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

Answer to Anonymous referee # 2

***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 #2 for the interesting and constructive comments regarding the manuscript bg-2011-379.

Our responses to referee's comments 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 paper reports on an interdisciplinary study of two sediment cores from the Western Mediterranean. Both cores exhibit a different sedimentation history due to different locations at the continental slope and different exposure to riverine input. The cores were analyzed and compared in terms of their sedimentological, chemical and microbiological composition. The authors try to correlate the physicochemical settings with the microbial diversity identified by both, molecular and cultivation-based analyses.***
***This kind of interdisciplinary approach is highly appreciated, usually shows synergistic effects and fits nicely to the scope of the journal.***
***However, such an approach leads to a high amount of data that have to be presented in a condensed way to guide the reader through the main findings of the manuscript.***
***For instance, I would concentrate on a few figures that indicate the correlation of geochemistry and microbiology. The age model in figure 2 is nice, but should be shifted to the supplementary section as the age is sufficiently indicated in figures 3 and 6. The same is true for most of the data of figures 4 and 5. The microbiologically important values of sulfate and salinity can be integrated in figure 3 while the calcium profile can be removed from this figure.***

As suggested by the referee, 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.
_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 (instead of the total number of identified bands).

***On the other hand, some of the data that were shifted to the supplementary section can easily be integrated in the main text. The methods for example are described twice. It would be better to combine the description of methods in the supplementary material and in the methods section in a condensed way. If standard techniques were used that were published elsewhere, please just cite them.***

As suggested by the referee, methods sections in the manuscript and methods section in SppOS were merged.

***My major methodological concern is the application of a nested PCR ahead of the DGGE analysis. Normally, there is no need for this. There are protocols for DNA extraction available to gain enough nucleic acids for a single-step PCR, even from much deeper subsurface samples. A nested PCR can dramatically change the DGGE profiles. A slightly preferential amplification might explain the high amount of Betaproteobacteria in the molecular analysis in comparison to the cultivation study. I can not understand why the DNA of Betaproteobacteria should be preserved in the "paleome" better than the DNA of other microorganisms.***

The use of a nested PCR in this study was necessary since single-step PCR amplification did not produced enough DNA for DGGE analysis and bands were generally weak without nested PCR (maybe because PCR inhibitors were present). Nested PCR prior to DGGE were previously used in the literature (Boon et al., 2002; Dar et al., 2005). The authors of this two publications suggested that the second PCR round did not changed neither the number nor the intensities of the DGGE bands, compared to a one-step PCR protocol with same primers.
The hypothesis of a preferential amplification of the group *Betaproteobacteria* is unlikely to occur since all samples were treated in same conditions (e.g. same mix for PCR amplifications; DGGE performed at the same time on two gels) and different betaproteobacterial relative abundances were observed within samples. An increase of the relative abundance of this group coincided with lithological sequences of terrestrial orgin (or characterized by a terrestrial input).
Moreover, *Betaproteobacteria* were already found to dominate marine-originated shallow subsurface (see Wang et al., 2008) or ocean crust (Mason et al., 2010) and ultramafic rocks (Brazelton et al., 2012). In all these publications, the authors explained their presence by two different hypotheses: (i) their capability to survive and to potentially degrade complex substrates, or (ii) their capability to maintain in a "DNA Paleome". The presence of a "DNA paleome" preserving betaproteobacterial DNA must be explored by performing total DNA extractions from environmental samples and also by performing DNA extractions on extracted cells from sediment matrices (as done for the cell counts; see Kallmeyer et al., 2008), and by comparing the diversity in samples subjected to both treatments, in order to confirm or to infirm this hypothesis.
For the moment, within Western Mediterranean Sea sediments we don't know if this group is active or dead, but this experiment might be considered for further studies.
In addition, we never said that the DNA of *Betaproteobacteria* was preferentially preserved in the "paleome": we are discussing the presence of this group because it is a common terrestrial lineage while many other lineages are ubiquitous and their presence is much more difficult to interpret.

***Also, a quantitative analysis based on a nested PCR is highly questionable.***

We agree with the referee's comment that PCR-DGGE approach is biased, like other PCR-based molecular techniques. However, while 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" and we do not perform a "true" quantitative comparison. Also, some studies (e.g. Boon et al., 2002; Dar et al., 2005; Roussel et al., 2009) 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.

***In the canonical correspondence analysis, the authors have identified environmental parameters that influence the microbial community structures. The availability of electron acceptors (sulfate and metal oxides) and the quality of electron donors (indicated by age?) strongly influence the community structure. However, other trace elements are probably proxies for the origin of the sedimentary material (fluvial, marine) and should only be interpreted that way. Please make this point more clear.***

As suggested by the referee, the paragraph 4.1.4 CCA analysis (page 275, lines 15-30, page276, lines 1-10) was modified as follows in order to make it clearer:

"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 were likely influenced by the porewater sulfate concentration and lithology (presence of sand in the upper 20 cm only), so obviously by the availability 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) that might be here influenced by the origin of the sedimentary material (fluvial, marine). 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 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 of CCA and of lithological analyses *versus* microbial abundances/ diversity show that microbial communities from the Mediterranean Sea sediments were stratified, and were likely to be affected by the availability of electron acceptors (sulfate and metal oxides), the quality of electron donors (indicated by age) and the lithology (e.g. grainsize, mineral composition, sedimentation rate), as previously observed in marine sediments on the Pacific Ocean Margin (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."

***Other comments:***

***Abstract: The statement, that the two cores can be calibrated with previously analyzed reference cores is at a relatively prominent position. However, except for the age determination, this is not really done in the paper.***

In our opinion, this statement is highly important since a calibration of our cores with previously analyzed reference cores allowed us for example, to use acquired data ($\delta^{18}O$, interpretations of climatic events during the last 20ka) on climatic events registered in sediment sequences within the

Ligurian Sea and to discuss this point. In order to consent referee's comment, the abstract was modified as follows: "Since both environments in this area were well-documented from paleoclimatic and paleooceanographic point of views, large data-sets were available and allowed to calibrate the investigated cores with reference and dated cores previously collected in the same area, and notably correlated to Quaternary climate variations."

***Introduction: What do you mean by "microbial effectives"? Please explain.***

"Microbial effectives" refer to total cell counts within marine sediments. The expression "microbial effectives" was replaced by "microbial abundances" (introduction, page 4, line 5).

***Methods section: Chapters 2.9.1 and 2.9.2: It is rather unusual to use bullet points here. Please describe the media composition in a "normal"text.***

Media compositions are now described in a "normal" text.

***Results section: Both cores have a rather complicated name and it would help to follow the results if the authors would refer the names to their origin a few times in the results section (e.g. "The Gulf of Lion core RHS-KS-33" and "The Ligurian Sea core KESC9-30").***

As suggested by the referee, the expressions "The Gulf of Lion core RHS-KS-33" and "The Ligurian Sea core KESC9-30" were inserted a few times in the results section.

***References: Too many. If you cite other publications, please focus on the most important ones and avoid long listings.***

As suggested by the referee, references list was reduced.

***Figures:***
***Figure 6: Please explain the line in the right part of the diagrams.***

The black line in the right part of the diagrams separates the *Betaproteobacterial* sequences from other bacterial sequences. Its meaning is now written in the figure legend.

***Figure 7: Showing a cluster analysis of DGGE profiles as a dendrogram only makes sense if defined clusters are visible.***

In order to concur referee's comment dendrograms were deleted.

***Figure 8. This figure can be removed. The quantification of Betaproteobacteria and the Chloroflexi can be integrated into Figure 6.***

We would like to keep this figure that we consider as a "key figure". Indeed, within this figure, we link the observed trend (*Betaproteobacterial* relative abundances correlating with lithology changes) with the climatic context that affected the sedimentation type and rate during the last 20ka, in the Western Mediterranean Sea. *Betaproteobacteria* and *Chloroflexi* curves cannot be introduced to figure 6 (Fig. 3, in the revised version) since figure 6 shows microbial diversity at each depth and within figure 8 the microbial diversity is plotted against sediment age. This curve allowed us to compare the presence of *Betaproteobacteria* and *Chloroflexi* to a specific climatic event (last glacial maximum, last deglaciation, Holocene) described in figure 8 A, G (Fig. 5, in the revised version).

***Supplementary material: I am not sure if this material will be formatted by the production editor. If not, please number the figures and write the captions directly under the figures.***

As suggested by the referee, supplementary material was restructured. In the revised version, all supplementary figures are now numbered and legends are written under each figure.

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