Biogeosciences Discuss., 9, 253–310, 2012
www.biogeosciences-discuss.net/9/253/2012/
doi:10.5194/bgd-9-253-2012

Biogeosciences Discussions

Paleoenvironmental imprint on subseafloor microbial communities in Western Mediterranean Sea Quaternary sediments

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Received: 11 October 2011 – Accepted: 5 December 2011 – Published: 9 January 2012

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

An interdisciplinary study was conducted to evaluate the relationship between geological and paleontological parameters and the bacterial and archaeal community structure of two contrasted subseafloor sites in the Western Mediterranean Sea (Ligurian Sea and Gulf of Lions). Since both depositional environments were well-documented in this area, large data-sets were available and allowed to calibrate the investigated cores with several reference and dated cores previously collected in the same area, and notably correlated to Quaternary climate variations. Molecular-based fingerprints showed that the Ligurian Sea sediments, characterized by an heterolithic facies with numerous turbidites from a deep-sea levee, were unexpectedly dominated by *Betaproteobacteria* (more than 70 %), at the base of the core mainly below five meters in the sediment. Analysis of relative betaproteobacterial abundances and turbidites frequency indicated that the microbial diversity was controlled by the important climatic changes occurring during the last 20 ka. This result was supported by statistical direct multivariate canonical correspondence analyses (CCA). In contrast, the Gulf of Lions core, characterized by a homogeneous lithology of upper-slope environment, was dominated by the *Bacteroidetes* group and in a lesser extent, by the *Betaproteobacteria* group. At both sites, the dominance of *Betaproteobacteria* coincided with increased terrestrial inputs, as confirmed by the geochemical measurements (Si, Sr, Ti and Ca). In the Gulf of Lions, geochemical parameters were also found to drive microbial community composition. Taken together, our data suggest that the palaeoenvironmental history of erosion and deposition recorded in the Western-Mediterranean Sea sediments has left its imprint on the structure/composition of the microbial communities during the late Quaternary.

1 Introduction

Several consistent lines of evidence suggest that environmental characteristics such as geological, geochemical, geophysical and sedimentological properties of sediments

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are important factors controlling the habitability of the subseafloor sediments. Generally, microbial abundances exponentially decrease with increasing sediment depth (Parkes et al., 1994; Parkes et al., 2000) particularly due to a decreasing reactivity of organic matter with depth (Middelburg, 1989). However, local increases in microbial activity and microbial effectives as well as population shifts, occur in response to specific geochemical conditions (e.g. basaltic aquifer, brine incursion, methane-sulfate transition zone, thermogenic gas) (Parkes et al., 1990; Cragg et al., 1992; D'Hondt et al., 2004; Parkes et al., 2005) and at lithological interfaces (e.g. porous ash layers vs. clay layers, diatom-rich sedimentary layers) (Inagaki et al., 2003; Parkes et al., 2005). The mechanical constrains of the sediments such as grain size and porosity are likely to drive the distribution of microorganisms (Rebata-Landa and Santamarina, 2006). Subseafloor microbial metabolic activities are controlled by the bioavailability of energy sources and electron acceptors, themselves correlated to fluid flow regimes (Cowen et al., 2003; D'Hondt et al., 2004; Engelen et al., 2008). In some places, the prokaryotic community composition is correlated to the bioavailability of organic matter substrate and/or sediment origin (terrigeneous or marine source) (Nunoura et al., 2009). In some Quaternary subseafloor sediments, microbial abundance and activity can be explained by the present hydrogeological situation and organic matter quality, while still reflecting to a certain extent the imprint of past environmental conditions via the sediment structure and geochemical composition (Beck et al., 2011).

If the environmental characteristics act upon the microbial diversity and activity, microorganisms have, conversely, a direct impact on their geological environment by increasing mineral dissolution, releasing organic/inorganic nutrients and metals that can be used for respiration, anabolism or synthesis of metal-enzymes, and by altering the elemental and ionic speciation and distribution, leading sometimes to mineral precipitation (Rogers and Bennett, 2004; Gleeson et al., 2007). Some studies suggested that the biogeographical distribution and diversity of the microbial communities in subsurface sediment is more influenced by the environmental control than by the geographical separation (Inagaki et al., 2006; Martiny et al., 2006).

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The main objective of this study was to determine if the sediment history and the paleoenvironment evolution of an area affected by strong climatic events and by high sedimentation rates was reflected in the present microbial community structure and composition. The Mediterranean Sea was chosen because of its small size, its particular sensitivity to climatic variations and because it was documented by a large literature (Rabineau et al., 2005 and references therein). The Western Mediterranean Sea continental margins in particular, offer an exceptional preservation of recent climatic changes with high sedimentation rates because of the youth of this small Liguro-provençal ocean (Rabineau et al., 2006; Sierro et al., 2009). In this region, the subseafloor microbial community composition, diversity and distribution are barely documented, contrarily to microbial communities from water column (Danovaro et al., 2010), surficial sediments (Polymenakou et al., 2005b; Polymenakou et al., 2009), brines (Yakimov et al., 2007; La Cono et al., 2011) or mud volcanoes (Daffonchio et al., 2006; Lazar et al., 2011). The aims of this study were (i) to investigate the subsurface microbial diversity associated with both climate-controlled turbiditic sequences (Var Ridge) and upper slope deposits (Gulf of Lions) in the Western Mediterranean Sea, and (ii) to examine the significance of sediments characteristics and of paleoenvironmental conditions in determining depth profile of microbial community composition.

2 Material and methods

2.1 Study sites and general sampling methods

The northern part of the Western Mediterranean Sea is characterized by two contrasted continental margins: the Gulf of Lions to the West with a wide and highly sedimented shelf, and the Ligurian margin to the East with a very narrow and by-passing shelf (Fig. 1a, b). Three physiographic provinces characterize the Gulf of Lions margin (Fig. 1c): a wide shelf (with a slope of 0.1°), 70 km wide at the most; a continental slope (1.5°) and a rise (0.9°) (Berne et al., 1999; Baztan et al., 2005). In contrast, the

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morphology of the coastal plain of Nice, in the Ligurian Sea (Fig. 1d), is characterized by a very narrow continental shelf, 2–3 km wide in average, and a steep continental slope (3.4 to 6.3°) leading to water depths of 2000 m at a distance of less than 20 km from the coast (Fig. 1d) (Pautot, 1981). Within the Ligurian Sea, the Var turbiditic system extends for 300 km, from the river mouth to the distal area at the base of the northern continental slope of Corsica (Savoye et al., 1993; Savoye et al., 1998; Migeon et al., 2000) (Fig. 1d). Cores RHS-KS-33 and KESC9-30 were respectively collected with gravity piston cores (Küllenberg type) on the outer shelf-upper slope (291 m water depth) of the western part of the Gulf of Lions and in the Ligurian Sea at the toe of the slope (2160 m water depth) on top of the Var sedimentary ridge (Table 1). Both cores were immediately sliced onboard and subsections (1 m) were stored and transported (3 days) at 4°C. In the laboratory, the core liners were aseptically opened and subsamples for microbial analysis were collected at depth interval of ca. 20 cm (core KESC9-30) and ca. 30 cm (core RHS-KS-33), from the inner part of the cores using cut-off sterile 2 ml syringes, as described elsewhere (Fulthorpe et al., 2011) (see Supplement).

2.2 Non-destructive analysis of the cores

After sampling for microbiology and geochemistry, the physical properties (GRA bulk density, magnetic susceptibility, and P-wave velocity) of the cores were measured non-destructively with the Geotek-MSCL (Multi-Sensor Core Logger; United Kingdom) and the density and porosity were determined (Richter et al., 2006). Subsequently, cores were split in half with an automatic core splitter, photographed and visually described according to the grain size (clay, silt, sand), structures (laminations and bioturbation) and content (shells, spots of organic matter, etc). Lithological logs were drawn as well as quantitative cumulative grain size curves. Semi quantitative elemental composition profiles of sediments were measured using a Core-Scanner (Avaatech, Netherlands), with a 1 cm step, a current of 0.2 mA, a voltage of 10 and 30 kV and counting time of 20 s.

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2.3 Sediment and pore water chemistry analyses

Porewater was obtained by centrifuging approximately 2 g of sediment for 30 min at 13 000 g at 4°C. The porewater was then collected in 2 ml Eppendorf tubes, acidified with nitric acid (65 %) at 1/1000 (v/v) and stored at 4°C until analysis. SO_4^{2-}, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+} concentrations were determined by ion exchange chromatography as described in Lazar et al. (2011). Total carbon (TC) content (percentage/dry weight) was analyzed by combustion in a LECO CS 125 carbon analyzer on the pelleted material (ca. 2 g) previously extracted and oven-dried (65°C) during 20 h. The total organic carbon (TOC) was measured in the same way after removing inorganic carbon by progressive and controlled acidification with HCl ..(Cauwet, 1981). Quantitative elemental chemical compositions of sediments were determined by Wavelength Dispersive X-Ray Fluorescence spectrometry (WD-XRF, S8 Bruker) on fused beads and compressed powder pellets for major and some trace elements, respectively, as previously described by El Maghraoui et al. (1999). Grain size analyses were performed with a Coulter LS130 laser microgranulometer. Samples (ca. 0.1 g) for grain size analysis were carefully collected in every observed *laminae* for the KESC9-30 core and at the same depths as the microbiological samples (every 30 cm on average) on the RHS-KS-33 core.

2.4 Age model

The age models were established by correlating the calcium records of the studied cores with the calcium records of well dated twin cores previously acquired at the same locations (Table 1), using the AnalySeries software (http://www.ncdc.noaa.gov/paleo/softlib/softlib.html) (Fig. 2). For core RHS-KS-33 we correlated the quantitative calcium profile with the calcium carbonates content of twin core MD99-2348 (Fig. 2a and b) (Sierro et al., 2009). Age model of core MD99-2348 is based on 13 calibrated AMS ^{14}C dates for the last 21 ka on (Beaudouin et al., 2005; Jouet et al., 2006; Sierro et al., 2009). For core KESC9-30 we correlated scanner calcium profiles with the same data

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of reference core KNI23 (Fig. 2c and d). Age model of core KNI23 is based on 5 calibrated AMS ^{14}C dates for the last 20 ka on KNI23 (Jorry et al., 2011). Both reference cores also had a high-resolution planktonic oxygen isotope stratigraphy on *Globigerina bulloides* (Sierro et al., 2009; Jorry et al., 2011) which enables close correlation to global climatic changes (Fig. 2).

2.5 Total cell counts

Total cell counts were performed according to the protocol of Kallmeyer et al. (2008), with some modifications. When necessary, prior to carbonate dissolution step, amorphous silicates were dissolved with hydrofluoric acid (HF) (Sigma-Aldrich, Deisenhofen, Germany) according to the protocol proposed by Morono et al. (2009). Cells were counted by epifluorescence microscopy, using a Leica DMZ2500 microscope, with a 100x Plan Fluotar objective and a L5 filter set (Ex. 480/40 Em. 527/30).

2.6 DNA extractions and PCR amplification

For each sample, the total community genomic DNA was extracted from ca. 2 g of wet sediment. Cell disruption was performed in a 6770 Freezer/Mill (Spex SamplePrep, NJ, USA) by grinding the sample into a liquid nitrogen chamber. At this step, the sample was first cooled for 2 min in liquid nitrogen then crushed for 1 min at a beating rate of 8. After cell disruption, samples were extracted with PCI (phenol/chloroform/isoamyl alcohol) protocol. This protocol was described elsewhere (Alain et al., 2011). The nucleic acid precipitation was enhanced by the addition of linear acrylamide (Gaillard and Strauss, 1990). In order to characterize global bacterial and archaeal community depth structure, a PCR-DGGE analysis targeting the V3-V5 region of the 16S rRNA gene was undertaken. Bacterial 16S rRNA genes were amplified in a nested PCR using the Bac8F/1492R (Lane, 1991) and then 341F-GC/907R (Casamayor et al., 2000) primer combinations. Archaeal 16S rRNA genes were amplified by nested PCR using the following primer combinations: Arc21F/Arc958R (DeLong, 1992) and then

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Arc344F-GC/Arc915R (Casamayor et al., 2000). The amplification was performed as described elsewhere (Roussel et al., 2009b). In order to limit contamination, all PCR manipulations were carried out under sterile conditions (PCR cabinet; CaptairBio, Erlab) and all disposable plastic wares were autoclaved and UV treated (40 min) prior to use. Negative controls (PCR mixture without DNA) were included in all sets of PCR reactions to provide a contamination check. A re-amplified negative control from the first round PCR plus a fresh negative control were also included.

2.7 DGGE and sequencing of DGGE bands

Denaturing gradient gel electrophoresis (DGGE) analysis was conducted for all sampled depth layers of the two cores as previously described (for details see Roussel et al., 2009a and the Supplement). Different denaturing gradients were used for bacterial and archaeal community structure (Supplement, Fig. S1, S2 and S3). Bands of interest were excised and then sequenced (Beckman-Coulter Cogenics, Stansted). In case of co-migration of different sequences generating a double chromatogram signal, the individual bands were reamplified with the same primer set and then migrated on a more tight DGGE (30–35 % and 40–50 %), as described by Gafan and Spratt in 2005. Sequences were compared to those present in the databases using the BLASTn algorithm (Altschul et al., 1990) and then aligned to their nearest neighbor using the programs CLUSTAL_X and PHYLIP version 3.69 as described elsewhere (Alain et al., 2008). Gene sequences are available at the GenBank/EMBL/DDBJ databases under the accession numbers HE586735 to HE586938 and HE589592 to HE589593.

2.8 Statistical analysis

Bacterial DGGE banding patterns were analyzed using Adobe Illustrator 10.0.3 (http://www.adobe.com) software so as to determine the exact position of a band on the gel and the ImageJ software package (http://rsbweb.nih.gov/ij/) so as to determine the intensity of each band, as described by Fromin et al. (2002). Unweighted Pair Group

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Method with Arithmetic Mean (UPGMA) dendrogram and Multidimensional Scaling (MDS) based on Bray Curtis similarities between normalized DGGE profiles (presence/absence and intensity matrix) were constructed by using the PRIMER v6.0 software (PRIMER-E, Ltd., UK). To investigate relationships between bacterial community structure and environmental parameters, we used a direct gradient approach, canonical correspondence analysis (CCA) using the software package CANOCO, version 4.5 for Windows as previously described (Ghiglione et al., 2008).

2.9 Enrichment and isolation of microbial strains

Enrichment cultures were performed, in triplicate, at three depth intervals of the RHS-KS33 core (Gulf of Lions): 109–114 cm, 478–481 cm and 698–703 cm, as described below.

2.9.1 Media for aerobic cultures

- Solid non-selective media for heterotrophic microorganisms (prepared with two different gelifying agents): Marine Broth 2216 media (BD Difco™) with agar (1.5 % w/v, Sigma-Aldrich, Deissenhofen, Germany) or Phytagel™ (0.8 % w/v, Sigma-Aldrich, Deissenhofen, Germany) as solidifying agents.
- Solid selective media for heterotrophs (amended with 4 different substrate combinations): based on artificial seawater (ASW) amended with one single substrate. The artificial seawater basis, contained, per litre: KBr (0.09 g), KCl (0.6 g), $CaCl_2$ (1.47 g), $MgCl_2 6H_2O$ (5.67 g), NaCl (30 g), $SrCl_2 6H_2O$ (0.02 g), $MgSO_4 7H_2O$ (5.62 g), NaF (0.002 g), and PIPES buffer (3 g). The pH was adjusted to 7.5. After autoclaving, the medium was cooled and the following solutions were added from sterile stock solutions: NH_4Cl (4.67 mM), KH_2PO_4 (1.5 mM), a solution of seven vitamins (1X) (Widdel and Bak, 1992), a trace element solution (1X) (Widdel and Bak, 1992) and a selenite-tungstate solution (1X) (Widdel and Bak, 1992). One of the following substrates was then added to the artificial seawater medium:

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acetate (10 mM), glucose (10 mM), cellulose (0.5 g l^{-1}) or chitin (0.5 g l^{-1}) (all from Sigma-Aldrich, Deissenhofen, Germany).

– Selective liquid media for heterotrophs (containing acetate or glucose): ASW supplemented with acetate or glucose at a final concentration of 10 mM.

2.9.2 Media for anaerobic cultures

– Liquid selective media for fermentative microorganisms (containing glucose or pyruvate): ASW prepared without sulfate and supplemented with glucose or pyruvate at a final concentration of 10 mM, and reduced with sterile sodium sulfide (1.2 mM).

– Selective liquid medium for nitrate-reducers: ASW flushed by dinitrogen and supplemented with 10 mM $NaNO_3$ and 10 mM acetate.

– Selective liquid media for sulfate-reducing microorganisms (amended with 3 different substrate combinations): ASW supplemented with substrate combinations. After autoclaving, the medium was reduced by addition of sterile sodium sulfide (1.2 mM) and then provided with either H_2/CO_2 (80/20, 2 bars) gas phase, pyruvate/fumarate (each at a final concentration of 10 mM) or acetate (final concentration of 10 mM). Production of sodium sulphide was checked through the colorimetric method of Cord-Ruwisch (Cord-Ruwish, 1985).

– Liquid selective media for methanogens (two substrates combinations): ASW basis prepared without sulfate, reduced with sodium sulphide (1.2 mM) and supplemented with a H_2/CO_2 (80/20, 2 bars) gas phase or acetate (final concentration of 10 mM).

The preparation of the anaerobic media and all the manipulations of the anaerobic cultures were performed in an anaerobic chamber (Coy Laboratory Inc.) or as described elsewhere (Widdel and Bak, 1992). All media contained resazurin (0.5 g l^{-1}) as an

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anaerobiosis indicator. The media were inoculated with 1 % sediment slurry and incubated for 3 months, in the dark, at 4 °C, 16 °C, 20 °C and 37 °C. As a negative control, uninoculated media were incubated under the same conditions. Growth was routinely monitored by observation of the Petri dishes or by optical microscopy.

Cultivation assays targeting members of the subclass *Betaproteobacteria* were performed with samples collected at depth intervals 45–52 cm, 101–105 cm, 251–256 cm, 422–427 cm, 583–587 cm, 752–757 cm and 808–813 cm for the core KESC9-30 and with samples collected at depth intervals 84–88 cm, 198–203 cm, 267–271 cm, 307–312 cm and 508–512 cm for the core RHS-KS-33. These assays were done in aerobic growth conditions on a modified R2A medium (Gomila et al., 2007), containing for 1 l of distilled water: $CaCl_2$ (0.1 g), $MgSO_4\,7H_2O$ (0.0492 g), NaCl (5 g), yeast extract (0.5 g), sodium pyruvate (0.3 g), acid hydrolysate of casein (0.5 g), pancreatic digest of casein (0.25 g), meat extract (0.3 g) and PIPES buffer (3.4 g). The pH was adjusted to 7.2 before autoclaving. After autoclaving, K_2HPO_4 (5 ml l^{-1}) and glucose (0.5 g l^{-1}) were added to the medium from 1M sterile stock solutions. Culture media were inoculated with 1 % sediment slurry and incubated at 25 °C in the dark. Positive enrichment cultures obtained on solid media were purified by repeated streaking on plates. The dilution-to-extinction technique was employed to isolate strains from the liquid cultures.

3 Results

3.1 Sediment structure and paleoenvironment

Sedimentary facies from core RHS-KS-33 were relatively homogeneous and dominated by clay-rich lithologies (<3.9 µm) with silty-clay *laminaes* within the first upper meter, a silt (<62 µm) rich layer at the top of the core (between 17–40 cm), and very fine to fine sand enrichment in the upper 20 cm.

Abundant organic matter spots and bioturbation were observed throughout the core (Fig. 3). TOC content of this core was low and ranged from 0.3 % to 0.5 % (Fig. 3). TC

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content ranged between 4.6 % and 5.6 % and its profile usually followed the Ca pattern. In this core, the Ca profile presented a maximum between 440 and 520 cm (Fig. 3). The sedimentological succession of core KESC9-30 presented major lithological changes compared to core RHS-KS-33. Core KESC9-30 showed alternations of clay rich layers, with abundant planktonic and benthic faunas, and coarse grained layers (silt to coarse sand layers) (Fig. 3). At the top, the core was dominated by numerous silty *laminae* (<62.5 µm) that evolved downcore to thick centimetric to pluri-centimetric, fine to medium sand turbidites (<500 µm) with locally erosional basal contact (Fig. 3). Clay rich intervals were interpreted as dominantly pelagic intervals while coarse layers were interpreted as turbidites (Fig. 3). TOC was low and ranged from 0.1 % to 0.5 %; alternations of organic carbon-rich intervals with carbon-poor intervals were closely correlated with grain-size pattern (Fig. 3). Lowest TOC values (<0.2 %) occurred in sandy layers, in particular below 600 cm, at the bottom of the core (Fig. 3). TC (between 3.5 % and 6 %) and Ca profiles were relatively scattered, especially below 600 cm, where the turbidites frequency increased significantly.

3.2 Geochemical composition of sediment and pore water

Along core RHS-KS-33 sediment elemental compositions were fairly homogeneous, with only minor shifts at the top and around 500 cm (Fig. 4, Table S1). Elemental compositions in core KESC9-30 varied downcore and generally followed the lithostratigraphy. Clay-rich intervals were characterized by small variations in the elemental composition of the sediment, compared to sandy-rich intervals. Below 600 cm, the silty turbidites were replaced by sandy turbidites (Fig. 4).

At both sites, pore water salinities were generally close to the sea water values (38 ‰) and locally increased to more than 42.5 ‰ (Fig. 5). Pore water salinities of the core KESC9-30 were high (>42.5 ‰) in the clay-rich layers (480–490 cm) and in silty turbidites (around 640 cm), but lower (mean = 39.4 ‰ after 500 cm) in sandy turbidites. Salinity profiles followed sodium and chloride profiles and sulfate decreased with depth. Sulfate profiles suggest that microbial sulfate reduction occurred at both

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sites. In addition, sulfate and salinity peaks observed on core KESC9-30 between 633 and 667 cm, suggest that seawater penetrated within this turbiditic layer. Similar to sulfate concentrations, magnesium concentrations decreased with depth.

3.3 Abundance, structure microbial community composition

Total cell numbers were determined by epifluorescence counts at, respectively, ten and seventeen depths in cores RHS-KS-33 and KESC9-30 (Fig. 6). Microbial abundances varied from 5.3×10^6 (12–17 cm) to 3.7×10^5 cells cm^{-3} (378-382 cm) in the Gulf of Lions and between 4.4×10^6 cells cm^{-3} (101–105 cm) and 6.6×10^5 (351-3-56 cm) in the Ligurian Sea. At the Gulf of Lions site, the total cell numbers slightly decreased with increasing depth, while at the Ligurian Sea site they remains relatively constant (Fig. 6). *Archaea* and *Bacteria* were present in both cores. Bacterial 16S rRNA genes were successfully amplified at all depths all along both cores, while archaeal 16S rRNA genes could be amplified from only four depth intervals of core RHS-KS-33 and fourteen depth intervals of core KESC9-30 (Fig. 6). Despite additional trials with different PCR conditions (with additional DNA extracts, decreased annealing temperatures, etc.), amplification of archaeal 16S rRNA genes from other depths remained unsuccessful. DGGE was used to determine the interval-depth patterns of the dominant members of microbial communities, and bands were excised to determine the taxonomic affiliations. In total, 131 DGGE bands from the two cores were sequenced and used for phylogenetic analyses. At least three bands were excised and sequenced for a given gel position. Bands with equivalent positions in different lanes always corresponded to the same sequence. 117 of the 131 sequenced bands showed 95 % or higher similarity to their closest relative within the GenBank database. Within the 611 bands observed on all DGGE gels, 63 % were identified, and used for construction of cumulative bacterial/archaeal plots at each depth (Fig. 6).

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3.3.1 Archaeal communities

The archaeal diversity of core RHS-KS-33 was exclusively composed of sequences closely related to the Miscellaneous Crenarchaeotic Group (MCG), an uncultivated lineage predominant in 16S rRNA archaeal clone libraries obtained from marine deep subsurface sediments (Fry et al., 2008; Teske and Sørensen, 2008) (Fig. 6). In core KESC9-30, nine different lineages were present along the core. The most abundant group was MCG (37.8 %) followed by the South African Gold Mine Euryarchaeotic Group 1 (SAGMEG-1; 21.6 %) (Takai et al., 2001b) and by *Halobacteria* (16.2 %). In addition, a number of minor uncultivated archaeal groups, including the Marine Group I (MGI) (DeLong, 1992), the Marine Benthic Group B (MBGB; synonymous with Deep-Sea Archaeal Group (DSAG) (Takai et al., 2001a)), the Marine Benthic Group C (MBGC) (Vetriani et al., 1999), the Marine Benthic Group D (MBGD) (Vetriani et al., 1999) and the SAGMEG-relatives were retrieved (each representing less than 5.4 % of the sequences). MGI were dominant at the top of the core, while other groups were present at different depths throughout the core (Fig. 6).

3.3.2 Bacterial communities

Bacterial communities presented much more diverse DGGE band profiles than archaeal communities, at both sites. The sequenced DGGE bands from the core RHS-KS-33 belonged to the lineages *Bacteroidetes, Proteobacteria* (classes *Alpha-, Beta- and Gammaproteobacteria*), *Actinobacteria and Firmicutes*. The phylum *Bacteroidetes* (46.3 % of total bacterial relative abundances) dominated almost all depth intervals, except the surface layers (12–17 cm) where it represented less than 20 % of the diversity. Within the *Proteobacteria*, the class *Betaproteobacteria* (28.3 %) was the second abundant lineage at this site with sequences belonging to three families (*Burkholderiaceae*, *Ralstoniaceae* and *Comamonadaceae*). *Betaproteobacteria* were encountered at almost all depths, except 84–88 cm and 307–312 cm (Fig. 6). Sequences belonging to the phylum *Actinobacteria* (11.8 %) grouped within one single

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family (*Propiniobacteriaceae*), while the phylum *Firmicutes* (9.6 %) was represented by three families (*Clostridiaceae*, *Veillonellaceae* and *Staphylococcaceae*). Sequences belonging to the classes *Alpha-* and *Gammaproteobacteria* rarely occurred at this site (<5 %). Overall, sequences of this core were closely related to ubiquitous microorganisms, previously recovered from marine or terrestrial environments contaminated with heavy metals (Selenska-Pobell et al., 2001; Akob et al., 2007; Bruun et al., 2010; Kampe et al., 2010), from soil (Phuong et al., 2008; Schouten et al., 2010), water, groundwater (Sahl et al., 2008) and petroleum crude oil samples (Yamane et al., 2008) (Fig. 6).

Bacterial communities of core KESC9-30 were composed of *Proteobacteria* (classes *Alpha-*, *Beta-* and *Gammaproteobacteria*), *Chloroflexi*, *Bacteroidetes* and candidate division OP8. The class *Betaproteobacteria* (57.1 %) was the dominant lineage at all depth intervals. The percentage of this group, changed with depth from about 48.9 % within the surface clay-rich layers to 71.2 % after 400 cm, within turbidites (Fig. 6). Sequences related to the class *Betaproteobacteria* grouped within four families (*Oxalobacteraceae*, *Burkholderiaceae*, *Ralstoniaceae* and *Comamonadaceae)* and the most abundant family was *Ralstoniaceae* (24.2 %), followed by *Comamonadaceae* (11.6 %). Within these families, sequences were affiliated to the genera *Ralstonia* and *Comamonas* two important taxa of soil microbial communities, capable of degrading recalcitrant compounds. Phyla *Gammaproteobacteria* (18.2 % of total) and *Bacteroidetes* (13.1 %) comprised, respectively, one family (*Moraxellaceae*) and two families (*Sphingobacteriaceae* and *Flavobacteriaceae*), and were distributed at different depths within this core. At this site, sequences were closely related to environmental sequences or isolates originating from soil (Zinger et al., 2009), underground water, waste water (Gomila et al., 2007), mines (Sahl et al., 2008) or mining lakes (Kampe et al., 2010). Most of them were related to strains able to degrade several classes of recalcitrant compounds. Another group retrieved only at this site was the phylum *Chloroflexi* that encompassed 7.1 % of the bacterial diversity. All detected sequences belonged to the subdivision II of this phylum (Hugenholtz et al., 1998) and were mainly present

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at shallow depths (from 3 cm to 286 cm) in dominated clay/silty-clay layers. These sequences were most closely related to clones obtained from marine subsurface sediments of Pacific Ocean Margin (Inagaki et al., 2006), Gulf of Mexico (Nunoura et al., 2009) and from tidal flat sediments from the Wadden Sea (Wilms et al., 2006) (over 95 % sequence similarity). The closest cultured relative to our sequences was the bacterium *Dehalogenimonas lykanthroporepellens* strain BL-DC-9, with a sequence similarity of 89 %, a strain capable to reductively dehalogenate 1,2,3-trichloropropane (Yan et al., 2009). Details for sequence similarities of excised DGGE bands are available as Supplement (Fig. S1, S2, S3).

3.4 Microbial cultures

In this study, two cultivation strategies were used. (i) First, enrichment cultures were performed from three depth intervals on the RHS-KS-33 core to isolate a broad spectrum of physiological types of marine microorganisms (Fig. 6, Table 2). (ii) Then, after we highlighted the dominance of *Betaproteobacteria* at both studied sites, a specific culture medium was designed (see Methods section) and enrichment assays were performed at different depth intervals, on both studied cores (Fig. 6), in order to cultivate representatives of this group. Generally, growth was detected at both sites, and a total of 75 strains were isolated on various substrates and cultivation conditions. Phylogenetic analyses revealed that the strains belonged to four lineages: *Alpha-*, *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*. In core RHS-KS-33, the largest number of isolates affiliated with the *Gammaproteobacteria* (54 strains, 73.97 % of the culture collection, Table 2). Most of them were closely related to the genera *Halomonas* (23 strains), *Pseudoalteromonas* (15 strains) and *Marinobacter* (9 strains), with over 99 % sequence similarity. In core RHS-KS-33, the results obtained in aerobic rich and selective solid media showed the presence of a largest diversity of cultivable prokaryotes at shallow depths than at the bottom of the core. Several liquid enrichment cultures targeting nitrate reducers or sulfate-reducers were obtained but could not be maintained until isolation, and no positive enrichment was obtained for methanogens. In

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contrast, all enrichment cultures designed for the growth of fermentative microorganisms were positive with a high turbidity, indicating the presence of this metabolism at each depth interval. Unexpectedly, cultures made on the modified R2A medium at 25 °C did not allowed isolating members of the class *Betaproteobacteria*. Phylogenetic analysis revealed that the isolated strains on this medium belonged to the lineages *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria* (Table 2).

3.5 Bacterial community structure and physicochemical variable ecological analysis

We first performed CCA using all physico-chemical parameters as constrained variables of the bacterial community diversity from DGGE fingerprints of core RHS-KS-33 samples. A strong Spearman's rank pairwise correlation between Ba and Sr/Ca ($R = 0.92$, $p < 0.01$) allowed us to use barium as a proxy of strontium/calcium to perform CCA together with the other physico-chemical parameters. We found that 44.8 % of changes in the DGGE profiles could be explained by a set of physic-chemical parameters including S, MgO, Sr, Ba and to a lesser extend Ni and K/Al (Table 3 and Fig. 7). The cumulative percentage of the first two canonical axes accounted for 57.3 % of this variance (Table 3). Subsequent axes accounted for less than 13 % of the variance each, and were not considered further. The first canonical axis was highly negatively correlated to sulfate (ca. –0.75), to a lesser extent to barium (ca. –0.5) and slightly positively correlated to strontium and potassium/aluminum (ca. 0.2). This first synthetic gradient clearly separated deep samples (below 500 cm) from shallower samples (from the top to 350 cm), indicating that their community composition varies with different concentrations of these chemical species. The second canonical axis was highly positively correlated with nickel and magnesium, clearly separating depth intervals (around 200 cm and around 400 cm) (Fig. 7).

The total variance of fitted core KESC9-30 samples DGGE data when using sulfate, age, iron and to a lesser extent manganese, strontium and titanium/calcium variables was 27.7 % (Table 3). The cumulative percentage of variance of the species –

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environment relationship indicates that the first and second canonical axes account for 23.5 % and 45.2 % of this variance, respectively. The first canonical axis was highly positively correlated with sulfate (ca. 0.80), to a lesser extent with iron, manganese, strontium, titanium/calcium (<0.4) and highly negatively correlated with age (ca. –0.80). For this core, Spearman's rank pairwise correlation revealed that Ti/Ca was a proxy for Fe/Ca, Ca/Al and Al_2O_3; Fe_2O_3 was a proxy for Zn and TiO_2 (Fig. 7). The first observed gradient clearly confirmed that the age of the sediments (which is also related to the source of sediments) clearly influence the structure and the composition of bacterial communities, below 400 cm. At shallower depths (100–300 cm), we observed that the bacterial community structure was driven by other factors than the sedimentary dynamics, such as sulfate concentration and to a lesser extent to some metals concentrations (Fig. 7).

4 Discussion

The study of a well-characterized area represents an opportunity to use numerous environmental data sets, contrarily to exploratory surveys. In this respect, the continuous and well-preserved depositional environment of the Western Mediterranean Sea is particularly well-documented (Rabineau et al., 2005). Thus, the cores RHS-KS-33 and KESC9-30 investigated here could be compared with several reference cores from the Gulf of Lions and the Ligurian Sea collected at the same water depth, showing that these cores are representative of their depositional settings (i.e. upper slope and deep levee respectively).

The vertical distribution of microbial communities in subsurface marine sediments of Western Mediterranean Sea was investigated by the DGGE fingerprinting method that allows processing and analysis of a great number of samples in a reproducible way. DGGE is a powerful tool for rapid monitoring of successive changes within microbial communities. In this study, several changes in the microbial community structure and composition of subsurface sediments were linked to changes in environmental conditions since the LGM as described hereafter.

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4.1 A microbial diversity impacted by the paleoenvironmental conditions and the geochemical sediment and pore-water compositions

The results of this study provide insights into identities of predominant microorganisms at two contrasted sites from the Western Mediterranean Sea. The total cell numbers in surface layers at both studied locations were generally one or two orders of magnitude lower compared to the universal depth distribution of subsurface bacterial populations proposed by Parkes et al. (2000) (Fig. 6). The possible environmental factors that control biomass, diversity and activity of subseafloor microbial communities have been broadly discussed (DeLong, 2004; Jørgensen and D'Hondt, 2006) and the importance of organic compounds driving microbial community densities in subseafloor sediments has often been pointed out (Lipp et al., 2008). These biomass values might be explained by the very low TOC contents (less than 0.5 %) (Parkes et al., 2000) and by the quality of the organic matter (OM). A paradigm commonly seen in the literature is that terrigeneous OM (decaying vegetation and fauna) is highly refractory in comparison to native marine OM (phytoplankton production) (Hedges et al., 1997). This might be the case here. For the time being, the role of terrigeneous organic matter and its fate in the ocean is still a subject of current debate (Hedges et al., 1997; Benner, 2004).

The structure and the composition of archaeal and bacterial assemblages in these samples are diverse and very different in terms of percentage of dominance from those encountered in other deep-sea sediment settings and locations within the Mediterranean Sea, such as surficial sediments (first centimeters) (Polymenakou et al., 2005b; Polymenakou et al., 2009), mud volcanoes (Lazar et al., 2011) or brines (Yakimov et al., 2007; La Cono et al., 2011).

4.1.1 Archaeal diversity

Fingerprinting analysis of archaeal and bacterial communities at both study sites revealed that archaeal OTU (operational taxonomic units) number was much lower than that for *Bacteria*, which is consistent with the shallow subseafloor sediments (Luna et

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al., 2004; Luna et al., 2009; Danovaro et al., 2010). Besides, archaeal diversity seems to be closely influenced by the lithology, since core RHS-KS-33, characterized by a homogeneous lithology, was dominated by a single archaeal group (the MCG) and core KESC9-30, characterized by a heterogeneous lithology, was characterized by nine different archaeal clusters. The archaeal diversity of both studied sites was typical of marine sediments. MCG dominated archaeal diversity at both sites. MCG were found to dominate marine sediments from different locations, notably from the Peru Margin (organic carbon rich, sites 1227, 1228, 1229) (Parkes et al., 2005; Inagaki et al., 2006; Sørensen and Teske, 2006; Webster et al., 2006) or the Sea of Okhotsk (ash layers) (Inagaki et al., 2003). They are likely to be heterotrophic anaerobes, capable of using complex organic substrates (Teske and Sørensen, 2008) and this may explain why they are so widely distributed in surface and subsurface, marine and terrestrial systems (Teske and Sørensen, 2008). Likewise, members of other archaeal clusters previously found in deep marine/terrestrial sediments such as SAGMEG, MBGB, MBGC, MBGD and MGI (Fry et al., 2008; Teske and Sørensen, 2008) were also found in the Ligurian Sea, at different depths, with various sediment composition (Fig. 6).

4.1.2 Bacterial diversity

In general, the bacterial DGGE libraries were very diverse at both sites. Ubiquitous lineages such us *Betaproteobacteria*, *Bacteroidetes* and *Gammaproteobacteria* were present at both sites, while *Actinobacteria* and *Firmicutes* were detected only in the Gulf of Lions and *Chloroflexi* only in the Ligurian Sea. This diversity is similar with diversities found in other studies on Eastern Mediterranean Sea sediments in which bacterial clone libraries were dominated by *Alpha-, Gamma-*, *Deltaproteobacteria, Acidobacteria*, *Planctomycetes*, *Actinobacteria*, *Bacteroidetes*, and few *Verrucomicrobia, Firmicutes, Betaproteobacteria*, and *Chloroflexi* (Polymenakou et al., 2005b; Polymenakou et al., 2009). Nevertheless, *Deltaproteobacteria*, *Planctomycetes* and *Verrucomicrobia* were not detected in this survey. Considering that the DGGE method detects preferentially the dominant groups, these groups may not be dominant in our

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study (Webster et al., 2003; Schaefer et al., 2007). Our results indicate that bacterial assemblages from the Western Mediterranean Sea sediments (dominated by *Betaproteobacteria*) are very different from those encountered in the Eastern Mediterranean Sea sediments (dominated by *Acidobacteria*). This finding is in agreement with previous studies where significant longitudinal differences were observed between the Western, Central and Eastern Mediterranean Sea, indicating a high regional variability (Polymenakou et al., 2005b, a; Heijs et al., 2008; Danovaro et al., 2010). One possible explanation is that microbial communities in coastal subseafloor environments are strongly influenced by geological and geochemical settings (Inagaki et al., 2006; Inagaki and Nakagawa, 2008; Nunoura et al., 2009; Danovaro et al., 2010). Furthermore, the difference in depositional environments which reflects the source and the bioavailability of organic carbon, may explain the different microbial communities in the Gulf of Lions and the Ligurian Sea (Nunoura et al., 2009). The dominance of a typical terrestrial group *(Betaproteobacteria)* at two studied sites is surprising since both sites have never been emerged despite sea-level changes.

4.1.3 Cultures

The cultural survey revealed that the collection of bacterial strains isolated from the Gulf of Lions was dominated by mesophilic or psychrophilic, aerobic heterotrophs belonging to the *Gamma-* and the *Alphaproteobacteria*. These results are consistent with temperatures measured *in situ* (13-14^{o}C, data not shown). The cultivation of aerobic or facultative anaerobic strains from deep sediment layers has been repeatedly reported in the literature from samples collected at 77, 171, 277 and 420 m.b.s.f (D'Hondt et al., 2004; Batzke et al., 2007; Parkes et al., 2009). In this study, the isolation of bacterial strains under aerobic conditions from anoxic sediments may be explained by the fact that some of these strains are facultative anaerobes (ex. *Halomonas, Marinobacter, Alcanivorax,* etc.) or that they are true aerobes that were inactive in situ ("in dormancy"...). Within the *Gammaproteobacteria*, most of the isolates were affiliated to the genus *Halomonas*, an ubiquitous halotolerant microorganism (Arahal and

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Ventosa, 2006). Unexpectedly, five isolates of *Alphaproteobacteria* were distantly related to their closest cultured representative (below 98.7 % 16R rRNA similarity, the threshold value for the delineation of a new species) (Stackebrandt and Ebers, 2006), indicating that these strains represent novel species (Table 3). Even though the microbial abundances and the total organic carbon content at both sites are quite low, both studied sites may support a heterotrophic microbial ecosystem, since a predominance of strains and sequences affiliated with heterotrophic taxa have been recovered. Molecular inventories have revealed the presence of numerous sequences affiliated with environmental clones from hydrocarbon-polluted or metal-contaminated habitats (e.g. Selenska-Pobell et al., 2001; Akob et al., 2007), and cultures led to the isolation of several heterotrophic taxa known for their ability to degrade refractory substrates (e.g. *Marinobacter, Halomonas*), suggesting that refractory substrates may somehow support this low biomass microbial ecosystem.

4.1.4 CCA analysis

A major issue of this study was to determine whether changes in dominant bacterial community structure with depth in the sediment cores were driven by their depositional paleoenvironment. Our CCA analysis regrouped bacterial communities according to their vertical distribution, driven by several combinations of environmental parameters, specific to each site (Fig. 7). Changes in bacterial community composition at the top of core RHS-KS-33 are likely influenced by the porewater sulfate concentration and lithology (presence of sand in the upper 20 cm only), so obviously by the presence of oxidants that can serve as terminal electron acceptor for respirations.

Middle depth distributions seem to be influenced by concentrations in magnesium and nickel, two nutriments up-taken by microorganisms from rocks and soils (Gadd, 2010). Depth distributions below 500 cm were influenced by strontium and potassium/aluminum, used to trace sediment sources, as these elements are present only in soil minerals (silicates) (Yarincik et al., 2000). In the Gulf of Lions, minerals such as micas (silicates) are common and were found in the twin core MD99-2348 (Rabineau

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et al., 2005). Distribution of bacterial communities in the Ligurian Sea is strongly influenced by the pore water sulfate concentrations at the top of the core, while below 400 cm, it is mostly impacted by the sediment age and turbidites frequency.

Altogether, our results show that microbial communities from the Mediterranean Sea sediments were stratified, and were likely to be affected by both lithological (e.g. grain size, mineral composition, sedimentation rate) and geochemical (e.g. pore water chemistry) characteristics, as previously observed elsewhere (Inagaki et al., 2006). Moreover, these results seem to be linked to paleoenvironmental conditions *via* sediment structure, organic matter quality and geochemical composition of sediments.

4.2 Imprint of climatic events on the microbial community structure/composition

4.2.1 Quaternary Climatic changes in the Western Mediterranean Sea

During the Quaternary, important climate changes occurred in Western Europe, with occurrence of large glaciations and major changes in sea-level, air temperature, and humidity, vegetation and oceanic currents that strongly impacted marine and terrestrial ecosystems. The two climatic extremes are represented by glacial periods with cold climate and low sea levels, and interglacial periods with warm climate and high sea-levels. During the Last Glacial Maximum (LGM; 18–26 ka cal BP), mountain glaciers covered a large part of the Alps, smaller glaciers also existed in the Pyrenees and the Massif Central (Fig. 1b) (Florineth and Schluchter, 1998; Antoine et al., 1999; Hinderer, 2001). The mountain glaciers have generated important physical erosion episodes, providing important volumes of sediments of various grain sizes, to be transported by rivers and ultimately deposited in the Gulf of Lions and the Ligurian Sea. Throughout the last glacial period, extreme cold events named Heinrich Events were recurrent every 7–10 ka (named Heinrich 1 to 5 for the last 50 ka) (Heinrich, 1988) (Fig. 8a, g). These events, identified in the North Atlantic Ocean and Nordic Sea sediments as ice-rafted detritus rich layers deposited from massive iceberg calving, were related to rapid

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retreat of ice sheets (Heinrich, 1988; Bond et al., 1992; Grousset et al., 1993; Elliot et al., 1998). At the time of Heinrich events, the prodigious amounts of freshwater added to the North Atlantic also resulted in a decrease in sea surface temperature and salinity in the Western Mediterranean Sea (Sierro et al., 2005; Cacho et al., 2006). The LGM was followed by a rapid warming and deglaciation initiated at 19 ka cal BP (Clark et al., 2004), with sea-level rise leading to present day interglacial conditions. This glacial to interglacial transition (named the last termination (T.I)) is of crucial importance because drastic changes occurred over a short period (from about 15 to 11 ka cal BP). However, the warming and deglaciation since the LGM was not linear, with some very rapid climate oscillations (Fig. 8a, g). Our investigations on Western Mediterranean sediments suggest that climatic events of the last 20 ka influenced the structure and composition of microbial communities.

4.2.2 Ligurian Sea

One piece of evidence for this hypothesis lies in the correlation between the *Betaproteobacteria* distribution and the high amount of frequent turbidites during LGM to the beginning of Holocene (10 ka) in marine deep sediments. Most *Betaproteobacteria* live in soil and freshwater environments (Methe et al., 1998), and only a small fraction is present in marine environments (Parkes et al., 2005), even in coastal sediments (Koizumi et al., 2003; Stevens et al., 2005; Kersters et al., 2006; Wilms et al., 2006; Fry et al., 2008). Unexpectedly, because the depositional environment of KESC9-30 is definitively a deep marine environment (2160 m) and has been deep even during LGM, we discovered a high percentage of *Betaproteobacteria* in the DGGE sequences of KESC9-30 sediments. When *Betaproteobacteria* are plotted against age, according to the age model previously described, one can observe that the relative abundance of this group shifts from 50–60 % within the surface clay-rich layers (from 10 to 11 ka cal BP) to 70–75 % in turbidites (20 to 11 ka cal BP) (Fig. 8h). The Var turbiditic system results from the short connection between terrigeneous sources and canyons which explains high sedimentation rates (~0.60 m ka^{-1}) (Piper and Savoye, 1993) recorded

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during the Holocene. *Betaproteobacteria* might have been transported by rivers and preserved since the last 20 ka, as extracellular DNA or as dead cells.

Furthermore, our attempts to cultivate this group were unsuccessful, which may signify that this group is maybe not active in marine sediments. In that case, *Betaproteobacteria* might represent relicts, and might be used as biomarker proxy for a terrigeneous input. These findings are congruent with a previous study where more than 60 % *Betaproteobacteria* were found in a marine-originated shallow subsurface layer (Wang et al., 2008). Wang et al. suggested that *Betaproteobacteria* were inoculated from the adjacent environments (terrestrial input through rainfalls) during historical events that influenced the sediment deposition in the area. *Betaproteobacteria* were shown to be dominant in other marine environments such as gas hydrate deposits from Cascadia Margin (Marchesi et al., 2001) or deep subsurface sediments from the Nankai Trough (Newberry et al., 2004), but their presence in these habitats was not discussed by the authors. Interestingly, sequences closely related to hydrocarbon-degrading *Betaproteobacteria* (genus *Ralstonia*) were found in the ocean crust (Mason et al., 2010); this led us to hypothesize that widespread communities could maintain over time, under certain conditions, in marine environments.

On the contrary, when *Chloroflexi* relative abundances is plotted against age, this group is present only in the upper sediment layers from 11 ka cal BP to present-day which corresponds to sedimentary sequences of marine origin with much less turbidites and more pelagic (typically marine) sediments (Fig. 8h). The majority of our sequences were affiliated with subdivision II of the *Chloroflexi*, a phylogenetic branch typical for deep subsurface habitats (Wilms et al., 2006; Inagaki and Nakagawa, 2008). The detection of typical deep-biosphere bacteria such as the *Chloroflexi* in early Holocene marine sediments and of *Betaproteobacteria* in LGM terrestrial-influenced sediments suggests that trends in microbial communities might reflect terrestrial vs. marine sediment inputs.

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4.2.3 Gulf of Lions

The Gulf of Lions sedimentary sequence is complex, despite the homogeneous lithology, because sedimentation rates in the area are strongly influenced by changes in sea levels (Sierro et al., 2009). At this site, Holocene sediments are highly condensed. This is due to a drastic change (around 14.8 ka ago) in the sedimentation rate from 2.5 m to less than 0.1 m in relation to a rapid sea-level rise and change of main depocenter from outer-shelf to the inner-middle shelf (Fig. 8a and Fig. 1) (Rabineau et al., 2005; Sierro et al., 2009). During LGM, the shoreline was positioned on the outer shelf-upper slope (i.e. on the edge of the present-day shelf-break), so the river outlet was very near the position of core RHS-KS-33. We therefore studied if the bacterial community structure and composition were influenced by climatic events that occurred during the LGM and just after LGM (but without major change in physical parameters grain-size, porosity, etc. . .).

Within these sediments, betaproteobacterial communities represented 28 % of the total relative abundance of *Bacteria* (Fig. 8b), a lower percentage than that encountered within the Ligurian Sea (Fig. 8h). Several peaks of *Betaproteobacteria* were observed during the LGM (Fig. 6 and Fig. 8b). In the Gulf of Lions, the LGM was characterized by the occurrence of cold and arid episodes (Heinrich events) (Jouet et al., 2006; Beaudouin et al., 2007). Between 20 ka and 17 ka, the sea level was low and marine sediments in the core were characterized by the proximity of the shoreline and the river mouth at that time and by an important terrestrial input (Fig. 1) (Rabineau et al., 2005). As in the case of the Ligurian Sea, the terrestrial input seems to influence the composition of microbial communities, since during this period, the *Betaproteobacteria* relative abundances increased (Fig. 8b). A clear peak of *Betaproteobacteria* (reaching nearly 50 %) was observed around 18.7 ka, at the same time calcium concentrations also showed a very prominent peak (Fig. 8d). Calcium abundance can be related either to the marine realm (through the abundance of microfossils with calcitic skeleton, e.g. Foraminiferas) or to the terrestrial realm when calcium exists in older compacted

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and outcropping sediments that can be transported by rivers. In our case, detailed studies of Foraminiferas (Sierro et al., 2009) showed that their concentration increased drastically around 14.8 ka when sedimentation rate decreased and created a condensed layer rich in Foraminiferas. This observation is also imaged in the grain size curve in Fig. 8c, since Foraminiferas have a silt-sand size. Furthermore, this peak in Ca, produced by Foraminiferas occurred together with a peak in Sr. At 18.7 ka (Fig. 8) any increase in grain size, in the amount of Foraminiferas (Sierro et al., 2009), or in the amount of Sr was observed. As a consequence, we inferred a continental source for the observed Ca peak (Fig. 8d). This assumption is sustained by the pollen analysis made on the same twin core that showed an important amount of reworked pollen at this depth (5 m, 18.7 ka with our age model) (Beaudouin et al., 2007; Sierro et al., 2009). Another line of evidence is given by pollen analysis on the same twin core that showed an important amount of reworked pollen at this depth (5 m, 18.7 ka with our age model) (Beaudouin et al., 2007; Sierro et al., 2009). The hypothesis of an increased terrestrial input is reinforced by two geochemical markers (Ti/Al, Fig. 8e, and Sr/Ca, Fig. 8f), commonly used in the literature to trace terrestrial sources in marine environments (Schnetger et al., 2000; Bayon et al., 2007). Elements present in detrital silicate minerals such as Fe, Ti or Al showed increased values synchronous to the Ca peak (Fig. 4). However, the constancy of Ti/Al ratios in this interval strongly suggests that the composition of the silicate detrital fraction did not change (Fig. 8e). Nonetheless, the negative peak in Sr/Ca ratios indicates an input of material that is relatively depleted in Sr, strongly indicating a carbonate fraction of terrestrial origin (Fig. 8f).

These different lines of evidence suggest that sediments discharged into the sea, from a drainage basin with a high amount of carbonate, and that this carbonate was preferentially eroded at that time. We can suggest that at the end of the LGM-beginning of H1, the Hérault canyon and interfluve area received sediments preferentially from the Hérault drainage basin, which had a higher amount of old carbonates, conversely to what is observed at present where sediments mostly originate from the Rhône River (Révillon et al., 2011). This terrestrial source of carbonate sediment would also be

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prone to the increase of *Betaproteobacteria*. In this case, the presence of *Betaproteobacteria* is independent of grain size as core RHS-KS-33 is completely homogeneous. After the Ca peak, the presence of *Betaproteobacteria* remained non negligible during the entire H1 events, with second order peaks that were difficult to interpret. Note that this site is much shallower than the VAR site, so that the position of the core is always under the influence of the river.

This study showed, for both sites, an unexpected high relative abundance of taxa with terrestrial microbial affinities within ancient lithological layers deposited in a marine environment. This result might be surprising considering the small size of microorganisms and the in situ porosity, since seismic profiles showed pockmarks (so fluid circulations) in the surrounding area of the core RHS-KS-33 (Fig. 1). Nevertheless, microbial surfaces necessarily interact with abiotic surfaces through weak interactions (ex. van der Waals, hydrogen, ionic), and this could explain why microbial communities maintained, at least partially, their composition since sediment deposition, despite fluid circulations.

4.2.4 Preservation of DNA or dead cells in marine sediments?

*S*ome taxa could be successfully cultured using low salinity medium (modified R2A medium), indicating either the occurrence of taxa of terrestrial origin or the presence of taxa with a large salinity range (e.g. *Halomonas* sp., *Pseudomonas* sp.). Conversely, as *Betaproteobacteria* could not be isolated from our samples, *Betaproteobacteria* sequences might come from fossilized DNA or dead cells entrapped in place since 10–20 ka. The long-term preservation of DNA and/or microorganisms has repeatedly, and sometimes controversially, been reported during the last decade, from various field data from different environments (halite, amber, sediments, evaporites, water samples), in facies as old as 100 million years (Inagaki et al., 2001; Fish et al., 2002; Inagaki et al., 2002; Inagaki et al., 2005; Souza et al., 2006). In 2005, Inagaki et al., proposed that some molecular genetics reflections of the past may be somehow stored in a so-called “DNA Paleome” under specific conditions where degradations are

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minimized. Preservation of DNA/dead cells over long periods of time in marine sediments is favored by the formation of organic-mineral complexes such as clay, silica or calcite, by the formation of resting stages, by anoxia, by low temperatures and by neutral or alkaline pH, while acidic pHs induce depurinations and thus increase DNA-clay interactions (Soina et al., 1994; Coolen and Overmann, 2007; Dong and Yu, 2007; Corinaldesi et al., 2011). The interdisciplinary approach of this study doesn't allow us to conclude whether *Betaproteobacteria* were preserved as DNA-mineral complex or fossilized cells, but support the hypothesis of the existence of a Paleome. Consequently, we hypothesize that the subseafloor microbial communities at the Gulf of Lions and at the Ligurian Sea reflect to some degree a genetic imprint of ancient paleoenvironment and paleoclimatic changes.

5 Conclusions

Our results indicate that the investigated Western Mediterranean Sea sediments in margin settings harbor a unique prokaryotic diversity, different from that described in the Eastern-Mediterranean Sea marine sediments and other spot locations within the Mediterranean Sea. Furthermore, it seems that within the Mediterranean Sea, regional settings, such as sedimentary dynamics and fluxes from the continent are controlling the prokaryotic diversity within marine sediments. The palaeoenvironmental history of erosion and deposition recorded in the Western-Mediterranean Sea sediments appear to have let its imprint on the structure/composition of the microbial communities for the late Quaternary. Our findings demonstrate that molecular signatures of terrestrial microbial are dominant in the lithological marine sequences affected by terrestrial inputs. Future investigations will need to consider more sites and more comprehensive methods (RNA extractions and metagenomics) to confirm the present findings. Such investigations will certainly provide new insights about the distribution of prokaryotic communities within marine sediments, and how these communities are influenced by the Earth history.

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Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/253/2012/bgd-9-253-2012-supplement.pdf.

Acknowledgements. We thank the crew and the chief scientists of the 2008 cruises (RHOSOS: S. Berne and B. Dennielou, ESSCAR9: P.Woerther and J.-F. Bourillet), aboard the R/V *Le Suroit* for the core recoveries. We further thank G. Lericolais, head of the Laboratory of Sedimentary Environments of Ifremer for offering us the possibility to use the laboratory for the core description, and the technical team (G. Flock and M. Rovere) for assistance during splitting, photography and physical properties analysis. We are grateful to the editor Tina Treude for her very constructive comments. This work was financially supported by the Joint Research Unit UMR 6197 (CNRS-Ifremer-UBO), by the Joint Research Unit UMR6538, by the project SEDAAMIC funded by the program INTERRVIE of the INSU (CNRS) and by the project PALEOMIC funded by the program "Projets Exploratoires 2011" of the Université de Bretagne Occidentale (UBO). M.-C. Ciobanu was awarded with a PhD scholarship by the French Ministry of Research and CAREX Project (Coordination Action for Research Activities on life in Extreme Environments).

The publication of this article is financed by CNRS-INSU.

References

Akob, D. M., Mills, H. J., and Kostka, J. E.: Metabolically active microbial communities in uranium-contaminated subsurface sediments, FEMS Microbiol. Ecol., 59, 95–107, doi:10.1111/j.1574-6941.2006.00203.x, 2007.

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9, 253–310, 2012

Alain, K., Tindall, B. J., Intertaglia, L., Catala, P., and Lebaron, P.: *Hellea balneolensis* gen. nov., sp nov., a prosthecate alphaproteobacterium from the Mediterranean Sea, Int. J. Syst. Evol. Micr., 58, 2511–2519, doi:10.1099/ijs.0.65424-0, 2008.

Alain, K., Callac, N., Ciobanu, M.-C., Reynaud, Y., Duthoit, F., and Jebbar, M.: DNA extractions from deep subseafloor sediments: novel cryogenic-mill-based procedure and comparison to existing protocols, J. Microbiol. Meth., 87, 355–362, 2011.

Alley, R. B., Marotzke, J., Nordhaus, W. D., Overpeck, J. T., Peteet, D. M., Pielke, R. A., Pierrehumbert, R. T., Rhines, P. B., Stocker, T. F., Talley, L. D., and Wallace, J. M.: Abrupt climate change, Science, 299, 2005–2010, doi:10.1126/science.1081056, 2003.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J.: Basic Local Alignment Search Tool, Journal of Molecular Biology, 215, 403–410, doi:10.1016/s0022-2836(05)80360-2, 1990.

Antoine, P., De Beaulieu, J. L., Bintz, P., Brugal, J. P., Girard, M., Guadelli, J. L., Morzadec-Kerfourn, M. T., Renault-Miskovsky, J., Roblin-Jouve, A., Schmider, B., Van Vliet-Lanoe, B., and Vigne, J. D.: La france pendant les deux derniers extrêmes climatiques, variabilité naturelle des environnements, 59 pp., 1999.

Arahal, D. R. and Ventosa, A.: The Family *Halomonandaceae*, in: The Prokaryotes. Proteobacteria: Gamma Subclass, 3 Edn., edited by: Dworkin, M., Springer, 2006.

Bard, E., Rostek, F., Turon, J. L., and Gendreau, S.: Hydrological impact of Heinrich events in the subtropical northeast Atlantic, Science, 289, 1321–1324, doi:10.1126/science.289.5483.1321, 2000.

Batzke, A., Engelen, B., Sass, H., and Cypionka, H.: Phylogenetic and physiological diversity of cultured deep-biosphere bacteria from equatorial Pacific Ocean and Peru Margin sediments, Geomicrobiol. J., 24, 261–273, doi:10.1080/01490450701456453, 2007.

Bayon, G., Pierre, C., Etoubleau, J., Voisset, M., Cauquil, E., Marsset, T., Sultan, N., Le Drezen, E., and Fouquet, Y.: Sr/Ca and Mg/Ca ratios in Niger Delta sediments: Implications for authigenic carbonate genesis in cold seep environments, Mar. Geol., 241, 93–109, doi:10.1016/j.margeo.2007.03.007, 2007.

Baztan, J., Berne, S., Olivet, J. L., Rabineau, M., Aslanian, D., Gaudin, A., Rehault, J. P., and Canals, M.: Axial incision: The key to understand submarine canyon evolution (in the western Gulf of Lion), Mar. Petrol. Geol., 22, 805–826, doi:10.1016/j.marpetgeo.2005.03.011, 2005.

Beaudouin, C., Suc, J. P., Acherki, N., Courtois, L., Rabineau, M., Aliosi, J. C., Sierro, F. J., and Oberlin, C.: Palynology of the northwestern Mediterranean shelf (Gulf of Li-

BGD
9, 253–310, 2012

ons): First vegetational record for the last climatic cycle, Mar. Petrol. Geol., 22, 845–863, doi:10.1016/j.marpetgeo.2005.03.005, 2005.
Beaudouin, C., Jouet, G. L., Suc, J. P., Berne, S., and Escarguela, G.: Vegetation dynamics in southern France during the last 30 ky BP in the light of marine palynology, Quat. Sci. Rev., 26, 1037–1054, doi:10.1016/j.quascirev.2006.12.009, 2007.
Beck, M., Riedel, T., Graue, J., Köster, J., Kowalski, N., Wu, C. S., Wegener, G., Lipsewers, Y., Freund, H., Böttcher, M. E., Brumsack, H.-J., Cypionka, H., Rullkötter, J., and Engelen, B.: Imprint of past and present environmental conditions on microbiology and biogeochemistry of coastal Quaternary sediments, Biogeosciences, 8, 55-068, doi:10.5194/bg-8-55-2011, 2011.
Benner, R.: What happens to terrestrial organic matter in the ocean?, Mar. Chem., 92, 307–310, doi:10.1016/j.marchem.2004.06.033, 2004.
Berne, S., Loubrieu, B., and equipe Calmar, E.: Canyons and recent sedimentary processes on the western Gulf of Lions margin. First results of the Calmar cruise, Comptes Rendus De L Academie Des Sciences Serie Ii Fascicule a-Sciences De La Terre Et Des Planetes, 328, 471–477, doi:10.1016/s1251-8050(99)80148-7, 1999.
Bond, G., Heinrich, H., Broecker, W., Labeyrie, L., McManus, J., Andrews, J., Huon, S., Jantschik, R., Clasen, S., Simet, C., Tedesco, K., Klas, M., Bonani, G., and Ivy, S.: Evidence for massive discharges of icebergs into the North-Atlantic ocean during the Last Glacial Period, Nature, 360, 245–249, doi:10.1038/360245a0, 1992.
Bruun, A. M., Finster, K., Gunnlaugsson, H. P., Nornberg, P., and Friedrich, M. W.: A Comprehensive Investigation on Iron Cycling in a Freshwater Seep Including Microscopy, Cultivation and Molecular Community Analysis, Geomicrobiol. J., 27, 15–34, doi:10.1080/01490450903232165, 2010.
Cacho, I., Shackleton, N., Elderfield, H., Sierro, F. J., and Grimalt, J. O.: Glacial rapid variability in deep-water temperature and delta O-18 from the Western Mediterranean Sea, Quat. Sci. Rev., 25, 3294–3311, doi:10.1016/j.quascirev.2006.10.004, 2006.
Casamayor, E. O., Schafer, H., Baneras, L., Pedros-Alio, C., and Muyzer, G.: Identification of and spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: Comparison by microscopy and denaturing gradient gel electrophoresis, Appl. Environ. Microbiol., 66, 499–508, doi:10.1128/aem.66.2.499-508.2000, 2000.
Cauwet, G.: Non-living particulate matter, in: Marine Organic Chemistry – Evolution, Composition, Interactions and Chemistry of Organic Matter in Seawater, edited by: Series, E. O.,

BGD
9, 253–310, 2012

71–89, 1981.
Clark, P. U., McCabe, A. M., Mix, A. C., and Weaver, A. J.: Rapid rise of sea level 19,000 years ago and its global implications, Science, 304, 1141–1144, doi:10.1126/science.1094449, 2004.
Coolen, M. J. L. and Overmann, J.: 217 000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment, Environ. Microbiol., 9, 238–249, doi:10.1111/j.1462-2920.2006.01134.x, 2007.
Cord-Ruwish, R.: A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria., J. Microbiol. Meth., 4, 33–36, 1985.
Corinaldesi, C., Barucca, M., Luna, G. M., and Dell'Anno, A.: Preservation, origin and genetic imprint of extracellular DNA in permanently anoxic deep-sea sediments, Mol. Ecol., 20, 642–654, doi:10.1111/j.1365-294X.2010.04958.x, 2011.
Cowen, J. P., Giovannoni, S. J., Kenig, F., Johnson, H. P., Butterfield, D., Rappe, M. S., Hutnak, M., and Lam, P.: Fluids from aging ocean crust that support microbial life, Science, 299, 120–123, doi:10.1126/science.1075653, 2003.
Cragg, B. A., Harvey, S. M., Fry, J. C., Herbert, R. A., and Parkes, R. J.: Bacterial biomass and activity in the deep sediment layers of the Japan Sea, Hole 798B, Proc. Ocean Drilling Prog. Sci. Results 127/128, 761–776, 1992.
D'Hondt, S., Jorgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K. U., Holm, N. G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S. D., Sass, H., Skilbeck, C. G., Aiello, I. W., Guerin, G., House, C. H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R. J., Schippers, A., Smith, D. C., Teske, A., Wiegel, J., Padilla, C. N., and Acosta, J. L.: Distributions of microbial activities in deep subseafloor sediments, Science, 306, 2216–2221, 306/5705/2216 [pii] doi:10.1126/science.1101155, 2004.
Daffonchio, D., Borin, S., Brusa, T., Brusetti, L., van der Wielen, P., Bolhuis, H., Yakimov, M. M., D'Auria, G., Giuliano, L., Marty, D., Tamburini, C., McGenity, T. J., Hallsworth, J. E., Sass, A. M., Timmis, K. N., Tselepides, A., de Lange, G. J., Hubner, A., Thomson, J., Varnavas, S. P., Gasparoni, F., Gerber, H. W., Malinverno, E., Corselli, C., Garcin, J., McKew, B., Golyshin, P. N., Lampadariou, N., Polymenakou, P., Calore, D., Cenedese, S., Zanon, F., Hoog, S., and Biodeep Sci, P.: Stratified prokaryote network in the oxic-anoxic transition of a deep-sea halocline, Nature, 440, 203–207, doi:10.1038/nature04418, 2006.
Danovaro, R., Company, J. B., Corinaldesi, C., D'Onghia, G., Galil, B., Gambi, C., Gooday,

BGD
9, 253–310, 2012

A. J., Lampadariou, N., Luna, G. M., Morigi, C., Olu, K., Polymenakou, P., Ramirez-Llodra, E., Sabbatini, A., Sarda, F., Sibuet, M., and Tselepides, A.: Deep-Sea Biodiversity in the Mediterranean Sea: The Known, the Unknown, and the Unknowable, PLoS One, 5, e11832 doi:10.1371/journal.pone.0011832, 2010.

DeLong, E. F.: Archaea in coastal marine environments, Proceedings of the National Academy of Sciences of the United States of America, 89, 5685–5689, doi:10.1073/pnas.89.12.5685, 1992.

DeLong, E. F.: Microbial life breathes deep, Science, 306, 2198–2200, doi:10.1126/science.1107241, 2004.

Dong, H. L. and Yu, B. S.: Geomicrobiological processes in extreme environments: A review, Episodes, 30, 202–216, 2007.

Ehlers, J.: Quaternary and Glacial Geology, Wiley, New York, 578 pp., 1996.

El Maghraoui, M., Joron, J. L., Etoubleau, J., Cambon, P., and Treuil, M.: Determination of forty four major and trace elements in GPMA magamtic rock reference samples using X-ray fluorescence spectrometry (XRF) and Instrumental Neutron Activation Analysis (INAA), Geostandards Newsletter, the journal of Geostandards and Geoanalysis, 23, 59–68, 1999.

Elliot, M., Labeyrie, L., Bond, G., Cortijo, E., Turon, J. L., Tisnerat, N., and Duplessy, J. C.: Millennial-scale iceberg discharges in the Irminger Basin during the last glacial period: Relationship with the Heinrich events and environmental settings, Paleoceanography, 13, 433–446, doi:10.1029/98pa01792, 1998.

Engelen, B., Ziegelmueller, K., Wolf, L., Kopke, B., Gittel, A., Cypionka, H., Treude, T., Nakagawa, S., Inagaki, F., Lever, M. A., and Steinsbu, B. O.: Fluids from the oceanic crust support microbial activities within the deep biosphere, Geomicrobiol. J., 25, 56–66, doi:10.1080/01490450701829006, 2008.

Fish, S. A., Shepherd, T. J., McGenity, T. J., and Grant, W. D.: Recovery of 16S ribosomal RNA gene fragments from ancient halite, Nature, 417, 432–436, doi:10.1038/417432a, 2002.

Florineth, D. and Schluchter, C.: Reconstructing the Last Glacial Maximum (LGM) ice surface geometry and flowlines in the central Swiss Alps, Eclogae Geologicae Helvetiae, 91, 391–407, 1998.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., Aragno, M., and Rossi, P.: Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns, Environ Microb., 4, 634–643, 358 [pii], 2002.

Fry, J. C., Parkes, R. J., Cragg, B. A., Weightman, A. J., and Webster, G.: Prokaryotic bio-

BGD
9, 253–310, 2012

diversity and activity in the deep subseafloor biosphere, Fems Microbiology Ecology, 66, 181–196, doi:10.1111/j.1574-6941.2008.00566.x, 2008.
Fulthorpe, C. S., Hoyanagi, K., Blum, P., and Scientists, E.: Methods: Geochemistry and microbiology, in: Proceedings of the Integrated Ocean Drilling Program, edited by: U.S. Implementing Organisation Services, T. A. M. U., Integrated Ocean Drilling Program Management International, Inc., , Texas, 22–23, 2011.
Gadd, G. M.: Metals, minerals and microbes: geomicrobiology and bioremediation, Microbiology, 156, 609–643, mic.0.037143-0 [pii], doi:10.1099/mic.0.037143-0, 2010.
Gaillard, C. and Strauss, F.: Ethanol precipitation of DNA with Linear Polyacrylamide as a carrier, Nucleic Acids Research, 18, 378–378, doi:10.1093/nar/18.2.378, 1990.
Ghiglione, J. F., Palacios, C., Marty, J. C., Mével, G., Labrune, C., Conan, P., Pujo-Pay, M., Garcia, N., and Goutx, M.: Role of environmental factors for the vertical distribution (0-1000 m) of marine bacterial communities in the NW Mediterranean Sea, Biogeosciences, 5, 1751–1764, doi:10.5194/bg-5-1751-2008, 2008.
Gleeson, D., McDermott, F., and Clipson, N.: Understanding microbially active biogeochemical environments, Adv. Appl. Microbiol., 62, 81–104, S0065-2164(07)62004-8 [pii], doi:10.1016/S0065-2164(07)62004-8, 2007.
Gomila, M., Bowien, B., Falsen, E., Moore, E. R., and Lalucat, J.: Description of Pelomonas aquatica sp. nov. and Pelomonas puraquae sp. nov., isolated from industrial and haemodialysis water, Int J Syst Evol Microbiol, 57, 2629–2635, 57/11/2629 [pii], doi:10.1099/ijs.0.65149-0, 2007.
Grousset, F. E., Labeyrie, L., Sinko, J. A., Cremer, M., Bond, G., Duprat, J., Cortijo, E., and Huon, S.: Patterns of ice-rafted detritus in the Glacial North-Atlantic (40 degrees-55 degrees-N), Paleoceanography, 8, 175–192, doi:10.1029/92pa02923, 1993.
Hedges, J. I., Keil, R. G., and Benner, R.: What happens to terrestrial organic matter in the ocean?, Org. Geochem., 27, 195–212, doi:10.1016/s0146-6380(97)00066-1, 1997.
Heijs, S. K., Laverman, A. M., Forney, L. J., Hardoim, P. R., and van Elsas, J. D.: Comparison of deep-sea sediment microbial communities in the Eastern Mediterranean, Fems Microbiology Ecology, 64, 362–377, doi:10.1111/j.1574-6941.2008.00463.x, 2008.
Heinrich, H.: Origin and consequences of cyclic ice rafting in the Northeast Atlantic-Ocean during the past 130,000 years, Quat. Res., 29, 142–152, doi:10.1016/0033-5894(88)90057-9, 1988.
Hinderer, M.: Late Quaternary denudation of the Alps, valley and lake fillings and 774 modern

BGD
9, 253–310, 2012

river loads, Geodin. Acta, 14, 231–263, 2001.
Hugenholtz, P., Goebel, B. M., and Pace, N. R.: Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity (Vol. 180, pg 4765, 1998), J. Bacteriol., 180, 6793–6793, 1998.
Hughen, K. A., Southon, J. R., Lehman, S. J., and Overpeck, J. T.: Synchronous radiocarbon and climate shifts during the last deglaciation, Science, 290, 1951–1954, doi:10.1126/science.290.5498.1951, 2000.
Inagaki, F., Takai, K., Komatsu, T., Kanamatsu, T., Fujioka, K., and Horikoshi, K.: Archaeology of Archaea: geomicrobiological record of Pleistocene thermal events concealed in a deep-sea subseafloor environment, Extremophiles, 5, 385–392, doi:10.1007/s007920100211, 2001.
Inagaki, F., Sakihama, Y., Inoue, A., Kato, C., and Horikoshi, K.: Molecular phylogenetic analyses of reverse-transcribed bacterial rRNA obtained from deep-sea cold seep sediments, Environ. Microbiol., 4, 277–286, doi:10.1046/j.1462-2920.2002.00294.x, 2002.
Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., Nealson, K. H., and Horikoshi, K.: Microbial communities associated with geological horizons in coastal subseafloor sediments from the sea of okhotsk, Appl. Environ. Microb., 69, 7224–7235, 2003.
Inagaki, F., Okada, H., Tsapin, A. I., and Nealson, K. H.: Research paper: Microbial survival – The paleome: A sedimentary genetic record of past microbial communities, Astrobiology, 5, 141–153, doi:10.1089/ast.2005.5.141, 2005.
Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., Nealson, K. H., Horikoshi, K., D'Hondt, S., and Jorgensen, B. B.: Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin, P. Natl. A. Sci. USA, 103, 2815–2820, 0511033103 [pii], doi:10.1073/pnas.0511033103, 2006.
Inagaki, F. and Nakagawa, S.: Spatial Distribution of the Subseafloor Life: Diversity and Biogeography, in: Links between Geological Processes, Microbial Activities & Evolution of Life: Microbes and Geology, edited by: Dilek, Y., Furnes, H., and Muehlenbachs, K., Mod. Appr. Sol. Earth S., 135–158, 2008.
Jégou, I.: Comment l'activité turbiditique est-elle enregistrée dans les dépôts de débordement de la ride sédimentaire du Var ?, Université de Bretagne Occidentale, Rapport de DEA, 2004.
Jørgensen, B. B. and D'Hondt, S.: Ecology – A starving majority deep beneath the seafloor,

BGD
9, 253–310, 2012

Science, 314, 932–934, doi:10.1126/science.1133796, 2006.
Jorry, S. J., Jegou, I., Emmanuel, L., Jacinto, R. S., and Savoye, B.: Turbiditic levee deposition in response to climate changes: The Var Sedimentary Ridge (Ligurian Sea), Mar. Geol., 279, 148–161, doi:10.1016/j.margeo.2010.10.021, 2011.
Jouet, G., Berne, S., Rabineau, M., Bassetti, M. A., Bernier, P., Dennielou, B., Sierro, F. J., Flores, J. A., and Taviani, M.: Shoreface migrations at the shelf edge and sea-level changes around the Last Glacial Maximum (Gulf of Lions, NW Mediterranean), Mar. Geol., 234, 21–42, doi:10.1016/j.margeo.2006.09.012, 2006.
Kallmeyer, J., Smith, D. C., Spivack, A. J., and D'Hondt, S.: New cell extraction procedure applied to deep subsurface sediments, Limnol. Oceanogr.-Meth., 6, 236–245, 2008.
Kampe, H., Dziallas, C., Grossart, H. P., and Kamjunke, N.: Similar Bacterial Community Composition in Acidic Mining Lakes with Different pH and Lake Chemistry, Microb. Ecol., 60, 618–627, doi:10.1007/s00248-010-9679-5, 2010.
Kaye, J. Z., Sylvan, J. B., Edwards, K. J., and Baross, J. A.: Halomonas and Marinobacter ecotypes from hydrothermal vent, subseafloor and deep-sea environments, Fems Microbiol. Ecol., 75, 123–133, doi:10.1111/j.1574-6941.2010.00984.x, 2011.
Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., and Stackebrandt, E.: Introduction to the Proteobacteria, Proteobacteria: Alpha and Beta Subclasses, The Prokaryotes, 3 Edn., edited by: Dworkin, M., Springer, 2006.
Koizumi, Y., Kojima, H., and Fukui, M.: Characterization of depth-related microbial community structure in lake sediment by denaturing gradient gel electrophoresis of amplified 16S rDNA and reversely transcribed 16S rRNA fragments, Fems Microbiol. Ecol., 46, 147–157, doi:10.1016/s0168-6496(03)00212-5, 2003.
La Cono, V., Smedile, F., Bortoluzzi, G., Arcadi, E., Maimone, G., Messina, E., Borghini, M., Oliveri, E., Mazzola, S., L'Haridon, S., Toffin, L., Genovese, L., Ferrer, M., Giuliano, L., Golyshin, P. N., and Yakimov, M. M.: Unveiling microbial life in new deep-sea hypersaline Lake Thetis. Part I: Prokaryotes and environmental settings, Environ. Microbiol., doi:10.1111/j.1462-2920.2011.02478.x, 2011.
Lane, D. J.: 16S/23S rRNA sequencing, in: Nucleic Acid Techniques in Bacterial Systematics, Stackebrandt, E., and Goodfellow, M., eds.Wiley John and Sons, New York, NY, 115–175, 1991.
Lazar, C. S., Parkes, R. J., Cragg, B. A., L'Haridon, S., and Toffin, L.: Methanogenic diversity and activity in hypersaline sediments of the centre of the Napoli mud volcano, Eastern

BGD
9, 253–310, 2012

Mediterranean Sea, Environ. Microbiol., doi:10.1111/j.1462-2920.2011.02425.x, 2011.
Lipp, J. S., Morono, Y., Inagaki, F., and Hinrichs, K. U.: Significant contribution of Archaea to extant biomass in marine subsurface sediments, Nature, 454, 991–994, nature07174 [pii], doi:10.1038/nature07174, 2008.
Luna, G. M., Dell'Anno, A., Giuliano, L., and Danovaro, R.: Bacterial diversity in deep Mediterranean sediments: relationship with the active bacterial fraction and substrate availability, Environ. Microbiol., 6, 745–753, EMI611 [pii], doi:10.1111/j.1462-2920.2004.00611.x, 2004.
Luna, G. M., Stumm, K., Pusceddu, A., and Danovaro, R.: Archaeal diversity in deep-sea sediments estimated by means of different terminal-restriction fragment length polymorphisms (T-RFLP) protocols, Curr. Microbiol., 59, 356–361, doi:10.1007/s00284-009-9445-4, 2009.
Marchesi, J. R., Weightman, A. J., Cragg, B. A., Parkes, R. J., and Fry, J. C.: Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis, Fems Microbiol. Ecol., 34, 221–228, doi:10.1111/j.1574-6941.2001.tb00773.x, 2001.
Martiny, J. B., Bohannan, B. J., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., Morin, P. J., Naeem, S., Ovreas, L., Reysenbach, A. L., Smith, V. H., and Staley, J. T.: Microbial biogeography: putting microorganisms on the map, Nat. Rev. Microbiol., 4, 102–112, doi:10.1038/nrmicro1341, nrmicro1341 [pii], 2006.
Mason, O. U., Nakagawa, T., Rosner, M., Van Nostrand, J. D., Zhou, J., Maruyama, A., Fisk, M. R., and Giovannoni, S. J.: First investigation of the microbiology of the deepest layer of ocean crust, PLoS One, 5, e15399, doi:10.1371/journal.pone.0015399, 2010.
Methe, B. A., Hiorns, W. D., and Zehr, J. P.: Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes, Limnol. Oceanogr., 43, 368–374, 1998.
Middelburg, J. J.: A simple rate model for organic-matter decomposition in marine-sediments, Geochim. Cosmochim. Ac., 53, 1577–1581, doi:10.1016/0016-7037(89)90239-1, 1989.
Migeon, S., Savoye, B., and Faugeres, J. C.: Quaternary development of migrating sediment waves in the Var deep-sea fan: distribution, growth pattern, and implication for levee evolution, Sediment. Geol., 133, 265–293, doi:10.1016/s0037-0738(00)00043-9, 2000.
Migeon, S., Mulder, T., Savoye, B., and Sage, F.: The Var turbidite system (Ligurian Sea, northwestern Mediterranean) – morphology, sediment supply, construction of turbidite levee and sediment waves: implications for hydrocarbon reservoirs, Geo-Mar. Lett., 26, 361–371,

BGD
9, 253–310, 2012

doi:10.1007/s00367-006-0047-x, 2006.
Morono, Y., Terada, T., Masui, N., and Inagaki, F.: Discriminative detection and enumeration of microbial life in marine subsurface sediments, ISME J., 3, 503–511, doi:10.1038/ismej.2009.1, ismej20091 [pii], 2009.
Newberry, C. J., Webster, G., Cragg, B. A., Parkes, R. J., Weightman, A. J., and Fry, J. C.: Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190, Environ. Microbiol., 6, 274–287, doi:10.1111/j.1462-2920.2004.00568.x, 2004.
Nunoura, T., Soffientino, B., Blazejak, A., Kakuta, J., Oida, H., Schippers, A., and Takai, K.: Subseafloor microbial communities associated with rapid turbidite deposition in the Gulf of Mexico continental slope (IODP Expedition 308), Fems Microbiol. Ecol., 69, 410–424, doi:10.1111/j.1574-6941.2009.00718.x, 2009.
Parkes, R. J., Cragg, B. A., Fry, J. C., Herbert, R. A., Wimpenny, J. W. T., Allen, J. A., and Whitfield, M.: Bacterial biomass and activity in deep sediment layers from the Peru Margin, Philos. T. R. Soc. A, 331, 139–153, doi:10.1098/rsta.1990.0061, 1990.
Parkes, R. J., Cragg, B. A., Bale, S. J., Getliff, J. M., Goodman, K., Rochelle, P. A., Fry, J. C., Weightman, A. J., and Harvey, S. M.: Deep bacterial biosphere in Pacific-Ocean sediments, Nature, 371, 410–413, doi:10.1038/371410a0, 1994.
Parkes, R. J., Cragg, B. A., and Wellsbury, P.: Recent studies on bacterial populations and processes in subseafloor sediments: A review, Hydrogeol. J., 8, 11–28, doi:10.1007/pl00010971, 2000.
Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., Kallmeyer, J., Jorgensen, B. B., Aiello, I. W., and Fry, J. C.: Deep subseafloor prokaryotes stimulated at interfaces over geological time, Nature, 436, 390–394, doi:10.1038/nature03796, 2005.
Parkes, R. J., Sellek, G., Webster, G., Martin, D., Anders, E., Weightman, A. J., and Sass, H.: Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG), Environ. Microbiol., 11, 3140–3153, doi:10.1111/j.1462-2920.2009.02018.x, 2009.
Pautot, G.: Morphollogic framework of the Baie-des-Anges (Nice-côte-d'Azur) – Instability model of continental – slope, Oceanol. Ac., 4, 203–211, 1981.
Phuong, N. M., Kang, Y., Sakurai, K., Iwasaki, K., Kien, C. N., Noi, N., and Son, L. T.: Ar-

BGD
9, 253–310, 2012

senic contents and physicochemical properties of agricultural soils from the Red River Delta, Vietnam, Soil Sci. Plant Nutr., 54, 846–855, doi:10.1111/j.1747-0765.2008.00312.x, 2008.

Piper, D. J. W., and Savoye, B.: Processes of late Quaternary turbidity current flow and deposition on the Var deep-sea fan, North-West Mediterranean Sea, Sedimentology, 40, 557–582, doi:10.1111/j.1365-3091.1993.tb01350.x, 1993.

Polymenakou, P. N., Bertilsson, S., Tselepides, A., and Stephanou, E. G.: Links between geographic location, environmental factors, and microbial community composition in sediments of the Eastern Mediterranean Sea, Microb. Ecol., 49, 367–378, doi:10.1007/s00248-004-0274-5, 2005a.

Polymenakou, P. N., Bertilsson, S., Tselepides, A., and Stephanou, E. G.: Bacterial community composition in different sediments from the Eastern Mediterranean Sea: a comparison of four 16S ribosomal DNA clone libraries, Microb. Ecol., 50, 447–462, doi:10.1007/s00248-005-0005-6, 2005b.

Polymenakou, P. N., Lampadariou, N., Mandalakis, M., and Tselepides, A.: Phylogenetic diversity of sediment bacteria from the southern Cretan margin, Eastern Mediterranean Sea, Syst. Appl. Microbiol., 32, 17–26, doi:10.1016/j.syapm.2008.09.006, 2009.

Rabineau, M., Berne, S., Aslanian, D., Olivet, J. L., Joseph, P., Guillocheau, F., Bourillet, J. F., Ledrezen, E., and Granjeon, D.: Sedimentary sequences in the Gulf of Lion: A record of 100,000 years climatic cycles, Mar. Petrol. Geol., 22, 775–804, doi:10.1016/j.marpetgeo.2005.03.010, 2005.

Rabineau, M., Berne, S., Olivet, J.-L., Aslanian, D., Guillocheau, F., and Joseph, P.: Paleo sea levels reconsidered from direct observation of paleoshoreline position during Glacial Maxima (for the last 500,000 yr), Earth Planet. Sci. Lett., 252, 119–137, doi:10.1016/j.epsl.2006.09.033, 2006.

Rebata-Landa, V. and Santamarina, J. C.: Mechanical limits to microbial activity in deep sediments, Geochem. Geophys. Geosys., 7, Q11006, doi:10.1029/2006gc001355, 2006.

Révillon, S., Jouet, G., Bayon, G., Rabineau, M., Dennielou, B., Hemond, C., and Berné, S.: The provenance of sediments in the Gulf of Lions, western Mediterranean Sea, Geochem. Geophys. Geosyst., 12, Q08006, doi:10.1029/2011gc003523, 2011.

Richter, T. O., Van der Gaast, S. J., Koster, B., Vaars, A. J., Gieles, R., De Stigter, H. C., De Haas, H., and Weering, V.: The Avaatech XRF Core Scanner: technical description and applications to NE Atlantic sediments, in: New Techniques in Sediment Core Analysis., edited by: Rothwell, R. G., Geological Society, Special Publications, London, 39–50, 2006.

BGD
9, 253–310, 2012

Rogers, J. R. and Bennett, P. C.: Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates, Chem. Geol., 203, 91–108, doi:10.1016/j.chemgeo.2003.09.001, 2004.

Roussel, E. G., Sauvadet, A. L., Allard, J., Chaduteau, C., Richard, P., Bonavita, M. A. C., and Chaumillon, E.: Archaeal Methane Cycling Communities Associated with Gassy Subsurface Sediments of Marennes-Oleron Bay (France), Geomicrobiol. J., 26, 31–43, doi:10.1080/01490450802599284, 2009a.

Roussel, E. G., Sauvadet, A. L., Chaduteau, C., Fouquet, Y., Charlou, J. L., Prieur, D., and Bonavita, M. A. C.: Archaeal communities associated with shallow to deep subseafloor sediments of the New Caledonia Basin, Environ. Microbiol., 11, 2446–2462, doi:10.1111/j.1462-2920.2009.01976.x, 2009b.

Sahl, J. W., Schmidt, R. H., Swanner, E. D., Mandernack, K. W., Templeton, A. S., Kieft, T. L., Smith, R. L., Sanford, W. E., Callaghan, R. L., Mitton, J. B., and Spear, J. R.: Subsurface microbial diversity in deep-granitic-fracture water in Colorado, Appl. Environ. Microbiol., 74, 143–152, doi:10.1128/aem.01133-07, 2008.

Savoye, B., Piper, D. J. W., and Droz, L.: Plio-Pleistocene evolution of the Var deep-sea fan off the French Riviera., Mar. Petrol. Geol., 10, 550–571, 1993.

Savoye, B., Nelson, H., Unterseh, S., Piper, D., and Rehault, J. P.: The Var Fan distal lobe (Ligurian Sea. Mediterranean): sidescan Facies, seismic architecture, nature of deposits. , I.A.S. 15th International Sedimentolgical Congress, Alicante, avril 1998Book of Abstracts, 701, 1998.

Schaefer, H., Ferdelman, T. G., Fossing, H., and Muyzer, G.: Microbial diversity in deep sediments of the Benguela Upwelling System, Aquatic Microb. Ecol., 50, 1–9, doi:10.3354/ame01164, 2007.

Schnetger, B., Brumsack, H. J., Schale, H., Hinrichs, J., and Dittert, L.: Geochemical characteristics of deep-sea sediments from the Arabian Sea: a high-resolution study, Deep-Sea Res. Pt. II, 47, 2735–2768, doi:10.1016/s0967-0645(00)00047-3, 2000.

Schouten, S., Middelburg, J. J., Hopmans, E. C., and Damste, J. S. S.: Fossilization and degradation of intact polar lipids in deep subsurface sediments: A theoretical approach, Geochim. Cosmochim. Ac., 74, 3806–3814, doi:10.1016/j.gca.2010.03.029, 2010.

Selenska-Pobell, S., Kampf, G., Flemming, K., Radeva, G., and Satchanska, G.: Bacterial diversity in soil samples from two uranium waste piles as determined by rep-APD, RISA and 16S rDNA retrieval, Antonie Van Leeuwenhoek International Journal of General and

BGD
9, 253–310, 2012

Molecular Microbiology, 79, 149-161, 10.1023/a:1010237711077, 2001.
Sierro, F. J., Hodell, D. A., Curtis, J. H., Flores, J. A., Reguera, I., Colmenero-Hidalgo, E., Barcena, M. A., Grimalt, J. O., Cacho, I., Frigola, J., and Canals, M.: Impact of iceberg melting on Mediterranean thermohaline circulation during Heinrich events, Paleoceanography, 20, Pa2019 10.1029/2004pa001051, 2005.
Sierro, F. J., Andersen, N., Bassetti, M. A., Berne, S., Canals, M., Curtis, J. H., Dennielou, B., Abel Flores, J., Frigola, J., Gonzalez-Mora, B., Grimalt, J. O., Hodell, D. A., Jouet, G., Perez-Folgado, M., and Schneider, R.: Phase relationship between sea level and abrupt climate change, Quat. Sci. Rev., 28, 2867–2881, 10.1016/j.quascirev.2009.07.019, 2009.
Soina, V. S., Vorobiova, E. A., Zvyagintsev, D. G., and Gilichinsky, D. A.: Preservation of cell structures in permafrost-A model for exobiology, in: Life Sciences and Space Research Xxv, edited by: Greenberg, J. M., Oro, J., Brack, A., Devincenzi, D. L., Banin, A., Friedmann, E. I., Rummel, J. D., Raulin, F., McKay, C. P., Baltscheffsky, H., Schwartz, A. W., Schidlowski, M., and Roessler, K., Adv. Space Res., 3, 237–242, 1994.
Sørensen, K. B., and Teske, A.: Stratified communities of active archaea in deep marine subsurface sediments, Applied and Environmental Microbiology, 72, 4596-4603, 10.1128/aem.00562-06, 2006.
Souza, V., Espinosa-Asuar, L., Escalante, A. E., Eguiarte, L. E., Farmer, J., Forney, L., Lloret, L., Rodriguez-Martinez, J. M., Soberon, X., Dirzo, R., and Elser, J. J.: An endangered oasis of aquatic microbial biodiversity in the Chihuahuan desert, Proceedings of the National Academy of Sciences of the United States of America, 103, 6565–6570, doi:10.1073/pnas.0601434103, 2006.
Stackebrandt, E. and Ebers, J.: Taxonomic parameters revisited: tarnished gold standards, Microbiology Today, 152–155, 2006.
Stevens, H., Stubner, M., Simon, M., and Brinkhoff, T.: Phylogeny of Proteobacteria and Bacteroidetes from oxic habitats of a tidal flat ecosystem, Fems Microbiol. Ecol., 54, 351–365, doi:10.1016/j.femsec.2005.04.008, 2005.
Takai, K., Komatsu, T., Inagaki, F., and Horikoshi, K.: Distribution of archaea in a black smoker chimney structure, Appl. Environ. Microbiol., 67, 3618–3629, doi:10.1128/aem.67.8.3618-3629.2001, 2001a.
Takai, K., Moser, D. P., DeFlaun, M., Onstott, T. C., and Fredrickson, J. K.: Archaeal diversity in waters from deep South African gold mines, Appl. Environ. Microbiol., 67, 5750–5760, doi:10.1128/aem.67.21.5750-5760.2001, 2001b.

BGD
9, 253–310, 2012

Teske, A. and Sørensen, K. B.: Uncultured archaea in deep marine subsurface sediments: have we caught them all?, Isme Journal, 2, 3–18, doi:10.1038/ismej.2007.90, 2008.
Vetriani, C., Jannasch, H. W., MacGregor, B. J., Stahl, D. A., and Reysenbach, A. L.: Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments, Appl. Environ. Microbiol., 65, 4375–4384, 1999.
Wang, J. J., Wu, Y. C., Jiang, H. C., Li, C. H., Dong, H. L., Wu, Q. L., Soininen, J., and Shen, J.: High beta diversity of bacteria in the shallow terrestrial subsurface, Environ. Microbiol., 10, 2537–2549, doi:10.1111/j.1462-2920.2008.01678.x, 2008.
Weaver, A. J., Saenko, O. A., Clark, P. U., and Mitrovica, J. X.: Meltwater pulse 1A from Antarctica as a trigger of the bolling-allerod warm interval, Science, 299, 1709–1713, doi:10.1126/science.1081002, 2003.
Webster, G., Newberry, C. J., Fry, J. C., and Weightman, A. J.: Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale, J. Microbiol. Meth., 55, 155–164, doi:10.1016/s0167-7012(03)00140-4, 2003.
Webster, G., Parkes, R. J., Cragg, B. A., Newberry, C. J., Weightman, A. J., and Fry, J. C.: Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin, Fems Microbiol. Ecol., 58, 65–85, doi:10.1111/j.1574-6941.2006.00147.x, 2006.
Widdel, F. and Bak, F.: Gram-negative mesophilic sulphate-reducing bacteria, in: The Prokaryotes, edited by: Balows, A., Trüper, H. G., Dworkin, M., Harder, W., and Schleifer, K.-H., Springer New-York, 3352–3378, 1992.
Wilms, R., Kopke, B., Sass, H., Chang, T. S., Cypionka, H., and Engelen, B.: Deep biosphere-related bacteria within the subsurface of tidal flat sediments, Environ. Microbiol., 8, 709–719, doi:10.1111/j.1462-2920.2005.00949.x, 2006.
Yakimov, M. M., La Cono, V., Denaro, R., D'Auria, G., Decembrini, F., Timmis, K. N., Golyshin, P. N., and Giuliano, L.: Primary producing prokaryotic communities of brine, interface and seawater above the halocline of deep anoxic lake L'Atalante, Eastern Mediterranean Sea, Isme Journal, 1, 743–755, doi:10.1038/ismej.2007.83, 2007.
Yamane, K., Maki, H., Nakayama, T., Nakajima, T., Nomura, N., Uchiyama, H., and Kitaoka, M.: Diversity and Similarity of Microbial Communities in Petroleum Crude Oils Produced in Asia, Biosci. Biotech. Bioch., 72, 2831–2839, doi:10.1271/bbb.80227, 2008.
Yan, J., Rash, B. A., Rainey, F. A., and Moe, W. M.: Isolation of novel bacteria within the Chloroflexi capable of reductive dechlorination of 1,2,3-trichloropropane, Environ. Microbiol.,

BGD
9, 253–310, 2012

11, 833–843, doi:10.1111/j.1462-2920.2008.01804.x, 2009.
Yarincik, K. M., Murray, R. W., and Peterson, L. C.: Climatically sensitive eolian and hemipelagic deposition in the Cariaco Basin, Venezuela, over the past 578,000 years: Results from Al/Ti and K/Al, Paleoceanography, 15, 210–228, doi:10.1029/1999pa900048, 2000.
Zinger, L., Shahnavaz, B., Baptist, F., Geremia, R. A., and Choler, P.: Microbial diversity in alpine tundra soils correlates with snow cover dynamics, Isme Journal, 3, 850–859, doi:10.1038/ismej.2009.20, 2009.

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Table 1. Location, bathymetry, length and age of studied and reference cores.

Core	Cruise	Latitude	Longitude	Water Depth(m)	Length (cm)	AMS ^{14}C date (cal ka)
Studied cores*						
KESC9-30	ESSCAR9	43°23′.016 N	07°44′.187 E	2160	828	*calc. age model
RHS-KS-33	RHOSOS	42°41′.596 N	03°50′.493 E	291	709	*calc. age model
Reference cores*						
KNI-23	NICASAR	43°23′.02 N	07°44′.19 E	2130	1052	(Jorry et al., 2011)
MD99-2348	IMAGES V	42°69′.300 N	03.84′.167 E	300	2400	(Sierro et al., 2009)

*Cores were recovered using a Küllenberg piston corer.

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Table 2. 16S ribosomal DNA sequence similarity analysis of cultured isolates from cores KESC9-30 and RHS-KS-33.

Sequence type	Accession n°	N° of isolates	Depth origin (cmbsf)*	Culture conditions	Closest cultured genus (% BLASTn identity)	Taxonomic affiliation
Gulf of Lions						
						Alphaproteobacteria
RHS-str.Ne D	HE589592	1	109	MB2216-agar, O_2, 20°C	*Pelagicola* sp. (96.5 %)	*Rhodobacteraceae*
RHS-str.108_Ne E	HE586857	1	109	MB2216-agar, O_2, 4°C	*Pelagicola* sp. (96.4 %)	*Rhodobacteraceae*
RHS-str.305	HE586858	6	109, 478	Acetate-agar, O_2, 20°C or 37°C	*Phaeobacter* sp. (99 %)	*Rhodobacteraceae*
RHS-str.307	HE586859	1	109	Acetate-agar, O_2, 20°C	*Phaeobacter* sp. (98 %)	*Rhodobacteraceae*
RHS-str.404	HE589593	2	109, 478	Glucose-agar, O_2, 20°C	*Phaeobacter* sp. (98 %)	*Rhodobacteraceae*
RHS-str.502	HE586860	2	109, 478	Pyruvate fermentation	*Sphingomonas* sp. (91 %)	
RHS-str.509	HE586861	2	478	Glucose fermentation, 20 or 37°C	*Sphingomonas* sp. (89 %)	
						Gammaproteobacteria
RHS-str.102_2EC	HE586862	1	109	MB2216-agar, O_2, 37°C	*Marinobacter* sp. (99 %)	*Alteromonadaceae*
RHS-str.103_3BL	HE586863	1	109	MB2216-agar, O_2, 37°C	*Marinobacter* sp. (99 %)	*Alteromonadaceae*
RHS-str.104_4JA	HE586864	2	109, 478	MB2216-agar, O_2, 37°C	*Idiomarina* sp. (99 %)	*Idiomarinaceae*
RHS-str.105_5CR	HE586865	3	109, 478, 698	MB2216-agar, O_2, 37°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.106_6PE	HE586866	2	109, 478	MB2216-agar, O_2, 20 or 37°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.7RO	HE586867	1	478	MB2216-agar, O_2, 16°C	*Shewanella* sp. (99 %)	*Shewanellaceae*
RHS-str.Or F	HE586868	2	109, 478	MB2216-agar, O_2, 4°C or 20°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.Br_G	HE586869	1	109	MB2216-agar, O_2, 4°C	*Pseudoalteromonas* sp. (99 %)	*Pseudoalteromonadaceae*
RHS-str.202_Mar A	HE586870	4	109, 478	MB2216-phytagel, O_2, 20 or 37°C	*Marinobacter* sp. (99 %)	*Alteromonadaceae*
RHS-str. 203_Cr B	HE586871	5	109, 478, 698	MB2216-phytagel, O_2, 20 or 37°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.204_Cr C	HE586872	2	109, 478	MB2216-phytagel, O_2, 37°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.402	HE586873	2	109, 478	Glucose-agar, O_2, 20°C	*Pseudoalteromonas* sp. (99 %)	*Pseudoalteromonadaceae*
RHS-str.403	HE586874	4	109, 478, 698	Glucose-agar, O_2, 20°C or 37°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.405	HE586875	4	109, 478	Glucose-agar, O_2, 20°C or 37°C	*Pseudoalteromonas* sp. (99 %)	*Pseudoalteromonadaceae*
RHS-str.X _19b	HE586876	1	109	Glucose-agar, O_2, 20°C	*Halomonas* sp. (99%)	*Halomonadaceae*
RHS-str.406	HE586877	2	109, 478	Glucose-agar, O_2, 20°C	*Pseudoalteromonas* sp. (99 %)	*Pseudoalteromonadaceae*
RHS-str.407	HE586878	3	109, 478, 698	Glucose-agar, O_2, 20°C	*Pseudoalteromonas* sp. (99 %)	*Pseudoalteromonadaceae*
RHS-str.409	HE586879	3	109, 478	Glucose-agar, O_2, 20°C or 37°C	*Pseudoalteromonas* sp. (99 %)	*Pseudoalteromonadaceae*
RHS-str.301	HE586880	2	109, 478	Acetate-agar, O_2, 20°C or 37°C	*Marinobacter* sp. (99 %)	*Alteromonadaceae*
RHS-str.302	HE586881	4	109, 478	Acetate-agar, O_2, 20°C or 37°C	*Halomonas* sp. (100 %)	*Halomonadaceae*
RHS-str.303	HE586882	1	478	Acetate-agar, O_2, 37°C	*Alcanivorax* sp. (99 %)	*Alcanivoracaceae*
RHS-str.304	HE586883	1	478	Acetate-agar, O_2, 20°C	*Marinobacter* sp. (99 %)	*Alteromonadaceae*
RHS-str.308	HE586884	1	478	Acetate-agar, O_2, 20°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
RHS-str.90	HE586885	1	84	Modified R2A medium, O_2, 25°C	*Aeromonas* sp. (99 %)	*Aeromonadaceae*
RHS-str.220	HE586886	1	198	Modified R2A medium, O_2, 25°C	*Pseudomonas* sp. (99 %)	*Pseudomonadaceae*
						Firmicutes
RHS-str.320	HE586887	1	307	Modified R2A medium, O_2, 25°C	*Bacillus* sp. (99 %)	*Bacillaceae*
						Actinobacteria
RHS-str.520	HE586888	1	508	Modified R2A medium, O_2, 25°C	*Brevibacterium* sp. (99 %)	*Brevibacteraceae*
Ligurian Sea						
						Gammaproteobacteria
ESC-str.110	HE586889	1	101	Modified R2A medium, O_2, 25°C	*Halomonas* sp. (99 %)	*Halomonadaceae*
ESC-str.770	HE586890	1	752	Modified R2A medium, O_2, 25°C	*Pseudomonas* sp. (99 %)	*Pseudomonadaceae*
						Firmicutes
ESC-str.50	HE586891	1	45	Modified R2A medium, O_2, 25°C	*Bacillus* sp. (99 %)	*Bacillaceae*
						Actinobacteria
ESC-str.590	HE586892	1	583	Modified R2A medium, O_2, 25°C	*Brevibacterium* sp. (99 %)	*Brevibacteraceae*

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Table 3. Summary of the results from canonical correspondence analyses of the bacterial community structure data when constrained by environmental variables.

Environmental variables	RHS-KS-33		KESC9-30	
Total inertia	1.754		3.703	
Sum of all canonical eigenvalues	0.783		1.061	
CCA explained variability*	44.74 %		28.65 %	
	Axis 1	Axis2	Axis 1	Axis2
Eigenvalues	0.269	0.180	0.231	0.198
Species-environment correlations	0.905	0.810	0.900	0.833
Cumulative percentage variance				
of species data	15.3	25.6	6.2	11.6
of species-environment relation	34.3	57.3	24.4	45.2
Monte Carlo test	*F-ratio: 1.88,*	*P-value: 0.002*	*F-ratio: 1.86,*	*P-value: 0.002*

*Sum of all canonical eigenvalues/Total inertia.

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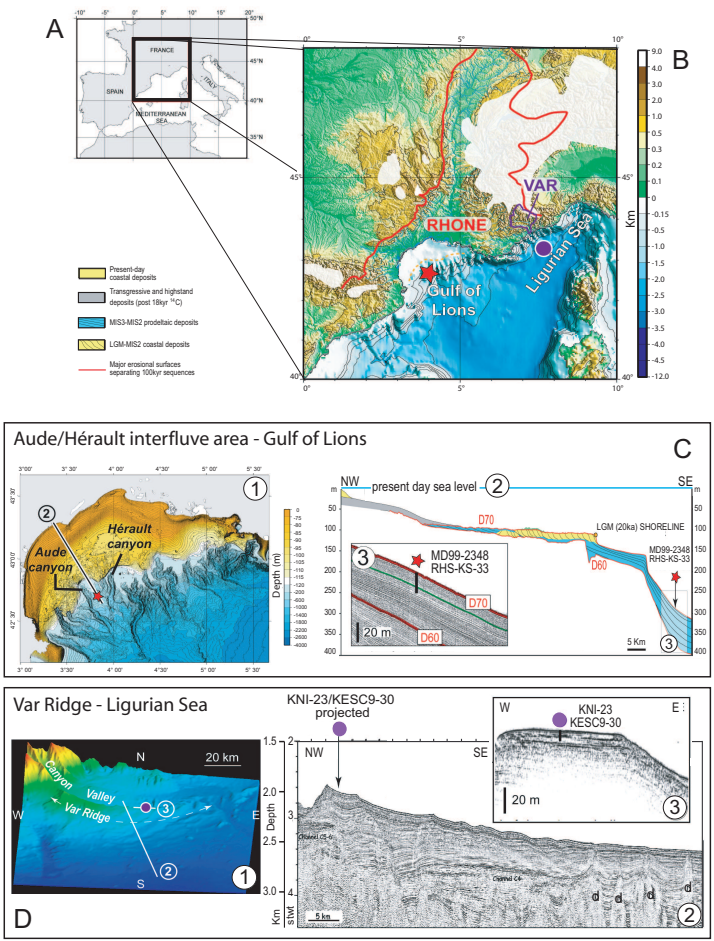

Fig. 1. Caption on next page.

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Fig. 1. Morphological and sedimentological background of the studied cores. **(A)** Location of study area within the Mediterranean Sea. **(B)** Map of the Southern France (modified from Rabineau et al., 2006), showing the continental drainage systems of the Gulf of Lions (red line) including mainly the Rhône, the pyreneo-languedocian rivers and Var (purple line) watersheds. In white: maximum extension of the mountainous glaciers during last glacial maximum (LGM-MIS2) (drawn and simplified using data from (Ehlers, 1996; Antoine et al., 1999). Red star: core RHS-KS-33 (Gulf of Lions), Purple dot: core KESC9-30 Ligurian Sea. **(C)** Gulf of Lions bathymetric map (1) and location of line drawing of Sparker profile shown in (2) (black line) on the upper slope between the Aude and Hérault canyons at the same location as twin core MD99-2348 (Beaudouin et al., 2005; Jouet et al., 2006; Sierro et al., 2009). (2) Line drawing of a Sparker seismic profile from the present day coast line to the outer shelf (modified from Rabineau et al., 2006, see location in (1)): RHS-KS-33 core sampled MIS2-early transgressive prodeltaic deposits. (3) Zoom of the Sparker seismic line in the area of RHS-KS-33, showing the non disturbed stratified seismic facies of the upper slope deposits. **(D)** The Var Ridge, in the Ligurian Sea. (1) 3-D block diagram (modified from (Jégou, 2004)) showing the morphology of the Var Ridge and location of the Sparker seismic profiles (white lines) in (2) and (3) and the location of KESC9-30 core, same as twin core KNI-23 (Migeon et al., 2000; Jorry et al., 2011) (purple dot). (2) Sparker seismic profile (modified from (Migeon et al., 2006)) illustrating the architecture of the Var Ridge and the relative position of core KESC9-30 on top of the ridge; (d): salt domes at the southern part of the ridge. (3) Zoom of a sub-bottom profile (3.5 kHz) showing the irregularly stratified facies of turbidites of the ridge (modified from (Jégou, 2004)).

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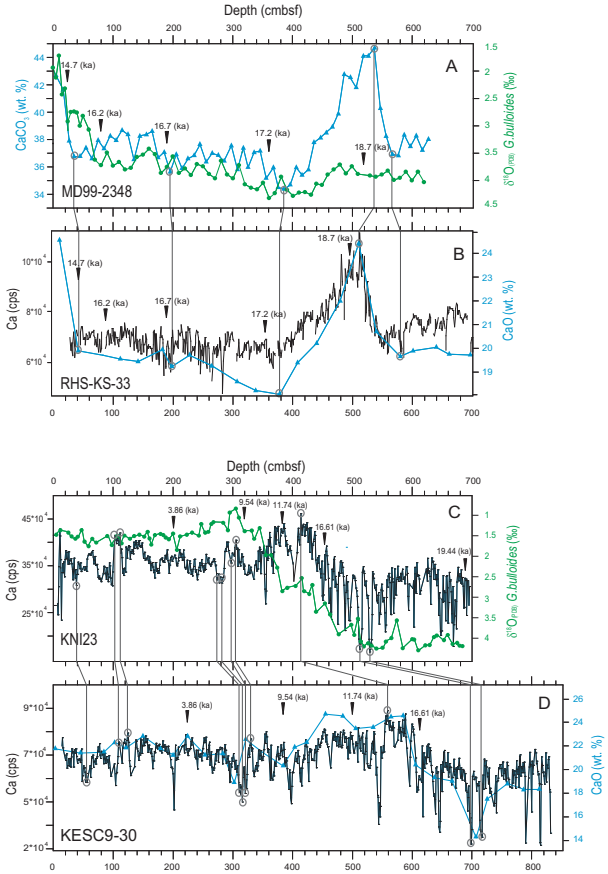

Fig. 2. Age model based on planktonic oxygen isotopes and AMS ^{14}C dates calibrated on the calcium records of twin cores: MD99-2348 and KNI23 (Sierro et al., 2009; Jorry et al., 2011). The green plain circles represent δ^{18}O *G. bulloides* record in MD99-2348 **(A)** and KNI23 **(C)** cores. The black arrows show the AMS ^{14}C dates (ka cal BP) in twin cores and transposed AMS ^{14}C dates in studied cores. The grey circles point up the calibration peaks of core correlations. Abbreviations: cmbsf, centimeters below the seafloor; cps, counts.

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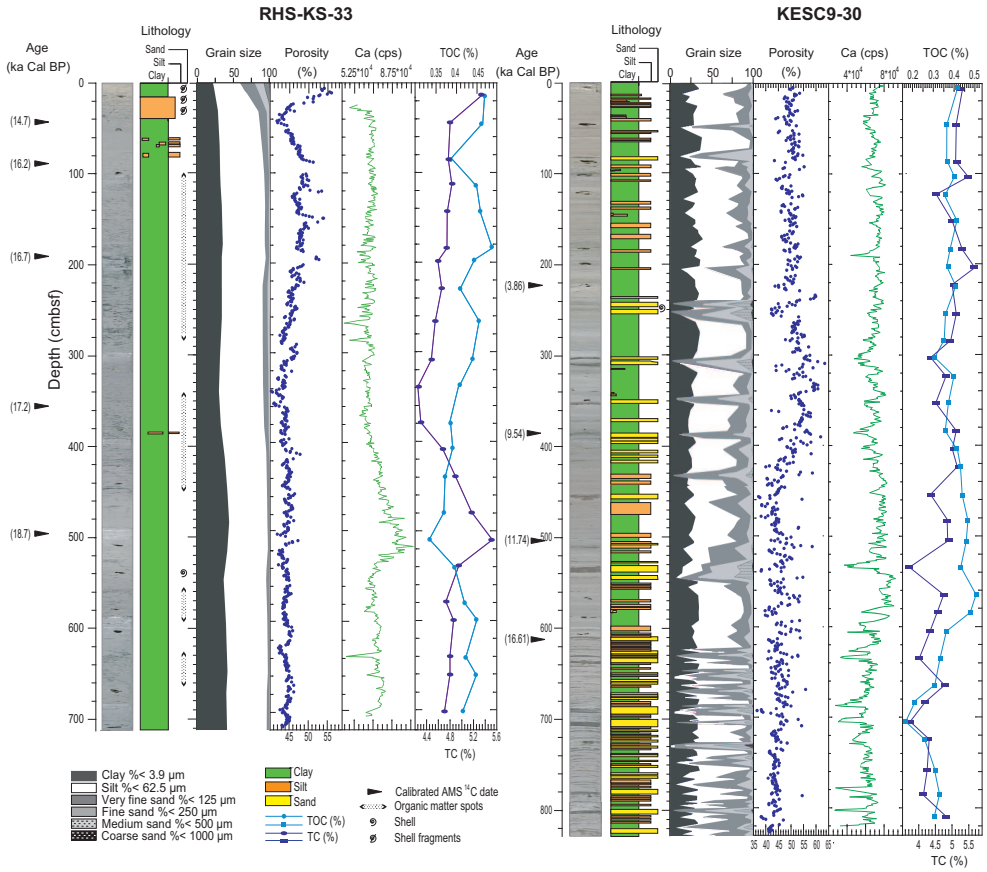

Fig. 3. Principal physicochemical characteristics of studied cores. (TC) total carbon, (clear blue), (TOC) total organic carbon (dark blue). Abbreviations: cmbsf, centimeters below the seafloor; cps, counts.

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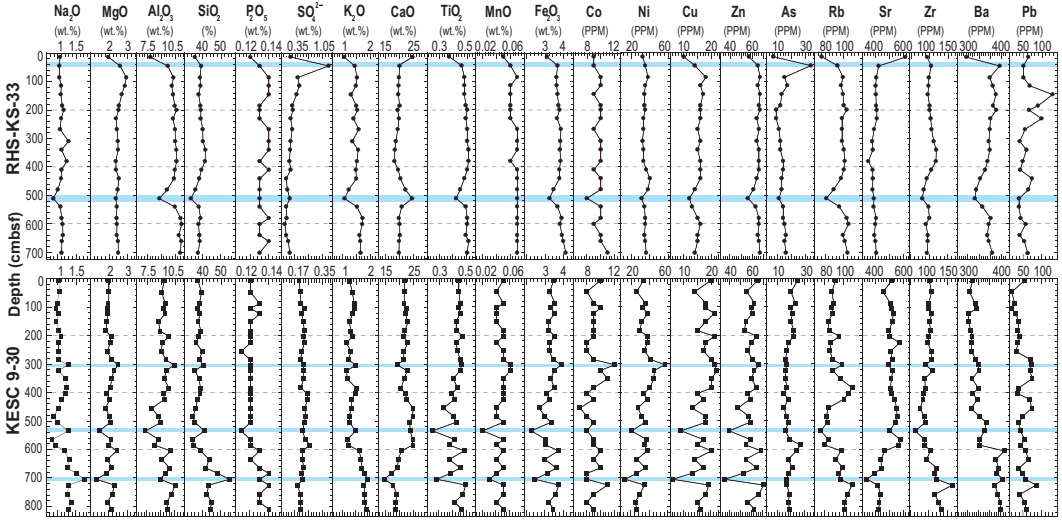

Fig. 4. Elemental composition of the sediment in cores RHS-KS-33 and KESC9-30. The black dots and the black squares represent contiguous samples of ~3 cm thick analyzed using WD-XRF. The blue blocks represent important changes in elemental composition profiles.

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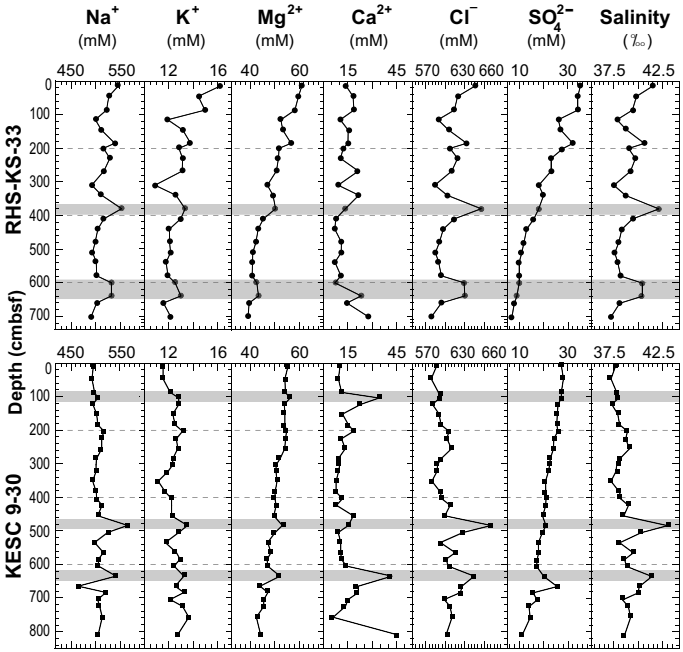

Fig. 5. Depth profiles of geochemical pore water measurements of studied cores. The grey blocks represent important changes of measured ions. Abbreviations: cmbsf, centimeters below the seafloor.

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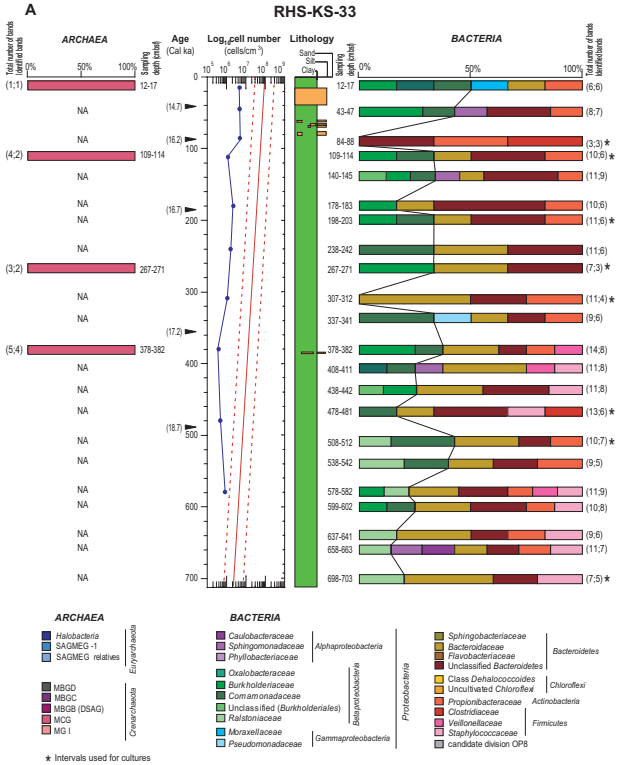

Fig. 6a. Depth distribution of the bacterial and archaeal community structures based on 16S rRNA gene, and total cell numbers compared with lithology, in the Gulf of Lions and the Ligurian Sea. The column diagrams represent the cumulative percentages of bacterial and archaeal phylotypes detected at two sites: **(A)** Gulf of Lions (RHS-KS-33); **(B)** Ligurian Sea (KESC9-30). The relative abundance of different families represents the sum of taxa detected after sequencing of DGGE bands. The phylogenetic affiliation of each band sequence was determined by similarity analysis. The total number of bands observed at each sampled interval and the total number of identified bands are given in parentheses, on the left of the bars. The solid red curve represents the general depth distribution of subsurface bacterial populations, with 95 % upper and lower prediction limits shown by dashed red curves, from Parkes and collaborators, 2000). Abbreviations: cmbsf, centimeters below the seafloor; NA, not amplified; SAGMEG-1, South African Gold Mine Euryarchaeotal Group 1; MBGD, Marine Benthic Group D; MBGB, Marine Benthic Group B; MCG, Miscellaneous Crenarchaeotic Group; MGI, Marine Group I.

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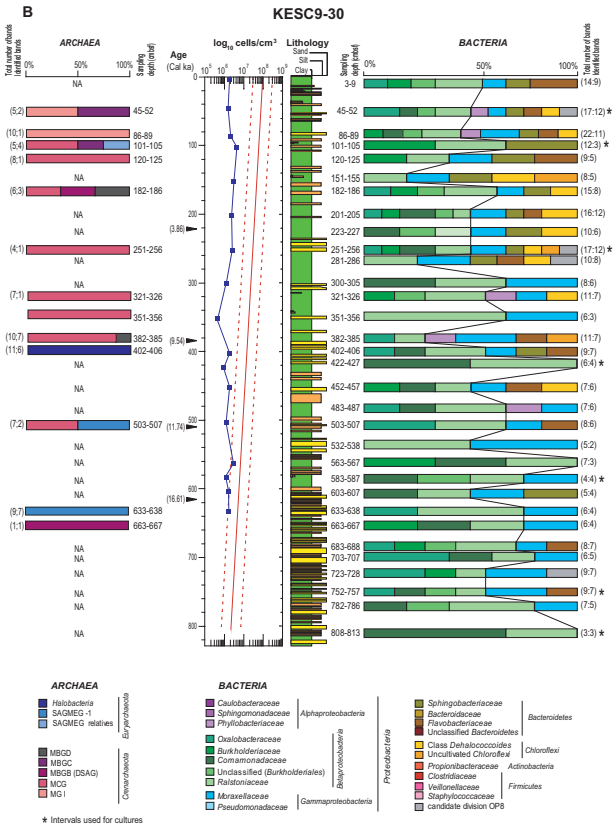

Fig. 6b.

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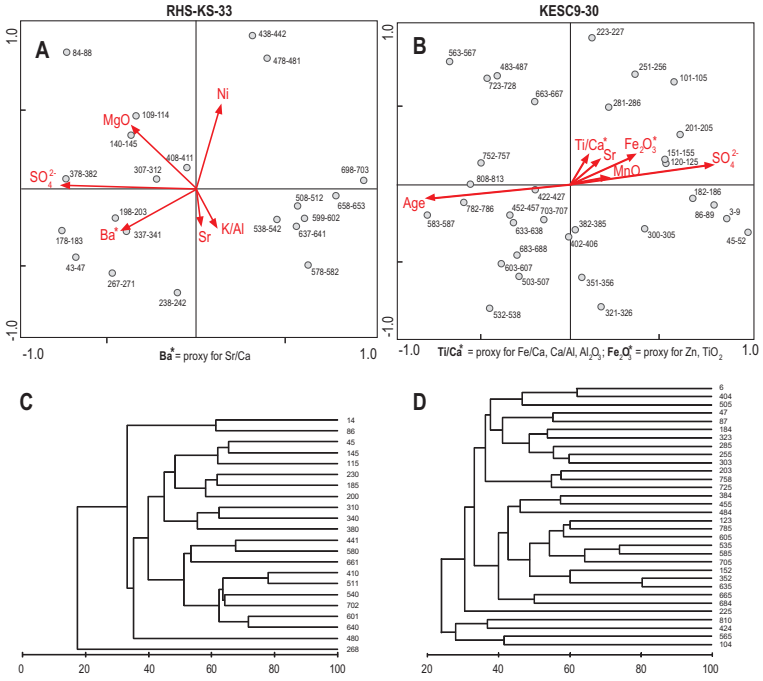

Fig. 7. Canonical correspondence analysis (DGGE bands intensity) and UPGMA dendrogram (presence/absence and intensity of DGGE bands) of bacterial community structure from core RHS-KS-33 and core KESC9-30. Red arrows point in the direction of increasing values of each variable. The length of the arrows indicates the degree of correlation with the represented axes. The position of samples relative to arrows is interpreted by projecting the points perpendicularly on the arrow and indicates the extent to which a sample bacterial community composition is influenced by the environmental parameter represented by that arrow. Samples from different depth intervals are indicated by light grey circles. Dendrograms indicate DGGE profile similarity (based on Bray Curtis distances) of different interval depths.

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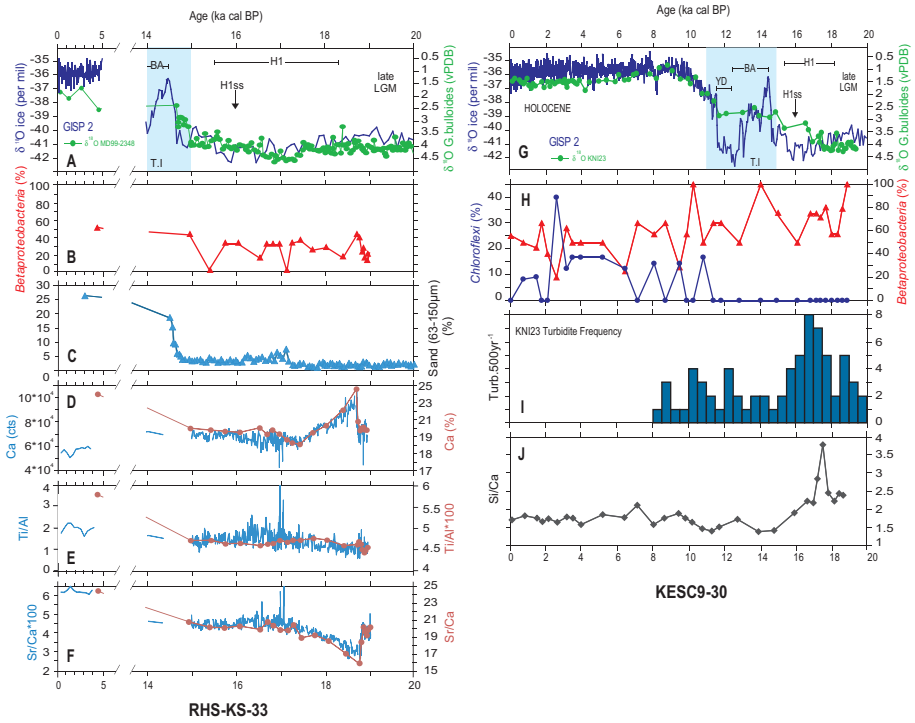

Fig. 8. Distribution of *Betaproteobacteria* during the Last Glacial Maximum and the Holocene in marine sediments from Western Mediterranean Sea (Ligurian Sea and Gulf of Lions). **(A, G)** $\delta^{18}O$ in GISP2, modified from (Jorry et al., 2011). **(B)** Distribution of *Betaproteobacteria* during the last 20 ka. **(C)** Distribution of sand (63–150 µm) during the last 20 ka. **(D)** Distribution of Ca concentrations during the last 20 ka. **(E)** Distribution of the ratio Ti/Al during the last 20 ka. **(F)** Distribution of the ratio Ca/Sr during the last 20 ka. **(H)** Distribution of *Betaproteobacteria* and *Chloroflexi* during the last 20 ka. **(I)** Turbidite frequency of overflow deposits observed on the Var Sedimentary Ridge, from 20 to 8 ka. **(J)** Distribution of the ratio Si/Ca during the last 20 ka. cts: counts; Abbreviations: late LGM, late Last Glacial Maximum (21 to 18.3 ka cal BP); H1,Heinrich 1 (15.5 to 18.3 ka cal BP, as defined in Bard et al. (2000)); H1ss, Heinrich 1 *stricto sensu* (centered at 16 ka cal BP, as defined in Heinrich (1988) and in Bard et al. (2000)); BA, Bølling-Allerød interval (between 14.5 and 12.5 ka cal BP; warm interval); YD, Younger Dryas (~12.5–11.5 ka cal BP; cold interval) (Bard et al., 2000; Hughen et al., 2000; Alley et al., 2003; Weaver et al., 2003). T.I, last climatic termination (blue block);

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