Dr. Karine Alain & Dr. Maria-Cristina Ciobanu
Laboratoire de Microbiologie des Environnements Extrêmes
UMR6197 CNRS-UBO-Ifremer
IUEM
Technopôle Brest Iroise
29280 Plouzané, FRANCE

Plouzané, April 2 nd, 2012

To: Dr. Tina Treude and the editorial office of Biogeosciences

Answer to Anonymous referee # 1

Manuscript : bg-2011-379, Paleoenvironmental imprint on subseafloor microbial communities in Western Mediterranean Sea Quaternary sediments

On behalf of the coauthors, we would like to thank the anonymous referee #1 for the interesting and constructive comments regarding the manuscript bg-2011-379. We think that we can clarify most points underlined in this review.

Our responses are reported point by point and changes in the text are located by the number of the corresponding page and lines into the discussion paper.

This manuscript reports interesting and relevant data on subsurface microbial diversity and its possible relation to geological parameters, it falls within the thematic scope of the Biogeosciences Journal, but requires to be restructured in a new revised version. Although interdisciplinary studies are very useful, this manuscript is an unusual amalgamation of data (geophysical data, geochemical and DGGE profiles) that tries to be held together by statistical analyses. I have scientific, methodological and presentation issues with this manuscript.

1/ The scientific question is confused and probably too ambitious compared to the data set. The aim was to determine if the paleoenvironement had any effect on the microbial community structure. This could be considered a little too simple, as the sedimentary history can influence microbial ecosystems either directly through biogeographical factors or indirectly through the modification of the environmental conditions (e.g. geochemical or physical conditions).

Even if the referee appreciates the contribution of this manuscript to the subsurface microbial diversity and its possible relationship to geological parameters, he/she seems to underappreciate the dataset presentation. In order to simplify the dataset presentation, some figures were split and/or moved to the supplementary online section (SppOS), as follows:
_Figure 1 (A,B,C,D) became Figure 1 (A,B); C,D seismic profiles were moved to SppOS.
_Figure 2 was moved to SppOS.
_Figure 3 was merged with sulfate and salinity profiles of Figure 5. Ca profile of Fig. 3 was removed. Remaining profiles of Fig. 5 were moved to SppOS.
_Figure 4 was moved to SppOS.
_Figures 6A and 6B became Figures 3A and 3B. In these novel figures, for more readability, bar charts/percentages were modified to include unsequenced bands, already presented in brackets.
_Figure 7 became Figure 4. Dendrograms were deleted.
_Figure 8 became Figure 5. In this figure, KNI23 turbidites frequencies were replaced with KESC9-30 turbidites frequencies. Relative abundances of identified *Chloroflexi* and *Betaproteobacteria* are now

expressed as a function of the total number of DGGE bands observed on gels (instead of the total number of identified bands).

As suggested by the referee, we propose to modify the manuscript as follows in order to focus our objectives on the presented dataset:
P258, lines 1-4, it is now written: "The main objective of this study was to explore the vertical variations in microbial diversity within Mediterranean Sea sediments with respect to lithological and some geochemical characteristics, reflecting themselves, in some extent, the paleoenvironmental conditions of sediment deposition".

As the microbiological and pure geochemical data set was limited, it was therefore very difficult to know if the microorganisms detected were adapted to their environment or simply deposited.

Knowing if the microbial communities are actives or not, and adapted to their sedimentary environment is an interesting question which we consider investigating in a future project. We needed firstly to explore subsurface microbial diversity and its potential relationships to sediment characteristics, which was exactly the main objective of our present study.

Each hypothesis was difficult to investigate as some of the data was missing and /or as 16S rRNA fingerprints based on extracted DNA do not discriminate living and dead cells or physiological characteristics. Although the geological and paleontological parameters were comprehensive, it was not possible to determine if the microbial community was deposited or metabolically active. This could have been compensated by the use of supplementary "geomicrobiological" data, such as more depth profiles (methane, hydrogen, ammonium, nitrate, sulfide), metabolic activities (sulfate reduction, methanogenesis, aerobic respiration), and at least the analysis of RNA, functional genes or using SIP (or even better by quantification methods using Q-PCR or FISH). A reference core outside any present or past terrigenous influence could also help to confirm the different hypothesis (as done in several similar studies).

As already written in the manuscript (page 258, lines 9 – 14), we lack microbial diversity data within marine sediments in western Mediterranean Sea. In this respect, the primary objective of this study was to provide initial information on the identity of dominant microbial groups in such environment and secondly to compare this diversity with some physicochemical parameters that inform on sedimentation context of studied areas during the last 20 ka. We think that DNA extractions and DGGE fingerprint method were suitable for these objectives since many other studies based on DNA extracts demonstrated a relationship with environmental or geochemical parameters (e.g. Parkes et al., 2005; Inagaki et al., 2006; Wilms et al., 2006; Nunoura et al., 2009) within subsurface sediments.
We agree that DNA do not discriminate between living or dead cells (as already written in the previous version of the manuscript: page 282, § 4.2.4 "Preservation of DNA or dead cells in marine sediments?") and does not give any indication about the activity of the detected cells. The use of PCR-DGGE method together with geochemical ratios (e., Ti/Al, Si/Ca; indicating sediment source), and with age measurements ($\delta^{18}O$; information on climate changes) allowed us to emphasize (within two different sedimentation contexts) a terrestrial origin of sediments to the fore in a marine environment strongly influenced by last deglaciation climate conditions, or by the sea-level variations.

With respect to referee's suggestion regarding the use of other geomicrobiological data, such as microbial activity measurements with radiotracers or depth profiles of chemical species, we agree that it would have been very interesting to include all these data in our study. However, as already mentioned in the methods section, studied cores were subsampled onshore. For this reason, these analyses could not be performed. Yet, gases analyses are done upon arrival of the core onboard and microbial activity measurements require specific storage. Furthermore, we also agree that (e.g. as

already written in our conclusions page 282, §5, line 24) it would be very interesting to complete the present findings with RNA data in a future work. In addition, it would also be interesting to perform nucleic acid extractions on extracted cells in order to discriminate between extracellular and intracellular DNA, but this will be an interesting approach for follow-up studies.

I think Beck and colleagues (2011), with a very similar objective, addressed the question using a more robust approach. I therefore suggest to either add some more "geomicrobiological" data using a quantitative method, e.g. Q-PCR (with specific Betaproteobacteria primers); or to restructure the manuscript as a simple description of the microbial diversity supported by geological data. The title should also be modified.

As a response to the referee's comment, please note that Beck and colleagues (2011) did not investigate the complex sedimentary context in terms of sources of sediment or of climate/sea level influence on microbial communities.
As suggested by the referee, we modified the title as follows: "Imprint of the sedimentological context on subseafloor microbial communities in Western Mediterranean Sea Quaternary sediments"

2/ My second concern is methodological and technical. The authors seem to ignore the biases and issues inherent to molecular techniques.

The biases and issues inherent to molecular techniques were previously discussed in several publications (e.g. Nubel et al., 1996; Rossello-Mora et al., 1999; Schäfer and Muyzer, 2001). In order to address this point, we added the following sentence to our manuscript:
Page 272, §4, line 25, it is now written: "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. This technique is commonly used in microbial ecology and is quite sensitive. However, this tool has some limitations: it is potentially affected by PCR-inherent biases, by the presence of multiple bands pattern for a single specie (Nubel et al., 1996) or by the occurrence of dissimilar sequences that may develop co-migrating behavior within a DGGE gel (Rossello-Mora et al., 1999). Even so, DGGE is a powerful tool for rapid monitoring of successive changes within microbial communities. In this study, bacterial DGGE profiles, statistical analyses with environmental parameters and excision and sequencing of DGGE bands allowed us to observe several changes in the microbial community structure and composition of subsurface sediments in Western Mediterranean sea, linked to changes in environmental conditions since the LGM as described hereafter."

2A/ Although DGGE is an excellent method to quickly and cost-effectively screen a large amount of samples, the data produced is very delicate to analyse as sensitivity is quite low (especially compared to metagenomics or clone libraries). This aspect is crucial, especially when using the presence or absence of a band to analyse the diversity. Additionally, the quantitative data, produced from the analysis of the intensity of DGGE bands obtained by nested PCR, is very uncertain at best. And 47% of the bands were unidentified ! It is therefore inaccurate to present the proportion of each phylotype and thus it should be presented differently. The quality of DGGE gels is quite poor as some lanes seem to be overloaded and inconsistent (what is that massive blank band across bacterial DGGE gels ?). It is not because the CCA analysis shows significant results that the data set used is robust. . . For these reasons, any quantitative analysis of the DGGE gels is probably not significant and should also be removed (CCA). I suggest that if the authors wish to analyse microbial diversity data using CCA, they should at least base their analysis on clone libraries or metagenomics data in order to have a proper estimation of the representability of the

diversity (as is done in most of the recent diversity papers using CCA using statistical parameters). Moreover, for all these reasons a rise from 50-60% to 70-75% in the Betaproteobacteria proportion is not significant and should be removed (Fig 8.).

Indeed, the DGGE method allows only an overview of the dominant microbial groups within a sample. For this reason, we used the term relative abundance (see legends of figures 3a and 3b) to describe the microbial diversity within this publication. We agree that the former version of figure 6 was somewhat misleading, while all information of interest was indicated on it. For a better visualization of the dataset, the bar charts/percentages of figure 6 were modified to include unsequenced bands (unsequenced bands appear now in light grey on the bar charts), and the legend figure was modified accordingly.

We think that the referee confused the dataset used for figures 6a and 6b (= Fig. 3a, 3b, in the revised version of the manuscript), and the dataset used for CCA analysis. CCA analyses were done only from bacterial nested PCR-DGGE fingerprints based on the intensity of all DGGE bands determined using ImageJ software. The presence of a weak band on a DGGE gel was overcome by taking into account the presence of bands with intensity superior to the background. Such treatment of fingerprint data is in accordance with the analysis of molecular fingerprints done by several authors (Schäfer and Muyzer, 2001; Fromin et al., 2002; Fry et al., 2006; Webster et al., 2007) in marine microbial ecology. We agree that this approach is biased. However, as all samples were treated in the same way, methodological drawbacks should not affect their comparison. For this reason, we use the terms “relative abundances” and “trends”. In addition, some studies demonstrated that the nested PCR-DGGE approach do not miss any major known group detected by a clone library of the same sample and that this technique represents a reasonably quantitative measure of the relative abundance of major taxa within an environmental sample (e.g. Boon et al., 2002; Dar et al., 2005).

Besides, the percentage of 47% of unidentified bands given by the referee is incorrect. In the manuscript, it is written that 63% of the bands were identified (*Archaea* + *Bacteria*), so 37% of the bands (*Archaea* + *Bacteria*) were unidentified. Among the *Bacteria*, 65.26% of the bands were identified for the core RHS-KS-33 and 68.47% of the bands were identified for the core KESC9-30. Besides, most of different band positions observed on bacterial DGGE gels were identified (see SppO figures S4 and S5).

The referee made another confusion concerning statistical analysis as CCA refers to the totality of the fingerprint. So, the method takes into account the intensity of all bands observed on DGGE gels and not the identified bands and the phylotype affiliations. Consequently, we think that the trends brought to light by CCA analysis are congruent with and reinforces trends observed by band sequencing approach. For all these reasons, we would like to keep CCA results.

The referee also suggested removing figure 8 (now Fig. 5). Within this figure, we propose an interpretation of our results. In this figure we first explain the environmental context that influenced the sediment deposition at each site. In the case of the Ligurian Sea the presence of turbidites was influenced by important climatic changes that occurred during the last 20ky (last glacial maximum and last deglaciation) and regarding to the Gulf of Lions site, the sediment deposition was mainly influenced by changes of sea level. Once each sedimentologial context explained by geochemical markers (Ti/Al, Sr/Ca, Si/Ca) we link microbial data (diagrams B and H) with the climatic context that affected the sedimentation type and rate during the last 20ka, in the western Mediterranean Sea. Since this figure represents the “key figure” of this study we would like to keep it. Nevertheless, this figure was modified by replacing KNI23 turbidites frequencies with KESC9-30 turbidites frequencies (newly calculated). In addition, relative abundances of identified *Chloroflexi* and *Betaproteobacteria* are now expressed as a function of the total number of DGGE bands (instead of the total number of

identified bands), for more readability and to be in accordance with the revised version of figure 6 (= novel Fig. 3).

2B/ The archaeal diversity is related to phylotypes found in marine sediments. If the bacterial diversity was controlled by climatic changes that occurred during the sediment history, why did it seem to affect only the bacterial community? A characterisation of the metabolic activities occurring (activity measurements, RNA based methods, functional genes. . ..) could help.

Indeed, this is a very good question that we already thought of. Unfortunately, it is difficult to answer since, in areas dominated by turbidites, we amplified *Archaea* from only three samples. In addition, several studies suggested that *Archaea* do not dominate over *Bacteria* in surficial sediments. Consequently, we cannot conclude.

2C/ Contamination is also a major issue when working on such low biomass environments. Nested PCR using bacterial primers is very sensitive to contamination, especially when working with low DNA concentrations as it is suggested by the use of nested PCR, and the very low cell counts. However, as no analysis of a blank DNA extraction was performed, I would strongly suggest to perform blank extractions and amplify them under exact conditions as described. This is mainly to confirm that the constant presence of some bacterial phylotypes (Betaproteobacteria) was not linked to contamination. Moreover, as there was no control of external contamination (e.g. seawater), the author should justify why they can rule out this possibility.

We agree that contamination is a major issue when working with low biomass samples. First of all, these samples are not extremely low biomass samples as we detected abundances between 3.7 10^5 and 5.3 10^6 cells cm^{-3} in our samples. As indicated in the manuscript (page 262, §2.6, lines 5 to 7), negative controls were included in all sets of PCR reactions to provide a contamination check, and the resulting negative controls were always negative.
Contaminations during DNA extraction were often showed to occur when using commercial DNA extraction kits. Here, DNA was extracted under a microbiological safety cabinet, using a new cryogenic method and with sterilized and filtered solutions. Blank DNA extractions were performed, and turned to be negative. In consequence, the following phrase “Blank DNA extractions were also performed on reagents (no sediment sample) with the same procedure.” was added in the “Materials & methods” section. This result strongly suggests that no contamination occurred during DNA extraction. In addition, external contamination during coring is unlikely to occur since the Küllenberg corer is a gravitational core that doesn’t need a continuous lubrication (mud + sea water) as in the case of drilling. Besides, subsampling was done in sterile conditions and sediment in contact with the core liner was removed. For these reasons, contamination during coring is uncertain to occur.

2D/ The 2g of sediment used for the DNA extractions seems to be a very limited amount for such a low biomass. Why didn’t the authors try to extract more sediment from the sample (e.g. several extractions pooled) to increase the DNA template and avoid having to use nested PCR ?

Several studies reported microbial diversity analyses from similar or inferior quantities of sediments (Parkes *et al*., 2007; Webster *et al*., 2010; Blazejak and Schippers, 2010; Beck *et al*., 2011), and several studies performed nested PCR (Fry et al., 2006; Roussel *et al.*, 2009a, 2009b; Webster *et al*., 2009). Sometimes nested PCR is used when PCR inhibitors (such as humic acids) are coextracted during nucleic acid extraction step, which might be the case here.

2E/ The very low cell counts, compared to the Parkes model in these terrigenous influenced sediments, is not sufficiently explained, especially when other sites with similar terrreginous TOC

show higher cell counts. I am therefore not convinced it is relevant to compare results that have been obtained with two different methods. Moreover the unusual absence of significant cell abundance decline with depth is not explained either.

Such biomasses were already observed in other surface sediments (see Nunoura et al., 2009, Gulf of Mexico). These authors suggested that the result is correlated to either the quality, quantity of organic matter or electron acceptors supplies. The significant cell abundance that decline with depth observed by Parkes and colleagues (2000) was significant for 0 to 1000 mbsf. Since our cores were only 8 meters long, a significant cell decline with depth is less observed.

2F/ I really appreciated that the authors used a culture based approach. However, it is always quite surprising to find that 95% of the isolated strains were cultured in heterotrophic aerobic conditions. Others studies also found a majority of facultative aerobs (e.g. Kopke 2005, Batzke 2007) but most of them were isolated under anaerobic conditions. The explanation of heterotrophs using refractory organic matter and suggesting that these microbial communities are adapted to their environment does not match the justification of constant low cell counts (suggesting deposition). Theses explanations should be harmonized. Could the authors also provide a comparison between the cultured and the molecular data, as studies using both approaches are seldom (this could be done in table 2). MPN analyses would be useful as they show quantitative results (e.g. Kopke 2005).

Our cultivation strategy based on the production of a strain collection (and not on MPN) is absolutely not quantitative, but purely qualitative. It demonstrates the presence of culturable aerobic heterotrophs, fermenters, nitrate-reducers and sulfate-reducers in the Gulf of Lions, as it is now written in the discussion section of the manuscript (page 274, §4.1.3, line 17), in addition to the results section (where it was already written). It is not surprising that 95% of the isolated strains were cultured in heterotrophic aerobic conditions, as strains with these metabolic capabilities are the more easily culturable ones and as many opportunistic prokaryotes (so-called “lab weeds”) are generally grown under these conditions.
The referee’s comment concerning the constant low cell counts *versus* culture results is quite confusing since one single culturable cell is sufficient to get a positive culture, independently of cell abundances in the natural sample (Alain and Querellou, 2009; Zengler, 2009).
As suggested by the referee, a comparison between molecular and cultural data is now provided in the manuscript (page 276, §4.1.3, line 14): “Molecular and cultural approaches gave the same results at the phylum level but showed numerous differences at the family level, as already observed in different locations”.

3/ My third concern is the data presentation (it looks like an IODP preliminary report). Data presentation is quite confusing leading to redundant and unnecessary data, as a consequence of the excessive number of figures (n=8) and Table (n=3). I suggest removing table 1 and splitting the relevant information between figure 1 and the method section. Try and simplify figure 1, I am not sure all the profiles are useful and the legend is confusing. As figure 2 is not necessary for the understanding of the manuscript, suppress it or move it to the supplementary data. Not all the data shown in figure 3 is explicitly used in the manuscript and is redundant with figure 6. Define what is really essential, try to merge figure 3 and 6. Some profiles in figure 4 and 5 are not used (Na+, K+, K2O ...), please remove the unnecessary and merge fig 4 and 5. The data in Table 3 can be included in the text. The “geological” data shown in figure 8 is interesting but as mentioned previously the percentages of Chloroflexi and Betaproteobacteria are probably not reliable and should be removed (also alter discussion section). Moreover, a bibliography listing 130 references (if counted right. . .) is unnecessary as this is not a review article.

As suggested by the reviewer, table 1 was moved to SppOS section and lengths and locations of the cores were inserted into the method section (page 258, §2.1, line 7). Please take note about new data presentation provided at the beginning of this document:
_The seismic profiles (C, D) of Figure 1 were moved to supplementary information section and the legend was simplified accordingly.
_Figure 2 is necessary to explain how the age model was established and represents one of the key figures of this manuscript. This figure permitted us to establish a correlation between the age and climatic events that occurred during the last 20ka (please see § 4.2.1 Quaternary Climatic changes in the Western Mediterranean Sea; (Jorry et al., 2011)). Nevertheless, as asked by the referee, Figure 2 was moved to supplementary information section.
_Please note that, for a better understanding of the dataset, it was the intention of the authors to present both geological and diversity data with depth, at a first glance, and then with age (last figure). This type of data presentation is frequently used in order to compare several data sets.

Furthermore, only the lithological sequences are common to both previous versions of figures 3 and 6 (now figures 2 and 3); previous figure 3 represents a description of the environmental context for each study site, while previous figure 6 shows microbial diversity data and microbial abundances as a function of lithology. For a better readability of the sedimentological data regarding to microbiological data, it is important to present the data in this way. We propose to move previous Figures 4 and 5 to SppOS. We do not agree to include data from Table 3 in the text as it will take even more space.

Regarding to the data presentation in previous Fig. 8 (now Fig. 5), as already mentioned, we would like to keep this figure. Our biggest concern is that the referee seems to completely ignore most of the points within the discussion section about important climatic changes (also presented in old figure 8: stratigraphy based on $\delta^{18}O$, turbidite frequency, geochemical proxies for terrestrial input: Ti/Al, Sr/Ca) that occurred during the last 20ka (last glacial maximum, deglaciation and Holocene) that strongly impacted the Western Mediterranean marine sediments accumulation.

Minor points:

_P255 L10-12: for the reasons mentioned above "dominated" should be replaced by indicating that there are consistently detected in the samples. This statement should also be altered elsewhere in the manuscript.
As suggested by the referee, "dominated" was replaced by "consistently detected in the samples".

P258 L24, P266 L16, P274 L26, P279 L13: remove "etc" and ". . ." (it is imprecise)
_P257 L20 : when and where were the cores collected (cruise, date, ship). I always find frustrating to have to look for method in the supplementary data. Please try and group everything in the methods section.

As suggested by the referee, the expressions "etc" and "…" were removed.
As asked by the referee, it is now written in the methods section when and where the cores have been collected ("Cores RHS-KS-33 (709 cm length; 42°41'.596N, 03°50'.493E) and KESC9-30 (828 cm length; 43°23'.016N, 07°44'.187E) …") and table 1 was moved to SppOS.

_P274-L20 : how was the temperature measured ?

The temperature of each core was measured at the bottom of the core upon arrival of the core on the ship (e.g. 15.4°C for the core KESC9-30). The water temperature was also measured to be of 13°C at 2160 m (water depth were the core KESC9-30 was taken). The *in situ* temperature was estimated from these two measurements to be between 13-14°C.

The text was modified as follows:
(page 258, §2.1, line 16)
"At both sites, the *in situ* temperature was estimated from the water temperature measurement (at 291 and 2160 m) and the temperature of each core (measured at the bottom of each core, upon arrival of the core on the ship). The estimated *in situ* temperature was of 13-14°C."

(page 274, §4.1.3, line 20)
"These results are consistent with *in situ* temperatures, estimated to be between 13-14°C"

_P278-L15 : Ralstonia along with other phylotypes are also regularly found in molecular genetic reagents (e.g. Webster 2006, p 70) !

Environmental sequences closely related to the genus *Ralstonia* were previously found in ocean crust (Mason et al., 2010) or ultramafic rocks (Brazelton et al., 2012). In these two publications, the authors performed negative controls for DNA extractions and PCR amplifications, and it is hard to believe that this group might be a "contaminant". Moreover, sequences belonging to *Betaproteobacteria* group were consistently detected within marine-originated shallow subsurface (e.g. Wang et al., 2008; § 4.2.2 Ligurian Sea) and the hypothesis that this group might be inoculated from adjacent environments was already suggested by the authors. The presence of the group Burkholderiales within marine environments might be explained by their capability to adapt to extreme conditions, since members or this group degrade hydrocarbons and are capable to oxidize H_2. For instance, within Western Mediterranean Sea sediments the activity of this group must be explored.

References

Alain, K., and Querellou, J.: Cultivating the uncultured: limits, advances and future challenges, Extremophiles, 13, 583-594, 10.1007/s00792-009-0261-3, 2009.

Beck, M., Riedel, T., Graue, J., Koster, J., Kowalski, N., Wu, C. S., Wegener, G., Lipsewers, Y., Freund, H., Bottcher, M. E., Brumsack, H. J., Cypionka, H., Rullkotter, J., and Engelen, B.: Imprint of past and present environmental conditions on microbiology and biogeochemistry of coastal Quaternary sediments, Biogeosciences, 8, 55-68, 10.5194/bg-8-55-2011, 2011.

Boon, N., De Windt, W., Verstraete, W., and Top, M. E.: Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specifc 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants, FEMS Microbiology Ecology, 39, 101-112, 2002.

Brazelton, W. J., Nelson, B., and Schrenk, M. O.: Metagenomic evidence for H_2 oxidation and H_2 production by serpentinite-hosted subsurface microbial communities, Frontiers in Microbiology, 2, 1-16, 10.3389/fmicb.2011.00268, 2012.

Dar, S. A. J. G., Kuenen, J. G., and Muyzer, G.: Nested PCR-denaturing gradient gel electrophoresis approach to determine the diversity of sulfate-reducing bacteria in complex microbial communities, Applied Environmental Microbiology, 71, 2325-2330, 2005.

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 Microbiol, 4, 634-643, 358 [pii], 2002.

Fry, J. C., Webster, G., Cragg, B. A., Weightman, A. J., and Parkes, R. J.: Analysis of DGGE profiles to explore the relationship between prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin, Fems Microbiology Ecology, 58, 86-98, 10.1111/j.1574-6941.2006.00144.x, 2006.

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, Proc Natl Acad Sci U S A, 103, 2815-2820, 0511033103 [pii]10.1073/pnas.0511033103, 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), Marine Geology, 279, 148-161, 10.1016/j.margeo.2010.10.021, 2011.

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, 10.1371/journal.pone.0015399, 2010.

Nubel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., Ludwig, W., and Backhaus, H.: Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis, Journal of Bacteriology, 178, 5636-5643, 1996.

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 Microbiology Ecology, 69, 410-424, 10.1111/j.1574-6941.2009.00718.x, 2009.

Parkes, R. J., Cragg, B. A., and Wellsbury, P.: Recent studies on bacterial populations and processes in subseafloor sediments: A review, Hydrogeology Journal, 8, 11-28, 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 sub-seafloor prokaryotes stimulated at interfaces over geological time, Nature, 436, 390-394, 10.1038/nature03796, 2005.

Rossello-Mora, R., Thamdrup, B., Schafer, H., Weller, R., and Amann, R.: The response of the microbial community of marine sediments to organic carbon input under anaerobic conditions, Systematic and Applied Microbiology, 22, 237-248, 1999.

Schäfer, H., and Muyzer, G.: Denaturing gradient gel electrophoresis in marine microbial ecology, Methods in Microbiology, 30, 425 – 468, 2001.

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, Environmental Microbiology, 10, 2537-2549, 10.1111/j.1462-2920.2008.01678.x, 2008.

Webster, G., Yarram, L., Freese, E., Koster, J., Sass, H., Parkes, R. J., and Weightman, A. J.: Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE, Fems Microbiology Ecology, 62, 78-89, 10.1111/j.1574-6941.2007.00372.x, 2007.

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, Environmental Microbiology, 8, 709-719, 10.1111/j.1462-2920.2005.00949.x, 2006.

Zengler, K.: Central Role of the Cell in Microbial Ecology, Microbiology and Molecular Biology Reviews, 73, 712-729, 10.1128/mmbr.00027-09, 2009.