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Biochemical and microbial features of shallow marine sediments along the Terra Nova Bay (Ross Sea, Antarctica)

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ABSTRACT

Shallow marine sediments were collected from seven stations (three of which located at Gerlache Inlet, two at Tethys Bay, one at Adelie Cove and one just beneath the Italian Research Base) along the Terra Nova Bay coast (Ross Sea, Antarctica). Their chemical, biochemical and microbiological properties were studied in order to provide further insights in the knowledge of this Antarctic benthic ecosystem. Overall, the organic carbon (OC) represented the major fraction of total carbon (TC) and displayed concentrations similar to or slightly lower than those previously measured in Antarctic bottom sediments. The biopolymeric carbon within OC ranged from 4.1% to 19.9% and showed a wide trophic range (65–834 $\mu g\ g^{-1}$ d.w.). Proteins (PRT) represented on average the main biochemical class contributing to labile organic carbon, followed by lipids (LIP) and carbohydrates (CHO). The activity of aminopeptidase, β-D-glucosidase, alkaline phosphatase and esterase was checked, giving the highest values at Tethys Bay and at the deepest water sediments. The principal component analysis, which was computed considering physical, chemical (elemental and biochemical sedimentary composition) and microbiological parameters (including bacterial abundance, ectoenzymatic activities, T-RFs richness and diversity indices), allowed to obtain two main clusters ("Tethys Bay" and "other stations"). Based on data obtained, two representative 16S rRNA clone libraries using samples from Tethys Bay and Gerlache Inlet were constructed. The sequences of 171 clones were compared to those available in public databases to determine their approximate phylogenetic affiliations. Both aerobic and anaerobic bacteria were disclosed, with the majority of them affiliated with the *Gamma-* and *Deltaproteobacteria*, *Bacteroidetes* and *Acidobacteria*. The occurrence of strictly anaerobic bacteria suggests that sediments might also undergo anoxic conditions that, in turn, could favor the accumulation of PRT in respect to CHO, assuming that fermentation of amino acids is slower than that of sugars from decomposing polysaccharides.

1. Introduction

Like other continental shelf systems, polar shelves are regions of intense biological activity and biogeochemical cycling. They are characterized by seasonally high primary production, large drawdowns of pCO_2, and intense sedimentation rates (Ducklow et al., 2008; Fonda Umani et al., 2005). In Antarctica, settling particulate matter is underexploited along the water column due to low temperature and low microbial activities (Christian and Karl, 1995; Fabiano and Pusceddu, 1998; Vetter and Deming, 1994), therefore resulting in the accumulation of organic detritus in the coastal belt. This, in turn, potentially supports high microbial biomass and activity (Fabiano and Danovaro, 1998). Both the consistence and quality of organic matter that accumulates in Antarctic marine may fuel the benthic biota, including microorganisms, during the whole year (Isla et al., 2006). This relies on the fact that the particle supply is restricted to a short-period of the spring–summer time, when primary production blooms and sea-ice release their solid load, rich in labile compounds (Fischer et al., 1988; Fabiano and Pusceddu, 1998; Isla et al., 2006; Nedwell et al., 1993; Pusceddu et al., 1999). Thus, the carbon cycling is modulated by pulsed organic inputs to sediments, which are strongly dependent on dramatic seasonal shifts in pelagic primary production (Mincks et al., 2005). In

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Antarctic coastal waters significant interannual variations in the source of organic carbon (OC) inputs to sediments have been also observed (Nedwell et al., 1993). However, in general OC is exported to deeper waters by pycnoclinic currents (Frignani et al., 2003). According to Smetacek et al. (1990), coastal waters at Terra Nova Bay function as a "loss-type" system, which is mainly dependent on the vertical flux of primary organic matter and fecal pellets. In this area, the organic carbon (OC) is likely of marine origin and the particle flux may enable the entire particulate matter pool to reach sediments within 2–3 days (Albertelli et al., 1998). According to Frignani et al. (2003), OC is mainly produced by autotrophic eukaryotes, especially diatoms, which grow at both the ice–water interface and in the water column. A large and persistent *Phaeocystis* bloom was detected at Terra Nova Bay in December–January, after which the community composition shifted towards a diatom-dominated assemblage, in relation with the shoaling of the mixed layer (Fonda Umani et al., 2005). In the Ross Sea, the biogenic silica flux from diatoms frustules was estimated around 11–17 g m^2 yr^{-1}; it is by far the most important biological contribution to sediments in this marine system (Frignani et al., 2003) and may represent the main source of labile organic carbon for heterotrophic bacteria growth. In particular, a substantial part of such a carbon is excreted as exo-polysaccharides (EPS) and glycoproteins (Bahulikar and Kroth, 2008). According to Pusceddu et al. (2000), whilst the organic matter quantity in sediments depended on the influxes from the water column, temporal changes of its biochemical composition were related to processes occurring within the benthic trophic chain. In polar shelves, the cold-adapted microbiota displayed mineralization rates and pathways comparable to those of temperate communities (Kostka et al., 1999), thus playing important roles in nutrient recycling and diagenesis (Bowman et al., 2003). The uptake of organic matter by the benthic community strongly depends on its species composition. Nevertheless, whilst the sedimentary biochemical composition, bacterial biomass and ectoenzymatic activities have been concurrently investigated in Antarctica (Fabiano and Danovaro, 1998), the bacterial community structure has never been considered as an additional ecologically important factor.

In this context, the present work was mainly aimed at understanding the relationship between the labile organic matter and the bacterial communities in Antarctic shallow sediments along the Terra Nova Bay coast, in order to provide further insights toward the knowledge of this Antarctic benthic ecosystem. The experimental strategy adopted in this work used a combination of chemical, biochemical and biomolecular techniques (T-RFLP and clone libraries), allowing the further integration of data coming from the characterization of organic matter, the determination of ectoenzymatic activities and the identification of the main bacterial groups inhabiting shallow sediments.

2. Materials and methods

2.1. Study area

The sampling area covered the sea zone of Terra Nova Bay (Ross Sea) between Gerlache Inlet and Adelie Cove (coordinates: 74°37′ to 74°46′S and 163°59 to 164°07′E). The bottom of both sites is characterized by a canyon. The coast along the Terra Nova Bay presents rocky cliffs, with occasional beaches which present large boulders. The seafloor from 20 to 40 m depth is primarily granitic rock, with softer substrates composed of coarse sands or gravels, whereas the substrate becomes finer between 30 and 70 m.

2.2. Collection and preliminary treatment of sediment samples

During the Austral Summer of 2004–2005 (25 January–13 February 2005), shallow sediment samples were collected by a box corer from seven stations along the Terra Nova Bay coast (Fig. 1). In details, stations A and B were located at Tethys Bay; station D was located just beneath the Italian Base pier where small vessels berth when the pack-ice edge recedes; stations E, F and G were at Gerlache Inlet, no far from the Campbell Glacier. Finally, station C, which was located at Adelie Cove (near a penguin rockery 9 km south form the Base), is part of the Antarctic Specially Protected Area (ASPA) no. 161 and can be considered a pristine site. The sampling depth ranged between 19 and 66 m.

Portions of sediment surface (1 cm) were aseptically collected from the box corer. Sediments were preliminary processed within approximately 2 h after sampling in the laboratory of the Italian Base, as follows. Sub-samples for the determination of the chemical and biochemical composition (including DNA content) were stored at −20 °C until processing. For the estimation of total bacterial number, sub-samples were fixed with filter-sterilized paraformaldehyde (final concentration, 2%) and kept at +4 °C until analyses. Sub-samples for the ectoenzymatic activities were kept at 4 °C until processing (within 3–4 h from sampling, see below).

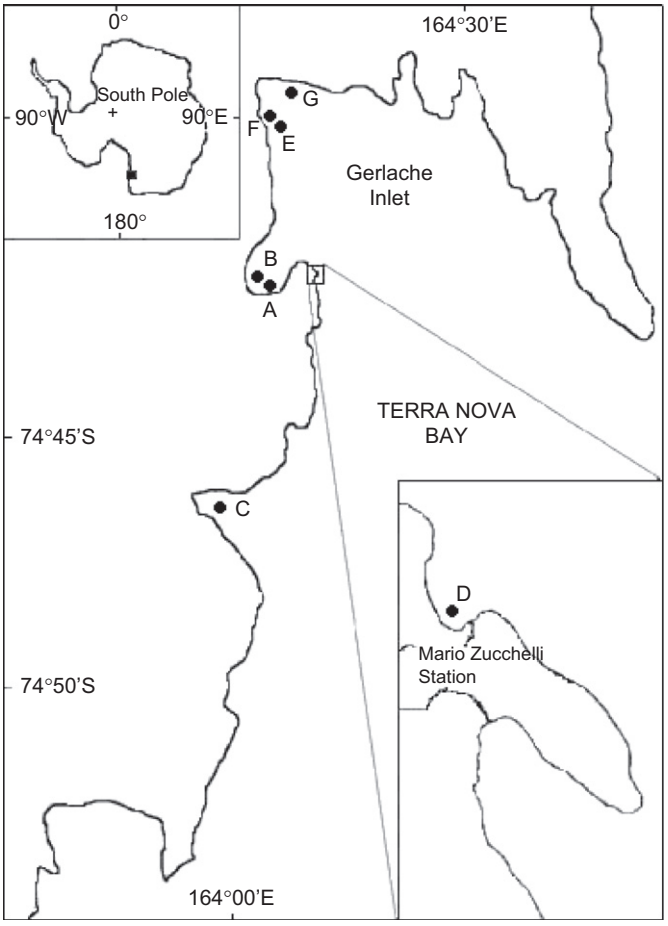

Fig. 1. Coastal marine area of Terra Nova Bay (Ross Sea) where sediment samples were collected. Stations A and B were located at Tethys Bay; station C was at Adelie Cove; station D was just behind the Italian Research Base "Mario Zucchelli"; stations E, F and G were located at Gerlache Inlet.

2.3. Chemical characterization of sediment samples

Sub-samples for chemical and biochemical analyses were freeze-dried and homogenized prior to be analyzed, paying attention to remove gravel and shell debris.

Sedimentary water content was estimated as the difference between wet and dry weight, determined by drying samples at 60 °C to constant weight.

2.3.1. Elemental composition of sediments

Total carbon (TC) and nitrogen (N) were determined by CHNSO element analyzer (Fisons 1108EA). Organic carbon (OC) was determined as total carbon after removal of carbonates with 8 N HCl. Inorganic carbon (IC) was calculated as the difference between TC and OC. N and P were determined in non-acidified samples. Acetanilide was used as standard for C and N determinations. Reproducibility was $\leq 3\%$ for C, and $\leq 5\%$ for N. P was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at 177 nm on 1 mol l^{-1} HCl extracts of combusted samples (Aspila et al., 1976). Accuracy evaluated on marine sediment reference material (BCSS) was $98.8\% \pm 4.8$; reproducibility of 5 mineralizations was $\leq 5\%$.

2.3.2. Biochemical composition of sediment organic matter

Sediment samples were analyzed for total protein (PRT), carbohydrate (CHO) and lipid (LIP) content to evaluate the polymeric components of organic matter.

PRT content was determined spectrophotometrically using Coomassie Brilliant Blue (Bradford, 1976; Mayer et al., 1986) after extraction of 4 g of freeze-dried sediment with 8 ml 0.5 N NaOH in an ultrasonic bath for 2 h at 40 °C (Nunn and Keil, 2006). After reaction with dye, absorbance was determined spectrophotometrically at 595 nm. PRT concentrations are reported in bovine serum albumin (BSA, Biorad) equivalents.

CHO content was determined by the Dubois method (Dubois et al., 1956) after extraction of 2.5 g sediment with 50 ml 1 N CH_3COOH for 4 h at 20–30 °C in ultrasonic bath (Mecozzi et al., 2000). Concentration of CHO was expressed in D(+)-glucose equivalents after reaction with 96% sulphuric acid and 5% phenol followed by spectrophotometric detection at 485 nm.

LIP were extracted overnight from 2 g of dried sediment by direct elution with chloroform and methanol (2:1 v/v) at 4 °C (Bligh and Dyer, 1959); LIP analysis was carried out by the charring method with H_2SO_4 at 200 °C for 15 min (Marsh and Weistein, 1966). Tripalmitin was used for standard solutions and the absorbance was determined at 375 nm.

All analyses were carried out in three replicates *per* sediment sample. For each biochemical class, results are expressed as *per* gram of sediment dry weight (d.w.).

For the evaluation of labile components of OC due to the three main biochemical classes, PRT, CHO and LIP were converted into carbon equivalents, assuming the respective conversion factors of 0.49, 0.40 and 0.75, respectively (Fichez, 1991). The sum of carbon equivalents of PRT, CHO and LIP constituted biopolymeric carbon (BPC) and was expressed as μgC g^{-1} sediment d.w.

2.4. Bacterial abundance and activity

2.4.1. Total cell counts

Before analysis, sediment slurries were prepared by diluting sediments 1000-fold in particle-free tetrasodium-pyrophosphate dispersing solution (0.1 M, 0.2 μm filtered), and shaking thoroughly. A 1–3 ml aliquot of the suspension was incubated with 4,6-diamidino-2-phenylindole (DAPI, final concentration 1 μg ml^{-1}) for 15 min in the dark, and filtered through a black polycarbonate membrane (0.2 μm pore size, 25 mm diameter, Isopore, Millipore, Milan, Italy). Cell counts were determined by counting stained cells in 20 randomly selected fields using a Zeiss Axioplan epifluorescence microscope with 365/420 nm excitation/emission filters. Data obtained were expressed as cells g^{-1} of sediment d.w.

2.4.2. Ectoenzymatic activities

The determination of the ectoenzymatic activities (EEAs) in sediment samples was performed immediately after sampling using fluorogenic substrates based on methylumbelliferone (MUF) (Hoppe, 1983; King, 1986) and methylcoumarinylamide (MCA) (Mayer, 1989). Aminopeptidase (Amp), β-D-glucosidase (Glu), alkaline phosphatase (AkP) and esterase (Est) were analyzed using the following substrates at a final concentration of 50 μM: leucine-MCA, β-D-glucopyranoside-MUF, phosphate-MUF and butyrate-MUF, respectively. The enzyme assays were carried on slurries prepared by diluting freeze-dried sediment 1:10 (w/v) in artificial seawater under continuous stirring and incubation in the dark at 5 °C. In particular, sediment sub-samples from the station A at Thetis Bay were used to test the influence of temperature on the potential hydrolysis rates; analyses were carried out at 5, 10, 15 and 30 °C. Five ml aliquots of each slurry were collected at five different fixed times in 2 h, boiled at 100 °C for 2 min, cooled in ice to stop the hydrolytic reaction, and centrifuged at 4000 g to recover the fluorescent dye released in the supernatant. Substrate hydrolysis rate was calculated in triplicate from slope of regression line. Solutions of MUF or MCA at different concentrations were added to slurries of each sample to build standard curves. Fluorescence was measured with a luminescence spectrometer (Perkin-Elmer LS 55) at 380/480 nm excitation/emission wavelength. Data obtained were expressed as nmoles of hydrolyzed substrate g^{-1} d.w. h^{-1}.

2.5. Bacterial population structure and composition

2.5.1. DNA extraction and PCR amplification of 16S rRNA genes

Total DNA from environmental samples for terminal restriction fragment length polymorphism (T-RFLP) analysis and clone libraries (see below) was extracted from 250 mg of starting material using a bead beating kit (Power Clean soil extraction kit, MOBIO laboratories) following the manufacturer's instructions. Three replicates were performed for each sample. DNA was quantified by a fluorimetric assay (Zipper et al., 2003, 2004). Genes coding for 16S rRNA were amplified by PCR in a reaction mixture (final volume of 50 μl) containing the following (final concentration): 1 × PCR buffer (Polymed), 2 mM $MgCl_2$, 2.5 mM of each deoxynucleotide triphosphate (Fermentas), 30 pmol (each) forward and reverse primers (MWG, Germany) and 2U *Taq* DNA polymerase (Polymed). Universal bacterial 16S rRNA primers 27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CTACGGCTACCTTGTTACGA-3′) were used to amplify nearly full-length 16S rRNA genes from a combination of three different DNA preparations. The primer 27F was labelled with tetrachlorofluorescein (TET) for T-RFLP analysis. Two μl (about 10 ng) of genomic DNA was used as template in the PCR reactions. PCR reaction conditions were as described by Di Cello et al. (1997) and Michaud et al. (2004). A PTC-100 Peltier Thermal Cycler (MJ Research Celbio) was used to perform PCR reactions. Amplification products of three parallel PCR reactions were combined and purified with the Wizard SV Gel and PCR Clean-Up system (Promega) prior to cloning and/or T-RFLP analysis, as described in the following sections.

2.5.2. T-RFLP analysis

The T-RFLP fingerprinting technique was used to estimate the genetic diversity of bacterial communities as described by Lukow et al. (2000) and Luna et al. (2006). We used two separate restriction digests for bacterial communities in order to obtain the fingerprinting information from different enzymes *per* sample. Approximately 300 ng of amplified 16S rRNA genes from each DNA sample were digested in duplicate with 10U of the restriction endonucleases *Hinf*I or *Taq*I (Fermentas) for 3 h at 37 and 65 °C, respectively. One μl of each sample was mixed with 12.5 μl of formamide and 0.5 μl of standard Rox 500 (Applied Biosystems). Reaction mixtures were denatured by heating at 95 °C for 3 min followed by an incubation at 0 °C for 5 min. Denatured DNA was loaded onto an automatic DNA sequencer (model ABI310, Applied Biosystems). T-RF profiles were analyzed with Genescan version 3.7 (Applied Biosystems). Only profiles with a cumulative peak height ≥ 5000 fluorescence units were used for the analysis. The detection threshold applied to T-RFLP profiles was calculated according to the approach suggested by Luna et al. (2006) and in our case was 0.32% of the total fluorescence.

The "abundance percentage" (Ap) of each T-RF was calculated as $Ap = ni/N \times 100$ where ni represents the peak area of one distinct T-RF and N is the sum of all peak areas in a given T-RFLP pattern (Lukow et al., 2000). The size of each T-RF was estimated by reference to the internal standard. Both sample replicates *per* enzyme were used when running the web-based tool T-Align (Smith et al., 2005) in order to generate a consensus profile containing only T-RFs present in both replicates by removal of non-common peaks. T-RFLP consensus profiles from different samples were subsequently aligned by using T-Align. Numbers and heights of peaks were used to characterize the microbial community diversity patterns. After enzymatic digestion of PCR amplicons, each T-RF can be defined as an operational taxonomic unit (OTU) within a community (Engebretson and Moyer, 2003).

2.5.3. Construction and screening of 16S rRNA gene libraries

To construct a library of PCR amplified 16S rRNA genes from DNA extracted from sediments, amplicons were ligated overnight at 4 °C into the pGEM-T Easy vector (Promega) and introduced by transformation into *Escherichia coli* DH5α competent cells. Ligation and transformation were carried out as recommended by the manufacturer (Promega). Transformed cells were selected onto Luria Bertani (LB) plates containing 100 μg ampicillin ml^{-1} (sodium salt), X-gal (80 μg ml^{-1}), and IPTG (0.5 mM), and incubated at 37 °C for 16 h. One-hundred white colonies from each library were randomly chosen and used for plasmid extraction with QIAprep spin miniprep kit (QIAgen). Purified plasmids were used in sequencing reactions with primer 27F. Sequencing reactions were performed using the BigDye terminator cycle sequencing reaction kit v1.1 (Applied Biosystems) and an ABI 3700 automated DNA sequencer (Applied Biosystems).

Electropherograms were visually inspected and sequences were assembled with BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) sequence alignment editor freeware (version 7.0.5.3). All assembled sequences were examined for chimeric artifacts using CHIMERA CHECK from the Ribosomal Database Project II (RDP) (Cole et al., 2005) and potentially chimeric sequences were discarded. Sequences were aligned on the Ribosomal Database Project (RDP-II) server and grouped according to sequence similarity using DOTUR software (Schloss and Handelsman, 2005), applying the furthest neighbor assignment algorithms with a PHYLIP distance matrix as input file. Sequences with 97% similarity were treated as a single OTU. Sequences (one for each OTU) were aligned with the 16S rDNA sequences of their closest cultured organisms from Ribosomal Database Project (RDP-II) and NCBI databases, using ClustalW (Thompson et al., 1994). Program alignments were evaluated by neighbor-joining using MEGA3 (Kumar et al., 2004). Phylogenetic inference and evolutionary distance calculations were generated using the Jukes–Cantor distance model; 1000 bootstrap replicates were used to obtain confidence estimates for the phylogenetic trees.

2.6. Nucleotide sequence accession numbers

The nucleotide sequences obtained were deposited in GenBank/DDBJ/EMBL databases under accession numbers from EU857836 to EU858006.

2.7. Data analysis

2.7.1. Statistical analysis

Comparison between groups for a significant difference of mean or rank values were performed after normality and variance tests by analysis of variance (ANOVA, Krustal–Wallis method) and the relative importance of each treatment group was investigated by *ad hoc* pairwise multiple comparison procedure (Tukey test). Calculations were carried out using SigmaStat software for Windows, version 3.1 (Copyright 1992–1995, Jandel Corporation). Data were also correlated to measure the associations between pairs of variable.

Physical (depth), chemical (elemental and biochemical composition), biological (enzymatic activities, log transformed bacterial abundances as well as richness, and the diversity indices computed on T-RFLP results) data were used for the principal component analysis (PCA).

Analysis of similarities (ANOSIM) was also carried out. Such analysis provides a method to assess statistical differences between groups in multivariate data sets, and produces a sample statistic, R, which represents the degree of separation between test groups (Clarke, 1993). A value close to 1 indicates the community composition is totally different, whereas a value of 0 indicates no difference. ANOSIM was used with the geographical location as factor.

Moreover, the influence of abiotic variables on the community structure was assessed using the BEST analysis in PRIMER 6 (Clarke and Gorley, 2006). The BEST analysis selects the environmental variables that may explain biotic patterns by using every possible combination of abiotic and biotic variables until it finds the 'best' fit.

2.7.2. Diversity indices

In order to investigate the bacterial diversity both for T-RFLP and clones outputs, the Margalef richness index (d; Margalef, 1958) as well as the Shannon–Wiener index (H'; Magurran, 1996), were calculated assuming that the number of peaks represented an indication of species distribution (ribotype richness) and that the peak heights represented the relative abundance of each bacterial species (Hughes et al., 2001). The Shannon index is a general diversity index that is positively correlated with species richness and evenness, and is more sensitive to change in abundance of rare species. Margalef richness index standardizes the number of species encountered against the total number of individuals encountered. Finally, the evenness (Pielou index, J), which reflects the relative importance of each taxon within the entire assemblage, was also computed (Danovaro et al., 2006).

In particular, for T-RFLP bacterial phylotype/genotype richness was expressed as the total number of peaks within each electropherogram, and the two indices were computed on the

basis of peak heights of each T-RF used as an estimator of relative abundance of each detected genotype. These calculations were performed using Primer 6 software, version 6β R6 (Copyright 2004, PRIMER-E Ltd.).

Coverage values were calculated in order to determine how efficiently the clone libraries described the complexity of the original bacterial community. The coverage (Good, 1953) value is given as $C=1-(n1/N)$ where $n1$ is the number of clones which occurred only once in the library (singletons).

Species richness based on the non-parametric richness estimator Chao1 (Chao, 1987) and abundance-based coverage estimators (ACE) (Chao and Lee, 1992) were calculated for the two clone libraries by EstimateS program (Version 7.5, http://viceroy.eeb.uconn.edu/EstimateS) (Colwell, 2005). Clone libraries diversity calculations were made on the basis of 3% difference between sequences.

3. Results

3.1. Sediment characterization

3.1.1. Elemental composition of sediment

The grain-size of sediments ranged from coarse to fine sands (Dunbar et al., 1985). Overall, water content ranged from 12.0% to 35.8% at station D and E, respectively. Low concentrations of both TC (range 0.15–1.08%) and N (0.01–0.12%) were detected in all samples (Table 1). The highest and lowest OC and TC values were determined at Gerlache Inlet (0.91% and 0.11% at stations E and F, respectively) with a significant increase with water depth, due to natural carbon exportation by pycnoclinic currents (Frignani et al., 2003). IC concentration was negligible at almost all the stations. P amounts resulted between 252 (station C at Adelie Cove) and 1425 $\mu g\ g^{-1}$ d.w. (station A at Tethys Bay). The organic molar carbon/nitrogen (C/N) ratio ranged from 6.88 to 20.99 (mean 11.31 ± 4.64). N content was correlated with OC ($r=0.971$; $P<0.001$; $n=7$) and PRT ($r=0.919$; $P<0.005$; $n=7$).

3.1.2. Biochemical composition of sediment organic matter

Biochemical features of Antarctic sediments are reported in Table 1. The highest values were measured at Tethys Bay (stations A and B). PRT concentrations ranged from 60 ± 5 μg (mean ± standard deviation; MD ± SD) to 1042 ± 45 μg in BSA equivalents g^{-1} d.w. LIP concentrations ranged from 50 ± 4 to 306 ± 19 μg in tripalmitin equivalents, while CHO values ranged from 5 ± 0.5 to 284 ± 21 μg in D(+)-glucose equivalents. For all the three analyses, ANOVA data indicated that there was a statistically significant difference ($P<0.001$). The PRT:CHO ratio was always >1, ranging from 1.4 to 12.1, whereas the LIP/CHO ratio was between 0.9 and 10.0.

DNA content resulted higher at Tethys Bay (stations A and B) than at the other stations, with the minimum value (0.05 μg DNA g^{-1} d.w.) recorded at station F at Gerlache Inlet.

The fraction of BPC in OC ranged from 4.1% to 19.9% at stations B and E, respectively, and the average was $9.6\% \pm 6.0$. The highest BPC values were detected in sediments from stations A (790.9 ± 36.7 μg C g^{-1} d.w.) and B (834.4 ± 40.0 μg C g^{-1} d.w.), where concentrations were approximately 2.5 times higher than in sediments from C, E and G, and approximately 10 times higher than in samples from D and F (Fig. 2).

When considering the whole dataset on the biochemical composition of sediment organic matter, PRT represented the dominant biochemical class contributing to labile organic carbon. This general trend was obtained for all stations. The average percentage composition of BPC was as regard to PRT $52.2\% \pm 10.7$, with a range from 33.9% to 64.6% (stations D and A, respectively). LIP accounted for 25.1–52.7% (stations A and F, respectively) (mean $36.5\% \pm 10.7$); the average of CHO was $11.3\% \pm 6.7$, with a range from 3.0% to 20.1% (stations F and C, respectively).

A significant correlation was found between PRT and LIP ($r=0.957$; $P<0.001$; $n=7$).

3.2. Bacterial abundance and activity

3.2.1. Total cell counts

Higher bacterial abundances were determined at Tethys Bay (station A: $3.6 \times 10^7 \pm 8.5 \times 10^4$ cells g^{-1} d.w.; station B: $4.3 \times 10^7 \pm 1.2 \times 10^5$ cells g^{-1} d.w.), Adelie Cove (station C: $3.9 \times 10^7 \pm 1.3 \times 10^5$ cells g^{-1} d.w.) and Malippo pier (station D: $3.3 \times 10^7 \pm 6.8 \times 10^4$ cells g^{-1} d.w.). By contrast, all stations located at Gerlache Inlet were characterized by slightly lower values (station E: $2.2 \times 10^7 \pm 1.8 \times 10^5$ cells g^{-1} d.w.; station F: $6.1 \times 10^6 \pm 1.1 \times 10^4$ cells g^{-1} d.w.; station G: $1.4 \times 10^7 \pm 4.2 \times 10^4$ cells g^{-1} d.w.). ANOVA results highlighted that there was a statistically significant difference ($P<0.001$), and the subsequent pairwise multiple comparison test (Tukey Test) confirmed that all samples were significantly different ($P<0.05$).

At almost all stations, a significant positive correlation was computed between total bacterial counts and both CHO ($r=0.838$; $P<0.05$; $n=7$) and PRT ($r=0.954$; $P<0.01$; $n=5$), with the exception in the latter case of stations C and D.

Table 1
Elemental and biochemical composition of Antarctic shallow sediments along the Terra Nova Bay.

Station	Bottom depth (m)	Elemental composition [a]							Biochemical composition[b]			
		WC (%)	TC (%)	OC (%)	IC (%)	N (%)	C/N	P ($\mu g\ g^{-1}$)	PRT	CHO	LIP	DNA
Tethys Bay												
A	20	28.0	0.50	0.50	nd	0.07	8.51	1425	1042 ± 45	204 ± 10	284 ± 21	2.47 ± 0.92
B	20	26.8	0.42	0.42	nd	0.07	6.88	1007	1034 ± 44	284 ± 19	306 ± 19	1.21 ± 0.21
Adelie cove												
C	27	22.3	0.31	0.35	nd	0.04	9.37	252	312 ± 23	157 ± 10	137 ± 7	0.66 ± 0.01
Malippo pier												
D	19	12.0	0.29	0.18	0.11	0.01	20.99	839	69 ± 5	48 ± 6	67 ± 5	0.03 ± 0.00
Gerlache inlet												
E	66	35.8	1.08	0.91	0.17	0.12	8.57	721	445 ± 28	77 ± 3	172 ± 8	0.33 ± 0.01
F	31	14.8	0.15	0.11	0.04	0.01	10.69	536	60 ± 5	5 ± 0.5	50 ± 4	0.05 ± 0.00
G	48	24.9	0.73	0.53	0.20	0.06	10.30	641	395 ± 30	41 ± 8	216 ± 19	0.91 ± 0.11

[a] WC: water content; TC: total carbon (TC); OC: organic carbon; IC: inorganic carbon (IC); N: total nitrogen; carbon/nitrogen molar ratio (C/N); P: total phosphorus; nd: not detectable.

[b] PTR: protein content (μg BSA g^{-1} d.w.); CHO: carbohydrate content (μg D-(+)-glucose g^{-1} d.w.); LIP: lipid content (μg tripalmitin g^{-1} d.w.); DNA: DNA content (μg DNA g^{-1} d.w.).

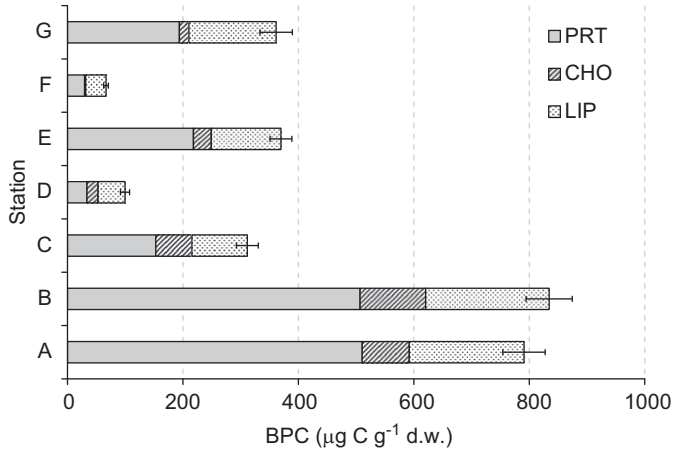

Fig. 2. Stacked histograms of sedimentary (1 cm) concentrations at each sampling station of three biochemical classes: proteins (PRT), carbohydrates (CHO) and lipids (LIP). Results are expressed as carbon equivalents to obtain biopolymeric content (BPC; $\mu gC\,g^{-1}$ sedimentary dry weight). Bars represent standard deviations of triplicate measurements.

3.2.2. Ectoenzymatic activities

Results on the EEAs are presented in Fig. 3. The highest potential Amp activities were found on average at stations A and B at Tethys Bay with 9.7 ± 0.7 and 10.6 ± 0.6 nmoles $g^{-1}\,h^{-1}$, respectively (Fig. 3a). Conversely, the lowest activities were detected at station F at Gerlache Inlet (0.3 nmoles $g^{-1}\,h^{-1}$ d.w.), which was characterized by coarse sand and showed the lowest bacterial abundance, and at station D at Malippo pier (0.7 nmoles $g^{-1}\,h^{-1}$ d.w.).

A similar pattern was followed by the Glu activities (Fig. 3b). The highest values were again determined in samples from stations A and B (7.0 ± 0.3 and 7.2 ± 0.4 nmoles $g^{-1}\,h^{-1}$ d.w., respectively) which were characterized by the highest carbohydrate content.

Est activities resulted about one order of magnitude higher than those of Amp, Glu and AkP in each sample (Fig. 3c). The Est:Glu ratio ranged from 25:1 (station A at Tethys Bay) to 947:1 (station D at Malippo pier); the Est:Amp ratio was 18:1 (station A at Tethys Bay) to 116:1 (station D at Malippo pier); finally, the Est:AkP ratio resulted between 8:1 and 55:1 (stations E and F at Gerlache Inlet, respectively). The highest Est values were found at stations A and B at Tethys Bay (205.0 ± 8.9 and 176.4 ± 14.1 nmoles $g^{-1}\,h^{-1}$ d.w., respectively), and at the deepest station G at Gerlache Inlet (164.8 ± 8.1 nmoles $g^{-1}\,h^{-1}$ d.w.). At stations C, D and E the average Est activity was 98.8 ± 6.1 nmoles $g^{-1}\,h^{-1}$ d.w. The lowest rate was measured at station F (28.2 ± 3.8 nmoles $g^{-1}\,h^{-1}$ d.w.) which was characterized by the lowest bacterial abundance.

Contrary to the other ectoenzymatic activities analyzed throughout this study, the highest AkP activity was found at stations B at Tethys Bay and E at Gerlache Inlet (13.9 ± 1.3 and 13.5 ± 0.5 nmoles $g^{-1}\,h^{-1}$ d.w., respectively) (Fig. 3d). The lowest activity (0.5 ± 0.1 nmoles $g^{-1}\,h^{-1}$ d.w.) was measured at station F. The average AkP activity at the remaining stations (A, C, D and G) was 4.1 ± 1.4 nmoles $g^{-1}\,h^{-1}$ d.w.

The ANOVA data computed for each individual EEA highlighted that there was an overall statistically significant difference ($P < 0.001$); the further pairwise multiple comparison test (Tukey Test) confirmed that almost all samples were significantly different ($P < 0.05$).

The EEAs determined at 5 °C provided the potential hydrolytic activity that may occur at each station in relation to the BPC biochemical classes. Furthermore, data obtained at four different

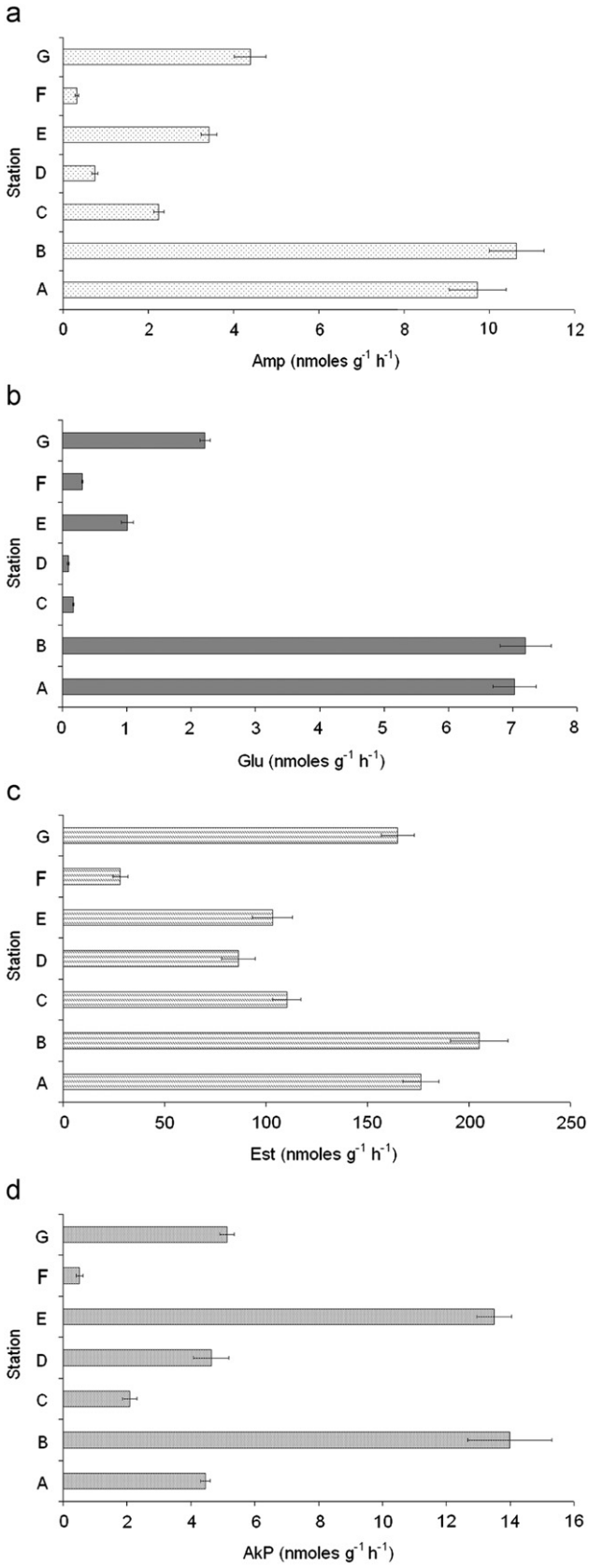

Fig. 3. Determination at 5 °C of ectoenzymatic activities (nmoles $g^{-1}\,h^{-1}$) in sediments collected along Terra Nova Bay. (a) amino-peptidase (Amp); (b) beta-glucosidase (Glu); (c) esterase (Est); (d) alkaline phosphatase (AkP). Bars represent standard deviations of triplicate measurements. Note the different scales.

temperatures (5, 10, 15 and 30 °C) on sediments from station A (Tethys Bay) are presented as an Arrhenius plot (Fig. S1). Such a test revealed that between 5 and 15 °C the Q_{10} was 3.1 for AkP, 2.78 for Glu, 2.51 for Est and 2.49 for Amp. Values determined at 30 and 15 °C were very similar one to each other.

3.3. Bacterial populations structure and composition

3.3.1. T-RFLP analysis

The size of fragments obtained by T-RFLP analysis ranged between 30 and 500 bp (not shown). The highest numbers of T-RFs were retrieved in sample G from Gerlache Inlet (79 and 70 with *Hinf*I and *Taq*I, respectively), whereas the lowest ones were disclosed in sample A from Tethys Bay (26 and 31 for with *Hinf*I and *Taq*I, respectively). The number of T-RFs found in the other stations was comprised between 29 and 70, and between 33 and 52 for *Hinf*I and *Taq*I, respectively (not shown).

All T-RFLP patterns were aligned as described in Section 2. The matrices obtained from the dataset of the two restriction digestions were used to calculate the Jaccard similarity coefficients that were used to perform clustering.

Cluster analysis computed for the *Hinf*I T-RF pattern revealed that the two Tethys Bay sampling stations (A and B) formed a unique cluster (Jaccard = 0.19), while other stations (namely G, C, E and F) constituted a more heterogeneous group. Within this latter cluster, stations E and F were the most similar (Jaccard = 0.23). Lastly, station D clustered as an outgroup being the most dissimilar sample with an average Jaccard index of 0.13.

Two well definite clusters were observed for the *Taq*I T-RF patterns. The first included stations A, B and F. Among these, the first two fell into a subcluster (Jaccard = 0.33). The second cluster, that included stations C, D, E and G, was composed of two subclusters: one composed of stations D and E (Jaccard = 0.32) and the second composed of stations C and G (Jaccard = 0.25).

Diversity indices (Fig. 4) computed between the T-RF patterns obtained using the two restriction enzymes highlighted that both Shannon and Margalef values were always higher for profiles from *Hinf*I than *Taq*I, even though they shared a similar trend, with an increase from stations A to G. As expected, the same trend was displayed by the Pielou index (not shown). Station A exhibited the lowest value of both richness and diversity, whereas station G was characterized by the highest ones.

Moreover, stations A and B (both at Tethys Bay) showed similar values of richness and diversity for both the used restriction enzymes. By contrast, indices computed between stations E and F (both at Gerlache Inlet) were significantly different, even though they were geographically very close.

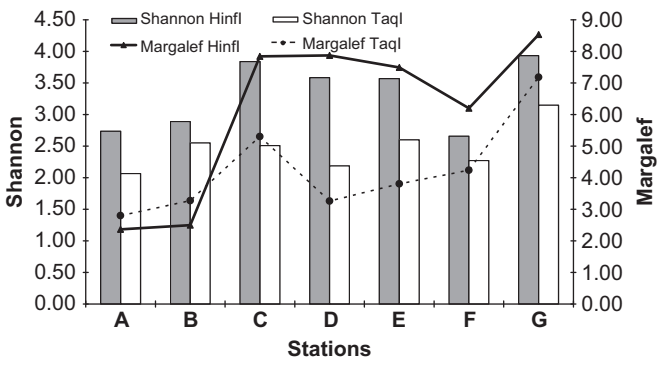

Fig. 4. Shannon and Margalef diversity indices computed between the T-RF patterns obtained using the restriction enzymes *Hinf*I and *Taq*I.

3.3.2. Principal component analysis (PCA)

The relationships existing among physical, chemical and biological parameters for each sampling station were analyzed by PCA. We considered the following eight parameters: sampling depth, sedimentary water content, sedimentary elemental composition (OC, total N and P), sedimentary biochemical classes (PRT, CHO, LIP and DNA), bacterial abundance, EEAs (AkP, Est, Glu and Amp), T-RF richness (for both enzymes) and diversity indices (for both enzymes). Data obtained are shown in Fig. 5.

The two principal components explained 99.1% of total variance (PC1: 86.4%; PC2: 12.6%). The first component PC1 from loading plots was directly correlated to a mixture of biological and biochemical parameters (PRT, CHO, LIP, BPC). However, none of these parameters appeared to weigh heavily. The second component was directly correlated to P, BPC and PRT.

MultiDimensional scaling (MDS) analysis confirmed results from PCA with a stress value of 0.01 (not shown). The subsequent multivariate analyses (ANOSIM) computed with geographical locations as factor [Tethys Bay (TB): stations A and B; Adelie Cove (AC): station C; Malippo Pier (MP): station D; Gerlache Inlet (GI): stations E, F and G], revealed that the community structure differed among the four considered areas (Global $R = 0.292$; $P < 0.001$). In particular, ANOSIM Pairwise Test highlighted that there was a statistically significant difference between TB and both AC and MP (R Statistic = 1; $P < 0.03$) and between TB and GI (R Statistic 0.33; $P < 0.001$). On the contrary, no significant difference resulted between GI and both AC and MP (not shown).

Finally, the BEST analysis was carried out to identify potential abiotic parameters responsible for differences in the community structure among the sampling stations. Data obtained revealed that the highest rank correlation ($\rho = 0.992$; $P < 0.001$) was due to the following five variables: P, PRT, CHO, LIP and Est.

3.3.3. Clone libraries

Two clone libraries were constructed for stations displaying the lowest and highest richness and diversity (stations A and G, respectively). Station A was located at Tethys Bay, while station G was at Gerlache Inlet.

A total of 173 16S rRNA gene sequences were obtained. Among these, two were found to be chimeric and were excluded from further analyses. All sequences with similarity $\geq 97\%$ were considered as belonging to the same OTU. In this way, the clone sequences from stations A (92 clones) and G (79 clones) were clustered into 51 and 54 OTUs, respectively. The clone coverage was 0.61 and 0.53 for stations A and G, respectively. Finally, the Chao1 and ACE richness estimators were computed for the two considered libraries: for station A Chao1 and ACE-1 were 127 ± 35.0 (95% confidence interval: 83.7–267.1) and 153.5 ± 51.5 (95% confidence interval: 92.0–311.9), respectively; for station G Chao1 was 109.1 ± 2.0 (95% confidence interval: 78.5–179.3) and ACE-1 was 151.6 ± 49.2 (95% confidence interval: 92.7–302.5). The diversity indices computed for the two clone libraries confirmed that the station G presented higher values of both Shannon and Margalef indices (3.9 and 12.8, respectively) than station A (3.2 and 11.1, respectively).

The phylogenetic analysis revealed the distribution of clones within nine and eleven major taxonomical groups of bacteria at stations A and G, respectively. A low percentage (4–6%) of sequences were closely related to unclassified bacteria. Eight of these taxa, including the over-represented ones (i.e. *Bacteroidetes*, *Gammaproteobacteria* and *Deltaproteobacteria*), were shared by the two stations (Fig. 6).

In both clone libraries, the phylum *Proteobacteria* constituted the largest fraction (48–49%) of detected sequences, with the *Gamma* and *Delta* subdivisions being the most abundant ones.

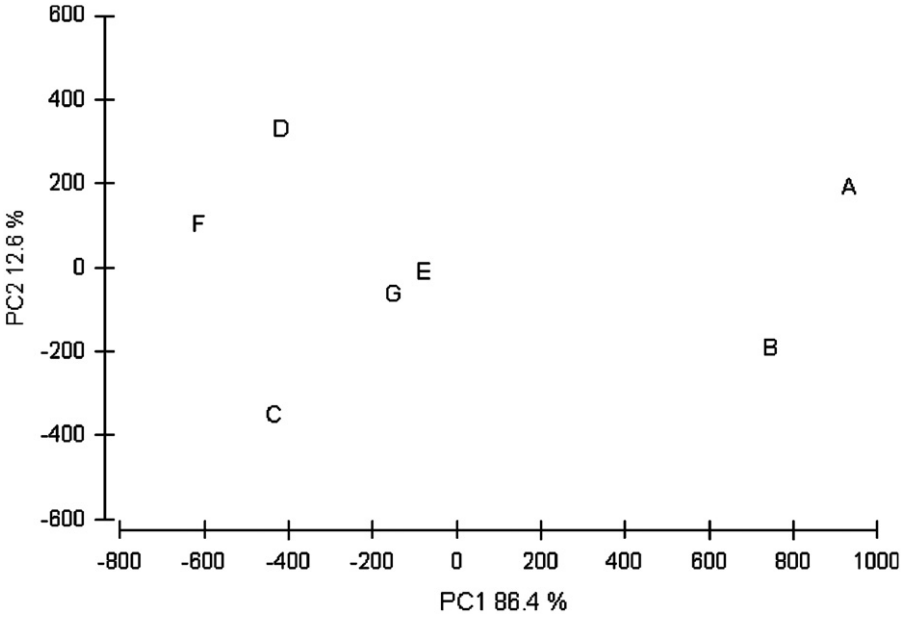

Fig. 5. Biplot of the PCA. Parameters and stations are plotted on the plane of the first two principal components (PC1 and PC2). The explained variance is shown on the axes.

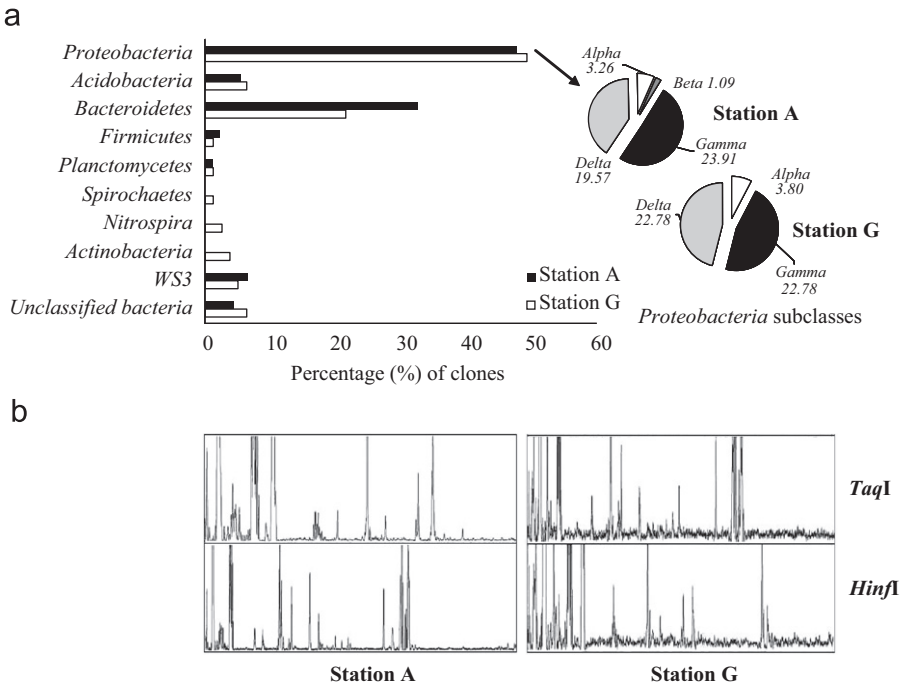

Fig. 6. Bacterial population structure and composition in sediments collected from the stations A (Tethys Bay) and G (Gerlache Inlet). (a) Schematic representation of different phylogenetic groups obtained from clustering of clone sequences of the two 16R rDNA clone libraries. (b) Comparison of 16S rDNA-based T-RFLP community fingerprint patterns obtained by digesting PCR products with *Taq*I and *Hinf*I.

Sequences affiliated with the *Gammaproteobacteria* constituted about 23–24% of clones and several proteobacterial phylotypes were only distantly related to known bacteria (Fig. S2) and most similar to uncultured *Gammaproteobacteria* related to the order *Chromatiales* and the family *Ectothiorhodospiraceae*. Among the *Deltaproteobacteria*, which represented 19% and 23% of clones at stations A and G, respectively, most sequences were affiliated with *Desulfosarcina* and *Desulfurophalus*, two genera including sulfate-reducing bacteria (Fig. S3). Only few clones belonged to *Alpha-* (three clones *per* station) or *Betaproteobacteia* (one clone detected at station A and affiliated to *Burkholderia* sp.).

The *Bacteroidetes* was the second most abundant phylogenetic group in both sediment samples (32.6% and 21.5% at stations A and G, respectively). Most sequences (86%) in such phylum belonged to the family *Flavobacteriaceae* and were affiliated with four major *Flavobacteriaceae* clades (Fig. S4): *Gelidibacter/Bizionia, Zobellia/Maribacter*, *Ulvibacter* and *Lutibacter*.

Two smaller groups common to both stations were those of the *Acidobacteria* (5–6%) (Fig. S5), with new (possible) species close to the *Halophaga* sp. group, and the WS3 division (5–6%). Sequences affiliated with the *Planctomycetes* and *Firmicutes* were scarcely represented in both sediment samples. Finally, few clones

representative of the *Actinobacteria* (three clones), *Nitrospira* (two clones) and *Spirochaetes* (one clone) divisions were detected exclusively at station G (Fig. S6).

4. Discussion

In this study the OC represented the major fraction of TC and displayed concentrations similar to or slightly lower than those previously measured in Antarctic bottom sediments (Isla et al., 2002, 2004, 2006; Masquè et al., 2002). The highest OC value could be dependent on the OC exportation to deeper water by pycnoclinic currents, as observed by Frignani et al. (2003). The C/N ratio generally remained around 9, thus resulting higher than the 6.625 (Redfield (1958)) ratio. This finding indicated the degradation of marine organic matter and a possible contribution of sea-ice. At station D, N content was comparably low, thus producing the highest C/N value. Overall, the C/N values were higher than those previously observed in bottom Antarctic sediments (DeMaster et al., 1996; Isla et al., 2002; Masquè et al., 2002). The correlation computed between N content and both OC and PRT suggested a biological origin of N.

Few studies have been carried out on the biochemical composition of the sedimentary organic matter in Antarctic sediments (e.g., Fabiano and Danovaro, 1998; Isla et al., 2002, 2004, 2006; Masquè et al., 2002; Pusceddu et al., 2000). The BPC content accounted for a low percentage of OC, even if it was summed to humic acid (14%; *data not shown*), which represented approximately 20% OC. Hence, the largest OC fraction remained uncharacterized. However, it has been demonstrated that the more refractory organic matter, which accumulates in Ross Sea sediments is mainly a relict with a mean age of 5200 yr BP and formed by recycled inert geopolymers originating from glacier erosion (Ohkouchi and Eglinton, 2008). Overall, BPC concentrations displayed a wide range of trophic conditions and resulted quite low compared with those previously determined in coastal sediments at Terra Nova Bay (Danovaro et al., 1999; Pusceddu et al., 2000). Such discrepancy might depend on several factors, including the interannual variability (Nedwell et al., 1993), differences in general environmental conditions at sampling time or patchiness of sedimentary organic matter concentrations, as supported by observations on the distribution of meio- and macro-benthic communities (e.g. Cattaneo-Vietti et al., 1997; Danovaro et al., 1999; Fabiano and Danovaro, 1999). The highest BPC values were determined at Tethys Bay that is generally covered by ice during the Austral Summer. However, it was exceptionally free from ice during the sampling period (25 January). A plausible explanation for this finding might be the organic input deriving from the ice-melting and the further phytoplankton bloom. It is plausible that BPC in the bay was mainly due to the metabolic activity of diatoms whose abundance in sediments was $1.16–2.83 \times 10^6$ cells g^{-1} (*data not shown*). This idea was supported by the relatively high PRT/CHO ratio (on average 4.4), which generally displays high values during early stage of a phytoplankton bloom, consistently with high amounts of freshly produced organic compounds (Pusceddu et al., 2000). Finally, the highest BPC values and ectoenzymatic activities at Tethys Bay were due to the geographical configuration of this semi-enclosed area, where OC remains entrapped instead to be exported by pycnoclinic currents to deeper waters (Frignani et al., 2003).

The OC speciation in PRT, LIP and CHO represents a useful tool to assess the labile portion of organic matter (Polymenakou et al., 2007). Overall, the main fractions of BPC were PRT and LIP and, consequently, high PRT/CHO and LIP/CHO ratios were measured. At temperate latitudes, sediments generally display a PRT/CHO ratio <1 (Isla et al., 2006). By contrast, throughout this study such a ratio showed always values >1, ranging from 1.4 to 12.1. This great PRT accumulation is apparently not common, since CHO are usually the main reservoir of labile compounds on the continental shelf (Köster and Meyer-Reil, 2001; Pusceddu et al., 2000). However, PRT predominance over CHO and LIP in sediments at higher latitudes has been previously reported for both coastal and deep areas at Terra Nova Bay (Danovaro et al., 1999; Fabiano and Danovaro, 1998; Pusceddu et al., 2000) and in the Eastern Weddell Sea (Isla et al., 2006). In this study, PRT probably accumulated in superficial sediments because anaerobic microbial activities were more prevalent and faster in fermenting CHO than amino-acids, and due to the fact that PRT tend to combine with refractory compounds (Covazzi Harriague et al., 2007).

The second major constituent of BPC, the LIP content (average 39.4%), was well correlated with PRT. LIP were readily degraded by esterases belonging to a class of enzymes that are commonly synthesized by heterotrophic aerobic and anaerobic bacteria. Diatoms and fecal pellets of zooplankton are assumed to be the most important carriers of LIP to Antarctic seabed sediments (Mühlebach and Weber, 1998). In particular, diatom aggregates can be responsible for the high nutritive quality (in terms of PRT and LIP contents) found in shelf sediments (Isla et al., 2006). In this study, the LIP/CHO ratio resulted twice higher than other Antarctic sediments (Fabiano and Pusceddu, 1998; Isla et al., 2006).

The high-molecular weight organic compounds that largely composed marine sediments must be hydrolyzed prior to the bacterial uptake throughout enzymatic activities. The activity of Amp, Glu, AkP and Est were temperature-dependent. Contrary to deep-water sediments, few reports exist on coastal sedimentary EEA, thus making difficult a comparison. As previously observed for sediments from temperate and high latitudes, Amp activity exceeded Glu activity, even if the Amp/Glu ratio resulted relatively lower than those previously reported (Danovaro et al., 2001; Fabiano and Danovaro, 1998; Poremba and Hoppe, 1995). Both Glu and Amp activities resulted generally similar to or higher than those measured in sediments from the Northern Adriatic and eastern Antarctica (Bowman et al., 2003; Danovaro et al., 2001), although lower than those determined in deep-water sediments of the Ross Sea (Fabiano and Danovaro, 1998). By contrast, Est activities resulted always higher than the other ectoenzymatic activities analyzed in shallow Antarctic sediments, although LIP were not predominant within BPC. This finding suggests that the mobilization of the different biochemical classes may proceed at different rates, independently from substrate concentrations. As previously observed by Fabiano and Danovaro (1998) for Ross Sea deep-water sediments, EEAs varied significantly among the stations investigated, thus suggesting a site-specificity. In addition, Amp, Glu and Est activities generally appeared to be related to the amounts of PRT, CHO and LIP, respectively. Conversely, AkP activities were correlated neither to total P in sediments nor to bacterial abundances, although this enzyme is considered as an alternative measure of biomass (Fabiano and Danovaro, 1998). In addition to the high concentration of P in sediments, the absence of correlations of the EEAs with microbiological and biochemical parameters might be dependent on the geochemical sedimentation rather than on the microbial activity.

Bacterial abundances resulted one to two orders of magnitude lower than those reported for deeper Antarctic sediments (Fabiano and Danovaro, 1998; Bowman et al., 2003). The bacterial distribution appeared to be strongly dependent on the availability of organic matter in sediments. This finding was strengthened by the significant statistical differences computed between stations with respect to the bacterial abundance values, which were

generally reflected by the BPC content. Based on the significant positive correlation obtained between total bacterial counts and PRT, it could assumed that part of N was very likely originated from microbial abundance.

In the present study, we report for the first time on the biodiversity and community structure of benthic microbial community inhabiting Terra Nova Bay, thus gaining further understanding of the under-investigated microbiology of coastal polar sediments. The analysis of the 16S rRNA gene clone libraries revealed a rich phylogenetic diversity and an evident sequences-sharing between stations. Overall, sequences fell into twelve major bacterial phylogenetic lineages including *Alpha-*, *Beta-*, *Delta-* and *Gammaproteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Planctomycetes*, *Spirochaetes*, *Nitrospira* and WS3. Among these, clones affiliated to *Spirochaetes*, *Nitrospira* and *Actinobacteria* occurred only in the Gerlache Inlet library. At both stations, *Proteobacteria* and *Bacteroidetes* were over-represented. These data are in agreements with previous observations on Arctic (Li et al., 2009; Ravenschlag et al., 1999; Tian et al., 2009) and Antarctic (Bowman et al., 2003; Bowman and McCuaig, 2003) sediments. The predominance of *Proteobacteria* was reported in most surface marine sediments in polar environments, where they represent $>50\%$ of the microbial biomass (Bowman et al., 2003; Li et al., 2009; Ravenschlag et al., 2001). Almost half of the proteobacterial clones were allocated in the *Delta* subclass (about 20% of the libraries). This finding is in disagreement with other investigations revealing *Gammaproteobacteria* as the most significant clades in marine sediments worldwide (Bowman and McCuaig, 2003; Inagaki et al., 2003; Li et al., 2009; Polymenakou et al., 2005). However, members of the *Deltaproteobacteria* are mainly sulfate-reducers and sulfate reduction may account for up to 50% of organic matter degradation in coastal marine sediments (Jørgensen, 1982). They were probably responsible for the sulfur, iron and phosphorous precipitates which coated the siliceous and alumino-silicate sediments analyzed throughout this study (as determined by energy dispersive X-ray analysis; *data not shown*). In particular, sequences were mainly affiliated with two genera, *Desulforhopalus* and *Desulfosarcina*, whose members have been detected in Antarctica (Bowman and McCuaig, 2003; Purdy et al., 2003), Svalbard (Arctic) (Knoblauch et al., 1999; Ravenschlag et al., 1999) and Japan Trench (Li et al., 1999), highlighting their ubiquitous presence in low-temperature marine sediments. The occurrence of such strictly anaerobic bacteria suggests that sediments might also undergo anoxic conditions that could favor the accumulation of PRT in respect to CHO, assuming that fermentation of amino acids is slower than that of sugars from decomposing polysaccharides (Neira et al., 2001). The anoxia at surface sediments is strengthened by the abundance of sequences affiliated to *Gammaproteobacteria Chromatiales* and *Ecthothiorhodospiraceae*. Sulfides from *Deltaproteobacteria* are probably metabolized by such anaerobic photosynthetic bacteria of the sulfur cycle. Modest microbial productivity may therefore contribute to BPC productivity, when shallow-water sediments are irradiated during the austral summer.

In both 16S rRNA gene libraries, a conspicuous presence of sequences affiliated with four *Flavobacteriaceae* marine clades was determined. This finding suggests that *in vivo* degradation of organic polymers in shallow-water Antarctic sediments may be mainly carried out by these bacteria, which are known for their ability to efficiently degrade many types of polysaccharides (Bowman and Nichols, 2005). Therefore, the concomitant occurrence of low CHO content, scarce Glu activity and high abundance of *Flavobacteria* may derive from previous carbohydrate consumption during the early summer algal bloom. Besides, low enzyme activity might be explained by the not prevalent concentration of cellulose-like polysaccharides (substrate for beta-glucosidase) in the analyzed sediments.

In conclusion, even though the highest concentrations of TC in sediments were determined at higher water depths, the chemical speciation of OC within BPC indicated that major differences between sediments were observed in the semi-enclosed Tethys Bay, where BPC accumulate due to its scarce exportation to deeper waters. The complex population structure consisted of aerobic and anaerobic heterotrophic bacteria with the occurrence of chemolithotrophs. Such a mixture demonstrated that surface sediments may alternate from oxidizing to anoxic conditions and *viceversa*, thus affecting BPC degradation. However, the *in situ* occurrence of a bacterial productivity based on S-cycle, especially during dark period, cannot be *a priori* excluded. Iron and sulphur precipitation on sediment particles was detected by EDX-SEM analysis (data not shown), suggesting that they might represent substrata supplying energy to chemolithotrophic bacteria.

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Appendix A. Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csr.2010.06.009.

References

Albertelli, G., Cattaneo-Vietti, R., Chiantore, M., Pusceddu, A., Fabiano, M., 1998. Food availability to an *Adamussium* bed during the austral summer 1993/94 (Terra Nova Bay, Ross Sea). J. Mar. Syst. 17, 425–434.

Aspila, K.I., Agemian, H., Chau, A.S.Y., 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101, 187–197.

Bahulikar, R.A., Kroth, P.G., 2008. The complex extracellular polysaccharide of mainly chain-forming freshwater diatom species from epilithic biofilms. J. Phycol. 44, 1465–1475.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phisiol. 37, 911–917.

Bowman, J.P., McCuaig, R.D., 2003. Biodiversity, community structural shifts, and biogeography on prokaryotes within Antarctic continental shelf sediment. Appl. Environ. Microbiol. 69, 2463–2483.

Bowman, J.P., Nichols, D.S., 2005. Novel members of the family Flavobacteriaceae from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. Int. J. Syst. Evol. Microbiol. 55, 1471–1486.

Bowman, J.P., McCammon, S.A., Gibson, J.A.E., Robertson, L., Nichols, P.D., 2003. Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Appl. Environ. Microbiol. 69, 2448–2462.

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cattaneo-Vietti, R., Chiantore, M., Albertelli, G., 1997. The population structure and ecology of the Antarctic scallop *Adamussium colbecki* at Terra Nova Bay (Ross Se, Antarctica). Sci. Mar. 61, 15–24.

Chao, A., 1987. Estimating the population size for capture–recapture data with unequal catchability. Biometrics 43, 783–791.

Chao, A., Lee, S.-M., 1992. Estimating the number of classes via sample coverage. J. Am. Stat. Assoc. 87, 210–217.
Christian, J.-R., Karl, D.M., 1995. Bacterial ectoenzymes in marine waters: activity ratios and temperature responses in three oceanic provinces. Limnol. Oceanogr. 40, 1042–1049.
Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143.
Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User manual/tutorial, PRIMER-E, Plymouth.
Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M., Tiedje, J.M., 2005. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res., D294–D296.
Colwell, R.K., 2005. EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. User's Guide and application published at: 〈http://purl.oclc.org/estimates〉.
Covazzi Harriague, A., Misic, C., Petrillo, M., Albertelli, G., 2007. Stressors affecting the macrobenthic community in Rapallo Harbour (Ligurian Sea, Italy). Sci. Mar. 71, 705–714.
Danovaro, R., Luna, G.M., Dell'Anno, A., Pietrangeli, B., 2006. Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. Appl. Environ. Microbiol. 72, 5982–5989.
Danovaro, R., Manini, E., Fabiano, M., 2001. Exoenzymatic activity and organic matter composition in sediments of the Northern Adriatic Sea: response to a river plume. Microb. Ecol. 44, 235–251.
Danovaro, R., Pusceddu, A., Mirto, S., Fabiano, M., 1999. Miofaunal assemblages associated with scallop beds (*Adamussium colbecki*) in the coastal sediments of Terra Nova Bay (Ross Sea, Antarctica). Antarct. Sci. 11, 415–418.
DeMaster, D.J., Ragueneau, O., Nittrouer, C., 1996. Preservation efficiencies and accumulation rates for biogenic silica and organic C, N and P in high-latitude sediments: the Ross Sea. J. Geophys. Res. 101, 18501–18518.
Di Cello, F., Pepi, M., Baldi, F., Fani, R., 1997. Molecular characterization of an n-alkane-degrading bacterial community and identification of a new species, *Acinetobacter venetianus*. Res. Microbiol. 148, 237–249.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Ducklow, H.W., Erickson, M., Kelly, J., Montes-Hugo, M., Ribic, C.A., Smith, R.C., Stammerjohn, S.E., Karl, D.M., 2008. Particle export from the upper ocean over the continental shelf of the west Antarctic Peninsula: a long-term record, 1992–2007. Deep Sea Res. 55, 2118–2131.
Dunbar, R.B., Anderson, J.B., Domack, E.W., Jacobs, S.S., 1985. Oceanographic influences on sedimentation along the Antarctic continental shelf. Antarct. Res. Ser. 43, 291–312.
Engebretson, J.J., Moyer, C.L., 2003. Fidelity of select restriction endonucleases in determining microbial diversity by terminal-restriction fragment length polymorphism. Appl. Environ. Microbiol. 69, 4823–4829.
Fabiano, M., Danovaro, R., 1998. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). Appl. Environ. Microbiol. 64, 3838–3845.
Fabiano, M., Danovaro, R., 1999. Meiofauna distribution and mesoscale variability in two sites of the Ross Sea (Antarctica) with contrasting food supply. Polar Biol. 22, 115–123.
Fabiano, M., Pusceddu, A., 1998. Total and hydrolizable particulate organic matter (carbohydrates, proteins and lipids) at a coastal station in Terra Nova Bay (Ross Sea, Antarctica). Polar Biol. 19, 125–132.
Fichez, R., 1991. Composition and fate of organic matter in submarine cave sediments; implication for the biogeochemical cycle of organic carbon. Oceanol. Acta 14, 369–377.
Fischer, G., Fütterer, D., Gersonde, R., Honjo, S., Ostermann, D., Wefer, G., 1988. Seasonal variability of particle flux in the Weddell Sea and its relation to ice cover. Nature 306, 363–365.
Fonda Umani, S., Montia, M., Bergamasco, A., Cabrini, M., De Vittor, C., Burba, N., Del Negro, P., 2005. Plankton community structure and dynamics versus physical structure from Terra Nova Bay to Ross Ice Shelf (Antarctica). J. Mar. Syst. 55, 31–46.
Frignani, M., Giglio, F., Accornero, A., Langone, L., Ravaioli, M., 2003. Sediment characteristics at selected sites of the Ross Sea continental shelf: does the sedimentary record reflect water column fluxes? Antarct. Sci. 15, 133–139.
Good, I.J., 1953. The population frequencies of species and the estimation of population parameters. Biometrika 40, 237–264.
Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. 11, 299–308.
Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannan, B.J., 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. Appl. Environ. Microbiol. 67, 4399–4406.
Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., Neaslon, K.H., Horikoshi, K., 2003. Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. Appl. Environ. Microbiol. 69, 7224–7235.
Isla, E., Masqué, P., Palanques, A., Guillén, J., Puig, P., Sanchez-Cabeza, J.A., 2004. Sedimentation of biogenic constituents during the last century in western Bransfield and Gerlache Straits, Antarctica: a relation to currents, primary production, and sea floor relief. Mar. Geol. 209, 265–277.
Isla, E., Masqué, P., Palanques, A., Sanchez-Cabeza, J.A., Bruach, J.M., Guillen, J., Puig, P., 2002. Sediment accumulation rates and carbon burial in the bottom sediment in a high-productivity area: Gerlache Strait (Antarctica). Deep Sea Res. 49, 3275–3287.
Isla, E., Rossi, S., Palanques, A., Gili, J.M., Gerdes, D., Amtz, W., 2006. Biochemical composition of marine sediment from the eastern Weddel Sea (Antarctica): high nutritive value in a high benthic-biomass environment. J. Mar. Syst. 60, 255–267.
Jørgensen, B.B., 1982. Mineralisation of organic matter in the sea-bed—the role of sulfate reduction. Nature 296, 643–645.
King, M.G., 1986. Characterization of b-glucosidase activity in intertidal marine sediments. Appl. Environ. Microbiol. 51, 373–380.
Knoblauch, C., Sahm, K., Jørgensen, B.B., 1999. Psychrophilic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov., and *Desulfotalea arctica* sp. nov. Int. J. Syst. Bacteriol. 49, 1631–1643.
Köster, M., Meyer-Reil, L.-H., 2001. Characterization of carbon and microbial biomass pools in shallow water coastal sediments of the southern Baltic Sea (Nordrügensche Bodden). Mar. Ecol. Prog. Ser. 214, 25–41.
Kostka, J.E., Thamdrup, B., Glud, R.N., Canfield, D.E., 1999. Rates and pathways of carbon oxidation in permanently cold Arctic sediments. Mar. Ecol. Prog. Ser. 180, 7–21.
Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5, 150–163.
Li, L., Kato, C., Horikoshi, K., 1999. Microbial diversity in sediments collected from the deepest cold-seep area, the Japan Trench. Mar. Biotechnol. 1, 391–400.
Li, H., Yu, Y., Luo, W., Zeng, Y., Chen, B., 2009. Bacterial diversity in surface sediments from the Pacific Arctic Ocean. Extremophiles 13, 233–246.
Lukow, T., Dunfield, P.F., Liesack, W., 2000. Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. FEMS Microbiol. Ecol. 32, 241–247.
Luna, G.M., Dell'Anno, A., Danovaro, R., 2006. DNA extraction procedure: a critical issue for bacterial diversity assessment in marine sediments. Environ. Microbiol. 8, 308–320.
Magurran, A.E., 1996. Ecological Diversity and its Measurement. Chapman and Hall, London.
Margalef, R., 1958. Information theory in ecology. Gen. Syst. 3, 36–71.
Marsh, J.B., Weistein, D.B., 1966. Simple charring methods for determination of lipids. J. Lipid Res. 7, 574–576.
Masquè, P., Isla, E., Sanchez-Cabeza, J.A., Palanques, A., Bruach, J.M., Puig, P., Guillèn, J., 2002. Sediment accumulation rates and carbon fluxes to bottom sediments at the Western Bransfield Strait (Antarctica). Deep Sea Res. 49, 921–933.
Mayer, L.M., 1989. Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. Limnol. Oceanogr. 34, 973–981.
Mayer, L.M., Shick, L.L., Setchell, F.W., 1986. Measurement of protein in nearshore marine sediments. Mar. Ecol. Prog. Ser. 30, 159–165.
Mecozzi, M., Dragone, P., Amici, M., Pietrantonio, E., 2000. Ultrasound assisted extraction and determination of carbohydrate fraction in marine sediments. Org. Geochem. 31, 1797–1803.
Michaud, L., Di Cello, F., Brilli, M., Fani, R., Lo Giudice, A., Bruni, V., 2004. Biodiversity of cultivable psychrotrophic marine bacteria isolated from Terra Nova Bay (Ross Sea, Antarctica). FEMS Microbiol. Lett. 230, 67–71.
Mincks, S.L., Smith, C.R., DeMaster, D.J., 2005. Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment "food bank". Mar. Ecol. Prog. Ser. 300, 3–19.
Mühlebach, A., Weber, K., 1998. Origin and fate of dissolved sterols in the Weddell Sea, Antarctica. Org. Geochem. 29, 1595–1607.
Nedwell, D.B., Walker, T.R., Ellis-Evans, J.C., Clarke, A., 1993. Measurements of seasonal rates and annual budgets of organic carbon fluxes in an Antarctic coastal environment at Signy Island, South Orkney Islands, suggest a broad balance between production and decomposition. Appl. Environ. Microbiol. 59, 3989–3995.
Neira, C., Sellanes, J., Levin, L.A., Arntz, W.E., 2001. Meiofaunal distributions on the Peru margin: relationship to oxygen and organic matter availability. Deep Sea Res. 48, 2453–2472.
Nunn, B.L., Keil, R.G., 2006. A comparison of non-hydrolytic methods for extracting amino acids and proteins from coastal marine sediments. Mar. Chem. 98, 31–42.
Ohkouchi, N., Eglinton, T.I., 2008. Compound specific radicarbon dating of Ross Sea sediments: a prospect for constructing chronologies in high-latitude oceanic sediments. Quat. Geochronol. 3, 235–243.
Polymenakou, P.N., Bertilsson, S., Tselepides, A., Stephanou, E.G., 2005. Bacterial community composition in different sediments from the eastern Mediterranean Sea: a comparison of four 16 S ribosomal DNA clone libraries. Microb. Ecol. 50, 447–462.
Polymenakou, P.N., Fragkioudaki, G., Tselepides, A., 2007. Bacterial and organic matter distribution in the sediments of the Thracian Sea (NE Aegean Sea). Cont. Shelf Res. 27, 2187–2197.
Poremba, K., Hoppe, H.G., 1995. Spatial variation of benthic microbiol production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. Mar. Ecol. Prog. Ser. 118, 237–245.

Purdy, K.J., Nedwell, D.B., Embley, T.M., 2003. Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting Antarctic sediments. Appl. Environ. Microbiol. 69, 3181–3191.
Pusceddu, A., Cattaneo-Vietti, R., Alberelli, G., Fabiano, M., 1999. Origin, biochemical composition and vertical flux of particulate organic matter under the pack ice in Terra Nova Bay (Ross Sea, Antarctica) during late summer 1995. Polar Biol. 22, 124–132.
Pusceddu, A., Dell'Anno, A., Fabiano, M., 2000. Organic matter composition in coastal sediments at Terra Nova Bay (Ross Sea) during summer 1995. Polar Biol. 23, 288–293.
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.
Ravenschlag, K., Sahm, K., Pernthaler, J., Amann, R., 1999. High bacterial diversity in permanently cold marine sediments. Appl. Environ. Microbiol. 65, 3982–3989.
Redfield, A.C., 1958. The biological control of chemical factors in the environment. Am. Sci. 46, 205–220.
Schloss, P.D., Handelsman, J., 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl. Environ. Microbiol. 71, 1501–1506.
Smetacek, V., Scharek, R., Nöthig, E.-M., 1990. Seasonal and regional variation in the pelagial and its relationship to the life history cycle of krill. In: Kerry, K.R., Hempel, J. (Eds.), Antarctic Ecosystems, Ecological Change and Conservation. Springer, Berlin Heidelberg, New York, pp. 103–114.
Smith, C.J., Danilowicz, B.S., Clear, A.K., Costello, F.J., Wilson, B., Meijer, W.G., 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiol. Ecol. 54, 375–380.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Tian, F., Yu, Y., Chen, B., Li, H., Yao, Y.-F., Guo, X.-K., 2009. Bacterial, archeal and eukaryotic diversity in Arctic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. Polar Biol. 32, 93–103.
Vetter, Y.A., Deming, J.W., 1994. Extracellular enzyme activity in the Arctic northeast water polynya. Mar. Ecol. Prog. Ser. 114, 23–34.
Zipper, H., Brunner, H., Bernhagen, J., Vitzthum, F., 2004. Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. Nucleic Acids Res. 32, e103.
Zipper, H., Buta, C., Lammle, K., Brunner, H., Bernhagen, J., Vitzthum, F., 2003. Mechanisms underlying the impact of humic acids on DNA quantification by SYBR Green I and consequences for the analysis of soils and aquatic sediments. Nucleic Acids Res. 31, e39.