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Interactive comment on "Structural elucidation and environmental distributions of butanetriol and pentanetriol dialkyl glycerol tetraethers (BDGTs and PDGTs)" *by* Sarah Coffinet et al.

Anonymous Referee #1

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In this article, the authors report on the structural identification of a recently discovered class of archaeal membrane lipids, the butanetriol and pentanetriol dialkyl glycerol tetraethers (BDGTs and PDGTs) using 1D and 2D NMR techniques on isolated BDGT-0. In addition, their occurrence and possible source organisms in contrasting environmental settings is discussed, notably in the light of the stable carbon isotopic composition of the biphytane alkyl chain (bp- 0) released upon ether cleavage compared to that of bp-0 from GDGTs, methane, TOC and DIC from the corresponding sediment samples. Overall, this manuscript is very well written, easy and pleasant to read, the data seem reliable and the interpretations are generally well argued and convincing. I have, however, a few (minor) points to be discussed and commented prior

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acceptance for publication in Biogeosciences. The first point deals with the NMR structural characterization of BDGT-0. The authors conducted top-quality work to isolate ca. 0.9 mg of BDGT-0 with a high purity, and use high tech NMR material to perform the 1D and 2D experiments necessary for full characterization. However, in the manuscript, the description of the NMR data is quite poor and very succinct, key points regarding the structural elucidation of AT LEAST the glycerol/butanetriol moieties deserving to be argumented in much greater details (e.g., the methyl group C4'should be present as a doublet. Is it the case? If so, what is the value of the coupling constant?). What kind of correlations proving the proposed structure can be used from the 2D NMR experiments? In the present state, strictly no arguments are provided to justify the proposed structure, and notably why the extra methyl group is located at C-3' and not elsewhere. Since this identification is of prime importance in the sense that it describes a novel compound series (cf. the title of the manuscript), more work is needed (see the paper from Sinninghe Damsté et al., 2002 in Journal of Lipid Research as an excellent example of NMR description for such complex compounds). Figures illustrating some key features could be added, either to the main manuscript, or as supplementary material. A second point deals with the possible biological origin(s) of the BGDTs and PDGTs. According to the authors, and given the d13C values of bp-0 released upon ether cleavage/hydrogenolysis of isolated IPL BGDT-0 and IPL GDGT-0, as compared to the d13C values of CH4, TOC and DIC in different sedimentary settings, BGDT-0 shows a d13C composition systematically more 13Cenriched than CH4. As pointed out by the authors, this indicates that the microorganisms producing BGDTs (and PGDTs) are not methanotrophs. It is then suggested that the d13C values determined for bp-0BGDT in the Black Sea and Rhone delta may be indicative of methanogens, whereas other source organisms with a different metabolism may produce BGDTs in the other settings. From the data presented, I agree that a methylotrophic source for BGDTs can be ruled out, but this is more or less all that can be deduced from the d13C values. Mixed sources (instead of methanogens) cannot be excluded in the case of the Black sea and Rhone delta sediments (methane itself likely originates from different producers), and a

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methanogenic origin cannot be ruled out in the case of the other sediment settings (cf. discussion from lines 240 to 252). From the abstract (lines 18-22), these possibilities in the different setting are presented as being more “clear-cut” as they really are. This should perhaps be reworded (in the abstract). In addition to these two main points, there are a few minor corrections (typos, mainly) to make. - Lines 18-22 (abstract): see previous comments about mixed sources in the Black sea and Rhone delta sediments - Line 123: DCM at 60 °C? In a sealed tube? Please specify. - Line 129: Is the second decimal value (0.03‰ meaningful in the case of d13C measurements? - Line 131: replace “analysis” by “composition”. - Line 168: “high-resolution one and two-dimensional . . .”. See also general comments for section 3.1, in which the NMR data (of the butanetriol moiety, at least) should be discussed in much greater details. - Line 288: replace “side-chains” by “isoprenoid chains”. - Line 288: If I’m right, in Elling et al., the extra methyl group reported is located on the glycerol moiety (“MeO-Archaeol”) and not on the isoprenoid chain. - Line 298: “butanetriol- or pentanetriol-based” - Lines 324-326: “suggesting a distinct role in the cell membranes”. What do the authors mean by this? Given the structure of BGDTs (hydrophilic head groups and hydrophobic isoprenoid chains), it is difficult to conceive a distinct role than “classical” GDGTs. . . And what is the relationship between the differences in the d13C composition of BDGTs and GDGTs and the membrane role? The differences in stable carbon isotopic composition can be attributed to different microorganisms producing BDGTs and GDGTs and having different metabolisms. - Line 364: Methanomassiliicoccus luminyensis: in italics - Line 365: add volume number (82) and page numbers (4505-4516) - Line 383: De Rosa - Line 405: replace “802” by “802-805” - Line 410: Add page numbers (3090-3095) after 63, and delete “6” - Line 431: Candidatus: in italics - Line 469: D14C - Line 469: delete “15” and add page numbers (3123-3137) - Table 1: To be revised and completed according to the first main comment. - Figure 5 (caption), Line 558: add “between dissolved C02 and DIC, considering. . .” after - 10.7‰

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