The authors investigate here a challenging issue about (i) the meaning of bacterial DNA signature found in deep sediments (DNA which might reflect both ancient and extant microbial populations), and (ii) the environmental processes (climatic conditions & lacustrine regimes, post depositional alterations) that shape sedimentary DNA.
The global strategy is based on the comparison of the genetic/taxonomic signatures of bacterial DNA in two well defined climatic intervals : an unproductive glacial environment (~25 000 years ago) and a productive one in the Holocene (~ 5000 years ago), these two sediment layers harboured active microbial populations as revealed by ATP measurements.
I agree that this approach allows to compare variations in DNA over the last 25 000 years in response to both past environmental conditions and geochemical evolution of the sediments; however it does not allow to conclude clearly about the post-depositional diagenetic processes. I have the feeling that the strategy applied in the study is not consistent with some of the goals (L15-19 p 18349) more particularly those concerning the effect of diagenesis.
I had difficulties to identify which data supports the conclusion about the effect of diagenesis processes. The authors have no possibility to know what the initial assemblages were (bacterial DNA at the moment of deposit) consequently most of the conclusions regarding the role of post depositional alterations are impossible to verify.
While some results and a large part of the discussion (part 4.2 from L 23p 18361, part 4.3, part 4.4 L 12-28) are of great interest, I would advise to moderate the conclusion regarding diagenesis processes.

I have several major comments regarding the DNA analysis (some of the problems listed hereafter can be fixed in the revised MS) :

- If the goal was to discriminate the active bacteria/archaea from the ancient bacterial DNA, the methodology could have been adapted to reach this goal. The sampling strategy allowed to work both on DNA and RNA (transcripts) in order to better discriminate active and inactive taxa (even if some limits exists also on the RNA approach, the transcripts are mainly associated to active taxa). It was a solution to consolidate the observations.
- The sequencing depth is very low (with only 11 to 43 DNA sequences per sediment layer). This low sequencing depth has to be taken into account in the interpretation of results, only the dominant taxa have been detected, these inventories are probably far from revealing the real diversity (especially for bacteria: we can see a higher number of DGGE bands than OTUs number for bacteria, this confirms the incompleteness of the sequencing inventory). Consequently it is hazardous to compute shanon index from this very partial diversity inventories. (P 18353).
- Somme points have to be clarified earlier in the MS (key information appears in the discussion p18364 L24) :

  The type of DNA extraction and consequently the type of DNA which was targeted: intracellular DNA, extracellular DNA orboh probably?

  The length of DNA fragments that were PCR amplified before cloning ? (long fragments should come mainly from intact inactive cells and active taxa, while short fragments should be represented mainly by ancient DNA).

The mat & met section has to be very precise regarding all the DNA analyses, even if previous publications exist (published DGGE results).

Specific comments:

L 6 – 9 p 18349 "Finally, the long-term persistence and activity of microbes in sediments following burial can further modify geochemical conditions via diagenesis, leading to selective preservation and modification of bacterial assemblages. »

I have the feeling that this sentence has to be clarified:
Saying 'modification of bacterial assemblages': which bacterial assemblage do you refer to ? the active bacteria or the global bacterial signal including both the DNA from past bacterial communities & active bacteria

P 18350 " Finally, we established archaeal clone libraries at regular intervals throughout the microbially-active sediments of the Holocene period to provide structural criteria of dfferentiation between extant and ancient populations."

The sequenced DNA can come either from active or past inactive archae ; I don't think the study provides criteria of differentiation between extant and ancient populations with the applied strategy and methods. I suggest the modification of this sentence.

P 18351 L 4 " We supplement these insights with a new 16S rRNA gene analysis of the total sedimentary DNA extracted from the whole Holocene record and one deep ancient LGM …"

Please provide detailed information about the method of extraction (total sed DNA ; it means both active and inactive , both intra and extracellular DNA).

P 18352 L 22 : "Shifts in productivity associated with lacustrine conditions were estimated from the ratio of total eukaryotic pigments to total prokaryotic pigments."

The diatoms pigments do not represent the whole amount of eukaryotic pigments (eukaryotes are represented not only by diatoms, I suppose some chlorophytes, cryptophytes, dinophytes, etc. were also present in the eukaryotic assemblage) consequently I would advise not to use 'eukaryotic when only diatoms are quantified ; please change 'eukaryotic pigments' by 'diatoms pigments' in the MS (L 10 p 18856 for instance)

P 18352 L 25 : "Detailed procedures for DNA extraction, PCR amplification and DGGE are described elsewhere "

The authors have at least to mention which type of DNA extraction they used ('total DNA' with classical commercial kits , extracting both intracellular DNA and a large part of extracellular DNA ?).
The targeted DNA region and especially their length have to be clearly mentioned (for bacteria and archae): this is of high importance: short DNA fragments might include more sequences associated to inactive taxa represented by extracellular DNA (fragmented DNA) while working on long fragment (> 500bp) should allow to detect less of these ancient taxa and proportionally more active taxa or bacterial DNA preserved in intact cells.

P 18353 " 83 bacterial clones and 228 archaeal clones were respectively selected from samples at 4.97 and 29.77m sediment depth and samples at 0.25, 0.55, 1.90, 2.51, 4.97, 7.81, 9.37 and 29.77m sediment depth

Please indicate clearly how many clones per sediment layer were analyzed; it is not really clear to me if 83 clones were selected for each depths (4.97 and 29. 77) or if 83 is the total for the two depths (the information is in the Fig 6 but it could mentioned here also). Same for archaeal clones

P 18358 L 2 "Together ATP, DAPI and DGGE profiles (Fig. 3e and f) provided evidence for viable microbial assemblages in the Holocene and LGM sediments."

I would suggest to remove this sentence. As the authors mentioned after, DGGE results provide no evidence for viable microbial assemblage, DGGE results provide only evidence for the presence of amplifiable DNA sequences. Moreover DAPI is not appropriate to discriminate dead or active cells.
Only ATP measurements provide evidence for the presence of active taxa (prokaryotes ?).

P 18258 L12 "In addition, we established six additional archaeal clone libraries established throughout the Holocene record to provide criteria of discrimination between ancient and recent 16S rRNA sequences"

not clear to me : What do you mean by ancient and recent sequences ? do you referrer to the dating of the sediment or, to the discrimination between active taxa in upper deposits vs inactive in deeper deposits. I don't think these data allow to discriminate active and inactive taxa.

Part 3.2.3 & 3.2.4 :

The comments regarding the presence / absence of bacterial and archeal groups in the different sediment layers have to be reported and discussed with cautions: the sequencing effort is insufficient to conclude on the microbial composition of sedDNA.

Discussion

p.18361 Part 4.2 preservation and interpretation of fossil DNA

This section could be shortened, avoiding repetitions of results and references to figures. I suggest to reduce this section

p.18364 L 24 – 28 "Finally, 16S rRNA gene sequences of *Archaea* (900 bp) and *Bacteria* (1400 bp) analyzed

This information is extremely important and have to be stated earlier in the MS (Mat et Met)

p.18365 DGGE band (150bp) …..

I am not sure to understand (I confess that I have not read the biblio reference to get the information): What size were the PCR products used to perform the DGGE ? not 150bp ?

P18366

I would suggest to moderate the discussion /conclusion in view of the low sequencing depth applied in this study

P18367

Since the strategy applied in this study is not really relevant to conclude about the magnitude of post depositinal alterations, I suggest to moderate the conclusion/discussion on this specific point (in view of the difficulty to disentangle the relative importance of post depositional alterations).

P18368 L21-22

This is indeed a very strong limitations to my point of view