Comment on bg-2021-284
Anonymous Referee #2

Referee comment on "Deposit feeding of a foraminifera from an Arctic methane seep site and possible association with a methanotroph revealed by transmission electron microscopy" by Christiane Schmidt et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-284-RC2, 2021

In this study, living individuals of the calcareous benthic foraminifera Nonionella labradorica were collected from active Arctic methane-emission site sediments and used for a one-day feeding experiment to investigate the uptake of the prokaryot methanotroph Methyloprofundus sedimenti. Transmission Electron microscopy pictures of the foraminiferal cytoplasm were analyzed to test the hypothesis that Nonionella labradorica is a deposit feeder and is able to ingest and feed on Methyloprofundus sedimenti. The presented TEM micrographs are of high quality, and show the deposit feeding character of Nonionella labradorica. But the feeding experiment itself demonstrates only week results.

The original scientific question of the manuscript fits into the field of biogeosciences, but the results presented do not fulfil this claim and the concept and data can only partly be described as novel. Therefore, no significant conclusions can be drawn with respect to the initial questions.

The scientific methods and assumptions are valid and clearly outlined, although I want to comment the following points:

- It is not clear for me why the authors put a lot of effort to the geochemical analyses and molecular genetics but did not show these data in detail and do not really link them to the experiment. Why they determined sulfate decline and the SMTZ if they collect their foraminifera from the 0-1 cm surface and feed them with methanotrophs from the water column? It would have been better to concentrate on the feeding experiment.
- If I understand correctly, the methanotroph food shows a natural labelling (line 63: methanotrophs produce the biomarker diplopterol, which has an extremely light δ13C signature (− 60 ‰)). It would have been of big advantage to use this kind of natural label or any other labelling to track a possible uptake by the foraminifera.
- The incubation time seems to have been planned too short, especially at these cold temperatures, because no uptake was observed.
- The authors mentioned a starvation phase before the experiment of 2-4h. Do they mean 2-4 days? Otherwise, 2-4 h are no starvation time, I would say. Foraminifera probably do not feed 24/7.
- The authors mention that for each experimental time point, 4 out of 5 foraminifera
were examined. Was the selection for these 4 purely random?

The results are not sufficient to support the interpretations and conclusions, and I want to comment the following points:

- The TEM analyses showed that all investigated specimens (collected in the field and in the experiment) contain degradation vacuoles containing clay/inorganics, which is a strong indicator for sediment uptake and deposit feeding in *Nonionella labradorica*, indeed. But this does not have something to do with the presented experiment, because sediment uptake took part before the individuals were placed in the petri dishes and the experiment started.
- The observed bacteria inside vacuoles or at the aperture area of the foraminifera are still few and not convincing, there seems no significant correlation. Living foraminiferal tests are surrounded by a sticky cytoplasm cover. Fig 4 shows sediment particles around the aperture. This means that specimens were not cleaned properly before placed in the petri dishes (contradict to the text in line 189). This kind of sediment may also be very sticky, as well as pseudopodia remnants around the aperture. Added methanotrophs may stick here by accident after some time. This seems to be more an artefact because of the experimental set up and should not be interpreted as an “association of foraminifera and methanotrophs” as noted in line 354 of the discussion.

The description of the experiment is very clear and allows reproduction. The authors give proper credit to related work and clearly indicate their own new contribution. The number and quality of the references is appropriate. The language is fine, although there are some minor mistakes in the text. The title reflects the contents of the paper, and the abstract provides a good summary. The introduction is very informative, but in parts drive off from the topic and raises high expectations in the experiment, e.g. that a direct link can be drawn from methanotrophic bacteria to the extremely light 13C-signals in foraminiferal tests. Concerning the supplementary material, I miss the data of the geochemical measurements.

The overall presentation is well structured and clear described. The TEM micrographs are of high quality, and show the deposit feeding character of *Nonionella labradorica*. This should be published! But I recommend publishing the data in a more foraminiferal-focused journal and changing the focus of the manuscript.