Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2020-349-RC2, 2020

Biogeosciences
Discussions

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

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Received and published: 3 December 2020

In this paper, Nadine Smit and co-authors investigate the lipid biomarker inventory in soils near a gas seep. They find a very high abundance of mycobacteria and 13C-depleted mycocerosic acids (MAs) near to the seep. The abundance of mycobacteria decreases away from the seep. This is accompanied by a corresponding increase in the carbon isotope composition of MA's. This implies that mycobacteria are utilising 13C-depleted substrates and demonstrates that MA's have the potential to provide new insights into carbon cycling within gas-rich environments.

This is a really lovely study with very few faults. The combined 16S rRNA/biomarker

approach is novel and the authors did an impressive job with the structural identification. I was also glad to see mass spectra in the figures – this will be very useful for other researchers! Overall, a really nice, well-written paper which should be published in Biogeosciences.

I do have a couple of suggestions:

1) You appear to have a unique, source-specific biomarker which can be used to study gas oxidation processes. Very cool! This is a useful addition to our "biomarker toolkit" and complements other "gas oxidation" proxies (e.g. BHPs or fatty acids which are specific to methanotrophs, or hopanoid carbon isotopes values which reflect the balance between heterotrophy and methanotrophy). However, you only identified MA's very close to the gas seep and they were absent in the 13m soil. Therefore, it is plausible that we might never find these lipids in paleo-record (at least using conventional GC-MS). Or am I wrong? Are there other sites/settings where these might be likely to occur? Perhaps in the marine realm? e.g. hydrothermal vent systems, mud volcanoes, cold seeps etc.

2) You mentioned that MA's have been found in ancient bones (ca, 20,000 years ago) but is there the potential for these lipids to be preserved further back in geological time? i.e. millions of year? And what putative degradation products might we expect to find in the geological record?

3) Did you look at the non-fatty acid fractions? Could there be any other diagnostic lipids hiding in the aliphatic fraction (for example)?

Minor comments:

Do we know why mycobacteria synthesise MA's with this weird branching pattern?

Have MA's been identified in any other bacterial strains? Or have people never looked?

Figure 3: although the abundance of mycobacteria are highest near the seep, the lowest del13C values actually occur 1.8m from the seep. One might (incorrectly) assume

that the lowest values would occur directly at the seep. Is there a suitable explanation for this?

Figure 2: I am not surprised that the C32 hopanoic acid dominates the non-seep samples – it appears to be the dominant hopanoic acid elsewhere (see Inglis 2018; https://www.sciencedirect.com/science/article/pii/S0016703718300036). However, I was intrigued by the C31 and C33 hopanoic acids. There doesn't appear to be any mention of these in the text – I wondered if: 1) you obtained del13C values from these lipids, and 2) what the putative source of these lipids are?

L39: I would also mention here (and elsewhere) that you can use: 1) specific bacteriohopanepolyols for methane oxidation (e.g. Talbot et al., 2016 OG; van Winden et al. 2012 GCA) and 2) hopanoid carbon isotopes (Inglis et al 2019 GCA, van Winden 2020 Geobiology) to probe methanotrophy in terrestrial environments.

L95: Sample collection = why 0.8 and 13.2m? Any methodological reasoning?

L339: alternatively, the -33 to -37 per mil values could reflect a mixed bacterial community (e.g. heterotrophs + methanotrophs; e.g. Inglis et al., 2019 GCA).

L350: apart from having Shc gene, any other evidence the mycobacteria synthesise hopanoids? Any existing cultures?

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