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Interactive comment on "Functional diversity of microbial communities in pristine aquifers inferred by PLFA – and sequencing – based approaches" *by* Valerie F. Schwab et al.

Anonymous Referee #2

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The authors have carried out an extensive range of chemical and biochemical analyses of pristine aquifer ground water collected in Germany in order to determine microbial diversity. Techniques such as FAME-GC-MS, isotope ratio MS and sequencing showed that the range of microorganisms depended on the groundwater chemistry. This is an interesting study combining both PLFA and PCR data to improve phylogenetic resolution. The follow comments should be addressed before final publication. Line 59 missing 2- on SO4 Line 64: please re-word try replacing "how exactly" with "it is not well understood how" Section 3.3: please describe how external contamination was avoided in the PLFA extractions and analysis. Particularly the 16:0, 16:1, 18:0,18:1 which are almost ubiquitous contaminants. This is particularly important as 16:0 was

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Discussion paper

shown to be significant in the PLFA distribution analysis. See: Yao, C.-H.; Liu, G.-Y.; Yang, K.; Gross, R.W.; Patti, G.J. Inaccurate quantitation of palmitate in metabolomics and isotope tracer studies due to plastics. Metabolomics 2016, 12 Did the authors check the specificity of the fractions with the SPE method used? Some approaches can see cross contamination with the GL and PL fractions. This could be easily check with standards of GL and PL. Line 184 Is there a reference for the SPE method used? Line 188 define PLOHs ml should be mL throughout Fatty acid quantitation: was there a standard for all fatty acids quantified. I see that a 19:0 fatty acid was used as an internal standard and the Thermo FAME mix as an external standard. Did this contain each FA of that was quantified? If not it is not possible to “quantify” the absolute concentrations of the 47 fatty acids. If there was a standard please state this as it is a key issue for fatty acid quantification. Each FA will have a different response factor. If there wasn’t then the mol% cannot be calculated. Peak areas relative to the internal standard could be used for the PCA however.

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-442, 2016.