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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 #3

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This manuscript is a very interesting study on an attempt to relate paleoenvironmental changes to prokaryotic diversity in Mediterranean sediments. The paper is generally well written and the ideas and most of the methodological work are well done. However, I do have concerns about the interpretation of the PCR-DGGE data and discussions based on this part of the study. Therefore I feel that this paper warrants publication in this journal but only after some important revision and careful consideration of the points raised below.

Cell numbers in these sediments are very low, most probably due to the low TOC values within these sediments. However, because of these very low cell numbers (i.e. lower

than predicted numbers based on other subsurface sediments) the authors should have taken great care over possible DNA contamination issues. These problems have been of great concern in assessing prokaryotic diversity in subsurface sediments. This is especially the case in determining bacterial diversity which is at a much greater risk from microbiological laboratory contamination.

Therefore, the authors should have included several blank DNA extractions in their studies (i.e. no sediment sample) to determine what, if any, the background contamination was from their extraction reagents/kits. Such problems become a further concern when nested PCR is used to assess microbial diversity, as was the case in this study. The lack of negative control DNA extractions in this study is especially concerning, as from my experience and from work by others (e.g. Tanner et al (1998) AEM 64:3110; Barton et al (2006) JMM 66:21; Webster et al (2006) FEMSEC 58:65), many of the identified bacterial types that dominate in these Mediterranean sediments are similar to those found as contaminants in DNA extractions due to lab reagents. The presence of low levels of exogenous DNA in extraction reagents also could be a reason why the authors were unable to culture Betaproteobacteria from the sediments. To ensure any further doubt over these results and to preclude publication in this journal it is essential the authors include some data on their blank DNA extractions and address this issue.

In addition, I would have also expected the authors to have undertaken DNA extractions on a larger sample size or have pooled replicate DNA extractions due to the low biomass in these samples in order to achieve higher DNA yields to prevent further problems associated with the PCR amplification of low DNA concentrations.

Further points regarding the PCR-DGGE work include, the gels shown in the suppl. data are generally quite poor in their resolution - with a lot of diffuse bands which I assume would make statistical analysis of them slightly challenging. Also the DGGE data shown in Figure 6 is somewhat misleading due to the authors expressing the data as percentages of each phylo group. This is incorrect as the percentages shown are only based on bands that have been excised and sequenced with a positive ID and

therefore do not include the large number of unsequenced bands. These unsequenced bands should be included within the bar charts/percentages as unsequenced or other bands to help the reader understand fully the data set.

e.g. 611 bands were observed by DGGE, but only 63% were identified. Also of interest and may be should be included is how many band positions does the 611 individual bands relate to, and how many of these band positions were sequenced for each site?

I also cannot help feeling that the authors have presented a lot of unnecessary contextual data as supplementary data that is not really relevant to some of the questions they want to address.

Minor Comments

P255 L8 change to: DNA-based fingerprints showed. . .

P255 L13 change to: ..diversity was as a result of the important. . ..

P256 L14 change to: In some sediments, the. . ...

P263 L5-25 what was the gas phase in anaerobic cultures for fermenters, SRBs with VFAs?

P266 PL13-14 the authors state that 14 depths were amplified by Archaea 16S rRNA gene PCR but Fig 6 clearly shows data from only 13 depths?

Interactive comment on Biogeosciences Discuss., 9, 253, 2012.