

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 #1

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The MS focuses on analysing the diversity in pristine aquifers via a combination of phospholipid derived fatty acids and DNA and RNA based Illumina amplicon sequencing. The author collected sample in eight different wells which all expressed different mineral, nutrient and redox levels. They showed that the diversity of the wells depend on the biogeochemical composition in those wells. The main issue I have with the research contained in this MS is the following: While the separation of different intact polar lipid classes using a silica column and chloroform (or dichloromethane), acetone and methanol is a commonly used technique since its development in the 50/60s it has been recently shown that the separation is incomplete. Heinzelmann et al. 2014 showed that a considerable portion of the glycolipids end up in the methanol (or so

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called phospholipid) fraction and a portion of the phospholipids ends up in the acetone aka glycolipid fraction. Assuming that phospholipids are the main lipids in cell membranes and are considered to reflect living biomass and glycolipids are mainly storage lipids with a longer lifetime after cell death in comparison to phospholipids, this incomplete separation will introduce biases into the data. Therefore, the PLFA fraction described in this MS will not reflect purely membrane lipids and therefore living biomass. Additionally, the fate of SQDGs is completely ignored. SQDGs have been shown to be part of the cell membrane under phosphate limiting conditions. Also, first results indicate that glycolipids play a role as membrane lipids in membranes in phototrophic microorganisms and therefore cannot be considered to be purely storage lipids. This bias has to be addressed in the MS before acceptance. Additionally, especially for someone who isn't too familiar with PCA plots this part of the results/discussions tends to be a bit confusing at times. For they make up a rather big part of the whole paper, please try to make it more clear. Minor comments L18-46: The abstract seems to be quite long, shorten it if possible L68: Not all living organisms have cell membranes consisting of phospholipid derived fatty acids. Cell membranes of archaea consists of etherbound isoprenoid chains and not phospholipid derived fatty acids. Additionally, Van Mooy et al. (2006+2009) showed that under phosphate limited conditions marine microorganisms produce sulphur containing SQDGs instead of phospholipids as membrane lipids. L74-90: This information is quite detailed and could as well be in a table L116: Include the description of the sampling sight into the Material and Methods section L179: In general, the extract obtained during Bligh-Dyer extraction is called Bligh-Dyer-extract or BDE Figure 1: Make it more clear which of the wells are HTU and HTL. I found it a bit confusing. Also make it a bit more clear in the Figure that you didn't sample from H1 and H2 (you never mentioned them in the text but in the figure they suddenly show up). Additionally, make it more clear which well is H3.1 or H3.2 etc.

Technical comments L59: Add an – to SO₄ L76: prefixes of fatty acids like iso (i) and anteiso (a or ai) should be in italic L143 and following: Either L or l, please be consequent throughout the text L154-155: Either litre or liter, be consequent throughout

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the text L170: I guess you mean CO₂ not CO₂- L171: Leave a space between mmol and L-1, in general be consequent about having a space between units or not L219: delete the – L280: insert space after - L289: insert space before ± L303: insert space before ± L312: the t in St should subscript L364-367: insert space after ± L402: There is an + missing for NH₄⁺ L403: It is either sulfate or sulphate. Please be consequent throughout the text L413: A dot is missing after the) L417: Concerning different sulphur species, please stick either to American (f) or British English (ph) L421: Remove the % after 1.1 L439: Subscript the t after Fe L507: Remove the C in 10MeC₁₂:0, check the whole text (be consequent about FA nomenclature throughout the text if you want to add the C or not) L508: Fe²⁺ not Fe₂⁺ L566: Remove the space before ‰ (check the whole text) L615-933: Reference list is not according to Journal style. Please change that. Also add doi when possible. Number of issues are missing. Also journal names should be abbreviated. L632: space missing after the : L659-660: I assume it is the same author. Please be consequent with the names L665: The 2 has to be in subscript L677 and following: Names of microorganisms have to be in italic L682: space missing before Nitrospira L692-694: Authors initials have to be the same L901: Capital D for Desulfovibrio L907: It is Sinninghe Damsté and not JSS (also check the name in all references for the é) Figure 2: it is HCO₃⁻ not HCO₃- Figure 3: It is Na

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