Revisions for BG-2021-284

We thank the reviewers for their thorough reading of our manuscript and their constructive comments. Below we have copied each review in full (in black text), and highlighted (main) reviewer comments in **black bold** text. We provide our response to them in orange text. Text quoted from the original manuscript is in grey and proposed changes based on the review are in blue.

Thanks to these requested comments and suggestions, we feel the manuscript has improved considerably and hope that our proposed revision will meet the criteria for publication in ***Biogeosciences***.

Sincerely,

Christiane Schmidt (on behalf of all authors)

Report of Reviewer #3

The manuscript „ Deposit feeding of a foraminifera from an Arctic methane seep…" by Christiane Schmidt and co-authors describes a feeding experiment with Nonionellina l. from a seep site with cultured methanotrophs (Methyloprofundus s.).

The methods are described clearly and great care has been taken to ensure the viability of the foraminifers. **Impressive photos of the foraminifera are presented.**

The experimental set-up seems to me **(as a non-expert for foraminifera)** a bit weak:

- There were 5 specimens in each set up, but results for only 4 are reported

The reason why we accessed only4 of 5 embedded ones, was that the fifth one was usually at a bad orientation for us to do the preparation. It had nothing to do with a selection of specimen and was entirely random, based on how the organisms were by chance oriented in rhe resin.

- The **incubation time and/or incubation temperature was too short or too low**, as hardly any feeding (bacteria in vacuoles or near the aperture) was observed. Unfortunately, an extended incubation or slightly warmer temperatures, with an extension or repetition of the experiment is not possible....

We discuss elsewhere in this Response to Reviewers the issue of experiment timing. Please see those passages.

- The **presence of storage granulas and of gram-negative cell walls in the observed bacteria is not specific for methanotrophs, only the ICMs are characteristic for methanotrophs**

We changed the abstract to only include the word internal characteristics (and the ICM as a characteristic for methanotrophs, as reviewer 3 suggested.

Abstract: Several foraminifera are deposit feeders that consume organic detritus (dead particulate organic material with entrained bacteria). However, the role of such foraminifera in the benthic food-web remains understudied. Foraminifera feeding on methanotrophic bacteria, which are ^{13}C-depleted, may cause negative cytoplasmic and/or calcitic $\delta^{13}C$ values. To test whether the foraminiferal diet includes methanotrophs, we performed a short-term (20-h) feeding experiment with *Nonionellina laboradorica* from an active Arctic methane-emission site (Storfjordrenna, Barents Sea) using the marine methanotroph *Methyloprofundus sedimenti*, and analyzed *N. labradorica* cytology via Transmission Electron microscopy (TEM). We hypothesized that *M. sedimenti* would be visible post experiment in degradation vacuoles, as evidenced by their ultrastructure. Sediment grains (mostly clay) occurred inside one or several degradation vacuoles in all foraminifers. In 24% of the specimens from the feeding experiment degradation vacuoles also contained bacteria, although none could be confirmed to be the offered *M. sedimenti*. Observations of the apertural area after 20-h incubation revealed three putative methanotrophs, close to clay particles based on internal characteristics. Further, we noted the absence of bacterial endobionts in all examined *N. labradorica* but confirmed the presence of kleptoplasts, which were often partially degraded. In sum, we suggest that *M. sedimenti* can be consumed via untargeted grazing in seeps and that *N. labradorica* can be generally classified as a deposit feeder at this Arctic site.

These methanotrophs were identified based on internal characteristics such as a type I stacked intracytoplasmic membranes (ICM), this was clarified in the results and discussion section.

Line 271 -73 As noted, *Methyloprofundus sedimenti* is characterized by a typical type I intracytoplasmic membrane (ICM). Other characteristics, which are not specific for methanotrophs included storage granules (SG) and a typical gram-negative cell wall (GNCW) (Fig. 2).

We have also shifted some of the description of the methods section to the results section, again stating only ICM was used for identification of methanotrophs..

3.2. Ultrastructure of methanotroph culture used in the feeding experiment

Old Manuscript 266-269, *Metyloprofundus sedimenti* is characterized by a typical type I intracellular stacked membrane (ISM), storage granules (SG) and typical gram-negative cell wall (GNCW) (Fig. 2). These features were used to identify M. sediment.

3.2. Ultrastructure of methanotroph culture used in the feeding experiment

Line 245-253 Transmission Electron Microscopy was performed on culture aliquots to allow morphological comparison to previously published work (Tavormina et al., 2015). *Methyloprofundus sedimenti* strain PKF-14 cells have a gram-negative cell wall, coccoid to slightly elongated shape and characteristic intracytoplasmic membrane (ICM) and storage granules (SG) (Fig 2c). Additionally, 16S rRNA gene sequencing was performed (data not shown) to confirm it to be similar to the published *Methyloprofundus sedimenti* (Tavormina et al., 2015). *Metyloprofundus sediment*i is characterized by a typical type I intracellular stacked membrane (ICM). Furthermore, it has storage granules (SG) and a gram-negative cell wall (GNCW), which are not only characteristica of methanothrophs (Fig. 2).

Furthermore, we deleted two sentences from section, 3.3.2 Ultrastructure of aperture-associated bacteria, to match the comments of the reviewer 3.

Old lines 289-290 Specimen E36, from the 20-h treatment, hosted another putative methanotroph showing three large SG (Fig. 5). Storage granules occur throughout this putative methanotroph (Fig. 5c).

And changed description of Figure 2.

Old lines 293-295 As noted, *Methyloprofundus sedimenti* is characterized by a typical type I intracytoplasmic membrane (ICM). Other characteristics that are not specific for methanotrophs were storage granules (SG) and typical gram-negative cell wall (GNCW) (Fig. 2).

New lines: 319-322 As noted, Methyloprofundus sedimenti is characterized by a typical type I intracellular stacked cytoplasmic membrane (ICSM). Other characteristica which are not specific for methanotrophs, were storage granules (SG) and typical gram-negative cell wall (GNCW) (Fig. 2).

Also the Figure caption of Figure 2 has been changed to reflect the comment of reviewer 2:

Before line 190: The characteristic features for methanotroph identification is the typical type I intracytoplasmic membrane (ICM). Furthermore, other internal structures visible are storage granules (SG), and a gram-negative cell wall (GNCW).

In the **discussion, the relation of the study to porewater chemistry is a bit superficial and not necessary for the experiment.**

We address this issue elsewhere in this Response to Reviewers. Please see those passages.

Also, the **discussion on the SMTZ and anaerobic methane oxidation is miss-leading,** as the foraminifera have been sampled from the sediment surface, and also Methyloprofundus is an aerobic methane oxidizer, presumable from the sediment surface.

We address this inclusion of sediment geochemistry elsewhere in our responses. Please see those passages.

As so few bacteria have been found in or in front of the foraminifera the conclusion that they can feed on them is not justified.

We addressed this issue in the response to the reviewers #2. In summary, the feeding experiment itself did not yield a strong result, as the putative methanotrophs could be also a remain form the field or from the cysts which had been removed before experiments We have changed the text to reinforce the fact that this species is a deposit feeder and we have put less emphasis on the results related to the experiment. However, the results of the observation with putative methanotroph are clearly visible in Figure 4, and 5, and deserve to be published. We frame the association with the methanotroph to be putative, and do not emphasize in the title that this evidence is due to the feeding experiment. We hope that these points will be sufficient for reviewer 3 to approve publication.