Biogeosciences Discuss.,
doi:10.5194/bg-2016-442-AC2, 2017

Biogeosciences Discussions
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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.

Valerie F. Schwab et al.

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Received and published: 29 January 2017

We thank referee #2 for the constructive and helpful comments. We will carefully consider each of them in the revised manuscript (RM).

Answer to general comments:

Line 59: S042- will be corrected Line 64: The sentence will be rewritten: "However, how exactly the composition and function of microbial communities in groundwater depend on hydrology, chemistry and the relationship to groundwater recharge dynamics is still not well understood"

Section 3.3: please describe how external contamination was avoided in the PLFA ex-

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tractions 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 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. Thank you for bringing this point of view: Yes, external contamination was avoided as much as possible. We will add this sentence in the PLFA extraction section "To minimize external contamination, all material (including filters) and glass in contact with the samples during extraction and purification were baked at 500 °C for 5h to remove organic contaminants. Only trace levels of 16:0 FAME have been detected in blank extracts".

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. Yes, as suggested, we checked the efficiency of the separation by simply running a glycolipid (digalactosyl diglyceride) and a phospholipid (1,2-dinonadecanoyl-sn-glycero-3-phosphatidyl-choline) standard thought the SPE column using the written protocol. No phospholipid derived FA (C17:0) and glycolipids derived FA (C17:2) was detected in the glycolipids and phospholipids fractions, respectively. See also answer to referee 1.

Line 184 Is there a reference for the SPE method used? No.

Line 188 define PLOHs; This will be changed to hydroxy-fatty acids ml should be mL: L will be written in all the RM

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.

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Peak areas relative to the internal standard could be used for the PCA however.

Thank you for bringing this important error in view. No commercial standard is available for ladderanes. Therefore, as suggested, we will use the relative peak area for PCA. See attached new PCA figures using relative peak areas (figures 4,5 and 6 of the RM). Changes in the RM will be made accordingly.

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

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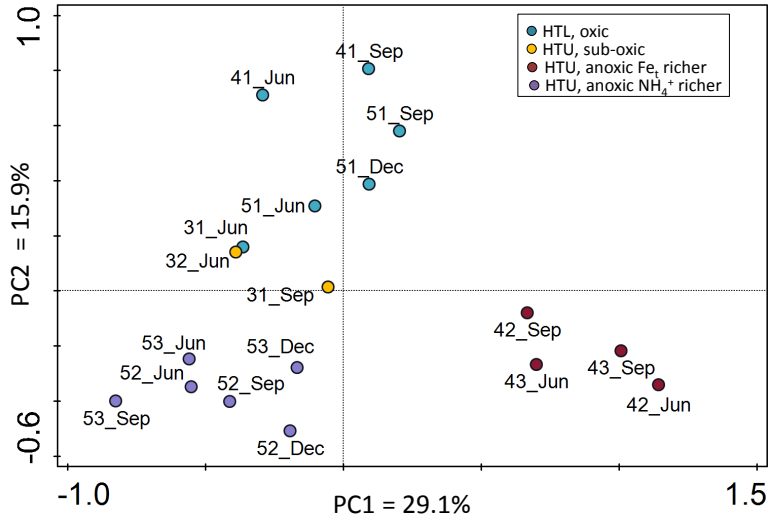

Figure 4: Principal component analysis (PCA) of PLFAs composition. The different wells are represented by dots with different colours: blue for oxic groundwater, yellow for sub-oxic/oxic groundwater, dark red and violet for anoxic groundwater richer in Fe_t and NH_4^+. Note the separation between the lower and upper aquifer (HTL and HTU, respectively) and the anoxic wells at location H4.2/4.3 and H5.2/5.3.

Fig. 1.

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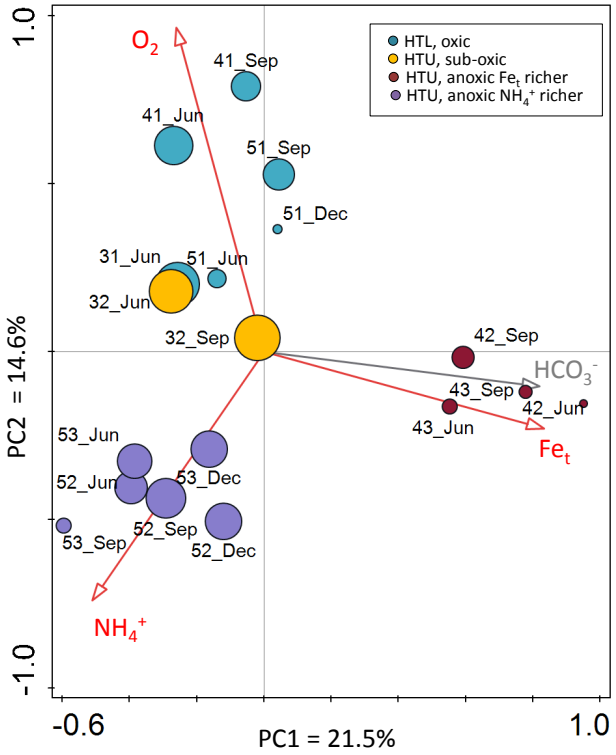

Figure 5: Redundancy analysis (RDA) of PLFAs, used as species, and the most significant environmental parameters O_2, NH_4^+ and Fe_t that explained 37.7% of the variability. The different wells are represented by dots with different colours: blue for oxic groundwater, yellow for sub-oxic groundwater, dark red and violet for anoxic groundwater richer in Fe_t and NH_4^+.

Fig. 2.

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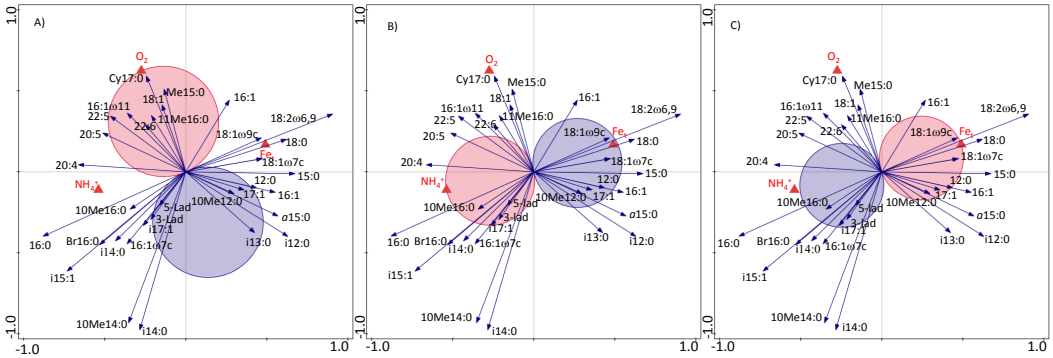

Significance test for variation partitioning

Tested Fraction	% of explained variation	F	P
a+b+c+d+e+f+g	100	3.1	0.002
a	21.0	2.2	0.008
b	19.8	2.1	0.034
c	16.9	1.9	0.016
a+d	---	3.1	0.001
b+e	---	3.6	0.004
c+f	---	3.5	0.002

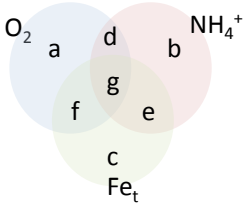

Figure 6: Variation partitioning t-value biplots showing the PLFAs significantly correlated with the environmental variables (A) O_2, (B) Fe_t and (C) NH_4^+. Results of the significance test of the variation partitioning are shown in the associated table. The PLFAs are represented by arrows projecting from the origin. Concentration changes, between sampling data, of a particular PLFA is significantly related to concentration changes of the environmental variables, when the arrow-tip of those PLFA is enclosed within circles. The arrow-tip is enclosed within the red circle for positive correlation and inversely within the blue circle for negative correlation.

Fig. 3.

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