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Biogeosciences Discussions

Interactive comment on "Paleoenvironmental imprint on subseafloor microbial communities in Western Mediterranean Sea Quaternary sediments" *by* M.-C. Ciobanu et al.

Anonymous Referee #1

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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 micro-

bial 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). 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. 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). 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.

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

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

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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.).

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.

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.

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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 ?

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.

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).

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

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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.

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.

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.

_P274-L20 : how was the temperature measured ?

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

References

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