

## Reply to comments of Anonymous Referee #1

Methane oxidizing seawater microbial communities from an Arctic shelf (bg-2017-410)

Christiane Uhlig, John B. Kirkpatrick, Steve D'Hondt, and Brice Loose

This is the first study to use next generation sequencing techniques to study methane oxidizing bacteria in Arctic seawater and sea ice, combining community analysis with measurement of methane oxidation rates and incubation experiments with varying methane concentrations and incubation time. Methane oxidizing bacteria can play an important role in reducing methane flux to the atmosphere, but relatively little is known about what controls their abundance and activity, so this paper makes a nice contribution to the literature. The methane-oxidizing microbial communities in seawater and sea ice are different, but in both environments their relative abundance is fairly low. Relative abundance remained low even after incubation at elevated methane concentrations, though total abundance increased. I have no major criticisms of this paper.

Reply: We would like to thank Referee #1 for the encouraging and helpful review. Please find our replies to the specific comments below.

Specific comments: P6, L25: how many contaminant sequences were removed, relative to total sequences?

Changed to: Contaminating sequences observed in kit and filter blanks accounted for 1.4% of total reads and were removed from all samples.

P9, L16: 16S rRNA gene

Changed to: "16S rRNA" corrected to "16S rRNA gene"

P14: I'm not sure this means copper couldn't be limiting methane oxidation

Reply: Thanks for this comment. It is true, that we cannot rule out that methanotrophs containing particulate MMO and soluble MMO were growing on limiting copper (resulting in the presence of *pmoA* genes) while the actual oxidation was performed by sMMO. We suggest to modify the statement to "Copper, which is essential for expression of particulate methane monooxygenase, can restrict MOB growth (Zhitovchenko et al., 1995; Avdeeva and Gvozdev, 2017). In the absence of copper, many MOB express a copper-independent soluble methane monooxygenase (Hakemian and Rosenzweig, 2007). Since we did neither determine copper concentrations, nor the expression of particulate and soluble methane monooxygenase, we cannot exclude that copper was limiting in our study."

Figure S5: Is there a list of candidate OTUs somewhere? Figure S5 doesn't show many of these very clearly- I can't even tell what group of proteobacteria is shown in the first panel. Any theories on what's going on with these organisms if they're not oxidizing methane?

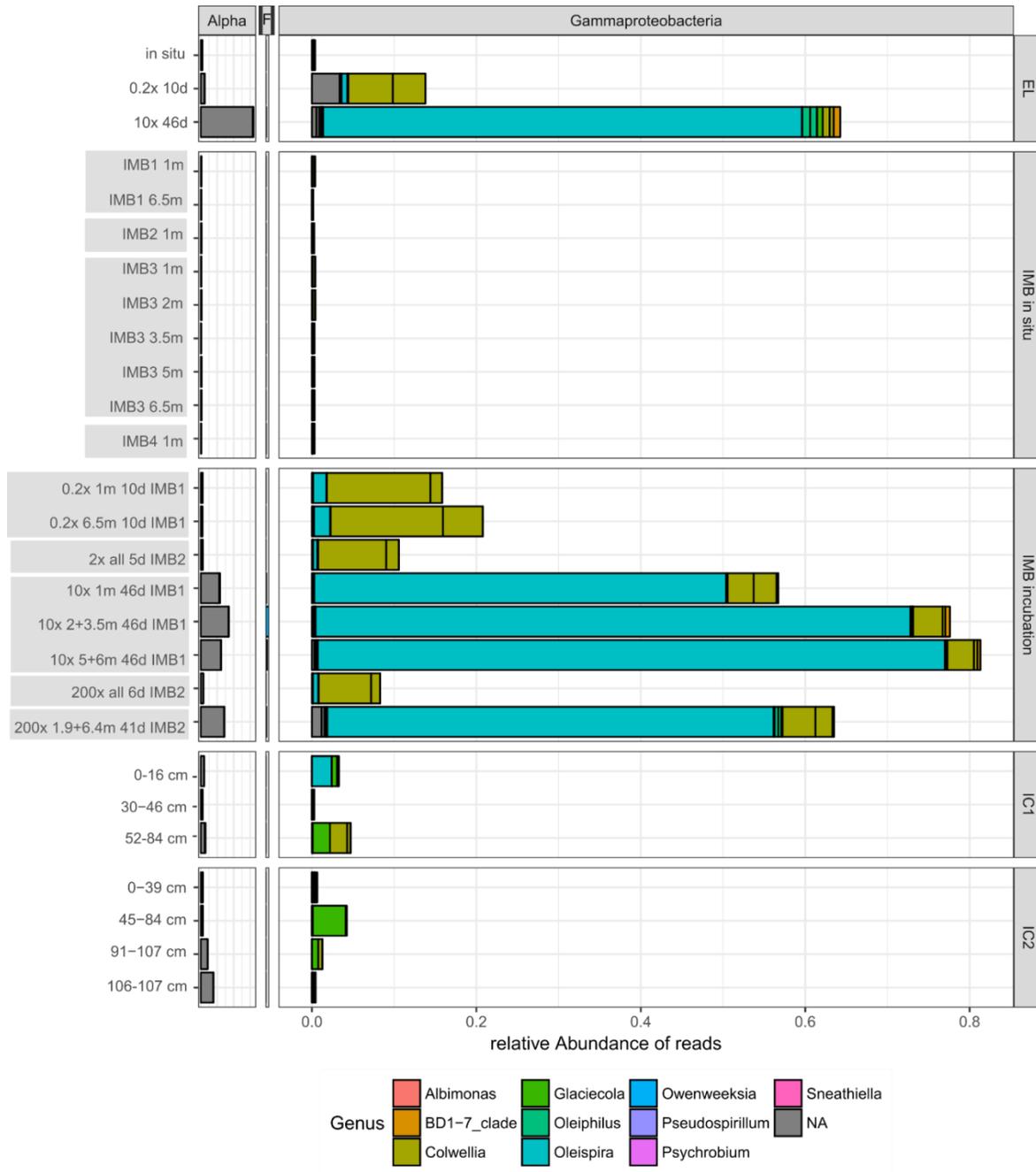
Reply: Thanks for the note. The labels of the graph were fixed and provided in the figure legend. A list of the candidate OTUs will be prepared for the revised version of the Supplementary Material. We hypothesize that those groups might be involved in cross-feeding on intermediate metabolites produced by the methanotrophs, which was for example observed for *Colwellia* (Jensen et al., 2008). But not the entire cell gain can be explained by methane as C-source. We thus suggest that the candidate OTUs might feed on available DOM, *Oleispira* for example is known to prefer complex organic substances (Yakimov, 2003). Although we aimed to remove OTUs favored by the "bottle effect", by removing types that were also abundant in 0.2x incubations, it is possible that the candidate OTUs are favored by the long incubations since the 10x and 200x incubations were incubated for about 41 to 45 days compared to 10 days for the 0.2x treatment.

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Figure S5:



**Supplementary Figure 5:** Relative abundances of differentially more abundant OTUs. The graph is subset for each sampling site or experiment in vertical direction, and for the different phylogenetic classes in horizontal direction; panels from left to right: Alphaproteobacteria, Flavobacteria < 4%, Gammaproteobacteria. X-axis scaling is identical for all panels. Not-normalized data was used to calculate relative abundances.

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