

The authors comprehensively characterized geochemistry and microbial communities of two seep sites in Monterey Bay, and observed very little DNA and RNA belonging to anaerobic methanotrophic archaea (ANME), particularly at Clam Field. They further supported this with gene quantification, microscopy, and sediment incubations under varying methane partial pressures, and posited that ANME may be outcompeted by other taxa in the presence of more complex hydrocarbons. They concluded that such a surprising absence of ANME has the potential to revise estimates of methane flux into the hydrosphere.

This manuscript is well-written, and the data are presented and interpreted in a logical order. I particularly appreciate how the authors contextualized their results as they presented them. I recommend accepting this manuscript once a few (mostly minor) details are clarified in the text. Hopefully my comments below are useful.

We appreciate this referee's thorough review, and we are happy to address their revisions and concerns in another draft. Our direct responses to their line-by-line comments are below in blue, with proposed textual additions in green.

Methods:

Lines 187-188: What was the minimum 16S sequencing depth of any sample? Were any samples discarded due to low read depth?

The minimum number of reads recovered per 16S rRNA gene sample (DNA) was 7,377, while the minimum number of reads recovered per 16S rRNA sample (cDNA) was 71. We will report these numbers in a future manuscript draft. No samples were discarded after sequencing, however in 3 16S rRNA samples (cDNA), the target did not amplify (and was therefore not sequenced). This is already shown in Fig. 4 – where bars are blank – and in Table S4. We will also add a clarification to the caption of Fig. 4.

Line 192: Please specify if the published *mcrA* sequences were manually compiled, or cite a relevant source.

The published *mcrA* sequences were manually compiled. This will be specified in a second draft.

Results

Line 295-296: Re “while the relative abundance of Bacteroidota decreased with sediment depth (Fig. 4a)” Just from looking at Fig. 4a, Bacteroidota look somewhat consistent with depth here- please justify with statistics or remove.

The decrease is extremely subtle in the plot but is clear in the values. We can therefore add the values (for all groups) to the sentence: “The relative abundances of Campylobacteria and Chloroflexi tended to increase with sediment depth in a core (by an average of 141% and 422%, respectively), while the relative abundance of Bacteroidota decreased (by an average of 53.4%) with sediment depth (Fig. 4a).”

Was no cDNA recovered from three of the samples at Clam Field seep-edge (Fig. 4a)?
Yes, correct! This is stated in Table S4, but we will make the following textual note to avoid confusion in the methods section: “At this stage, 16S rRNA genes and *mcrA* genes were successfully amplified from all 45 sediment horizons, while 16S rRNA was successfully amplified from 42 of 45 sediment horizons and *mcrA* transcripts were successfully amplified from 11 of 45 sediment horizons (Table S4).” We will also add a clarification to the caption of Fig. 4.

Line 336 “Seep-SRB1, the group containing many known obligate ANME symbionts”
Please provide a citation or two.

Thanks for pointing this out. We will provide the following four citations that provide evidence of ANME/Seep-SRB1 symbiosis: Knittel et al., 2003; Schreiber et al., 2010; Skenner et al., 2017; Metcalfe et al., 2021.

Lines 356-358: If I’m understanding correctly, the two highly abundant rows corresponding to ANME-2c in Extrovert Cliff both represent one ASV that cannot be resolved between the two reference sequences (and so its relative abundance is split between the two of them). I think it would minimize confusion if this were described more explicitly (particularly in the figure legend).

Yes, you’re understanding correctly! This sentence is already in the methods section: “Relative abundances of ASVs placed on internal tree nodes were divided among tip nodes associated with that internal node.” However, we will add a similar explanation to the figure caption for clarity. The caption will now include the following text: “Heatmap values were calculated by adding the relative abundance of all ASVs assigned to each tip node by EPA-ng (v. 0.3.8). (ASVs assigned to internal nodes were evenly divided among all tip nodes associated with that internal node. See methods.)”

Line 431: Please briefly justify the comparison to US Atlantic Margin seeps in particular: were they simply the most convenient to compare because similar data were collected by the same research group?

Yes, the USAM comparison sites were convenient to compare to because they were collected by the same research group, and all sampling, DNA/RNA extraction, sequencing, and sequence processing methodologies were standardized. In a future draft, the sentence will read: “To directly compare Monterey Bay seep microbial communities with canonical seep communities, we compared them to those of four seeps along the U.S. Atlantic Margin (USAM), which were sampled and sequenced with the same methodologies.”

Discussion

Lines 478-483: I see some similar phrasing between this and the third paragraph of the introduction of the Semler et al 2022 AEM study. This feels borderline unnecessarily picky to point out, given the same first author, but I mention it just in case the editor or publisher disagrees with me.

Thank you for mentioning this. We will reword this statement in a future draft, so it conveys the same meaning but reads: “This community is primarily composed of ANME archaea (including ANME-1a, -ab; ANME-2a, -2b, -2c, -2d; and ANME-3) and SRB (including members of the Seep-SRB1 and Seep-SRB2 in the Desulfobacterales, *Desulfobulbus* and Seep-SRB4 in the Desulfobulbales; and thermophilic HotSeep-1). Sulfide-oxidizing and aerobic methane-oxidizing Gammaproteobacteria, as well as the putatively methanotrophic JS1 lineage of Atribacterota, are also abundant at seeps.”

I understand that the lack of methane-dependent sulfate reduction and inability to enrich ANME in the clam field incubations would be considered a “negative” result, but why not comment on the Desulfobacterota that increased over time (Fig. S5?) Could these be the hydrocarbon degraders implicated in 553-555, and/or are they particularly good at thriving in sulfidic conditions?

Given the lack of methane-dependent sulfide production, and given that we did not add any non-methane hydrocarbons to our incubation headspace, we believe that the sulfate reducers enriched in these incubations do not make a living off of hydrocarbon degradation – they are instead thriving under increasingly sulfidic conditions. However, we will clarify in the text that the comparisons displayed in Fig. S5 were only comparisons between timepoints, not between methane headspace treatments; comparisons of T=6 WITH methane vs. T=6 WITHOUT methane yielded no significant taxa enrichments or un-enrichments, indicating that while communities changed over time, the presence of methane did not seem to cause these differences.