

***Interactive comment on “Different methanotrophic potentials in stratified polar fjord waters (Storfjorden, Spitsbergen) identified by using a combination of methane oxidation techniques” by S. Mau et al.***

**S. Mau et al.**

helge.niemann@unibas.ch

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Author: Interactive comment on “Different methanotrophic potentials in stratified polar fjord waters (Storfjorden, Spitsbergen) identified by using a combination of methane oxidation techniques” by S. Mau et al.

Anonymous Referee #1: This manuscript gives the results of a study evaluating the distribution of CH<sub>4</sub> oxidizing activity in a polar fjord using radioactive tracers, stable isotopes and molecular approaches. The authors correctly point out that measure-

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ments of CH<sub>4</sub> oxidation in ocean waters are comparatively rare, especially in high latitude environments. The study is also noteworthy from the perspective that multiple approaches are used to evaluate CH<sub>4</sub> oxidation. In my view the title of the manuscript is a bit confusing and perhaps should be changed.

Author: We suggest the following title, which we believe is less confusing: Vertical distribution of methane oxidation and methanotrophic potential in stratified waters of the arctic fjord Storfjorden (Svalbard, Norway)

Anonymous Referee #1: I interpret CH<sub>4</sub> oxidation potential to indicate the maximum rate of CH<sub>4</sub> oxidation, that is the zero order (substrate saturated) rate in the Michaelis sense. As in most studies of CH<sub>4</sub> oxidation in low CH<sub>4</sub> systems, a true “tracer” experiment cannot be performed, where the added isotope does not significantly affect the substrate pool (exception: Pack et al. cited herein). The additions here significantly increased the concentration of available substrate forcing back-calculation of the rate at the ambient substrate concentration from first order rate constants. However, radioactive CH<sub>4</sub> additions were not sufficient to elicit a zero order response; half-saturation constants for CH<sub>4</sub> oxidation are generally several  $\mu\text{M}$ , compared with the  $<500\text{nM}$  levels observed here, even after. Thus, the measured rates are not potential rates, nor are they an estimate of rates at the in situ CH<sub>4</sub> concentration, until adjusted via the first order rate constant.

Author: The reviewer is correct that the <sup>14</sup>C-CH<sub>4</sub> additions significantly increased total CH<sub>4</sub> concentrations (by about 450 nM compared to  $<80\text{ nM}$  CH<sub>4</sub> background concentrations). However, <sup>3</sup>H-CH<sub>4</sub> amendments increased the overall CH<sub>4</sub> concentrations only by 1-2 nM (as explained in the manuscript), thus, raising background concentrations only slightly. Certainly, using <sup>14</sup>C-CH<sub>4</sub> at (roughly) natural abundance levels as described by Pack et al. would even have been better to investigate methanotrophic activity at in situ CH<sub>4</sub> concentrations, but processing techniques are firstly very cost-intensive and were 2ndly not available for the present study. As explained in the introduction and discussion, our aims were to identify methane oxidation at near ambient-

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and at elevated CH<sub>4</sub> concentrations. The elevated substrate concentrations (~5-fold by addition of <sup>14</sup>C- CH<sub>4</sub>) led to a substantial increment in methanotrophic activity in water layers that are periodically subjected to high CH<sub>4</sub> concentrations, but not in those where CH<sub>4</sub> concentrations are generally low. We used the term 'potential' to describe this increment, which we found in some water layers. Therefore, CH<sub>4</sub> oxidation potential is not synonymous with the maximum uptake rate. We used potential to refer to methane oxidation that can increase if more substrate is available. It is true that by using only two different substrate concentrations (adding 2 nM and 450 nM), a kinetic study yielding half saturation constant (*k<sub>m</sub>*) and maximum reaction velocity (*v<sub>max</sub>*) cannot be done. We referred to the Michaelis-Menten concept to provide an explanation for our results showing an increment in methanotrophic activity at (artificially) elevated substrate concentrations in water layers that are periodically subjected to high CH<sub>4</sub> concentrations, while we couldn't find this in water masses where CH<sub>4</sub> concentrations are generally low. We believe that this justifies the conclusion that the methanotrophic community inhabiting the water masses that are periodically exposed to high CH<sub>4</sub> concentrations is adapted to high CH<sub>4</sub>/substrate levels, while the community inhabiting the water mass with generally low CH<sub>4</sub> concentrations is not adapted to metabolize additional CH<sub>4</sub>. This could be related to a low enzymatic *k<sub>m</sub>*. Low *k<sub>m</sub>* values (which were found to range between 10 nM-10 μM; e.g. Baani and Liesack, 2008, Bender and Conrad, 1992) could indeed explain this phenomenon. However, the available *k<sub>m</sub>* values from the literature were determined from organisms found in terrestrial or freshwater environments or from cultured bacteria, which most likely do not represent the still rather unknown marine communities. Furthermore, the enzymatic *k<sub>m</sub>* may not be the same as the apparent cell/community-based *k<sub>m</sub>* (see discussion by Button, 2010). We would thus like to refrain from further discussion of kinetic aspects and keep the Michaelis-Menten kinetic as an interpretative tool of our results.

Anonymous Referee #1: The authors give great detail in the Methods about recovering respired CH<sub>4</sub> and CH<sub>4</sub> incorporated into biomass or released as dissolved organic matter. However, it is not made clear whether the reported rates are for total CH<sub>4</sub>

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consumed or simply the fraction recovered as CO<sub>2</sub>, i.e. respired. On a similar note, since the assimilated CH<sub>4</sub> was fractionated, it may be useful and insightful to report the fraction incorporated into microbial biomass.

Author: The reported rates are given as total CH<sub>4</sub> consumed and metabolized to either CO<sub>2</sub> or organic carbon. We will clarify this in a revised manuscript and provide an overview of the fraction of <sup>14</sup>C in the organic C pool.

Anonymous Referee #1: The manuscript would benefit by placing the results in the context of other marine and even freshwater studies. The authors give a wonderful compendium of measured CH<sub>4</sub> oxidation rates in Table 1 (referenced only in the Introduction). This could be referenced again in a rate comparison in the Discussion.

Author: This is an excellent suggestion. We will incorporate a discussion on previous findings of water column MOx rates.

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**BGD**

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