

Dear Dr. Marcel van der Meer,

Please find attached the corrected version of our manuscript entitled “Functional diversity of microbial communities in pristine aquifers inferred by PLFA – and sequencing – based approaches” by Valerie F. Schwab et al.

We found the comments of the referees constructive and helpful, and carefully considered each of them in this revised manuscript (RM). Below please find our point-by-point responses to the referee comments. Changes are reported in yellow background in the RM.

Best regards

Valerie F. Schwab

Answers on the general comments from referee 1:

Possible bias due to an incomplete separation between the glycolipids, betaine lipids, sulfoquinovosyldia-cylglycerols (SQDGs) and phospholipids fractions was mentioned in the introduction lines 83 to 87 and lines 94 to 106.

Additionally, the efficiency of the separation between glycolipids and phospholipids was tested running a glycolipid (digalactosyl diglyceride) and phospholipid (1,2-dinonadecanoyl-sn-glycero-3-phosphatidyl-choline) standard through the SPE column using the written protocol. This was mentioned lines 196-202.

Answers on the minor comments from referee 1:

Text related to PCA analyses was clarified; lines 255-256, lines 312-322 and lines 339-344.

The abstract has been slightly shortened.

Lines 66-67: This sentence has been corrected

Lines 74-90: The description about the possible source of the PLFA was deleted from the introduction and we added Table 1.

The description of the sampling site has been included into the Material and Methods.

Lines 183-184: TLE was changed to BDE

Figure 1 was clarified and the sentence line 122 was added

SO₄²⁻ has been corrected throughout the RM

prefixes of fatty acids like iso and anteiso (a or ai) were put in italic

l was changed to L throughout the RM

CO₂⁻ was corrected throughout the RM

mol has been changed to % relative abundance (see comments referee 2)

S_t was corrected throughout the RM

NH₄⁺ was corrected throughout the RM
sulphate and sulphur were corrected throughout the RM
space after % was removed throughout the RM
Fe_t was corrected throughout the RM
The C in 10MeC12:0 (and in other PLFAs) was removed throughout the RM
Fe²⁺ was corrected throughout the RM
Reference list was changed according to Journal style. Doi was added, author name was corrected
and name of species was noted in italic. However, I will need indication how I shuo
Figure 2: HCO₃⁻ was corrected
Figure 3: Na⁺ was corrected

Answers on the general comments from referee 2:

Line 57: S04²⁻ has been corrected
Line 62-64 the sentence has been rewritten.
To descript how we avoided external contaminations we added line 176- 178
Results of the test about the efficiency of the separation between the glycolipids and the
phospholipids were added line 196-202.
Line 191 PLOHs was changed to hydroxy-fatty acids
ml was changed to mL throughout the MS
As mol% cannot be calculated, as suggested by the referee 2, the PLFAs were quantified
relatively the internal standard C19:0 and then converted in % for the PCA analyses. This was
noted Line 234-235 and Line 242
% PLFAs have been changed in annexed Tables and PCA and RDA were re-evaluated using %
PLFAs (new figures 4,5 and 6).