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ENVIRONMENTAL MICROBIOLOGY

Diel Rhythm Does Not Shape the Vertical Distribution of Bacterial and Archaeal 16S rRNA Transcript Diversity in Intertidal Sediments: a Mesocosm Study

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Abstract In intertidal sediments, circadian oscillations (i.e., tidal and diel rhythms) and/or depth may affect prokaryotic activity. However, it is difficult to distinguish the effect of each single force on active community changes in these natural and complex intertidal ecosystems. Therefore, we developed a tidal mesocosm to control the tidal rhythm and test whether diel fluctuation or sediment depth influence active prokaryotes in the top 10 cm of sediment. Day- and nighttime emersions were compared as they are expected to display contrasting conditions through microphytobenthic activity in five different sediment layers. A multiple factor analysis revealed that bacterial and archaeal 16S ribosomal RNA (rRNA) transcript diversity assessed by pyrosequencing was similar between day and night emersions. Potentially active benthic *Bacteria* were highly diverse and influenced by chlorophyll *a* and phosphate concentrations. While in oxic and suboxic sediments, *Thaumarchaeota* Marine Group I (MGI) was the most active archaeal *phylum*, suggesting the importance of the nitrogen cycle in muddy sediments, in anoxic sediments, the mysterious archaeal C3 group dominated the community. This work highlighted that active prokaryotes organize themselves vertically within sediments independently of diel fluctuations suggesting adaptation to physicochemical-specific conditions associated with sediment depth.

Keywords *Archaea* · *Bacteria* · Active community · Intertidal mudflat · Mesocosm · Diel cycle · Microphytobenthic biofilm

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Introduction

Diversity, activity, and abundance of bacterial and archaeal communities in coastal mudflats vary greatly in space and time because of sharp gradients in salinity, temperature, nutrient concentration, oxygen, and redox potential [1–4]. Other factors, such as depth [2, 5] and/or diel rhythm (i.e., day/night conditions) [6], may influence microbial community composition in intertidal zones. In these areas, the vertical distribution of prokaryotic communities depends on climatic factors such as storms, precipitation [7], tide, wind, or wave action [8–10] as well as variations of biotic and other abiotic determinants such as salinity and temperature [11], organic carbon availability [12–14], inorganic nutrient content [15], bioirrigation and bioturbation [16–18], and microphytobenthic activity [19, 20].

The high productivity recorded in intertidal mudflats [21] is partly due to the formation of a transient microphytobenthic biofilm that can develop at the surface during daily tidal exposure periods [22–24]. Studies have shown that

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microphytobenthos (MPB) impacted the prokaryotic community structure and activity by producing large amounts of extracellular polymeric substances [25] but also by uptaking large quantities of nutrients during the day [19, 26]. Few studies have investigated the fate of the organic matter produced and its impact on prokaryotic community composition during nighttime high tide [8, 9]. At high tide, Thornton et al. [27] observed a drastic uptake of ammonium during the day compared to the night, suggesting shifts in the microbial groups involved in the nitrogen cycle. Moreover, a modeling approach in a subtidal estuary revealed a strong decrease in nitrification and denitrification rates associated with an increase in photosynthesis during the daytime [20], consistent with competition between MPB and prokaryotic communities for nutrient uptake [19]. All of this highlighted the importance of considering the co-occurrence of microphytobenthos and prokaryotes for understanding the ecological drivers of active bacterial and archaeal community structure. Together, these studies have revealed that intertidal systems experience drastic changes during the tidal or/and diel cycle and highlight the complexity of microbial interactions in a spatially heterogeneous and temporally varying habitat.

By considering a simplified intertidal system (i.e., at low tide), we aimed at testing whether microphytobenthic activity and/or depth represented by the variation in environmental conditions shapes active prokaryotic assemblages over a 24-h period. We hypothesized that, at low tide, prokaryotic 16S ribosomal RNA (rRNA) transcript diversity may be different between day and night conditions as a result of an intense benthic primary production by diatoms during the day. The mix of regional and local factors that influence prokaryotic community composition in marine ecosystems and their relations are difficult to evaluate with in situ surveys [28]. Thus, we designed a tidal mesocosm experiment, with day and night exposure, to assess bacterial and archaeal 16S rRNA transcript diversity from five different depths of intertidal muddy sediments.

Experimental Procedures

Sampling and Tidal Mesocosm Setup

Muddy sediment samples (mean grain size = 11.7 μm ± 1.63 μm) were collected in April 2013 on the ridges at low tide in the intertidal mudflat of Marennes-Oléron Bay (France, N 45° 54′ 53″, W 01° 05′ 23″). Cores from 0 to 10 cm below sediment surface (bsf) were sliced into five layers (0–0.5, 0.5–1, 1–2, 2–5, and 5–10 cm). Next, in the laboratory, each layer was sieved by hand without seawater addition through 500-μm-pore-sized mesh to remove macrofauna (i.e., grazers and bioturbators). The experimental PVC cores (height = 12 cm; diameter = 12.5 cm) were reconstituted layer by layer and arranged in the main tank according to previous work [29] (Fig. 1a).

The tidal mesocosms consisted of a main tank and an overflow tank (Fig. 1a). Two identical tidal mesocosms were designed to study both day and night low tides (Fig. 1b). For the daytime mesocosm (Day, Fig. 1b), the experiment was started at 4 am with a simulated high tide; 15 h of light was followed by a dark cycle (light/dark 15:9, corresponding to the field light/dark conditions in April 2013) with a low or high tide every 6 h that was simulated by gradually rising or lowering the water level. For the nighttime mesocosm (Night, Fig. 1b), the experiment was started at 4 am with a simulated high tide; 15 h of dark was followed by a light cycle (light/dark 15:9) with a low or high tide every 6 h. The cores were placed in the dark submitted to tidal cycle in the mesocosm for a 2-week period before the experiment for nutrient equilibration. Water collected from Marennes-Oléron Bay was filtered through a 0.2-μm mesh and used for the overflow and recirculation system. After the settling period of 2 weeks, the cores were inoculated using 500-μm-sieved fresh MPB biofilm (chlorophyll *a* concentration = 118.96 and 87.19 μg g^{-1} sediment (sed) dry weight (DW), in the Day and Night experiment, respectively) from the sampling site (0–0.5 cm bsf) [30] and fixed diel and tidal rhythms were applied. The core sampling was carried out at low tide (1 PM, Fig. 1b) in the two mesocosms 5 days after MPB inoculation at the maximum MPB biofilm growth period. Three cores were randomly harvested from each mesocosm and treated as biological replicates, and the corresponding five layers were collected separately. All abiotic and biotic parameters were analyzed from the three replicate cores at each depth in the two mesocosms as described below except for RNA extraction (two cores were used as biological duplicates).

Abiotic and Biotic Parameters

The depth temperature profiles were measured with five 3.1-cm-length Hobo sensors (Hobo Pro V2, Bourne, MA, USA) fixed on a homemade stick that was vertically pushed into a specific sediment core to stabilize the sensors at five depths (0.5, 1, 2, 5, and 10 cm below the sediment surface).

The pore water pH and salinity were immediately measured on a supernatant after sediment centrifugation (15 min, 3000×*g* at 8 °C) with a pH probe (Eutech Instruments PC150, Landsmeer, The Netherlands) and a conductivity meter Cond 3110, TetraCon 325, WTW, Weilheim, Germany), respectively.

The chlorophyll *a* concentration (Chl *a*), used as a proxy of algal biomass, was quantified with fluorimetry (640 nm, Turner TD 700, Turner Designs, USA) using 50 mg of freeze-dried sediment extracted in the dark at +4 °C with 90% acetone (mixed by repeatedly turning) and centrifuged (10 min, 3500×*g*, +8 °C). The Chl *a* concentrations were

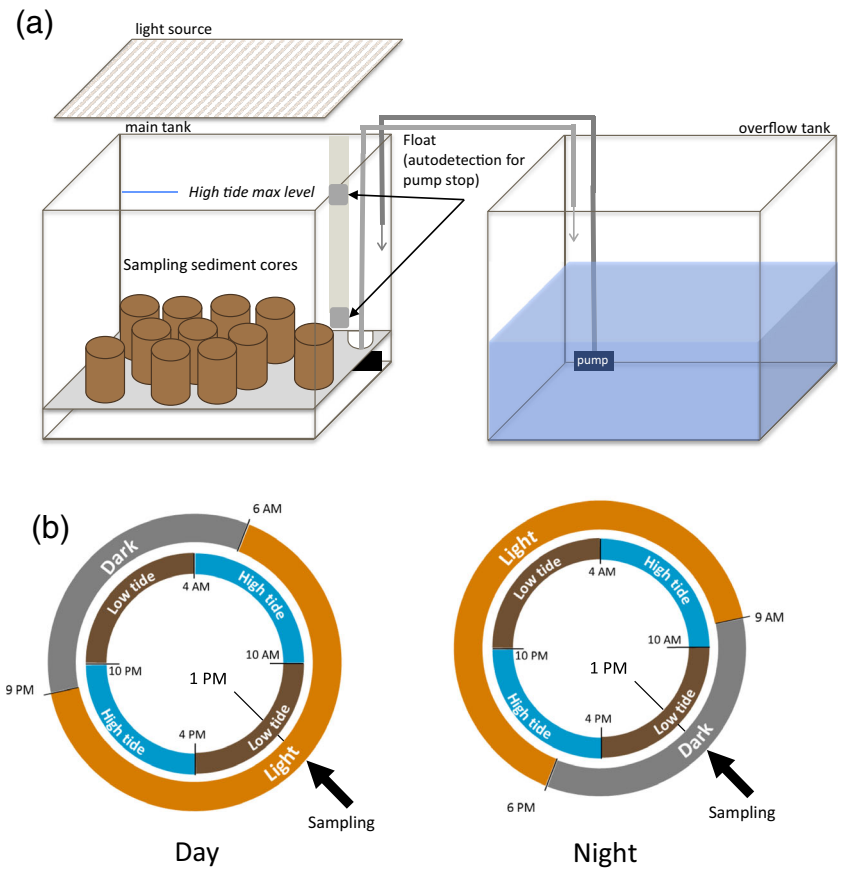

Fig. 1 Schematic view of the experimental design. **a** One mesocosm consisted of two tanks: a main and overflow tank. **b** Schedule for the Day and Night mesocosms

expressed as microgram per microgram sediment DW according to Lorenzen [31].

RNA Extraction

RNA was extracted for each depth from two biological duplicate cores of the two mesocosms (n = 20) with 5 g of liquid nitrogen-frozen sediment using a Power Soil™ Total RNA Isolation Kit (MOBIO, CA, USA) following the manufacturer's recommendations. Isolated RNAs were quantified using fluorimetry (NanoDrop, ND-800, Thermo Scientific, USA). The RNA samples were tested for the presence of contaminating genomic DNA using PCR (b341F-518R for *Bacteria* [32] and 931F-M1100r for *Archaea* [33]; Online Resource Table S1) and then reverse transcribed with random hexamers using SuperScript III (Invitrogen, Life Technologies, USA).

454 Pyrosequencing and Bioinformatic Analysis

Amplification of the V3-V5 region of the 16S rRNA transcripts was performed for all the extracted samples (n = 20) using the following primer set: for *Bacteria* 563F and 907R [34] and for *Archaea* 519F [35] and 915R [36] (Online Resource Table S1). Libraries were created for all five layers and the two mesocosms (the duplicates were pooled before sequencing, n = 10). Pyrosequencing was accomplished using the GATC platform (Konstanz, Germany) with a Roche 454 GS-FLX system with titanium chemistry. All of the sequences were checked against the following quality criteria: (i) no Ns; (ii) quality score ≥27 and >30 for *Bacteria* and *Archaea*, respectively, according to the PANGEA process [37]; (iii) a minimum sequence length of 200 bp; and (iv) no sequencing error in the forward primer. After removing the primer sequence, the putative chimeras were detected using UCHIME [38]. The remaining reads were clustered at 97% similarity threshold [39] with UCLUST [40]. The representative operational taxonomic units (OTUs) were used to build phylogenetic trees with FastTree [41] for the main taxonomic groups with reference sequences. These references were extracted to the SSUREF SILVA 119 [42] according to the following criteria: length >1200 bp, quality score >75%, and a pintail value >50. The taxonomic annotation was conducted with the nearest neighbor (NN) method. This process was implemented in the pipeline Phylogenetic Analysis of Next-generation AMplicons (PANAM, https://github.com/panammeb/) and is described more in detail in a related study [43]. To retain and compare the largest possible number of samples, the datasets were resampled down to 13,000 and 800 sequences for *Bacteria* and *Archaea*, respectively (normalization process).

The resulting datasets consisted of 25,600 OTUs (at 97% threshold) for *Bacteria* and 1065 OTUs (at 97% threshold) for *Archaea*. Singletons and plastids were removed for further analyses resulting in a dataset containing 9664 bacterial OTUs and 465 archaeal OTUs.

Statistical Analysis

All values are presented as the mean (±standard error), and statistical analyses were performed with R software (R Core Team, 2013). The most influential environmental parameters measured driving both the bacterial and archaeal OTU_{97}-relative abundance dataset were identified using a forward selection procedure [44] with the function forward.sel in the package "packfor" [45].

A multiple factor analysis (MFA) was performed [46] using the package FactoMineR [47] to evaluate the distribution of bacterial and archaeal 16S rRNA transcript relative abundance (Online Resource Fig. S1). The overall analysis involved a principal component analysis (PCA) applied to the entire table, in which each column of group *i* was standardized by the inverse of the first eigenvalue of the separate PCA of group *i*. Thus, the MFA was run using normalized bacterial and archaeal relative abundance (through the 16S rRNA transcripts abundance) datasets as two different groups of variables. The 16S rRNA transcript distributions in both bacterial and archaeal datasets were not impacted by the normalization process (Procrustes correlation = 0.98, $P < 0.01$) by the removal of either plastids or singletons (Procrustes correlation = 0.94–0.98, $P < 0.01$). A third group of variables, containing the plastid abundance (resulting from sequencing dataset) and significant environmental parameters identified by the forward selection, was then added as additional variables to the MFA final ordination (Online Resource Table S2).

Additionally, to explore the mean influence of a given factor (i.e., sediment depths), we decomposed the total inertia (Online Resource Fig. S1) of the standardized table using between- and within-class analyses with the functions dudi.pca, bca, and wca in the "ade4" package [48].

Results

Experimental Conditions at Low Tide in the Daytime Mesocosm (Day) and Nighttime Mesocosm (Night)

In the sediment surface, the photosynthetic active radiation was 231 μmol photons m^{-2} s^{-1} (±46 μmol photons m^{-2} s^{-1}) during the simulated daytime low tide. The ambient temperature varied between daytime and nighttime due to light warming, and the mean temperature was 31.2 °C (±6.3 °C) and 20.2 °C (±0.7 °C), respectively. The sediment temperature was 25.7 °C (±0.3 °C) during the Day and 19.9 °C (±0.1 °C) during the Night experiment (Online Resource Fig. S2). The salinity varied from 32.0 (±0.2) (Day) to 30.5 (±0.3) (Night), and the pH exhibited depth variations ranging from 6.8 to 7.6 in both Day and Night (Online Resource Fig. S2).

Microphytobenthic Biofilm

Microphytobenthic biomass and potential activity was estimated through the quantification of chlorophyll *a* concentration (Online Resource Fig. S2) and a record of 16S rRNA plastid transcripts, respectively. In the surface sediment (upper 0.5 cm), the chlorophyll *a* concentration was higher in the Day treatment (173.6 ± 20.8 μg g^{-1} sed DW) than in the Night treatment (90.1 ± 4.2 μg g^{-1} sed DW). These surficial concentrations represent an increase of 46 and 3% of chlorophyll *a* content during Day and Night experiments, respectively, compared to the initial concentration of chlorophyll *a* added in each experiment. Then, the chlorophyll *a* concentration decreased with increasing depth (i.e., 28 μg g^{-1} sed DW after 2 cm bsf (Online Resource Fig. S2). This result could be congruent with a lower relative abundance of 16S rRNA transcript plastid-affiliated sequences in the Night treatment. During the Day exposure, these plastid-related sequences were abundant between 0 and 1 cm bsf, representing 56.7% of relative abundance of 16S rRNA transcript plastid-affiliated sequences in comparison to 39.4% during the Night exposure.

A Specific Distribution of Potentially Active Bacterial and Archaeal Community Structures Revealed by a Multiple Factor Analysis

A forward selection of 17 environmental variables highlighted the factors explaining a significant part of differences in bacterial and archaeal transcript diversity. The bacterial 16S rRNA transcript distribution was better explained by chlorophyll *a* and phosphate concentrations, while archaeal 16S rRNA transcript distribution was better explained by depth and pH (Online Resource Table S2). Then, a MFA was used to investigate the distribution of both bacterial and archaeal 16S rRNA transcripts (Fig. 2 and Online Resource Fig. S2). The first dimension of the ordination discriminated the five sediment layers from the surface to deep layers (Fig. 2a). A between-class analysis coupled to the MFA revealed that than 66% of the total MFA inertia was due to sediment depth rather than Day/Night exposure, indicating that the diversity of both bacterial and archaeal 16S rRNA transcripts was largely structured along a vertical gradient in the sediments (Fig. 2). This diversity was almost identical during the Day and Night treatments (5% total MFA inertia explained by the Day/Night factor; Fig. 2).

The bacterial taxa that contributed the most to the first dimension were δ-*Proteobacteria* (4.14% contribution of the

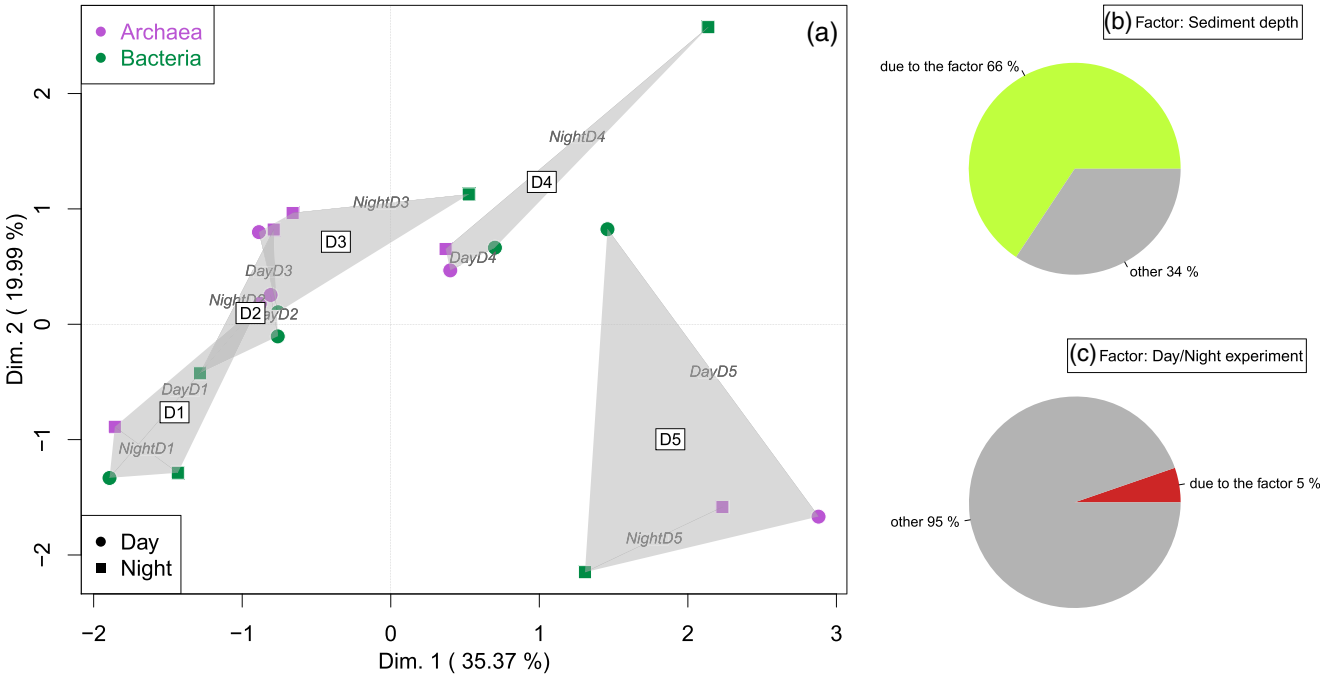

Fig. 2 Individual map from the multiple factor analysis (MFA) and inertia due to each factor. **a** Each sample name represents the barycentre of the two positions according to the dataset: *Bacteria* (*green*) and *Archaea* (*purple*). The samples from Day experiment are circles and Night experiment samples are in *squares*. Sediment depths (D1 = 0–0.5 cm; D2 = 0.5–1 cm; D3 = 1–2 cm; D4 = 2–5 cm; D5 = 5–10 cm) are highlighted using ordihull from the "vegan" package [49]. Pie charts (**b** and **c**) show the inertia of the MFA due to each factor: **b** sediment depth and **c** Day/Night experiment

variable to the first dimension), *Deferribacteres* (3.80%), environmental group (i.e., BHI80-139, Candidate division OD1, Candidate division OP3, Candidate division TM7, Candidate division WS3, EM19, GOUTA4, Hyd24-12, JL-ETNP-Z39, LD1-PA38, NPL-UPA2, TA06, and TM6) (3.64%), *Chloroflexi* (3.56%), and *Armatimonadetes* (3.13%) (Online Resource Fig. S3).

The archaeal taxa that contributed the most to the first dimension of the MFA were archaea group C3 (6.88% contribution of the variable to the first dimension), *Euryarchaeota* Rice Cluster V (6.63%), *Euryarchaeota* LDS group (5.92%), *Euryarchaeota Thermoplasmata* (5.82%), and *Crenarchaeota* Marine Benthic Group B (5.76%) (Online Resource Fig. S3).

Bacterial 16S rRNA Transcript Diversity

Coverage ranged from 71.8 to 88.4%, with maximum coverage recorded at the surface. Both the coverage values (Online Resource Table S3) and rarefaction curves (Online Resource Fig. S4a) indicated that we assessed a large part of the active bacterial community. Chao1 index indicated that richness was higher in lower sediment layers (i.e., from 1 to 10 cm below the sediment surface) than in the upper one (i.e., from 0 to 1 cm bsf) during the Day exposure (Online Resource Table S3). During the Night exposure, richness was higher (from 11,625 to 13,709 estimated OTUs) from 1 to 5 cm bsf (Online Resource Table S3).

After the removal of singletons and plastid-related sequences, the bacterial dataset consisted of 9663 OTUs (represented by 87,805 sequences). Bacterial sequences were related to 42 phyla affiliated with *Proteobacteria*, *Planctomycetes*, *Firmicutes*, and *Acidobacteria* (65.63, 5.36, 4.16, and 3.08% of the sequences, respectively, Fig. 3).

The *Proteobacteria* transcript abundance ranged from 54.04 to 77.22% of the sequences. Together, the γ-*Proteobacteria* and the δ-*Proteobacteria* dominated the proteobacterial assemblage (from 70.94 to 82.17% of the proteobacterial-affiliated sequences). The γ-*Proteobacteria* represented 38.90% of the proteobacterial-affiliated sequences and were mainly retrieved in the sediment surface, from 0 to 2 cm bsf, with the exception of the Night treatment between 5 and 10 cm bsf. The δ-*Proteobacteria* represented 36.86% of the total proteobacterial sequences and were mainly retrieved in the deeper horizon (from 2 to 10 cm bsf). The α-*Proteobacteria* sequences were less abundant, accounting for 13.76 to 22.26% of the proteobacterial sequences, and their abundances were maximal from 1 to 5 cm bsf. The β- and ε-*Proteobacteria*, were represented by less than 5% of the total proteobacterial sequences (Fig. 3).

Planctomycetes abundance ranged from 3.40 to 8.55% of the transcript relative abundance in each sample, except for the Day treatment's surficial sediments (0–0.5 cm bsf), in which they represented only 1.64%. *Planctomycetes* consisted in *Phycisphaerae* (43.86%) and *Planctomycetacia* (18.07%).

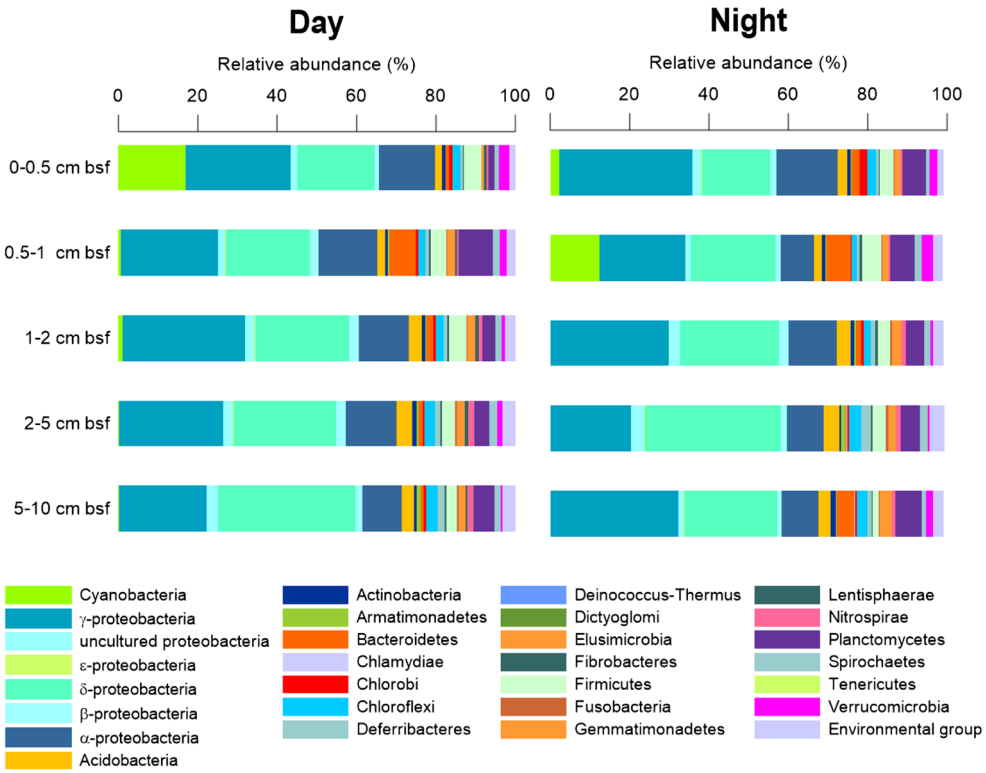

Fig. 3 Distribution of bacterial 16S rRNA transcripts. The relative abundance of the 16S rRNA transcripts and the affiliation of the total bacterial operational taxonomic units (OTUs 97%) at the phylum level (except for *Proteobacteria*, class level) between the two Day and Night treatments among the five layers below the sediment surface (bsf). Environmental group contained BHI80-139, Candidate division OD1, Candidate division OP3, Candidate division TM7, Candidate division WS3, EM19, GOUTA4, Hyd24-12, JL-ETNP-Z39, LD1-PA38, NPL-UPA2, TA06, and TM6. Note that plastids originated from microalgae were removed from the dataset

A large portion of the *Planctomycetes*-affiliated sequences fell into environmental groups such as the vadinBA30 marine sediment group. Interestingly, no anammox-like *Planctomycetes* (*Brocadiales*) were recorded in our dataset.

Archaeal 16S rRNA Transcript Diversity

For the archaeal 16S rRNA transcripts dataset, the rarefaction curves indicated that the sequencing depth captured almost all of the diversity present in the natural community (Online Resource Fig. S4b). Chao1 index showed that during both the Night and Day exposures, sediment layers from 1 to 2 cm below the surface, richness was higher (Online Resource Table S2). Archaeal sequences were affiliated with *Thaumarchaeota*, *Crenarchaeota*, *Bathyarchaeota* (all part of the TACK superphylum [50]), and *Euryarchaeota* (Fig. 4).

Between 0 and 5 cm bsf, *Thaumarchaeota* dominated the potentially active archaeal assemblage (from 53 to 83% of the transcript abundance). *Thaumarchaeota* were mainly represented by Marine Group I (MGI), ranging from 4.96 to 83.20% of the total transcript number, in the deeper and the surface layers, respectively. To a lesser extent, *Halobacteriales* were retrieved, ranging from 3.01 to 23.61% of the total transcript number, and the poorly known Rice Cluster V was represented from 0.79 to 9.44% of the total transcript abundance, with maximal occurrence in the surficial horizon (between 0 and 5 cm bsf).

Between 5 and 10 cm bsf, a contrasting picture was observed, with a decrease in the abundance of the thaumarchaeal transcripts (less than 12.2% of the total sequences) in favor of the archaeal group C3 (from 73.6 to 77.8%), also called MCG15 [51].

Discussion

Previous research has reported that temperature and salinity impact the microbial communities in marine systems [52] and global surveys have attempted to explore the overall microbial diversity and distribution in oceans [53–55]. In this study, we have developed an original tidal mesocosm experiment to explore the effect of diel rhythms and/or sediment depth on the relative abundance of both bacterial and archaeal 16S rRNA transcripts originating from five sediment depths of an intertidal mudflat incubated at two different conditions. This work highlighted a greater impact of sediment depth rather than diel cycle on potentially active prokaryotes in intertidal sediments colonized by a microphytobenthic biofilm. While experimental studies such as mesocosms do not replicate environmental conditions exactly, they consisted in valuable systems to simplify complex environments. By controlling specific environmental variables, mesocosm experiments allowed investigating local-scale community dynamics [28] but also elucidating microbial response to the given test conditions. In the present work, the similarity between Day and Night in terms of pH or

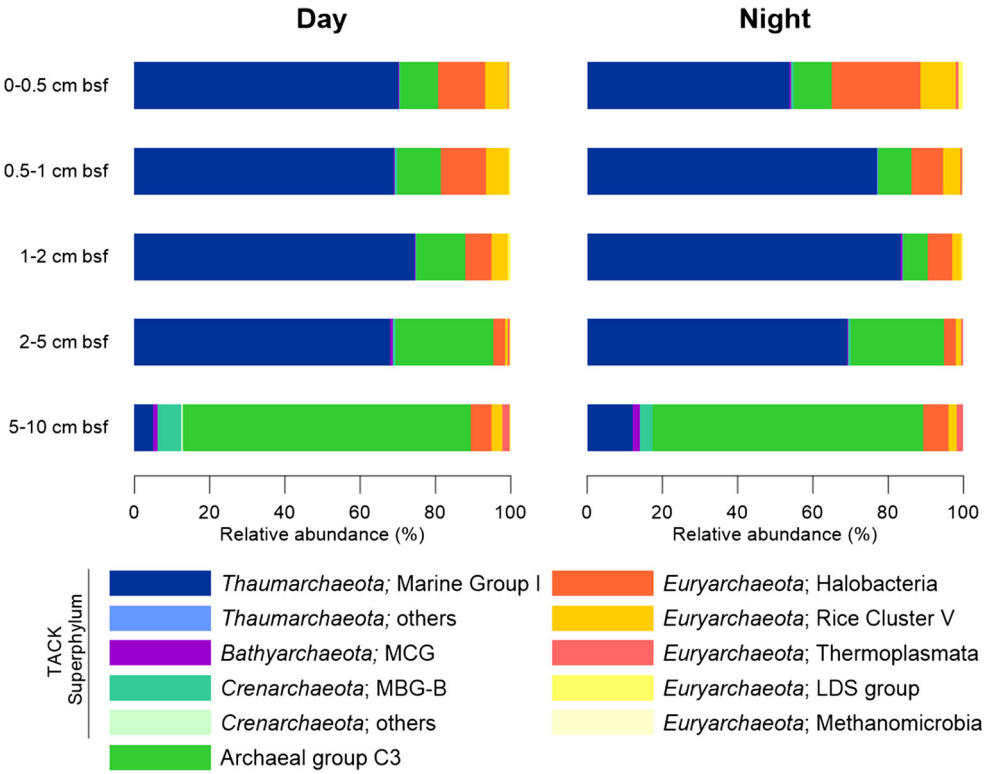

Fig. 4 Distribution of archaeal 16S rRNA transcripts. The relative abundance of the 16S rRNA transcripts and the affiliation of the total archaeal operational taxonomic units (OTUs 97%) at the class level between the two Day and Night treatments among the five layers below the sediment surface (bsf)

prokaryotic abundance may indicate that there was no tank-induced variation and that the system was valuable for investigating the impact of specific factors.

One important issue of the procedure used for this mesocosm experiment was that the cores used in this study were not kept intact from the field as macrofauna was removed by sieving the sediment by depth and the cores were then reassembled. This uncommon reassembling procedure inspired by Michaud et al. [29] allows a faster recovery of nutrient vertical profiles. While it could affect the diversity of bacterial and archaeal 16S rRNA transcripts, the core reassembly procedure may impact both Day and Night cores by the same way and thus do not interfere in the interpretation of the results.

The results indicated that microphytobenthic (MPB) abundances decreased with depth according to light penetration (data not shown) and oxygen content (representing from 39 to 60% of the bacterial transcript relative abundance between 0 and 1 cm bsf and representing less than 20% of bacterial transcript relative abundance from 1 to 10 cm bsf). The best blast match of these plastid transcripts sequences suggests that the microphytobenthic communities mainly belong to the diatom order *Naviculales* (nblast best hits: AF514848, AF514855, AF514847; Poulin et al. [56]). Interestingly, during the Night exposure, the plastids were primarily retrieved from 0.5 to 1 cm bsf, suggesting that the diatoms might not migrate to the sediment surface. This phenomenon may have resulted in the absence of the vertical migration of MPB in the mesocosm system during night, which is congruent with a peak in cell division in the aphotic zone of the sediment [26].

This study highlighted that bacterial and archaeal 16S rRNA transcript diversity used as a proxy of potentially active bacterial and archaeal community layout is not modified by the diel cycle. The use of this proxy should be interpreted with caution considering the environmental parameters and specific taxa due to some inconsistent relationships between 16S rRNA and activity [57]. However, previous work reported the value of examining communities at the 16S rRNA transcripts [58, 59]. The MFA indicated that the diel rhythm had a weak effect on both potentially active *Archaea* and *Bacteria* (5% total inertia, Fig. 2c) and that the prokaryotic communities were mainly shaped by sediment depth (66% of total inertia of the MFA, Fig. 2b). These issues are crucial because it means that a same pool of microorganisms incubated in two mesocosms and sampled at two different conditions did not follow a neutral random distribution but can exhibit a similar active organization with vertical-driven assemblages. A limited number of archaeal and bacterial phyla, including the *Proteobacteria*, *Thaumarchaeota* Marine Group I, *Euryarchaeota* Rice Cluster V, or Group C3, explained a large part of the variance and thus represented the key phyla in the vertical assemblage distribution. This vertical configuration could be the reflection of microorganism adaptation and cooperation facing to their environment. The forward selection of the most influential environmental parameters (Online Resource Table S2) revealed that potentially active *Bacteria*

were mainly influenced by the chlorophyll *a* and phosphate concentrations. Interestingly, the phosphate concentrations were already found to be an important factor influencing enzymatic activities and abundance of prokaryotes in the same intertidal mudflat [60]. In the current study, phosphate concentrations were correlated with bacterial taxa mainly retrieved in deeper layers between 2 and 10 cm bsf and this could be related to a phosphate release by some proteobacterial taxa [61]. Data obtained in the present work suggested that archaeal 16S rRNA transcript relative abundances were mainly related to the pH in the sediments. However, the lack of cultured representative for many bacterial and archaeal environmental groups limits the physiology characterization of their adaptive abilities.

Proteobacteria largely dominated the potentially active bacterial assemblage, with most of the 16S rRNA transcript sequences affiliated with δ-, γ-, and α-*Proteobacteria*. The retrieval of δ-*Proteobacteria* has been largely reported in coastal and estuarine sediments [53, 62] and may also be potentially active in intertidal mudflats. Moreover, the retrieval of OTUs affiliated with *Desulfobacteraceae* (from 0.82 to 8.02%) and *Desulfobulbaceae* (from 0.72 to 7.04%), which are known to be involved in the sulfur cycle [63], supports the idea that this biogeochemical cycle is important in intertidal sediments. Indeed, some are commonly retrieved in sedimentary ecosystems [64], whereas others, such as *Desulfobulbus*, could oxidize sulfide through a filamentous cable [65]. Sequences affiliated with the unclassified *Desulfuromonadales* were also retrieved in the present work, and this environmental group has been previously reported in contaminated sediments and has in some cases been strongly correlated with heavy metals such as Fe, Cu, and Cr [62]. Among the γ-*Proteobacteria*, most of the sequences were related to the *Dasania* genus, even in the lower anoxic sediment samples (between 5 and 10 cm bsf), although this genus is known to contain obligatory aerobic *Bacteria* [66]. Thus, we hypothesized that this lineage might have developed tolerance mechanisms to anoxia or might contain phylotypes that are able to grow in anaerobic conditions.

Our study also indicates the importance of potentially active *Planctomycetes* [67]. These *Bacteria* inhabit aquatic and terrestrial ecosystems but also inhabit ultra-dry soils [68], marine stromatolites [69], acid peat bogs [70], and hot springs [71]. In recent decades, this lineage has become of specific interest for microbial ecologists because these taxa have been determined to be important players in the nitrogen cycle through anaerobic ammonia oxidation [72–76]. However, anammox-like *Brocadiales* were not found in our dataset and qPCR based analyses on *hszA* gene did not provide evidence of anammox process in this work (data not shown) which are congruent with previous work showing that all *Planctomyctes* were not implicated in this process [76].

Our study also suggested the presence of potentially active *Firmicutes*. Related transcript sequences were affiliated with gut commensal groups such as *Ruminococcaceae* and *Lachnospiraceae*. However, in a large study, Zinger et al. [53] also reported this type of *Bacteria* in coastal sediments and hypothesized a terrestrial origin. This terrestrial-related group may be dispersed by episodic events such as river flooding, storms, or discharge from the catchment area before sampling for the experiment occurred.

Considering potentially active archaeal assemblages, a clear segregation was observed between 0 and 5 cm bsf where *Thaumarchaeota* 16S rRNA transcript numbers dominated (6.9–26.4% transcripts), while from 5 to 10 cm bsf, the archaeal group C3 dominated (73.6–77.8% transcripts). In this study, each sample was extracted in duplicates and then pooled before sequencing. While replicates should give robustness at the findings, all the issues of this study are congruent as Day and Night experiments were very similar and confirmed that the diversity described is reliable. As an important evidence, this strong shift in archaeal 16S rRNA transcript diversity was observed in the two independent Day and Night experiments.

The *Thaumarchaeota* Marine Group I is involved in the nitrification process in diverse ecosystems such as deep waters [77–79], estuarine and coastal waters [58, 80, 81], marine sediments [82, 83], lakes [84, 85], and soils [86, 87]. The abundance of *Thaumarchaeota* transcripts in the sediment of an intertidal zone strengthened the idea that the nitrogen cycle is of main influence in this environment [88, 89]. This finding was congruent with the increase in nitrate, which is the final product of nitrification, from 0 to 5 cm bsf. Moreover, previous studies conducted in microcosm experiments in a French mudflat [90] indicated a dominance of potentially active *Bathyarchaeota* or Miscellaneous Crenarchaeotic Group (MCG) [91] and contrasted with another study conducted in a peatland in which an archaeal active assemblage was dominated by methanogens [92]. In our study, the archaeal group C3 dominated from 5 to 10 cm bsf (73.6–77.8% transcripts). Several recent studies have reported its presence in hydrothermal vents [93, 94], methane seeps, cold seeps [95, 96], estuarine sediments [90], and lakes [85]. Although this group has been retrieved in diverse environments, it has rarely been recorded as abundant or active in any ecosystem. Despite the ubiquity of the archaeal group C3 in various sediments, little is known about its functional role in ecosystems.

Conclusion

Coastal sediments exhibit some of the highest levels of microbial diversity in the marine realm because they are located at the interface of terrestrial, aerial, and aquatic systems and are nutrient-rich environments with a large proportion of anoxic/

oxic niches. In intertidal systems, the inhabiting prokaryotes can be subjected to extreme changes in environmental conditions, leading to strong modifications of their metabolism. In particular, over a 24-h period, both the diel and tidal rhythms influence the photosynthesis process enacted by benthic microalgae (i.e., microphytobenthos), thus driving the availability of oxygen and photosynthesis-derived products. In this context, using a tidal mesocosm, we demonstrated that this diel cycle did not impact the bacterial and archaeal 16S rRNA transcript diversity. Active *Bacteria* and *Archaea* were diverse, suggesting the capacity of this system to promote diverse ecosystem functions. Our work indicates that *Bacteria* and *Archaea* organize themselves in specific active assemblages vertically along sediment depth independently of diel fluctuations. These specific active assemblages were recorded with predominance of taxa such as *Thaumarchaeota* MGI or *δ-Proteobacteria Desulfobulbus* involved in nitrogen and sulfur cycles, respectively. We thus hypothesize that the nitrogen and sulfur cycles may influence the presence of these specific active assemblages in a stringent zone where organic matter is less labile in order to maintain competitiveness. This work also highlighted the importance of considering less characterized groups, such as the archaeal C3 group that may be important factors in ecosystem functioning.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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