

Interactive comment on “Mats of psychrophilic thiotrophic bacteria associated with cold seeps of the Barents Sea” by S. Grünke et al.

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The authors presented a comprehensive analysis of bacterial communities of deep sea cold seeps and set thereby the basis for further studies. Even in those days we still need those describing studies. However, I highly appreciate that the authors are starting to explain the situation using the environmental conditions and comparison to other habitats with related properties.

However, doing deep sea research, I miss some data on the depth of the used study areas.

Reply: We appreciate the helpful comments made by this reviewer to improve this manuscript. The depth and location of each habitat is given in Supplementary Table 1.

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In addition, some explanation of the relevance of those chosen sampling sites for comparison reasons to other cold seeps would be helpful not readers being less familiar to cold seep research.

Reply: The investigated types of cold seeps represent three different kinds of geological seep structures, i.e. mud domes above gas chimneys, pockmarks and mud volcanoes. Our reasoning of sampling was to compare thiotrophic mats from active seeps lying in very cold (polar) deep-sea waters to those of temperate or warm deep sea areas. We have clarified this in line 60 and added another explanation in line 92 f.

In addition, I would highly appreciate some discussion of the limitation of the analyses due to experimental bias given by the used methods (ie. pressure changes during samplings, “community changes” due to sample treatment).

Reply: Sediment samples used for bacterial community analyses were recovered with conventional multiple corers and push cores. An advantage of studying polar systems is that the cold temperatures remain the same for the entire water column, different from e.g. tropical deep-sea sampling, where cores warm up during retrieval. We had no tools for retrieval of pressurized cores, however, we could still detect viable filaments of e.g. *Beggiatoa* after recovery (Table 3, lines 419f.), as well as living and active protists (data not shown). We now added an additional observation to discuss this (line 122f.) One potential artefact was disturbance caused by degassing of cores. We achieved very good and reproducible results with letting gas-disturbed cores sit for 1-2 days close to in situ temperature to allow geochemical gradients and bacterial mats to re-establish (line 112f.). Again, based on the composition and morphological appearance of the mats as well as the sulphide profiles (deBeer et al., 2006), we found the same types as before. Hence, we believe that core retrieval and pressure changes did not noticeably alter the communities for our DNA-based analyses and also did not significantly influence our observations on the sensitivity of the mat-forming thiotrophs towards temperature changes. Potential biases introduced into our analyses may result from the PCR method itself and, regarding the identification of the

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large mat-forming thiotrophs like *Beggiatoa* and *Thiomargarita*, from the use of universal bacterial primers. Difficulties in DNA extraction and amplification of their 16S rRNA genes, though, have been previously described (Schulz, 2006; Girnth et al., 2011) and are still an issue in present analyses. With the help of the comprehensive study by Salman et al. (2011) very recently published, it may now be possible to construct specific primers for the different giant mat-forming thiotrophs of the family *Beggiatoaceae*, helping to improve the identification of these bacteria. Regarding this study, though, this information was not yet available at its start and conventional bacterial primers were found to be most convenient for the presented community analyses.

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