2014-08-17

Effect ofplankton-derivedorganic matter on themicrobial community of coastal marine sediments

Journal of Experimental Marine Biology and Ecology, v. 461, p.257-266, 2014
http://www.producao.usp.br/handle/BDPI/46597

Journal of Experimental Marine Biology and Ecology 461 (2014) 257–266

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Effect of plankton-derived organic matter on the microbial community of coastal marine sediments

Paula Carpintero de Moraes *, Diego Castillo Franco, Vivian Helena Pellizari, Paulo Yukio Gomes Sumida

Instituto Oceanográfico, Universidade de São Paulo, Praça do Oceanográfico, 191, Cidade Universitária, 05508-120 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 14 August 2014
Accepted 17 August 2014
Available online xxxx

Keywords:
Bacterial diversity
DGGE
Marine sediment
Plankton-derived OM

ABSTRACT

An experimental study was carried out to observe the microbial response to two different plankton-derived organic matter inputs in a coastal sedimentary community of Ubatuba, São Paulo, SE Brazil. The organic enrichment experiment was conducted in order to test experimentally the stimulus of the sediment prokaryotic community after the input of labile material simulating an algal bloom reaching the sea floor. A total of 57 corers (two treatments: the diatom *Phaeodactylum tricornutun* and the phytoflagelate *Tetraselmis* sp. and a control) were maintained for a total of 30 days in constant temperature, circulation and oxygenation. After the addition of algae an increase in oxygen consumption was observed, accompanied by an increase of prokaryotic total and live density, showing an immediate response from the community to the input of labile material in the sediment. Analyses of molecular fingerprints of bacterial communities by denaturing gel gradient electrophoresis (DGGE) showed differences in bacterial community composition between both treatments and control just after algae addition. This was well evidenced after bacteria genomic libraries analyses that showed differences in diversity and dominance between treatments. In general, *Gammaproteobacteria* was the most diverse and abundant group in the sediment samples. However, the addition of phytoflagellates led to a shift in dominance in favor of *Alphaproteobacteria* while diatom input led to a greater bacterial diversity.

1. Introduction

Marine sediments represent some of the most complex microbial habitats on Earth, and the benthic microbial community plays an important role in the marine biogeochemical cycles. This is a result of their high abundance (>10^8 cells per gram) and their key function in mediating and regulating the transformation and speciation of major bioactive elements. Several factors have been described to influence sediment bacterial community (Bordalo, 1993; Ikenaga et al., 2010), such as temperature (Kirchman et al., 2005), organic matter quality (Bissett et al., 2007, Powell et al., 2003) and the benthic community (Wobus et al., 2003). However, determining which factors are the most important has been difficult because of the interactions among them.

Large amounts of particulate organic matter (POM) are deposited in marine sediments, which stimulate biogeochemical degradation by microorganisms (Henrichs, 1992). In coastal and shelf areas this process is enhanced because these are sites of intense productivity (ca. 30%), sedimentation and burial of organic carbon (Duarte et al., 2004; Wollast, 2002). Sinking and deposited POM serves as a high quality food source for many forms of marine life, including the benthos (Graf, 1992), being a potentially important structuring mechanism in soft sediments. Microorganisms react quickly to the sedimentation of phytoplankton by an increase in their biomass, density and productivity, which usually accompany increases in sediment chlorophyll (Pfannkuche et al., 2000; Rusch et al., 2003; Smith et al., 2002). However, possible changes in the composition and diversity of benthic bacterial communities in response to such inputs are not completely known (Franco et al., 2007; Rink et al., 2007).

Several experimental studies have demonstrated that different monomeric and polymeric components of dissolved organic carbon, whether through direct supply or by experimentally induced phytoplankton blooms, may select specific sub-communities or populations of bacterioplankton (Abell and Bowman, 2005; Lebaron et al., 1999; Riemann et al., 2000). The specific composition of organic matter in various algae also seems to be an important factor in selecting communities and distinct populations of planktonic bacteria (Grossart et al., 2005; Schäfer et al., 2002). The study and knowledge of the association between different types of labile POM (or different planktonic organisms) reaching the sediment and the response of specific microbial communities need further investigation since the sediment bacteria are of great importance for the recycling and decomposition of organic matter in both sediment and water columns.

The aim of the present study was to investigate the response of benthic microbial communities to POM input derived from phytoplankton production. Three hypotheses were checked: 1. Organic matter inputs increase the density and organic matter consumption of the heterotrophic

* Corresponding author. Tel.: +55 11 30916543.
E-mail address: pamoraes@usp.br (P.C. Moraes).

http://dx.doi.org/10.1016/j.jembe.2014.08.017
0022-0981/

microbial community; 2. The intensity and velocity of the microbial response to phytoplankton inputs are dependent on the phytoplankton species; 3. The diversity and community composition change after the addition of different phytoplankton species. To assess these responses, we carried out sediment-water flux experiments in Nov/Dec 2010 where sediment characteristics and bacterial community were monitored for a total of 30 days after the POM addition of two different microalgae to the sediment surface.

2. Material and methods

2.1. Sample site characteristics

The Southeast Brazilian Continental Shelf is characterized by a mesotrophic/oligotrophic regime. Primary production and sedimentation patterns are mainly influenced by the interaction of three water masses driven primarily by the direction and intensity of winds (Castro-Filho et al., 1987; Mahiques et al., 2004). From November to March prevailing east-northeasterly winds induce the intrusion of the nutrient-rich South Atlantic Central Water (SACW) in subsurface layers enhancing primary production through phytoplankton blooms, especially of diatoms (Castro-Filho et al., 1987; Gaeta et al., 1999; Metzler et al., 1997). Between April and October, the prevalence of southerly winds favors the retreat of the SACW and the higher frequency of cold fronts homogenizes the water column. During this period, resuspension and nutrient regeneration are the main factors driving primary productivity in the region, which is dominated by phytoflagellates. The lack of large rivers in the area limits the contribution of terrestrial organic carbon, indicating a minimal terrestrial contribution to the organic matter of the sediment, which is mainly derived from autochthonous sources (i.e. phytoplankton and microphytobenthos) (Yoshinaga et al., 2008).

2.2. Sediment sampling and experiment preparation

Samples were taken at mid-shelf depths just offshore Ubatuba, at the north coast of São Paulo state, SE Brazil. The sediment was collected with a multicorer in a transition area between coastal and oceanic waters (23° 36.679′S–44° 58.598′W; 40 m depth) in November 2010. Sediment cores were sliced in four depth horizons (0–2, 2–5, 5–10 and 10–20 cm) and the sediment from each slice was homogenized, sieved in 1 mm mesh to remove the macrofauna and larger particles, and remounted in acrylic corers with 10 cm inner diameter and 20 cm sediment column. A total of 57 corers were maintained in three separated systems (two treatments and one control) in the dark and with constant oxygenation and recirculation in a temperature-controlled room (19 °C).

We have chosen the phytoflagellate *Tetraselmis* sp. and the diatom *Phaeodactylum tricornutum* to perform the experiment, since they are common and abundant species in the study area and are routinely cultivated in our home institution algae cultivation facility. Before the addition, chlorophyll-a and total organic matter of the cultures were measured and an equivalent amount of chlorophyll of each algae was added to the treatments in a total of five times the maximum found in the region (2.77 mg m^{-3}). The total organic matter added to the sediment was 0.0048 g for *Phaeodactylum* and 0.0032 g for *Tetraselmis*. *P. tricornutum* is a pennate diatom and unlike other diatoms the cell wall is essentially organic (see Tesson et al., 2009), while *Tetraselmis* sp. is a small green flagellate.

After five days of acclimation, three corers were taken for the initial analysis (t = 0) and microalgae were added to the sediment surface. Thus, both treatments received the same amount of chlorophyll-a from different microalgae. The microcosms were maintained for 30 days after the introduction of algae. Sampling was performed six times (t = 1, 2, 5, 10, 20 and 30 days after treatment) for every set of three corers for each treatment and control groups. Dissolved oxygen fluxes were measured first in the dark, and the first centimeter was sampled for sedimentary (phytopigments, total organic matter and total organic carbon) and microbial analyses.

2.3. Oxygen measurements

Three corers from each treatment and control were randomly selected each time for the determination of dissolved oxygen and oxygen fluxes across the sediment–water interface. The cores were tightly sealed during flux incubations and a magnetic stirrer driven by an external rotating magnet maintained a continuous water circulation at a rate below the resuspension limit. Incubations of 3 h were performed in darkness, and water samples for dissolved O_2 were taken at the start (before inserting lids) and end (after removing lids). The standard Winkler technique was used for O_2 analysis (Strickland and Parsons, 1972).

2.4. Sediment characteristics

Total sediment organic matter (TOM) was obtained through calcination in a muffle furnace (500 °C) for 3 h (Byers et al., 1978). Phytopigments in the sediment were extracted in 100% acetone for 24 h at −10 °C. Absorbance was measured at 665 and 750 nm before and after acidification (0.1 N HCl) using a spectrophotometer (Plante-Cuny, 1978). Chlorophyll-a (chl-a) and phaeopigment (phaeo) concentrations were calculated applying the equation proposed by Lorenzen (1967), corrected for sediments. Total sediment organic carbon (TOC) was estimated by the Walkley–Black titration method modified for sediments (Gaudette et al., 1974).

2.5. Prokaryotic density

Each sediment replicate (ca. 1 cm^3) was added to 9 ml of 0.2 μm prefiltered seawater formaldehyde (2% vol/vol.) and stored at 4 °C until analysis. Total direct and viable counts were obtained using the Live/Dead (L/D) BacLight Viability Kit (L-7012, Molecular Probes, Eugene, OR) (Haugland, 1996; Quéric et al., 2004). For a gentle separation of living bacterial cells from sediment grains the samples were diluted 10,000 times after a vortexing period of 10–15 min. 2 ml of the diluted samples was transferred into capped polyethylene vials and stained with 3 ml of a 2:1 mixture of both stains (6.68 M SYTO 9 and 20 M propidium iodide), prepared just prior to use, and incubated for 15 min at room temperature. The sample was filtered onto a 0.2 μm pore-size black polycarbonate filter, and the filters were mounted with low-fluorescent mounting oil (provided with the viability kit) and examined by epifluorescence microscopy. A total of 400 cells were counted in each sample.

2.6. DNA extraction, PCR amplification and DGGE

Samples collected for analysis of microbial community were kept at −20°C until analysis. DNA extraction from sediment samples was performed using the PowerSoil DNA Isolation Kit (MoBio Laboratories, CA, and USA) according to the manufacturer′s specifications. The extracted DNA was quantified and purity checked by absorbance measurements with a spectrophotometer.

Hipervariable region 3 (V3) of the 16S rRNA gene was amplified by the primer set for bacteria 335FGC-518R (Ahn et al., 2006; Klammer et al., 2008). The 50 ml PCR mixture contained 0.5 ml of the primer set (20 mM each), 5 ml 10× PCR Buffer (Invitrogen), 1.5 ml $MgCl_2$ (50 mM), 0.4 ml dNTP (100 mM), 1 U *Taq* polymerase (Platinum, Invitrogen) and 2 ml of DNA templates (approximately 20 ng) and completed with sterilized ultrapure water. PCR amplification was performed by using a Mastercycler (Eppendorf). Amplification conditions were as follows: 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min with the final extension step at 72 °C for 10 min. All PCR products were checked by 1% (w/v) agarose gel electrophoresis.

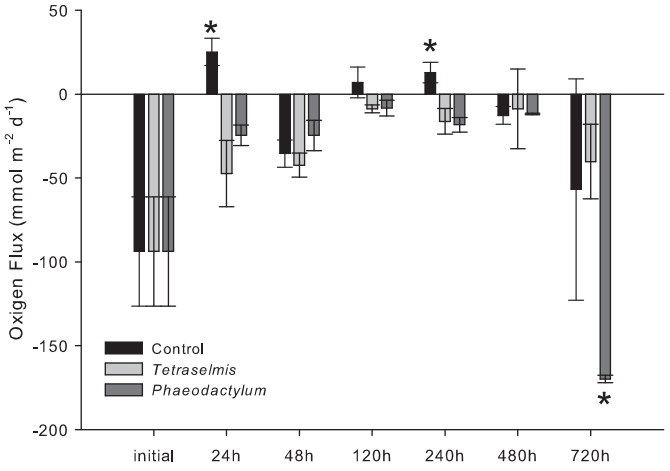

Fig. 1. Oxygen fluxes (mmol m^{-2} d^{-1}) across the sediment–water interface (mean ± standard error) within the experimental time (hours). (*p < 0.05, ANOVA, Tukey).

In order to reduce sample numbers, PCR amplicons from replicates of sediment were pooled before loading on DGGE. Prior to pooling, a DGGE was run on the individual samples to ensure acceptable reproducibility (results not shown). DGGE was performed with 8% (w/v) acrylamide gel which contained a linear chemical gradient ranging from 35% to 60% denaturant (100% denaturant 7 M urea, and 40% formamide). Similar amounts of PCR products were loaded into the gel and electrophoresed in 1× TAE buffer at 60 °C and 60 V for 16 h, using a Dcode Universal Mutation Detection System (Bio-Rad Laboratories, Richmond Calif). After electrophoresis, the gel was stained with silver nitrate modified from Sanguinetti et al. (1994) and the image obtained with a scanner.

2.7. Cloning, sequencing, and phylogenetic analysis of 16S rRNA gene fragments

The dominant and most intense DGGE bands (30 bands) were excised from the gel and incubated in sterilized ultrapure water for 30 min at 70 °C. 1 ml of supernatant was used to reamplify the band with the same PCR program as that used for DGGE. A mobility check of the amplified was performed to confirm whether the position of the bands was the same as that original by replicating DGGE analysis using the identical conditions. Cycles sequencing was conducted using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) by the company Genomic Engenharia Molecular, using the 518r primer.

A total of four samples (treatments after 24 h and 48 h the algae addition) were chosen to clone library analysis. PCR products (primers 27f–1401r, Lane, 1991) were cloned with a TOPO TA cloning kit (Invitrogen Ltd., Paisley, UK) according to the manufacturer's instructions. Cloning products were re-amplified in PCR with primers M13F-1401r. PCR amplified vector inserts of the correct size were purified with PureLink™ PCR Purification Kit (Invitrogen). A total of 80 cloning products of each sample were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) by the company Genomic Engenharia Molecular, using the universal primer T7.

DNA sequences were assembled with Bio-Edit Sequence Alignment Editor and all trimming, clustering and classification were performed in Mothur (Schloss et al., 2009). All sequences obtained were edited to exclude the primer sequences, and checked for chimerical structures using the DECIPHER (Wright et al., 2011). Sequences were aligned in Mothur using the greengenes template (http://greengenes.lbl.gov) and a distance matrix was generated. Sequences were clustered into OTUs using the farthest neighbor algorithm at 5% dissimilarity. Representative sequences from OTUs at a 0.05 distance were obtained and classified using the RDP reference files (http://www.mothur.org/wiki/Taxonomy_outline) and Wang approach. Similar results were obtained after comparison with sequences deposited in the GenBank using the Blast algorithm (http://www.ncbi.nlm.nih.gov/BLAST).

2.8. Statistical analysis

The variables were analyzed for normality using the Kolmogorov–Smirnov test. Differences between the treatments and times of samples were assessed by parametric analysis of variance (one-way ANOVA), if normality was observed, and with a non-parametric test (Kruskal–Wallis) when necessary (BIOSTAT 4.0). Relationships between the variables were tested with Pearson correlation analysis for all samples (n = 18). Canonical redundancy analysis (RDA; Canoco 4.5; Ter Braak and Smilauer, 2000) was applied to evaluate the influence of the abiotic factors in prokaryotic abundance and prokaryotic total density and live density was used as a response matrix (18 samples; 2 variables) and abiotic parameters as an explanatory matrix (18 samples; 5 variables).

DGGE bands were analyzed using BioNumerics (6.1). Similarities between the tracks were calculated by using Dice coefficient (SD) (unweighted data based on band presence or absence) and band-independent, whole-densitometric-curve based Pearson product-moment correlation coefficient (r) and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering.

In order to establish which variables best explained the variability in DGGE profile, a canonical correspondent analysis (CCA; CANOCO 4.5) was applied. The statistical significance of relationship between genetical diversity (DGGE profile) and biotic and abiotic variables was assessed using the Monte Carlo test (9999 permutations).

Sequences of the clone libraries were used for statistical analysis in Mothur (Schloss et al., 2009). The clusters were made at 5% dissimilarity

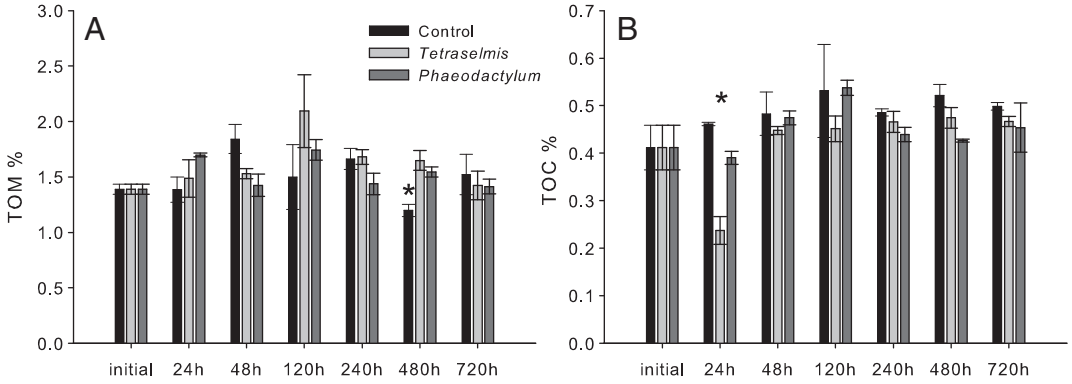

Fig. 2. Total organic matter (A. MOT) and total organic carbon (B. TOC) in the first sedimentary layer (0–1 cm) (mean ± standard error) within the experimental time (hours). (*p < 0.05, ANOVA, Tukey).

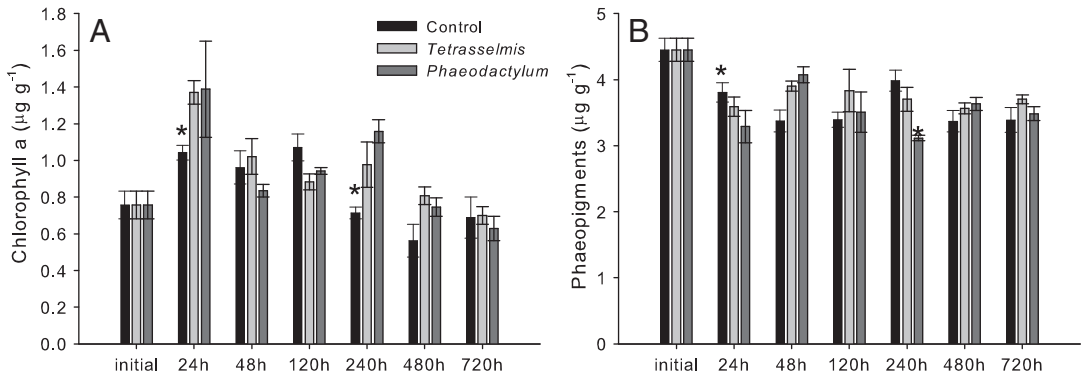

Fig. 3. Phytopigment distribution (mean ± standard error) in the first sedimentary layer (0–1 cm) within the experimental time (hours). (*p < 0.05, ANOVA, Tukey). A. chlorophyll a and B. phaeopigments.

cut-off and served as the OTUs for generating predictive rarefaction models and for calculating the richness indices estimator Chao1 and Shannon's diversity index. The LIBSHUFF analysis was performed for pair-wise comparisons in each library to determine the significance of differences between clone libraries using the LIBSHUFF function available in Mothur. The *P* value was estimated by 10,000 random permutations of sequences between libraries.

3. Results

3.1. Oxygen and sediment characterization

Oxygen uptake increased significantly after the addition of algae compared to the control (Fig. 1). Control experiments, on the other hand, showed either a discrete production or low consumption rates of oxygen during most of the experiment (Fig. 1). Total organic matter (TOM) values did not significantly change after the algae addition and no significant temporal differences were observed in either treatments or control (Fig. 2A). Total organic carbon was very constant during the experimental time. A significant decrease in the values was observed 24 h after *Tetraselmis* addition (Fig. 2B).

After 24 h a significant increase in chlorophyll-a followed by a decrease after 48 h in both treatments were observed (Fig. 3A; Table 1). The treatment with *Tetraselmis* showed a constant decrease reaching the lowest values 120 h after the start of the experiment. For the *Phaeodactylum* treatment and in the control, this happened only after 480 h (Fig. 3A; Table 1). Phaeopigments were distributed more homogeneously throughout the experimental period (Fig. 3B, Table 1). A significant decrease was observed after 24 h of algal addition in both treatments, but not in the control (Fig. 3B, Table 1). No correlation was observed between the variables.

3.2. Prokaryotic density

The density of prokaryotes at sediment surface (0–1 cm) ranged from 5.9 to 10.7×10^9 cells ml^{-1}, with higher values in treatments compared to the control (Fig. 4A). Densities of live cells were similar to total density ranging from 3.7 to 7.2×10^9 cells ml^{-1} (Fig. 4B). After 24 h of the addition an increase in prokaryote density, mainly in the treatment with *Tetraselmis*, which was significantly higher than the density in the control (Fig. 4A) was observed. Both treatments showed significantly higher values than the control in almost all samples times (Fig. 4A, B). Temporally, we observed an increase in the density of prokaryotes 24 h after the addition of *Tetraselmis* and 48 h after the addition of *Phaeodactylum* (Fig. 4A, Table 2). The percentage of live cells observed in this study was always high ranging from 51 to 82%. In both treatments an increase of live cells with time, which was not observed in the control (Fig. 4B, Table 2) was observed.

A significant positive correlation was observed only between total prokaryotic density and chl-a ($r = 0.504$, $p = 0.001$). The RDA performed with both live and total prokaryotic density explained 33.6% of the total sample variance (Fig. 5). Chl-a and phaeopigments were the only significant parameters that explained the prokaryotic density variability (Fig. 5).

3.3. DGGE pattern and bacterial diversity

The community structure of sediment bacteria in the experiment was compared based on the DGGE analysis of 16S rRNA gene fragments. The DGGE patterns showed that changes in time are more important than between treatments (Fig. 6). Between 25 and 34 bands were observed in sediment samples and a higher number of bands were found in the control in the first days of experiment, decreasing with time. In treatments, however, the number of bands increased with time. Community composition could be separated in six groups with more than 70% of similarity in the UPGMA cluster analysis (Fig. 6). The first group was composed by samples initial, and control and treatments after 24 h, and the second group included the treatments after 48 h (Fig. 6). The third group included the control 48 and 120 h and treatments 120 h, and the fourth, fifth and sixth groups clustered all samples with 240, 480 and 720 h, respectively (Fig. 6).

Among the total bands, 10% occurred only in treatments, being mainly observed in the first days after the algae addition. Control showed 3% of exclusive bands, treatment with *Phaeodactylum* showed 5% of the bands occurring exclusively with this treatment, while no band occurred only when *Tetraselmis* was added.

The CCA of 16S rRNA gene DGGE data explained only 47.9% of the data variation in the first two axes (Fig. 7). CCA showed that time

Table 1
ANOVA statistical analysis results regarding the comparison of the values of surface chlorophyll a and phaeopigments (mg g^{-1}) in the experimental time. Significant differences $p < 0.05$.

	Chlorophyll a		Phaeopigments	
	p	Tukey ($p < 0.05$)	p	Tukey ($p < 0.05$)
Tetraselmis	0.00	Initial ≠ 24 h; 24 h ≠ 120 h, 240 h, 480 h, 720 h	0.03	Initial ≠ 24 h, 480 h
Phaeodactylum	0.00	Initial ≠ 24 h; 24 h ≠ 480 h, 720 h; 120 h ≠ 720 h; 240 h ≠ 480 h, 720 h	0.00	Initial ≠ 24 h, 120 h, 240 h, 720 h; 48 h ≠ 240 h
Control	0.00	480 h ≠ 24 h, 48 h, 120 h; 120 h ≠ 720 h	0.00	Initial ≠ 48 h, 120 h, 480 h, 720 h

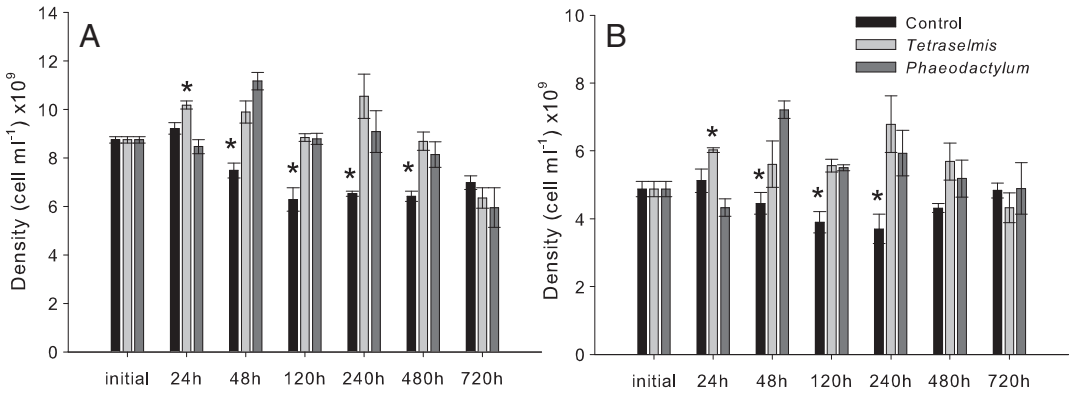

Fig. 4. Prokaryotic total density (A) and live density (B) (mean ± standard error) in the first sedimentary layer (0–1 cm) within the experimental time (hours). (*$p < 0.05$, ANOVA, Tukey).

was more important for changes in the community than treatments. According to the Monte Carlo analysis only chl-a and phaeopigments significantly correlated with general community structure.

Partial sequencing of 30 bands excised from DGGE profiles was determined (Fig. 6), and the taxonomic data are presented in Table 3. Most of the bands could be assigned to the *Gammaproteobacteria*, followed by *Alpha- and Deltaproteobacteria*, *Firmicutes* and *Bacterioidetes* (Table 3). Three bands occurred in all samples (A1, 2; B; G1, 2), and were assigned as *Bacteroidetes*, *Deltaproteobacteria* and *Gammaproteobacteria*, respectively. Three bands appeared in almost all samples (L, M and X), bands L and M were *Gammaproteobacteria* and band X, assigned as *Deltaproteobacteria* (Fig. 6; Table 3). Two bands (C and F) only occurred in the first days of the experiment and were assigned as *Gammaproteobacteria* (Fig. 6; Table 3).

3.4. Bacterial community composition analysis of 16S rDNA clone libraries

A total of four samples were explored for bacterial diversity and composition (treatments after 24 and 48 h after the addition), and 80 clones were sequenced from each clone library. After checking the sequence quality and eliminating the chimeras, a total of 260 sequences were used for the analysis (71 T24h, 70 P24h, 61 T48h, 58 P48h). Sequences were clustered into 178 OTUs with more than 95% identity, and rarefaction curves were done for each sample (Fig. 8). The highest bacterial richness (Chao 1 estimator) was found in treatment with *Tetraselmis* after 48 h (T48h), but this sample showed the lowest diversity (Shannon′s index that takes into account the richness and evenness of OTU distribution), and the highest diversity was found in the treatment with *Phaeodactylum* after 48 h (P48h) (Table 4).

The partitioning of bacterial diversity among the samples was analyzed using LIBSHUFF analysis (10,000 randomizations). The results showed that most of the groups were significantly different ($P < 0.0001$), with the exception of the community structure of T48h that was similar to P48h (Table 5).

The phyla composition of each clone library is shown in Fig. 9. All bacterial 16S rDNA clone libraries were diverse, including sequences affiliated with 11 phyla previously detected in marine sediments. The dominant phylum in all samples belonged to *Proteobacteria*, representing 76% of total sequences. Members of all classes of the *Proteobacteria* were found, and *Gamma-*, *Delta-*and *Alphaproteobacteria* were found in all samples and accounted for 31%, 18% and 26% respectively of the total OTUs.

Gammaproteobacteria was the most abundant group, with a high number of species in all samples. *Alphaproteobacteria* was the second most numerous group and also showed representatives in all samples, with *Tetraselmis* 24 h and 48 h presenting the majority of the sequences belonging to this group (Fig. 9). The samples of the *Phaeodactylum* treatment (P24h and P48h) showed the Deltaproteobacteria as one of the most representative groups (Fig. 9).

Bacteroidetes, the second most abundant phylum, was found in all samples, totaling 8% of the sequences and presented a high number of OTUs in P24h representing 15% of the sequences in the sample (Fig. 9). *Cyanobacteria*, *Cloroflexi*, *Actinobacteria*, *Fusobacteria* and *Planctomycetes* represented less than 4%, 3%, 3%, 1% of the total sequences each. The other phyla found were represented by one or two OTUs among all the sequences. *Actinobacteria*, *Cloroflexi* and *Planctomycetes* were not found only in samples P24h and T48h (Fig. 9). All other phyla were found in only one or two samples. At phylum level, the treatment with *Phaeodactylum* was more diverse than that with *Tetraselmis* (Fig. 9).

4. Discussion

4.1. Density and metabolic microbial response

Blooms of phytoplankton are common in many coastal areas and can provide massive inputs of organic matter into coastal sediments, triggering a dynamic response in the microbial community. Previous studies have shown that the prokaryotic community of the sediment quickly grows after the arrival of organic matter (Danovaro et al., 1999; Rusch et al., 2003).

Variations in the sedimentary parameters found in the present study suggest the consumption of particulate organic matter added to the sediment, especially of chl-a, which was rapidly consumed within 48 h. Kristensen and Holmer (2001) point out that most labile POM embedded in the marine sediment is quickly consumed, generally within the first six weeks. Similarly, plankton-derived dissolved organic matter has a half-life of 10 to 16 h in coastal sediments (Chipman et al., 2010).

The enrichment of the sediment promptly stimulated oxygen consumption, which is in accordance with the findings of other experimental and in situ studies (Cook et al., 2007; Enocksson, 1993). Since

Table 2
ANOVA statistical analysis results regarding the comparison of the values of surface total and live prokaryotic density (cell ml^{-1}) in the experimental time. Significant differences $p < 0.05$.

	Total density		Live density	
	p	Tukey ($p < 0.05$)	p	Tukey ($p < 0.05$)
Tetraselmis	0.00	24 h, 48 h, 120 h, 240, 480 h ≠ 720 h	0.07	
Phaeodactylum	0.00	24 h, 120 h, 240; 480 h ≠ 720; initial, 24 h, 480 h ≠ 40 h	0.01	Initial, 24 h, 720 h ≠ 48 h
Control	0.00	Initial, 24 h ≠ 120 h, 240 h, 480	0.03	Initial, 24 h ≠ 120 h, 240 h

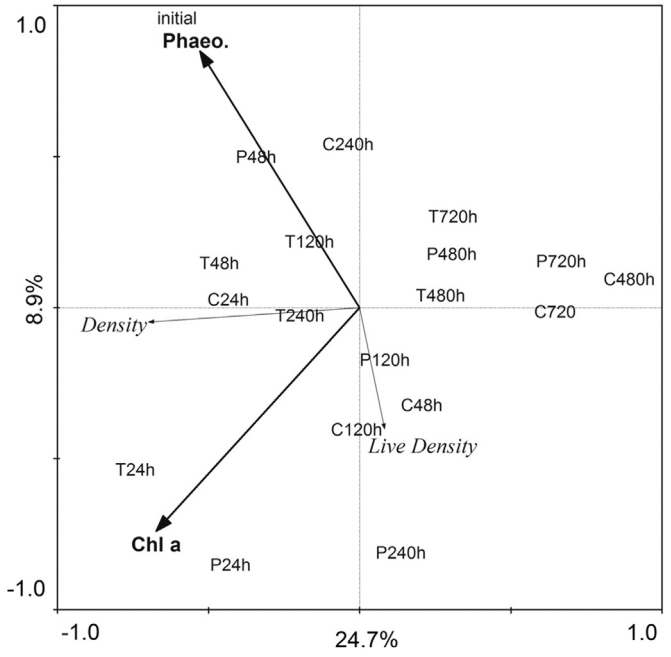

Fig. 5. Redundancy detrimental analysis (RDA) concerning the influence of abiotic variables (n = 19, 5 variables) in the prokaryotic density (n = 19, 2 variables) distribution. T = *Tetraselmis*, P = *Phaeodactylum* and C = control.

oxic respiration and sulfate reduction are generally considered the main pathways of organic matter degradation in coastal sediments (Canfield et al., 2005), our results suggest a higher degradation in treatments with the addition of algae. Additionally, live prokaryotes increased significantly after algae addition in comparison with controls. Therefore, as previously hypothesized, we believe that microorganisms responded to the stimulus, consuming labile OM (microalgae) soon after their arrival on the sediment surface.

The two different algae additions to the sediment generated different responses of the microbial community both in speed and magnitude. So, the intensity and velocity of the microbial response to phytoplankton inputs are dependant on the phytoplankton species. Immediate responses of prokaryotes to phytoflagellate addition are probably related to the nature of cells, which may suffer lysis much quicker than diatom cells, releasing DOM to the environment. Aidar et al. (1993) argue that phytoflagellates found in Ubatuba have a higher concentration of chlorophyll in comparison with diatoms, conferring a higher lability of the phytodetritus generated by these microalgae (see also Gaeta et al., 1999). However, this statement is misleading since chlorophyll cannot be considered the sole proxy for lability. In fact, Godói (2013) found that lipid contents are much higher and diverse in *P. tricornutum* than in *Tetraselmis* sp. used in the present study. In fact, the amount of OM generated by diatoms was greater than that of phytoflagellates with equal amounts of chlorophyll-a. Slower responses of the diatom treatment are likely to be related to its thecae, which may hinder microbial action (Hansen and Josefson, 2003).

4.2. Bacterial diversity and community composition variations

Microbial communities may respond to a varying supply of substrates either by physiological adaptation and/or by changes in the community composition. Even small additions of organic substrates may trigger a shift in the composition of the microbial community and an accompanying change in the bacterial growth efficiency (DelGiorgio and Cole, 1998). In the water column, bacterial community composition varies with the algal species and its physiological state (e.g. Grossart et al., 2005; Schäfer et al., 2002). However, this is not completely known for the sediment microbial community. Our study has shown that sediment bacteria present shifts in abundance and composition that are tightly linked to the nature of the supply of organic matter, and are mainly related to changes in chlorophyll in the sediment.

Although the analysis of microbial diversity based in clone libraries may not fully represent the natural community due to biases associated with the formation of chimeras and mutations induced by PCR, and the

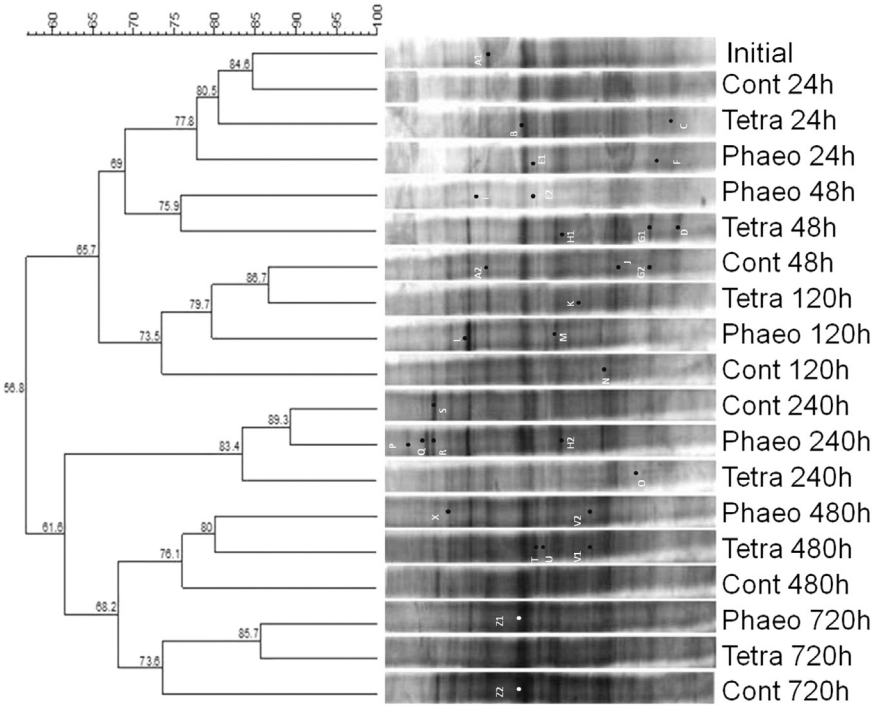

Fig. 6. Cluster analyses of DGGE banding patterns of sediment bacteria in the samples using UPGMA. Similarity matrix calculated using Pearson correlation. DGGE fingerprints of 16S rRNA gene amplified from DNA extracted from the first sedimentary layer showing the excised bands (letters A to Z).

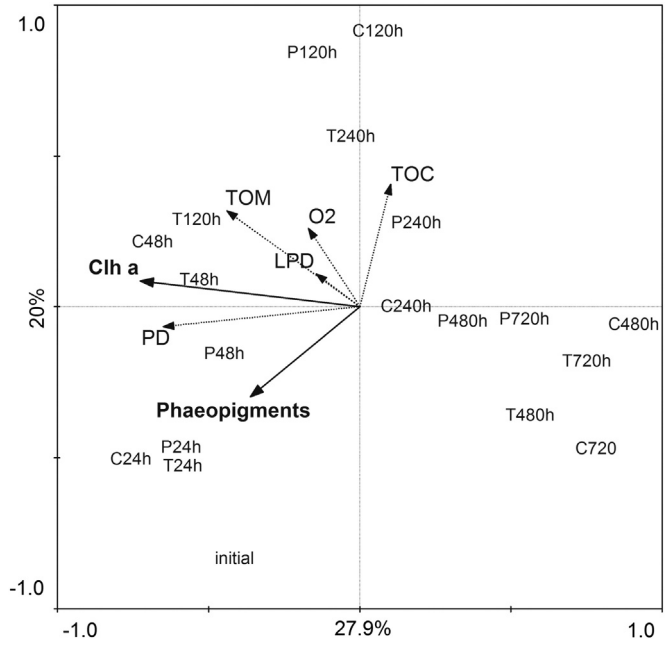

Fig. 7. Canonical correspondence analysis (CCA) performed with PCR-DGGE profiles of the sediment samples and the measured sedimentary variables (chl a, phaeopigments, TOM, TOC, O2 fluxes, and total (PD) and live (LPD) prokaryotic densities). T = *Tetraselmis*, P = *Phaeodactylum* and C = control. Bold lines are significant variables according to the Monte Carlo permutation test ($p < 0.05$) in the DGGE profile.

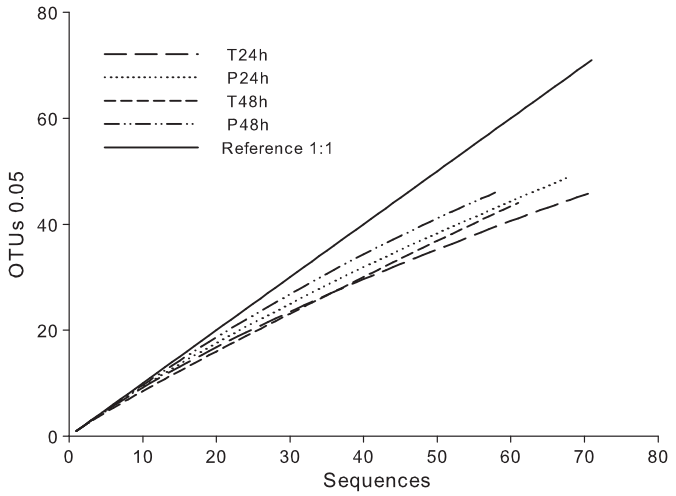

Fig. 8. Rarefaction analysis of 16S rDNA sequence heterogeneity in clone libraries from the sediment samples. T = *Tetraselmis*; P = *Phaeodactylum*.

weakness of the method in amplifying low density microorganisms, the method remains effective to identify the most abundant microorganisms in the ecosystem (Polymenakou et al., 2009). Despite the difficulty evaluating the actual microbial diversity in marine systems and the small number of sequences of each sample analyzed in the present study, we found a high diversity of bacteria phyla in the coastal sediments of Ubatuba.

Both the DGGE analysis and genomic libraries show an initial change of the bacterial community in sediments due to the addition of different types of algae. The comparison between the libraries (Table 5) and DGGE analyses (Fig. 6) showed differences between the samples, especially after 24 h of algal addition. Libraries showed that different bacterial groups dominate the bacterial community after the addition of different algae. Whereas the addition of phytoflagellates stimulates specific groups of bacteria (i.e. *Alphaproteobacteria* and *Gammaproteobacteria*), diatom addition generates the formation of more diverse assemblage of bacteria.

Gammaproteobacteria was the dominant group in sediment samples, which is consistent with previous studies that showed that this group has been frequently detected in marine sediments and often accounts for the largest proportion of clones in marine sediment libraries (Lai et al., 2006; Ravenschlag et al., 2001; Zeng et al., 2005). The *Gammaproteobacteria* is probably the most active bacteria involved in the degradation of fresh particulate organic matter (Chipman et al., 2010; Gihring et al., 2009), especially that derived from phytoplankton (Mills et al., 2008; Rusch et al., 2003). The response of this group mainly to diatoms may be related to the presence of diatom exudates (Pete et al., 2010; Puddu et al., 2003).

Alphaproteobacteria is usually found in high densities in the water column of coastal regions (Stevens et al., 2005), but not in marine sediments. In sediments, this group is generally associated with high productivity coastal areas, where it can attain elevated levels of species richness (Ikenaga et al., 2010; Polymenakou et al., 2009). Our *Tetraselmis* treatments generated a positive response of *Alphaproteobacteria* similar to what is normally found in the plankton. Here, members of this group are highly associated with phytoplankton bloom events and frequently associated with the colonization and hydrolysis of particles (Fandino et al., 2001; Grossart et al., 2005; Rink et al., 2007).

Table 3
Phylogenetic affiliation of genotype detected in the first sedimentary layer of the experimental samples by PCR-DGGE and sequence analysis of DGGE bands.

Sample	Total no. of bands detected	Band	Phylogenetic affiliation
Initial	19	A1	*Bacteroidetes*
Control 48 h		A2	
Tetraselmis 24 h	19	B	*Deltaproteobacteria*
Tetraselmis 24 h	5	C	*Gammaproteobactria*
Tetraselmis 48 h	6	D	*Alphaproteobacteria*
Phaeodactylum 24 h	13	E1	*Gammaproteobactria*
Phaeodactylum 48 h		E2	
Phaeodactylum 24 h	5	F	*Gammaproteobactria*
Tetraselmis 48 h	19	G1	*Gammaproteobactria*
Control 48 h		G2	
Tetraselmis 48 h	9	H1	*Gammaproteobactria*
Phaeodactylum 240 h		H2	
Phaeodactylum 48 h	11	I	*Bacteroidetes*
Control 48 h	11	J	*Alphaproteobacteria*
Tetraselmis 120 h	11	K	*Alphaproteobacteria*
Phaeodactylum 120 h	15	L	*Gammaproteobactria*
Phaeodactylum 120 h	15	M	*Gammaproteobactria*
Control 120 h	13	N	*unclassified Bacteria*
Tetraselmis 240 h	9	O	*Alphaproteobacteria*
Phaeodactylum 240 h	3	P	*Firmicutes*
Phaeodactylum 240 h	6	Q	*Gammaproteobactria*
Phaeodactylum 240 h	9	R	*Gammaproteobactria*
Control 240 h	4	S	*Bacteroidetes*
Tetraselmis 480 h	3	T	*Gammaproteobactria*
Tetraselmis 480 h	9	U	*Gammaproteobactria*
Tetraselmis 480 h	8	V1	*Firmicutes*
Phaeodactylum 480 h		V2	
Phaeodactylum 480 h	16	X	*Deltaproteobacteria*
Tetraselmis 720 h	3	Z1	*Gammaproteobactria*
Phaeodactylum 720 h		Z2	

Table 4
Observed bacterial richness and diversity estimators based on 95% OTU clusters. T = *Tetraselmis*; P = *Phaeodactylum*; Sobs = observed richness.

	Sobs	Chao-1	Shannon	Coverage
T24h	46	108.33	3.61	0.52
P24h	49	172.50	3.72	0.43
T48h	44	192.20	3.41	0.36
P48h	46	89.15	3.77	0.41

Table 5
LIBSHUFF analysis of the community structures of the samples at an OTU definition level of 95%. T = *Tetraselmis*; P = *Phaeodactylum*.

	T24h		P24h		T48h		P48h	
	dCXYScore	P^*	dCXYScore	P^*	dCXYScore	P^*	dCXYScore	P^*
T24h			0.031395	<0.0001	0.03854	<0.0001	0.07118	<0.0001
P24h	0.02097	<0.0001			0.04462	<0.0001	0.03550	<0.0001
T48h	0.01037	<0.0001	0.026091	<0.0001			0.00469	0.0317
P48h	0.04368	<0.0001	0.039931	<0.0001	0.01959	<0.0001		

* The significance *P* value should be below the critical threshold (0.05/8 = 0.0062).

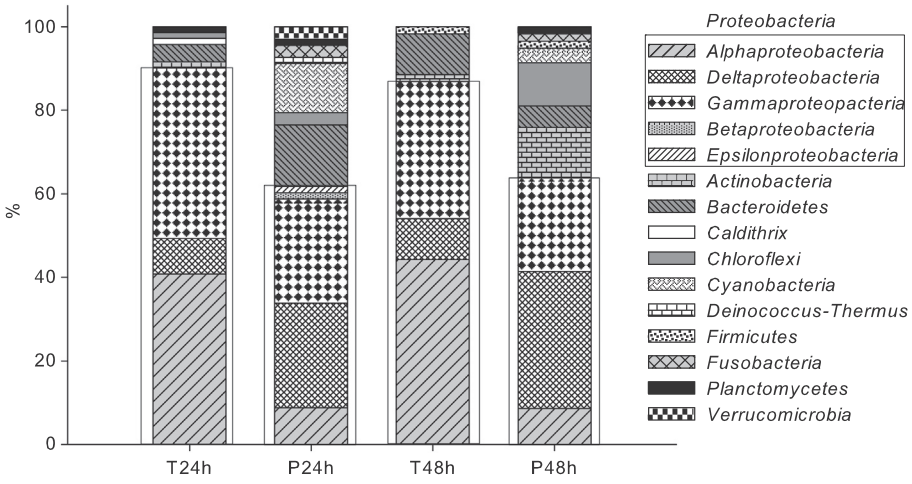

Fig. 9. Relative sequences abundance of dominant bacterial phylotypes in the sediment samples. T = *Tetraselmis*; P = *Phaeodactylum*.

Deltaproteobacteria has been suggested as a representative bacterial lineage in benthic environments and is a predominant group in marine sediments (Du et al., 2011; Feng et al., 2009; Pachiadaki et al., 2010). In the present study, we found only two bands from this group, but the bands were observed in almost all sediment samples and with a high intensity. Additionally, it was the dominant group in samples with diatom treatment. Most *Deltaproteobacteria* are sulfate reducers, one of the most important metabolism pathways in marine sediments that occur in anaerobic conditions (López-García et al., 2003; Ravenschlag et al., 2000). Although sulfate-reducing bacteria are expected to be confined to anoxic environments, they are also present in the oxic surface layer in high numbers, as observed in our samples (Sahm et al., 1999; Sass et al., 1997). This value is very similar to what we observed in the present study. Moreover, sulfate reducing bacteria metabolism is very diverse and they may use a variety of carbon resources being widespread in different habitats (Devereux et al., 1992; Elsabé et al., 2012).

Bacteroidetes are also found in sediment samples and seem to be an important group in the degradation of phytoplankton-derived carbon in sediments (Lai et al., 2006; Mills et al., 2008; Rusch et al., 2003). During phytoplankton blooms members of *Bacteroidetes* and *Alphaproteobacteria* are the most responsive groups to the changing organic matter field in the water column and also in changes in the composition of the phytoplankton community (Fandino et al., 2001; Grossart et al., 2005; Riemann et al., 2000; Rink et al., 2007).

Other groups with a significant presence in some samples were the *Actinobacteria* and *Cloroflex* particularly in P48h. Few studies have reported a significant contribution of *Actinobacteria* in marine sediment libraries (Stach & Bull, 2005; Ward and Bora, 2006). However, it has been observed that *Actinobacteria* are a small but significant group composing the marine system, with a wide distribution and persistent occurrence in marine sediments (Piza et al., 2004; Ward and Bora, 2006; Polymenakou et al., 2009; Elsabé et al., 2012). *Cloroflexi* group members were also previously found in marine sediments, always presenting a few representatives (Elsabé et al., 2012; Ghosh et al., 2010; Polymenakou et al., 2009).

5. Conclusion

This study demonstrated that the arrival of material derived from marine phytoplankton to the sediment leads to a rapid response of the sediment microbial community, both numerically and metabolically. Different planktonic organisms can lead to different responses of the sediment bacterial community, either by changes in total microbial density and/or changes in the community structure. Phytoflagellates lead to a faster increase in prokaryotic density stimulating mainly members of the *Alphaproteobacteria*. Diatom, on the other hand, leads to a higher increase of density and a wider diversity of bacterial phyla.

Acknowledgments

We are grateful to the Master and crew of the R/V Veliger II and the staff of the Research Station "Clarimundo de Jesus" (Ubatuba). We thank Thomas Edison da Silva, Maria Clara Argeiro, Arthur Z. Guth and all students from the Benthic Dynamics Laboratory for field assistance. This work was supported by FAPESP (São Paulo Research Foundation) grant nos. 2008/04594-4 (PYGS) and 2008/08244-8 (PCM). **[SS]**

References

Abell, G.C.J., Bowman, J.P., 2005. Colonization and community dynamics of class *Flavobacteria* on diatom detritus in experimental mesocosms based on Southern Ocean seawater. FEMS Microbiol. Ecol. 53, 379–391.

Ahn, J.H., Kim, M.C., Shin, H.C., Choi, M.K., Yoon, S.S., Kim, T., Song, H.G., Lee, G.H., Ka, J.O., 2006. Improvement of PCR amplification bias for community structure analysis of soil bacteria by denaturing gradient gel electrophoresis. J. Microbiol. Biotechnol. 16, 1561–1569.

Aidar, E., Gaeta, S.A., Gianesella-Galvão, S.M.F., Kutner, M.B.B., Teixeira, C., 1993. Ecossistema costeiro subtropical: nutrientes dissolvidos, fitoplâncton e clorofila-a e suas relações com as condições oceanográficas na região de Ubatuba, SP. Pub. Esp. Inst. Oceanogr. São Paulo 10, 9–43.

Bissett, A., Burke, C., Cook, P.L.M., Bowman, J.P., 2007. Bacterial community shifts in organically perturbed sediments. Environ. Microbiol. 9, 46–60.

Bordalo, A.A., 1993. Effects of salinity on bacterioplankton, field and microcosm experiments. J. Appl. Bacteriol. 75, 393–398.

Byers, S.C., Mills, E.L., Stewart, P.L., 1978. A comparison of methods to determining organic carbon in marine sediments, with suggestion for a standard method. Hydrobiology 58, 43–47.
Canfield, D.E., Thamdrup, B., Kristensen, E., 2005. Aquatic Geomicrobiology. Elsevier, (656 pp.).
Castro-Filho, B.M., Miranda, L.B., Miyao, S.Y., 1987. Condições hidrográficas na plataforma continental ao largo de Ubatuba: variações sazonais e em média escala. Bolm. Inst. Oceanogr São Paulo 35 (2), 135–151.
Chipman, L., Podgorski, D., Green, S., Kostka, J., Cooper, W., Huettel, M., 2010. Decomposition of plankton-derived dissolved organic matter in permeable coastal sediments. Limnol. Oceanogr. 55 (2), 867–871.
Cook, P.L.M., Veuger, B., Böer, S., Middelburg, J.J, 2007. Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. Aquat. Microb. Ecol. 49, 165–180.
Danovaro, R., Marrale, D., Della Croce, N., Parodi, P., Fabiano, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic-benthic coupling. J. Sea Res. 42, 117–129.
DelGiorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 29, 503–541.
Devereux, R., Kane, M.D., Winfrey, J., Stahl, D.A., 1992. Genus- and group-specific hybridization probes for determinative and environmental studies of sulfate-reducing bacteria. Syst. Appl. Microbiol. 15, 601–609.
Du, J., Xiao, K., Huang, Y., Li, H., Tan, H., Cao, L., Lu, Y., Zhou, S., 2011. Seasonal and spatial diversity of microbial communities in marine sediments of the South China Sea. Antonie Van Leeuwenhoek 100, 317–331.
Duarte, C.M., Middelburg, J.J., Caraco, N., 2004. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2, 1–8.
Elsabé, J.M., Brüchert, V., Fuchs, B.M., 2012. Vertical shifts in the microbial community structure of organic-rich Namibian shelf sediments. Afr. J. Microbiol. Res. 6 (17), 3887–3897.
Enocksson, V., 1993. Nutrient recycling by coastal sediments: effects of added algal material. Mar. Ecol. Prog. Ser. 22, 245–254.
Fandino, L.B., Riemann, L., Steward, G.F., Long, R.A., Azam, F., 2001. Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. Aquat. Microb. Ecol. 23, 119–130.
Feng, B.W., Li, X.R., Wang, J.H., Hu, Z.Y., Meng, H., Xiang, L.Y., 2009. Bacterial diversity of water and sediment in the Changjiang estuary and coastal area of the East China Sea. FEMS Microbiol. Ecol. 70, 80–92.
Franco, M.A., Mesel, I. De, Demba Diallo, M., Van der Gucht, K., Van Gansbeke, D., van Drijsvwijh, P., Costa, M.J., Vincx, M., Vanaverbeke, J., 2007. Effect of phytoplankton bloom deposition on benthic bacterial communities in two contrasting sediments in the southern North Sea. Aquat. Microb. Ecol. 48, 241–254.
Gaeta, S.A., Ribeiro, S.M.S., Metzler, P.M., Francos, M.S., Abe, D.S., 1999. Environmental forcing on phytoplankton biomass and primary productivity of the coastal ecosystem in Ubatuba region, southern Brazil. Rev. Bras. Oceanogr. 47, 11–27.
Gaudette, H.E., Flight, W.R., Toner, L., Folger, D.W., 1974. An inexpensive titration method for the determination of organic carbon in recent sediments. J. Sed. Petrol. 44, 249–253.
Ghosh, A., Dey, N., Bera, A., Tiwari, A., Sathyaniranjan, K.B., Chakrabarti, K., Chattopadhyay, D., 2010. Culture independent molecular analysis of bacterial communities in the mangrove sediment of Sundarban, India. Saline Syst. 6, 1–11.
Gihring, T.M., Humphrys, M., Mills, H.J., Huettel, M., Kostka, J.E., 2009. Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands. Limnol. Oceanogr. 54, 1073–1083.
Godói, A.C., 2013. Remineralização da matéria orgânica sedimentar em resposta à simulação de processos oceanográficos. (Dissertação de Mestrado). Universidade de São Paulo, Instituto Oceanográfico, (92 pp.).
Graf, G., 1992. Benthic–pelagic coupling—a benthic view. Oceanogr. Mar. Biol. 30, 149–190.
Grossart, H.P., Levold, F., Allgaier, M., Simon, M., Brinkhoff, T., 2005. Marine diatom species harbour distinct bacterial communities. Environ. Microbiol. 7, 860–873.
Hansen, J.L.S., Josefson, A.B., 2003. Accumulation of algal pigments and live planktonic diatoms in aphotic sediments during the spring bloom in the transition zone of the North and Baltic Seas. Mar. Ecol. Prog. Ser. 248, 41–54.
Haugland, R.P., 1996. Assays for cell viability, proliferation and function. In: Spence, M.T.Z. (Ed.), Handbook of Fluorescent Probes and Research Chemicals. Molecular Probes, Eugene, OR, pp. 365–398.
Henrichs, S.M., 1992. Early diagenesis of organic matter in marine sediments: progress and perplexity. Mar. Chem. 39, 119–149.
Ikenaga, M., Guevara, R., Dean, A.L., Pisani, C., Boyer, J.N., 2010. Changes in community structure of sediment bacteria along the Florida coastal everglades marsh–mangrove–seagrass salinity gradient. Microb. Ecol. 59, 284–295.
Kirchman, D.L., Malmstrom, R.R., Cottrell, M.T., 2005. Control of bacterial growth by temperature and organic matter in the Western Arctic. Deep-Sea Res. II 52, 3386–3395.
Klammer, S., Knapp, B., Insam, H., Dell'Abate, M.T., Ros, M., 2008. Bacterial community patterns and thermal analyses of composts of various origins. Waste Manag. Res. 26, 173–187.
Kristensen, E., Holmer, M., 2001. Decomposition of plant materials in marine sediment exposed to different electron accepters (O2, $NO3^-$, and SO_4^{2-}), with emphasis on substrate origin, degradation Kinetics, and the role of bioturbation. Geochim. Cosmochim. Acta 65, 419–433.
Lai, X., Zeng, X., Fang, S., Huang, Y., Cao, L., Zhou, S., 2006. Denaturing gradient gel electrophoresis (DGGE) analysis of bacterial community composition in deep-sea sediments of the South China Sea. World J. Microbiol. Biotechnol. 22, 1337–1345.
Lebaron, P., Servais, P., Troussellier, M., Courties, C., Vives-Rego, J., Muyzer, G., Bernard, L., Guindulain, T., Schäfer, H., Stackebrandt, E., 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat. Microb. Ecol. 19, 255–267.
López-García, P., Duperron, S., Philippot, P., Foriel, J., Susini, J., Moreira, D., 2003. Bacterial diversity in hydrothermal sediment and epsilonproteobacterial dominance in experimental microcolonizers at the Mid-Atlantic Ridge. Environ. Microbiol. 5, 961–976.
Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346.
Mahiques, M.M., Tessler, M.G., Ciotti, A.M., Silveira, I.C., Sousa, S.H.M., Figueira, R.C.L., Tassinari, C.C.G., Furtado, V.V., Passos, R.F., 2004. Hydrodynamically driven patterns of recent sedimentation in the shelf and upper slope off Southeast Brazil. Cont. Shelf Res. 24, 1685–1697.
Metzler, P.M., Glibert, P.M., Gaeta, S.A., Ludlam, J.M., 1997. New and regenerated production in the South Atlantic off Brazil. Deep-Sea Res. 44, 363–384.
Mills, H.J., Hunter, E., Humphrys, M., Kerkhof, L., Mcguinness, L., Huettel, M., Kostka, J.E., 2008. Characterization of nitrifying, denitrifying, and overall bacterial communities in permeable marine sediments of the northeastern Gulf of Mexico. Appl. Environ. Microbiol. 74, 4440–4453.
Pachiadaki, M.G., Lykousis, V., Stefanou, E.G., Kormas, K.A., 2010. Prokaryotic community structure and diversity in the sediments of an active submarine mud volcano (Kazan mud volcano, East Mediterranean Sea). FEMS Microbiol. Ecol. 72, 429–444.
Pete, R., Davidson, K., Hart, M., Gutierrez, T., Miller, A.E.J., 2010. Diatom derived dissolved organic matter as a driver of bacterial productivity: the role of nutrient limitation. J. Exp. Mar. Biol. Ecol. 391, 20–26.
Pfannkuche, O., Sommer, S., Kähler, A., 2000. Coupling between phytodetritus deposition and the small-sized benthic biota in the dep Arabian Sea: analyses of biogenic sediment compounds. Deep-Sea Res. II 47, 2805–2833.
Piza, F.F., Prado, P.I., Manfio, G.P., 2004. Investigation of bacterial diversity in Brazilian tropical estuarine sediments reveals high actinobacterial diversity. Antonie van Leeuwenhoek. 86, 317–328.
Plante-Cuny, M.R., 1978. Pigments photosynthétiques et production primaire des fonds meubles néritiques d'une région tropicale (Nosy-Bé, Madagascar). Trav. Doc. ORSTOM 96, pp. 1–359.
Polymenakou, P.N., Lampadariou, N., Mandalakis, M., Tselepides, A., 2009. Phylogenetic diversity of sediment bacteria from the southern Cretan margin, Eastern Mediterranean Sea. Syst. Appl. Microbiol. 32, 17–26.
Powell, S.M., Bowman, J.P., Snape, I., Satark, J.S., 2003. Microbial community variation in pristine and polluted nearshore Antarctic sediments. FEMS Microbiol. Ecol. 45, 135–145.
Puddu, A., Zoppini, A., Fazi, S., Rosati, M., Amalfitano, S., Magaletti, E., 2003. Bacterial uptake of DOM released from P-limited phytoplankton. FEMS Microbiol. Ecol. 46, 257–268.
Quéric, N.V., Softwede, T., Arntz, W.E., 2004. Application of a rapid direct viable count method to deep-sea sediment bacteria. J. Microbiol. Methods 57, 351–367.
Ravenschlag, K., Sahm, K., Knoblauch, C., Jorgensen, B.B., Amann, R., 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. Appl. Environ. Microbiol. 66, 3592–3602.
Ravenschlag, K., Sahm, K., Amann, R., 2001. Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). Appl. Environ. Microbiol. 67, 387–395.
Riemann, L., Steward, G.F., Azam, F., 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microbiol. 66 (2), 578–587.
Rink, B., Seeberger, S., Martens, T., Duerselen, C., Simon, M., Brinkhoff, T., 2007. Effects of phytoplankton bloom in a coastal ecosystem on the composition of bacterial communities. Aquat. Microb. Ecol. 48, 47–60.
Rusch, A., Huettel, M., Reimers, C.E., Taghon, G.L., Fuller, C.M., 2003. Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sands. Microb. Ecol. 44, 89–100.
Sahm, K., Knoblauch, C., Amann, R., 1999. Phylogenetic affiliation and quantification of psychrophilic sulfate reducing isolates in marine arctic sediments. Appl. Environ. Microbiol. 65, 3976–3981.
Sanguinetti, C.J., Dias Neto, E., Simpson, A.J.G., 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17, 915–919.
Sass, H., Cypionka, H., Babenzien, H.-D., 1997. Vertical distribution of sulfate-reducing bacteria at the oxic–anoxic interface in sediments of the oligotrophic Lake Stechlin. FEMS Microbiol. Ecol. 22, 245–255.
Schäfer, H., Abbas, B., Witte, H., Muyzer, G., 2002. Genetic diversity of 'satellite' bacteria present in cultures of marine diatoms. FEMS Microbiol. Ecol. 42, 25–35.
Schloss, P.D., et al., 2009. Introducing mothur: open source, platform independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.
Smith Jr., K.L., Baldwin, R.J., Karl, D.M., Boetius, A., 2002. Benthic community responses to pulses in pelagic food supply: North Pacific Subtropical Gyre. Deep-Sea Res. I 49, 971–990.
Stach, J.E., Bull, A.T., 2005. Estimating and comparing the diversity of marine actinobacteria. Antonie Van Leeuwenhoek. 87 (1), 3–9.
Stevens, H., Brinkhoff, T., Simon, M., 2005. Composition of free-living, aggregate-associated and sediment surface-associated bacterial communities in the German Wadden Sea. Aquat. Microb. Ecol. 38, 15–30.
Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis, Fish. Res. Board Can. Bull., Canada, 2nd ed.
Ter Braak, C.J.F., Smilauer, P., 2000. CANOCO Reference Manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca NY.
Tesson, B., Gaillard, C., Martin-Jézéquel, V., 2009. Insights into the polymorphism of the diatom *Phaeodactylum tricornutum* Bohlin. Bot. Mar. 52, 1–13.

Ward, A.C., Bora, N., 2006. Diversity and biogeography of marine Actinobacteria. Curr. Opin. Microbiol. 9, 279–286.
Wobus, A., Bleul, C., Maassen, S., Scheerer, C., Schuppler, M., Jacobs, E., Roske, I., 2003. Microbial diversity and functional characterization of sediments from reservoirs of different trophic state. FEMS Microbiol. Ecol. 46, 331–347.
Wollast, R., 2002. Continental margins—review of geochemical settings. In: Wefer, G., Billett, D., Hebbeln, D., Jørgensen, B.B., Schlüter, M., van Weering, T.C.E. (Eds.), Ocean Margin Systems. Springer.
Wright, E.S., Yilmaz, L.S., Noguera, D.R., 2011. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl. Environ. Microbiol. 78 (3), 3717–3725.
Yoshinaga, M.Y., Sumida, P.Y.G., Wakeham, S., 2008. Lipids biomarkers in surface sediments from unusual coastal upwelling area from the SW Atlantic Ocean. Org. Geochem. 39, 1385–1399.
Zeng, R., Zhao, J., Zhang, R., Lin, N., 2005. Bacterial community in sediment from the western Pacific Warm Pool and its relationship to environment. China Environ. Sci. 48, 282–290.