

Interactive comment on “Novel hydrocarbon-utilizing soil mycobacteria synthesize unique mycocerosic acids at a Sicilian everlasting fire” by Nadine T. Smit et al.

Nadine T. Smit et al.

nadine.smit@nioz.nl

Received and published: 9 December 2020

We thank the associate editor, Dr. Naeher, for taking the time to consider our manuscript and provide helpful questions and suggestions for improvement. Below, we respond to the detailed comments.

- Line 99-109: Could you please note why you use BDE for extraction and saponification instead of other methods? Using BDE is a great method to prevent decomposition of some lipids, but then you undertake base hydrolysis? Did you also make a comparison of your results by looking at intact polar lipids/PLFAs as well? For the saponification step, is there a reason why you adjusted the pH to 5 and not lower than that?

We used the BDE extraction method to extract as many compound classes (including intact polar lipids) as possible out of the Censo soils. Aliquots of our lipid extracts were used for different types of analysis, such as analysis of bacteriohopanepolyols and intact polar lipids which require BDE extraction. The results of these ongoing analyses will be presented in follow-up research papers. The focus of this study is the identification of the unique mycocerosic acids and base hydrolysis (applied to release fatty acids from much more structurally complex intact polar lipids) was the method of choice for this work. We adjusted the pH to 5 since in our experience we get the best yields for PLFA turnover using this pH value in our lab. Please see also previous studies from our lab for example:

Heinzelmann, S.M., Bale, N.J., Villanueva, L., Sinke-Schoen, D., Philippart, C.J.M., Sinninghe Damsté, J.S., Schouten, S., van der Meer, M.T.J., 2016. Seasonal changes in the D/H ratio of fatty acids of pelagic microorganisms in the coastal North Sea. *Biogeosciences* 13, 5527-5539.

- Lines 197-198: Are you able to you add an approximate number to show how much/percentage the uncultured mycobacteria represent?

The relative abundances of assigned reads in the 16S rRNA gene amplicon sequencing is 34.1 % (Censo 0m) and 8.5 % (Censo 1.8m). This is listed already in Table 1, but we will include these numbers in the text in the revised manuscript.

- Lines 217-219: Could you please add more details about these “explorative searches”? Or even a reference to provide more information about the synthesis of biohopanoids in mycobacteria? In combination with comment reviewer (G. Inglis): L350: apart from having Shc gene, any other evidence the mycobacteria synthesise hopanoids? Any existing cultures?

Unfortunately, we cannot provide more detailed information as the analysis of the Censo mycobacteria metagenome was not part of this study. However, preliminary BLAST search for the shc gene in *M. simiae* mycobacteria showed the potential for

[Printer-friendly version](#)[Discussion paper](#)

hopanoid production, while other more explored mycobacteria like *M. tuberculosis* do not possess these genes. Moreover, pathogenic mycobacteria are able to synthesize sterols instead of hopanoids as described in these papers:

Lamb, D.C., Kelly, D.E., Manning, N.J., Kelly, S.L., 1998. A sterol biosynthetic pathway in *Mycobacterium*. *FEBS letters* 437, 142-144.

Podust, L.M., Poulos, T.L., Waterman, M.R., 2001. Crystal structure of cytochrome P450 14 α -sterol demethylase (CYP51) from *Mycobacterium tuberculosis* in complex with azole inhibitors. *Proceedings of the National Academy of Sciences* 98, 3068-3073.

- Line 333: Could you note briefly why the $\delta^{13}\text{C}$ values of methane and ethane are quite enriched in these seeps? Even if the background information is surely provided in the cited study and known to many readers of your manuscript, I think it could be useful for the reader that is not very familiar with this, because methane is commonly highly depleted in other settings.

The information is provided in more detail in section 2.2 on the study area. Methane and ethane derive from overmature marine source rocks and are of thermogenic origin resulting in a very dry gas with $\text{C}_1/(\text{C}_2+\text{C}_3)$ ratios >100 (Grassa et al., 2004). The thermogenic origin of these gases is responsible for the enriched $\delta^{13}\text{C}$ values at the Censo seep. Hence, in contrast to other seeps, microbial sources for methane and ethane are not important. We will add this info in the revised manuscript.

We will address the reviewers and editor comments in detail in a revised manuscript.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-349>, 2020.

Printer-friendly version

Discussion paper

