightly, fluctuating between $+3.0$ to $+5.3\%$ below 4 mbsf. Again, the terrestrial layers are exceptions (M21: $-1.0$ to $+1.8\%$, M1: $+1.6\%$).  

Statistical relationships between 16S gene abundances and geochemical data. To assess the significance of observed trends in BARs in relation to geochemical data, we performed several statistical analyses. Using Kruskal-Wallis tests with Mann-Whitney post-hoc tests, we compared archaeal and bacterial 16S gene abundances and BARs between four biogeochemically defined zones (bioturbation zone, sulfate zone, SMTZ, and methane zone). Based on the uniform $^{210}$Pb$\text{supp}$ profiles at M1 and M5, which indicate macrofaunal sediment mixing to at least 14 cmbsf, the sediment layer from 0–14 cmbsf was conservatively defined as the bioturbation zone. The sulfate zone extended from below 14 cmbsf to the SMTZ, the SMTZ was the interval in which concentration ratios of sulfate to methane ranged from 2:1 to 1:2, and the methanogenesis zone was the zone below the SMTZ where methane accumulated to millimolar concentrations. As a result of site-specific differences in microbial activity, the depths of the sulfate zone, SMTZ and methane zones vary between stations (Supplementary Table 1, Supplementary Fig. 2). Kruskal-Wallis tests revealed that mean archaeal 16S gene abundances do not differ significantly between any of the biogeochemical zones, except between the sulfate zone and the methane zone (Table 1). By contrast, mean bacterial 16S gene abundances, and BARs are significantly higher in bioturbated sediments than in the other biogeochemical zones. Mean bacterial 16S gene abundances and BARs are also

| Archea | Bioturbation zone | Sulfate zone | SMTZ | Methane zone |
|-------|------------------|-------------|------|-------------|
| Bioturbation zone | / | 0.30 | 0.38 | 0.13 |
| Sulfate zone | 0.30 | / | 0.17 | $<0.05$ |
| SMTZ | 0.38 | 0.17 | / | 0.69 |
| Methane zone | 0.13 | $<0.05$ | 0.69 | / |

| Bacteria | Bioturbation zone | Sulfate zone | SMTZ | Methane zone |
|----------|------------------|-------------|------|-------------|
| Bioturbation zone | / | $<0.01$ | $<0.01$ | $<0.01$ |
| Sulfate zone | $<0.01$ | / | $<0.05$ | $<0.01$ |
| SMTZ | $<0.01$ | $<0.05$ | / | 0.38 |
| Methane zone | $<0.01$ | $<0.01$ | 0.38 | / |

| BAR | Bioturbation zone | Sulfate zone | SMTZ | Methane zone |
|-----|------------------|-------------|------|-------------|
| Bioturbation zone | / | $<0.01$ | $<0.01$ | $<0.01$ |
| Sulfate zone | $<0.01$ | / | $<0.01$ | $0.005$ |
| SMTZ | $<0.01$ | $<0.01$ | / | 0.09 |
| Methane zone | $<0.01$ | $<0.005$ | 0.09 | / |

Table 1. Comparison of bacterial and archaeal 16S gene abundances and BARs across four biogeochemical zones based on Kruskal-Wallis tests with Mann-Whitney post-hoc tests. Statistically significant p-values are shown in bold. Only stations from which all 4 biogeochemical zones were reached by coring were included (M24, M26, M27A, M29A, M1 (2009), and M5-2012).
but are less evident at a non-bioturbated control site in Aarhus Harbor. The dominance of bacterial 16S genes
archaeal and bacterial abundances and BARs below the depth of bioturbation (Fig. 4a,b, Supplementary Fig. 4b).
Table 2). In addition to the biogeochemical zone, TOC and TN data suggest that OM content is a main driver of
δ13C-TOC and decreases to circa 1:1 in the SMTZ and methane zone (Supplementary Fig. 2, Supplementary
dances of archaeal and bacterial 16S genes approach an average ratio of 2:1, a ratio that remains stable throughout
below (14–20 cmbsf), macrofaunal particle mixing and irrigation cease, and relative abundances of archaeal and
reworking and bioirrigation (Figs 2 and 3). Below (~6–14 cmbsf), as macrofaunal activity gradually decreases
gradients in 210Pbunsupp,
indicate that the dominance of Bacteria over Archaea in surface sediment might be the outcome of benthic mac-
Our results are in line with previous studies showing bacterial dominance in surface sediments22, 24–26 and similar
population sizes of Archaea and Bacteria in subsurface sediments7, 9, 12 (Fig. 1). Yet, our results for the first time
show that Archaea are better adapted to life under chronic energy

Method checks. Due to well-documented DNA extraction and PCR biases, which may affect the results of
PCR-based quantifications of microorganisms, we performed additional method checks (for details see ‘Method
comparisons’ in Supplementary Results). We compared bacterial and archaeal 16S gene abundance data to the
same data obtained with a widely used commercial DNA extraction kit and using two additional bacterial and
two additional archaeal 16S gene primer combinations. Independent of the DNA extraction method and primer
choice, the overall trends in bacterial and archaeal 16S genes were confirmed (ref. 14, Fig. 10c,d; Supplementary
Fig. 6). We also compared total 16S gene copy numbers to cell count data, to determine whether 16S gene copy
numbers were reproducible using a different microbial quantification method. Total 16S gene abundance trends
showed good agreement with the cell count data (Supplementary Fig. 8).

Discussion
Our results are in line with previous studies showing bacterial dominance in surface sediments22, 24–26 and similar
abundance profiles. Consequently, the bioturbation depth determined from δ13C-TOC displayed trends in relation to the
δ13C-TOC. Since both reworking and bioirrigation are spatially and temporally

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population sizes of Archaea and Bacteria in subsurface sediments7, 9, 12 (Fig. 1). Yet, our results for the first time
indicate that the dominance of Bacteria over Archaea in surface sediment might be the outcome of benthic mac-
rofaunal activity. BARs range from 10 to 60 in the top sediment layer (0–6 cmbsf), where the absence of depth
gradients in 210Pb, δ13C-TOC, DIC and sulfate concentrations indicate high rates of macrofaunal sediment
reworking and bioirrigation (Figs 2 and 3). Below (~6–14 cmbsf), as macrofaunal activity gradually decreases
with depth, bacterial 16S gene abundances decrease by one order of magnitude, whereas archaeal 16S gene
abundances increase (Figs 1 and 2, Supplementary Fig. 2). A clear increase in δ13C-TOC over the bioturbated interval
(Fig. 4d) suggests a shift in OM composition from the highly bioturbated surface layer to deeper non-bioturbated
sediments. Further below (14–20 cmbsf), macrofaunal particle mixing and irrigation cease, and relative abundances
of archaeal and bacterial 16S genes approach an average ratio of 2:1, a ratio that remains stable throughout
the sulfate zone and decreases to circa 1:1 in the SMTZ and methane zone (Supplementary Fig. 2, Supplementary
Table 2). In addition to the biogeochemical zone, TOC and TN data suggest that OM content is a main driver of
archaeal and bacterial abundances and BARs below the depth of bioturbation (Fig. 4a,b, Supplementary Fig. 4b).

The observed trends in archaeal and bacterial abundances are replicated across all 8 stations in Aarhus Bay,
but are less evident at a non-bioturbated control site in Aarhus Harbor. The dominance of bacterial 16S genes
in bioturbated sediment is, moreover, independent of primer choice (Supplementary Fig. 6) and reproducible
with a commercial DNA extraction kit15. Comparisons between total abundances of 16S genes and total cell
abundances from four stations indicate that 16S gene copy numbers per cell are between 1–5 in most samples,
with site-specific averages ranging from 2.0 to 4.3 (Supplementary Fig. 8b). These values are typical of microbial
populations in other environments35, 36 and indicate good agreement between two independent quantification
methods.

The mechanisms by which sediment macrofauna might affect archaeal and bacterial gene abundances in surface
sediments are diverse. Valentine27 proposed that Archaea are better adapted to life under chronic energy
limitation than Bacteria, and that Bacteria on the other hand are better adapted to dynamic and variable environ-
ments where energy limitation is not permanent. These notions may help explain why macrofaunal activity selects
for bacteria-dominated communities in surface sediments. Macrofaunal reworking strongly increases microbial
energy availability by introducing labile OM from overlying water and the sediment surface to deeper layers, and
by converting large organic particles into more microbially accessible small organic particles and dissolved OM36, 37, 38.
Bioirrigation introduces high-energy electron acceptors, e.g. O2 and nitrate, which increase rates of OM
degradation and promote chemical and chemolithotrophic reoxidation of reduced metals, sulfide, and ammon-
ium produced by microbial respiration39–41. Since both reworking and bioirrigation are spatially and temporally
variable processes, bioturbation inevitably converts surface sediments into a heterogeneous and dynamic environment where a stable redox zonation is absent. Translated to patterns we observe in Aarhus Bay, macrofauna may favor dominance of Bacteria in bioturbated surface sediments by enhancing the input and availability of both OM and high-energy electron acceptors, and by increasing physiological stress through redox fluctuations. Archaea may in turn increase relative to Bacteria in deeper sediments with little to no bioturbation due to chronic energy limitation, resulting from the predominance of recalcitrant OM and low-energy electron acceptors, such as sulfate and CO₂, or due to reduced physiological stress resulting from a more stable redox potential.

The notion of enhanced energy availability due to macrofaunal input and breakdown of OM in surface sediment is consistent with our OM data and with previous studies indicating a decrease in cell–specific sulfate reduction rates below 10 cm at M1. Though our TOC, TN, or C:N data show no clear trend in relation to BARs, our δ¹³C-TOC profiles show a clear shift from bioturbated surface to non-bioturbated subsurface sediment that matches the depth and rates of sediment reworking based on ²¹⁰Pb data (Fig. 4). δ¹³C-TOC values of −24 to −21‰ in surface sediment (Figs 2i,j and 4d) are consistent with a mainly phytoplankton origin of OM, whereas the shift to values of −20 to −18‰ in deeper, non-bioturbated layers indicates an increased contribution of a different OM pool with higher δ¹³C-TOC values. This OM pool likely includes seagrass, which has a typical δ¹³C-TOC range of −15 to −3‰, is resistant to microbial degradation, consisting largely of recalctrant polymers such as cellulose, hemicellulose, lignin, and cutin, and is widely found in Aarhus Bay (Zostera marina).

Selective feeding and mineralization of labile, more rapidly biodegradable phytoplankton-derived OM over more recalcitrant OM from seagrass would explain the observed increase in δ¹³C-TOC from the sediment surface downward through the bioturbation zone. Bacterial dominance in bioturbated sediments would then be promoted by higher growth rates of Bacteria compared to Archaea in the presence of labile OM.

Support for a prominent role of bioirrigation in Aarhus Bay comes from past measurements of the depth distributions of aerobic and anaerobic respiration reactions at M1, model calculations which suggest that ~90% of sulfate in sediment of M1 and M5 is introduced by bioirrigation, and from our DIC and sulfate data. In the SML, where BARs are the highest (Figs 1 and 2), bioirrigation activity is the highest based on sulfate and DIC concentrations that are close to those in overlying seawater (Fig. 2g,h). Below the SML, the slopes of the sulfate concentration profiles and the δ¹³C-DIC profiles indicate reduced but significant introduction of seawater by bioirrigation down to ~20 cmbsf (Fig. 2g–j). If bioirrigation is the main driver of the observed distributions in bacterial and archaeal gene abundances in surface sediments, then the higher BARs in bioturbated sediments could result from increased energy availability due to import and regeneration of high-energy electron acceptors or enhanced physiological stress due to redox fluctuations caused by fluctuating concentrations of these same high-energy electron acceptors.

If enhanced energy input and/or redox fluctuations due to macrofauna explain the dominance of Bacteria over Archaea within the bioturbated surface layer, then what controls the abundances of Bacteria and Archaea in sediments underlying the bioturbation zone? Our data indicate that two factors are important: the biogeochemical zone, i.e. sulfate zone, SMTZ, or methanogenesis zone, and the total amount of OM. Given that sulfate-dependent anaerobic oxidation of methane (AOM) is a quantitatively important process in SMTZs, that is only performed by Archaea, and that methanogenesis is the dominant respiration pathway in sulfate-depleted sediments of the methanone zone, and also only performed by Archaea, it is not surprising that BARs are higher in sulfate zones, where AOM and methanogenesis are less important, compared to SMTZs or methane zones. More interesting is, perhaps, that the quantitative importance of OM content (TOC, TN) in controlling archaeal and bacterial abundances in subsurface sediments appears to exceed the quantitative importance of OM composition (C:N, δ¹³C-TOC) (Supplementary Fig. 4b). A possible explanation is that energy availability, and thus energy substrate availability, to microorganisms in subsurface sediments is directly related to OM content. Accordingly, differences in OM lability/reactivity or nutrient content may be less important in subsurface layers, because the bulk of OM is highly recalcitrant, having been selectively stripped of readily bioavailable and nutrient-rich OM fractions by diagenetic processing while passing through shallow layers.

While the use of qPCR on archaean and bacterial 16S genes provides an efficient means to identify depth trends in abundances of the domains Archaea and Bacteria, insights into possible changes in archaeal and bacterial community structure are not obtained. Using the same archaean and bacterial primer combinations as in the qPCR primer comparison (for details see ‘Additional Primer Checks’ in Supplementary Methods and Supplementary Fig. 7), we therefore sequenced 16S genes of Archaea and Bacteria from 5 depth horizons at M1. Independent of primer pair, we observe a clear shift in archaean and bacterial community composition from bioturbated to non-bioturbated sediment (Fig. 5, Supplementary Fig. 8). For Archaea, the main vertical changes in community composition are the disappearance of Marine Group I Thaumarchaeota from bioturbated sediment to underlying horizons (Fig. 5a). Furthermore, Methanomicrobia, which account for a small fraction of reads in the bioturbation and sulfate zones, become more prominent in the SMTZ and methane zone. Neither of these changes is surprising. Marine Group I Thaumarchaeota consist of aerobic ammonia- and urea-oxidizing Archaea that are widespread in marine water columns and presumably extend their range into surface sediments thanks to bioirrigating macrofauna which regularly import O₂ into these layers. Methanomicrobia consist of methanogenic and anaerobic methanone-oxidizing Archaea. Methanogens are mainly found in sediments where respiration reactions involving O₂, nitrate, metal oxides, or sulfate, which have higher energy yields than methanogenesis, are absent, whereas anaerobic methane oxidation occurs mainly in SMTZs in marine sediments. The vertical changes in dominant bacterial groups are even more dramatic than those in Archaea (Fig. 5b). Acidobacteria, Bacteroidetes, Deltaproteobacteria, and Gammaproteobacteria account for ~80% of sequence reads in the bioturbated layer, and for <20% of sequence reads in underlying horizons. By contrast, in the four subsurface horizons, the bacterial community consists to ~70% of Chloroflexi, Planctomycetes, Candidate Division J1 (Atribacteria), Candidate Division OD1, and Candidate Division OP8. Though a division of these groups into functional guilds is confounded by the metabolic diversity within certain groups, e.g. Delta- and Gammaproteobacteria, and limited
Figure 5. Archaeal and bacterial community compositions in the bioturbation zone (5 cmbsf), the sulfate zone (80 cmbsf), the SMTZ (160 cmbsf), the shallow methane zone (310 cmbsf), and in a freshwater peat layer within the deep methane zone (1055 cmbsf) at M1. Archaeal 16S gene libraries were produced using the primer pairs A1 (Arc806F - Arc958R), A2 (Arc806F - Arc915Rmod), and A3 (Arc915Fmod - Arc1059R), while the bacterial libraries were produced using the primer pairs B1 (Bac8F - Bac338Rabc), B2 (Bac806F_mod2 - Bac908R), and B3 (Bac908F - Bac1075R). Operational Taxonomic Units (OTUs; 97% cutoff) were assigned to class level for Archaea and to phylum level for Bacteria using Mothur, based on an alignment of amplicons to the 16S rRNA reference sequences in SILVA Release 119. See Supplementary Methods for more information.
knowledge on others, e.g., Chloroflexi, Bacteroidetes, and the Candidate divisions, the clear shift in bacterial community composition from bioturbated surface sediments to underlying horizons that are cut off from fresh OM and high-energy electron acceptor inputs by macrofauna is apparent.

Interestingly, despite the observed changes in BARs from the sulfate zone to the methane zone at M1, the fraction of typical marine sulfite reducing Bacteria, such as Deltaproteobacteria and Firmicutes, is small in non-bioturbated sediment of the sulfate zone (≈10%) and does not change from the sulfate to the methane zone. Furthermore, methanogens also only account for a small fraction of archaeal sequence reads in the methane zone (≈5–25%). This trend of sulfate reducers and methanogens accounting for only minor fractions of the total microbial community in sulfate reducing and methanogenic subsurface sediments has been documented previously. It suggests that the change in BARs from the sulfate zone to the methane zone at M1, and possibly other stations in Aarhus Bay, is not solely driven by population trends among sulfate reducers and methanogens, but that the population sizes of other groups of Bacteria and Archaea are changing as well.

By quantifying archaeal and bacterial 16S gene abundances, we are ultimately aiming to quantify relative abundances of Archaea and Bacteria. This approach has several caveats, however. Mean ± Standard deviations of 16S gene copies per cell differ between these domains, with mean bacterial (average ± SD: 4.12 ± 2.75 copies cell$^{-1}$) exceeding mean archaeal values (average ± SD: 1.61 ± 0.88 copies cell$^{-1}$) by a factor of 2.5 (https://rrndb.umms.med.umich.edu/). This results in uncertainties whenever bacterial and archaeal cell abundances are estimated by quantifications of 16S genes. For instance, in the sulfate zone and SMTZ, where bacterial 16S genes are on average twice as abundant as archaeal 16S genes, actual abundances of bacterial and archaeal cells may be in the same range. In the methane zone, where 16S gene abundances of Archaea and Bacteria are roughly equal, archaeal cells may even outnumber bacterial cells. Moreover, if bacterial groups with high average 16S gene copy numbers per cell, such as Acidobacteria (average ± SD: 1.38 ± 0.48 copies cell$^{-1}$), Bacteroidetes (average ± SD: 3.21 ± 1.74 copies cell$^{-1}$), and Deltaproteobacteria (average ± SD: 2.70 ± 1.33 copies cell$^{-1}$); these latter groups account for 10–15%, 8–30% and 20–30% of bacterial 16S gene sequences in the bioturbated layer, respectively. Furthermore, if 16S gene copy numbers were substantially higher in Bacteria inhabiting bioturbated surface sediments compared to subsurface sediments, then our calculated 16S genes per cell at M1 should clearly decrease with depth; however, we see no decrease in mean 16S genes per cell with sediment depth (Supplementary Fig. 8b). Consequently, our data indicate that the vastly higher bacterial than archaeal 16S gene abundances reflect a strong numerical dominance of Bacteria over Archaea in the bioturbated layer and are not the result of higher bacterial 16S gene copy numbers per cell.

In conclusion, our study indicates that bioturbation is a key driver of microbial community structure in marine surface sediments. Previous studies have documented shifts in relative abundances of Archaea and Bacteria similar to the ones we observe in surface sediments, but not linked these changes to bioturbation. Other studies have documented differences in microbial biomass and community structure between burrow walls and surrounding sediment. Our data suggest that bioturbation is a key driver behind the observed shifts in BARs in surface sediments, and strongly influences microbial community structure not only in burrow walls but throughout the entire bioturbated sediment interval. The observed changes in BARs in Aarhus Bay match the typical depth interval of macrofaunal activity in marine sediments. Given that it is a globally important process that dominates particle transport in marine surface sediments and occurs from organic-rich coastal environments to oligotrophic ocean gyres, bioturbation could be a widely overlooked driver of microbial community structure in marine surface sediments worldwide. The strong shifts in BARs from the bioturbated surface layer to the underlying non-bioturbated layers suggest that the lower parts of bioturbation zones represent an important ecological transition, in which surface sediment microbial communities change to phylogenetically and physiologically distinct subsurface communities. Future studies involving closely monitored manipulation experiments will reveal how macrofaunal influences on OM content, electron acceptor availability, and redox fluctuations affect microbial community structure, and how the influence of bioturbation on microbial community structure depends on macrofaunal species composition, macrofaunal feeding mode, and the broader environmental setting.

Methods

**Sediment sampling.** All stations in central Aarhus Bay were sampled by gravity corer and by Rumohr Lot corer in October 2009 or May 2010 (Supplementary Table 1). Use of gravity corers enabled recovery of sediments down to depths of 3–11 m. Use of 1-m Rumohr corers enabled the recovery and high-resolution sampling of undisturbed surface sediments to depths of 0.5–0.8 m. Sediment sampling for analyses of archaeal and bacterial 16S gene abundances was done every 8 cm in Rumohr cores and every 10–20 cm in gravity cores.

Stations M1 and M5 were revisited in March 2012 for additional sampling with a Rumohr corer to study relationships between archaeal and bacterial 16S gene abundances and macrofaunal bioturbation in surface sediments at high depth resolution. The sampling resolution was every 2 cm in the upper 20 cm, every 4 cm from 20–40 cm, and every 8 cm below. Additionally, a 15-cm push core was taken in Aarhus Harbor in June 2012 and sectioned at 2-cm depth intervals.

**Site descriptions.** Aarhus Bay is a shallow semi-enclosed embayment on the transition between the North Sea and the Baltic Sea, characterized by 6–7 m thick methane-rich Holocene mud overlying late glacial clay-silt
and till and early Holocene peat\textsuperscript{31,38}. According to \textsuperscript{14}C-dating of marine bivalve and gastropod shells, the postglacial marine transgression which led to fully marine conditions started in Aarhus Bay about 8,500 years ago\textsuperscript{31,39}. Most of the anaerobic microbial mineralization of OM is coupled to the reduction of sulfate and only a few percent to the production of methane\textsuperscript{31}. Sulfate reduction rates are highest in the uppermost anoxic sediment and show a subsurface peak in the SMTZ\textsuperscript{33,66}.

The eight stations of this study are from the central basin of Aarhus Bay (Supplementary Fig. 1): M21 and M22A have no significant methane accumulation in the cored interval. M24 and M26 have shallow methane production but no free CH\textsubscript{4} gas in the cored interval, and M27A, M29A, M1, and M5 have shallow methane production and free CH\textsubscript{4} gas at variable depths within the cored interval (Supplementary Table 1). Sediments at all stations consist of organic-rich mud with two exceptions: M21 has organic-poor glacial sand below \textasciitilde2 m sediment depth, whereas M1 has early Holocene peat below 10.5 m sediment depth. With the exception of M21, depth profiles of pore water concentrations of sulfate, methane, dissolved inorganic carbon (DIC) and other ions, TOC, total organic nitrogen (TON), $\delta^{13}$C-TOC, $\delta^{15}$N-TN, and C:N, and rates of anaerobic mineralization of OM have been published (M22A-M29A\textsuperscript{32,35}, M1\textsuperscript{60,61}, M5\textsuperscript{33,66}).

All eight Aarhus Bay stations show visual evidence of bioturbation based on sediment color, and presence of burrows and macrofauna. These observations are consistent with sulfur, iron, and manganese balance calculations and $^{210}$Pb\textsubscript{unapp} distributions indicating that surface sediments are geochemically influenced by bioturbation that maintains oxidizing, yet anoxic conditions\textsuperscript{31}. Macrofaunal densities are high (2800 \pm 700 individuals/m\textsuperscript{2}), and the benthic fauna is dominated by bivalves (\textit{Abra alba}, \textit{Mysella bidendata}, \textit{Corbula gibba}, \textit{Macoma calcarea}), polychaetes (e.g., \textit{Terebellides stromi}, \textit{Nephys ciliata}, \textit{Nephys sp}., \textit{Heteromastus filiformis}, \textit{Pectinaria sp}.), and echi"noderms (e.g., \textit{Ophura albida}, \textit{Echinocardium cordatum})\textsuperscript{36,61}. An overview of the size, burial depth and feeding mode of these macrofauna is shown in Supplementary Table 3. Surface deposit and suspension feeding bivalves (mainly \textit{A. alba}) are by far the most abundant group, accounting for \textasciitilde50–90\% of macrofaunal individuals and \textasciitilde40–2–95\% of total macrofaunal biomass\textsuperscript{64}. Polychaetes are the second most abundant group, accounting for \textasciitilde5–30\% of all individuals and \textasciitilde1–60\% of total biomass, and are dominated by the genus \textit{Nephys}. A site in Aarhus Harbor (AHB) served as a bioturbation control site due to the absence of visible bioturbation.

Detailed descriptions of the Rumohr cores taken in March 2012 from M1 and M5, and the push core from AHB are provided in the Supplementary Methods.

**Sediment DNA extraction.** Samples for DNA extraction were taken by sterile, cut-off syringes and stored at \textasciitilde80 °C. DNA was extracted according to Lever \textit{et al} \textsuperscript{34} using a protocol that had been developed during extensive extraction tests with Aarhus Bay sediment. After initial removal of sDNA, i.e. dissolved DNA and DNA that was likely adsorbed to sedimentary matrices and became dissolved after soaking in an alkaline phosphate buffer, cells were chemically lysed via lysis protocol III, washed with chloroform-isoamylalcohol, precipitated by ethanol-NaCl solution with linear polyacrylamide (LPA) as a co-precipitant (20 \textmu{}L of each primer, 2 \textmu{}L of each master mix, 2 \textmu{}L of molecular-grade water. Plasmids of 16S genes from Methanosarcina-affiliated archaea and Sphingomonadales-affiliated bacteria were used as standards. Standard series covered gene concentrations of 10\textsuperscript{5}–10\textsuperscript{10} copies \mu{}L\textsuperscript{1}. The thermal cycler protocol consisted of (1) enzyme activation and initial denaturation at 95°C for 5 min; (2) 45 cycles of (a) denaturation at 95°C for 30 s, (b) annealing at 55°C for 30 s, (c) elongation at 72°C for 10 s (Archaee) or 15 s (Bacteria), and (d) fluorescence measurement at 78°C (Archaee) or 80°C (Bacteria) for 15 s; and (3) a stepwise melting curve from 95°C to 55°C in 1 min to check for primer specificity. All standards and samples were analyzed in triplicate. To check for PCR inhibition, we compared gene quantifications of original extracts to 1:10 dilutions. Due to slight PCR inhibition in original extracts, values of 1:10 dilutions were used to estimate the abundances of 16S genes in samples.

**Quantification of archael and bacterial 16S genes.** Abundances of archael and bacterial 16S genes were determined on a Roche LightCycler 480. Archaeal genes were quantified using the primer sets Arch 806F\textsuperscript{65} (\texttt{ATT AGA TAC CCS BGT AGT CC}) and Arch 958R\textsuperscript{66} (\texttt{GCG GTC GTT GAM TCC AAT}), while bacterial genes were quantified using 8Fmod\textsuperscript{68} (\texttt{AGA GTT TGA TYM TGG CTC AG}) and 338Rabc\textsuperscript{68} (\texttt{GCG WCC CGT AGG WGT}). Reaction volumes of 20 \textmu{}L consisted of 1 \times Light Cycler Mastermix (Roche), 1 \textmu{}g DNA template, and molecular-grade water. Plasmids of 16S genes from Methanosarcina-affiliated archaea and Sphingomonadales-affiliated bacteria were used as standards. Standard series covered gene concentrations of 10\textsuperscript{5}–10\textsuperscript{10} copies \mu{}L\textsuperscript{1}. The thermal cycler protocol consisted of (1) enzyme activation and initial denaturation at 95°C for 5 min; (2) 45 cycles of (a) denaturation at 95°C for 30 s, (b) annealing at 55°C for 30 s, (c) elongation at 72°C for 10 s (Archaee) or 15 s (Bacteria), and (d) fluorescence measurement at 78°C (Archaee) or 80°C (Bacteria) for 15 s; and (3) a stepwise melting curve from 95°C to 55°C in 1 min to check for primer specificity. All standards and samples were analyzed in triplicate. To check for PCR inhibition, we compared gene quantifications of original extracts to 1:10 dilutions. Due to slight PCR inhibition in original extracts, values of 1:10 dilutions were used to estimate the abundances of 16S genes in samples.

**Microbial cell counts.** Microbial populations at M22A, M27A, M29A, M1, and M5 were also quantified by cell counts on cores taken in May 2010. Cells were extracted using a protocol optimized for Aarhus Bay sediments\textsuperscript{35}, stained with Sybr Green I, and enumerated by epifluorescence microscopy with an automated stage and slide-loader combined with an image analysis software program\textsuperscript{69}. Cell abundances were then compared to the sum of archael and bacterial 16S genes measured by qPCR. Whenever cell abundances were not available from the same depth as qPCR data, data from the closest available depths were compared (depth difference: \textasciitilde4 cm for samples above 1 mbsf, and \textasciitilde13 cm for samples below 1 mbsf).

**Geochemical analyses.** Methods for the sampling and quantification of sulfate, methane, DIC, and $\delta^{13}$C-DIC in cores taken in October 2009 and May 2010 are described in ref. \textsuperscript{32}. Two Rumohr Lot cores were sampled at each station, one for methane quantification, DNA extraction, and cell counts, the other for sulfate, DIC, and $\delta^{13}$C-DIC. One gravity core was taken at each station, which was used for all of the above geochemical analyses, DNA extractions, and cell counts.

Sulfate, DIC, and $\delta^{13}$C-DIC measurements on samples obtained from M1 and M5 in March 2012 and the Aarhus Harbor reference site were done in the same way, except that porewater samples were obtained by centrifugation rather than by rhizons and processed as follows. For sulfate measurements, 1.5-mL Eppendorf tubes
were filled with sediment and centrifuged for 10 min at 10,000 × g and 4 °C. The supernatant was transferred and immediately acidified and stripped of hydrogen sulfide by gentle bubbling with CO₂ (g) for 1 min, after which samples were frozen at −20 °C until analysis. DIC samples were obtained by filling up 50-mL Falcon tubes with sediment, centrifugation for 10 min at 5,000 × g, followed by filtration of supernatant through 0.22-μm pore size polyethersulfone membrane filters (Cronus biotech Ltd.) into 2-mL borosilicate vials that were filled completely and stored at +4 °C until analysis. Methane sampling and analyses on March 2012 cores was done as explained in ref. 32.

TOC, TN, 813C-TOC, and 815N-TN were sampled and analyzed as described previously. Samples were always taken in direct vicinity to samples used for DNA extractions within the same Rumohr Lot or gravity core.

Radioisotope measurements and modeling. 210Pb (half-life: 22.3 years), 226Ra (1,600 yrs), and 137Cs (30.2 years) radioactivity were measured on Rumohr cores taken at M1 and M5 in March 2012 and analyzed via gamma-spectrometry. 2-cm depth intervals were pooled and dried as described for TOC. Measurements were carried out on a Canberra ultralow-background Ge-detector. 210Pb was measured via its gamma-peak at 46.5 keV, 226Ra via the granddaughter 214Pb (peaks at 295 and 352 keV), and 137Cs via its peak at 661 keV. 210Pbunapp was calculated by subtracting supported 210Pb values based on 226Ra decay from measured 210Pb values. Long-term (>1000 years) sedimentation rates at both stations were determined by 14C (half-life: 5,730 years) analyses on bivalve shells (Rasmussen et al., unpubl.) and indicate net erosion at M1, where surface sediments have an extrapolated age of 2,800 yrs, and a net sedimentation rate of 1 mm yr⁻¹ at M5. The mixing coefficient, D₀, was calculated using the following equation from Huh and Su, assuming a net sedimentation rate of 0 mm yr⁻¹ at M1 and 1 mm yr⁻¹ at M5 and a constant D₀ in the layer, in which the concentration of 210Pbunapp decreases exponentially with depth

\[ C = C₀ \exp((-S - (S² + 4 \lambda D₀b³)/2D₀) Z) \]

where C = activity of the radionuclide (Bq kg⁻¹ dwt) at depth z (cm), C₀ = activity at the surface, λ = radioactive decay constant (0.0311 yr⁻¹), D₀ = mixing coefficient (cm² yr⁻¹), S = sedimentation rate (cm yr⁻¹). For a review on the use of radionuclides to measure rates of macrofaunal sediment reworking, we recommend Maire et al. and the references within.

Statistical analyses. To determine whether archaeal and bacterial 16S genes, BARs, and cell abundances differed significantly between the four biogeochemical zones, we performed Kruskal-Wallis tests with Mann-Whitney post-hoc tests using the software PAST (http://folk.uio.no/ohammer/past/). We used station-specific averages for each biogeochemical zone (Supplementary Table 2) in our calculations. All treeline analyses and calculations of R² were done in Microsoft Excel. Spearman Rank Correlation tests were performed using the free, open-source online statistical package Wessa.net (http://www.wessa.net/).

Archaeal and bacterial community compositions at M1. To examine the archaeal and bacterial community composition change at the four biogeochemical zones, we sequenced the 16S gene PCR products generated by three archaeal and three bacterial primer combinations from five depths at M1 (5, 80, 160, 310 and 1,055 cmbsf) using an Ion Personal Genome Machine (PGM™) system (Ion Torrent, Life Technologies). For further details, see the Supplementary Methods.

References

1. Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C. & D’Hondt, S. Global distribution of microbial abundance and biomass in subseafloor sediment. Proc. Natl. Acad. Sci. USA 109, 16213–16216, doi:10.1073/pnas.1203849109 (2012).
2. Parkes, R. J. et al. A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere: Geosphere interactions. Mar. Geol. 352, 409–425, doi:10.1016/j.margeo.2014.02.009 (2014).
3. Lloyd, K. G. et al. Predominant archaea in marine sediments degrade detrital proteins. Nature 496, 215–218, doi:10.1038/nature12033 (2013).
4. Evans, P. N. et al. Methane metabolism in the archaeal phylum Bathynarchaeota revealed by genome-centric metagenomics. Science 350, 434–438, doi:10.1126/science.aac7745 (2015).
5. Meng, J. et al. Genetic and functional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. The ISME journal 8, 650–659, doi:10.1038/ismej.2013.174 (2014).
6. Rinke, C. et al. Insights into the phylogeny and coding potential of microbial dark matter. Nature 499, 431–437, doi:10.1038/ nature12352 (2013).
7. Lloyd, K. G., May, M. K., Kevorkian, R. T. & Steen, A. D. Meta-analysis of quantification methods shows that archaea and bacteria have similar abundances in the subseafloor. Appl. Environ. Microbiol. 79, 7790–7799, doi:10.1128/AEM.02990-13 (2013).
8. Schippers, A. & Neretin, L. N. Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. Environ. Microbiol. 8, 1251–1260, doi:10.1111/j.1462-2920.2006.01019.x (2006).
9. Webster, G. et al. Subsurface microbiology and biochemistry of a deep, cold-water carbonate mound from the Porcupine Seabight (IODP Expedition 307). Environ. Microbiol. 11, 239–257, doi:10.1111/j.1462-2920.2008.01759.x (2009).
10. Parkes, R. J. et al. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature 436, 390–394, doi:10.1038/nature03796 (2005).
11. Biddle, J. F. et al. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proc. Natl. Acad. Sci. USA 103, 3846–3851, doi:10.1073/pnas.0600351010 (2006).
12. Schippers, A., Kock, D., Höft, C., Köweker, G. & Siegert, M. Quantification of microbial communities in subsurface marine sediments of the Black Sea and off Namibia. Front. Microbiol. 3, 1–11, doi:10.3389/fmicb.2012.00016 (2012).
13. Buongiorno, Y. et al. Inter-laboratory quantification of Bacteria and Archaea in deeply buried sediments of the Baltic Sea (IODP Exp. 347). IEM Microbiol. Ecol. doi:10.1093/femsme/fsx007 (2017).
14. Lever, M. et al. A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. Front. Microbiol. 6 (2015).
Molari, M., Giovannelli, D., D’Errico, G. & Manini, E. Factors influencing prokaryotic community structure composition in sub...

Sahm, K. & Berninger, U. G. Abundance, vertical distribution, and community structure of benthic prokaryotes from permanently...

Valentine, D. L. Adaptations to energy stress dictate the ecology and evolution of the Archaea.

Klappenbach, J., Dunbar, J. M. & Schmidt, T. M. rRNA operon copy number reflects ecological strategies of bacteria.

Lauro, F. M. The genomic basis of trophic strategy in marine bacteria.

Davies, P., Morvan, C., Sire, O. & Baley, C. Structure and properties of fibres from sea-grass (Zostera marina).

Boström, C. J., Polanski, M., Ekblom, M., Hederstedt, M., Berntsson, L. & Walkusz, W. Biodiversity of benthic microbial communities in bioturbated coastal sediments is controlled by geochemical microniches.

Johansen, B. B. Biogeochemistry of chemoautotrophic bacteria. In: The Prokaryotes (eds Shlegel, H. G. & Bowien, B.). Springer (2014).

Jørgensen, B. B. Biogeochemistry of chemoautotrophic bacteria. In: The Prokaryotes (eds Ehleringer, J. & Rundel, P.). Springer-Verlag (1989).

Christensen, B., Vedel, A. & Kristensen, E. Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding (Nereis virens) polychaetes. Mar. Ecol. Prog. Ser. 192, 203–217, doi:10.3354/meps192203 (2000).

Jørgensen, B. B. Biogeochemistry of chemooautotrophic bacteria. In: Autotrophic bacteria (eds Shleger, H. G. & Bowien, B.). Science Technical Publishers, Madison, W. H and Springer-Verlag (1989).

Hoehler, T. M. & Jørgensen, B. B. Microbial life under extreme energy limitation. Nat. Rev. Microbiol. 11, 83–94, doi:10.1038/nrmicro2939 (2013).

Fry, B. & Sherr, E. B. δ13C measurements as indicators of carbon flow in marine and freshwater ecosystems. In: Stable isotopes in ecological research (eds Ehleringer, J. & Rundel, P.). Springer-Verlag (1989).

Gacia, E., Duarte, C. M. & Middelburg, J. J. Carbon and nutrient deposition in a Mediterranean seagrass (Posidonia oceanica) meadow. Limnol. Oceanogr. 47, 23–32, doi:10.4399/lo.2002.47.1.0023 (2002).

Davies, P., Morvan, C., Sire, O. & Bale, C. Structure and properties of fibres from sea-grass (Zostera marina). J. Mater. Sci. 42, 165, 297–309, doi:10.1007/s10853-006-0546-1 (2007).

Boström, C. et al. Distribution, structure and function of Nordic eelgrass (Zostera marina) ecosystems: implications for coastal management and conservation. Aquat. Conserv.: Mar. Freshwat. Ecosyst. 24, 410–434 (2014).

Swan, B. K. et al. Genomic and metabolic diversity of Marine Group I Thaumarchaeota in the mesopelagic of two subtropical gyres. PLoS one 9, e93380, doi:10.1371/journal.pone.0093380 (2014).

Whitman, W. B., Bowen, T. L. & Boone, D. R. The Methanogenic Bacteria. In: The Prokaryotes (eds Rosenberg, E., DeLong, E., Lory, S., Stackebrandt, E. & Thompson, F.). Springer (2014).

Knittel, K. & Boetius, A. Anaerobic oxidation of methane: progress with an unknown process. Annu. Rev. Microbiol. 63, 311–334, doi:10.1146/annurev.micro.61.080706.093130 (2009).

Lever, M. A. Functional gene surveys from ocean drilling expeditions - a review and perspective. FEMS Microbiol. Ecol. 84, 1–23, doi:10.1111/j.1574-6941.2013.012051 (2013).

Bertics, V. J. & Ziebis, W. Biodiversity of benthic microbial communities in bioturbated coastal sediments is controlled by geochemical microniches. The ISME journal 3, 1269–1285, doi:10.1038/ismej.2009.62 (2009).
52. Papaspyrou, S., Gregersen, T., Kristensen, E., Christensen, B. & Cox, R. P. Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes (Nereis diversicolor and N. virens). *Mar. Biol.* 148, 541–550, doi:10.1007/s00227-005-0105-3 (2006).
53. Stief, P. & De Beer, D. Bioturbation effects of Chironomus riparius on the benthic N-cycle as measured using microsensors and microbiological assays. *Aquat. Microb. Ecol.* 27, 175–185, doi:10.3354/ame027175 (2002).
54. Boudreau, B. P. Mean mixed depth of sediments: The wherefore and the why. *Limnol. Oceanogr.* 43, 524–526, doi:10.4399/lo.1998.43.3.0524 (1998).
55. Middelburg, J. J., Soetaert, K. & Herman, P. M. J. Empirical relationships for use in global diagenetic models. *Deep-Sea Res. (I Oceanogr. Res. Pap.)* 44, 327–344, doi:10.1016/S0967-0637(96)00101-X (1997).
56. Teal, L. R., Bulling, M. T., Parker, E. R. & Solan, M. Global patterns of bioturbation intensity and mixed depth of marine soft sediments. *Aquatic Biol.* 2, 207–218, doi:10.3354/ab00052 (2008).
57. Meischner, D. & Rumohr, J. A light weight high momentum gravity corer for subaqueous sediments. *Senckenb. Marit.* 6, 105–117 (1974).
58. Laier, T. & Jensen, J. B. Shallow gas depth–contour map of the Skagerrak-western Baltic Sea region. *Geo-Mar. Lett.* 27, 127–141, doi:10.1007/s00367-007-0066-2 (2007).
59. Jensen, J. B., Bennik, O., Lemke, W. & Kuijpers, A. The Storebælt gateway to the Baltic. *Geol. Surv. Denmark Greenl. Bull.* 7, 45–48 (2004).
60. Leloup, J. et al. Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): Abundance and diversity related to geochemical zonation. *Environ. Microbiol.* 11, 1278–1291, doi:10.1111/j.1462-2920.2008.01855.x (2009).
61. Holmikvist, L., Fertelman, T. G. & Jørgensen, B. B. A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochim. Cosmochim. Acta* 75, 3581–3599, doi:10.1016/j.gca.2011.03.033 (2011).
62. Langerhuus, A. T. et al. Endospore abundance and di- amino acid modeling of bacterial turnover in holocene marine sediment (Aarhus Bay). *Geochim. Cosmochim. Acta* 99, 87–99, doi:10.1016/j.gca.2012.09.023 (2012).
63. Glud, R. N., Gundersen, J. K., Røy, H. & Jørgensen, B. B. Seasonal dynamics of benthic O2 uptake in a semienclosed bay: Importance of diffusion and faunal activity. *Limnol. Oceanogr.* 48, 1265–1276, doi:10.4399/lo.2003.48.3.1265 (2003).
64. Fallesen, G. *The ecology of macrozoobenthos in Aarhus Bay, Denmark*. PhD thesis, University of Stirling (1994).
65. Takai, K. & Horikoshi, K. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl. Environ. Microbiol.* 66, 5066–5072, doi:10.1128/AEM.66.10.5066-5072.2000 (2000).
66. DeLong, E. F. Archaea in coastal marine environments. *Proc. Natl. Acad. Sci. USA* 89, 5685–5689, doi:10.1073/pnas.89.12.5685 (1992).
67. Juretschko, S. et al. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitroscococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. *Appl. Environ. Microbiol.* 64, 3042–3051 (1998).
68. Daims, H., Brühl, A., Amann, R., Schleifer, K. H. & Wagner, M. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. *Syst. Appl. Microbiol.* 22, 434–444, doi:10.1016/S0308-8146(99)00053-8 (1999).
69. Morono, Y. & Inagaki, F. Automatic slide-loader fluorescence microscope for discriminative enumeration of subselafloor life. *Sci. Drill* 9, 32–36, doi:10.5194/sd-9-32-2010 (2009).
70. Huh, C. A. & Su, C. C. Sedimentation dynamics in the East China Sea elucidated from 219Pb, 137Cs and 239,240Pu. *Mar. Geol.* 160, 183–196, doi:10.1016/S0025-3227(99)00020-1 (1999).
71. Maire, O. et al. Quantification of sediment reworking rates in bioturbation research: a review. *Aquat Biol* 2, 213–218, doi:10.3354/ab00053 (2008).
72. Hammer, Ø., Harper, D. A. T. & Ryan, P. D. Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 9–18 (2001).
73. Wessa, P. Free statistics software, Office of Research Development and Education, version 1.1.23–r7, http://www.wessa.net (2017).

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**Author Contributions**

M.A.L. designed the research; X.C., T.J.A., Y.M., and M.A.L. performed the research; X.C., T.J.A., Y.M., F.I., B.B.J., and M.A.L. analyzed the data; X.C. and M.A.L. wrote the paper with significant input from all other co-authors.

**Additional Information**

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